

**PRINT ISSN : 2395-6011**  
**ONLINE ISSN : 2395-602X**

**INTERNATIONAL JOURNAL OF SCIENTIFIC  
RESEARCH IN  
SCIENCE & TECHNOLOGY**

**VOLUME 2, ISSUE 4, JULY-AUGUST-2016**



Web Site : [www.ijsrst.com](http://www.ijsrst.com)

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# **International Journal of Scientific Research in Science and Technology**

**Print ISSN: 2395-6011 Online ISSN : 2395-602X**

**Volume 2, Issue 4, July-August-2016**

**International Peer Reviewed, Open Access Journal  
Bimonthly Publication**

**Published By**

**Technoscience Academy**

**(The International Open Access Publisher)**

**Email: [info@technoscienceacademy.com](mailto:info@technoscienceacademy.com)**

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# Micropropagation Photoautotrophic *Kalanchoe pinnata* in Water and Humus with use of Natural Light, and Determination of Total Flavonoids: A Review

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## ABSTRACT

Micropropagation is a vegetative propagation method widely studied in many different plant species, being a mode in tissue culture, the one that has found widespread and proven practical applications. Among the advantages of its use is the possibility of obtaining various plants from an initial explant, regardless of the season, besides the reduction of time and area required for the species propagation. The development of photoautotrophic micropropagation systems with natural light usage, emerge as potential possibilities to increase the micropropagation efficiency and help reduce costs, making it commercially viable for active principles and phytotherapeutics production. Thus, the flavonoid content contained in *Kalanchoe pinnata* plant extract, can base the pharmacological activity of it when used by population, for the cure and prevention of various types of chronic noncommunicable diseases (NCDs).

**Keywords:** *Kalanchoe Pinnata*, Micropropagation, Photoautotrophic, Flavonoids, Cost Reduction

## I. INTRODUCTION

### Photoautotrophic Micropropagation

Micropropagation is a vegetative propagation method widely studied in many different plant species, being the mode in tissue culture, the more widespread and has found proved practical applications [50]. Among the advantages of its use is the possibility of obtaining various plants from an initial explant, regardless of the season, besides the reduction of time and area required for the species propagation. Moreover, due to best sanitary conditions by cultivation of meristems previously treated by thermotherapy to eliminate diseases, there is the reproduction of the mother-plant genotype usually with fidelity during multiplication, which allows the propagation of species difficult to propagate by other methods.

The development of photoautotrophic micropropagation systems (production of micropropagules without addition of sucrose in the culture medium and under ambient conditions favoring photo-synthesis) [47], with natural light usage, emerge as potential possibilities to increase micropropagation efficiency and help reduce costs, making it commercially viable.

Studies have shown that light can determine the growth direction of plants (phototropism). It is able to determine chloroplasts [21, 22] and plant organs (photomorphogenesis) [21] differentiation, especially the leaf, the most susceptible organ to environmental changes [23, 24], and in other substances production, such as flavonoids [21]. This fact raises the possibility of studying various light sources effects at different wavelengths, focusing on plants in order to verify and modulate the amount of synthesized substances.

The light also strongly influences hormones biosynthesis. Vegetal hormones are molecules responsible for the seedlings development, present in low concentrations [26]. These substances act in all morphogenetic processes, including those induced by light. Studies have shown that gibberellin levels in events like disestiolation, germination and tuberization are directly linked to light, regulated by phytochrome or cryptochromes. The indole acetic acid (IAA) and cytokinins levels in disestiolation are also regulated by phytochrome. The light is able to regulate abscisic acid (ABA), ethylene and brassinosteroids levels in a phytochromes and cryptochromes dependent process [32]. The interaction between light, photoreceptors and plant hormones has a strong influence on plant life, even regulating tissues and organs development, that is, morphogenesis.

Studies have shown that the relationship between light and flavonoid biosynthesis is also closely linked. Flavonoids provide coloring for plants ranging from lilacto blue. The ability of these molecules to absorb wavelength in UV range gives to these substances a protection function against damage caused by this type of radiation [36, 37] and microorganisms or fungi [36].

Other authors have shown that different spectral ranges differently influence the development of plant organs, leading to changes in several anatomical characteristics such as leaf thickness, leaf area, stem diameter, stomata and trichomes density [53, 54, 55]. Besides morphological and anatomical features, light may influence secondary metabolites production such as flavonoids. The flavonoid biosynthesis occurs by secondary and mixed pathways (shikimate and acetate pathways), being regulated by blue and UV light in a process mediated by cryptochromes. In this process occurs the expression of the enzyme phenylalanine ammonia lyase, essential for the first step of fenilpropanoídica and chalcone synthase pathways, the first flavonoid biosynthesis pathway [38]. Due to these molecules protective action against microorganisms in plants, it makes them a target to pharmacological studies using plants.

The perception of color and the amount of substances that plants produce, as a rule, are related to spectral quality of light they receive. The solar radiation that reaches the Earth covers a spectral range extending

from 290 nm (ultraviolet) to 4000 nm (infrared) [21]. Many of these wavelengths are absorbed by ozone in the atmosphere, by the atmospheric oxygen and CO<sub>2</sub>. On average, 45% of the radiation coming from the sun are in the range from 380 nm to 710 nm [21]. It is in this spectral range that is the visible light, composed of seven spectral ranges, the seven rainbow colors.

In this range, there are also the wavelengths used by plants for photosynthesis (photosynthetically active radiation, FRG) [21]. However, light is not only utilized by the plant as a source of energy, it also controls the plant growth and development through signals. Plants are able to monitor the intensity, quality, direction and duration of light. As already mentioned, the solar radiation is decisive in many physiological processes such as stem elongation, germination, stomatal conductance, chlorophyll synthesis.

Due to its characteristics of sessile beings and their autotrophic nature, dependent on light energy, plants have developed environmental recognition mechanisms realizing the variations in the quantity and quality of light they receive, according to the vegetation cover in a particular location which can be closed or opened, light is gradually assimilated by layers of superimposed sheets.

Thus, the canopy energy is greater than the lower layers, meaning that the light reaching the soil is richer in wavelengths than that reaching the canopy, that is, it tends to contain more light in the red range. In addition, a few feet below the topsoil, only reaches lengths corresponding to the extreme red [21]. This effect is called radiation attenuation [21]. Different species require different amounts of light to its perfect development. Hence it is so important that plants recognize the different quantities and qualities of light where they are located.

In this manner, plants can regulate their development in order to seek light. This recognition process is through special molecules called photoreceptors, able to recognize different wavelengths. The processes regulated by light are categorically divided into two main classes: phytochrome mediated, red light receptors and cryptochromes mediated, blue light / UV-A receptors [26]. The phytochrome are the most characterized photoreceptors and form a family of

five proteins (phytochrome A, B, C, D and E) of approximately 125 kDa [27].

Each phytochrome has two forms, in particular spectrally reversible: one that absorbs red light (Pr - biologically inactive) and one that absorbs extreme red wavelength (Per - biologically active). Pr, when absorbs light in the 620-680 nm range is converted to the Per, this by absorbing radiation in the 700-800 nm range becomes Pr. The balance between the two forms, given by red and extreme red proportion in the environment light, will cause physiological responses in the plant [28, 21, 27].

The greater the amount of far red, the plant is more shaded. The amount of red and far red that the plant receives also indicates the seasons (long or short days).

This information determines the plants aspects such as germination, flowering, maintenance or not plumular hook, chlorophyll synthesis and circadian rhythm. Although in most cases, responses regulated by phytochrome are linked to red light, some studies show dependent responses of blue and UV-A light [29,30].

The cryptochromes are a family of blue / UV-A light photoreceptors extremely important during disestiolation of plants grown in the dark [31]. They are involved in the inhibition of stem growth processes, cotyledon expansion and chlorophyll synthesis. There have been shown that cryptochromes act in coordination with phytochromes in several processes and its action is temperature dependent [31]. However, phytochrome and cryptochromes, along with other photoreceptors, are not the only substances that act in photomorphogenic events. Other substances act on plant tissue, translating in them environmental light conditions. These substances are plant hormones.

It is possible to observe that among the environmental factors that most influence the plant development is the lighting. Studies have indicated that in addition to the amount of light received, the spectral quality of it is also important as it can induce morphoanatomic answers, for example, it can cause different red and extreme red ratios altering the structure of the mesophyll [24] or the stomata density optional components. The standard medium used universally for micropropagation of medicinal

[33]. The leaf is the organ that most responds to environmental radiation, being therefore chosen by many authors as object of study of spectral quality and quality effect in plants [34, 33, 24, 22, 35]. Different plant species are adapted to different lighting conditions, with an amount of light to be optimum to perform photosynthesis. Plants usually have morphoanatomic features related to these different light environments. Such characteristics provide the best use of the incident light and protection of photosynthetic apparatus [21]. During its life, plants can be exposed to different lighting conditions, so it is possible to notice that most plants have developed adaptation mechanisms, mainly anatomical, of the individual to the new climatic conditions [22]. This flexibility occurs even at a cellular level, as in the case of different organizations and developmental changes in mesophyll chloroplasts [21, 22].

Some advantages of photoautotrophic micropropagation at the expense of natural light compared to the conventional method of micropropagation include plant growth increase. Due to the removal of sucrose from the culture medium, there are improvements in the physiological characteristics of the plant, once the cultivation environmental conditions are more natural, reducing plant stress during acclimatization, increasing the percentage of survival of seedlings [48, 49, 50], elimination of lighting costs and reduced costs for repairs and maintenance, and also possibility of use of simplified facilities reducing construction costs [51].

In conventional micropropagation, the heterotrophic or fotomixotrónica nature of plant growth is directly or indirectly responsible for most of the factors related to the cost of production of micropropagated plants [50]. Explants are cultured in flasks without gas exchange and with high relative humidity (about 98%), high ethylene concentration, low CO<sub>2</sub> concentration (which decreases from 3.000 to 1 mol 9.000µmol the dark period to less of 100µmol mol<sup>-1</sup> during the photoperiod), and low photon flux density of photosynthetically active. That means that low lightning (40 - 50µmol m<sup>-2</sup> s<sup>-1</sup>) and with sucrose as a major source of metabolic energy [52], once the explants show low photosynthetic rate [50]. The nutrient medium used is composed of essential and

plants is MS medium (Murashige and Skoog) [56], which has as essential nutrients: inorganic salts, carbohydrates, vitamins and growth regulators.

Many explants or in-vitro plants have the ability to grow photoautotrophically, i.e. without sucrose in the culture medium and under environmental conditions, which promote photosynthesis [50]. The photosynthetic process, contrary to what occurs in the respiratory chain, need an external source of energy, without which it does not occur, showing that there is a strong relationship between light and plants, photosynthetic organisms. Since, during photosynthesis, light is not only a regulation factor, it acts as an important component in the biochemical reaction [21], being able to interfere in several physiological factors, such as chlorophyll production, stem stretching regulation, in enzymes production and other substances such as anthocyanins, a type of flavonoid [21]. In this sense, it is understood that the light is a determining factor for plants to be able to, from inorganic molecules, synthesize carbohydrates.

### ***Kalanchoe pinnata* Description**

The Brazilian flora is extremely rich, because in it there are thousands of species of medicinal plants, and among these plants there is *Kalanchoe pinnata*, which despite being widely used by the population, still has few studies proving its medicinal properties. It is a perennial plant, therefore easily found in several climates and regions of the world, and easy handling due to their morphological characteristics (Table 2).

The different species of the genus *Kalanchoe* (Crassulaceae family) are known in popular medicine in many countries as it can be observed in Table 1. As used in treatment of inflammatory processes and in several diseases. This fact favors the search for new bioactive molecules. Among the main active principles of *Kalanchoe pinnata*, there are the polyphenols [59], which are flavonoids, antioxidants, mucilage and others (Table 3). Among the present flavonoids, there is quercetin, which has shown significant action in the leishmaniasis treatment [41].

Quercetin and kaempferol are flavonoids widely spread throughout the plant kingdom and

have significant anti-inflammatory action, that can be attributed to inhibition of the enzymes phospholipase A2 (PLA2), 8 lipo-oxygenases, cyclooxygenase and inhibition of nitric oxide production, through modulation of enzyme iNOS [35, 37, 59].

**Table 1- Taxonomy of *Kalanchoe pinnata* (Lamarck) Persoon:**

<b>Kingdom</b>	Plantae (Plantas)	
<b>sub Kingdom</b>	Tracheobionta (vascular plant)	
<b>Super division</b>	Magnolioliophyta (Flowering plant)	
<b>Class</b>	Rosidae	
<b>Order</b>	Saxifragales	
<b>Family</b>	Crassulaceae Stonecrop family [39]	
<b>Genre</b>	<i>Kalanchoe</i>	
<b>Species</b>	<i>Kalanchoe pinnata</i> (Lam.) Per [14]	
<b>Synonyms</b>	Verea pinnata, Crassuvia floripendia, C. calyculata, Cotyledon calycina, Bryophyllum calycinum, Crassula pinnata, Sedum madagascariense, B. germinans, C. rhizophilla. [13]	
<b>Source</b>	Uncertain. It is believed to be the Mauritius Islands, Africa, India and Indian Ocean islands	
<b>Distribution</b>	It is distributed throughout India and grown in wild gardens in the hills of northern and western India, Deccan and Bengal [18]. In Brazil, they can be found from Sao Paulo to Bahia, mainly in the coastal zone.	
<b>Regional Names</b>	Brazil	Saião, Folha-da-fortuna, Coirama, Roda-da-fortuna, Folha-da-costa, Folha-grossa, Erva-da-costa
	India	Zakhm-hayat
	Arabia	kushnulhayat
	Walking stick	Koppata
	Sanskrit	Asthi-bhaksha
	telugu	Simajamudu
	Tamil	Ranakalli
	kannada	Ganduklinga
	malayalam	Elamurunga
Persian and Urdu	Chubehayat [17, 19]	

**Table 2 :** Morphological characteristics of *Kalanchoe pinnata*:

<b>Dimension</b>	Sublenhosa plants, perennial fleshy, 1.5 meters high; succulent stems, hermaphrodite, tubular, penduladas, pale green or yellow reddish.
<b>Seeds</b>	Oblong small soft - ellipsoid, striatum bad, soft. The leaves often produce, at the ends of the lateral nerves, buttons furnished with roots, stems and leaves, which drop off at the same time become new plants [13].
<b>Sheets</b>	Juicy, Dimensions: length 7-20cm 4-9cm wide on average, 10 to 25 leaves, or occasionally when lower generally have 8-12 and 6-8 in size, the upper usually 3-5 or sometimes 7 - leaflets, long and pointed, the united petioles by a ridge around the stem. Leaflets ovate or elliptical, serrated [17].
<b>Fruits</b>	With 10-14 mm long, produce numerous seeds per fruit in closed calice and corolla. [13]
<b>Trunk</b>	succulent stems, hollow, rarely branched. [13]

One of the main features of *Kalanchoe pinnata* is its ease in micropropagation in aquatic environments and on land, having high adaptability to different climates and easy handling conditions (Table 3). Due to these factors, it was decided to replace the synthetic medium MS [56] for water and earth through composted with natural bio-fertilizer and humus, and bio-organic materials used in biofertilizers, provided from waste solids reuse fruit.

According to Fukuoka [59], the natural fertilization is defined by natural conditions, soil regeneration with the soil itself, returning to the soil what it has given, such as fruits, vegetables and grains. The soil is enriched progressively mind, and the content of natural mind-nutrients present is balanced with the help of natural fertilizer from the own culture developed in this soil. In Table 3 below the optimal growth conditions for the *Kalanchoe pinnata*.

**Table 3.** Characteristics of a better climate adaptation and management:

<b>Life Cycle</b>	Perennial
<b>Climate</b>	Enjoy warm weather
<b>luminosity</b>	Enjoy light full sun and half-shade
<b>pruning</b>	Not necessary, but cut dry parts can be carried out, and to contain the spread, remove new growth if you want
<b>Cultivation</b>	It must be used substrate that has good drainage, which does not accumulate water which will cause the death of the plant. Mixing Tip: two pieces of coarse sand construction, a part of common garden and land part of the plant. It supports soil with low humidity, and also develops in water
<b>Fertilization</b>	At the site preparation, apply about two NPK tablespoons of 04-14-08 formula per square meter
<b>Propagation</b>	Vegetative from seedlings, and shoot buds formed along the sheet margins, producing young plants [40]
<b>Medicinal plant</b>	Her daughter medicinal properties have been used in the treatment of various diseases.
<b>Chemical constituents</b>	Mucilage, tannins, organic acids, minerals, quercetin glycosides [41, 43]. bufadienólídeos [44, 45].
<b>Toxic plant</b>	Special care should be taken with small children, pets and master-mind with grazing animals
<b>Use</b>	Quite showy ornamental effect, is very well in rock gardens forming together with other succulents

The objective of the research was to investigate the development of the crop in water and soil with humus (natural biofertilizers) of *Kalanchoe pinnata* leaves, (Lam Pers), kept under natural light (photoperiod).

Extracts of developed sheets were evaluated by spectrophotometry, as the concentration of flavonoids, which is, the minimum necessary to justify the

medical activities of the extracts of *Kalanchoe pinnata* leaves described in Table 4.

**Table 4:** Medicinal Uses Worldwide:

<b>Brazil</b>	Furunculosis [20], Intestinal problems, arthritis, ulcers, athlete's foot, abscesses, bubos, bronchitis, burns, calluses, conjunctivitis, corns, dermatosis, coughs, earaches, eczema, fever, urinary insufficiency, rheumatism, itch, glaucoma, infections, headache, kidney stones, scurvy, tumor, wart, sedative, whooping cough, wounds, insect stings, lymphatic disorders, mouth sores, respiratory infections, mouth sores, erysipelas [10]. Immunosuppressant, leishmaniasis [41, 43], diabetes [41, 42, 43] hemostatic, antiseptic, healing topical, inflammation (stomach pain) [15, 16]	
<b>USA</b>	Chicken pox, stomachache, fevers [10]	
<b>Mexico</b>	Inflammations, wounds, eye infections, headaches, menstrual disorders [10]	
<b>Ecuador</b>	Bruises, broken bones [10]	
<b>Guatemala</b>	Diarrhea, pain, skin problems, aches [10]	
<b>Nicaragua</b>	aches, burns, colds, pain, fever, headache, respiratory infections, coughs, childbirth [10]	
<b>Bangladesh</b>	Coughs, fever, constipations, mucus, epilepsy [11]	
<b>Peru</b>	Bacterial infections, boils, broken bones, ulcers, urethritis, sore, skin problems, nausea, migraine, intestinal problems, eye infections, epilepsy, gas, headache, heartburn, inflammation, cancer (lymphoma), bronchitis, conjunctivitis, coughs, earaches [10]	
<b>South America</b>	Asthma, tumors, headaches, colds, earaches, chest colds, sores, strains [10]	
<b>Nigeria</b>	Coughs, earaches, pimples, inflammation, eczema, cut umbilical cord in new born baby [7]	
<b>West Indies</b>	Ulcers, menstrual disorders, urinary disorder, hypertension [10]	
<b>Other places</b>	Arthritis, asthma, burns, bruises, constipation, malnutrition, headaches, migraines, nephritis, respiratory infections, earaches, diabetes, paralysis, rheumatism, swelling [10], and to induce vomiting of blood, expel worms, cut umbilical cord in new born baby [7]	
<b>Vietnam</b>	Anti-inflammatory and antibacterial [8]	
<b>India</b>	Orissa	Diarrhea [5, 2]
	Karnataka	Leaf juice externally, applied to scabies and leucoderma and leaf decoction applied over, cuts to stop bleeding [10, 12]
	Maharashtra	The leaves are used against cough dysentery [4]
	in Himalaya	Leaves are applied on wound, bruises, insect bite, swelling [9]
	Arunachal Pradesh	Leaf extract is taken in empty stomach is used in the treatment urinary bladder stones and fever in children's [3]

## II. METHODS AND MATERIAL

### A. Humus preparation

The papaya, banana, orange, mandarin and mango residue were gathered and milled with the aid of an organic waste processor in order to form a biomass of fruits. They were then added to water ratio gradient in order to obtain the best uniformity for the process of fermentation the kefir grains, for cleaving complex sugars and free reducing sugars, providing among these microelements to facilitating uptake by plants. During

the fermentation, the fruit biomass was placed in glass containers covered with fabric. Subsequent to fermentation, biomass was added to the earth for the humus formation, through composting method. Fermented biomass was evaluated to their chemical composition of trace elements as well as dosage of total ash, moisture, protein, carbohydrates, glucose, fructose, fiber and lipids.

### B. Determination of the chemical composition of biomass fruit

The chemical composition of biomass (consisting of papaya, orange, mango, banana and tangerine waste)



was determined according to the methodology of the AOAC [57]: moisture (925.45); protein (960.52); lipids (920.39) and ash (923.03). The conversion factor used for determining the protein content was 6.25. The energy value was calculated using the general factors of Atwater and considering the energy from dietary fiber (DF) [58]. The analyzes were performed in triplicate and the results expressed in g per 100 g of sample in the integral base. The DF content was determined according to AOAC method 991.43 [57]. The analysis was done in quadruplicate and the results expressed as g / 100g sample basis. Dosages of glucose and fructose were made in triplicate by Waters chromatograph column for detection of sugars (Shodex SC 1011); refractive index detector; Mobile phase: EDTA-Ca 0,187g / l. Chromatography conditions: column temperature: 72 ° C; detector temperature: 45C, mobile phase flow: 0.6 ml / min. Detector sensitivity 32, Volume injection: 10µL, running time: 30 minutes.

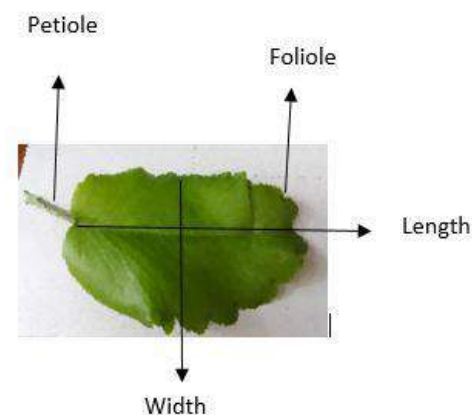
### C. Photoautotrophic Micropropagation

The leaves of *Kalanchoe pinnata*, initially developed in soil, were transferred to individual containers in which were placed submerged in water. The jars were kept at room temperature, covered by a synthetic porous fabric (TNT), provided direct daylight (photoperiod). Initially, the dimensions and leaflets of each sheet were measured. After a period of seven to ten days of permanence in the same water, the size of sprouts from the leaflets and their roots were measured as shown in Fig. 1, and the water was changed. Each bud, with its leaves and roots were transferred to the humus (prepared from biomass fruit fermented with kefir and composted). It was transferred 0.5 ml of *K. pinnata* extract solution into a 25 mL volumetric flask and was added 0.100 mL of 10% aluminium chloride with 4.3 ml of grain alcohol. We adopted the same experimental procedure dilutions, described below, for the preparation of the absorbance of the response curve as a function of the concentration (mg / ml) ranging from 60 to 260 (mg / mL) of total flavonoids equivalent of catechin using the equation of the line from the calibration curve of the standard secondary reference catechin being evaluated linearity.

#### Linearity

To check the linearity, it was elaborated analytical curve with the stock solution of standard secondary reference

with soil). Then, the micropropagation development monitoring was made, measuring the dimensions of the roots and formation, and the growth of buds in relation to the cultivation time.



**Figure 1.** Illustrative Photo *Kalanchoe pinnata*. Source: Author's photo

### D. Preparation of the *Kalanchoe pinnata* leaves extract

There were extractions of leaves developed to evaluate the concentrations of flavonoids. The alcoholic extract of 100 g of cereal leaf was macerated in ethanol, and transferred quantitatively into 100 mL volumetric flask, and the final volume with grain alcohol.

### E. Determination of total flavonoid, catechin equivalents

catechin (purity = 98%) in the concentration of 500,0µg / ml in cereal alcohol. From the stock solution dilutions were prepared at concentrations of 0.084; 0.05; 0.028; 0.017; 0.0076 g / L, which was reacted with 0.100 mL of 10% aluminum chloride solution (v / v), completing the volume with ethanolic grain alcohol. After 40 minutes of rest, we proceeded to read in a spectrophotometer at 510 nm using as white cereal alcohol solvent plus aluminum chloride solution. Elaborated the analytical curve, checking the linearity of the method, using visual observation and appropriate statistical analysis, obtaining - the equation of the line and the linear correlation coefficient. Equation 1 is the equation of the calibration curve of the standard secondary reference

catechin, where  $y$  is absorbance and  $x$  is the concentration of standard secondary reference catechin:

$$y = 10.121x - 0,0475 \quad (1)$$

$$r^2 = 0,9936$$

From the obtaining of a line to the calibration curve and the correlation coefficient ( $r^2$ ) .9936 was verified the linearity of the method.

### III. RESULT AND DISCUSSION

#### A. Quantification of equivalent total flavonoids catechin in *Kalanchoe pinnata* extract.

In Table 5 it can be seen the results of quantification of total flavonoids, catechin equivalents in *Kalanchoe pinnata* extract, with an average of 42.39 mg / mL.

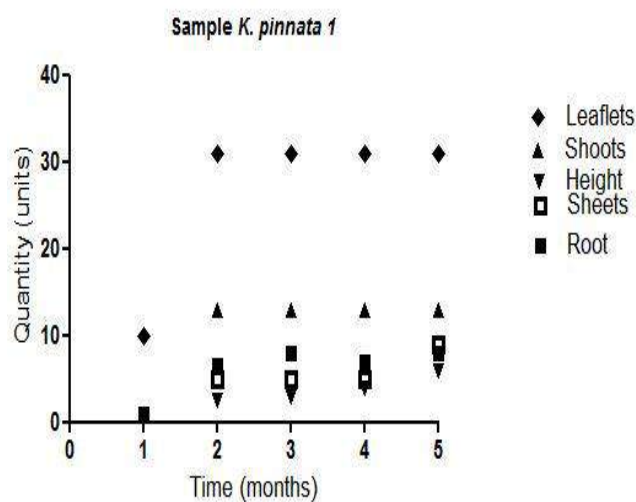
**Table 5** – spectrophotometric dosage of the equivalent total flavonoids catechin present in *Kalanchoe pinnata* extract to 510nm. **EKP:** *Kalanchoe pinnata* extract. **CF:** concentration of total flavonoid, catechin equivalents.

Concentration of EKP (µg/mL)	Absorbance	CF µg/mL)
60,0	0,2230	17,34
100,0	0,3320	28,00
140,0	0,4219	36,00
180,0	0,5543	50,00
220,0	0,6030	54,00
260,0	0,7456	69,00
<b>Average: 42,39</b>		

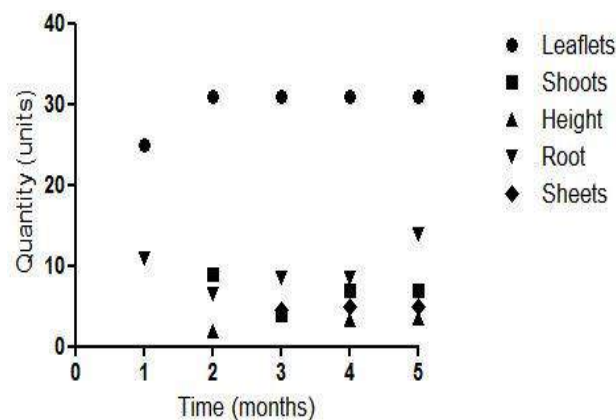
#### B. *Kalanchoe pinnata* Micropropagation

Water-soaked sheets produced roots from all the leaflets, and buds (buds) with leaves in most leaflets, from 7 to 10 days. In a similar period, the sheet cultivated on earth developed, on average, two shoots 10 days and 40 days from the main stem height reached an average of 8 cm from each bud, 8 new sheets for each sprout dimensions Similar to that of the mother sheet. In water, the leaves are multiple and diminutive dimensions, three times smaller, and remains in this size and had to be transferred to new culture in water or on land.

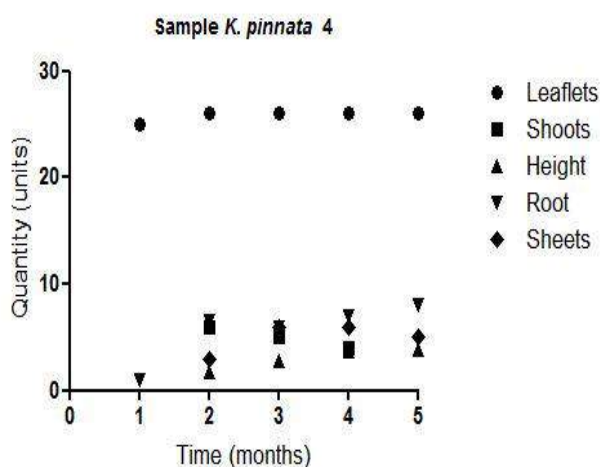
#### C. Statistics of *K. pinnata*.micropropagation



**Figure 2.** *K. pinnata* with 11.5cm long and 6.5cm wide; ANOVA:  $p < 0.05$  \*\*\*



**Figure 3.** *K. pinnata* with 15.7 cm long and 10 cm wide; ANOVA:  $p < 0.05$  \*\*\*



**Figure 5.** *K. pinnata* with 14cm long and 9 cm wide; ANOVA:  $p < 0.05$  \*\*\*

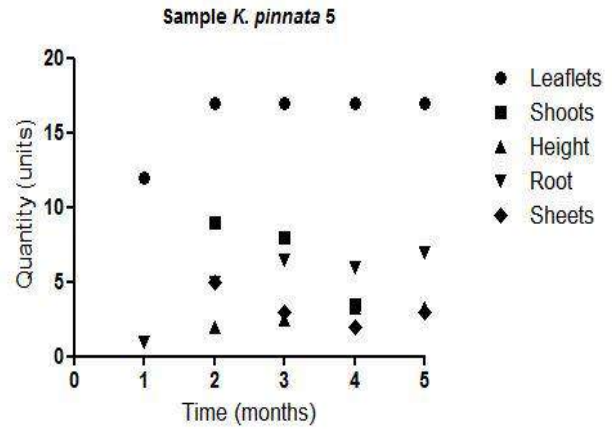
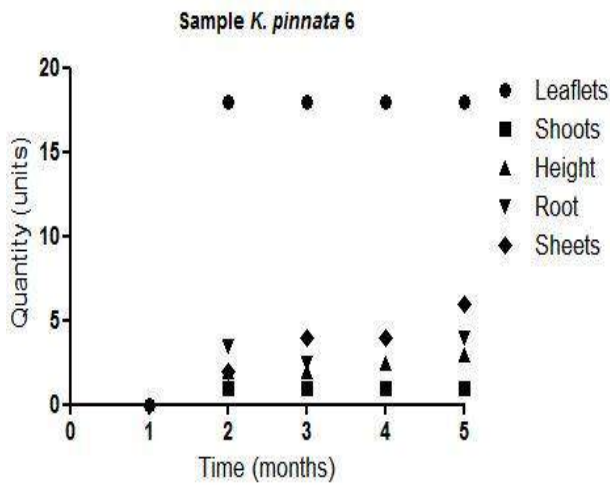


Figure 6. *K. pinnata* with 11 cm long and 5.5 cm wide; ANOVA:  $p < 0.05$  \*\*\*

Figure 7. *K. pinnata* with 18cm in length and 12.5 in width; ANOVA:  $p < 0.05$  \*\*\*

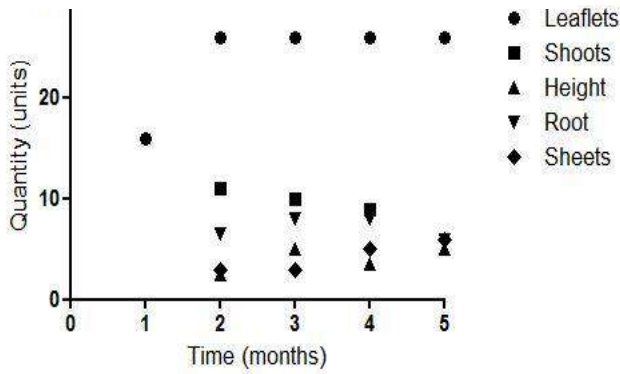


Figure 4. *K. pinnata* in length and 13cm 9.7 cm wide; ANOVA:  $p < 0.05$  \*\*\*

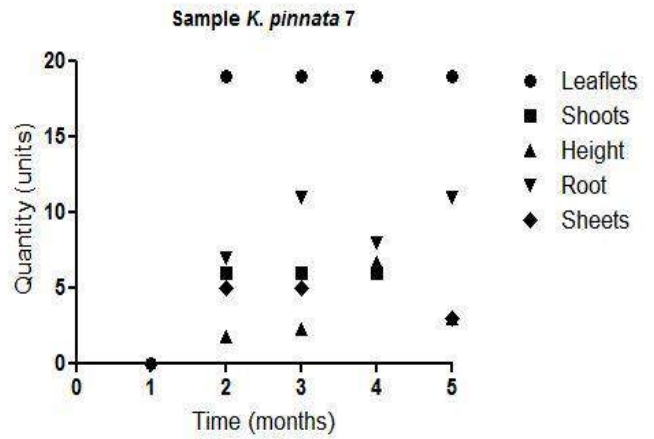


Figure 8. *K. pinnata* with 10,5cm long and 5,4wide; ANOVA:  $P < 0, 05$  \*\*\*

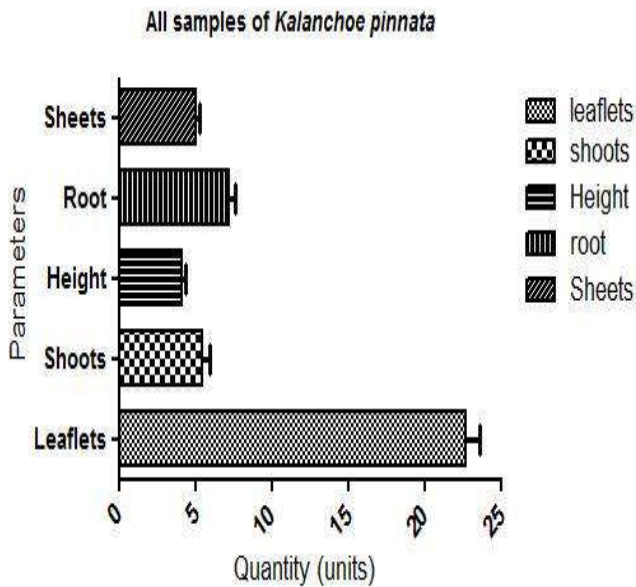
## IV. CONCLUSION

The micropropagation of *Kalanchoe pinnata* proved to be auspicious, since it presented a high capacity for reproduction by fotoautotrophic micropropagation in water with the use of natural light, and soil humus with derived bio-organic fertilizer. Observe a satisfactory value the concentration of micronutrients present in the biomass of fruits needed for plant nutrition, this fact makes possible the replacement of synthetic media, which implies lower costs allowing obtaining of one rich source of active ingredients for herbal, water extracts (poultices) or cereal alcohol (tinctures).

The photoautotrophic plant micropropagation, in addition to increasing the growth of in vitro explants, also minimizes the risk of microbial contamination, reduces production costs, improves the physiological characteristics of the plant and facilitates their acclimatization to ex vitro conditions due to have been grown in conditions natural.

## V. ACKNOWLEDGEMENTS

Agency CAPES, Prof. Dr. Marco Antônio Sthephano (FBT-FCF-USP), Prof.<sup>a</sup>. Dra. Marina Ishii (FBT-FCF-USP), Dr<sup>a</sup> Aline de Oliveira Santos (FBA-FCF-USP), Dr<sup>a</sup> Caroline Matos de Mello (IPT- USP)



**Figure 9.** *K. pinnata* (all sheets with varying sizes) ANOVA:  $P < 0.05$  \*\*\*

### A. Determination of the chemical composition of biomass fruit

**Table 6 - Proximate analysis of biomass of fruits**

Biomass fruit (g/100 g)	(I)	(II)	(III)	(IV)	Average
Carbohydrates	4	4,9	4,8	--	4,56
Lipids	0,02	0,03	0,02	--	0,023
Proteins	0,4	0,5	0,8	--	0,56
Ash	2	1,98	2	--	1,99
Humidity (%)	89	89	89	--	89
Fibers	5	4,8	4,9	4,9	4,9
Glucose (g/L)	7,756	7,54	7,15	--	7,483
Fructose (g/L)	9,107	8,80	8,31	--	8.756

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# Therapeutic Management of Staph Spp Skin Infection in Labrador

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## ABSTRACT

A three years old black male Labrador was presented with clinical sign of alopecia, pruritus, eczema on dorsal region of paw of right forelimb and chin region. Antibiotic sensitivity test of skin scraping revealed the infection of coccal bacteria undifferentiated from staphylococcus spp., with highly sensitivity to gentamycin. Intramuscular injection of genticyn along with immunomodulation therapy resulted in remission of clinical signs and improvement of healthy condition.

**Keywords:** Staphylococcus, Gentamycin, Pyoderma.

## I. INTRODUCTION

Skin diseases are the most common health problems in dogs. The condition of dog's skin and coat are important indicator of its general health. Skin disorders of dogs vary from acute, self-limiting problems to chronic or long-lasting problems requiring lifelong treatment. They also need to be differentiated on the basis of being of primary or secondary in nature, making diagnosis complicated <sup>[1]</sup>. The skin is a milieu for controlled bacterial growth. Skin supports the growth of commensally bacteria, which protect the host from pathogenic bacteria. Environmental and local factors, host immunity, and organism adherence and virulence are intricately related to systemic infection. *Staphylococcus aureus* and *Streptococcus pyogenese* notoriously pathogenic in the skin (Chiller, *et al.*, 2001). Among this Staphylococcal infection is a common skin disorder of the dog. Infections may be superficial (folliculitis), deep (furunculosis), or both. *Staphylococcus intermedius* is the most commonly isolated bacterium (Scott, *et al.*, 2001) which was isolated from 88.6% and 49.4% of skin and ear samples, respectively, during the years 1992 through 1997, and frequency of isolation remained unchanged (Petersen, *et al.*, 2002). Despite their frequency of occurrence, many pyodermas are misdiagnosed or improperly managed (Lhrke, P. J., 1987).

Chronic skin infections leads to pyoderma which includes symptoms like scaling, crustiness, hair loss, and the development of papules, pustules, and pus/discharge on the skin. It can be tentatively diagnosed by visual examination but definitive diagnosis may require examination of hair and discharge, and skin cultures of the lesions (Michael Dym, Holistic & Conventional Veterinarian (Bio).

### History and Clinical Examination:

A 3 years old male Labrador was presented to the Teaching Veterinary Clinical Complex, OUAT, Bhubaneswar, with the history of alopecia, pruritus, Eczema on dorsal surface of paw of right forelimb and also on chin region. On physical examination serosanguineous fluid was found to be oozing out of it. Skin scraping examination didn't reveal any presence of parasitic infection like mites but antibiotics sensitivity test suggested coccal infection undistinguishable from Staphylococcus species which is highly sensitive to Gentamycin and Amikacin.

### Treatment

Prior to clinical test the dog was treated with Inj. Ivermec- 1ml s/c ly (Ivermectin @ 1 ml upto 20kg body weight), Lixen (Cephalexin-600mg @ 1 tab per 20 kg B.wt) 1tab daily for 3 days, Cetrizine- 1 tab orally daily



for 3 days, Curabless Ointment- 25gm to be applied liberally and Multistar Pet- 1phl, 10ml orally twice. Following antibiotic sensitivity test, on fourth day it was treated with Inj. Genticyn- 2ml i/m ly (Gentamycin) daily for three days, Curable ointment and Multistar Pet to continue. On day 7, a substantial clinical improvement was noted, based on complete lack of pus and blood oozing out of it.

### Discussion

Ivermec was given as ecto-endo parasiticide for the treatment of any parasitic infection, lichen contain cephalixin antibiotic given for the treatment of bacterial infection causing Soft tissue infection, Pyoderma (Skin infections due to coagulase positive Staphylococci), cetirizine was given as antiallergic medication, Multistar Pet is multivitamin which was given to enhance the immune system and for regeneration of healthy epithelium, curabless contain terbinafine hydrochloride(antifungal), ofloxacin (antibacterial), ornidazole (antiprotozoan) and clobetasol propionate(corticosteroid used to treat various skin disorders including eczema and psoriasis) which was given for the treatment of various types of skin problems.



**Figure 1.** Moist Eczema of the chin region



**Figure 2.** Pyoderma with pus and blood on the dorsal surface of paw region of right forelimb (acral granuloma)



**Figure 3.** Antibiotic sensitivity test showing bacterial sensitivity to Gentamycin.

## II. CONCLUSION

Use of sensitive antibiotics along with supplementation of systemic immunomodulation therapy will be more effective in localized canine pyoderma cases compare to conventional routine treatment.

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# Tree Gummosis a Thread for Mango Production in Northern Senegal

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## ABSTRACT

In Senegal mango is one of the most important fruit crops. Mango trees are planted in a large part of the territory and produced in different cropping systems. Setting up mango orchards represents a great contribution to the national production effort. However, infestations of the vegetative organs of the trees by diseases, in addition to fruits infestations, are a major constraint to the development of mango plantations. The identification of the causal agents for any disease is a prerequisite for corrective actions. In the Lampsar orchard, an outbreak of gummosis was observed in almost all plots of mango trees, with greater intensity in the lower parts of the field. However a variety of symptoms was recorded. Some plants showed cracking of the stem bark and gum exudation (gummosis), while others, exhibited only the cracking of the stem bark. The symptoms were most frequently located below the grafting point and/or on the junction point of the graft and the rootstock. The incidence of the disease varied from a plot to the other, ranging from 0.59% to 8.5%. The diagnosis, made for 30 fungal isolates obtained from samples collected in the field, allowed identifying *Lasiodiplodia* sp as a causal agent, thanks to the presence of its characteristic pycnidia and pycniospores. Recommendations were made for the control of this fungus in the orchard.

**Keywords:** *Lasiodiplodia* sp, *Mangifera Indica*, Sénégal, Tree Gummosis.

## I. INTRODUCTION

The mango (*Mangifera indica* L.) is one of the major fruit crops of tropical and subtropical regions. According to FAO (2011), the production of tropical fruits was estimated at over 82.2 million tons in 2009. The mango presents one of the main crops with 39% of world production of tropical fruits. In Senegal, mango production is experiencing a big growth with an increase of acreages allocated to this crop and a modernization of mango orchards as well. Mango represents 60% of fruit production in the country with an annual production of 150,000 tons harvested on a land surface of about 41,000 ha (ASEPEX, 2012). Although the soil and climatic conditions of the country offer a great potential for the expansion of mango production (USAID, 2006), this value chain is facing a lot of constraints related to phytosanitary quality of both tree and fruit stages. Postharvest rotting of fruits due mainly to anthracnose

and fruit fly cause important losses. In addition, the appearance of a disease causing gum exudation from the trunk of the trees in the north of the country accentuates the pest pressure on the plant and thus affects the entire value chain. This disease has been reported in mango orchards from different parts of the world sometimes in relation to mango decline (Malick *et al.*, 2005). The most specific symptom of the disease, one that earned him his name, is the flow of brown gum, along the trunk and branches (Vanderweyen, 1974). Several fungi are associated with tree gummosis, among them, are listed : *Phytophthora palmivora* (N'diaye *et al.*, 2011), *Lasiodiplodia theobromae*, *Ceratocystis fimbriata*, *F. solani*, *Phoma* sp, *Ceratocystis omanensis* (Khanzada *et al.*, 2004b; Al Adawi *et al.*, 2006; Iqbal *et al.*, 2007; Saeed *et al.*, 2011; Haougui, 2013). The objective of this study was to identify the causing agent of the disease and assess its incidence in order to better target an adequate control method.

## II. METHODS AND MATERIAL

### 1. Study Site

The mango orchard where the disease broke out, is located in the zone of Lampsar, 30 km east of the main city of Saint-Louis (16°06'N latitude, 16°20'W longitude and 29 m altitude). The climate is of Salelian type and characterized by low rainfall (less than 300 mm in the year). The soil is sandy but the proximity of the river Senegal has impacted deeply on the landscape and provides fresh water for irrigation.

The studied orchard covers 50 hectares of land, of which about 40 ha are cultivated, the remaining 10 hectares are represented by driveways and various facilities. It is divided into 20 plots of 2 ha each. Almost all mango trees were planted in 2013 on rows with 7 m interval. In each row trees are planted in lines with 5 m spacing. Plot 10 and 19 were set up 2 years later, in 2015. The plantation design resulted in a population of 572 trees per plot populated with only mango trees of the variety Kent. Planting was performed by first sowing mango kernels let to grow in the nursery. The seedlings, used as rootstock were thereafter grafted with scions from mature mango trees of the variety kent only. The orchard is equipped with drip irrigation system. This is set in a way to feed each mango tree with twenty drippers emitting 5L per hour each around each around the tree. Each plot is irrigated every 2 days for 50 minutes. Fertilization is performed through the irrigation system allowing the supply of nutrients in soluble form in the irrigation water. The nutrients are delivered to the trees with the drippers on the basis of fertilization program. A mechanical weed control with an offset is performed on the driveways and between the rows as necessary. It is done manually under the canopy of the trees. Several phytosanitary treatments were carried out in the orchard, likewise, an insecticide was sprayed in May 2015 and lime paintings were applied to the trunk of the mango trees to control fungi.

### 2. Collection of data

A questionnaire was submitted to the head of the orchard. Thereafter, an assessment of the diseases was carried out in the orchard and covered systematically all plants of the plots. Parameters like the presence of

cracking or gumming were recorded. Disease severity was scored on a scale of 0 to 3, and the location of the symptoms with respect to the grafting point was also recorded. The topography of the different plots in the orchard was taken into account in terms location on upland or lowland areas in the orchard.

### 3. Sampling

Thirty samples were collected from diseased plants by peeling the bark on parts of the trunk showing disease symptoms. Twenty other samples were taken from the trunk of symptomless plants. The fragments were then taken to the laboratory for isolation of pathogens.

### 4. Isolation of Fungi

The isolation of pathogenic fungi responsible for the disease was performed at the front of progression of developing disease symptoms according to the methodology described by Diédhiou *et al.* (2007). The samples were first soaked in a 2% solution of sodium hypochlorite for 1 minute. A sterilized scalpel incision is made in order to cut a small fragment of bark at the front progression of disease. The fragment is placed in a petri dish containing PDA (Potato Dextrose Agar) supplemented with 100 ppm of chloramphenicol. The petri dishes are incubated at room temperature in the dark. After 48 h, the mycelium growing out of the fragment is transplanted into fresh petri dishes. When the pure cultures are obtained, petri dishes are incubated for 3 to 6 weeks to allow sporulation. Features of fruiting bodies like spores and pycnidia were used for the identification of fungi.

### 5. Statistical analysis

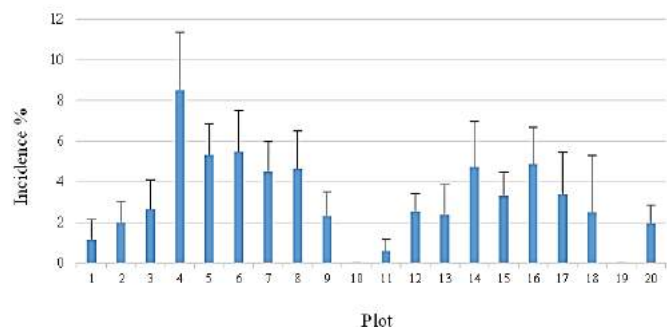
The data were submitted to an analysis of variance (ANOVA) using the SigmaStat ® software with a confidence interval of 95%. Mean values was separated through Tukey's tests pairwise comparisons.

## III. RESULT AND DISCUSSION

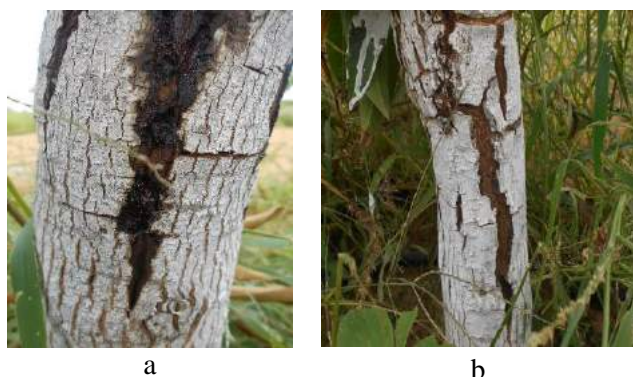
### 1. Incidence of disease

Gum exudation from the trunk of mango trees was observed in all plots except for plots 10 and 19. The

incidence of the disease ranged from 0 in plot 10 to 8.5% in plot 4 (Figure 1). Some of the diseased plants exhibited cracking of the bark of the trunk accompanied by an exudation of gum (Figure 2a). The other type of symptoms was characterized by only the cracking of the bark on the trunk of trees (Figure 2b). The highest rate of cracking was observed at the plot 4 (8.5%). The younger trees in plots 10 and 19 were apparently healthy.



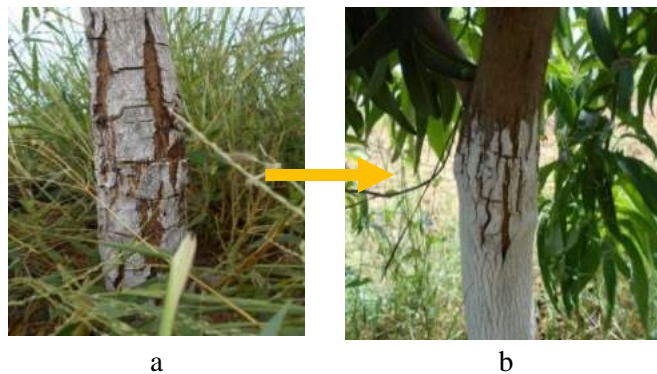
**Figure 1:** Incidence of gummosis in the plots of the orchard (n = 14, p ≤ 5%) (in the plot, there are 14 rows representing each a replication)



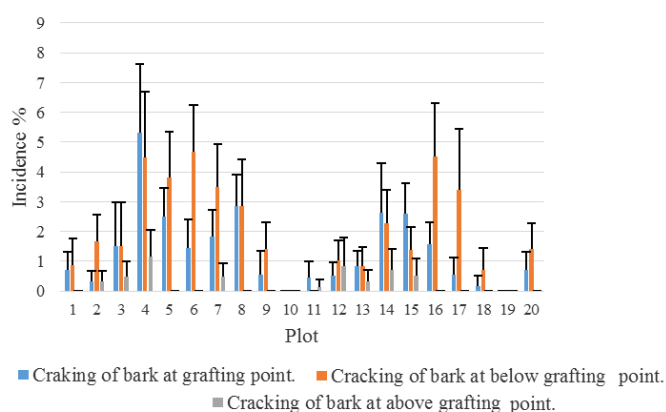
**Figure 2:** Trunk of mango trees showing cracking and exudation of gum (a) and cracking of the bark (b)

Either gum exudation and/or cracking of the bark of the trunk of the trees were mainly located either below the grafting point (figure 3a), or at the grafting point (figure 3b).

For most of the cases, cracking of the bark and/or gum exudation were often located on the grafting point and below the graft point (figure 4). The proportion of plants with symptoms above the grafting point was low. The incidence per plot was variable but reached a maximum of 5.33% in plot 4 for symptoms on the grafting point. The highest incidence for symptoms appearing below the grafting point was also recorded in the same plot 4.

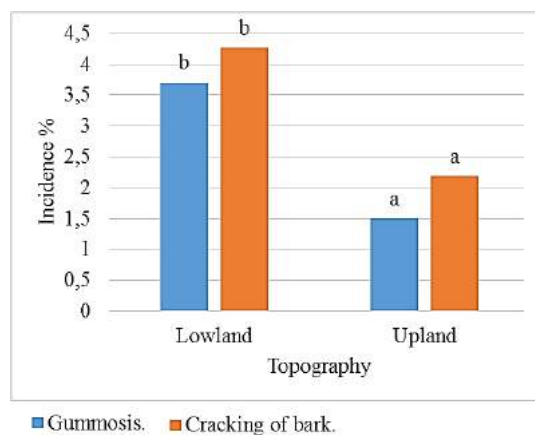


**Figure 3:** Plants with symptoms of gummosis at the grafting point (b) and below the graft point (a)



**Figure 4:** frequency of gummosis symptoms with respect to the grafting point

The incidence of gum exudation and cracking of the bark was significantly higher in plots located in the lowland portions of the field 3.7 % and 4.3% respectively against 1.5% and 2.4% for plants in the upland areas (figure 5).



**Figure 5:** incidence of gummosis and cracking of the bark in the lowland and upland areas of the orchard.

## 2. Pathogenic agents

All fungal isolates from the samples collected in the field were initially white to smoky gray with a soft aerial mycelium on PDA. This mycelium color turned thereafter black on both sides of the Petri dishes. On the upper side, shiny black fruiting bodies that match the pycnidia were gradually visible. The conidia from the pycnidia were initially hyaline, unicellular and subovoïde to ellipsoid, with granular content. At maturity, the conidia become dark brown and cinnamon, with a thick wall and presented a central septum (figure 6). These features are specific for fungi of the genus *Lasiodiplodia*.



**Figure 6 :** conidia (pycniospores) of *Lasiodiplodia* sp under compound microscope

## DISCUSSION

*Lasiodiplodia* sp was found as the causal agent causing gum exudation as well as cracking of the bark of the mango trees in the orchard. All macroscopic and microscopic features of colonies, pycnidia, and conidia were characteristic for the genus *Lasiodiplodia*. However among all species of *Lasiodiplodia*, *L. theobromae* is the one always associated with diseases of mango. It is described as a cosmopolitan fungus that causes various diseases on about 500 plant species (Pedraza *et al.*, 2013). *L. theobromae* is found mainly in tropical and subtropical regions in mango production area. It was reported as a pathogen of mango worldwide and is associated with several diseases such as mango decline, canker and dieback (Ismail *et al.*, 2012). According to Khanzada *et al.*, (2005) *L. theobromae* is a soil-borne fungus that causes both diseases on standing trees and on mango fruits during storage. *L. theobromae* was identified as causal agent for mango gummosis back

in 1992 (Narasimhudu & Reddy, 1992). Khanzada *et al.*, (2004a) reported that mango decline and gummosis in Sindh (Pakistan), were also caused by *L. theobromae*. The same fungus was found associated with all symptoms of dieback of the mango tree (Iqbal *et al.*, 2007; Khanzada *et al.*, (2004b). The disease is characterized by burning twigs, gum bleeding and splitting of the bark. Diedhiou *et al.*, (2007) and Haggag *et al.*, (2010) also identified *L. theobromae* as the causal agent of stem end rot of mango fruits after harvesting.

*Lasiodiplodia* sp was also isolated from several samples from apparently healthy mango trees in the orchard. A similar case was reported by Muniz *et al.*, (2011) who found *L. theobromae* in tissues of asymptomatic cashew plants. This could explain the absence of symptoms in plants used as controls. According to Muniz *et al.*, (2011), *L. theobromae* is able to live in plant tissues without causing apparent symptoms. Following external signals due to biotic or abiotic stress, symptoms begin to appear and the disease is established. In the case of the orchard studied, water and fertilizers were provided to individual trees abundantly. In addition, smaller younger trees were still symptomless.

The incidence of gummosis varied across the different plots in the orchard. This variability could also be related to the topography. In fact, a higher infestation rates were observed for plots in the low areas of the orchard. This could be explained by the presence of a moist microclimate favorable for the development of pathogens. Harsh climatic conditions and high humidity are favorable for the development of mango gummosis (N'diaye *et al.*, 2011). Such conditions were found zone where the orchard is located.

Gum exudation from trees may be observed alone or in combination with other symptoms in mango orchards (Iqbal *et al.*, 2007; Shahbaz *et al.*, 2009; Malik *et al.*, 2005). In the present study, mango gummosis was combined with cracking of the bark on the trunk for some of the infected plants. This combination was observed for other plant species like neem tree (Khalil, 2012) and citrus (Graham and Timmer, 2003). In other cases, gum exudation is associated with dieback (Iqbal *et al.*, 2007; Shahbaz *et al.*, 2009; Malik *et al.*, 2005).

The combination of gum exudation with other disorders on the mango tree could be an indication that they share

a common causal agent (Iqbal *et al.*, 2007). In the present study, the symptoms were mainly observed on the rootstock. It is known that *L. theobromae* is a soil-borne fungus (Khanzada *et al.*, 2005), which is preserved in the soil, on plants debris, etc. The rootstock is therefore more exposed to the pathogen and should be more susceptible to develop the disease in a conducive environment. The genotype of the rootstock could then play an important role in plant susceptibility to the disease. If resistant rootstocks could be found, this may help reduce the importance of the potential damage of the disease. The questionnaire conducted with the orchard manager revealed however, that any mango varieties were used as rootstock making any further investigation in this direction, not relevant. Surveys conducted in the region showed no other mango orchard with gummosis. It is important to mention that the variety kent is not common in the region. In addition, all other orchards are of traditional type, with trees planted and left without further care until harvest period.

#### IV. CONCLUSION

Gummosis disease was observed in almost all plots of this single orchard, with widely varying incidences between plots and higher levels infestation in the lower parts. Results of the present study would suggest that *Lasiodiplodia sp* is the causal agent of gummosis diseases of mango in Senegal. Isolation of the pathogen from asymptomatic plants, confirms its endophyte character. Further studies would be necessary to identify not only the species of *Lasiodiplodia* associated of the gummosis of mango in Senegal, but also the sensitivity of different mango.

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# Isolation and Identification of Bacteria from Different Soil Samples of Telangana and Andhra Pradesh States

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## ABSTRACT

The word agriculture is more related to natural activity and to nature like plant life cycle, ecosystem, green earth, vegetables, fruits, soil, water & natural environment. Plants have a number of relationships with fungi, bacteria, and algae. These are known to deliver a number of benefits including plant nutrition, disease resistance, tolerance to adverse soil and climatic conditions. These techniques have proved to be successful biofertilizers that form a healthy relationship with the roots. Biofertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers.

In the present studies various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The places from where these soil samples were collected include Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kurnool, Kadapa, Gudipadu areas of Andhra Pradesh State. Most of the soils thus obtained were seem to be supporting agriculture actively. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. Characterization and microscopic observation of the culture was done and the bacteria identified were *Streptomyces sp.*, *Staphylococcus sp.*, *Azotobacter sp.*, *Leptothrix sp.*, *Derxia sp.*, *Bacillus sp.*, and *Sphaerotilus sp.* Of the above isolated organisms *Azotobacter sp.*, *Streptomyces sp.* and *Bacillus sp.* are familiar to be used as biofertilizers in various crop fields. *Derxia sp.* is a Gram negative, nitrogen fixing bacteria but not much known as a biofertilizer, which seems to be very interesting to scientist to work on it. Hence it is important to study its role in agriculture.

**Keywords:** Soil samples, Biofertilizers, Bacteria, Crop fields, Farmers.

## I. INTRODUCTION

One of the major concerns in today's world is the pollution and contamination of soil. <sup>[1]</sup> The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. While manure, cinder and iron making slag have been used to improve crops for centuries, the use of biofertilizers is arguably one of the great innovations. <sup>[2]</sup> <sup>[3]</sup> Minerals, organic components and microorganisms are three major solid components of the soil. They profoundly affect the physical, chemical, and biological properties and processes of terrestrial systems. Biofertilizer are the products containing cell of different types of beneficial microorganisms. Biofertilizers are classified as Nitrogen

fixers which includes Bacteria like *Rhizobium*, *Azotobacter*, *Mycobacterium* and *Blue Green Algae* like *Anabaena*, *Nostoc* etc. Further depending upon the fixing capacity they are divided as Phosphate solubilizing fertilizer, Plant growth promoting Rhizobacterim (PGPR), Sulphur solubilizing microbes etc.

Biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting

rhizobacteria "(PGPR).<sup>[4]</sup> These are some of bacteria that can grow in the root environment and be effective on plant growth. Mechanisms that can promote plant growth include production of phytohormones, biological nitrogen fixation and increased solubility of insoluble elements in soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, biofertilizers do not contain any chemicals which are harmful to the living soil.

Several soil bacteria help the crops to fix the atmospheric nitrogen and the organic phosphate. Hence these potential bacteria would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers.<sup>[5]</sup> It also means that nature grows only when there is balance of activities between natural things only like animals, plants, microbes and environment! These potential bacteria as biological fertilizers can be important components of integrated nutrient management.

## II. METHODS AND MATERIAL

### A. Collection of soil samples

Various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The places from where these soil samples were collected include Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kadapa, Kurnool, Gudipadu areas of Andhra Pradesh State. Most of the soil thus obtained was seem to be supporting agriculture actively. The land was dug as deep as 10 – 15 cm for the collection of soil samples. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. The soil samples were wetted from time to time to help in the sustenance of the microorganisms as moisture is also a very important factor that plays an important role in the survival of microorganisms.

### B. Characterization of the soil samples

The soil samples were then studied for the soil type, colour and the crops that they support for cultivation. The soils were then typed as red soil and black soil. Crops like Green leafy vegetables, Onion, Turmeric, Ground nuts, Rice, Pea plants and Tomato were being cultivated on these soils. The soils were then labelled and segregated according to the crops and place from where they were obtained.

### C. Preparation of culture media

Nutrient agar media was used for primary isolation and Jensen agar media ,William and Kusters media for their sub culturing.

### D. Isolation and characterization of bacteria

Serial dilution agar plating was done for the isolation of soil microbes. Suspension was diluted up to  $10^{-10}$ . The aliquots were cultured for bacteria. For primary isolation of bacteria Nutrient broth, Nutrient agar media and specific medias like Jenson's media and William and Kusters media for the growth of *Azotobacter sp.* and *Actinomyces sp.* were used. The inoculum was spread with the help of a spreader evenly on the media. The inoculated plates were then incubated at 37°C for 24 h. Growth of the microorganisms is observed in the form of turbidity and colony growth respectively. Characterization and microscopic observation of the culture was done.

## III. RESULT AND DISCUSSION

One way to increase crop yield is using the beneficial microorganisms. Biofertilizers help the crops to fix the atmospheric nitrogen and the organic phosphate. They maintain a healthy symbiotic relationship with the crops thus helping to increase the yield of the crops. This symbiotic relationship proves beneficial both to the organism contributing for their sustenance and also in turn increases the soil fertility thereby helping to obtain an increased, disease free, resistant crop type <sup>[6]</sup>. Biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum* and blue green algae(BGA) have been in use for a long time. And also there must be some organism having direct or indirect association with

plants which need to be investigated thoroughly. For this one has to study the organism present in the different soil samples of various plants.

In the current studies various soil samples were collected from actively cultivated lands after a thorough survey of the areas. The first step of this project is to isolate and identify bacteria from different soil samples like Gandipet, Shamirpet areas in Hyderabad, Chevella on the outskirts of Hyderabad and Adilabad district of Telangana State and Kurnool, kadapa, Gudipadu areas of Andhra Pradesh State. Most of the soils thus obtained were seem to be supporting agriculture actively. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. Characterization and microscopic observation like Gram staining, Spore staining, Capsular staining and biochemical test were performed. It is evident from the Table 1. the organism isolated and identified from Telangana State of Gandipet black soil rice field is *Streptomyces sp.*, to confirm this organism it was inoculated in to Williams and kusters media which had shown the orange pigmented smooth, entire mucoid and translucent colonies, characteristic feature of *Streptomyces*. According to literature *Streptomyces sp.* isolated (Tinatin Doolotkeldieva et al., 2015)<sup>[7]</sup> from the rhizosphere has improved the composition of rhizosphere microflora, attracting saprophytic microorganisms: ammonificators and oligotrophs. The presence of the biocontrol microorganism *Streptomyces sp.* in the rhizosphere plays an important role in enhancing the growth and development of useful groups, such as nitrogen-fixing bacteria<sup>[7]</sup>. Organism identified from Tomato plants of Shamirpet black soil is *Staphylococcus sp.* Chevella black soil, showed the growth of *Azotobacter sp.* in pea plant and the organism isolated from leafy green vegetables of Adilabad red soil are *Leptothrix sp.* and *Azotobacter sp.*

*Leptothrix sp.* is a sheathed filamentous bacterium that can generally be found in different types of aquatic environments with sufficient organic matter. It is known to be capable of oxidizing both iron (II) and manganese (II), unlike other sheathed bacteria. Crops inoculated with *Azotobacter* and *Azospirilla* reviewed by Wani (1990) indicated that Pearl millet and Sorghum, which are grown as dry land crops showed 11-12% increased yields due to inoculations.<sup>[8]</sup> Table 2. shows that an

organism isolated from Andhra Pradesh State of Kurnool black soil Rice is *Azotobacter sp.* and from Ground nut is *Derxia sp.* The organism *Azotobacter sp.* are Gram-negative bacteria<sup>[9]</sup> found in neutral and alkaline soils, in water and in association with some plants. It plays a vital role in every ecosystem, it is found worldwide, in climates ranging from extremely northern Siberia to Egypt and India.<sup>[10]</sup> It is used as biofertilizers for the development of various vegetable plants such as mustard, maize, wheat, cotton etc. The most dominant *Azotobacter sp.* which was isolated from Chevella, Adilabad and Kurnool districts is confirmed by inoculating in to specific Jensen agar media. *Derxia* is a genus of Gram-negative, nitrogen-fixing bacteria from the family of Alcaligenaceae.<sup>[11] [12] [13]</sup> Rennie (1980) examined sugarcane rhizosphere soil samples from Brazil and found them to contain equal populations of *Derxiagummosa*, *Enterobacter cloacae*, *Bacillus polymyxa* and *Azotobacter vinelandi*.<sup>[14]</sup> And organism isolated from Ground nuts of Kadapa red soil are *Bacillus sp.* and *Staphylococcus sp.* Literature shows that *Bacillus* and *Staphylococcus sp.* bacteria have potential to fix atmospheric nitrogen, able to produce IAA with the range of  $15.13 \pm 0.2$  to  $33.1 \pm 0.2$   $\mu\text{g/ml}$  when supplemented with 100  $\mu\text{g/ml}$  of tryptophan and showed some P-solubilizing activity which have been claimed by manufacturers.<sup>[15]</sup> Sample collected from Gudipadu black soil of Onion and Turmeric crop field identified as *Bacillus sp.* and from Ground nuts are *Sphaerotilus sp.* and *Bacillus sp.*

*Sphaerotilus sp.*<sup>[16]</sup> is a filamentous bacterium that is covered in a tubular sheath and can be found in flowing water and in sewage and waste water treatment plants. A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant growth promoting rhizobacteria or PGPR (Schroth and Hacock, 1981). PGPR<sup>[17]</sup> belong to several genera e.g. *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas sp.*, *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas sp.* (Weller, 1988). Several soil bacteria and fungi notably species of *Pseudomonas*, *Bacillus*, *Penicillium* and *Aspergillus* etc., secrete organic acids and lower the pH in their vicinity to bring about solubilization of bound phosphates in soil (Sundara Rao and Sinha, 1963)<sup>[18]</sup>. Habibi et al. (2011) strongly suggested that using

biofertilizers (combined strains) plus half a dose of organic and chemical fertilizers have resulted in the greatest grain yield and oil yield in medicinal pumpkin<sup>[19]</sup>. Studies showed that the inclusion of wheat plant with PGPR increased the growth characteristics of wheat<sup>[20]</sup>.

The second step of the project is to check potential use of these bacteria as biofertilizers on various crop fields. Furthermore, the phosphate solubilization capacity and nitrogen fixing ability of *Azotobacter* and other

organisms isolated from various soils are to be tested and employed as biofertilizers during field trials on various crop plants which will be taken up as next phase of project work. Biofertilizer containing those microorganisms would play a great role in crop improvement. Crop yield can be increased by 20-30% if they are used properly. Hence these potential bacteria would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers.<sup>[21]</sup>

**Table 1:** Organism isolated from Telangana State.

Area / Type of soil		Type of crop	Media used	Colony morphology	Microscopic Observatios	Organism identified
G a n d i p e t	B l a c k  S o i l	Rice	Nutrient agar	Orange pigmented smooth, entire mucoid and translucent colonies.	<u>Gram-positive</u> , filamentous bacteria with well-developed vegetative hyphae with branches. Spore surfaces were hairy, smooth, and spiny .	<i>Streptomyces sp.</i>
		Rice	Willium and kusters media	Orange pigmented smooth, entire mucoid and translucent colonies.	<u>Gram-positive</u> , filamentous bacteria with well-developed vegetative hyphae with branches. Spore surfaces were hairy, smooth, and spiny .	<i>Streptomyces sp.</i> is confirmed
Shamipet Black Soil		Tomato Plant	Nutrient agar	Golden yellow colonies	Gram positive cocci in clusters	<i>Staphylococcus sp.</i>
C h e v e l l a	B l a c k  S o i l	Pea plant	Nutrient agar	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative thick sporulating bacilli arranged singli.	<i>Azotobacter sp.</i>
		Pea plant	Jensen agar media	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative, thick sporulating bacilli arranged singli.	<i>Azotobacter sp.</i> is Confirmed
A d i l a b a d	R e d  S o i l	Green leafy vegetables	Nutrient agar	Non pigmented medium sized, round, entire margin colonies.	Gram negative filamentous network.	<i>Leptothrix sp.</i>
		Green leafy vegetables	Nutrient agar	Non pigmented, smooth, mucoid and translucent colonies.	Gram negative, thick sporulating bacilli arranged singli.	<i>Azotobacter sp.</i>

**Table 2:** Organism isolated from Andhra Pradesh State

Area / Type of soil	Type of crop	Media used	Colony morphology	Microscopic Observatio	Organism identified
Kurnool Soil	Ground nut	Nutrient agar	Small, mucoid, pin pointed, opaque, smooth.	Gram negative slightly curved rods with rounded ends. Cells appear as dividing pairs.	<i>Derxia sp.</i>
	Rice	Nutrient agar	Non pigmented smooth, mucoid and translucent colonies.	Gram negative thick sporulating bacilli arranged singly.	<i>Azotobacter Sp.</i>
Kadapa Soil	Ground nut	Nutrient agar	Non pigmented pale, smooth mucoid and opaque.	Gram positive thick sporulating bacilli.	<i>Bacillus sp.</i>
	Ground nut	Nutrient agar	Round, flat, serrated margins, yellow pigmented colonies.	Gram positive Cocci arranged in cluster.	<i>Staphylococcus sp.</i>
Gudipadu Soil	Onion, Turmeric and Ground nut	Nutrient agar	Non pigmented pale, smooth, mucoid and opaque colonies.	Gram positive thick sporulating bacilli.	<i>Bacillus sp.</i>
	Ground nut	Nutrient agar	Large irregular, mucoid, rough and opaque colonies.	Gram negative sheated bacilli	<i>Sphaerotilus sp.</i>

#### IV. CONCLUSION

The purpose of this study was to investigate the bacteria associated with different soil samples of various crop fields of Telangana and Andhra Pradesh States. The bacteria isolated and identified were *Streptomyces sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Azotobacter sp.*, *Leptothrix sp.*, *Derxia sp.*, *Bacillus sp.*, and *Sphaerotilus sp.* Of the above isolated organism there is no evidence of using *Leptothrix sp.* and *Sphaerotilus sp.* as biofertilizers. *Azotobacter sp.* is used as biofertilizers for the development of various vegetable plants. *Derxia sp.* is a Gram negative, nitrogen fixing bacteria but not much known as a biofertilizer, which seems to be very

interesting to scientist to work on it. Hence it is important to study its role in agriculture. Furthermore, the phosphate solubilization capacity and nitrogen fixing ability of *Azotobacter*, *Derxia* and other organisms isolated from various soils are to be tested and employed as biofertilizers during field trials on various crop plants which will be taken up as next phase of project work.

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# Biological Control of Bark Eating Caterpillar *Inderbela Quadrinotata* in Indian Gooseberry

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## ABSTRACT

Field trials were carried out to study the efficacy of different entomopathogens, commercial bio-pesticides and plant extract against bark eating caterpillar (*Inderbela quadrinotata* wlk.) on Indian gooseberry (*Emblica officinalis*). All the treatments show remarkable variations against the moth. Among the treatments the bio-pesticides were found superior over entomopathogens in frass reduction of the caterpillar. The result reveals that neem extracts shows maximum frass reduction of the larvae followed by *Bacillus thuringiensis* and *Beauveria bassiana* over control. Further, the efficacy test of entomopathogens reveals that *Fusarium moniliformae* was found comparably the best among the other tested fungal isolates in frass reduction. The use of biopesticides preferably the entomopathogens in pest management, is widely acceptable as it is safer and economical maintaining the ecological balance of the nature.

**Keywords:** Bark Eating Caterpillar, Biological Control, Plant Extract, Entomopathogens, Indian Gooseberry.

## I. INTRODUCTION

The bark eating caterpillar (*Inderbela quadrinotata* wlk.) a moth of order Lepidoptera, with wood boring habits, the most prevalent in Asia Pacific region (34%) (FAO, 2009), affects nearly fifty plant species in tropical and sub-tropical India. The caterpillar damages varieties of fruit trees in central India (CROPSAP, 2013) including Indian gooseberry (*Emblica officinalis*), and thus a pest of national significance (AESAs, 2014). Although bark caterpillar is apolyphagous generally considered of minor importance in forestry, however is a major pest of Indian gooseberry (Mathew, 1997; Bharpoda et al 2009). The infected plant generally shows symptoms in form of large dark-brown masses of chewed wood and faecal matter near the forks. The larva bores into the bark making tunnel of nearly 20 cm. depth take refuge during the day and eats the bark of the tree during night. Bark damaged by the larva results in inhibiting translocation of cell sap, and reduces growth of host species (Sashidharan et al 2010) along with adverse impact on fruiting of the plant.

The infestation of pest generally starts in the month of April with the emergence of moths (Pathak, 2003). The damage due to this pest prevails more or less but maximum during rainy season over rest of the season in a year (Sasidharan and Varma, 2008; Bharpoda et al 2009). The temperature plays an important role in population variations of the pest, thus the insect-tree host-natural enemy relationship can help in developing timely control management of the larvae (Sasidharan and Varma, 2008). The incidence of pest is also influenced due to elevation, varying 0 to 98% which is negligible in high elevation, followed by mid and maximum in low areas (Gupta et al 2014). The age of plant species and availability of appropriate alternative host plants are significant factors for survival of a residual pest population (Mathew, 1997).

The pest control using pesticides are found effective but create toxicity in the environment. The Agro-ecosystem analysis (AESAs) focusing bio-intensive techniques based IPM shows least damage to the ecosystem, and has emerged as a better alternative of pest management programme in various countries (FAO, 2016). Among

the integrated management approaches, biological control is favourable in terms of cost-benefit ratio, combating insecticide-resistant pests and minimizing the practice of chemical pesticides (Bale et al., 2008). Biological control has also gained maximum acceptance for management of major pests as they restore the natural balance through meaningful human intervention (Ballal and Verghese, 2015). A large number of *Fusarium* species are entomopathogenic known for their abundance in nature and can be easily cultured and mass produced, and causes high insect mortalities along with high host specificity without damaging to plants (Teetor-Barsch and Roberts, 1983). The microbial biocontrol agent shows different mechanisms of action (Fravel, 2005) and are natural enemies devastating the vast population of the pest with no hazardous effect on human health and the environment (Khan et al 2012). Therefore, the present work is based on evaluation of various biocides, plant extract and entomopathogens as a part of biological control system and their potentiality in effective management of bark eating pest of Indian gooseberry.

## II. METHODS AND MATERIAL

The experiment was conducted in a nursery, in Jabalpur district of Madhya Pradesh, in central India (23°10'N 79°56'E) during year 2014. The experimental plot contains 105 trees of *E. officinalis* distributed in 21 rows of 5 trees each. The bark eating larvae infected with entomopathogenic fungi was collected from the nursery and brought for further laboratory study.

### Larval Cultures and Isolation of Entomopathogens

The infected larvae were put on moist and sterilized paper on petridishes for the germination of entomopathogen for 72 hrs. The entomopathogenic fungal isolates used in this study were obtained as natural infections in host insects. Three species of entomopathogenic fungi viz. *Fusarium oxysporum*, *F. moniliformae* and *Aspergillus niger* were identified with the help of standard mycological literature (Booth, 1971; Ellis, 1971; Grove, 1937; Sutton, 1980).

### Pathogenicity Test

The isolates were grown on Potato Dextrose Agar (PDA) at various concentrations for 10-15 days. The harvesting of conidia were carried out by scraping the surface of the agar using sterile spatula, and rinsing it with distilled water containing 0.1% Triton X-100. The conidial suspension was filtered to remove debris. The conidia were determined using hemocytometer (*neubauer improved* cell counting chamber) and the suspensions were adjusted to  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  to prepare required volume for each isolate. The conidial suspensions of the isolates were applied by pipetting onto the larva. The *F. oxysporum* isolate was applied at concentrations of  $1.6 \times 10^{-3}$ ,  $1.6 \times 10^{-5}$ ,  $1.6 \times 10^{-6}$ ,  $1.6 \times 10^{-7}$ ,  $1.6 \times 10^{-8}$  cell  $\text{mL}^{-1}$ , *F. moniliformae* isolates at  $1.5 \times 10^{-3}$ ,  $1.5 \times 10^{-5}$ ,  $1.5 \times 10^{-6}$ ,  $1.5 \times 10^{-7}$ ,  $1.5 \times 10^{-8}$  cell  $\text{mL}^{-1}$  and *A. niger* isolates at  $1.8 \times 10^{-3}$ ,  $1.8 \times 10^{-5}$ ,  $1.8 \times 10^{-6}$ ,  $1.8 \times 10^{-7}$ ,  $1.8 \times 10^{-8}$  cell  $\text{mL}^{-1}$  along with one control plate in each experiment. Finally the petri-plates were tightly sealed and incubated at  $25 \pm 1^\circ\text{C}$ , 70% RH and larvae were examined for pathogenicity at regular interval of one week for four weeks. It was observed that *Fusarium. oxysporum* at  $1.6 \times 10^{-6}$ , *Fusarium moniliformae* at  $1.5 \times 10^{-6}$  and *Aspergillus niger* at  $1.8 \times 10^{-6}$  cell  $\text{mL}^{-1}$  concentrations was found to be effective against larvae and kept for further experimental studies.

### Field Trials

Two field trials were laid out in triplicates in randomized block for testing the efficacy of biocontrol agents along with control, against bark eating pest of Indian gooseberry. In first trial three commercial biopesticides including Deuteromycotina fungi *Fusarium oxysporum* (fungal suspension) @  $1.7 \times 10^7$  cell  $\text{mL}^{-1}$  and *Beauveria bassiana* (Trade name- Boverin) (1% w/w) @  $25 \text{ g L}^{-1}$ , and a spore forming bacteria *Bacillus thuringiensis* (var. kurstaki) (Btk) (Trade name-Dipel BL) @  $25 \text{ g L}^{-1}$ , along with aqueous extract of Neem @ 10% were tested against the larvae. The bio-pesticides were prepared on the basis of their active spore formulation and were sprayed by Hand Sprayer (cap. 5lit). The second trial was conducted to evaluate the entomopathogenic activity of three in vitro isolated fungi with their effective dose of pathogenicity on the larvae. *Fusarium oxysporum* @  $1.6 \times 10^6$ ,



cell mL<sup>-1</sup>, *F. moniliformae*@1.5 ×10<sup>-6</sup> cell mL<sup>-1</sup>and *Aspergillus niger*@ 1.8 ×10<sup>-6</sup> cell mL<sup>-1</sup>, and solvent extract of *Cleistanthus collinus* prepared in petroleum ether@10%, were applied on the infected tree. The observations on the reduction of frass made by *I. quadrinotata* were recorded 3 7 10 and 15 DAT in both the trials. The effects of various treatments on bark eating caterpillar were determined using ANOVA and treatment means were separated using the LSD test.

### III. RESULT AND DISCUSSION

#### 1. Results

##### Effect of commercial biopesticides and plant extract on frass reduction

The efficacy of fungal suspension, commercial bio-pesticides and plant extract was studied and all the treatment shows significant frass reduction of *I. quadrinotata* in compared to control plot (p<0.05) (Table 1). There was a lower production of frass where neem extract was applied and in contrast, the frass formation progressed a little more in other treatments. The pooled mean data revealed that neem extract @10% was found significantly effective, followed by *Bacillus thuringiensis* @25g L<sup>-1</sup> in reduction of frass 15 DAT. The Larvae treated with fungal suspension of *Fusarium oxysporum* and *Beauveria bassiana* also shows frass reduction to a satisfactory percentage over control at 15 DAT.

**Table 1.** Effect of bio-pesticides and plant extract in frass reduction (%)

Treatment	Dose	Frass reduction (%)*
<i>F. oxysporum</i>	1.7x10 <sup>-7</sup> cell mL <sup>-1</sup>	60.83
<i>B. bassiana</i>	25g L <sup>-1</sup>	68.52
<i>B. thuringiensis</i>	25g L <sup>-1</sup>	70.27
Neem Extract	10%	79.5
Control		0.00
SEm ±		3.0201
CD at 5%		6.5802

\*mean of three replications

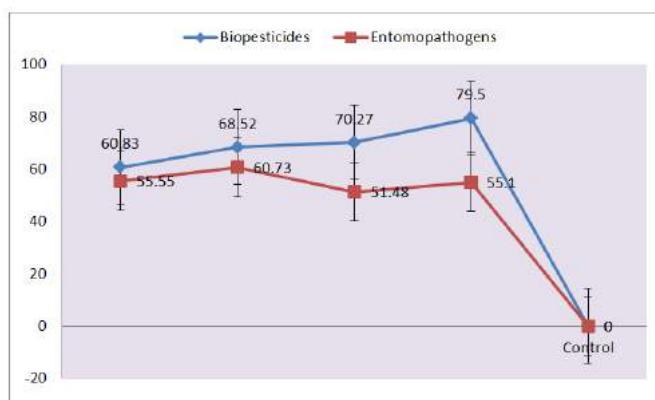
##### Effect of entomopathogens and plant extract on frass reduction

The pathogens isolated from the infected larvae were bio assayed for entomo-pathogenic activity against *I. quadrinotata* and were found effective in frass reduction. The assessment of the efficacy of three isolates and solvent petroleum ether plant extract shows significant variations from the control treatment (Table 2).

**Table 2.** Entomopathogenic activity of isolated pathogen in frass reduction (%) 15 DAT

Treatment	Dose	Frass reduction (%)*
<i>F. oxysporum</i>	1.6 ×10 <sup>-6</sup> cell mL <sup>-1</sup>	55.55
<i>F. moniliformae</i>	1.5 x 10 <sup>-6</sup> cell mL <sup>-1</sup>	60.73
<i>A. niger</i>	1.8 ×10 <sup>-6</sup> cell mL <sup>-1</sup>	51.48
<i>C. collinus</i>	10%	55.1
Control		0.00
SEm ±		7.6727
CD at 5%		17.693

\*mean of three replications



**Figure1:** Comparison of various treatments in frass reduction (%) of larvae, 15 DAT. Values expressed as mean SEM± over control

Among all the treatments *Fusarium moniliformae* @ 1.5 × 10<sup>-6</sup> cell mL<sup>-1</sup> shows maximum frass reduction. The suspension of the fungal isolates and petroleum ether extract of *Cleistanthus collinus* shows remarkable differences due to effects of mycoses in the frass of the larvae. The fungal suspension *F. oxysporum* @ 1.6 ×10<sup>-6</sup>

cell mL<sup>-1</sup> and *Cleistanthus collinus* extract were found at par with each other in minimizing frass formation by the larvae 15 DAT. The comparative study of all the treatments reveals that neem extract was found to be significantly superior among all the treatments in frass reduction of *I. quadrinotata* larvae (Figure 1).

## 2. Discussion

The use of bio-control agents and plant extracts in pest management in fruit trees was found significant. The present study reveals that neem extract was found significantly superior among all the treatments in frass reduction of the larvae. Sundararaj (2014) advocated the use of maximum quantity of neem based products as an integral component for pest management due to its feasibility. Kurosawa et al (2012) reported that emulsion form of nano organic pesticide prepared from Azadiractin A, B and Limonoids in combination with Earthen Pot Irrigation System (EPIS) resulted 50-90% reduction in apical twig, gall maker, leaf roller, bark and shoot borer and termites in Indian gooseberry in arid areas. Satti et al (2013) observed that botanical treatment of Neem and Argel at ten days interval has potentiality in controlling green leaf caterpillar (*Noorda blitealis* Walker), in drumstick tree (*Moringa oleifera* Lam.). The application of biocontrol agents in combination with biofertilizers for Fusarium wilt of *Delbergia sissoo* (Singh et al., 2002) and with organic manure against Fusarium wilt of *Gmelina arborea* (Singh et al 2003) was reported to be effective, which support the present work. Tounsi et al (2005) reported that the wild strain of *B. thuringiensis*, kurstaki individually as compared to toxin mixtures, have more effective biopesticidal action against the Mediterranean flour moth, *Ephestia kuehniella*. Mohan et al (2014) determined toxicity of *Bacillus thuringiensis* (*Bt*) kurstaki against important lepidopterous insect pests of agricultural importance. Similarly, the effectiveness of the entomopathogenic fungi *Beauveria bassiana* has been reported against the larvae of *Polyphylla fullo* (Erlar and Ozgur Ates, 2015), cotton bollworm (*Helicoverpa zea*) (Lopez and Sword, 2015) Colorado potato beetle *Leptinotarsa decemlineata* (Wraight and Ramos, 2015) and tobacco caterpillar (*Spodoptera litura*) (Kaur et al 2011).

The efficacy of entomopathogenic fungi against bark eating caterpillar was found to be encouraging. In the

present study the efficacy of *Fusarium sps.* and *Aspergillus sps.* was less significant in frass reduction as compared to plant extract and biocides which may be due to their weak pathogenesis on diseased lepidopteran forest pests as reported by Draganova et al (2013). The entomopathogenic activity of the pathogens on various other pests gave promising results. Abd El- Ghany et al (2012) observed that crude extract of *F. oxysporum* was found effective in controlling Greater wax moth *Galleria mellonella* (49.48%). Similarly, the entomopathogenic activity of *F. oxysporum* observed on southern green stink bug (*Nezara viridula*) of rice shows 51.96% mortality (Dutta et al 2013). Under laboratory conditions *Aspergillus niger* causes mortality of Red spider mite, *Oligonychus coffeae* Nietner of tea up to 91.11% (Mazid et al 2015).

## IV. CONCLUSION

The present research concludes that biological management of bark eating caterpillar in Indian gooseberry trees, the bio-pesticides appeared to be a promising candidate however the fungal isolate were also effective and thus needs a wide investigation. The Integrated pest management using plant extract, bio-pesticides, entomopathogenic fungi is environmental friendly, feasible and widely accepted. Future investigation in search of new biological control methods with the integration of modern scientific techniques can lead to develop ecofriendly pest control model.

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# Correlation of Knowledge and Metabolic Syndrome in Container Crane Operator

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## ABSTRACT

This study aimed to know the correlation between knowledge and incident of metabolic syndrome in container crane operators. The design of the study was observational method and conducted by cross sectional approach in an international container terminal facilities company in Surabaya. The independent variable was knowledge of metabolic syndrome using questionnaires method. The dependent variable was metabolic syndrome using International Diabetes Federation criteria. The number of population was 68 container crane operators and 40 operators as the samples were taken randomly. The results showed that knowledge did not have significant correlation with metabolic syndrome incident. Considering the study's result, the company needs to find other cause of the metabolic syndrome in their container crane operator population and find effective health promotion in metabolic syndrome prevention.

**Keywords:** Knowledge, Metabolic Syndrome, Crane Operator.

## I. INTRODUCTION

Cardiovascular disease, diabetes mellitus and stroke are degenerative diseases that allegedly afflict more and more workers in the world. These diseases can cost the company's health and reduce the quality of life of the workers. The diseases may also be an underlying factor cause of workers unsafe action. Unsafe actions may lead to work accidents.

Metabolic syndrome is a collection of symptoms that consist of glucose intolerance, insulin resistance, central obesity, dyslipidemia, and hypertension which can be a risk factor for cardiovascular disease and diabetes mellitus [1, 2]. Researchers believe that low physical activity and unhealthy lifestyles are related to metabolic syndrome in workers. To increase crane operator awareness in low physical activity and unhealthy lifestyles effects, the company held seminar about metabolic syndrome in 2015.

Health seminar in health promotion has important role in the company's occupational health and safety programs. The purpose of seminar is to give knowledge about metabolic syndrome. The knowledge is expected to change worker health's behavior. A container crane operator must have good condition of health including sufficient physical strength, endurance, agility, coordination, and reaction speed to meet the demands of working as a container crane operator.

## II. METHODS AND MATERIAL

This study aimed to know the correlation between knowledge and incident of metabolic syndrome in container crane operators. This study was designed as an observational study and conducted by cross sectional method. Forty container crane operators as respondents were obtained by using simple random sampling technique. The dependent variable in this study was metabolic syndrome using International Diabetes Federation criteria, while the independent variable was worker's knowledge about metabolic syndrome. This

study data get through questionnaires and direct measurements of blood pressure, waist circumference, fasting triglyceride (TG), high density lipoprotein (HDL) and blood glucose. The ethics committee for Research Project in Airlangga University, Surabaya, Indonesia approved the study. The study was held in an international company that provides container terminal facilities in Surabaya, Indonesia.

### Validity and Reliability

The questionnaires instrument was validated by 13 operators excluded from the respondents. Cronbach's Alpha reliability method was adopted to determine the internal consistency of the instrument. A reliability coefficient of 0.91 was obtained.

### III. RESULT AND DISCUSSION

Knowledge of metabolic syndrome data obtained by calculating the total score of the correct answers. Each correct answer is worth 1 score. Table 1 show that the average score of container crane operator knowledge was 8.03. The lowest score is 0 and the highest score of 13 out of 18 questions. Based on the calculation of 95% Confidence Interval, the average metabolic syndrome knowledge in the population of PT. X container crane operator was 7 to 9 score.

**Table 1.** Distribution of Respondents Knowledge Score

Variable	Mean	Median	Modus	SD	95% CI
Knowledge Score	8,03	8	8	2.64	7.18-8.87

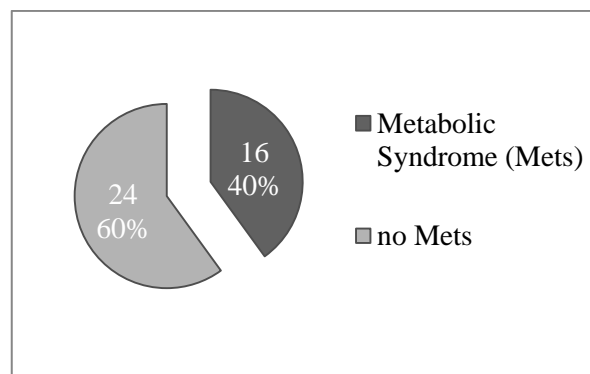
For correlation analysis, knowledge variable was transformed into categorical variable. As seen in Table 2, the respondents knowledge score most frequents were less than 9 point (23 men).

**Table 2.** Distribution of Categorical Data in Knowledge Score Variable

Data		Frequency	Percentage (%)
Knowledge Score	≤8	23	57.5
	>8	17	42.5
Total		40	100

Based on the examination results of the respondents fasting TG, HDL, blood sugar, blood pressure and abdominal circumference added with history of blood pressure medications, history of treatment of cholesterol as well as diabetes mellitus, the study found the number of respondents who have metabolic syndrome according IDF criteria as much as 40% or 16 of the total 40 respondents as seen in Fig 1.

Dyslipidaemia was the most frequent result came from 32 people or 82.5%, then the second rank was the abdominal circumference (above 90 cm for Asian ethnic) which was found in 30 people or 75% of total respondents, followed hypertension and blood sugar levels above normal (hyperglycaemia).



**Figure 1.** Percentage of Metabolic Syndrome Incidents

Correlation between knowledge score and metabolic syndrome incident was analysed using Chi-square test and  $\alpha$  0.05. Chi-square analysis was due to the non-parametric data category. This analysis aims to examine differences in the proportion of two or more groups of samples [3, 4]. Knowledge of the metabolic syndrome that  $\leq 8$  score had fewer metabolic syndrome incidences than  $> 8$  score as seen in Table.

**Table 3.** Correlation between Knowledge Score and Metabolic Syndrome

Variable	Mets		N (%)	p-value	Correlation
	Yes	No			
<b>Knowledge Score</b>					
>8	10 (58.8%)	7 (41.2%)	17 (100%)	0.08	Not Significant
	6 (26.1%)	17 (73.9%)	23 (100%)		
≤ 8					

Occupational health care in PT. X had health education in metabolic syndrome theme which was attended by representatives of employees in 2015. This study found that many respondents could only answer questions less than 9 questions. Percentage of metabolic syndrome in operator who can answer  $\leq 8$  questions and  $> 8$  question was 26.1% and 58.8% and showed no significant correlation.

Metabolic syndrome can be prevented with a workers healthy behaviour. Benjamin Bloom theory, quoted by Notoatmodjo [5] stated that health behaviour could be shaped by knowledge along with an attitude, and practice. Knowledge has 6 levels intensity, which are knowing, understanding/comprehension, application, analysis, synthesis, and evaluation. Data analysis showed that the operator with a high score had greater risk of metabolic syndrome. This result might be caused the operator was on the knowing level and did not understand also able to evaluate the hazards of the metabolic syndrome.

Lawrence Green theory mentioned that health problems caused by behavioural factors and non-behavioural factors. Though with increasing one's knowledge did not necessarily lead to behaviour change. Changes in healthy behaviour required an enabling factor and reinforcing factors [5]. According to Rowe, *et al* cited by [6], enabling factors are health facilities availability, availability of infrastructure to support the success of the program. In this study, enabling factors could be achieved by providing the sports infrastructure for operators, health care and referrals system. Reinforcing factors could be given through the figure of someone who had influence in the company and provided continuous monitoring of workers health.

Notoatmodjo also added that a person's health behaviours related his socio-economic level, culture, religion, education and experience [5]. Knowledge could not associate with the occurrence of the metabolic syndrome directly be caused of a lot of other factors involved. Operators with knowledge score above 8 points might be at risk of metabolic syndrome due to unsuccessful influence figure health workers in health presentation class, the absence of supporting healthy habits infrastructure, his culture, his socio-economic, education and experience. Similarly, the operator that had score knowledge under 8 points more likely to have metabolic syndrome risk factors from the opposite of the previous explanation.

#### IV. CONCLUSION

There is no significant correlation between knowledge and metabolic syndrome incident. The company owner must find the cause of metabolic syndrome incident among workers and the worker themselves should aware of the importance having healthy behaviour and prevention of metabolic syndrome.

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# Lane Detection for Departure using Hough Transform for Driving System

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## ABSTRACT

The Hough transform is technique of feature extraction used for analysis of images, computer vision, and digital image processing. The purpose of the technique is to find imperfect instances of objects within a certain class of shapes by a voting procedure. This voting procedure is carried out in a parameter space, from which object candidates are obtained as local maxima in a so-called accumulator space that is explicitly constructed by the algorithm for computing the Hough transform.

A Hough Transform Based Lane Detection for Driving System has been developed to aid a driver in the lane departure decision-making, to reduce a loss of concentration and to prevent an accident while driving. In this paper, we propose a method for detecting the lanes by using a webcam camera to record the road as a video file (.avi) and using Hough Transform method to detect the lanes in an image. The results show the lane detection in various line road conditions. According to the results, the drivers can use this information to increase their safety of driving, especially when making the decision of lane changing.

**Keywords:** Computer Vision, Voting Procedure, Hough Transform, Driver Assistance Systems, Standard Hough Transform

## I. INTRODUCTION

Vision based street lane detection and tracking is an important process for Driver Assistance Systems, which can reduce the risk of car accidents. Various lane detection and tracking methods have been proposed but situations like strongly differing illuminations, and unmarked or partly marked lanes are still challenging. Now a days, the growing volume of the traffic all around the world requires higher levels of the traffic safety on the road. There are so many unsafe driving cars that the driver required to be highly careful while driving. Important for driver is being careful when he/she is changing lane, especially in new driver who absolutely cannot keep too much information at once and has no confidence for driving. Driver may lose concentration and control over car. In fact, human behaviors are indeed hard to recognize, predict and handle by current available equipments. Therefore, a monitoring and warning system focusing on behaviors of the vehicle is

needed while the car is moving on the road. A Hough Transform Based Lane Detection for Driving System was developed to find the way that can reduce the loss of views to the frontstreet.

The Hough Transform, one of the easy techniques for image processing, used for detecting road lanes within the image. This system is designed to work in conjunction with the general principles of the webcam image processing. The system helps the driver by keeping track of movements of a vehicle through the lanes. The System proposed in this paper is comprehensive system which uses road information collected using camera. Firstly, the acquired video file is extracted to image frames. Secondly, the image frames are specifically divided into road part from the information of webcam camera. Thirdly, RGB color image is converted by image enhancement to grayscale image. Threshold is applied to evaluate the grayscale image into a binary image. Fourthly, the Hough

transform method is applied to detect the lanes in the road image and finally, lane departure is predicted from obtain Hough Line Pattern. The concept in this paper proposes complete system to monitor the position of a vehicle with respect to road lanes for improved driving safety.

## II. METHODS AND MATERIAL

### Frame Differencing

Frame Differencing is an easiest simple method for finding objects which got subtracted from one frame to another, in which all the different objects of interest called foreground responsible for this process to be applied to detect moving objects. In this method, the algorithms cannot be used because the frame differencing were not able to detect the object when it is stationary, even it intrude along the defensive line at that time.

### Image Processing

Digital Image Processing converts image data into digital data. The system receives image data, calculates output digital image data and then store image data into the computer's memory that can be performed by reservation memory of the machine in the form of an array. The value in each array represents the quantity of pixel. The position of the image is determined by the position of the array.

### Principle for splitting video into image

Video file is a computer file that contains digitized video. The video which has high motion will be having the high numbers of frames per second (frame rate). Video files can be split into frame, used for process the image and stored in a three dimensional array.

### Cropping an image

Crop tool is used to extract road part within an image by using the 'imcrop' function that can specify the size and position as parameters by specifying the crop rectangle as a four-element position vector.



(A) Original Image



(B) Road Part Image

**Figure 1.** The result of cropping road part image.

### Image Enhancement

This process changes the color image (RGB) to a grayscale image. Its analysis detects objects in the image frames into the process. If using the color image in the work, the process will be slow because the process must access all of the chromaticity. Therefore, it would be easier to access the grayscale image.

### Morphological Operations

Morphological operations affect the form, structure or shape of an object in an image. These operations are performed on binary images and used in pre or post processing. For this paper, Morphological operations assist segmentation. They are used to cleanup the image including clear border objects, remove small objects and also reduce noises in the image.

### Hough Transform

The Hough transform is a popular technique which can be used to isolate features of a particular shape within an image. In this work, it is used for detecting line road as lane detection. There are 2 methods for computing the Standard Hough transform (SHT) of the binary image BW, which is an algorithm of parameter matrix whose



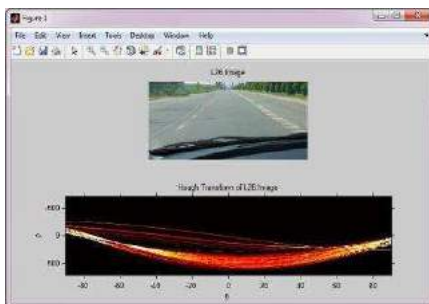
rows and columns correspond to rho and theta values respectively. The first method is given below:

$$[H, \theta, \rho] = \text{hough}(BW) \quad (1)$$

For detect lines in the image. The second method is adding parameter name and value pairs in the same equation as follow:

$$[H, \theta, \rho] = \text{hough}(BW, \text{ParameterName}, \text{ParameterValue}). \quad (2)$$

When Parameter Name is 'Rho Resolution', specify a real scalar value between 0 and norm (size (BW)), to determine the spacing of the Hough transform bins along the rho axis. The default value is 1. When Parameter Name is 'Theta', specify a vector of Hough transform theta values. The acceptable range of theta values is  $-90^\circ \leq \theta < 90^\circ$ .



**Figure 2.** Detect line road by using the Hough transform

### Houghlines

This method is used to extract line segments in the image based on Hough transform. There are 2 functions to describe the meaning of parameter and how to search the line segment. The first function is given below:

$$\text{lines} = \text{houghlines}(BW, \theta, \rho, \text{peaks}) \quad (3)$$

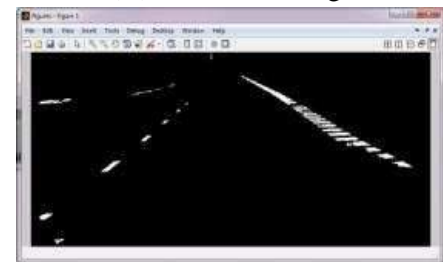
Where BW associated with particular bins in a Hough transform. theta and rho are vectors returned by function hough. peaks is a matrix returned by the hough peaks function which contains row and column coordinates of the Hough transform. Secondly, using parameter/value pairs as follow:

$$\text{lines} = \text{houghlines}(\dots, \text{param1}, \text{val1}, \text{param2}, \text{val2}) \quad (4)$$

It specifies the distance between two line segments with the same Hough transform bin. If its distance less than the value specified, the hough lines function will merge the line into a single line, and Minlength parameter which specifies whether merged line should be kept or left.



(A) Road Part Image



(B) Image after Extract Line Segment

**Figure 3.** Example of Hough lines

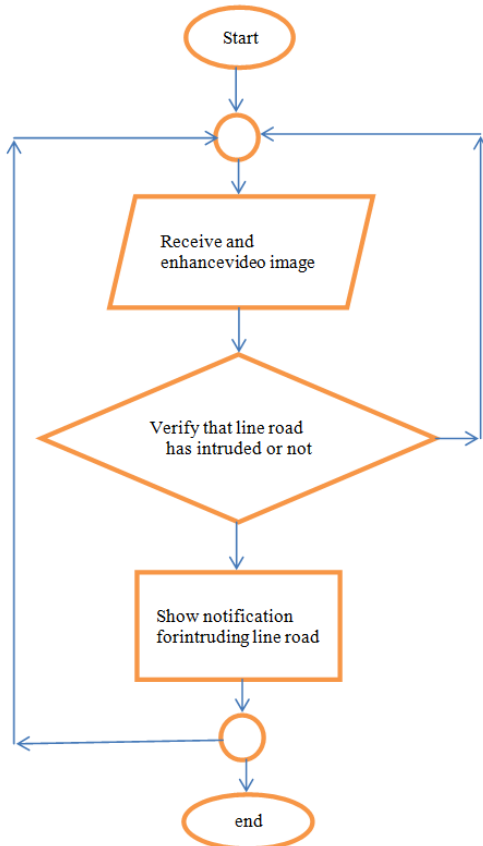
### Proposed Method for Hough Transform

In this paper, the main components of driving system detection based on the Hough transform are webcam camera and computer for storing imagedata. Firstly, the system saves image data from webcam camera and then transfers it into frame grabber processing the computer. Secondly, in recording data, real times applied to image data (.avi). Then the program which analyses the image data in a video signal will be taking the recorded data to further processing. The idea of this project is to provide a computer to monitor the image of line road that has intruded. The surveillance will be notified when car crosses the line. The processes are described below:

The main components of driving system detection based on the Hough transform are described as below:

Step 1: Receiving image: the developed system is able to acquire images from video files, which are already saved in a form of .avi video. The image will be the same size as the resolution of the camera that used to record without any additional configuration.

Step 2: Splitting image: The system analyzes the video file to apply to the video frames splitting process by applying the data of video files and then the system specifies the variable to divide sub frames in the variable of array image to the frame.



**Figure 4.** Frame work of driving system detection based on the Hough transform.

Step 3: Cropping image: Specifying the area of interest by using crop tool to extract the region of road part within the image. It can specify the size and position as parameters by specifying the crop rectangle as a four-element position vector.

Step 4: Image enhancement (from RGB color to grayscale image): In this process, the system conducts the frame, which stored in the storage module, to convert RGB color image into grayscale image. After this process, we have the grayscale image. As shown in Fig 5.



(a) Road part image

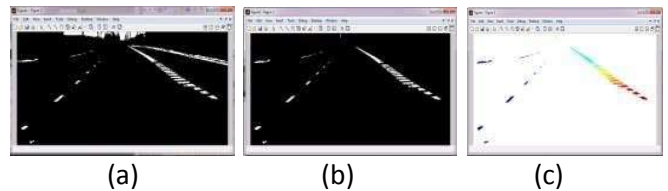


(b) Grayscale image

**Figure 5.** The results of image enhancement from converting RGB color image (road part) to grayscale image.

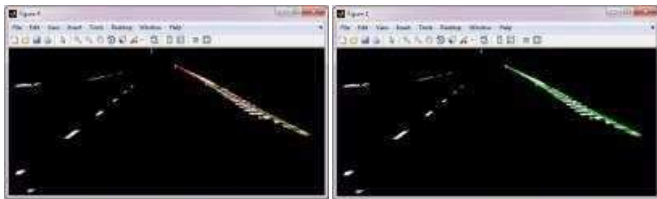
Step 5: Converting grayscale image to binary image: Threshold is an appropriate method that uses to convert the grayscale image to binary.

Step 6: Segmenting and removing objects: Using the opening operation, Morphological Operations which can assist segmentation and remove objects which are not structuring element. As shown in Fig 6.



**Figure 6.** The image received by Morphological Operations, (a) is cleaned image, (b) is image with clear border objects and (c) is all remaining connected regions. It was showed by color.

Step 7: Defining the analytic area within the image: The system conducts the binary image to define detection or surveillance area within the image by using the Hough transform which can identify straight line. We set green color to detect line and red color to detect the longest line segment as shown in Fig 7.



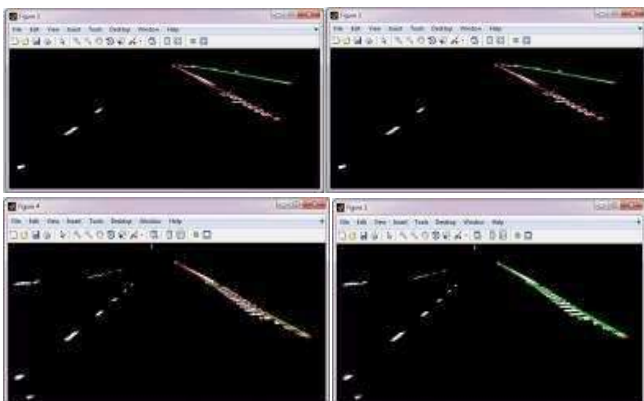
(a) red color (b) green color

**Figure 7.** The results of final detected lines

Step 8: Computing Hough transform: From step7, the system operates the defined segment to analyze the Hough transform. The original image is determined by Hough function. After computing, we get the Hough transform in red color to show the line parameters of its algorithm in the image.

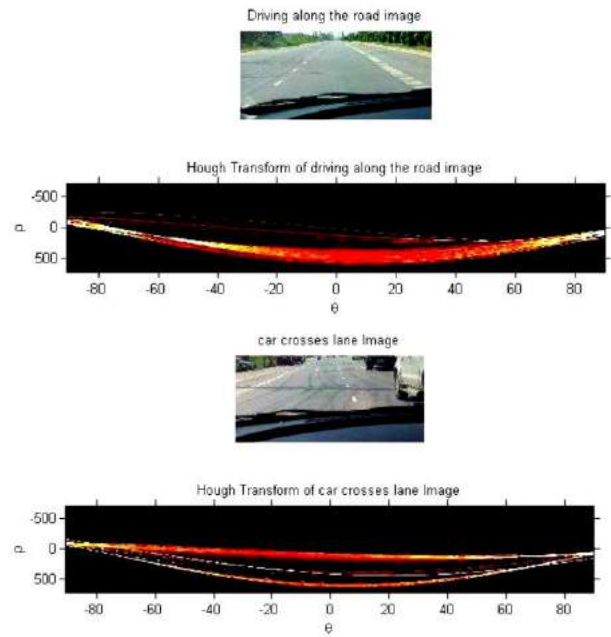
### III. RESULT AND DISCUSSION

A Hough Transform Based Lane Detection for Driving System has been operated by setting the webcam camera to monitor the movement of vehicle through the lanes, segmenting the video file to the image, converting RGB color image to grayscale, setting threshold to make a binary image, using Morphological Operations and defining the analytic areas by using Hough transform. In this section we will explore the experimental results of the performance of the lane detection algorithm in various environments and describe its algorithm based on its process. We test the lane detection while driving along the lanes which have 2 types of lanes; dashed line and solid line. It also has 2 colors; white and yellow lane color. We proposed test conditions as follows:



**Figure 8.** Lane detection results by Hough transform

This test condition shows the detected lane boundaries which are highlighted by green color. The longest detected lane boundaries are highlighted in redcolor. From the experimental results, this algorithm can detect any kind of lanes with various test conditions.



This test condition shows the red line parameters in Hough transforms. We show the original image in 2 Situations; intruded lane and not intruded lane.

## IV. CONCLUSION

In this paper, we proposed a method for helping the drivers in the lane departure decision-making based on the Hough transform by detecting lanes. The adopted lane detection method was consisted of image processing, morphological operations, dynamic threshold, Hough lines and Hough transform. Its advantages of Hough transform are easy to use, lowcost and also effective in detect lines form the image. Experimental results reveal the efficiency of the performance of the lane detection algorithm in various environments. In further research, we will focus on how to detect the lane correctly in various situations, and how to arouse driver's attention by setting the sensor in a car.

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# Specific Activity and Production Yield of Neutron Induced Cross Section Reactions for Hafnium Isotopes at 14.5MeV

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## ABSTRACT

Specific activity and total production yield at the end of irradiation (EOI) (i.e. Saturation) have been calculated. Projected production yields from irradiation of Hafnium target element induced (as a function of irradiation time) by 14.5MeV neutron at  $1 \times 10^9$  neutron/cm<sup>2</sup>.s neutron flux. Different irradiation time have been selected for the periods (1, 60, 3600, 86400, 172800 s) showing the saturation state for each reaction. The analyzing of a complete energy range has been done starting from threshold energy for each reaction. The cross sections are reproduced in fine steps of incident neutron energy with 0.01MeV intervals with their corresponding errors. The recommended cross sections for available experimental data taken from EXFOR library have been calculated for all the considered neutron induced reactions for Hf (Z=72; A=176-180) isotopes. The calculated results are analyzed and compared with the experimental data.

**Keywords:** EXFOR, Incident Neutron, 14MeV, Recommended Cross Section, Specific Activity.

## I. INTRODUCTION

The excitation functions in (n,2n), (n, $\alpha$ ), and (n,p) reactions measured for Hf (Z=72, A=176-180) with the aid of EXFOR library have been evaluated in the present work for the exact estimation of the cross sections among different authors. This paper describes Specific Activity, Production Yield and Neutron Induced Cross Section Reactions for Hafnium Isotopes at 14.5MeV neutron energy. The systematic of such reactions, neutron induced reactions was discussed in Smith D.L. et al. (1989) [1], Van D. N. et al. (2008) [2], Noguere G. et al. (2009) [3] and Junhua L. et al. (2011) [4]. The present work concerns the induced neutron cross section reactions. Recommended formulas for the evaluation of cross sections for these reactions were derived using EXFOR experimental data for different authors describing the emission or neutron capture in nuclear reactions. The parameters of formulas were fitted with minimum chi squared from the analysis of available experimental data.

Radioisotopes are produced in a nuclear reactor by neutron induced reaction from different neutron sources

(nuclear reactor, neutron generator) by exposing appropriate target material to the neutrons in the reactor, causing a nuclear reaction to occur which leads to the production of desired radioisotope. The factors which decide the type of nuclear reaction that takes place and the rate of production are [5]:

1. The energy of the neutrons and the neutron flux.
2. The characteristics and quantity of the target material.
  - a. Substances which are explosive, pyrophoric, and volatile, etc. are not permitted to be irradiated in neutron sources.
  - b. Targets should be stable under irradiation conditions.
  - c. Isotopically pure target gives high specific activity radioisotopes.
  - d. The physical form of the target should be such that the neutron flux depression is minimum.
  - e. The target should be in a suitable chemical form for post irradiation processing.
  - f. Usually target in metallic form are preferred.
  - g. If the target is hygroscopic; it is preferable to preheat the target prior to encapsulation.

- The activation cross-section for the desired reaction. The radioisotopes have numerous applications in medicine, agriculture, industry and pure research.

## II. METHODS AND MATERIAL

### 1. Recommended Cross Section

The available measured data from EXFOR library for the cross section of the above mentioned reactions measured for Hf ( $Z=72$ ,  $A=176-180$ ) have been plotted, interpolated and recalculated in different fine steps and for different energy ranges of incident neutron by using Matlab-8.1 in order to calculate the recommended cross section for each mentioned reaction. This can be described in the following steps:

- The interpolations for the nearest data for each energy interval as a function of cross sections and their corresponding errors have been done using Matlab-8.1.
- The sets of experimental cross sections data are collected for different authors and with different energy ranges. The cross sections with their corresponding errors for each value are re-arranged according to the energy interval 0.01MeV for available different energy range for each author.
- The normalization for the statistical distribution of cross sections errors to the corresponding cross section values for each author has been done.
- The interpolated values are calculated to obtain the recommended cross section which is based on the weighted average calculation according to the following expressions [6]:

$$\sigma_{w.a.} = \frac{\sum_{i=1}^n \frac{\sigma_i}{(\Delta\sigma_i)^2}}{\sum_{i=1}^n \frac{1}{(\Delta\sigma_i)^2}} \quad (1)$$

Where the standard deviation error is:

$$S.D. = \frac{1}{\sqrt{\sum_{i=1}^N \frac{1}{(\Delta\sigma_i)^2}}} \quad (2)$$

Where  $\sigma_i$  : is the cross section value.  $\Delta\sigma_i$  : is the corresponding error for each cross section value.

Fig.s 1 to 3 illustrate the recommended cross sections for the above mentioned reactions as calculated in the present work compared with EXFOR library. It is clear in the caption of each figure, the refry of authors name are arranged according to the year of measured data are listed with the present calculated recommended cross section. The results are in good agreement with the measured data.

### 2. Specific Activity And Production Yield of Radioisotopes

The activity of a certain sample is the number of radioactive disintegrations per sec for the sample as a whole. The specific activity, on the other hand, is defined as the number of disintegrations per sec per unit weight or volume of sample. The unit of activity is the Becquerel (Bq), which is defined as a decay rate of one disintegration per second (dps). The fundamental equation to calculate the activity produced in a target is described by a first order differential equation [5,7]:

$$\frac{dN}{dt} = N_{tot}\phi\sigma_{act} - \lambda N \quad (3)$$

Where:

$\frac{dN}{dt}$  : is the production rate per second.

$N$  : is the number of activated atoms.

$N_{tot}$  : is the total number of target atoms.

$\phi$  : is the neutron flux (number of neutrons per  $\text{cm}^2$  per second).

$\sigma_{act}$  : is the activation cross section ( $1\text{barns}=10^{-24} \text{cm}^2$ ) refers to the production of the particular radioactive species.

$\lambda$  : Decay constant.

The activity of radionuclide formed at any time during or at the end of irradiation is obtained by integration of equation (3) [5,7].

$$A = \lambda N = N_{tot}\phi\sigma_{act}(1 - e^{-\lambda t}) \quad (4)$$

Where:

$$N_{tot} = \frac{mN_{av}a}{M} \quad (5)$$

Therefore, the specific activity is:

$$A_{SP.act.} = \frac{N_{av} \phi a \sigma_{act} (1 - e^{-\lambda t})}{M} \quad (6)$$

$m$  : Mass of the target material in grams.

$N_{av}$  : Avogadro's number ( $6.023 \times 10^{23}$  atom/mole).

$a$  : Isotopic abundance of the target isotope.

$M$  : Atomic mass of target material in atomic mass unit.

$t$  : Time of irradiation in sec.

The nuclear reaction cross sections are of considerable importance in optimizing the production process of a radioisotope. In principle, the well-known activation equation is applicable to all activation processes, induced by neutron [8].

### III. RESULT AND DISCUSSION

Nuclear reactions leading to radioisotope production yield studied in this paper are:

#### 1- (n, $\alpha$ ) reaction

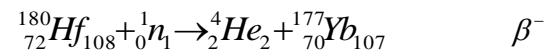
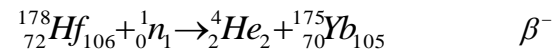
In the following cases: For (n,  $\alpha$ ) reactions, some of them lead to a product with a certain half-life and intensity which decays by beta emission to the isotope of interest, as shown in Table I, the following details of decay modes for (n,  $\alpha$ ) reactions are [9]:

**Table I:** Experimental (IAEA)[10] and calculated recommended cross sections at 14.5MeV with the activation product yields of Hafnium target element at ( $1 \times 10^9$  n/cm<sup>2</sup>.s) fast neutron flux, and the properties of their products.

Target	Reaction	Cross Section (mb)	Activation Yield (Bq per 1 $\mu$ g) at Different Irradiation Time						Properties of Product		
			1 sec	1 min	1 hour	1day	20 days	saturation	$T_{1/2}$	$E_{\gamma}(MeV)$	$I_{\gamma}(\%)$
$^{176}_{72}Hf_{104}$	$^{176}_{72}Hf_{104}(n,2n)^{175}_{72}Hf_{103}$	2000(EXP.)	4.07E-8	2.44E-6	1.46E-4	3.5E-3	6.39E-2	0.3523	70d	0.0545	54
		2000(pw)	4.07E-8	2.44E-6	1.46E-4	3.5E-3	6.39E-2	0.3523		0.0615	14
$^{178}_{72}Hf_{106}$	$^{178}_{72}Hf_{106}(n,\alpha)^{175}_{70}Yb_{105}$	2.1(EXP.)	3.69E-9	2.21E-7	1.32E-5	2.94E-4	1.86E-3	1.93E-3	4.19d	0.054	2.9
		2.91(pw)	5.11E-9	3.06E-7	1.83E-5	4.07E-4	2.6E-3	2.7E-3		0.1135	1.9
	$^{178}_{72}Hf_{106}(n,p)^{178m}_{71}Lu_{107}$	1.02(EXP.)	5.15E-7	3.04E-8	8.07E-4	sat	sat	9.36E-4	21m	0.0932	17.4
		0.63(pw)	3.17E-7	1.87E-5	4.98E-4	sat	sat	5.77E-4		0.2135	81.4
$^{180}_{72}Hf_{108}$	$^{180}_{72}Hf_{108}(n,2n)^{179m}_{72}Hf_{107}$	690(EXP.)	.....	.....	.....	.....	.....	.....	18.7s	.....	.....
		21.43(pw)	9.18E-4	2.25E-2	sat	sat	sat	2.53E-2		0.15	17.2
$^{180}_{72}Hf_{108}$	$^{180}_{72}Hf_{108}(n,\alpha)^{177}_{70}Yb_{107}$	2.2(EXP.)	2.64E-7	1.58E-5	7.97E-4	sat	sat	2.61E-3	1.9h	1.08	4.7
		0.91(pw)	1.09E-7	6.56E-6	3.3E-4	sat	sat	1.08E-3		0.3436	85

(n, $\alpha$ ) Reactions:

Decay Modes [9]:

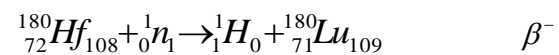
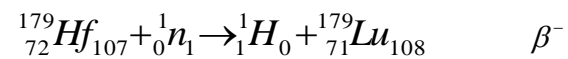
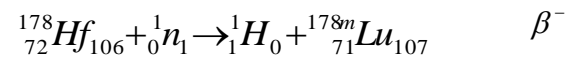


2- (n,p) reaction

In some cases (n,p) reaction leads to a product with a certain half-life and intensity which decays by gamma emission to the isotope of interest as shown in Table I for the following reactions:

(n,p) Reactions :

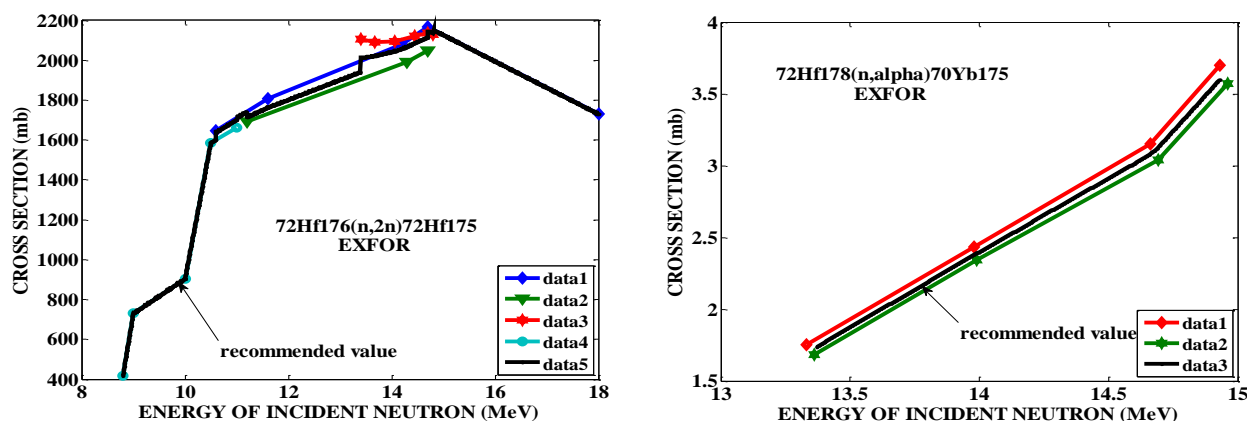
Decay Modes [9]:



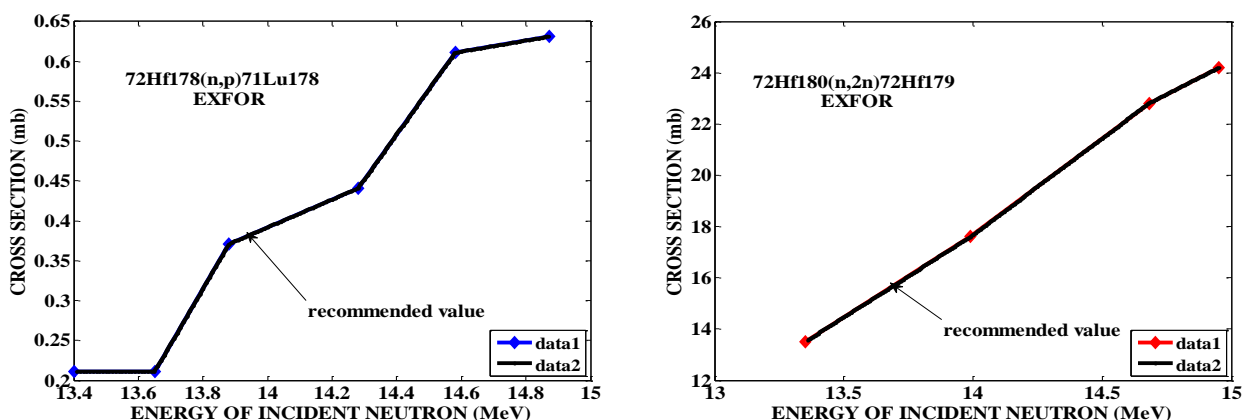
It is clear from Table I that (n, $\alpha$ ) and (n,p) reactions for each product are described by combining with gamma emissions . The study of the systematic cross sections for (n,2n) reaction which are listed in Table I gives products with gamma emission decay mode.

Table I list the calculated specific activity and total production yield at the end of irradiation (EOI) (i.e. Saturation). The projected production yields from irradiation of Hafnium target element induced (as a function of irradiation time) by 14.5MeV neutron at  $1 \times 10^9$  neutron/cm<sup>2</sup>.s neutron flux values are shown in Fig. (4). Different irradiation time have been selected for the periods (1, 60, 3600, 86400, 172800 s) showing the saturation state for each reaction.

Fig. (4) show that the growth of activity in a target under irradiation increases exponentially and reaches a saturation value limited by the neutron flux for a given weight of the target element. The results show good agreement in most of the reactions, but there is a discrepancy for some reactions between the experimental and calculated values, because there are differences in the type of the detector used, experimental technique, and the cross sections even if the cross section value of a certain author is in a good agreement with the experimental value.

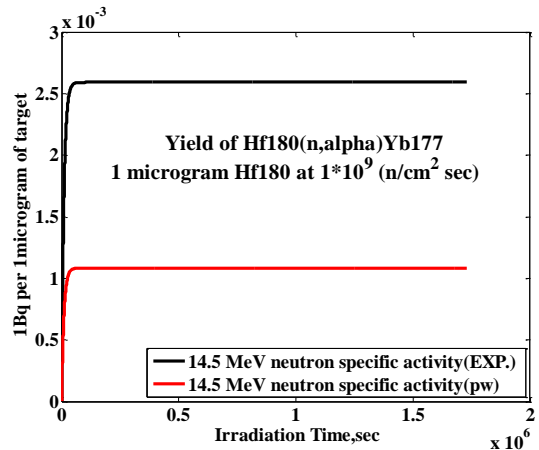
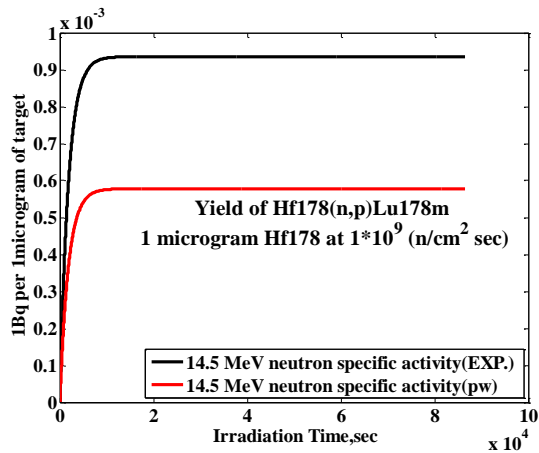
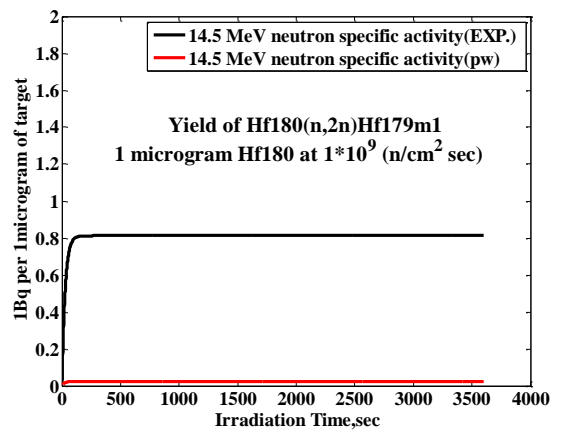
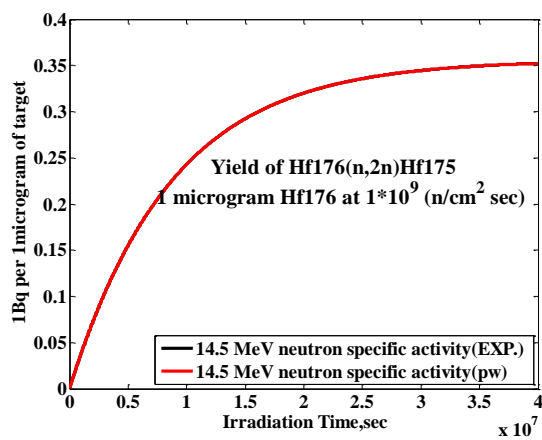
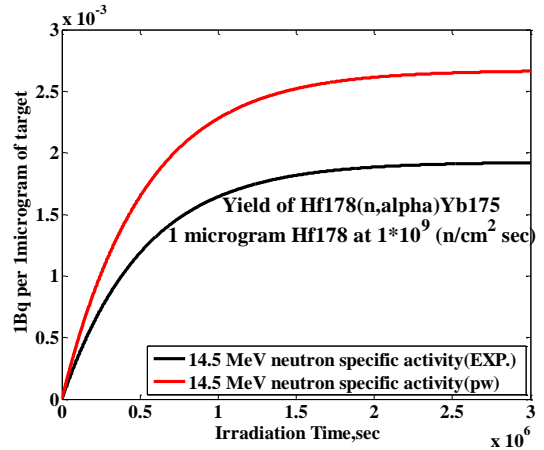
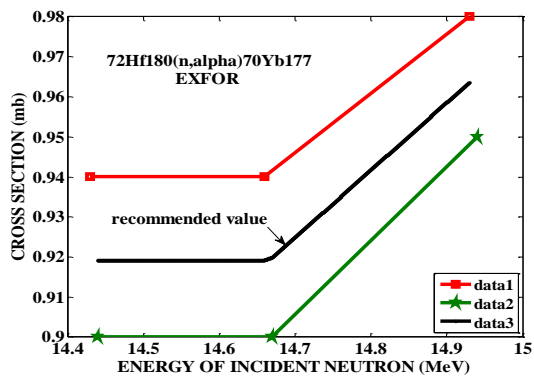


**Figure 1.** Recommended cross section compared with EXFOR Library versus the energy of incident neutron. Left side: for the  $^{72}\text{Hf}176(n,2n)^{72}\text{Hf}175$  reaction; Data 1: [11] LuHanlin et al.(1999). Data 2: [12] Kiraly B. et al.(2001). Data 3: [13] ZhuChuan-Xin et al.(2010). Data 4: [14] Serris M. et al.(2012).Data 5: present work (pw). Right side: for  $^{72}\text{Hf}178(n,\alpha)^{70}\text{Yb}175$  reaction; Data in right side: Data 1: [15] Konno C. et al.(1990). Data 2: [16] Konno C. et al.(1993). Data 3: present work (pw).



**Figure 2.** Recommended cross section compared with EXFOR Library versus the energy of incident neutron. Left side: for  $^{72}\text{Hf}178(n,p)^{71}\text{Lu}178$  reaction; Data 1: [17] Murahira S. et al.(1995). Data 2: present work (pw).Right side: for  $^{72}\text{Hf}180(n,2n)^{72}\text{Hf}179$  reaction; Data in right side Data 1: [16] Konno C. et al.(1993). Data 2: present work (pw).





**Figure 4:** Calculated and experimental [10] activation for the production yields of 72Hf Target elements by neutron irradiation of targets as a function of irradiation time at  $1 \times 10^9$  (n/cm<sup>2</sup>.s) fast neutron flux with 1microgram of target element.

## IV. CONCLUSION

We have evaluated the neutron induced nuclear cross section data of spherical hafnium isotopes for considerable energy ranges. The calculated recommended cross sections are in good agreement with experimental data. The reliability in this work is to estimate the specific activity and production yield for the energy  $E=14.5\text{MeV}$  for  $^{72}\text{Hf}$  ( $A=176-180$ ) isotopes target elements of neutron induced reactions. The results confirm that the comparison of calculated and experimental cross sections is especially important because reaction calculations have to be used to estimate important cross sections for applied purposes, which are difficult to measure.

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# Steam-Microwave on Reduction of Microbial Level of Red Pepper and Compared with Gamma Irradiation

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## ABSTRACT

Red pepper is utilized as a single flavor or in combination with other spices. This spice contains capsaicin, Carotenoid pigment and high Ascorbic acid level which have proper health and Anti-cancer effects on it. Normally this product is replete with microbial load and pathogens prior to applying any decontamination methods that could threaten human life. So creating a new approach for microbial decontamination of whole dry red pepper is obligatory, which is applying steam and microwave together. Here, saturated steam and microwave heat is used for 10 minutes with 720 watts and for 90 seconds respectively, while microbial load, enzymatic activities, moisture content and color alternation were monitored. Finally it was observed that processed red pepper contains the minimal microbial load and lacks any enzymatic activities, even in comparison with applying gamma irradiation with 5K Gy in which enzyme activity was noticeably seen.

**Keywords :** Red pepper, Irradiation, Microbial load, Microwave, Capsaicin

## I. INTRODUCTION

Red Pepper is planted all over the world and is used both in fresh and dry forms. This product basically is used for creation of desired red color along with a sharp taste, it has been known that (Kim et al. 2014; Rico et al. 2010). Red Pepper contains a significant amount of A and C vitamins. Its active ingredient that describes the sharp taste is known as capsaicin which has high impact on reduction of body fat mass and cholesterol levels, it was reported that (Song et al. 2014). Red Pepper production has risen 21% since 1994 and plays an important role in all kinds of food, dressings and seasonings, it was reported that (Neetoo & Chen 2012). Red Pepper contains large amounts of carotenoids and ascorbic acid which has high anticancer effects, it was reported that (CRC handbook of medicinal spices.). Before applying any decontamination method, this useful material contains lots of microbial loads along with huge amounts of mold, yeast, and big varieties of bacterial loads, it has been known that (Kim et al. 2014; Rico et al. 2010), and is considered as a contaminator and even infectious substance in food materials, it was reported

that (Neetoo & Chen 2012). It specifically leads to microbial spoilage such as Salmonella and E. Coli's activities in uncooked materials, thus this contamination needs to be reduced to its lowest amount available, it was reported that (Rico et al. 2010).

In Mexico's market, 10 out of 27 samples of red pepper are infected by Salmonella. Furthermore, US witnessed one of the greatest epidemic spreads of Salmonella caused by raw red pepper usage. Most such pathogenic substances have their least activity in dry situations. On the contrast they would have their maximum activity in humid and watery circumstances which leads to endangering consumers' health, it has been known that (Neetoo & Chen 2012; Song et al. 2014). Nowadays, legal and financial acts are concentrated on either developing and enhancing microbes de-off techniques based on preservation quality, consumers' health, and safety. Common preservative techniques are as follow:

A. Utilizing Ethylene Oxide fumigation, which is being used for a long time but banned in many countries since their cancer-causing effects, it has been known that

(Kim et al. 2014; Song et al. 2014). The reason lies underneath the accelerated combination of Ethylene Oxide and Chloride or Bromide in order to generate stable mutagenic cancer-causing compositions (i.e. 2-ChloroEthanol and 2-BromoEthanol), it was reported that (Taylor et al. 2014).

B. Using UV lamps in production and packing lines which its decontamination effect is not that efficient which itself is the consequence of UV's low penetration rate, it was reported that (Kim et al. 2014).

C. Using Ozone would be considered as a safe solution because of its appropriate anti-bacterial effects along with having no garbage and toxin as well, and being decomposed completely in face of Oxygen. This will lead to termination of Microorganisms by means of oxidation of cellular components. However, its usage is not efficient in fatty substances since its oxidative rate is measured to be more than standard thresholds, it was reported that (Oliveira et al. 2013).

D. Utilizing Gamma irradiation, from 2 up to 7 KGy has efficient and significant effect on decontamination of all kinds of spices. Although, other factors such as public fear and high costs, leads to cold reception in many countries, it has been known that (Song et al. 2014; Kim et al. 2014).

E. using heat treatment supported by saturated steam is an efficient approach which performs sterilization properly. However, it leads to unwanted organoleptic and nutritional effects in the specified spice. Moreover, there is a mechanism needed for drying the wet pepper, it was reported that (Kim et al. 2014).

F. Microwave treatment, Microwave's heat has lots of application among food industry, which in controlled situations can act as a beneficial approach in order to reduce microbial load, leading to high performance and qualified product compared with other common heat treatments, it has been known that (Puligundla et al. 2013; Hamoud-Agha et al. 2014).

Losing the quality, including color, odor, texture and taste, is related to remained plant's enzymatic activity such as Polyphenol oxidase, Peroxidase and Lipoxygenase. This negative impact emerges boldly in

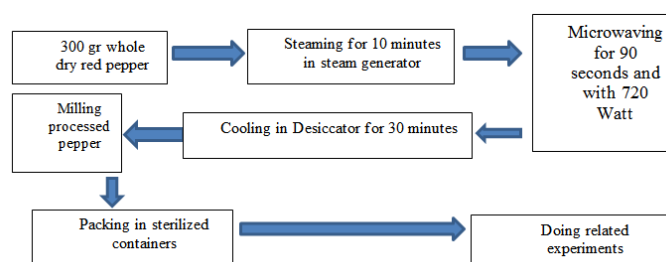
their utilization of other food products and leads to product's quality loss. Hence, this enzymatic activity needs to be ceased through heat treatment and not through other non-thermal approaches including fumigation with Ethylene Oxide gas, Gamma irradiation, UV radiation and etc., it was reported that (Schweiggert et al. 2005). Preservation techniques is designed in a way that, with reduction in Pathogen vegetative cells and Spore form, it would be possible to reach to a qualified healthy and safety level. Most of these preservative techniques are developed for highly moisturized products. However dry substances have lots of application in food industry. So, for increasing microbial quality concentrating on dry materials such as spices must be applied. On the other hands, decontaminating dry materials is really troublesome due to adjustment of existed microflora with low moisture content. Heat resistant in micro-organisms depends on water activity (aw) and atmosphere's relative humidity equilibrium. Water existence is the most effective factor in microbial heat resistance. Since, heat resistance in dry environments is existed in much greater amounts rather than in Aqueous Solutions, it was reported by (Fine & Gervais 2004).

## II. METHODS AND MATERIAL

### A. Samples Preparation

Initially, 3 samples had been made out of a 110 KG pepper's batch, each 300 grams, then mixed thoroughly and randomly divided it into 3 parts again. Each part was used in 3 approaches for a mixture of steam and microwave process, one for Gamma irradiation up to 5 KGy and the last one for control.

1) Sample preparation for the steam-microwave process Fig. 1:



**Figure 1:** Sample preparation for the steam-microwave process

It can be concluded that, this process has two stages:

- Steaming with a Feller (from Germany) steam generator machine, in order to condition, reduce microbial load and cease enzymatic activities.
- Microwaving with a MC-3022 snr machine produced by LG, in order to dry the conditioned red pepper, continue both microbial load reduction and cease enzymatic activities.

## 2) Sample preparation for Pepper Irradiating process

Following milling the second batch and putting them in sterilized containers, they are sent for Gamma irradiation up to 5 KGy for about 34 min and 12 second. Source host configurations of Irradiation device are as follows:

- Dos Rate: 2.56 Gy/Sec
- Transit Rate: 7.86 Gy

After irradiating the second batch, they are sent to laboratory for microbial and chemical experiments.

## 3) Sample preparation for control

Control sample is the one with no applied processes and is just milled and packed with means of comparing with two other samples.

## B. Microbial experiments

### 1) Total Plate Count Agar based on Iran's National Standard (5272):

The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of PCA (Merck, Germany) which has a rotational movement, then flocculating the specified culture and finally transporting it into Incubator in an upside down position for 72 hours in 30°C.

Counts the number of colonies with following formula:

$$N_E = Y/d$$

d: coefficient of initial dilution suspension.

Y: Average number of existed colonies in two plates.

### 2) Counting Mold and Yeast below 0.6 water activity based on Iran's National Standard (10899-3):

This experiment is performed in a Yeast extract-Dextrose-Chloramphenicol (YGC) culture medium. The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of YGC (Merck, Germany) which has a rotational movement, then flocculating the specified culture and finally transporting it into Incubator in an upside down position for 5 days in 25°C.

Counts the number of colonies with following formula:

$$N_E = Y/d$$

d: coefficient of initial dilution suspension.

Y: Average number of existed colonies in two plates.

### 3) Counting Coliform based on Iran's National Standard (9263):

Its culture medium is Crystal violet, neutral red and bile lactose agar (VRBL). The counting process for specified colonies is performed by a standard procedure which includes adding prepared 0.1 dilution of Pepper suspension to the culture medium of VRBL (Merck, Germany) which has a rotational movement, then flocculating the specified culture, supported by adding 4 ml from VRBL to each plate, and finally transporting it into Incubator in an upside down position for 24 hours in 30°C.

### 4) Counting E.coli based on Iran's National Standard (2946):

This process is performed by adding 1 ml of initial suspension to 9 ml of Lauryl sulphate broth. The Inoculated tubes are held in incubator in 37°C in periods of 24 and 48 hours. In case of gas production in Durham tubes, EC and all supplementary tests are held, otherwise the report is sent as negative.

## C. Physicochemical experiments

### 1) pH measurement based on Iran's National Standard (2852):

10 grams of red Pepper is mixed with 50 ml of distilled water, followed upon measuring its pH by a pH meter

electrode (Jenway, Germany) and after fixing its number, it will be reported.

- 2) Moisture measurement based on Iran's National Standard (2705) for calculating Total Solid (TS):

After weighing 5 grams of the sample (M2) and the container with its lid (md), it will be put in oven (Memert, Germany) for 120 minutes, followed by cooling in desiccating machine for 30 minutes. Then, it will be weighed (ml) upon which the dried sample mass (M1) is extracted by below formula:

$$M1=ml-md$$

In order to calculate moisture percentage following formula can be used:

$$W_{H_2O} = \left[ 1 - \frac{m_1}{m_2} \right] * 100$$

- 3) Qualitative determination of enzymatic activity (Catalase and peroxidase) based on Iran's National Standard (48):

On the cross section of a gram of different treatments, a drop of 3.0 or 5.0 per cent hydrogen peroxide solution (Merck, Germany) is added. With emergence of pink colour tinged with orange, or creation of lots of bubbles, the existence of Catalase and peroxidase enzymes is assured in the specific sample.

- 4) Peroxide test based on Iran's National Standard (4179):

First oil in pepper is extracted with cold extraction, and then 5 grams of the oil is mixed with 50 ml of solution (acetic acid (Sigma Aldrich, US) + iso-octane (Merck, Germany) which is prepared in a 3 to 1 proportion. Then, 0.5 ml of saturated potassium iodide (KI) (Merck, Germany) is added to the container. Then the container is left in a dark cabinet for 1 minute. Following stage, 100 ml of distilled water is added to the container with a few drops of starch (Kimia Gostar, Iran) (1 to 5 percentage starch solution).

A prepared sample which is now in dark colour, will be under the affection of titration with 0.01 N sodium thiosulfate (Sigma Aldrich, US) up until it becomes colourless. Besides, Titration of the control is performed.

Thiosulfate amount consumed by the control is subtracted from the amount consumed by the sample.

Amount of proxide:

$$\frac{N * (S - B) * 1000}{W}$$

Hereby the parameters are as followed:

S: Thiosulfate volume consumed by the sample

B: Thiosulfate volume consumed by the control

N: Thiosulfate Normality

W: Sample Weight

#### D. Machinery Tests

- 1) Colorimetric measurement with Hunter lab system  
a\*, b\* and L\* colour space is a model which was approved in 1976 by International Commission on Illumination light for describing the relationship between the visible colours by eye in which L\* defines brightness (between 0 to 100), a\* represents red and greenish colour (+a for red and -a for green) and b\* explains yellow and blue colours (+b for yellow and -b for blue), that both a\* and b\* are from -120 to +120.

#### E. Statistical Analysis

By using Microsoft Excel in representing and packing the raw data and utilizing one-sample T-test from SPSS software the data was summarized, interpreted and observed properly.

### III. RESULT AND DISCUSSION

#### A. Total Solid results with $P < 0.01$ assumption

According to Fig. 2, Steam-microwave process (B), had the lowest TS compared to the control (C) and irradiated sample (A), which B's TS is less than about 0.64 of control sample averagely, Rico et al.(Rico et al. 2010) have shown that.

Employing heat steam-microwave process leads to increase in Moisture content and the reason lies underneath the facts of water absorption during steaming process and Failure in returning the moisture to its initial amount during Microwave process. In spite of the fact that these changes are considered as really minute (about 0.6%) and would have no effects on pepper's preservative and organoleptic properties.

In irradiated sample, TS rose to a small extent and this is due to the fact that there were some structural failures in molecular combinations, subsequently some moisture loss during Irradiation.

#### B. pH results with $P < 0.01$ assumption

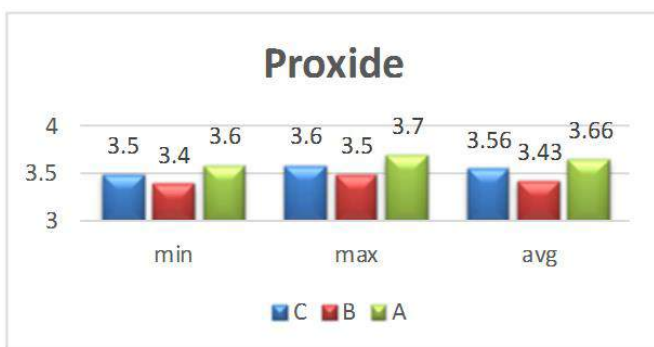
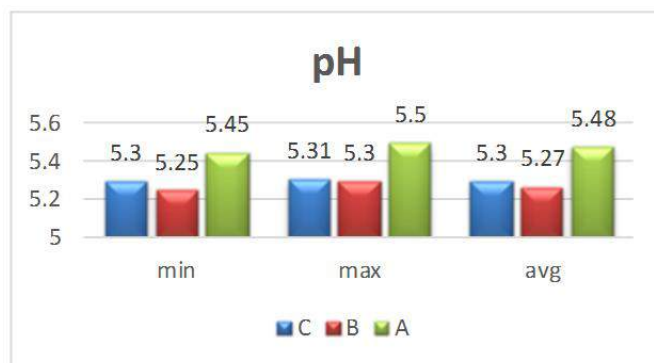
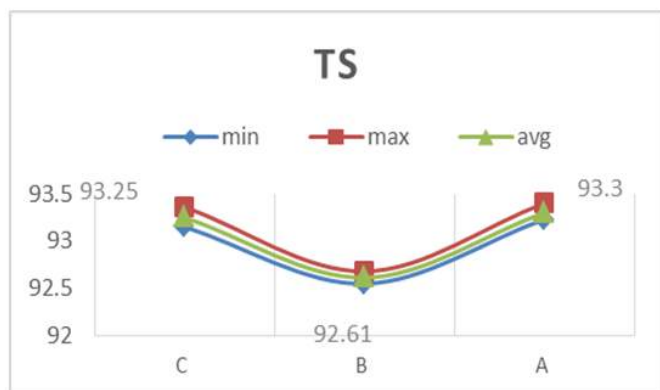
According to Fig. 2, pH alterations after both A and B processes had negligible differences to control sample, have been reported (Rico et al. 2010; Lu et al. 2011; Valero & Cejudo 2014) and there was only 0.2 increases in pH value by utilizing Gamma irradiation in average.

pH alterations for both two processes compared to control sample were minimal. In other hand, Gamma Irradiation had a slice increase for 0.2 which might be because of broke Amine compounds followed by pH increase.

#### C. Peroxide index (meq/Kg o2) results with $P < 0.01$ assumption

It was observed in Fig. 2, that Peroxide index in all samples was tightly to each other, however there was an insignificant increase in Peroxide amount for Irradiation sample (A), Aziz et al. (Aziz et al. 2002) reported that and those which were used in steam-microwave process had the least Peroxide amount among all other.

Peroxide index in both A and B approaches were highly similar to each other and even to control sample (C), but there was some negligible decrease in irradiation technique which was due to the fact that oil content oxidation is happened in irradiation.



**Figure 2 :** TS, pH, and peroxide amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample)

#### D. Coliform results with $P < 0.01$ assumption

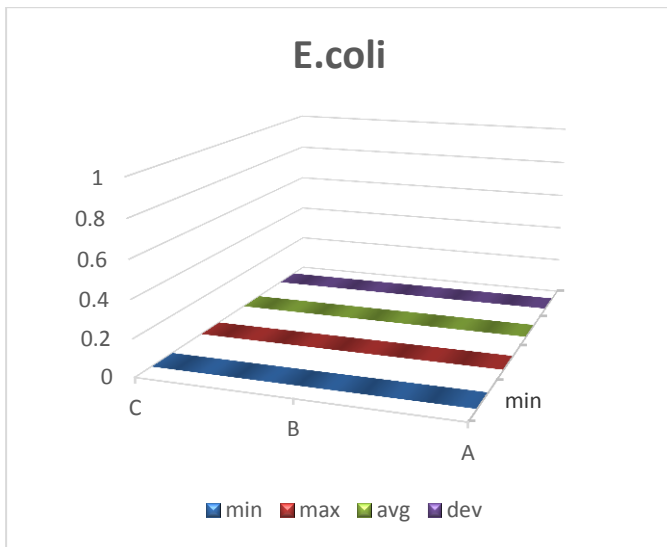
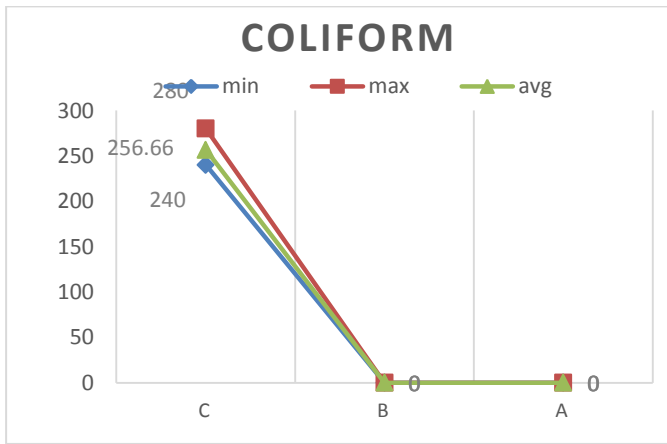
According to Fig. 3, Coliform amount, in the control sample (C) is about 256 averagely, while in Steam-microwave process (B) and Irradiated sample (A), this factor was reached to zero, have been reported (Kim et al. 2014; Aziz et al. 2002; Schweiggert et al. 2005) .

In both steam-microwave (B) and Gamma Irradiation (A) approaches, Coliform had gained well-received desirable zero amounts which are conceived as their high effective intensity in Coliform termination.

#### A. E.coli results with $P < 0.01$ assumption

As it is observed in Fig. 3, E.coli amount was about zero in control sample, consequently in processed samples (A and B) there would not be any E.coli bacteria, it has been known that (Hamoud-Agha et al. 2014; Song et al. 2014) result.

There was no evidence on E.coli existence in the control sample (C), and consequently there were same results for other two approaches (A and B).



**Figure 3 :** Coliform and E.coli amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample).

**B. Total Plate Count Agar (TPCA) results with  $P < 0.01$  assumption**

According to Fig. 4, There was high amounts of TPC in our initial sample (C), nearly about 1287, but with employing the steam-microwave process (B) for 3 times, this number is reduced to about 56 colony, have been reported (Rico et al. 2010; Aziz et al. 2002; Schweiggert et al. 2005) .

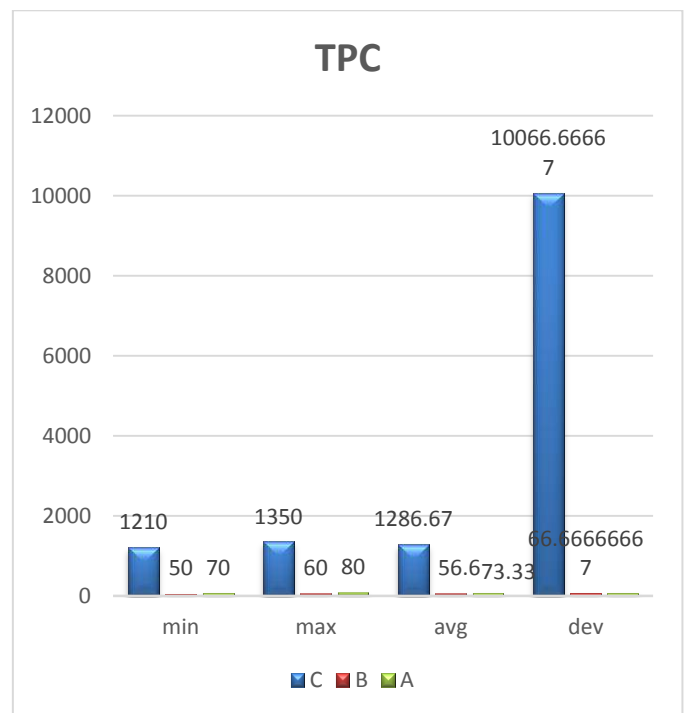
Besides, by applying irradiation this number is reduced to about 73 colonies, it has been known that (Aziz et al. 2002; Rico et al. 2010).

The most significant reduction of TPC was seen in steam-microwave process which on average decreased the number from 1286 in control sample (C) to 56, which is followed by next technique in second position, Gamma Irradiation that lessened the number to 73 units of TPC.

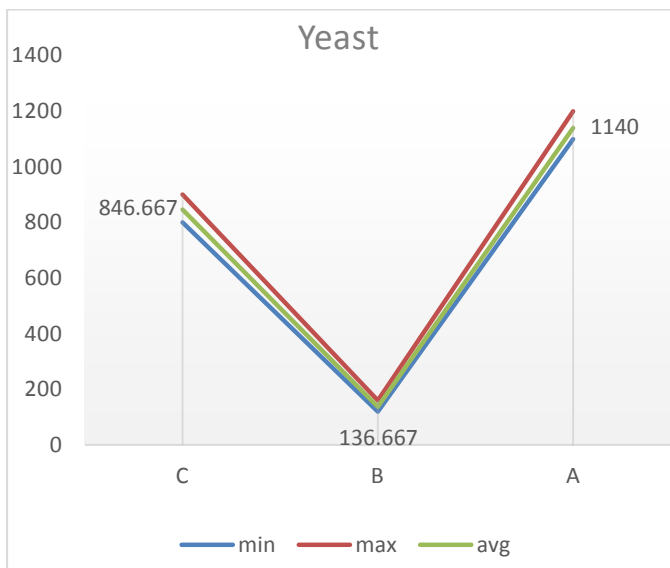
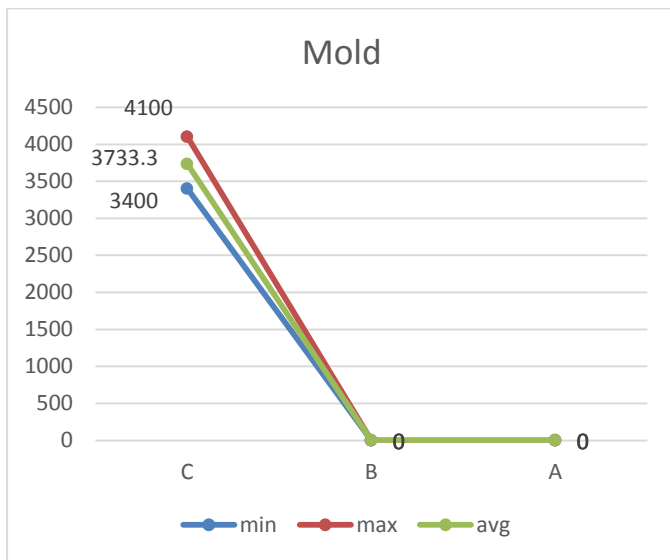
**C. Mold results with  $P < 0.01$  assumption**

According to Fig. 4, Initial Mold count on our control sample (C) was about 3733, but with employing the steam-microwave process (B) or applying Gamma irradiation with 5 KGy, there was no other signs of mold observed in culture media, it has been known that (Schweiggert et al. 2005; Rico et al. 2010) .

Mold values in control sample was high in amounts initially, for about 3737, but with applying steam-microwave (B) and Gamma Irradiation (A) upon processing its amount declines drastically to the state of no mold in both samples which explains highly responsive behaviour of mold destruction in both processes.







**Figure 4 :** TPC, Mold, and yeast amount curve for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample).

#### D. Yeast results with $P < 0.01$ assumption

According to Fig. 4, There was high amounts of Yeast in our initial red pepper sample (C), nearly about 846, however by employing the steam-microwave process (B), this number is reduced to about 136, it has been known that (Schweiggert et al. 2005; Rico et al. 2010). Besides, by applying irradiation with 5 KGy in 3 times, this number is increased to about 1140, in contrast with the data, Rico et al. (Rico et al. 2010) have shown that, due to lower treatment dose irradiation.

The lowest yeast amount is referred to steam-microwave process (B) in which the amount was reduced from 846 of control sample (C) to 136 units. However, employing Gamma Irradiation approach (A), led to its soar to 1140 which was due to the fact that Irradiation causes germination of yeast cells. This malfunction creates disastrous problems which itself is the key factor in spoilage and fluff in future products.

#### E. Hunter Lab Colorimetric results

##### 1) $L^*$ (Brightness factor) with $P < 0.01$ assumption

According to Fig. 5, In steam-microwave approach  $L^*$  amount was about 44 which was reduced from the control sample value by 3 units, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result, although there was a 3 unit increase on this factor by employing Gamma Irradiation (A) approach compared to control sample (C), Song et al. (Song et al. 2014) have shown that.

##### 2) $a^*$ (Red factor for red pepper) with $P < 0.01$ assumption

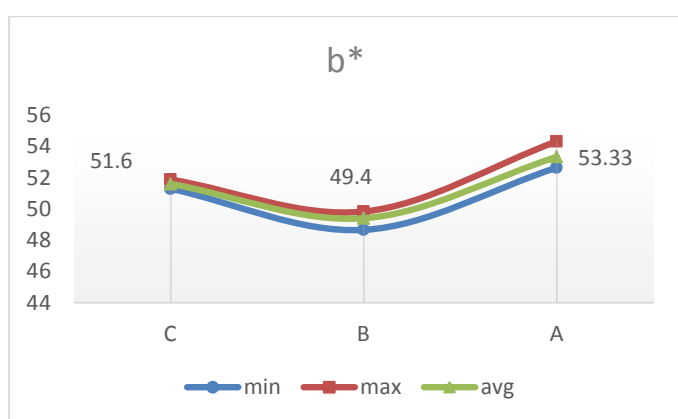
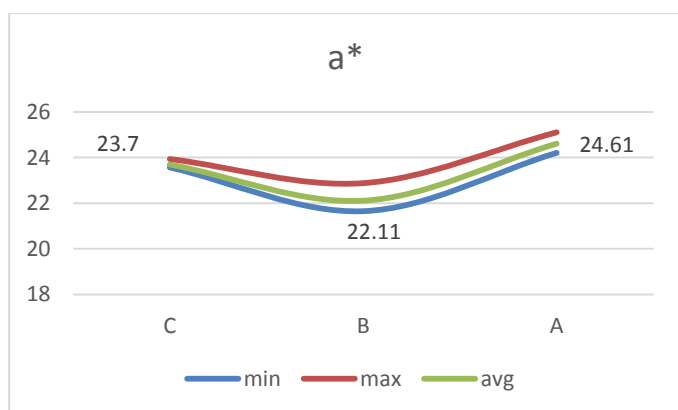
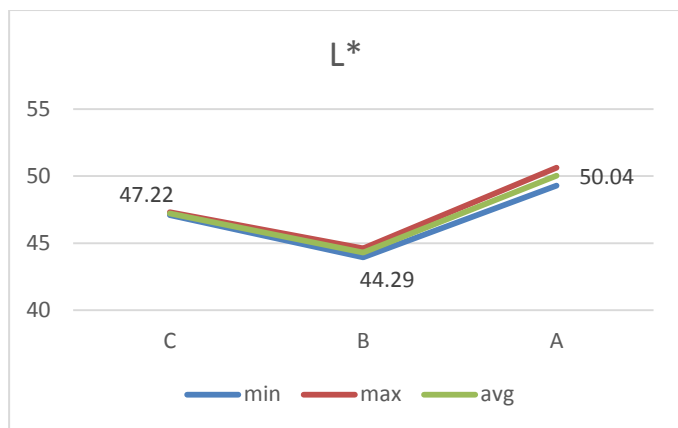
According to Fig. 5, On average,  $a^*$  index was about 22.11 in the steam-microwave approach (B), which had a reduction compared to control sample (C) with its amount near about 23.7, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result, although in Gamma Irradiation approach (A) this index was increased in small amounts to 24.61, Song et al. (Song et al. 2014) have shown that.

##### 3) $b^*$ (Yellow factor for red pepper) with $P < 0.01$ assumption

According to Fig. 5,  $b^*$  index had a decrease in the steam-microwave approach (B), and its amount declined from 51.6 for Control sample (C) to 49.4, have been reported (Schweiggert et al. 2005; Rico et al. 2010; Chang et al. 2010) result. Although in Gamma Irradiation approach (A) this index was increased for about 5 units which lead to more yellow color in its output, Song et al. (Song et al. 2014) have shown that.

All in all, steam-microwave approach had tiny effect on reduction of brightness ( $L^*$ ), red color ( $a^*$ ) and yellow

color ( $b^*$ ) factors, which is due to the fact that there are some destructions in colour pigments during steaming and microwaving processes. In other hand, irradiation led to minuscule upward trend in all color factors that are the result of destruction of some pigments during Gamma irradiation along with de-polymerization of substances and structural unpacking.



**Figure 5:**  $L^*$ ,  $a^*$ , and  $b^*$  amount for 3 samples (A: gamma irradiation sample, B: steam-microwave process and C: control sample)

#### F. Existence Quality Test of Enzymatic Activity

According to the table 1, both control and Gamma irradiated samples had enzymatic activities which led to negative effects on its future products; however the output from steam-microwave process lacked any enzymatic activities. Schweiggert et al. (Schweiggert et al. 2005) has shown that.

Table 1: Existence Quality Test of Enzymatic Activity.

Condition	Control	Steam-Microwave	Gamma Irradiated
Enzymatic Activity			
No Enzymatic Activity			

#### IV. CONCLUSION

Steam-microwave process (B), had the lowest TS compared to the control (C) and irradiated sample (A), pH alterations after both A and B processes had negligible differences to control sample, Peroxide index in all samples was tightly close to each other; however there was an insignificant increase in Peroxide amount for Irradiation sample (A), Coliform amount, in the control sample (C) is about 256 averagely, while in Steam-microwave process (B) and Irradiated sample (A), this factor was reached to zero, E.coli amount was about zero in control sample, consequently in processed samples (A and B) there would not be any E.coli, The most significant reduction of TPC was seen in steam-microwave process, The most significant reduction of mold was seen in steam-microwave process, The lowest yeast amount is referred to steam-microwave process (B) However, employing Gamma Irradiation approach (A), led to its soar, All in all, steam-microwave approach had tiny effect on reduction of ( $L$  ( $a^*$ ) and ( $b^*$ ) factors,. In other hand, irradiation led to minuscule upward trend in all colour factors and both control and Gamma irradiated samples had enzymatic activities which led to negative effects on its future products; however the output from steam-microwave process lacked any enzymatic activities.

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# Preserving Privacy Using Attribute Based Access Control in Data Stream

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## ABSTRACT

Data security is a very broad area that addresses many issues, like legal and ethical issues regarding the right to access certain information. The sensitive data is accessible to authorized users only. The database security is based on the Access Control Mechanism (ACM) and the Privacy Protection Mechanism (PPM). The Access Control Mechanisms (ACM) is used to ensure that only information is available authorized to users. The authorized user can only access the authorized data. The privacy protection mechanism (PPM) is a general method used to transform the original data into some anonymous form to prevent from accessing owners sensitive information. There are numerous methods to provide the privacy for the sensitive data. In existing literature, the Access Control mechanism allows Role Based Access using Time Based Sliding Window Query. It protects the user information from the unauthorized access. PPM meets privacy requirement through k-anonymity it provides better privacy for the sensitive information which is to be shared. The privacy is achieved by the high accuracy of the user information. To protect data, the anonymization method is one of the best privacy protection mechanisms. The anonymization process will transform the sensitive information to some anonymized form using K-anonymity,  $\ell$ -diversity. The PPM needs to satisfy an additional constraint namely the Imprecision bound for each selection predicate. The imprecision bound reduced the delaying for publishing data stream. The challenge is to optimize the delay in publishing data stream, if the delay in publishing query is reduced then the imprecision bound is satisfied. Proposed system is an accuracy based access control using Attribute Based Access Control with Tuple Based Sliding Window Query. and PPM with the  $\ell$ -diversity. The  $\ell$ -diversity method is an extension of the k-anonymity method, it is more efficient than the k-anonymity method. It avoids the attacks like background knowledge attack and others in k-anonymity method.

**Keywords:** Access Control Mechanisms, Privacy Protection Mechanism, Information Security, Top Down Selection Mondrian, Attribute Based Access Control, Total Minimum Imprecision

## I. INTRODUCTION

In the field of Information Security, the data security refers to protecting data from unauthorized users. It is based on confidentiality, integrity and availability. To accessing a DataStream, it is not

possible to control the order in which data arrive, nor feasible to storing data. The data owners may not be willing to exactly processing the true value of their data due to various reasons, mostly privacy consideration and accuracy of data accessing. However, the unauthorized user can also take

advantages of this to accessing the data. Therefore it is need to user has a better access control mechanism to provide both security and privacy.

## II. METHODS AND MATERIAL

### 1. Literature Review

1. To provide better Access control mechanism to ensure both security and privacy of the sensitive information, Access control mechanism for Data Stream Management System (DSMS) provide access of authorized part of the stream to each user. **Zahid Pervaiz, Arif Ghafoor, Fellow, Walid G. Aref et.al** uses a Role Based Control Policy (RBAC) [1] defines the authorized view of the data stream for each role.

2. Role based access control gives permission to the users to access data based on their role. For Relational data **Nagabhushan, Arif Ghafoor, Zahid Pervaiz et al** defines [2] selection predicates query technique is savailable to role while the privacy requirement is satisfy.

3. The stream data offers query processing over continues and sequencing data for data publishing and the windowing techniques generally emphasize on the streaming data. **T.Ghanem, A.Elmagarmid, P.Larsen and w.Aref et al.** proposed the predicate window query processing for streaming data [3].The access control uses Role based techniques to satisfy minimum Imprecision bound using the time based sliding window query [1].

4. To maintain the privacy of data it is need to minimize the imprecision of aggregate for all queries. The imprecision bound is a resulted value which determines the amount of imprecision that can be tolerated for each query. Privacy preserving mechanism needs to sum of false negative and false positive is less than imprecision bound. **Zahid Pervaiz, Arif Ghafoor, Fellow, Walid G. Aref et.al** proposed the Top Down Selection Mondrian (TDSM) used to minimize imprecision bound for rational data [2].

5. The Data anonymization is one of the important privacy protection techniques. It transforms the sensitive information to some anonymzed form. **J. Cao, B.**

**Carminath, E. Ferrai and K. Tan et al.** presents the continues anonymzing data stream [4]. To anonymize data use generalization will replace the sensitive information with border range. **C. Clifton and T. Tesa et al** proposed the differential privacy model using generalization [5]. **L. Sweency** represents an anonymization method to preserve a privacy of data. i.e. k-Anonymity [6]. To prevent uncontrollable information loss and affects the accuracy of crowd sourcing database **Saiwo, Xi aloi, Sheng Wang et al** proposed K-Anonymity for crowd sourcing database [7].

### 2. Proposed Work

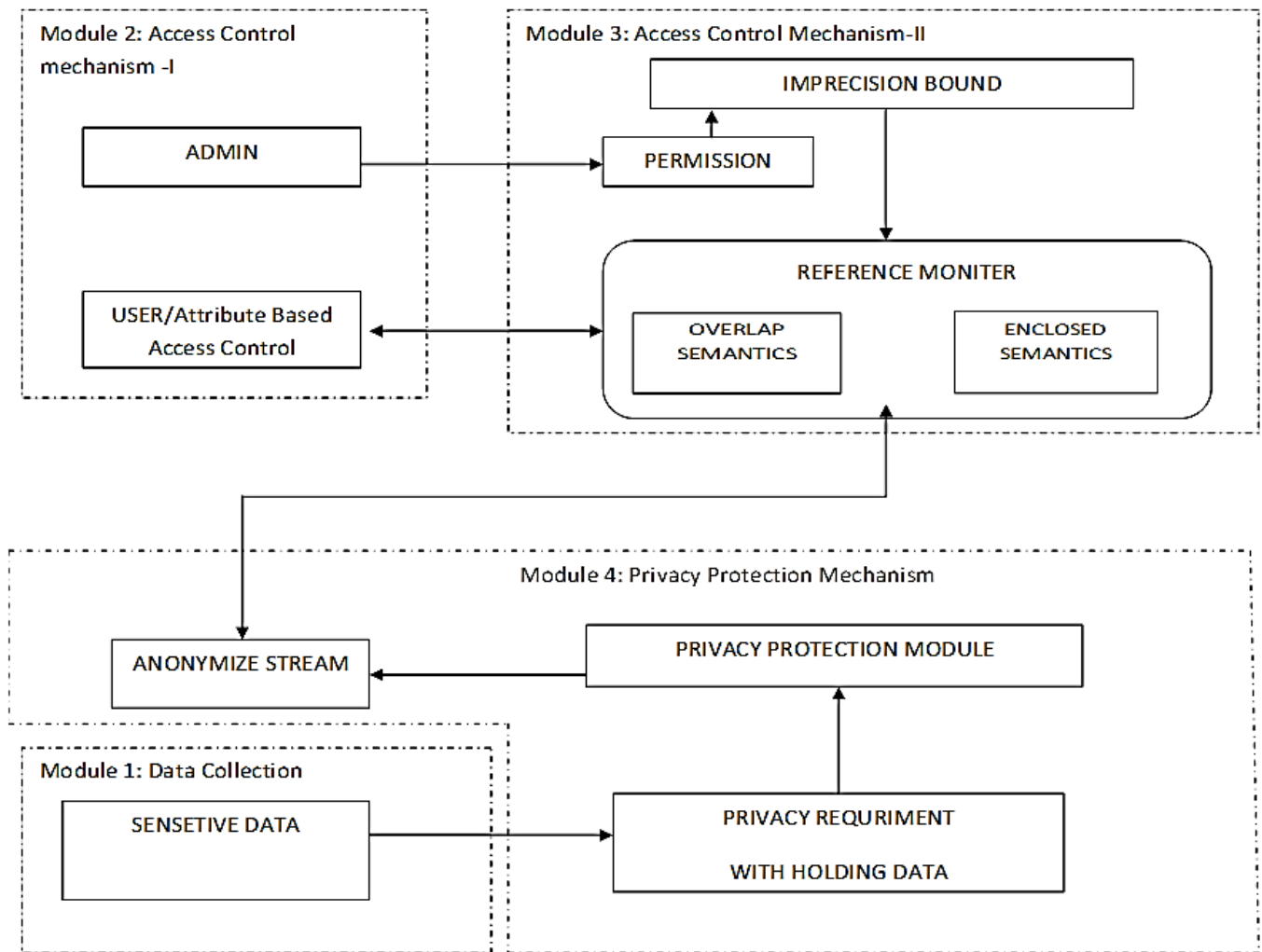
In proposed system, the access control mechanism allows only authorized queries predicates on sensitive data using Attribute Based Access Control (ABAC) policy. It defines an access control paradigm whereby access rights are granted to users through the use of policies which combine attributes together. The policies can use any type of attributes (user attributes, resource attributes, environment attribute etc.).The imprecision bound is a threshold value which determines the amount of imprecision that can be tolerated for each query. The access control policy administrator sets the imprecision bound using overlap semantics and enclosed semantics for each predicate Tuple Based Sliding Window Query. This query meets the minimum imprecision bound, means it prevents and solves the query violation problem. The proposed system formulate query generation module of the system using tuple based sliding window query that offers overlap and enclosed semantics. The proposed system refers data anonymization using the  $\ell$  -diversity.  $\ell$  -diversity method reduces the granularity of representation of the data,  $\ell$  -diversity can still defend against background knowledge that is unknown to the data publisher. The  $\ell$  -diversity method is an extension of the k-anonymity method.

The following techniques is used are as follows,

1. To use Attribute Based Access Control Policy for precession bounded access over DataStream
2. Implementation of heuristics algorithm for satisfying the imprecision bounds.
3. To use Tuple Based Sliding Window Query for accessing data to maintain accuracy.
4. To use  $\ell$  -diversity method for anonymization of DataStream instead of k-anonymity

The proposed system provides following dynamic policy management modules:

### 3. Proposed Architecture



**Figure 1:** Architecture of Attribute based Access Control System

#### ✓ Data Collection

In this module, the proposed system refers sensitive information in the form of streaming data. DataStream offers a continues data arrive in a system for storing and processing.

#### Access Control Mechanism I

The Access control mechanism-I is divide into two parts are as follows:

1) **User/attribute:** In this framework user accessing the DataStream using attribute Based Access Control (ABAC) technique. It provides a high level of flexibility that promotes security and information sharing. ABAC is composed of a set of Users, a set of attributes, and a set of Permissions.

2) **Admin Permissions:** System administrator set the permission to imprecision bound for each query, user-to-attribute assignments, and attribute-to permission assignments.

#### Access Control Mechanism II

The Access Control Mechanism –II is divided into two parts are as follows:

1) **Imprecision Bound:** It ensures that the authorized data has the desired level of accuracy. The imprecision bound can be used to meet the privacy requirement. The privacy protection mechanism is required to meet the privacy requirement according to the imprecision bound for each permission.

2) **Reference monitor:** A predicate Tuple Based Sliding Window Query is evaluated for a DataStream by including all the stream tuples that satisfy the query predicate. For predicate evaluation over an anonymized DataStream will be used reference monitor, including all the tuples in equivalence classes that overlap the query predicate range.

### Privacy Protection Mechanism:

The privacy protection module anonymized the data using  $\ell$ -diversity to meet privacy requirements and imprecision constraints on predicates set by the access control mechanism.

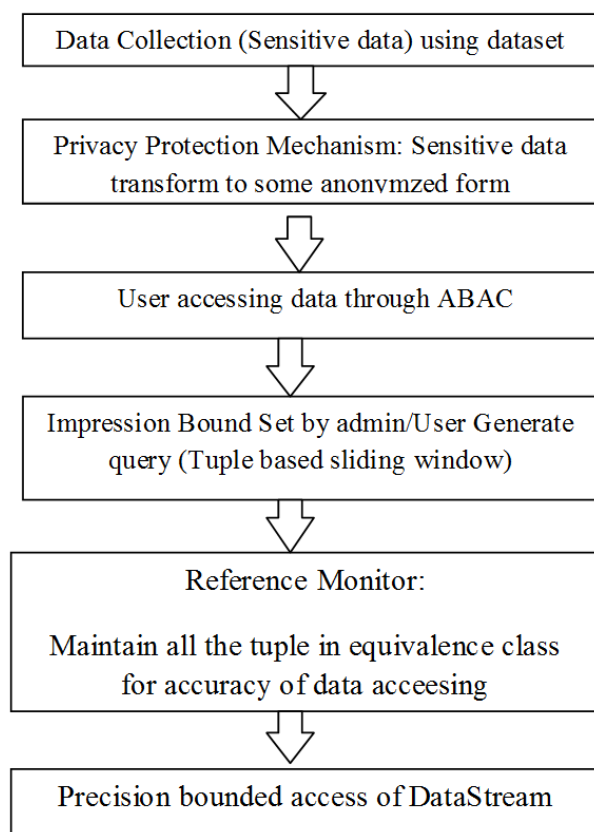
#### ✓ Recursive $\ell$ diversity:

Recursive  $(c, \ell)$ -Diversity. In a given  $q^*$ -block, let  $r_i$  denote the number of times the  $i$ th most frequent sensitive value appears in that  $q^*$ -block. Given a constant  $c$ , the  $q^*$ -block satisfies recursive  $(c, \ell)$ -diversity if  $r_1 < c(r_1 + r_2 + \dots + r_m)$ . A table  $T^*$  satisfies recursive  $(c, \ell)$ -diversity if every  $q^*$ -block satisfies recursive  $\ell$ -diversity. We say that 1-diversity is always satisfied. Now, both entropy and recursive  $\ell$ -diversity may be too restrictive. To see why let us first look at entropy  $\ell$ -diversity. Since  $-x \log(x)$  is a concave function, it can be shown that if we split a  $q^*$ -block into two sub-blocks  $q^*_a$  and  $q^*_b$  then  $\text{entropy}(q^*) \geq \min(\text{entropy}(q^*_a), \text{entropy}(q^*_b))$ . This implies that in order for entropy  $\ell$ -diversity to be possible, the entropy of the entire table must be at least  $\log(\ell)$ . This might not be the case, especially if one value of the sensitive attribute is very common – for example, if 90% of the patients have “heart problems” as the value for the “Medical Condition” attribute. This is also a problem with recursive  $\ell$ -diversity. It is easy to see that if 90% of the patients have “heart problems” as the value for the “Medical Condition” attribute then there will be at least one  $q^*$ -block where “heart problems” will have frequency of at least 90%. Therefore if we choose  $c < 9$  in Definition 4.2, no generalization of the base table will satisfy recursive  $(c, \ell)$ -diversity

### 4. Implementation Steps

The implementation steps are as follows:

- Step 1. The proposed system uses a continues and sequencing data . In step I the system uses DataStream.
- Step 2. In Step II the privacy protection mechanism deals with sensitive data.it transfer the sensitive data into some anonymize form using  $\ell$  - diversity.
- Step 3. In access control mechanism -I user access the accurate data using attribute based access control.
- Step 4. In access control mechanism –II generate the tuple based sliding window query for precision access of data and admin set the permission to maintain imprecision bound.
- Step 5. The reference Monitor maintains all the tuples in equivalence class for accuracy of data accessing.
- Step 6. In this step ,attributed based access control using tuple based sliding window query maintain precision bound access of the DataStream.



### 5. Scope

Role Based Access Control policy cannot be ensures permission on sequence of operation need to be controlled. In proposed system, Attribute-based access

control (ABAC), provides a high level of flexibility that promotes security and information sharing.

The Total Minimum Imprecision (TIM) algorithm use to meet the desired level accuracy for relational data accessing. In proposed system, the heuristics algorithm will be used for streaming data to satisfy precision bounded access.

The purpose of access control is to ensure that each user access only the authorized information. This semantic suggested in reference monitor, the Role Base Access Control (RBAC) uses the time based sliding window query. To prevent an overlapping data and maximum response time of query evolution, the proposed system refers Tuple Based Sliding Window Query with use of overlap and enclosed semantics.

The k-anonymity is the anonymization techniques convert the sensitive information to some anonymized form using generalization and suppression. The proposed system uses  $\ell$ -diversity method.  $\ell$ -diversity is a form of group based anonymization that is used to preserve privacy in data sets by reducing the granularity of a data representation.

## 6. Methodology

There are following techniques and algorithm in proposed methodology:

- ✓ **Data Collection:** The proposed system stores the sensitive data in the form of DataStream. Accessing a DataStream is concerned with extracting knowledge represented in non-stopping, continues and ordered sequence of information.
- ✓ **Access Control Mechanism I:** The framework of Access Control Mechanism -I uses Attribute-based access control (ABAC), provides a high level of flexibility that promotes security and information sharing. ABAC also overcomes some of the problems associated with Role Based Access Control (RBAC).RBAC cannot be ensure permission on sequence of operation need to be controlled. Using ABAC policy mining algorithm we prevent privileges unwieldy.
- ✓ **Access Control Mechanism II:** The purpose of Access Control Mechanism-II is used to Tuple

Based Sliding Window Query technique. Privacy is achieved using cost of accuracy and imprecision is introduces in the authorized information under an access control policy. In proposed system a heuristic algorithm set the minimum imprecision bound. Query impression bound is the total impression acceptable for query predicate and present by the access control administrator.

## 4. Privacy Protection Mechanism:

Privacy protection mechanism includes data anonymization. The data anonymization is the process that transforming sensitive data to some anonymized form. The proposed system used  $\ell$ -diversity, it is techniques better than k-anonymity. It has strong background knowledge and maintains lack of diversity. The approach for preserving privacy is based on data anonymization.

## Experimental Setup & Implementation:

1. To implement above system some pre work to do.
2. To Fetch the dataset from a file and import to the database.
3. A privacy preserving module preserves the data using l- diversity.
4. The reference monitor uses the top down heuristics algorithm for accuracy of data.
5. The ABAC policy uses the data accessing through attribute.

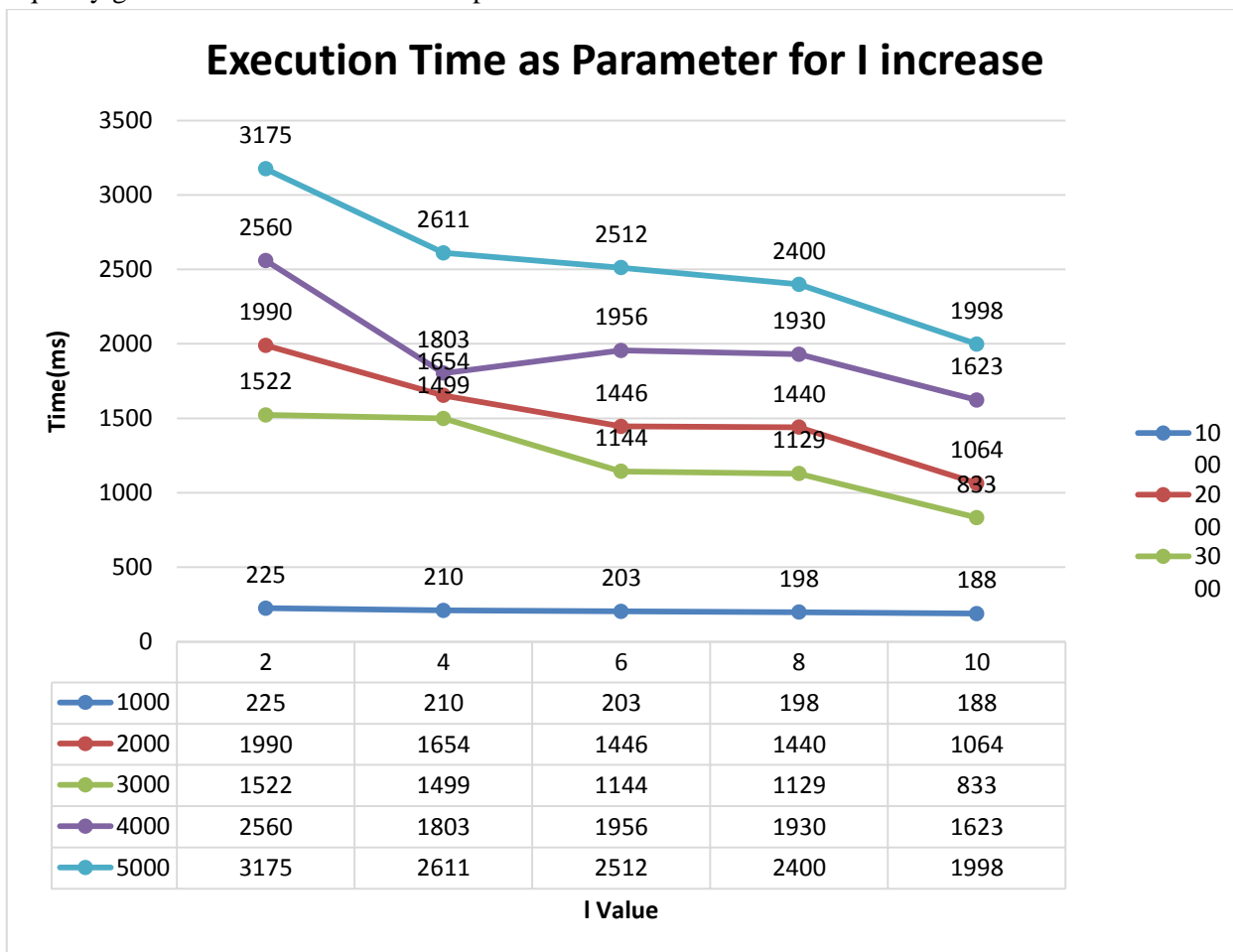
## III. RESULT AND DISCUSSION

We fetch and perform really insertion of record into database around then into frame work the normal execution time means what number of time used to insertion of record (computed in millisecond) of the both l-distinct and l – recursive technique. Framework of l-recursive in graph 1.

In a general view, the insertion of record having two different background procedure of database. In this checking methodology checker don't have a clue about the substance of client's tuple. In second stage, framework really redesigns the database relies on upon the consequence of the user. Sometimes the insertion or



updating fizzled in l recursive database then it holds up insertion.  
 until l quality gets to be sure and different tuples fail the



#### IV. CONCLUSION

In this, we focus on a privacy preserving of l-diversity database. We have presented two secure techniques l – distinct and l recursive for database anonymization techniques for protecting individual's privacy. The Authorized user accessing the database using ABAC policy for better accuracy.

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# Physical and Chemical Changes in the Soluble Fraction of Eggs from Hens Fed With *Pleuroncodes Planipes* (Red Crab), Stored for Different Lengths of Time At Different Temperatures

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## ABSTRACT

As an abundant resource on the coast of Baja California, Mexico, the crustacean known as *red crab* (*Pleuroncodes planipes*) may represent an alternative for use in the formulation laying hens' diet because of the crab's protein content. It has been reported that diets containing 4% red crab meal (RCM) generate eggs with satisfactory sensorial characteristics. However, changes in the physical and chemical characteristics of the water-soluble components that alter the biological value of eggs might occur during storage. Therefore the aim was to determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed with *Pleuroncodes planipes* meal (RCM). To determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed with a standard diet and with a diet with 4% (RCM), 90 hens were divided into two treatments: Control and RCM (4%). During four weeks of testing, production variables were evaluated. Eggs from each treatment were collected to assess the following factors: Haugh Units (HU), pH and levels of crude protein, essential amino acids and water-soluble vitamins after 0, 15 and 30 days at 4° and 20°C. The results were analyzed using a 2x2x2 factorial design, and the difference between means was compared using Tukey's test. The results showed that there was no significant difference in production performance between the treatment groups. During the storage period, there was a decrease in the CP content (49.8%/15 days – 49.2%/30 days) and HU (60.16/15 days – 52.62/30 days) and an increase in the pH (8.73/15 days – 9.32/30 days). The effects of temperature and egg storage time on the CP content (49.2%/20°C – 50.2%/4°C) and HU (56.82/20°C – 71.05/4°C) were different ( $P < 0.05$ ). The effects of diet and egg storage time resulted in differences ( $P < 0.05$ ) in the CP content (49.2 Control – 50.2 RCM) and HU (62.91 Control – 64.96 RCM). Adding 4% RCM to the diets of laying hens did not affect the studied productive parameters, but changes were observed in the water-soluble part of the egg in a double interaction (times and temperatures). The eggs presented changes inherent to the egg metabolism, modifying the variables studied.

**Keywords:** Red Crab Meal, Egg Quality, Storage

## I. INTRODUCTION

Researchers at the Northwest Center for Biological Research (known by its Spanish acronym, CIBNOR) have been studying the biology, ecology and utilization of red crab (*Pleuroncodes planipes*) for several years, and the results show that this species can be considered

the most abundant benthic decapod in Mexico. Data from Balart [1] and Aurióles and Pérez [2] reported an abundance of approximately 300,000-500,000 t/year and a maximum density of up to 40 red crabs/m<sup>2</sup> or higher for the coast of the Baja California peninsula. To date, this species has not been commercially exploited in Mexico. These authors suggest that 40,000 t of benthic

pelagic red crabs could be captured during the initial phase of the fishery without decreasing the amount available; creating a sustainable fishery that allows the harvest of biomass for industrial exploitation. Therefore, it is important to develop technologies for red crab exploitation and to evaluate the resulting products in various applications. Today, this type of red crab is widely studied for its use in the poultry industry as an ingredient in poultry diets, where it constitutes an alternative source of protein, fatty acids and pigments [3, 4, 5, 6, 7].

Mexico ranks first in the world in egg consumption (22 kg per capita per year or 305 eggs per person per year) [8]. Furthermore, the Mexican Norm NMX-FF-079-SCFI-2004 [9] regarding poultry products, fresh chicken eggs and method specifications considers an egg to be fresh at the 15 days old; however, eggs are often stored for longer periods and the different temperatures. During this period, the air space within the egg increases due to loss of water, and a change in pH occurs. This change and the effect of the compounds in new raw materials added to the bird's diet can affect the chemical and physical quality of eggs [10, 11].

According to the results published by Carrillo et al. [6] and Carranco et al. [7], the inclusion of between 3 and 6% of RCM in the diets of laying hens is probably adequate for obtaining high-quality eggs because higher levels cause changes in the sensorial characteristics of eggs. Starting from the above inclusion criterion, this study was designed to determine whether the physical and chemical properties of the water-soluble portion of eggs from hens fed a standard diet and a diet with 4% of red crab meal undergo modifications when stored at 4° and 20° for 0, 15 and 30 days.

## II. METHODS AND MATERIAL

Red crab meal (RCM) was provided by CIBNOR (The Northwest Center for Biological Research), Guaymas Unit, Sonora, Mexico. Subsequently, the meal was transported to the City of Mexico to the Department of Animal Nutrition Dr. Fernando Pérez-Gil Romo at the National Institute of Medical Sciences and Nutrition Salvador Zubirán, where it was stored in black plastic bags and frozen (-20°C) until use.

### A. Preparation of diets and test with birds

The procedure for the use of birds was according to The Technical Specifications for the Production, Care and use of Laboratory Animals [12].

This test was conducted at the CEIEPAV (Center for Teaching, Research and Extension in Poultry Production), in the School of Veterinary Medicine of the National Autonomous University of Mexico.

The sorghum-soybean meal-based diet and the 4% RCM diet were formulated to meet the nutrient requirements of the National Research Council [13] for laying hens through Nutrión Windows™ (Version 5.0 Pro), a computerized system for feed formulation (Guadalajara, Jal., Mexico).

Ninety Isa-Brown laying hens (32 wk age, with mean body weight of 1560 ± 20 g) were selected from 300 hen flock based on similar weights and production rate. After a 2-wk of adaptation, the selected hens were divided into 2 groups of 45 birds each, which consisted of five replications of nine birds each. The experimental period lasted 4 wk (34 – 38 wk of age). The treatments consisted of a control diet (Control) and a diet containing 4% RCM. Throughout the experimental periods, feed and water available to allow for ad libitum consumption. During this period, the productive parameters (egg production, egg weight, feed conversion and feed intake) were measured. At the end of 4 weeks, 250 eggs were collected from each treatment group and stored as follows: day 0 (50 eggs), 15 days at 20°C (50 eggs), 15 days at 4°C (50 eggs), 30 days at 20°C (50 eggs), and 30 days at 4°C (50 eggs).

### B. Haugh Units (HU)

For the fresh eggs and the eggs stored at 20°C and 4°C for 15 and 30 days, the HU were determined using automated equipment (Technical Service and Supplies, Inc., England, UK). The system consists of a microprocessor (QCM+) connected to a digital scale and a micrometer for measuring albumen height. The QCM+ collects data from online tools and displays a reading, which is transferred to a computer equipped with egg quality-assurance software. The HU were calculated from the albumen height and the egg weight using the same processor.

### C. Chemical Analysis

The following analyses were performed on the RCM according to standardized techniques published by the Association of Official Analytical Chemists [14]: crude protein (method 968.06), total ash (method 942.05), ether extract (method 920.39), minerals (Ca, Na and Mg) (methods Cap. 4-40), water-soluble vitamins (B<sub>1</sub>, B<sub>2</sub> and niacin) by high performance liquid chromatography (HPLC) (methods 970.65 and 942.23), gross energy of RCM, was determined a total combustion using an adiabatic bomb calorimeter (Parr 1755, Parr Instrument Company, Moline, IL, USA), and the amino acid profile by HPLC (hydrolyze the sample into its component amino acids, and derivative the amino acids using AccQ-Fluor Reagent) (WatersAccQtag Manual, Manual No. WAT052874, April 1993).

The pH of the egg samples fulfilling the desired age and storage conditions was performed using a Hanna Instrument pH-210 potentiometer, and the egg were subsequently mixed (yolk and albumen) and lyophilized. Using the lyophilized egg, analyses of crude protein, essential amino acids and water-soluble vitamins were performed according to the techniques described above.

### D. Statistical Analysis.

The data of the productive parameters tests were analyzed using a completely randomized design (ANOVA), and the differences between means were tested using Tukey's test with a confidence level of 95%. The analyses were conducted using SPSS version 11.0 for Windows (SPSS Inc., Chicago IL, USA).

The statistical model was:  $Y_{ij} = \mu + \tau_i + \epsilon_{ij}$ , where  $Y_{ij}$  = the response variable,  $\mu$  = the experimental mean,  $\tau_i$  = the effect of the  $i$ -th treatment,  $\epsilon_{ij}$  = the experimental error.

The data of the physical and chemical analyses of the water-soluble fraction of the egg were analyzed according to a 2x2x2 factorial design with the following factors: treatment, time and temperature. Tukey's test was used to perform a pairwise comparison of the means with a confidence level of 95%, using SPSS version 11.0 for Windows (SPSS Inc., Chicago IL, USA).

The statistical model was:  $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijk}(l)$ , where  $Y_{ijk}$  = the response variable,  $\mu$  = the experimental mean,  $\alpha_i$  = the effect of the  $i$ -th diet treatment,  $\beta_j$  = the effect of the  $j$ -th time treatment,  $\gamma_k$  = the effect of the  $k$ -th temperature treatment,  $(\alpha\beta)_{ij}$  = the effect of the interaction of treatment and time,  $(\beta\gamma)_{jk}$  = the effect of the interaction of time and temperature,  $(\alpha\beta\gamma)_{ijk}$  = the interaction of treatment, time and temperature,  $\epsilon_{ijk}(l)$  = the experimental error.

## III. RESULT AND DISCUSSION

### A. Chemical composition of the red crab meal (RCM)

The results of the approximate chemical analyses are presented in Table 1; the most abundant fractions were crude protein (33.74%), total ash (20.24%) and Mg (9.70 mg/100 g). Due to the high protein content found in this unconventional raw material, it was important to evaluate the content of essential amino acids. The experimental diets were formulated based on the chemical and amino acid compositions of the meal (Table 2).

TABLE I  
Composition of red crab meal  
(*Pleuroncodes planipes*) used in this study

(g/100 g) <sup>1</sup>	RCM
Moisture	9.13 ± 0.07
Ash	20.24 ± 0.03
Ether extract	7.29 ± 0.01
Crude protein	33.74 ± 0.14
Total carbohydrates	29.58
Gross energy (kcal/100 g)	255.7 ± 0.08
Calcium	7.97 ± 0.05
Sodium	1.18 ± 0.08
Magnesium	9.70 ± 0.96
Amino acid composition g aa/100g protein	
Isoleucine	3.98 ± 0.01
Leucine	6.55 ± 0.01
Lysine	10.02 ± 0.02
Methionine	1.55 ± 0.01
Cystine	1.98 ± 0.02
Phenylalanine	4.31 ± 0.02
Tyrosine	4.08 ± 0.03
Threonine	3.55 ± 0.01
Valine	5.35 ± 0.03
Arginine	3.68 ± 0.02
Histidine	6.80 ± 0.02
Alanine	6.35 ± 0.03
Aspartic acid	9.18 ± 0.03
Glutamine	14.55 ± 0.01
Glycine	7.56 ± 0.01
Proline	5.15 ± 0.03
Serine	3.42 ± 0.02
Tryptophan	1.27 ± 0.02

<sup>1</sup>The values are the mean ± standard deviation of six samples.

TABLE II  
Composition of the experimental layer diets

Ingredients (as fed g/kg)	Control	40 g/kg Red crab meal
Sorghum	673.957	701.255
Red crab meal	0.000	40.000
Calcium carbonate	107.159	92.204
Soybean meal	182.800	150.932
Calcium phosphate 1821	11.931	9.356
Vegetal oil	10.000	0.000
Salt (NaCl)	3.626	0.290
Premix <sup>a</sup>	2.500	2.250
L-Lysine HCl	2.426	0.000
DL-Methionine	2.249	1.163
Mycotoxin sequestrant	1.000	1.000
L-Threonine	0.703	0.000
Avelut powder <sup>b</sup>	0.500	0.500
Choline chloride 60%	0.500	0.500
Bacitracin-zinc	0.300	0.300
Avired <sup>c</sup>	0.200	0.000
Antioxidant	0.150	0.000*
<b>Calculated analysis (g/kg diet)</b>		
ME (kcal/kg)	2715.1	2715.1
Crude protein	150.0	151.0
Methionine	4.56	3.94
Methionine + cystine	6.90	6.90
Total calcium	41.78	39.81
Available phosphorus	3.40	3.40
Sodium	1.50	1.50
Lysine	8.60	9.84
Threonine	6.20	6.42
Tryptophan	1.91	1.74

<sup>a</sup> The premix provided the following per kg of diet: retinyl acetate, 12000 IU; cholecalciferol, 2500 IU; DL- $\alpha$ -tocopheryl acetate, 30 mg; menadione, 2 mg; thiamine, 2.25 mg; riboflavin, 7.5 mg; pyridoxine, 3.5 mg; cyanocobalamin, 0.02 mg; D-pantothenic acid, 12.5 mg; biotin, 0.125 mg; folic acid, 1.5 mg; Zn, 50 mg; Cu, 12 mg; I, 0.3 mg; Fe, 110 mg; Se, 0.1 mg; Mn, 110 mg.  
<sup>b</sup> Saponified xanthophylls of Aztec marigold (yellow, 15 ppm).  
<sup>c</sup> Red xanthophylls (canthaxanthin, 10 ppm).  
\* Due to the carotenoids contained.

### B. Production variables

No statistically significant differences ( $P > 0.05$ ) in the productive parameters measured were found (Table 3).

TABLE III

The effect of dietary supplementation with RCM on the production performance of laying hens between 34 and 38 weeks of age<sup>1</sup>.

	Number of eggs/hen	Egg weight (g)	Feed conversion ratio (g feed/g egg)	Feed consumption (g/hen/day)
Control	88.35 ± 6.26	64.20 ± 1.30	2.07 ± 0.11	118.0 ± 2.54
RCM	87.22 ± 7.78	62.5 ± 1.23	2.07 ± 0.15	112.74 ± 4.2

<sup>1</sup> The data are means of five replicates of nine hens each with the SD. No significant differences were found.

### C. Crude protein content (CP), pH and Haugh Units (HU) in eggs

Table 4 shows the results of the response of RCM addition, days and temperature storage on the crude protein content (CP), pH and HU of the egg. Showing a significant difference ( $P < 0.05$ ) in CP, pH and HU between days and temperatures storage.

With respect to temperature were found significant difference ( $P < 0.05$ ) in CP and HU, but not in pH ( $P < 0.05$ ). For the days of storage, there were differences ( $P < 0.05$ ), with CP and HU decreasing and the pH increasing as storage time went on ( $P < 0.05$ ).

The effect of temperature and egg storage time on CP, pH and HU, showing a significant difference ( $P < 0.05$ ) for CP between temperatures and not between storage days. The pH and HU showed differences for either of the measured variables ( $P < 0.05$ ).

The effects of diet and egg storage time on CP, pH and HU showing significant differences for both diet and storage days ( $P < 0.05$ ).

TABLE IV  
The effect of RCM addition storage days and storage temperature on egg Crude Protein content, pH and Haugh Units.

	Diet		
	Crude Protein content (g/100 g)	pH	Haugh Units
Control	49.2 <sup>b</sup>	8.48 <sup>a</sup>	62.91 <sup>b</sup>
RCM	50.2 <sup>a</sup>	8.53 <sup>a</sup>	64.96 <sup>a</sup>
SEM	0.27	0.06	1.56
Temperature			
Baseline*	48.8	7.35	76.14
20°C	49.2 <sup>b</sup>	8.44 <sup>a</sup>	56.82 <sup>b</sup>
4°C	50.2 <sup>a</sup>	8.50 <sup>a</sup>	71.05 <sup>a</sup>
SEM	0.19	0.04	1.1
Storage days			
15	49.8 <sup>ab</sup>	8.73 <sup>b</sup>	60.16 <sup>b</sup>
30	49.2 <sup>b</sup>	9.32 <sup>a</sup>	52.62 <sup>c</sup>
SEM <sup>d</sup>	0.27	0.06	1.56
Significance			
Diet	0.0002	0.4216	0.0158
Temperature	0.0037	0.0761	0.0001
Storage days	0.0033	0.0001	0.0001
Diet * Temperature	0.1101	0.1907	0.9504
Diet * Storage days	0.0001	0.5193	0.0055
Temperature*Storage days	0.0100	0.1343	0.0001
Diet *Temperature*	0.0515	0.1886	0.9767
Storage days			

<sup>a,b,c</sup> Means in each column followed by different subscript letters differ significantly.

<sup>d</sup> Pooled standard error of mean \*Baseline (fresh eggs without storing)

#### D. Amino acids and Water-soluble vitamin

There were no significant differences ( $P > 0.05$ ) on the content of the studied amino acids or the water-soluble vitamins ( $B_1$ ,  $B_2$  and niacin).

In this study, the value obtained for crude protein (33.74%) in RCM was lower than the values reported by Charley [15] for crab meal (47.2%) and by Castro et al. [5] for red crab (39.9%). The RCM showed a high level of total ash (20.24%). However, this value is lower than that reported by other authors (28.9-33%) [16, 17, 18]. This discrepancy can be explained by the environment in which these crustaceans live, rich in minerals, in particular Mg, which is an element that is present in high concentrations in all crustaceans, ranging from 10 to 50 mg/100 g [19]. The value of ether extract (7.29%) was greater than that reported by [15] of 4.9%. This variability is likely due to the season of capture and the fact that this meal was composed of males and females. Castro et al. [5] reported that females have a higher lipid content, requiring stored energy for reproduction.

The gross energy obtained in this study was 255.7 kcal/100g, a similar value to that of meat meal (271.5 kcal/100g) that has also been used as an ingredient in diets for laying hens [16]. Crustaceans obtain their energy mainly from protein catabolism, so the metabolism of these organisms is different from land animals [16].

With respect to the essential amino acids for birds, most were found in higher quantities compared to those reported by Toma and Meyers [20]. Additionally, Quintero [21] published higher values for the amino acids reported in this study, with the exception of histidine, valine and lysine. According to the formulation of diets for birds [13], it was only necessary to add the amino acids methionine, threonine and lysine to the control diet, unlike the diet containing RCM, which is rich in lysine (10.02 g aa/100 g protein) and to which only methionine was added.

The objective of this study was to determine the effect of storage conditions on the water-soluble fraction of eggs from hens fed a standard diet and a diet containing 4% RCM. The pH has a value of 7.6 on a freshly laid egg and rises to 8.5 after 24 hours at 20°C, reaching values

of 9 to 9.4 after several days. In this study, the pH increased as storage time went on (7.35 – 9.32), which is consistent with the pattern reported by Li -Chan et al. [22], who found that in a fresh egg (at the time of laying) the pH ranged from 7.6 to 8.5. The eggs stored for 15 days at different temperatures showed pH values between 7.35 and 9.32. These values are similar to those reported by these authors: the pH after three days of storage at 3°C was 9.18 and after 21 days was 9.4. The pH of the egg (mainly of the albumin) is an important factor in the control of the rheological properties of gels formed during the heat treatment (80°C). The pH depends on the balance between the dissolved carbon dioxide, bicarbonate ions and carbonate ions governed by the partial pressure of carbon dioxide from the external environment. The bicarbonate ion concentration increases as the carbonate concentration decreases [23]. In addition, the pH is related to the action of proteins and the reactivity of the sulfhydryl groups. As the pH increases in the egg during storage, the elasticity of the gel, the penetration force and the viscosity index decrease [24]. This change increases the fragility of the yolk by the dispersion of albumin, which could affect the functional properties of the egg through the formation of foam.

Both the physical and chemical egg qualities are important, especially when new ingredients are incorporated in the formulation of the birds' diet. Through various laboratory analyses, the changes experienced by the samples, both fresh and during storage, can be assessed, specifically regarding their physical (egg weight and HU) and chemical (protein, pH, amino acids and vitamins) characteristics, which influence their quality and the preference of the consumer. As reported by Grobas and Mateos [25], the water-soluble components of egg show little variation when the ingredients of the bird's diet are changed; however, these authors did not consider the changes due to the storage conditions and time.

In hens, daily consumption of the amino acids needed to procedure egg protein is more important than the total amount of protein. The amino acids that are important for the birds were quantified in RCM. Methionine, lysine and threonine are considered the limiting amino acids in poultry diets; in RCM, the lysine content was greater than that of methionine, such that it was only

necessary to add methionine to the diet. Therefore, the reduction of one of these amino acids negatively affects the production of albumin in the eggs, decreasing the size of the egg over time. Egg protein is considered high quality, and the essential amino acid ratio depends on the genetic line of the bird. Egg protein has been used as a reference for other types of food for human consumption. Therefore, it is important that the bird's diet contains appropriate levels of essential amino acids for maintaining biological and commercial egg quality [26].

The Mexican Norm NMX-FF-079-SCFI-2004 (regarding poultry products, fresh chicken eggs and test methods specification) [9] states the physical characteristics and specifications to be met by "classified fresh chicken eggs" that are produced and/or sold within the country. In this standard, five categories for fresh chicken eggs based on the egg weight and size are recognized and should be applied to all classifications of consumption (Extra Large = greater than 64 g, Large = 60-64 g, Medium = 55-60 g; Small = 50-55 g, and Pewee = less than 50 g). According to these categories, both the control eggs (63.8 g/initially to 60.1 g/20°C and 63 g/4°C) and the RCM eggs (64.2 g/initially to 61.4 g/20°C and 63.2 g/4°C) had weights that were within the "Large egg weight" category after 30 days in storage.

Likewise, fresh chicken eggs for cooking are classified in grades according to the following specifications: the scale of Haugh Units (HU) ranges from 0 to 110. The interpretation of these units is an aid to determine the laying time: lower values indicate older eggs. Thus, this classification is as follows: Mexico Extra = more than 60 HU; Mexico 1 = from 61 to 70 UH; Mexico 2 = from 31 to 60 UH and, out of classification = less than 30 HU. In general, both the control eggs and the RCM (62.91 and 64.96 HU, respectively) were classified as Mexico 1 (>5.5 mm height and albumin and >70 HU for eggs freshly laid), decreasing after 15 and 30 days at 4°C to rank as Large eggs (>4.2 mm and 61-70 HU) and after 30 days at 20°C as Medium eggs (>3.0 mm and 31-60 HU). In this study, a decrease in HU was observed as an effect of diet, storage time and temperature; this decrease was most notable for eggs stored for 30 days at 20°C. This change is explained by natural phenomena that occur in a perishable product that is not consumed at the laying time. This change can be understood as a

decrease in albumen quality that is reflected in the HU and manifested by the liquefaction of the dense albumen, which results in the loss of the internal structure and the spatial organization of the layers of the albumen and the yolk. The mechanisms responsible for this change are complex and not well defined, but all involve alterations of proteins. The phenomena can be described as follows [27]: a) partial destruction of the lysozyme-ovomucin complex due to the deactivation of lysozymes; b) destruction of the electrostatic bonds between the amino groups of the lysine residues of the lysozyme and the carboxyl groups of the sialic acid in the ovomucin when the pH increases; c) dissociation of ovomucin subunits, mainly of  $\beta$ , which is favored by the passage of divalent ions from the albumin to the yolk of the eggs during storage; d) modification of the structure of the ovomucin, especially the  $\beta$  subunit, which changes the type of the lysozyme-ovomucin complex, due to the increase in pH and; e) partial degradation of the O-glycosidic bonds, which results in the solubilization of ovomucin- $\beta$  and releases hexoses, hexosamines and sialic acid by an alkaline  $\beta$ -elimination process.

The above mentioned phenomena are related to the release of carbon dioxide from the interior of the egg, which tends to balance its concentration with the partial pressure of surrounding air, resulting in an increase in pH. The changes accelerate significantly when the egg is stored at room temperature. This acceleration occurred in eggs stored at 20°C for 15 and 30 days; when they were opened, it was observed that the yolk had moved from its original position (in the center), shrunk and flattened, and that the cell membrane (vitelline) broke easily. All of these changes are associated with alterations in the proteins present in the albumin [27]. Note that although there were no differences in the composition of water-soluble vitamins, their values become important in nutritional labeling [28].

#### IV. CONCLUSION

We conclude that the productive parameters are not affected by an addition of 4% RCM to the diets of laying hens. However, changes were observed in the CP content, pH and HU due to the chemical and physical changes that occur in the eggs stored at 20°C for 30 days.



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# Measurements of Radon Concentrations and Dose Assessments in Chemistry Department-Science College- Al-Mustansiriyah University, Baghdad, Iraq

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## ABSTRACT

Measurements of radon gas concentrations with their progeny and the annual effective dose indoor the building of Al-Mustansiriyah University College of Science-Chemistry Department have been carried out by using time-integrated passive radon dosimeters solid state nuclear track detector CR-39 technique. The detectors with 1cm x1cm have been distributed over 58 places and suspended for sitting (1m) and standing (1.75m) positions in each location under study. The dosimetric measurements are made over a period of 100 days from 30 January 2014 to 10 May 2014. The calibration process has been done using radium-226 source with known activity radiation. It has found that the indoor radon gas concentrations varying from  $35.220 \pm 5.935 \text{ Bq/m}^3$  to  $71.673 \pm 8.466 \text{ Bq/m}^3$  with an average value  $49.129 \pm 6.969 \text{ Bq/m}^3$  at 1m, and varying from  $31.794 \pm 5.639 \text{ Bq/m}^3$  to  $68.246 \pm 8.261 \text{ Bq/m}^3$  with an average value  $45.487 \pm 6.696 \text{ Bq/m}^3$  at 1.75m which are within the worldwide limits  $148 \text{ Bq/m}^3$  (EPA, 2003) and  $200\text{-}300 \text{ Bq/m}^3$  (ICRP, 2009). The annual effective dose of the inhalation exposure to radon gas has been estimated and this vary from  $0.370 \text{ mSv/y}$  to  $0.753 \text{ mSv/y}$  with an average value  $0.516 \text{ mSv/y}$  at 1m, and varying from  $0.334 \text{ mSv/y}$  to  $0.717 \text{ mSv/y}$  with an average value  $0.478 \text{ mSv/y}$  at 1.75m which are within the worldwide permissible limits  $3\text{-}10 \text{ mSv/y}$  (ICRP, 1993). The potential alpha energy concentration found to vary from  $3.808 \text{ mWL}$  to  $7.748 \text{ mWL}$  with an average value  $5.311 \text{ mWL}$  at 1m and vary from  $3.437 \text{ mWL}$  to  $7.378 \text{ mWL}$  with an average value  $4.918 \text{ mWL}$  at 1.75m which are less than the recommended value  $53.33 \text{ mWL}$  (UNSCEAR, 1993). The lung cancer cases per million person per year vary from 6.664 to 13.562 per million person per year with an average value 9.296 per million person per year at 1m and vary from 6.016 to 12.913 per million person per year with an average value 8.607 per million person per year which are less than the recommended range 170-230 per million person per year (ICRP, 1993). The number of decays per-minute using swabs measurements technique have been used for selected units within two swabs from building materials walls for each unite, with area of  $100 \text{ cm}^2$  using Ludlum 3030, the average of three swabs measurements have been calculated. Hence, the effectiveness of emitted alpha particles from the walls has been calculated to be varied from  $0.00556$  to  $0.02222 \text{ Bq/cm}^2$  with an average value  $0.01154 \text{ Bq/cm}^2$  at 1m and  $0.00000 \text{ Bq/cm}^2$  to  $0.01667 \text{ Bq/cm}^2$  with an average value  $0.00983 \text{ Bq/cm}^2$  at 1.75m respectively which is within the permissible limit  $0.04 \text{ Bq/cm}^2$  (Danial, 2010).

**Keywords:** Indoor Radon gas; Natural radioactivity; CR-39 detector; Annual effective dose; Ludlum 3030

## I. INTRODUCTION

Radon was the fifth radioactive natural element being found as a noble gas, which was discovered by the German Physicist Friedrich Ernst Dron in (1900), who called it Niton. It has been called radon since (1923). Its atomic number is 86 and mass number is 222 in the

periodic table [1, 2]. Radon is a gas that comes from the radioactive decay of either uranium-235 and 238 or thorium-232. All of the gaseous radon members of the three primordial series headed by U-235, U-238, and Th-232 which are common naturally occurring are radioactive alpha particle emitters [3]. The decay of radon, with uranium-238, goes through four inter-

mediate states to form radium-226 which has a half-life 1600 years. Radium-226 then decay to form radon-222 gas. Radon's half-life is 3.82 days, which provides sufficient time for it diffuse through soil and into homes, where it further disintegrates to produce the more radiologically active radon progeny (radon daughters) [2]. The name radon is Rn-222 isotope in order to distinguish it from other two natural isotopes; called thoron Rn-220 (alpha emitter of 55.6s half Life) and action Rn-219 (alpha emitter of 3.96 s half-Life) because they originate in the thorium and actinium series, respectively. Because of these properties the measurements of the alpha dose delivered from action Rn-219 and thoron Rn-220 would be the primary concern but so far these situations have been rare [2]. Because Rn-222 gas moves freely in the indoor environment, it becomes a human health hazard. If its concentration in indoor air becomes high, radon and its decay products can be inhaled and cause lung cancer. Many previous studies of indoor radon concentration have been performed with widely used of solid state nuclear track detectors (SSNTDs) [4, 5, 6, 7, 8, 9, and 10]. However, in this work the indoor radon gas concentrations using CR-39 detectors have been measured in the department of chemistry in college of science of Al-Mustansiriyah University, at which a radioactive sources are usually used in their laboratories.

## II. METHODS AND MATERIAL

### A. Area of the Study

Baghdad city is located in the Middle of Iraq and it is the capital of the Republic of Iraq. Its location of latitude 33.316666 and longitude 44.416668 it is located about 34meters above sea level, with a total area nearly of 204.2 km<sup>2</sup> and a population nearly of 7665292inhabitants, see Fig. 1 Baghdad city has a desert climate characterized by extreme heat during the day, an abrupt drop in temperature at night, and slight, erratic rainfall. The temperature is moderate at 12°C in winter and 33°C in summer. Its lands are flat and levelled in areas linked to waters from the Tiger River. AL- Mustansiriyah University is located in the Southern of Baghdad City, see Fig. 2. The science college and their buildings are shown in Fig. 3. The three figures are taken from Google earth.



Fig. 1. Baghdad City.



Fig. 2. AL- Mustansiriyah University.



Fig. 3. The science college.

### B. Measurement Technique

Radon concentrations are measured using 68 plastic cups (i.e. dosimeters) prepared with solid state nuclear track detectors CR-39 of 500µm thick, density 1.36 gm/cm<sup>3</sup>, UK issued, and 1x1 cm<sup>2</sup> area are distributed inside the rooms and laboratories of department of chemistry. 34 detectors of 68 are suspended at 1m and the others are suspended at 1.75m. Each dosimeter is to be made of a plastic cup of height 4.5cm, the diameter of the bottom is 3cm and that of the top is 4.5cm, with a circular hole of diameter 1cm in the centre, Fig. 4a shows the typical CR-39 track detectors. The hole is covered by a piece of sponge sealed into the interior surface of the lid. The detector CR-39 is fixed in the bottom of the dosimeter as shown in Fig. 4b. The detectors are left for a period of three months from 30 January 2014 to 30 April 2014. The exposed detectors are collected from different locations and etched chemically in 6.5M NaOH solution at 60°C for 6 hours. The chemical etching process to the CR-39 detectors has been done in order to show the alpha-particles tracks from <sup>222</sup>Rn. Optical microscope with magnification of 400X by an objective (4x, 10x, 40x and 100x) and two eye pieces (10x) with digital video camera of 5MB resolution and connected with a personal computer to show and counting the alpha damage tracks formed on the detectors.



Fig. 4a. Typical CR-39 track detectors seen in the present work for chemistry department.

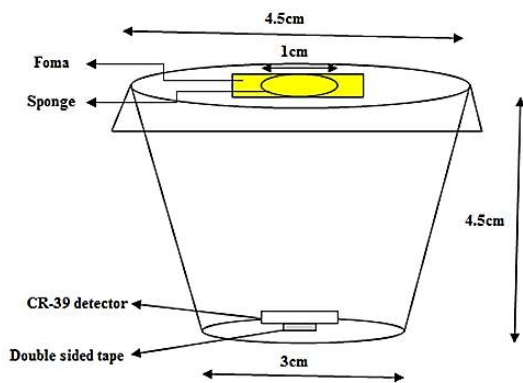


Fig. 4b. Plastic holder design (i.e.dosimeter) used in the present work.

### C. Ludlum3030

The Ludlum3030 alpha-beta counter is powered by main supply of AC voltage. The instrument features a built-in detector, ZnS (Ag) adhered to plastic scintillation material, tube 5.1cm (2inch) diameter, magnetically shielding photomultiplier, window 0.4mg/cm<sup>2</sup> aluminized molar, active and open area 20.3cm<sup>2</sup>, efficiency alpha 32% <sup>230</sup>Th, 39% <sup>238</sup>U, 37 % <sup>239</sup>Pu, high-voltage power supply, adjustable count time periods, and a click-per-event audio with adjustable volume. A pulse height analyser is employed to provide information to the two independent counters. Filter paper for qualitative analysis made in Germany by diameter 4.5cm, used to take samples from the walls of the chemistry department building of the college of science.

### D. Chemical Etching

For the preparation of etching solution, the weight of (NaOH) has been calculated as follows [11]:

$$W = W_{eq} \times N \times V \quad (1)$$

Where:  $W$  is the weight of NaOH needed to prepare for a given normality.  $W_{eq}$  is the equivalent weight of NaOH (i.e. the summation of the atomic weight of Na, O and H). i.e.  $W_{eq} = 22.98977 + 15.9994 + 1.00794 = 39.99711 = 40$ .  $N$  is the normality =6.25.  $V$  is the volume of distilled water (1 liter).  $W = 40 \times 6.25 \times 1 = 250$  gm of NaOH in 1 liter of distilled water.

### E. Microscopic Viewing

After etching and drying process the detectors have a viewing by optical microscope so as to get on the alpha particles tracks by selecting right zoom (the ability of magnification is equal to 1000 related to objective lens and camera with magnification 100 and 10 respectively) and to count the tracks per unit area. Especially glass slide have been used to calibrate the dimensions of pictures. The camera has been connected with a microscope to photograph promised tracks. The camera is connected with the computer to show pictures effects on a computer screen. The calibration of software have been done to calculates the area of field view on a glass slide in front of the lens object-oriented and to calculate the length and width of the picture, then calculate the area and divides the average number of tracks ( $N_{ave}$ ) for the (sample X) calculated on the area of field view per unit mm<sup>2</sup> ( $A$ ) to get the track density. Ten attempts (pictures) have been taken for each sample, otherwise to calculate the average number of tracks obtained for each sample. The track density ( $\rho$ ) has been calculated by using the following equation [12, 13]:

$$\text{Track density } (\rho) = \frac{\text{average number of total tracks } (N_{ave})}{\text{area of field view } (A)} \quad (2)$$

### F. Calibration of CR-39 Detector

The calibration of CR-39 detector has been carried out by using the standard source of radium <sup>226</sup>Ra with radioactivity ( $A=0.1\mu\text{Ci}=37000\text{Bq}$ ) at manufacturing data 1-03-1982, which emits radon gas <sup>222</sup>Rn. After correction the activity to  $A=36481.13\text{Bq}$  at 30-09-2013 and dose rate at 1cm is  $63.611\mu\text{Sv/hr}$  using Rad Pro software. The CR-39 detector and the standard source of radium <sup>226</sup>Ra were placed at the special container used in the present work with a cylindrical shape and volume 0.11m<sup>3</sup> diameter 40cm and height 85cm as shown in Fig. 4c.

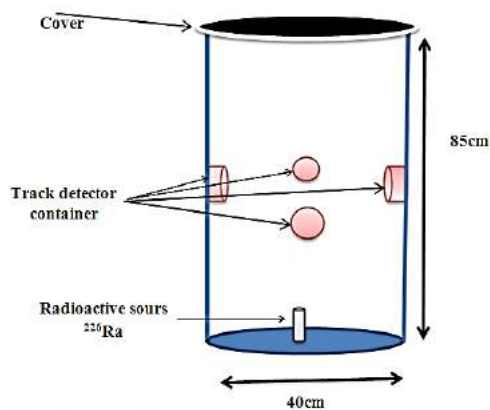


Fig. 4c. Container (cylindrical shape) used for the calibration in the present work.

The activity of radon gas ( $A_{\text{Radon}}$ ) inside the container at any time can be calculated by using the following equation [14]:

$$A_{\text{Radon}} = A_{\text{Radium}} \times (1 - \exp(-\lambda_{\text{Radon}} \times t)) \quad (3)$$

Where:  $t_{1/2}({}^{222}\text{Rn}) = 3.8253$  day.  $A_{\text{Radium}} = 36481.1\text{Bq}$  is the activity of radium-226 as a standard source.  $\lambda_{\text{Radon}}$  is the decay constant of radon-222  $= 0.1812 \text{ day}^{-1}$ .  $t$  is the exposure time in day. The exposure time of detectors was with different times (0.25, 0.5, 0.7, and 1 day), then the radon exposure determined by [15]:

$$E_s (\text{Bq. day/m}^3) = [A_{\text{Radon}} (\text{Bq}) / V (\text{m}^3)] \times t (\text{day}) \quad (4)$$

Where  $E_s$  is the radon gas exposure (i.e. concentration) in standard source.  $A_{\text{Radon}}$  is the radioactivity of  ${}^{222}\text{Rn}$  calculated by Eq. (3).  $V$  is the container volume in  $\text{m}^3$ ;  $t$  is the exposure time in day. Fig. 5 shows the relation between the track density ( $\rho_s$ ) and the radon exposure ( $E_s$ ).

$$\text{Slope} = \rho_s / E_s \quad (5)$$

Where  $\rho_s$  is the track density of standard source ( $\text{tracks/mm}^2$ ).  $E_s$  is the radon exposure of standard source ( $\text{Bq/m}^3$ ).days = ( $\text{Bq/m}^3$ ) by multiplying with (0.25, 0.5, 0.75, and 1 day).

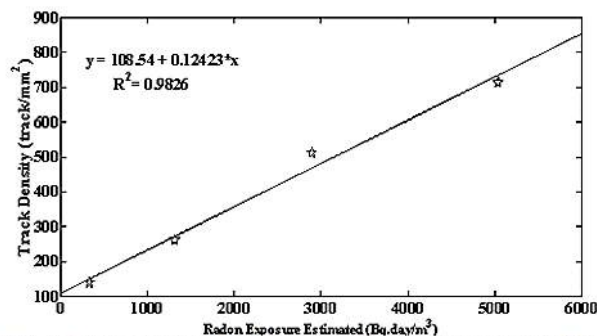


Fig. 5. Calibration curve for CR-39 detector. Track density ( $\text{track/mm}^2$ ) as a function of Radon exposure ( $\text{Bq.day/m}^3$ ).

## G. Calculation of Radon Exposure

After microscopic viewing process has been done to calculate the track density. Radon gas  ${}^{222}\text{Rn}$  concentration indoor has been measured by comparison between track densities on the detector around the unknown sample and that of the standard calibration source, from the following relation [15]:

$$\frac{E_x (\text{sample})}{\rho_x (\text{sample})} = \frac{E_s (\text{standard})}{\rho_s (\text{standard})} \quad (6)$$

i.e.  $E_x = E_s (\rho_x / \rho_s)$ . Where  $E_x$  is the radon gas exposure in unknown sample ( $\text{Bq/m}^3 \cdot \text{day}$ ).  $\rho_x$  is the track density of unknown sample ( $\text{tracks/mm}^2$ ). Therefore, from the slope  $= \rho_s / E_s$ ,  $E_x$  can be estimated as:

$$E_x = \rho_x / \text{Slope} \quad (7)$$

## H. Determination of Radon Concentration

Radon concentration in surrounding air is measured in terms of  $\text{Bq/m}^3$ , since the most regulate reference levels are specified in this unit. Determinations of radon concentration indoor buildings of the Department of Physics-College of Science are carried out by the following equation [16, 17]:

$$C_{\text{Rn}} (\text{Bq/m}^3) = \frac{E_x (\text{Bq.day/m}^3) \left(\frac{\rho}{t}\right)}{\rho_x} \quad (8)$$

Where  $\rho$  is the track density (number of track / $\text{mm}^2$ ) of distributed detectors.  $t$  is the exposure time (days) of distributed detectors. Comparable method is also obtained for track detectors techniques to determine the calibration constant (factor). This is obtained by dividing

the track density by the total exposure of radon source. Then Eq. (8) of radon exposure becomes [18, 19]:

$$C(\text{Bq}/\text{m}^3) = \frac{1}{\text{slope}} \left( \frac{\rho}{t} \right)_{\text{det}} \quad (9)$$

Since

$$\frac{1}{\text{slope}} = \frac{E_s(\text{Bq}\cdot\text{d}/\text{m}^3)}{\rho_s(\text{track}/\text{mm}^2)}; \text{slope} = \frac{\rho_s(\text{track}/\text{mm}^2)}{E_s(\text{Bq}\cdot\text{d}/\text{m}^3)} \quad (10)$$

Where the slope is the calibration factor in terms of  $(\text{track}\cdot\text{mm}^{-2}/\text{Bq}\cdot\text{day}\cdot\text{m}^3)$ .

## I. Dose Assessment Indoor Radon

### A. The annual effective dose

The annual effective dose,  $H_E$  due to radon inhalation, which corresponds to the values of indoor air radon concentrations, was calculated according to the following expression [20]:

$$H_E = C_{Rn} \times E_q \times T \times 9\text{nSv}(\text{Bq}\cdot\text{h}\cdot\text{m}^{-3})^{-1} \quad (11)$$

Where  $C_{Rn}$  is the average indoor air radon concentration, in  $\text{Bq}/\text{m}^3$ .  $E_q$  is the indoor equilibrium factor between radon and its progeny ( $=0.4$ ) (Wahl, 2007) [20].  $T$  is the exposure time to this concentration, in hours, and  $9\text{nSv}(\text{Bq}\cdot\text{h}\cdot\text{m}^3)^{-1}$  is the dose conversion factor.

### B. Potential Alpha Energy Concentration

The Potential Alpha Energy Concentration (PAEC) in terms of (WL) units was obtained using the relation [8, 21]:

$$\text{PAEC}(\text{WL}) = E_q \times C_{Rn}/3700 \quad (12)$$

### C. Lung Cancer Cases per Year per Million Person

The lung cancer cases per year per million people (CPPP), was obtained using the relation [4, 21, 22]:

$$(\text{CPPP}) = H_E \times (18 \times 10^{-6} \text{mSv}^{-1}\cdot\text{y}) \quad (13)$$

Where  $18 \times 10^{-6} \text{mSv}^{-1}\cdot\text{y}$  is conversion factor.

## D. Alpha particles emitted of surfaces

Specific activities of various radionuclides, disintegrations per minute per 100 square centimetres ( $\text{dpm}/100 \text{cm}^2$ ) used to measure alpha emitted of surfaces of an object, such as concrete or metal [23].

$$\text{Alpha emitted } (\text{Bq}/\text{cm}^2) = \text{dpm}/6000 \quad (14)$$

Where (dpm) is equal to  $(\text{Bq}/60)$ . Table 1 tabulated the location name and the cods at 1m and 1.75m in the department of chemistry laboratories building. While Fig. 6 shows the block diagram for the chemistry department laboratories building.

Table 1 Department of chemistry laboratories building.

Sequence	Location Name	Coding at 1m	Coding at 1.75m
1	Laboratory of Higher Studies Lab1	Ch1a2	Ch1a1
2	Library / Department of Chemistry	Ch2a2	Ch2a1
3	Teaching Staff Room No.1	Ch3a2	Ch3a1
4	Higher Studies Hall	Ch4a2	Ch4a1
5	Laboratory of Spectra2 G- LabA1	Ch5a2	Ch5a1
6	Teaching Staff Room No.2	Ch6a2	Ch6a1
7	Unit of Humanitarian Studies	Ch7a2	Ch7a1
8	Laboratory of Spectra1	Ch8a2	Ch8a1
9	Teaching Staff Room A1	Ch9a2	Ch9a1
10	Laboratory of Research of Inorganic	Ch10a2	Ch10a1
11	Laboratory of Prepare Diagnostic	Ch11a2	Ch11a1
12	Laboratory of Organic Chemistry	Ch12a2	Ch12a1
13	Laboratory of Organic Chemistry of Higher	Ch13a2	Ch13a1
14	Teaching Staff Room No.3	Ch14a2	Ch14a1
15	Laboratory of Analytical Chemistry	Ch15a2	Ch15a1
16	Inorganic Chemistry Laboratory LabA4	Ch16a2	Ch16a1
17	Inorganic Chemistry Laboratory for Higher	Ch17a2	Ch17a1
18	Laboratory of Physical Chemistry	Ch18a2	Ch18a1
19	Laboratory of Analytical Chemistry for	Ch19a2	Ch19a1
20	Laboratory of Industrial Chemistry for	Ch20a2	Ch20a1
21	Industrial Chemistry Laboratory	Ch21a2	Ch21a1
22	Laboratory of Analytical Chemistry LabA7	Ch22a2	Ch22a1
23	Laboratory of Research of Analytical	Ch23a2	Ch23a1
24	Laboratory Biochemistry	Ch24a2	Ch24a1
25	Laboratory of Biochemistry/Preparation	Ch25a2	Ch25a1
26	Physical Chemistry Laboratory for Students	Ch26a2	Ch26a1
27	Teaching Staff Room No.4	lost	lost
28	Teaching Staff Room No.5	lost	lost
29	Teaching Staff Room No.6	lost	lost

Note: a1=1m; a2=1.75m

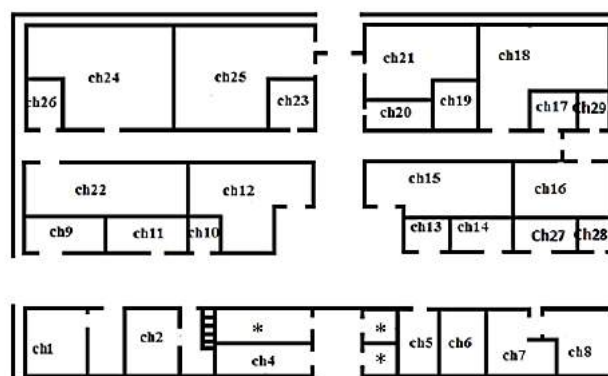


Fig. 6 Block diagram for the chemistry department.  
\* No suspended track.

### III. RESULT AND DISCUSSION

Radon exposure for such long time is necessary in order to obtain relatively a good number of tracks and to be counting statistically. The calibration factor used for conversion of track density (track/mm<sup>2</sup>.day) to radon concentrations in (Bq/m<sup>3</sup>) is 0.12423track.mm<sup>-2</sup> per Bq.day.m<sup>-3</sup>. The background has been calculated by calculating and subtracting from the track density of each track detector which was exposure to a period of measurement. The radiological risk associated with indoor radon exposure and relevant regulation has been evaluated. Regulations vary greatly between countries. The United States of America (USA) use a reference level of 148Bq/m<sup>3</sup> for dwellings and 400Bq/m<sup>3</sup> for workplaces (USEPA, 2004) [24]. The European Union (EU) accepts the recommended action level, the radiation level above which preventive action must be taken, included in the International Commission on Radiological Protection (ICRP, 1965) [25] of between 500 and 1500Bq/m<sup>3</sup> given by Kavasi et al., 2006 [26], and ICRP, 2009 [27] has identified the limit of radon concentration for the population to be (200-300 Bq/m<sup>3</sup>) (ICRP, 2009) [27]. In the United Kingdom (UK) the Health and Safety Executive (HSE) given by Kendall et al., 2005 [28] has adopted a radon action level of 400Bq/m<sup>3</sup> for workplaces. Also the limit is populated to be (148Bq/m<sup>3</sup>) in Environmental Protection Agency (EPA) (EPA, 2003) [29]. In Hungary the action level for workplaces is 1000Bq/m<sup>3</sup> (Kendall et al., 2005) [28]. While Israel uses a reference level between 40 and 200Bq/m<sup>3</sup> (Akerblom, 1999) [30]. There are no specific regulations in Iraq for indoor radon levels in either dwellings or workplaces. Department of Chemistry Building consists of several unites including laboratories preliminary studies and laboratories graduate addition to the private rooms of the masters. Among 68 suspended detectors, 6 have been lost. The results of the measurements at 1m and 1.75m for the department of chemistry are as follows:

#### A. Measurements at 1 meter for Department of Chemistry

Figs. 7, 8, 9, and 10 show the measured radon concentrations, the annual effective dose, potential alpha energy concentrations, and the lung cancer per year million person for different locations in the building of

department of chemistry at 1m. The minimum radon concentration is recorded at location (Ch16a1), inorganic chemistry laboratory lab A4, with a value 35.220±5.934Bq/m<sup>3</sup>, and the maximum value of radon concentration recorded at location (Ch3a1), teaching staff room no.1, with a value of 71.672±8.465Bq/m<sup>3</sup>, with an average value 49.129±7.009Bq/m<sup>3</sup>. The results are less than the limits (200-300Bq/m<sup>3</sup>) (ICRP, 2009) [27], and (148Bq/m<sup>3</sup>) (EPA, 2003) [29]. The measurements of radon concentrations from 30Bq/m<sup>3</sup> to 40Bq/m<sup>3</sup> are at locations: Ch16a1=35.219, Ch17a1=37.823, Ch21a1=39.741, Ch22a1=35.904, Ch23a1=37,412, Ch24a1=36.589 and Ch26a1=35.356Bq/m<sup>3</sup>. The measurements of radon concentrations from 40Bq/m<sup>3</sup> to 50Bq/ m<sup>3</sup> are at locations: Ch1a1=41.523, Ch8a1=41.797, Ch10a1=46.868, Ch11a1=46.045, Ch13a1=44.264, Ch14a1=47.553, Ch17a1=47.964 and Ch20=40.975. The measurements of radon concentrations from 50Bq/m<sup>3</sup> to 60Bq/m<sup>3</sup> are at locations: Ch4a1=59.612, Ch5a1=57.694, Ch6a1=57.009, Ch9a1=53.583, Ch18a1=57.968, Ch19a1=54.697. The measurements of radon concentrations from 60Bq/m<sup>3</sup> to 70Bq/ m<sup>3</sup> are at locations: Ch2a1=63.038, Ch7a1=61.257, Ch12a1=65.368 and Ch25a1=60.435. The measurements of radon concentrations from 70Bq/m<sup>3</sup> to 80Bq/m<sup>3</sup> are at location: Ch3a1=71.672. The values of the indoor annual effective dose vary from 0.370mSv/y in (Ch16a1) to 0.753mSv/y in (Ch3a1) with an average value 0.516mSv/y which is less than the lower limit of the admissible range 3-10mSv/y (ICRP, 1993)[31]. The values of the potential alpha energy concentration were found to vary from 3.808mWL in (Ch16a1) to 7.748mWL in (Ch3a1) with an average value 5.311mWL which is less than the admissible value 53.33 mWL given by UNSCEAR, 1993 [3]. The values of lung cancer cases per million person per year vary from 6.664 in (Ch16a1) to 13.565 in (Ch3a1) with an average value 9.296 per million person which is less than the lower limit of the admissible range 170- 230 per million person per year (ICRP, 1993) [31].

#### B. The measurements at 1.75 meter for Department of Chemistry

Figs. 11, 12, 13 and 14 show the measured radon concentrations, the annual effective dose, potential alpha energy concentrations, and the lung cancer per million



person per year for different locations in the department of chemistry building at 1.75m. The minimum radon concentration is recorded at location (Ch17a2), inorganic chemistry laboratory for higher studies A4, with a value  $(31.793 \pm 5.638 \text{Bq/m}^3)$ , and the maximum radon concentration recorded at location (Ch3a2), teaching staff room no.1, with a value of  $68.246 \pm 2.46 \text{Bq/m}^3$ , with an average value  $45.487 \pm 6.768 \text{Bq/m}^3$ . All results are within the worldwide limits  $200\text{-}300 \text{Bq/m}^3$  (ICRP, 2009) [27], and  $148 \text{Bq/m}^3$  (EPA, 2003) [29]. The measurement radon concentrations from  $30 \text{Bq/m}^3$  to  $40 \text{Bq/m}^3$  are at locations: Ch10a2=33.163, Ch11a2=33.026, Ch13a2=36.864, Ch14a2=35.904, Ch16a2=32.204, Ch17a2=31.793, Ch20a2=35.356, Ch21a2=37.960, Ch22a2=35.356, Ch23a2=36.864 and Ch26a2=36.041  $\text{Bq/m}^3$ . While the measurement radon concentrations from  $40 \text{Bq/m}^3$  to  $50 \text{Bq/m}^3$  are at locations: Ch1a2=45.634, Ch5a2=43.442, Ch9a2=49.197, Ch15a2=44.127, Ch19a2=47.964, and Ch24a2=41.386  $\text{Bq/m}^3$ ; and the measurement radon concentrations from  $50 \text{Bq/m}^3$  to  $60 \text{Bq/m}^3$  are at locations: Ch2a2=55.090, Ch4a2=53.171, Ch7a2=53.034, and Ch25a2=55.364  $\text{Bq/m}^3$ . Then the measurement radon concentrations from  $60 \text{Bq/m}^3$  to  $70 \text{Bq/m}^3$  are at locations: Ch3a2=68.246, Ch6a2=67.698, Ch12a1=67.013  $\text{Bq/m}^3$ . Fig. 15 shows the radon concentrations at 1m and 1.75m for different locations in the laboratories building of the chemistry department. The minimum value recorded at location (Ch17a2) with a value of  $31.793 \pm 5.638 \text{Bq/m}^3$  and the maximum value recorded at location (Ch3a1) with a value  $71.673 \pm 8.466 \text{Bq/m}^3$ . All results are within acceptable limits  $200\text{-}300 \text{Bq/m}^3$  (ICRP, 2009) [27], and  $148 \text{Bq/m}^3$  (EPA, 2003) [29]. The indoor annual effective dose varies from  $0.334 \text{mSv/y}$  in (Ch17a2) to  $0.717 \text{mSv/y}$  in (Ch3a2) with an average value  $0.478 \text{mSv/y}$  which is less than the lower limit of the admissible range  $3\text{-}10 \text{mSv/y}$  (ICRP, 1993) [31]. The potential alpha energy concentration were found to vary from  $3.437 \text{mWL}$  in (Ch17a2) to  $7.378 \text{mWL}$  in (Ch3a2) with an average value  $4.918 \text{mWL}$  which is less than the admissible value  $53.33 \text{mWL}$  given by UNSCEAR, 1993 [3]. The lung cancer cases per million person per year vary from 6.016 in (Ch17a2) to 12.913 in (Ch3a2) with an average value 8.607 per million person per year which is less than the lower limit of the admissible range (170-230) per million person per year (ICRP, 1993) [31].

### C. Measurements of the Alpha particles emitted from wall surfaces

For this process, we need to use a filter paper which has specifically designed to take swabs from the surface of the walls inside rooms, since the walls consisting of building materials. The swabs have been taken from 52 swabs from the wall surfaces with squared area  $(10 \times 10) \text{cm}^2$  by scanning all this area with a filter paper. The measurements have been done by insert the filter in the Ludlum3030 device for a period of one minute. This procedure will be repeated three times for each filter respectively, to take the average value then using Eq. (14) to calculate the emitted alpha particles. Fig. 16 shows the results of measurements of alpha particles emitted from the wall surfaces in the chemistry department building by using Ludlum3030. The minimum value is  $0.000 \text{Bq/cm}^2$  recorded at locations (Ch5a2, and Ch17a2), laboratory of spectra2 G-Lab A1, and inorganic chemistry laboratory for higher students A4 respectively. The maximum value  $0.02222 \text{Bq/cm}^2$  recorded at location Ch12a1 (the laboratory of organic chemistry) and Ch25a1 (the laboratory of biochemistry preparation room) respectively. The average value is  $0.01154 \text{Bq/cm}^2$  for sitting position and  $0.00983 \text{Bq/m}^2$  for standing position. Therefore, all results are lower than the recommended limit  $0.04 \text{Bq/cm}^2$  given by Daniel, 2011 [32].

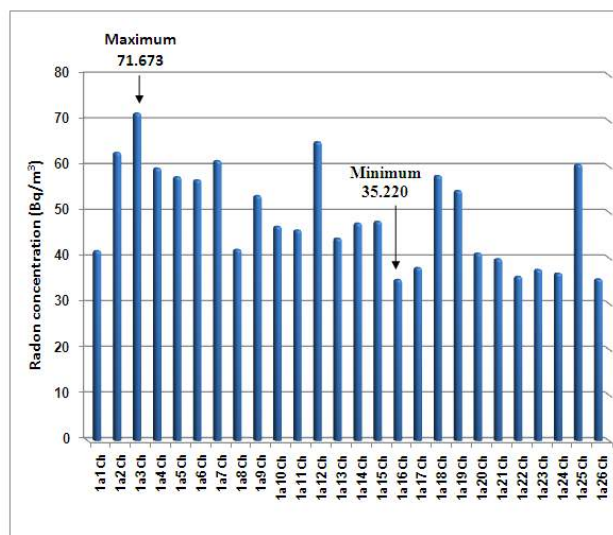


Fig. 7. Radon concentrations at 1m for different locations at department of chemistry.

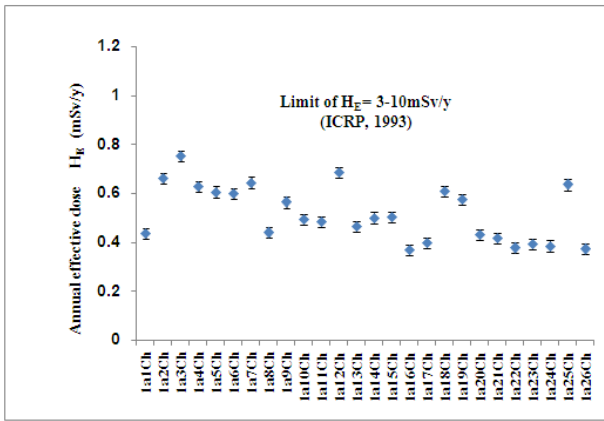


Fig. 8. Annual effective dose at 1m for different locations at department of chemistry.

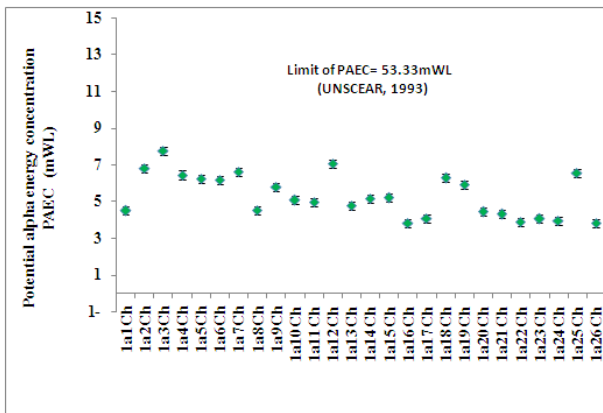


Fig. 9. Potential alpha energy concentration at 1m for different locations at department of chemistry.

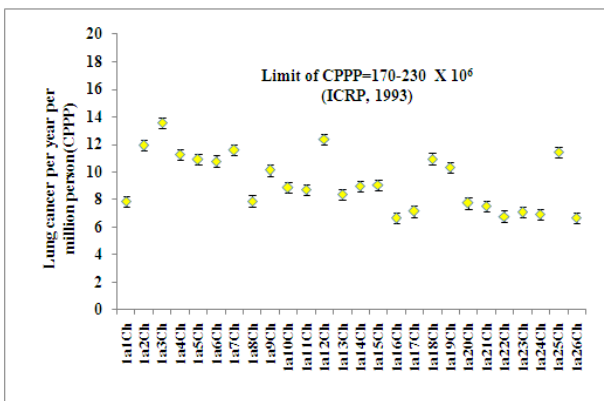


Fig. 10. Lung cancer cases per year per million person at 1m for different locations at department of chemistry.

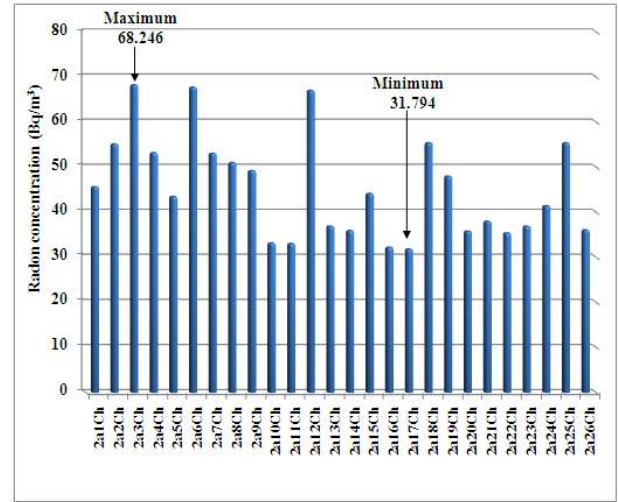


Fig. 11. Radon concentrations at 1.75m for different locations at department of chemistry.

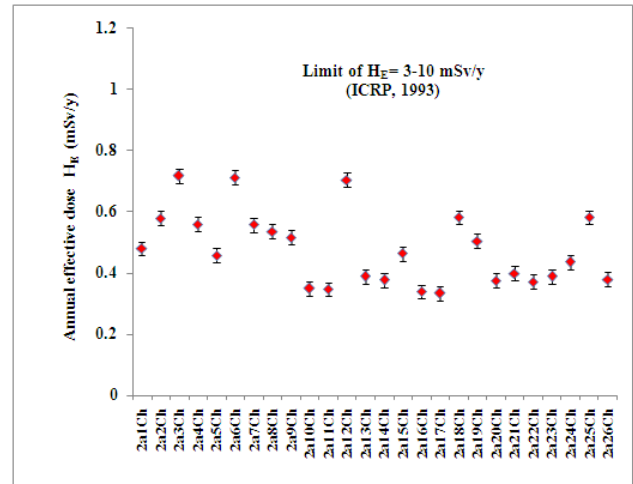


Fig. 12. Annual effective dose at 1.75m for different locations at department of chemistry.

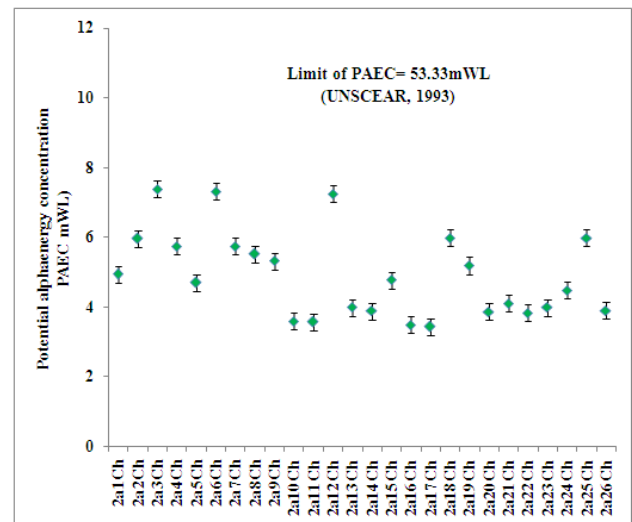


Fig. 13. Potential alpha energy concentration at 1.75m for different locations at department of chemistry.

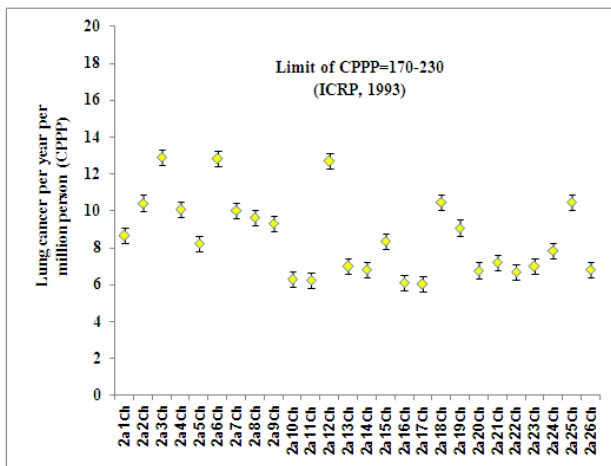


Fig. 14. Lung cancer cases per year per million person at 1.75m for different locations at department of chemistry.

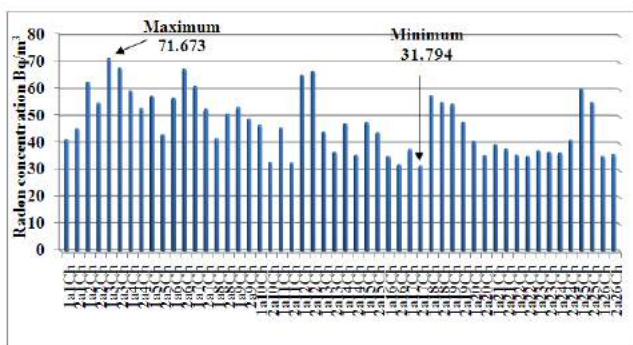


Fig. 15. Radon concentrations at 1m and 1.75m for different locations at department of chemistry.

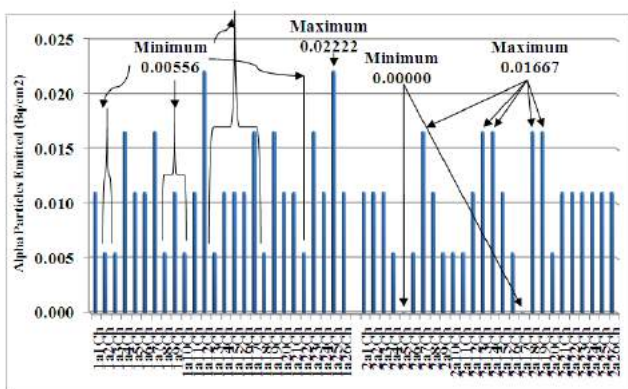


Fig. 16. Alpha particles emitted from surfaces in the department of chemistry at 1m and 1.75m.

#### IV. CONCLUSION

The maximum recorded value of higher radon concentrations in the laboratory building of Chemistry Department is  $71.673 \pm 8.466 \text{ Bq/m}^3$  in (Ch12a2) the laboratory of organic chemistry, and the minimum value is  $31.794 \pm 5.639 \text{ Bq/m}^3$  in (Ch17a2) the at inorganic chemistry laboratory for higher students A4 for standing

position respectively. Both results are lower than the recommended worldwide ( $200\text{-}300 \text{ Bq/m}^3$ ) (ICRP, 2009), and ( $148 \text{ Bq/m}^3$ ) (EPA, 2003). The maximum indoor annual effective dose, potential alpha energy concentration, and lung cancer cases per million person per year are  $0.704 \text{ mSv/y}$ ,  $7.245 \text{ mWL}$ , and  $12.680$  in (Ch12a2) the laboratory of organic chemistry for sitting position respectively. While the minimum values are  $0.334 \text{ mSv/y}$ ,  $3.437 \text{ mWL}$ , and  $6.016$  in (Ch17a2) the inorganic chemistry laboratory for higher students A4 for standing position respectively. Which are less than the lower limit of the admissible limit range  $3\text{-}10 \text{ mSv/y}$  (ICRP, 1993),  $53.33 \text{ mWL}$  given by (UNSCEAR, 1993), and the admissible limit range  $170\text{-}230$  per million per person per year (ICRP, 1993). The maximum measured alpha particles emitted values from the wall surfaces are  $0.02222 \text{ Bq/cm}^2$  at (Ch12a1, and Ch25a1) laboratory of organic chemistry and laboratory of biochemistry preparation room respectively for sitting position. The results are lower than the recommended worldwide limit ( $0.04 \text{ Bq/cm}^2$ ). All results are within the worldwide acceptable limits and there are no health risks.

#### V. REFERENCES

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# Demodulation of a Single Interferogram based on Bidimensional Empirical Mode Decomposition and Hilbert Transform

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## ABSTRACT

The phase calculation is a powerful measurement technique that allows the reconstruction of 3D profiles from interferogram intensity. In this article a new phase extraction algorithm is presented. This algorithm uses the BEMD technique (Bidimensional Empirical Mode Decomposition) for the evaluation of the phase distribution from a single uncarrier interferogram. The proposed method requires the numerical addition of a high spatial frequency carrier and application of the wavelet transform of the Fourier transform. An evaluation was made through a numerical simulation on simulated and real fringes to validate and confirm the performance of the proposed algorithm. The main advantage of this technique is its ability to provide a metrological solution for the fast dynamic analysis.

**Keywords:** Interferometry, Fourier Transform, Bidimensional Empirical Mode Decomposition, dynamic analysis, Carrier Superposition, Phase Retrieval, wavelet transform.

## I. INTRODUCTION

Optical interferometry techniques are widely used as tools for measuring micro-displacement and microstrain in several areas both scientific and industrial. [1]

These non-destructive methods are based on the measurement of small variation of the optical path using interference phenomena. Optical interferometry techniques are many and varied: classical interferometry, speckle interferometry, holography, fringe projection technique, shearography, etc. [2]

The physical quantity to be measured is modulated by a phase which is characterized by a deformed fringe system in an intensity image. The phase extraction algorithms are designed to calculate the phase distribution coding this image [3].

This research is based on the phase extraction from a single uncarrier interferogram using BEMD technique (Bidimensional Empirical Mode Decomposition). We

present here a phase calculation algorithm based on this method for calculating modal intrinsic functions bidimensional (BIMFs) followed by a digital phase shift by Hilbert Transform for each BIMF. Then, a spatial digital carrier has been superimposed followed by an application of the continuous wavelet transform or Fourier transform giving access to the phase distribution across a local analysis of intensity images.

## II. THE EMPIRICAL MODE DECOMPOSITION

The EMD decomposition base is intrinsic to the signal. The extraction of the oscillating components called empirical modes (IMF Intrinsic Mode Functions) is non-linear, but their recombination is linear. As EMD has no analytical formulation, it is defined by an algorithm and a process called sifting [4-6] for decomposing the signal to the Empirical Modes.

To calculate the IMFs of a signal  $x(t)$  we follow the following algorithm:

1. Extract local maxima and minima of the signal  $x(t)$

2. Interpolating the maxima sets and the minima sets (cubic splines, for example), to get the upper envelopes (lower envelopes) :  $e_{\min}(t)$ ,  $e_{\max}(t)$

3. calculate the mean of envelopes:

$$m(t) = \frac{e_{\min}(t) + e_{\max}(t)}{2}$$

4. subtract the mean envelope of the input signal

$$h(t) = x(t) - m(t)$$

5. If  $h(t)$  is an IMF, the residue is  $r(t) = x(t) - h(t)$  and the new signal will be  $x(t) = h(t)$ ,

6-If  $h(t)$  is not a IMF, the new signal will be

$$x(t) = r(t),$$

For that  $h(t)$  is a IMF, we must have a extrema between several zero.  $\sum \text{extrema} + \sum \text{zero} \geq 1$  and the mean of the upper and lower envelopes is zero

The signal  $x(t)$  can be written as:

$$x(t) = \sum_1^n IMF_i(t) + r(t) \quad (1)$$

The scope of the EMD technique to two-dimensional signals (BEMD) follows the same steps for the extraction of the BIMFs and the residue.

### III. HILBERT TRANSFORM

In mathematical and signal theory, the Hilbert transform, denoted  $H$ , of a real variable function  $f(t)$  is obtained by

convolution of  $f(t)$  with  $h(t) = \frac{1}{\pi t}$  [7].

$$H[x(t)] = x(t) * \frac{1}{\pi t} = \int_{-\infty}^{+\infty} x(\tau)h(t - \tau)d\tau = \frac{1}{\pi} \int_{-\infty}^{+\infty} \frac{x(\tau)}{t - \tau} d\tau \quad (2)$$

Moreover, the Hilbert transform may be interpreted as the output of a invariant linear system as input  $x(t)$ , which is a impulse response system  $\frac{1}{\pi t}$ . This is a mathematical tool widely used in signal theory to describe the complex envelope of the real magnitude modulated by a signal.

Its Fourier transform is expressed as follows:

$$TF\{H[x(t)]\} = H(f) \times X(f) \quad (3)$$

With

$$H(f) = TF\{h(t)\} = -j\text{sgn}(f) \quad (4)$$

Where  $\text{sgn}(f)$  is the "sign" function such as:

$$\text{sgn}(f) = \begin{cases} 1, & \text{si } f > 0 \\ 0, & \text{si } f = 0 \\ -1, & \text{si } f < 0 \end{cases} \quad (5)$$

Hilbert transform has the effect of turning of  $90^\circ$  the negative frequency component of  $x(t)$  and  $-90^\circ$  the positive frequency component. The Hilbert transform does not change the amplitude  $F(k)$ , it only changes the phase.

Hence the Hilbert transform is equivalent to a filter altering the phases of the frequency components by  $90^\circ$  positively or negatively according to the sign of frequency.

However, the direct application of this transform to interferogram to carry out the phase shift sometimes introduces a sign ambiguity problem can be corrected by a guided method of fringe orientation interferogram [8].

Applying the Hilbert transform to equation (1):

$$H[x(t)] = H\left[\sum_{i=1}^n IMF_i(t) + r(t)\right] = \sum_{i=1}^n H(IMF_i(t) + H(r(t))) \quad (6)$$

Therefore, the Hilbert of a signal  $x(t)$  is the sum the Hilbert of all IMF components and the residual  $r(t)$ .

Otherwise, with EMD decomposition (BEMD), we can shift a signal (image) with  $\frac{\pi}{2}$ .

### IV. THE FOURIER TRANSFORM

The intensity at a point of the interferogram at coordinates  $(x, y)$  is expressed as follows:

$$I(x, y) = I_0(x, y)[1 + V(x, y)\cos(my + \phi(x, y))] \quad (7)$$

With,  $I_0$ ,  $V$  and  $\phi$  are respectively the average intensity, the visibility factor and the desired phase.  $m$  is the

modulation rate of the high frequency spatial carrier respecting the following condition:

$$m \succ \left| \frac{\partial \phi}{\partial y} \right|_{\max} \quad (8)$$

The above expression can be written differently in the following form:

$$I(x, y) = I_0(x, y) + c(x, y)e^{imy} + c^*(x, y)e^{-imy} \quad (9)$$

Where

$$c(x, y) = \frac{I_0(x, y)V(x, y)}{2} e^{i\phi(x, y)} \quad (10)$$

The one-dimensional Fourier transform is performed on Equation (7) along the line x. The spectrum of the thus obtained interferogram is expressed as:

$$\hat{I}(x, k) = \hat{I}_0(x, k) + \hat{C}(x, k - m) + \hat{C}^*(x, k + m) \quad (11)$$

Where  $\hat{I}$ ,  $\hat{I}_0$ ,  $\hat{C}$  and  $\hat{C}^*$  are respectively the Fourier Transform of  $I$ ,  $I_0$ ,  $c$  and  $c^*$

After filtered two components  $\hat{I}_0$  and  $\hat{C}^*$ , and shifted the component  $\hat{C}$  to the origin. The inverse Fourier transform is applied to  $\hat{C}$  for obtain  $c(x, y)$ .

The real and imaginary parts of  $c(x, y)$  are given by

$$\text{Re}[c(x, y)] = b(x, y)\cos(\phi(x, y)) \quad (12)$$

$$\text{Im}[c(x, y)] = b(x, y)\sin(\phi(x, y)) \quad (13)$$

The desired phase distribution will be evaluated using the arctangent of the quotient Equation (13) on Equation (12):

$$\phi(x, y) = \text{arctg} \left( \frac{\text{Im}[c(x, y)]}{\text{Re}[c(x, y)]} \right) \quad (14)$$

## V. THE CONTINUOUS WAVELET TRANSFORM (CWT)

Unlike the overall processing the Fourier analysis, the wavelet transform allows a local study of the optical information modulating the interference signal  $I(x, y)$ .

By applying the wavelet transform to signal  $I(x, y)$ , we will have:

$$W(x, a, b) = \int_{-\infty}^{+\infty} I(x, y)\psi_{a,b}^*(y)dy \quad (15)$$

Where  $\psi_{a,b}$  are the wavelets: very special elementary functions constructed from the wavelet mother  $\psi(y)$  by translation and dilation [4-6] such as:

$$\psi_{a,b}(y) = \frac{1}{\sqrt{a}}\psi\left(\frac{y-b}{a}\right) \quad (16)$$

Where  $a \neq 0$  : The dilation parameter (scale) and  $b$ : the translation parameter.

Therefore

$$W(x, a, b) = \frac{1}{\sqrt{a}} \int_{-\infty}^{+\infty} I_0(x, y)[1 + V(x, y)\cos(my + \phi)] \quad (17)$$

Exploiting the localization of the wavelet and assuming a slow variation of  $I_0$  and  $V$ , the Parseval identity leads to

$$W(x, a, b) = \frac{I_0(x, b)V(x, b)\sqrt{a}}{2} \times \left[ \left( \hat{\psi} \left( a \left( m + \frac{\partial \phi}{\partial y} \right) \right) \right)^* e^{i\phi(x, b)} + \left( \hat{\psi} \left( -a \left( m + \frac{\partial \phi}{\partial y} \right) \right) \right)^* e^{-i\phi(x, b)} \right] \quad (18)$$

Choosing a mother wavelet that its Fourier transform is null in the negative frequencies (in our case complex Morlet), the second term of (18) can be neglected. So the wavelet transform becomes



$$W(x, a, b) = \frac{I_0(x, b)V(x, b)\sqrt{a}}{2} \times \left( \hat{\psi} \left( a \left( m + \frac{\partial \phi}{\partial y} \right) \right) \right)^* e^{i\phi(x, b)} \quad (19)$$

The modulus and the phase arrays can be calculated by the following equations:

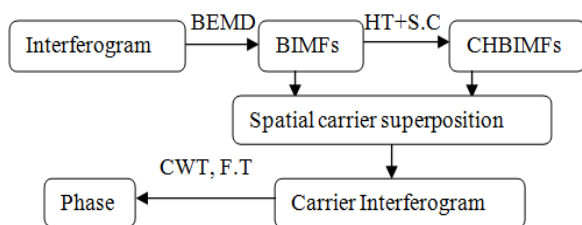
$$abs(a, b) = |W(a, b)| \quad (20)$$

$$\phi(a, b) = \arctan \left( \frac{\text{Im}(W(a, b))}{\text{Re}(W(a, b))} \right) \quad (21)$$

To compute the phase of the row, the maximum value of each column of the modulus array is determined and then its corresponding phase value is found from the phase array [9]. By repeating this process to all rows of the fringe pattern, a wrapped phase map is resulted and needs after to be unwrapped [10].

## VI. INTERFEROGRAM ANALYSIS

Our method of analysis of an interferogram can be outlined by the following chart (Figure 1)



**Figure 1:** The flow chart of interferogram analysis method

After applying the BEMD on our interferogram, we get the BIMFs, on which we apply the Hilbert Transform to digitally shift with  $\frac{\pi}{2}$ . These shifted BIMFs will be later corrected to remove their sign ambiguity and the new images are called CHBIMFs.

By adding all BIMFs and CHBIMFs, we find the two BIMF and CHBIMF images respectively.

Using a suitable modulation rate  $m$ , we combine digitally  $SIMF(x, y)$  and  $CHBIMF(x, y)$  with the matrix

$\cos(my)$  and  $\sin(my)$  to derive the carrier interferogram IP as following :

$$IP(x, y) = \cos(my) * SIMF(x, y) - \sin(my) * SCHIMF(x, y) \quad (22)$$

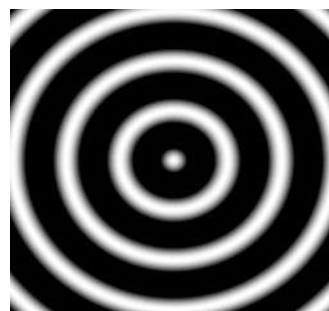
By using the Fourier Transform or wavelet Transform, we can obtain simply the phase distribution.

## VII. SIMULATIONS AND RESULTS

In this work, we apply our algorithm on an interferogram with size  $256 \times 256$  pixels whose distribution is given by:

$$I(x, y) = 1 + \cos(\phi(x, y)) \quad (23)$$

and illustrated in figure 2

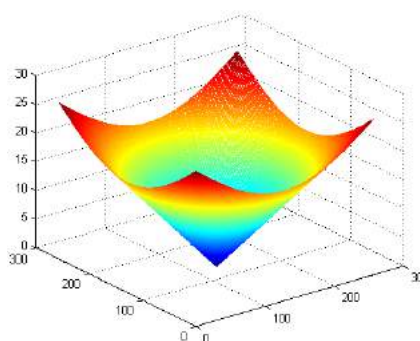


**Figure 2:** The simulated interferogram

with  $\phi(x, y)$  the phase variation that have expression:

$$\phi(x, y) = 0.15 * \sqrt{(x-128)^2 + (y-128)^2} \quad (24)$$

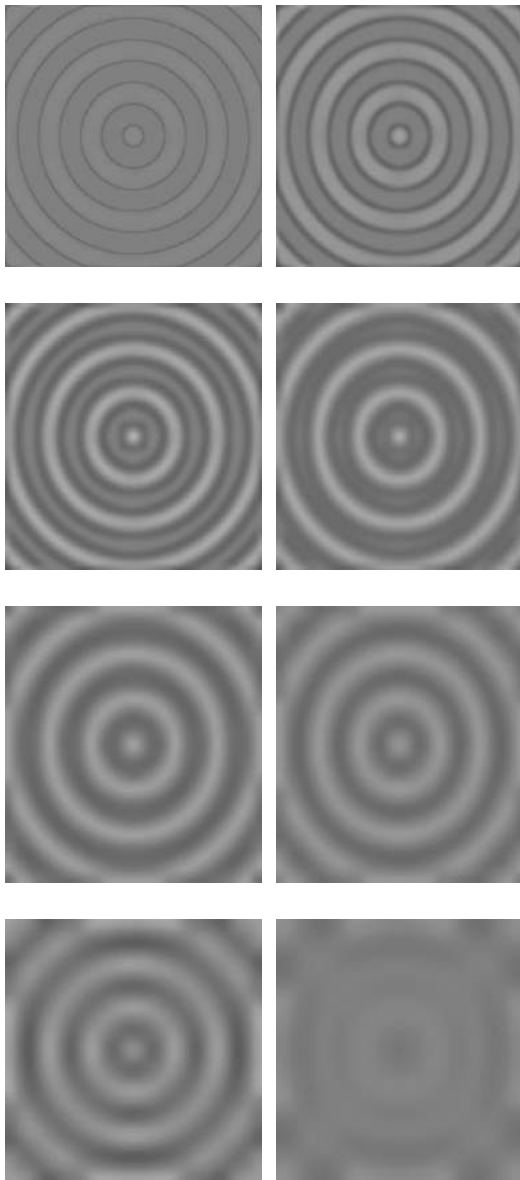
shown in Figure 3



**Figure 3:** The theoretical phase distribution

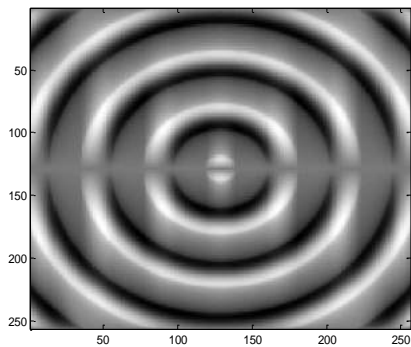
With  $x$  and  $y$  are the pixel coordinates in the image

The Figure 4 shows the BEMD decomposition of the interferogram obtained by numerical simulation.



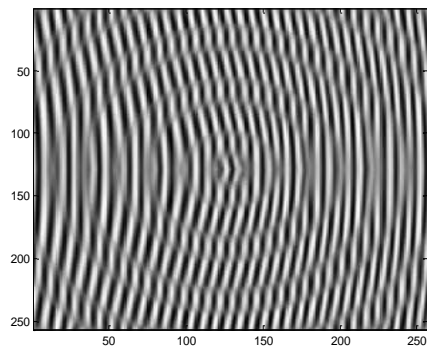
**Figure 4:** BEMD decomposition of interferogram (BIMFs)

After that, the Hilbert Transform is applied to each BIMF followed by a sign correction. Figure 5 shows the sum of all corrected BIMFs. It's a  $\pi/2$  phase shifted interferogram.



**Figure 5:**  $\frac{\pi}{2}$  phase shifted interferogram.

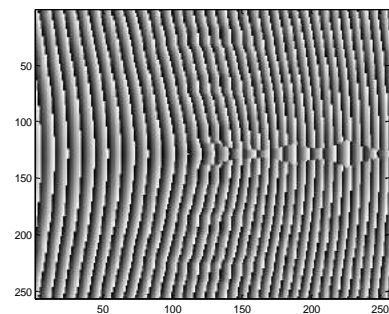
After combining the two phase shifted fringe patterns numerically with  $\sin(my)$  and  $\cos(my)$ , we have obtained the modulated fringe pattern shown in Figure 6 with modulation rate value 0.8 radian/pixel.



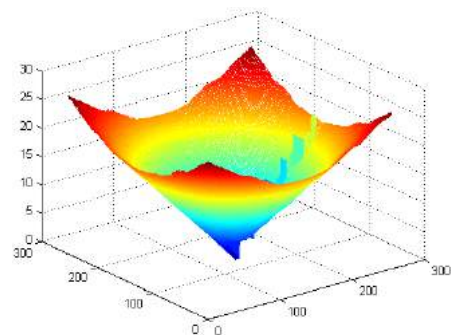
**Figure 6:** The carrier interferogram

The wavelet method was applied to the new carrier fringe pattern to retrieve the phase map of Figure 7.

Figure 8 illustrates the retrieved continuous phase map after the unwrapping process.



**Figure 7:** The wrapped phase map



**Figure 8:** The retrieved phase map

The algorithm generated by our method was used to calculate the desired phase image with an RMS error (Root Mean Square) equal to 2.26 rad, and this by using only one uncarrier interferogram.

## VIII. APPLICATION ON REAL FRINGES

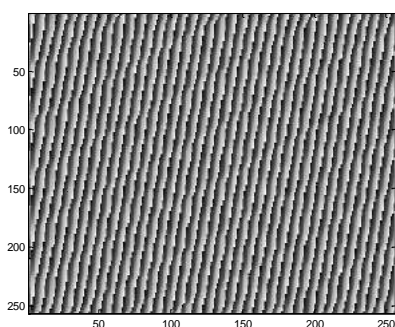
To illustrate the use of our method for real applications, we tested its performance on a rough ground finish, aluminium surface from a hard disk drive assembly of the company 4D Technology.

Figure 9 shows the real fringes of Hard Drive at aluminum.



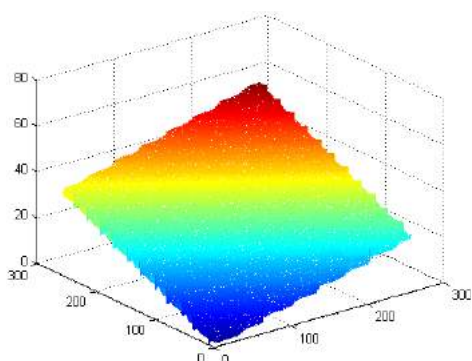
**Figure 9:** Real fringes of the hard drive enclosure

By applying the same steps as before, we find the wrapped phase distribution by using the Fourier Transform (FT) (Figure 10):



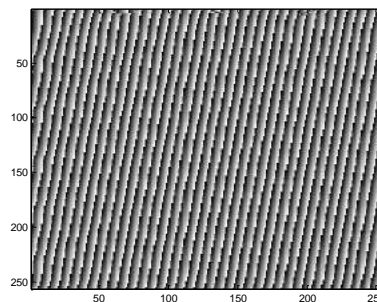
**Figure 10:** The wrapped phase by F.T

Figure 11 shows the distribution of the continuous phase obtained after unwrapping process.



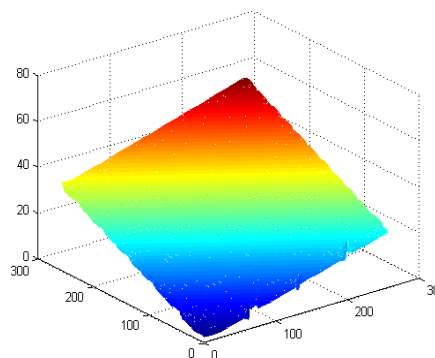
**Figure 11:** The unwrapped phase distribution by F.T

And if using the continuous wavelet transform (CWT), we find the wrapped phase distribution shown in figure 12.



**Figure 12:** The wrapped phase by CWT.

The figure 13 shows the distribution of continuous phase after unwrapping method.



**Figure 13:** The unwrapped phase distribution by CWT with both methods we obtained the same phase distribution

## IX. CONCLUSION

In this paper, we have presented a new algorithm of phase retrieval from a single uncarrier interferogram with closed fringes. This was achieved by numerical superposition of the spatial carrier by Empirical Mode Decomposition and Hilbert Transform, followed by fringes demodulation using Continuous Wavelet Transform or Fourier Transform.

The numerical simulation validated the reliability of the method on simulated and real fringes by estimating the phase distribution with good accuracy even with the closed fringes.

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# Protective Effect of Umbelliferone against Doxorubicin Induced Cardiotoxicity in Wistar Albino Rats

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## ABSTRACT

Doxorubicin (DOX) is widely used as chemotherapeutic agent. Usefulness of this agent is limited due to its cardiotoxic effects. The aim of this study was to investigate the curative effect of Umbelliferone (UMB) against Doxorubicin (DOX) induced cardiotoxicity in albino rats. Biomarkers like LDH, AST, ALT, ALP, CK, along with heart weight index, antioxidant enzymes, lipid profiles and histological examination were assessed to determine the cardiotoxicity effect. DOX alone (25mg/kg bw intraperitoneally three times per week for two weeks) administered group exhibited significant increase in LDH, AST, ALT, ALP, CK, FC, FFA and TG levels and decrease in Enzymatic and Non enzymatic antioxidants activities when compared to control group. Oral administration of Umbelliferone (UMB) (30mg/kg bw) treatment daily for two weeks along with DOX significantly attenuated the increased serum biomarkers, reduced hyperlipidemia and restored the tissue antioxidant activities when compared to DOX alone treated group. Histology examination also clearly showed that UMB significantly inhibited the cardiac damage induced by DOX. Based on the results, this study clearly indicates that UMB used as a potent compound against cardiotoxicity induced by DOX.

**Keywords:** Doxorubicin, Umbelliferone, Cardiotoxicity

## I. INTRODUCTION

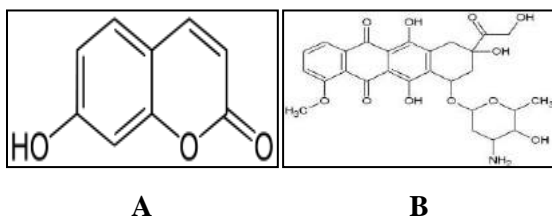
Globally Doxorubicin (DOX) used as an anticancer drug because of its antineoplastic antibiotic effect, this has used to treat the several cancers including leukemia and solid tumours (1). Long term usage of this drug causes severe side effects, which includes predominantly cardio toxicity later that may lead to irreversible cardiomyopathy and heart failure (2). Administration of anticancer agents adversely affect the antioxidant haemostasis in the cellular levels thereby generation of abnormal free radicals which lead to oxidative stress that causes tissue damage (3).

Myocardial infarction or heart attack is the major disease that causes predominantly death from all over the world. When blood supply is insufficient to myocardium, death of cardiomyocytes occurs, a condition known as ischemia. Prolonged ischemia leads to necrosis, which is

called as myocardial infarction. Currently many medicines are used to treat myocardial infarction that has so many side effects. Dietary occurring natural compounds play a key role in treating various diseases, including cardiac diseases. Herbal formulations are safer than modern drugs which lead to herbal preparations. World Health Organization also recommended the uses of herbal medicines as an alternative medicine in the developing countries (4).

Coumarin is a benzopyrene structured compound derived from the plant sources. Coumarin is extensively distributed in the entire parts of the plant, especially high amount in flowers, stem, and roots (5). Coumarins are vast group of natural compounds, among umbelliferone were predominantly found in the edible fruits of Golden apple *Aegle marmelos* (Rutaceae) (6). It is a yellowish-white crystalline solid, slightly soluble in hot water and high soluble in ethanol (7).

UMB contains wide spectrum of pharmaceutical effects including anti-lipidemic (8), anti-diabetic and anti-hyperglycemic (9), anti-inflammatory (10), radio protective (11), and antioxidant activity (12). In addition, recent studies revealed that UMB have significant hepatoprotective effect (13). But still, there is scarce information about the cardio protective effect of UMB. Hence this study was designed in such a way to elucidate the role of UMB on experimentally induced cardio toxicity.



**Figure 1.** Structure of (A) Umbelliferone (C<sub>9</sub>H<sub>6</sub>O<sub>3</sub>) and (B) Doxorubicin (C<sub>27</sub>H<sub>29</sub>N<sub>0</sub>O<sub>11</sub>)

## II. METHODS AND MATERIAL

### Source of chemicals and drugs

Doxorubicin was purchased from Hi Media laboratories, India. Umbelliferone (7-Hydroxy coumarin) was purchased from Sigma-Aldrich, Bangalore. All other chemicals used which were of analytical grade obtained from SRL/TCI/HIMEDIA laboratories, India.

### Experimental animals

Male Albino Wistar rats weighing about 150–160 g were procured from TANUVAS, Chennai, India and all the experiments were designed and conducted according to the Institutional Animal Ethics Committee approved guidelines. The animals were maintained in clean, sterile cages, well ventilated room with controlled temperature (25±2°C) and were acclimatized to 12-h light and dark cycles. Animals were fed with commercially available standard rat pelleted feed (M/S Hindustan Foods Ltd, Bangalore, India) throughout the experimental period. The animals were free access to food and water.

### Experimental design

The rats were divided into four groups, each group consisting of six animals.

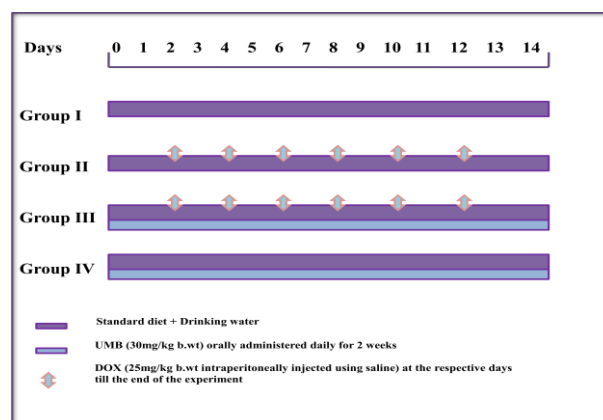
**Group I** Control rats were given 10% DMSO which served as a vehicle control through the experimental period along with standard diet and drinking water.

**Group II** Rats were injected DOX (25mg/kg bw, intraperitoneally using saline) three times per week for two weeks (14)

**Group III** Rats were administered with Umbelliferone orally (30mg/kg bw dissolved in 10% DMSO) daily for two weeks along with the same time DOX were injected intraperitoneally three times per week for two weeks period.

**Group IV** Rats were administered with UMB orally (30mg/kg bw dissolved in 10% DMSO) daily for two weeks.

At the end of the experimental period, the animals were fasted overnight and anesthetized with Ketamine (90 mg/kg bw) and Xylazine (10 mg/kg bw) and sacrificed followed by cervical decapitation. Blood were collected and allowed to coagulate at room temperature for 30 min. Serum were separated by centrifugation at 3000 rpm for 15 min at 4°C. The heart were immediately excised and washed in ice-cold saline. The heart tissues were sliced and homogenized into 0.1 M Tris–HCL buffer (pH 7.4). The tissue homogenates were centrifuged at 1000 rpm for 10 min at 4°C and the supernatants were collected and assessed for various parameters.



**Figure 2.** Schematic representation of experimental design Biochemical parameters

### Estimation of marker enzymes

Activities of Lactate dehydrogenase (LDH) (15), Aspartate transaminases (AST) (16), Alanine tansaminases (ALT) (16), alkaline phosphatase (ALP) (17) and Creatine kinase (18) were assayed in the serum of experimental animals.

## Estimation of enzymic and non-enzymic antioxidants

The data were analysed with SPSS/10 Software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of <0.05 were considered to indicate the statistically significance. All the results were expressed as mean  $\pm$  standard error (SE) for six animals in each group.

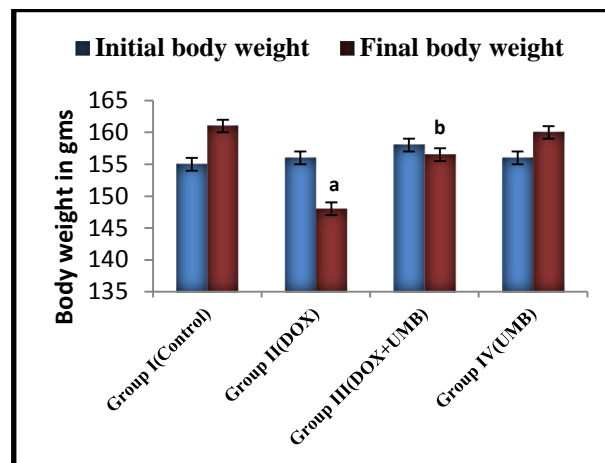
## III. RESULT AND DISCUSSION

### 1. RESULT

#### Effect of UMB on body weight, Heart weight and relative heart weight

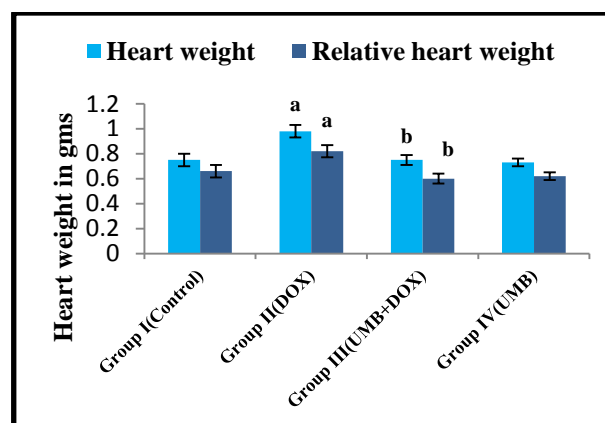
Fig 3 shows the initial and final body weight of the control and experimental group of animals. In DOX administered group II animals there is drastic reduction in the final body weight, whereas UMB+DOX administered group III animals showed significant increase in the final body weight when compared with DOX alone group II animals. In addition there is no significant difference found in the final body weight of UMB alone treated animals when compared to control animals.

Fig 4 shows the heart weight and relative heart weight of control and experimental animals. DOX administered animals showed increase in the heart weight and relative heart weight. UMB+DOX treated animals exhibited the significant decrease in the heart weight and relative heart weight when compared with the DOX induced animals. There are no significant changes between heart weight of the control animals and UMB alone treated animals.



(<sup>a</sup>Compared with control and <sup>b</sup>Compared with DOX)

Figure 3. Effect of UMB on body weight during experimental group of animals



(<sup>a</sup>Compared with control and <sup>b</sup>Compared with DOX)

Figure 4. Effect of UMB on Heart weight and relative heart weight during experimental group of animals. UMB decreased the levels of serum marker enzymes in DOX induced cardiotoxicity animals

Table 1 shows the effect of UMB on the levels of marker enzymes LDH, AST, ALT, ALP and CK in the serum of control and experimental group of rats. The marker levels were significantly increased in DOX induced animals when compared with control animals. Whereas UMB+DOX treated animals significantly decreased the levels of marker enzymes when compared with DOX induced animals. No significant changes observed between control and UMB alone treated animals.

### UMB treatment increased the levels of HDL cholesterol

Table 2 shows the serum lipoprotein fractions such as LDL, HDL and VLDL of control and experimental groups of animals. DOX administered animals showed significant reduction in the HDL levels and the same increased in the LDL/VLDL when compared with control animals. Whereas UMB+DOX treated animals significantly restored the HDL levels and decreased in the LDL/VLDL levels when compared to DOX induced animals.

### UMB alleviate the antioxidant activities during DOX induced cardiotoxicity

Table 3 and 4 indicates the enzymatic and non-enzymatic antioxidant activities in the heart of the

control and experimental groups. DOX induced animals showed significant decrease in the activities of enzymatic antioxidants such as SOD, CAT, GPx, GR and GST when compared with control animals. Whereas UMB+DOX supplemented animals showed significant increase in the activities of these enzymes when compared with DOX induced animals.

Non enzymatic antioxidants such as GSH, G6PD, VIT C, VIT E and VIT A also found significantly decreased activities during DOX induced animals when compared with control animals. In UMB+DOX treated animals, there is significant increase in the activities of GSH, G6PD, VIT C, VIT E and VIT A when compared with DOX induced animals. No significant change was observed in UMB alone treated animals when compared with control animal.

**Table 1:** Effect of UMB and on the activities of marker enzymes in serum of control and experimental group of rats

Groups	LDH	AST	ALT	ALP	CK
Control	87.33 ± 6.32	108.6 ± 9.16	74.83± 5.26	19.42 ± 1.03	10.46 ± 0.97
DOX	178.32 ± 14.67 <sup>a</sup>	158.5 ± 13.05 <sup>a</sup>	286.35 ± 21.84 <sup>a</sup>	73.53 ± 5.11 <sup>a</sup>	27.64 ± 1.98 <sup>a</sup>
UMB+DOX	111.23 ± 8.45 <sup>b</sup>	124.1 ± 11.04 <sup>b</sup>	128.43 ± 7.62 <sup>b</sup>	48.13 ± 3.06 <sup>b</sup>	13.23± 0.73 <sup>b</sup>
UMB	89.11 ± 7.05	104.9 ± 8.99	72.32 ± 5.04	18.46 ± 1.69	10.14 ± 0.57

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at p<0.05 compared with <sup>a</sup>Compared with control and <sup>b</sup>Compared with DOX. Units CK: μmol of phosphorus/h/mg protein; LDH, AST and ALT: μmol of pyruvate/h/mg protein; ALP: KA U/l.

**Table 2:** Effect of UMB on the activities serum lipoprotein fraction of control and experimental group of rats

Groups	LDL	HDL	VLDL
Control	16.12 ± 1.05	40.77± 3.29	26.14 ± 2.11
DOX	32.16 ± 2.02 <sup>a</sup>	26.16 ± 1.80 <sup>a</sup>	42.66 ± 4.09 <sup>a</sup>
UMB+DOX	20.65 ± 1.01 <sup>b</sup>	38.14 ± 2.68 <sup>b</sup>	35.56 ± 2.68 <sup>b</sup>
UMB	15.67 ± 1.22	40.29 ± 3.11	25.62 ± 1.95

Results are expressed as mean SD for six rats each group. Statistically significance at p<0.05; Results are compared with <sup>a</sup>Compared with group 1 and <sup>b</sup>Compared with group 2. Units— mg/dl



**Table 3.**Effect of Umbelliferone on the activities of antioxidant enzymes in the heart of control and experimental groups of rats

Groups	SOD	CAT	GPx	GR	GST
Control	3.86 ± 0.17	69.06 ± 5.39	19.23 ± 1.41	2.99 ± 0.10	1.86 ± 0.12
DOX	1.68 ± 0.07 <sup>a</sup>	37.45 ± 2.92 <sup>a</sup>	11.96 ± 0.87 <sup>a</sup>	0.74 ± 0.05 <sup>a</sup>	0.59 ± 0.04 <sup>a</sup>
UMB+DOX	2.32 ± 0.09 <sup>b</sup>	54.84 ± 4.23 <sup>b</sup>	16.28 ± 1.43 <sup>b</sup>	1.46 ± 0.11 <sup>b</sup>	1.43 ± 0.09 <sup>b</sup>
UMB	3.67 ± 0.16	68.38 ± 4.96	18.56 ± 1.47	2.69 ± 0.10	2.11 ± 0.14

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at p<0.05 compared with

<sup>a</sup>Compared with control and <sup>b</sup>Compared with DOX. Units: SOD in units/mg protein, CAT in μmol of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein, GPx in μmol of GSH utilized/min/mg protein, GR in μmol of NADPH oxidized/min/mg protein and GST in μmol of CDNB-GSH conjugate formed/min/mg protein. G6PD in μmol of NADPH oxidized/min/mg protein.

**Table 4.**Effect of Umbelliferone on the activities of non-enzymatic enzymes in the heart of control and experimental group of rats

Groups	GSH	G6PD	VITAMIN C	VITAMIN E	VITAMIN A
Control	5.92 ± 0.45	2.36 ± 0.13	1.84 ± 0.13	2.83 ± 0.25	3.16 ± 0.10
DOX	1.89 ± 0.07 <sup>a</sup>	0.58 ± 0.04 <sup>a</sup>	0.66 ± 0.05 <sup>a</sup>	1.43 ± 0.08 <sup>a</sup>	0.88 ± 0.02 <sup>a</sup>
UMB+DOX	3.75 ± 0.29 <sup>b</sup>	1.83 ± 0.06 <sup>b</sup>	1.38 ± 0.07 <sup>b</sup>	2.32 ± 0.16 <sup>b</sup>	2.28 ± 0.18 <sup>b</sup>
UMB	5.66 ± 0.39	2.27 ± 0.12	1.71 ± 0.10	3.01 ± 0.20	3.54 ± 0.09

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at p<0.05 compared with <sup>a</sup>

Compared with control and <sup>b</sup>Compared with DOX. Units: VitC in mg/g of wet tissue, VitE in mg/g of wet tissue, VitA in mg/g of wet tissue and GSH in μg/mg protein.

**Table 5.**Effect of UMB on cardiac lipids of control and experimental groups of rats

Groups	Free cholesterol	Esterified cholesterol	Phospholipids	Free fatty acids	Triglycerides
CONTROL	3.47 ± 0.29	2.34 ± 0.12	14.88 ± 1.21	3.19 ± 0.21	3.23 ± 0.18
DOX	6.34 ± 0.52 <sup>a</sup>	0.54 ± 0.04 <sup>a</sup>	09.37 ± 0.45 <sup>a</sup>	7.11 ± 0.59 <sup>a</sup>	6.89 ± 0.44 <sup>a</sup>
DOX+UMB	4.08 ± 0.29 <sup>b</sup>	1.78 ± 0.11 <sup>b</sup>	12.87 ± 0.68 <sup>b</sup>	4.31 ± 0.27 <sup>b</sup>	4.96 ± 0.52 <sup>b</sup>
UMB	3.39 ± 0.24	2.16 ± 0.16	14.66 ± 0.89	3.06 ± 0.29	3.17 ± 0.24

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at p<0.05 compared with

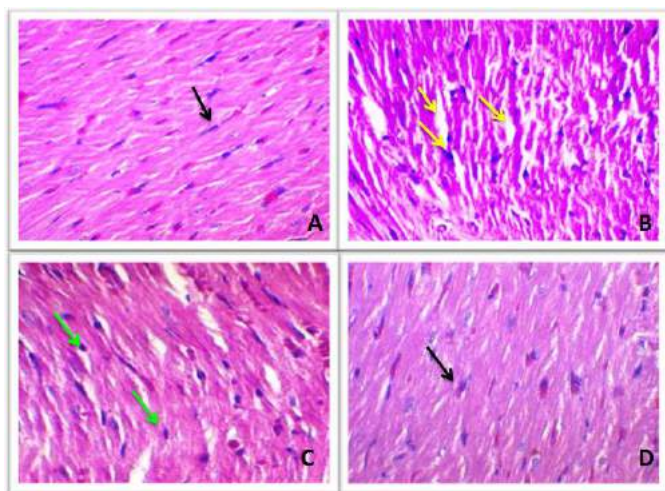
<sup>a</sup>Compared with control and <sup>b</sup>Compared with DOX Units: mg/dL

## Effect of UMB on cardiac lipid status

Table 5 shows the levels of free cholesterol, esterified cholesterol, phospholipids, free fatty acids and Triglycerides. DOX induced animals showed significant increase in the content of free cholesterol, free fatty acids, triglycerides and decreased levels of phospholipids, esterified cholesterol were observed. Whereas UMB+DOX administered animals found significant reduction in the levels of free cholesterol, free fatty acids, triglycerides and at the same time, increased in the levels of esterified cholesterol, phospholipids were observed when compared to DOX induced animals.

## Histology examination

Haematoxylin and Eosin stained (H&E) sections of heart were assessed under a light microscope (40x). Control animals (Fig 5A) and UMB alone administered animals (Fig 5D) showed normal architecture of myocardial fibres and myocytes. (Fig 5B) Doxorubicin administered animals showed myocyte swelling, severe damage in myocardial fibres and hyalinization in the heart. Umbelliferone + Doxorubicin treated animals (Fig 5C) almost showed normal myocytes and less damage in myocardial fibres.



**Figure 5.** Photomicrographs (40x) obtained from the heart sections A. Control group, B. DOX alone group, C. UMB + DOX group, D. UMB alone group. (—▶) Shows normal myocytes and cardiomyofibres (—▶) Shows swollen myocytes and damaged myocardial fibres (—▶) Shows normal myocytes and less myocardial fibres damage

## 2. DISCUSSION

Doxorubicin is an anthracycline, which is used for the treatment of various malignant and non-malignant tumors. The use of doxorubicin as chemotherapeutic drug has been limited due to its diverse toxicities, including cardiac, renal, haematological and testicular toxicity (34). It has been reported doxorubicin induced cardio toxicity mediated by lipid peroxidation and inhibition of fatty acids and oxidation in cardiac tissues (35). Several studies reported, anticancer therapies adversely affect the physiological homeostasis of different organ functions during cancer treatment. Doxorubicin induced myocardial damage have been well established in animal models.

The present study delineates the protective role of UMB in DOX induced cardio toxicity. Experimentally DOX challenged animal models severely causes oxidative stress mediated free radical generation that causes cardio toxicity. The metabolic action of DOX is initially converted into semiquinone form in the cardiac tissue, which is a toxic, short lived metabolite that interacts with molecular oxygen and initiates a cascade of reaction leading to ROS generation (36).

In this present study, administration of DOX drastically reduced in the final body weight and significantly increased the heart weight. Similarly DOX administration adversely affects the tissue by increase in the serum markers LDH, AST, ALT, ALP, and CK-MB. Umbelliferone challenged along with DOX animals significantly increase the body weight and attenuated the tissue damage thereby preventing the membrane enzymes leakage into the serum, this shows the protective effect of UMB in myocytes against cardiotoxicity (37).

Clinical investigations also suggest that increased oxidative stress associated with DOX causes cardiomyopathy that leads to heart failure (11). DOX and its mebolites reduced the oxidant level that leads to accumulate abnormal free radical generation in the heart tissue (38). In this study, the oxidative stress associated tissue injury caused by DOX results significant decreased in the activities of SOD, CAT, GPx, GR, GST and UMB along with DOX treatment restored the

enzymic antioxidant levels which clearly shows the beneficial effect against DOX induced cardiotoxicity (39). Non-enzymic antioxidants also play an excellent role in protecting the cells from oxidative damage. GSH an ubiquitous tripeptide which act as a free radical scavenger (39). Decreased level of GSH impairs the ability of cells thereby enhance in the levels of LPO. Lipid soluble antioxidant like Vitamin E which is present in the cellular membranes plays an important role in the suppression of free radicals (40). Vitamin C is a free radical scavenger, act as an antioxidant in recycling of Vitamin E. Cellular levels of vitamin C and vitamin E were maintained in the active form by GSH. In this study, DOX group shows reduced activities of Vitamin C, A, E and has pronounced GSH depletion were observed. UMB along with DOX treated animals showed significant increase in the levels of non enzymic antioxidants activities which may be due to ability of UMB quenched the free hydroxyl and superoxide radicals.

Hypercholesterolemia, hypertriglyceridemia is a well-known risk factor in cardiovascular disease. Heart attack risk can be reduced by lowering the cholesterol levels and the alteration in the lipoprotein transport and metabolism affects the physiological context of changes in plasma lipids (42). In this study DOX group significantly revealed increase in the levels of cholesterol and triglycerides. Interestingly UMB supplementation along with DOX completely attenuated the increased cholesterol and triglycerides levels. Biochemical data were further confirmed the histopathological studies from the cardiac tissues. UMB along with DOX treated animals showed the minimal damage by maintaining the cardiomyocytes and myofibres neatly to the normal architecture than the DOX animals (43). From the above, all the data revealed that the UMB has the potential to cure the cardiotoxic effect produced by the DOX.

#### IV. CONCLUSION

The present findings clearly shows the protective effect of UMB on DOX induced cardiotoxicity through maintaining systemic antioxidant activities, cellular membrane integrity and reducing hyperlipidemia conditions. Further studies are in the underway to investigate the intricate mechanism.

#### V. ACKNOWLEDGEMENT

First author Sathesh Kanna Velli, Project fellow gratefully acknowledges the UGC for the financial assistance provided in the form of UGC-UPE fellowship.

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# The Characteristics of Physico-Chemical and Sensory Properties of Bran and Pumpkin Flours Substitution of Simulation Chips

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## ABSTRACT

Snacks are food consumed between the times of the main meal. Indonesian people have a habit of eating a snack, and it is a business opportunity for the snack industry. Most snack on the market contain a lot of monosodium glutamate (MSG), to reduce the use of MSG, it can use local foodstuffs such as bran and fruit pumpkin that has a complete nutritional content, source of dietary fiber, and antioxidants. By processing the bran and pumpkin fruit into flour, it can be used as raw material in the processing of the simulation chips. The purpose of this study was to determine the effect of substitution proportion of TB and TLK to the physico-chemical properties and sensory quality of simulation chips. The design used was completely randomized design (CRD) with four replications for each treatment. Treatment substitution of TB consists of 7 levels: 0%, 5%, 10%, 15%, 20%, 25%, and 30% of the total flour, whereas substitution of TLK consists of 7 levels, namely 10%, 20 %, 30%, 40%, 50%, 60% of the total flour. The best results obtained on substitution treatment of F4 with TB were as much as 15% and 30% of TLK which has a water content of 3.71%, ash content of 5.09%, the protein content of 10.67%, fat content of 16.94%, carbohydrate content of 63.58%,  $\beta$ -carotene with 2.81  $\mu\text{g/g}$ , vitamin E of 0.59 mg / 100 g, TDF of 10.64% db, the antioxidant capacity of 668.52 ppm GAEAC, and IC 50 amounted to 145.19 mg / mL. Sensory acceptance is based on the overall quality attributes of a score of 4.13 (quite like to like).

**Keywords:** Flour, Bran, Pumpkin, and Chips Simulation

## I. INTRODUCTION

Indonesian people have a habit of eating a snack. Snacks are light meals consumed between main meals. Snack consumption is generally more or less 2-3 hours between main meal times, which are at 10 am and 4 pm. The habit of eating snacks is one of the business opportunities for the snack industry, as evidenced by the increasingly diverse types of snacks that have flooded the market, based on wheat flour, soy flour, chocolate, fruit, and nuts.

Snacks on the market is still dominated by dense foods contain a lot of calories and *monosodium glutamate* (MSG) (Pajajaran University, 2012). Eating snacks with high MSG content in large quantities and on an ongoing basis can cause health problems. To address this, now healthy snack began much talked about, because people began to realize the importance of the quality of food consumed to maintain health. The criteria of healthy

snacks are to contain vitamins, protein, and fiber food (Kompas, 2009). Consumption of healthy snacks can provide extra energy to move, and help meet the energy needs until the main meal.

Chips are generally made in an intact form in accordance with the raw materials, such as banana, cassava, and potato chips. Besides the chips processed products, there are also other types of product chips, known as the simulated chips. Simulated Chips are a snack that is processed by shaping the dough, made of thin sheets, sliced, dried and fried (Matz, 1984). Processing chips in dough forms possible using raw materials that have high nutritional value, containing dietary fiber, and antioxidants to produce a healthy snack. To get healthy snack products, it is preferably using raw materials derived from nature without using additional materials made from chemicals. One type of local food that can be used are rice bran and fruit pumpkin. Bran and pumpkin contains a complete

nutrient (carbohydrate, protein, fat, minerals and vitamins) as a source of dietary fiber and antioxidants. To facilitate the consumer in processing chips, rice bran, and pumpkin fruit can be processed into flour.

Bran is rice mill waste, which is generally used as cattle feed, while rice bran can be used as a food ingredient, they are natural healthy foods and as sources of antioxidants (Ramezanzadeh *et al.*, 1999 and Iqbal *et al.*, 2005). Components of essential substances in bran is a food fiber, as a source of calcium mineral of 500-700 mg/100g and magnesium at 600-700 mg/100g, contains vitamin B complex, vitamin E, essential fatty acids and amino acids (Astawan, 2009; Damayanthi *et al.*, 2010; and Pasha *et al.*, 2002).

Pumpkin including types of vegetables that can be grown in the lowlands to high, between 0-1500 m above the sea level (Hendrasty, 2003), generally the fruit of pumpkin can be grown in the tropics and sub-tropics (Kulkarni *et al.*, 2013). Pumpkin is a source of carotenoids, pectin, mineral salts, vitamins and other bioactive substances, such as phenolic compounds (Cerniauskiene *et al.*, 2014). The yellow color of pumpkin showed  $\beta$ -carotene compound that can be used as a food ingredient alternatives to increase the amount of  $\beta$ -carotene which the body needs daily (Usmiati *et al.*, 2005).

Products of simulated chips that have been researched are simulated chips of low fat bran flour substitution by Damayanthi and Listyorini (2006), the addition of 10% low fat bran flour is the best formula of simulated chips, but from the tests of sensory to the flavor of low fat bran flour simulated chips has left slightly bitter taste. To improve the taste of simulated chips, it can be done with pumpkin flour substitution that has flavor and sweeter aroma than the bran flour.

Utilization of bran flour (TB) and pumpkin flour (TLK) with other supporting materials produce healthy snacks that contain dietary fiber and antioxidants. Research on the substitution of bran flour with pumpkin flour in food products has not been widely conducted. The purpose of this study was to determine the influence of the proportion of the addition of bran flour (TB) and pumpkin flour (TL) to the physical properties (yield, hardness, and color), chemical (proximate, amylose,

vitamin E,  $\beta$ -carotene, dietary fiber, and total antioxidant), and sensory quality of the simulated chips.

## II. METHODS AND MATERIAL

### A. Materials

Bran used was obtained from farmers in Tabanan regency, while the pumpkin fruit is obtained from farmers in the Sub-district of Petang, Badung Regency, Bali. Additional materials used consisted of flour, green beans, onions, garlic, salt, refined sugar, baking powder, and margarine.

Chemicals used consisting of sulfuric acid ( $H_2SO_4$ ), boric acid ( $H_3BO_3$ ), HCL 0.02 N, hexane, Na-phosphate 0.1M, 0.1 ml of the enzyme amylase, the enzyme pepsin, pancreatic enzyme, 4M HCL, NaOH, 1,1-diphenyl-2-2picrylhydrazyl (DPPH), petroleum ether, ascorbic acid, ethanol, alcohol, methanol, potassium hydroxide,  $\alpha$ -tocopherol, methyl alcohol, and nitrite. All chemicals used for the analysis was obtained from Merk (Darmstadt, Germany).

Equipment used in the oven (with the brand of Shellab-USA, Type: 1370 FX), microwave oven with Kris brands with specification: 230V-50 Hz, 1400 W with a frequency of 2450 MHz, and the Genesys spectrophotometer 10S UV-VIS.

### B. Methods

This study was conducted in January through March 2014. The processing of simulated chips was conducted at the Laboratory of the Institute for Agricultural Technology (BPTP) Bali, while the chemical analysis was conducted at the Laboratory of Food Technology of Agriculture, University of Udayana, the Center for Postharvest Laboratory Testing Bogor, and the Laboratory of Food and Nutrition Faculty of Agricultural Technology of UGM.

### Sample Preparation

Rice bran is derived from paddy varieties of Cigeulis. The drying of the rice bran was conducted by using microwave oven (OM) on the power of 200 watt for 15 minutes. Fruit pumpkin originated from the species of

*Cucurbita moschata* (yellow squash or pumpkin), dried using OM to the power of 300 watt for 4 hours.

The processing of the simulated chips was made by mixing all the ingredients into doughs that can be formed. Subsequently made of thin sheets and molded round. The drying of simulated chips using an oven at 60°C for 2 hours (until dry). Simulated chips that have been dried were fried at a temperature of 190°C for 21 seconds.

Substitution treatment of TB and TF on simulated chips is the addition of TB consists of seven levels: 0%, 5%, 10%, 15%, 20%, 25%, and 30% of the total flour. While the addition of TLK consists of seven levels: 0%, 10%, 20%, 30%, 40%, 50%, and 60% of the total flour. The design used in this research is CRD (completely randomized design) which is repeated four times in each treatment.

## C. Chemical Analysis

### 1. Proximate Analysis

Proximate analysis was conducted on water content and ash content using the oven method (Apriyanto, 1989), protein content using the Micro-Kjeldahl (Apriyanto, 1989), fat content using Soxhlet (Apriyanto, 1989), and the carbohydrate content using carbohydrate by difference (Apriyantono, 1989).

### 2. Analysis of Food Fiber

Analysis of dietary fiber content was conducted by using the Multi-Enzyme (Asp *et al*, 1983). The analysis was conducted using three types of enzymes, the amylase enzyme, the enzyme of pepsin and the pancreatic enzymes.

### 3. Analysis of Vitamin E

Analysis of the levels of vitamin E was calculated using HPLC. 2 g sample was weighed, ethanol and saponified with KOH, then extracted with petroleum ether. Results of concentrated sample was vaporized in the evaporator, the rest of the concentrates was dissolved in 2 ml of methanol. Chromatography using the mobile phase methanol : water (97:3) with a flow rate of 1.2 ml/min. Quantitative determination of vitamin E using UV at a

wavelength of 295 nm, the column at a temperature of 24°C.

## 4. Antioxidant Capacity

Test of antioxidant capacity was conducted by using the DPPH (Blois, 1985). Antioxidants of gallic acid are used as a comparison. The crude extract sample was dissolved in methanol P.A. to obtain a concentration of 200, 400, 600, and 800 ppm. Extract solution and the solution antioxidant of Gallic acid derived respectively 4.50 ml and treated with 500 mL of 0.1 mM DPPH solution in a different test tube. The reaction reached at 37°C for 30 minutes and then the measure of the absorbance was measured using a spectrophotometer at a wavelength of 517 nm. Antioxidant capacity is determined by a standard curve of gallic acid in reducing free radical DPPH 0.1 mM by the formula of:

$$\text{Antioxidant capacity (ppm)} = \frac{X \text{ (mg/L)} \times \text{fp} \times \text{TV (L)} \times 1000000}{w \text{ (mg)}}$$

Notes : X = concentration of gallic acid  
standard regression equation  
fp = dilution factor  
TV = total volume  
w = sample weight

Absorbance of the reference solution is measured to perform the calculation of percent inhibition. The reference solution prepared by reacting 4.50 ml of methanol with 500 mL of 0.1 mM DPPH solution in a test tube. IC 50 value searched by using a standard curve equation of % inhibition as the y-axis and the concentration of antioxidants as a fraction of the x-axis. IC 50 is calculated by entering a value of 50% inhibition in a standard curve equation as the y-axis is then calculated the value of x as the concentration of IC 50. IC 50 value declared the concentration of the sample solution needed to reduce DPPH free radicals by 50%. Inhibition of the sample extract against 0.1 mM DPPH radical is determined by the formula of :

$$\% \text{ inhibition} = \frac{\text{absorbance of the blank} - \text{sample absorbance} \times 100\%}{\text{absorbance of the blank}}$$

## 5. Sensory Test

The sensory tests used were the test of description, test of preferences (hedonic) and test of ranking methods of Soekarto (1995). The tests were carried out by using 10 panelists on the description of the quality attributes of color, aroma, flavor, texture, taste and the after taste. The rating scale of the simulation chips quality attributes are: grade 1 = very weak; grade 6 = very severe. The preference or hedonic test was performed by using 40 semi-trained panelists to assess the parameters of color, aroma, texture, taste, and overall acceptance of the simulation chips used in the assessment. There are 6 grades in the hedonic test assessment, namely 1 = strongly dislike, 2 = dislike, 3 = somewhat dislike, 4 = somewhat like, 5 = like, and 6 = very like. The ranking test was used to determine the most preferred formulations; rank 1 indicates the most preferred product. Assessment is done by 40 semi-trained panelists. Panelists of semi-skilled and well-trained recruits are students of Food Technology of the Udayana University.

### Statistical Analysis

The observed data were analyzed by analysis of variance (ANOVA) using SPSS 16. Results of analysis of variance were significantly different ( $p < 0.05$ ) followed by a further test of Duncan's multiple range test (DMRT).

## III. RESULT AND DISCUSSION

### Antioxidant capacity and IC 50

Results of analysis of variance of simulation chips substitution of TB and TLK showed significant differences ( $p < 0.05$ ) on antioxidant capacity and IC 50. Increased antioxidant capacity of simulation chips was comparable with the addition of TB and TLK, but the reverse condition occurred in IC 50, in which IC 50 values decreased with the increasing substitution of TB and TLK (Table 1). The high antioxidant capacity in simulation chips was caused by TB contains the antioxidant capacity of 92.13 ppm GAEAC, and TLK contains the antioxidant capacity of 184.40 ppm GAEAC.

**Table 1.** The Average antioxidant capacity and IC 50 simulation chips with TB and TLK substitutions

Treatment (TB% : TLK%)	Antioxidant Capacity (ppm GAEAC)	IC 50 (mg/mL)
F1 (Control)	284.21 ± 74.04 <sup>a</sup>	393.36 ± 28.46 <sup>c</sup>
F2 (5:10)	428.68 ± 51.52 <sup>b</sup>	239.27 ± 9.81 <sup>d</sup>
F3 (10:20)	545.00 ± 24.68 <sup>c</sup>	185.83 ± 7.57 <sup>c</sup>
F4 (15:30)	668.52 ± 80.94 <sup>d</sup>	145.19 ± 15.16 <sup>b</sup>
F5 (20:40)	685.09 ± 80.72 <sup>d</sup>	138.45 ± 14.86 <sup>ab</sup>
F6 (25:50)	721.88 ± 55.43 <sup>d</sup>	126.66 ± 5.94 <sup>ab</sup>
F7 (30:60)	761.28 ± 42.50 <sup>d</sup>	120.59 ± 4.20 <sup>a</sup>
kk (%)	10.56	7.51

*Description :* Figures followed by the same letter in each column, indicating no different at the level of 5% Duncan test

IC50 value of simulation chips decreases with the increasing of TB and TLK substitutions. The highest IC 50 value was found in the F1 treatment of 393.36 mg/mL and the lowest was for the treatment F7 of 120.59 mg/mL. According to June *et al.*, (2003), IC 50 values of simulation chips on the treatment of F2 to F7 at 120.59 mg/mL to 239.27 mg/mL have the ability to moderate antioxidant activity, whereas treatment F1 has the capability of weak antioxidant activity with the value of 393.36 mg/mL. This indicates that the substitution of TB and TLK on simulated chips has the potential of free-radical scavengers, with an average concentration of 120.59 mg/mL have been able to ward off free radicals by 50%.

### The contents of the IDF, SDF and TDF

Results of analysis of variance of the simulation chips significantly affect ( $p < 0.05$ ) the insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF), as presented in Table 2. The increased levels of IDF, SDF, and TDF of simulation chips were comparable with the addition of TB and TLK. This is because the TB contain the IDF by 30.51% db, SDF 6.31% db, and TDF of 36.82% db while TLK contained IDF of 5.00% db, SDF of 10.21% db, and TDF of 15.22% db.

**Table 2.** The average of IDF, SDF and TDF simulation chips, with the substitution of TB and TLK



Treatment (TB% : TLK%)	IDF (% db)	SDF (% db)	TDF (% db)
F1 (Control)	3.90± 0.05 <sup>a</sup>	3.09 ± 0.42 <sup>a</sup>	6.77± 0.22 <sup>a</sup>
F2 (5:10)	4.75± 0.37 <sup>a</sup>	3.54± 0.28 <sup>ab</sup>	7.89± 0.10 <sup>b</sup>
F3 (10:20)	6.68 ± 0.35 <sup>b</sup>	3.96± 0.63 <sup>bc</sup>	10.54± 1.03 <sup>c</sup>
F4 (15:30)	6.62± 0.43 <sup>b</sup>	4.02± 0.08 <sup>bc</sup>	10.64± 0.50 <sup>cd</sup>
F5 (20:40)	6.94± 0.74 <sup>bc</sup>	4.07± 0.26 <sup>c</sup>	11.01± 0.89 <sup>cde</sup>
F6 (25:50)	7.49± 0.59 <sup>cd</sup>	4.08 ± 0.15 <sup>c</sup>	11.57± 0.69 <sup>de</sup>
F7 (30:60)	7.76± 0.48 <sup>d</sup>	4.17± 0.06 <sup>c</sup>	11.93± 0.44 <sup>e</sup>
kk (%)	7.57	7.57	6.35

Description : Figures followed by the same letter in each column, indicating no different at the level of 5% Duncan test

The content of TDF of simulation chips from the results of the research can be used as a source of dietary fiber because it contains TDF more than 6 g/100 g, the results of this research is in accordance with the statement of Friska (2002), which said food is the source of fiber if it contains 3-6 g/100 g of TDF.

### Vitamin E and β-carotene

The results of the analysis of the simulation chips variant significantly affect ( $p < 0.05$ ) on the content of vitamin E and β-carotene. The content of vitamin E and β-carotene increased with increasing addition of TLK for TB and TLK because TB is rich in vitamin E and TLK containing β-carotene (Table 3). The increasing amount of vitamin E and β-carotene was due to TB raw materials of Cigeulis varieties contain vitamin E amounted to 2.05 mg/100 g. The same was said by Pongjanta (2006), that there was an increase in the content of β-carotene on bread, cake, and cookies at 10-50% substitution of TLK.

**Table 3.** The Average of Vitamin E and β-carotene simulation chips substitution with TB and TLK

Treatment (TB% : TLK%)	Vitamin E (mg/100 g)	β-carotene (μg/g)
F1 (Control)	0.16 ± 0.07 <sup>a</sup>	0.25 ± 3.23 <sup>a</sup>
F2 (5:10)	0.25 ± 0.02 <sup>a</sup>	1.49 ± 36.41 <sup>b</sup>
F3 (10:20)	0.36 ± 0.07 <sup>a</sup>	2.52 ± 23.87 <sup>c</sup>
F4 (15:30)	0.59 ± 0.15 <sup>b</sup>	2.81 ± 17.28 <sup>cd</sup>
F5 (20:40)	0.71 ± 0.18 <sup>bc</sup>	3.06 ± 10.83 <sup>d</sup>
F6 (25:50)	0.80 ± 0.10 <sup>c</sup>	3.71 ± 28.33 <sup>e</sup>
F7 (30:60)	0.88 ± 0.09 <sup>c</sup>	4.13 ± 28.18 <sup>f</sup>
kk (%)	16.68	9.23

Description : Figures followed by the same letter in each column, indicating no different at the level of 5% Duncan test

### The Content of Proximate

Results of analysis of variance substitution treatment of TB and TLK of simulation chips significantly affect ( $p < 0.05$ ) on moisture, ash, protein, fat, and carbohydrates, as shown in Table 4. The highest water content of the simulation chips was in treatment of F1 (5.26 %), followed by water content dropped with the increasing substitution of TB and TLK. This was due to reduced content of gluten on simulation chips. Gluten is found in wheat flour water served as a binder and forming the dough elasticity. The water content of simulation chips was also affected by the water content of TB and TLK used. The water content of TB that is used has a moisture content of 10.54% and TLK water containing of 7.64%, lower than the water content of wheat flour by 14.5%.

The results of this research are consistent with the results of research by Silvi *et al.*, (2011), on a wet noodle products, substitution of wheat flour with 20% TLK has a water content of 32.12% lower compared with controls, at 35.42%. The same thing happened to sausage products, where fortification with TLK can lower the water levels of sausage (Agus *et al.*, 2009). According to the SNI, the water content of the chips in general is a maximum of 5-6%, while the moisture content of simulation chips of the study has met the SNI standards.

**Table 4.** The average content of the proximate of simulation chips with TB and TLK substitution

Treatment (TB% : TLK%)	Water Content (%)	Ash Content (%)	Protein Content (%)	Fat Content (%)	Carbohydrate content (%)
F1 (Control)	5.26 ± 1.14 <sup>c</sup>	4.24 ± 0.19 <sup>a</sup>	9.43 ± 0.92 <sup>a</sup>	14.05 ± 1.76 <sup>a</sup>	67.02 ± 2.94 <sup>c</sup>
F2 (5:10)	4.63 ± 1.08 <sup>c</sup>	4.30 ± 0.32 <sup>a</sup>	10.30 ± 0.92 <sup>ab</sup>	15.74 ± 0.71 <sup>b</sup>	65.02 ± 1.29 <sup>bc</sup>
F3 (10:20)	3.90 ± 0.44 <sup>ab</sup>	4.73 ± 0.21 <sup>b</sup>	10.78 ± 0.74 <sup>b</sup>	16.69 ± 1.13 <sup>bc</sup>	63.89 ± 1.63 <sup>ab</sup>
F4 (15:30)	3.71 ± 0.48 <sup>ab</sup>	5.09 ± 0.19 <sup>c</sup>	10.67 ± 0.51 <sup>b</sup>	16.94 ± 0.96 <sup>abc</sup>	63.58 ± 0.93 <sup>ab</sup>
F5 (20:40)	3.51 ± 0.53 <sup>ab</sup>	5.44 ± 0.20 <sup>d</sup>	11.09 ± 0.60 <sup>b</sup>	17.49 ± 0.81 <sup>cd</sup>	62.48 ± 0.89 <sup>a</sup>
F6 (25:50)	3.52 ± 0.41 <sup>ab</sup>	5.53 ± 0.19 <sup>d</sup>	11.20 ± 0.52 <sup>b</sup>	18.09 ± 0.69 <sup>cd</sup>	61.66 ± 0.88 <sup>a</sup>
F7 (30:60)	3.03 ± 0.61 <sup>a</sup>	5.76 ± 0.21 <sup>d</sup>	11.26 ± 0.52 <sup>b</sup>	18.48 ± 0.57 <sup>d</sup>	61.47 ± 0.72 <sup>a</sup>
kk (%)	18.46	4.46	6.52	6.06	2.37

Description : Figures followed by the same letter in each column, indicating no different at the level of 5% Duncan test

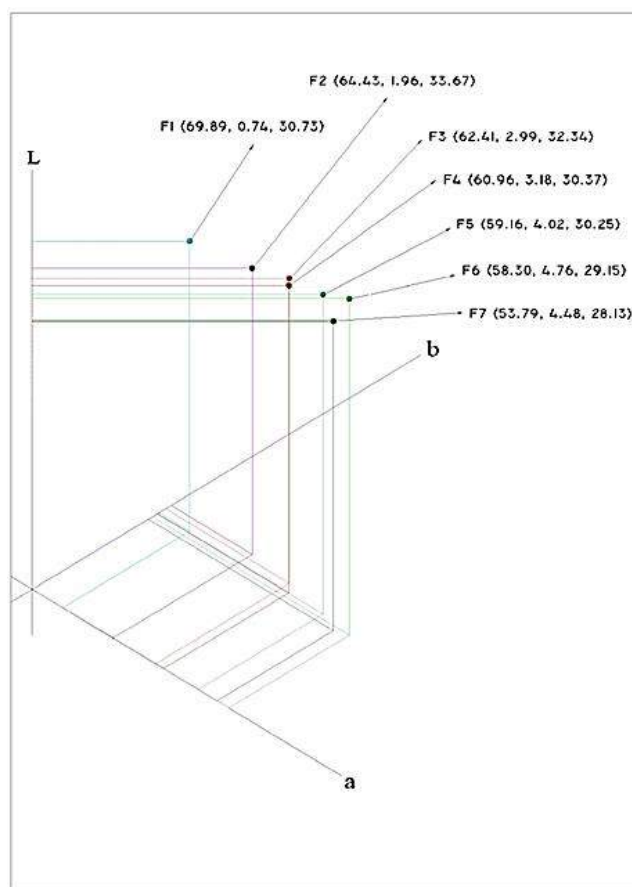
Ash, protein, and fat contents of the simulation chips increased with the increasing substitution of TB and TLK (Table 4). This is because the ash, protein, and fat contents of TB were greater than the wheat flour. The research results of Damayanthi and Listyowati (2006), showed that there was an increase in ash content of 3.01% and amounted to 7.96% protein on the simulation chips with the addition of 20% low-fat TB. The same results were found in biscuit products substitution of wheat flour with TLK by Kulkarini and Joshi (2013), who mentioned that there were increased levels of fat in biscuits with the increasing substitution of TLK. On the contrary the carbohydrate levels dropped with higher substitution of TB and TLK.

Carbohydrate content of the simulation chips decreased significantly, with the increasing substitution of TB and TLK (Table 4). The same results were expressed by El-Demery (2011), stating substitution to 20% of TLK on toast, which contain carbohydrates by 51.38% lower than in the controls (57.03%). Statement of Damayanthi and Listyorini (2006), the low-fat simulation chips are TB control carbohydrate content (73.48%) is higher than the carbohydrate content of simulation chips with the addition of 20% of low fat TB, amounting to 71.48%. It is also stated by See *et al.*, (2007) in baked goods, with the addition of 15% carbohydrates of TLK by 42.44%, lower than in the controls, at 46.28%.

### Physical Analysis

The results of the analysis of the physical properties of simulation chips significantly effect ( $p < 0.05$ ) on the color parameters of L, a, and b, as shown in Figure 1. Color L means brightness that indicates the value of L decreases with the increasing substitution of TB and TLK. Values of L simulation chips ranged from 53.79 to 69.89 that show the brightness range from 0-100. L color change is proportional to the reduction in the moisture content of the simulation chips. Chips with low water content has a dense texture and hard, so that when the frying process uses high temperatures, the water in the material and the surface is out in the form of water vapor and simultaneously hardening occurs on the surface of the material that causing the dark color (Jamaluddinet *et al.*, 2011 and Wibawa *et al.*, 2006). The color L decreased is allegedly due to the mailard

reaction at high temperature (Jamaluddinet *et al.*, 2011 and Norfezah, 2013) and because TLK has a high sugar content (Norfezah, 2013).

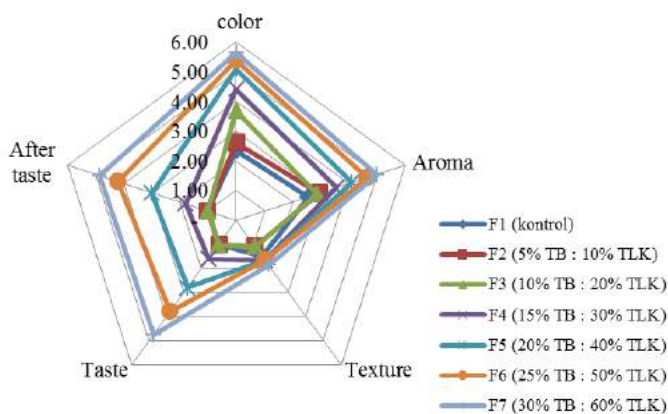


**Figure 1.** Graph Values of L, a, and b of simulation chips by substitution of TB and TLK

The a value of simulation chips increased with increasing addition of TB and TLK. The value of a, is the dimension of red-to-green. Simulating chips product has a positive value between 0.74 to 4.76 which is denoted by the red color. Simulating chips have a value of b, between 28.13 to 33.67 which have a positive value between 0-70 so it is denoted as the yellow color (Figure 1).

### Sensory Test

Sensory tests used in products of simulation chips are description test, test of preferences (hedonic), and test of ranking. The test results description of sensory attributes to determine the effect intensity of each attribute of the experiment, as presented in graphical form of Spider web (Figure 2).



**Figure 2.** Spider web graph description assessment of simulation chips with the addition of TB and TLK

Description about the yellow color and aroma increases with the increasing addition of TB and TLK, this was due TLK has a stronger color and aroma than TB. The texture of simulation chips hardened with the increasing addition of TB and TLK. While the increase in TB and TLK additions can increase the intensity of flavor and the after-taste of the simulation chips to bitter taste. It is associated with a color change of b toward yellow-brown due to high frying temperatures, so that the water at the surface and inside the material evaporated in large quantities and the remain is hard solids that can affect the taste of the product (Figure 2).

Results of analysis of hedonic test on the variants of simulation chips significantly affect ( $p < 0.05$ ) on color, aroma, texture, taste, and overall acceptance (Table 5). Assessment of the panelists using hedonic test (preference) of the color, aroma, texture, taste, and overall acceptance of simulation chips was even lower with the increasing addition of TB and TLK. Panelists did not like the dark color of simulation chips; it is because there was damage of carotenoids, and because of the reaction of mailard due to high sugar levels of TLK.

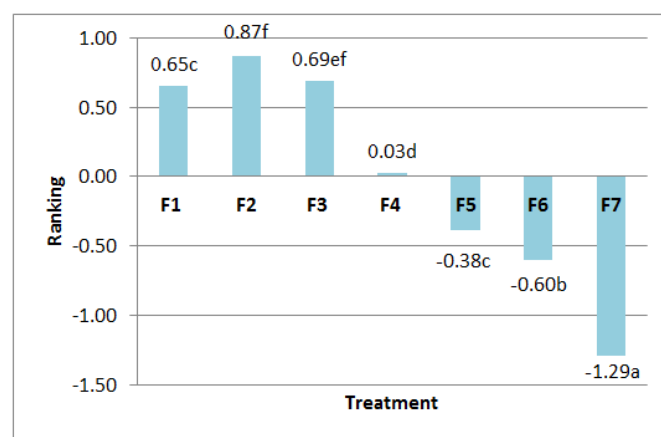
**Table 5.** The average of hedonic test of simulation chips with TB and TLK substitution

Treatment (TB% : TLK%)	Color	Aroma	Texture	Taste	Overall Acceptance
F1 (Kontrol)	5.05 ± 0.98 <sup>e</sup>	4.80 ± 1.02 <sup>cd</sup>	4.73 ± 1.19 <sup>c</sup>	4.83 ± 0.96 <sup>d</sup>	4.85 ± 0.89 <sup>d</sup>
F2 (5:10)	4.55 ± 0.84 <sup>d</sup>	4.88 ± 0.84 <sup>cd</sup>	4.83 ± 0.81 <sup>c</sup>	4.50 ± 0.99 <sup>cd</sup>	4.68 ± 0.76 <sup>d</sup>
F3 (10:20)	4.85 ± 0.83 <sup>de</sup>	4.88 ± 0.72 <sup>d</sup>	4.98 ± 0.73 <sup>c</sup>	4.95 ± 0.88 <sup>d</sup>	4.93 ± 0.71 <sup>d</sup>
F4 (15:30)	3.63 ± 0.92 <sup>c</sup>	4.33 ± 0.89 <sup>bc</sup>	4.50 ± 0.99 <sup>bc</sup>	4.28 ± 1.06 <sup>c</sup>	4.13 ± 1.02 <sup>c</sup>
F5 (20:40)	3.18 ± 1.03 <sup>b</sup>	4.15 ± 1.07 <sup>bc</sup>	4.10 ± 1.08 <sup>ab</sup>	3.15 ± 1.00 <sup>b</sup>	3.38 ± 0.98 <sup>b</sup>
F6 (25:50)	2.78 ± 1.02 <sup>ab</sup>	3.88 ± 1.32 <sup>ab</sup>	3.88 ± 1.26 <sup>ab</sup>	2.75 ± 1.23 <sup>ab</sup>	2.93 ± 1.12 <sup>a</sup>
F7 (30:60)	2.38 ± 1.25 <sup>a</sup>	3.48 ± 1.55 <sup>a</sup>	3.68 ± 1.44 <sup>a</sup>	2.43 ± 1.28 <sup>a</sup>	2.63 ± 1.21 <sup>a</sup>
kk (%)	26.36	25.19	25.05	27.75	24.70

Description : Figures followed by the same letter in each column, indicating no different at the level of 5% Duncan test

Panelists overall assessment is strongly influenced by the flavors of the simulation chips, because the taste plays an important role whether the food product is accepted by the panelists. Winarno (1992), stating that the preference and overall acceptance of the food in the mouth is the chemical interaction with the receptor through a process that is complicated and complex, so through this process a favorite food can be known through perception.

The results of test analysis on ranking of simulation chips of TB and TLK substitutions can be seen in Figure 3. Treatment of F2 was based on assessment of the panelists that achieved the first rankings, but not significantly different ( $p > 0.05$ ) from treatment with F3. The second ranking is the treatment of F3 and not significantly different ( $p > 0.05$ ) from treatment with F1. Treatment F4 achieved the fourth ranking that significantly affect ( $p < 0.05$ ) on the treatment of F1, F2, F3, F5, F6, and F7. While treatment F5, F6, and F7 are not preferred by the panelists because it tends to be dark in color, hard texture, and taste bitter.



**Figure 3.** Test chart ranking of simulation chips with the treatment of TB and TLK addition

Description : TB treatment (%); TLK (%); where F1 (0:0); F2 (5:10); F3 (10:20);

F4 (15:30); F5 (20:40); F6 (25:50); and F7 (30:60)

## The Best Method Selection

The decision-making was to determine the best treatment by using the method of Effectiveness Index. The principle of this method is to compare the measured parameters, namely proximate, antioxidant capacity, IC 50, dietary fiber,  $\beta$ -carotene, objective color, and sensory testing. The best alternative is to compare the value of chemical analysis and sensory test by looking at the value corresponding to the desired goal in this research.

Effectiveness Index analysis of the chemical analysis of simulation chips that have the highest Total Value of Products (TVP) was on F7 treatment followed by treatments of F6, F5, F4, F3, F2 and F1. While on the TVP that based on sensory testing, treatment F3 has the highest TVP, followed by treatments of F1, F2, F4, F5, F6 and F7 (Table 6). By comparing the TVP chemical analysis, sensory testing and the expected goals, the F4 treatment is selected as the best treatment. This is because the F4 treatment contains antioxidants,  $\beta$ -carotene, dietary fiber, and high nutrient content and by sensory was acceptable by the panelists. Although the real value of TVP chemical analysis of 0.64 (the fourth ranking) and the sensory value of TVP 0.62 (the fourth ranking), with the assumption that if the treatment of F7 with a value of the highest TVP of 0.67 which was selected but not accepted by the panelists because it has the lowest TVP of 0.00.

**Table 6.** Selection of the best methods of simulation chips with TB and TLK substitution

Treatment (TB% : TLK%)	Total Value of Product (TVP)	
	Chemical Analysis	Sensory Test
F1 (Control)	0.34	0.92
F2 (5:10)	0.45	0.88
F3 (10:20)	0.57	0.98
F4 (15:30)	0.64	0.62
F5 (20:40)	0.63	0.34
F6 (25:50)	0.66	0.17
F7 (30:60)	0.67	0.00

## IV. CONCLUSION

The conclusions of this research are :

1. The best formula of characteristics of physico-chemical properties of simulation chips is on the F4 treatment, with the addition of TB by 15%, and TLK by 30%. Chemical characteristics of the simulation chips have a water content of 3.71%, ash content of 5.09%, the protein content of 10.67%, fat content of 16.94%, and the carbohydrate content of 63.58%.
2. Simulation chips with the best formula contains  $\beta$ -carotene of 2,81 g, vitamin E of 0.59 mg/100 g, the IDF amounting to 6.62% db, SDF 4.02% db, TDF of 10.64% db, antioxidant capacity amounted to 668.52 ppm GAEAC, and IC 50 amounted to 145.19 mg/mL.
3. Characteristics of the physical properties of the best formula simulation chips is based on objective measurement of color, that it has value of L of 60.96 (brightness range 0-100), value of a of 3.18 (as the red color), and the value of b 30.37 ( as a yellow-to-blue).
4. Sensory characteristics of simulation chips best formula is based on test description of the color with the intensity of 4.40 quality attributes (light yellow to yellow), the intensity of the aroma quality attributes at 3.55 ( weak to strong aroma), the intensity of the texture quality attributes at 1.65 ( hard to crisp texture), the intensity of flavor quality attributes of 1.60 (a savory to bitter taste), and quality attributes mouth-feel intensity of 1.80 (kind of like to like).
5. The panelists assessment of the hedonic test on simulation chips on the F4 treatment, on the color of 3.63 (somewhat like, to like), on aroma of 4.33 (somewhat like to like), on the texture of 4.50 (somewhat like to like), on the taste of 4.28 (somewhat like to like), and the overall acceptance of 4.13 (somewhat like to like). The assessment of the panelists was based on the ranking test shows that the F1, F2, F3, and F4

formulas are preferred by the panelists compared with F5, F6 and F7 formulas.

6. Treatment F4 is the best treatment with TVP on chemical analysis of 0.64 and sensory analysis of 0.62.

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# Bioinformatics Tools for Protein Analysis

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## ABSTRACT

Understanding of the relationship between amino acid sequence and three dimensional structure of protein is one of the main objects of bioinformatics, if the relationship is known; it can be helpful for prediction of the protein structure from its amino acid sequence. In this paper, you will learn the fundamentals of structures of proteins, and we will discuss the basic tools and databases for protein visualization and classification. On the basis of such reviews and discussions, you will be able to understand how to predict protein structure and function from its amino acid sequence. The protein structure databases discussed in this paper are such as Protein Data Bank, NCBI Structure Database (MMDB). The Protein structure visualization databases and tools discussed here are such as Cn3-D, Chemscape Chine, Rasmol and Protein Explorer, SWISS PDB Viewer, Mage and Kinemages, and PDBsum. The protein structure alignment discussed are such as VAST and DALI Server. The Domain Architecture databases discussed here are CDD, CDART. Discussed tools for plotting protein-ligand interaction is Ligplot and the protein classification approaches are SCOP and CATH.

**Keywords:** Bioinformatics Tools, Protein Prediction, Protein Visualization, Protein Classification, Protein Function.

## I. INTRODUCTION

### A. Protein Structure Overview

Proteins are biomolecules made up chains of 20 different amino acid residues. For proper performance of particular biochemical function, a certain number of amino acid residues are essential and appearance of lower limit for a functional domain size is around 40-50-residues. The range for size of the protein is from this lower limit to several hundred residues in multi-functional proteins. Proteins normally consist of thousands of atoms arranged in a 3-dimensional structure that is specific for each type of protein. While a protein is formed, it folds itself into a complicated 3-dimensional shape. Each protein consist of one folded shape, and regularly folds into it, normally in less than one second. That complex folded shape directs how the protein functions and also how it performs its interaction with others molecules. For making up of each protein, a gene in the DNA of living cells should code the particular sequence of amino acid. Synthesis of protein is not done without its mRNA being present but

persistence of a protein is possible in the cell when its mRNA is no longer present. However the abundance of mRNA may be present but there is no message translation into protein unless genetically required. There is thus no proper correlation between protein and its mRNA in a living cell at any given time. Protein synthesis is a very complex process. Ribosomes employed as protein factories, RNA forms bridge in ribosomes and work as support structures as well as protein forming machinery.

There are four level of organization during protein structure analysis: primary structure is the chain of amino acid sequence of the protein. Every protein owns a unique amino acid sequence. Secondary structure :spatial arrangement of protein framework without its side chain conformation. Tertiary structure is three dimensional structure of entire protein. Quaternary structure refers to 3-D structures of proteins that consist of two or more polypeptide chains, referred as sub-units. In primary structure of protein, the sequence is held together by covalent peptide bond, in 3-D structure of

protein, the structure held together by variety of bonds such as: ionic bonds, hydrogen bonds, covalent bonds and hydrophobic interactions. Proteins and their structures are made up of polypeptides and domains(motifs). A short conserved region in a protein is called as motif and domain refers to a distinct portion of a protein estimate to fold independently of the rest of the protein and has its own function.

In primary structure of proteins, amino acids are linked together via the peptide bonds that are made up of the reaction of alpha –carboxyl group of one amino acid with alpha –amino group of another amino acid. For characterization of a protein its unique amino acid is an important part. For obtaining information about primary structure of protein we can use PROWL. This site will provide you with information about amino acid properties, their allowed bond geometry, probability for being interior residue versus exterior residues and which amino acid substitution are likely to maintain function and structure. This site also gives other information required for prediction of protein structure and function based on primary structure.

In secondary structure variety of structural elements are present such as alpha-helix, Beta sheet and random coil. In addition loops and turns, folds and motifs are present. Each of these structures can be predicted using different software which will be discussed in the next step.

In Tertiary structure, the arrangement of the polypeptide in 3-D far from its linear sequence is represented. This arrangement is as result of interactions between R-groups via van der waals, hydrogen and hydrophobic, ionic bonding. Motifs and domains are appeared in this structure level. Some examples are: Hairpin Beta-motif, Greek Key motif, Beta-alpha-beta motif.

Domains also appear in tertiary structure level. Polypeptide chains are more than 200 amino acid in length and fold into two or more compact globular cluster are known as domain. usually there are three types of domains: alpha domain, Beta domain and alpha/Beta domains. In quaternary structure, those proteins with more than one polypeptide chains are considered like haemoglobin.

Classification of protein based on structure: There are four types for classification of protein based on the structure such as type 1: alpha (Predominantly or core, exclusively alpha helices) example: Bundle and non-bundle, Beta(predominantly or core, primarily beta strand) example: Roll Barrel, Sandwich, single sheet, Alpha/Beta (Predominantly alternating Alpha-helix and beta-stand) example :Beta Alpha Beta motif, Alpha and Beta (Alpha helices and beta strand regions as separate grouping) example : Anti –parallel Beta sheet.[1-6].

## II. METHODS AND MATERIAL

### A. Protein structure Databases

Protein Data Bank (PDB) : PDB is a very large universal storage place of processing and distribution of 3-dimensional structure data of macromolecules. the information in PDB derived from variety tools and experiments like NMR, X-ray crystallography, microscopy, cryoelectron and theoretical modeling,. Accommodations of the database for users are access to structural data, providing methods for visualizing the structure and downloading structural information.[7]

NCBI Structure Database (MMDB): It includes database of 3D structure of biomolecules which experimentally determined. Most of these data derived from X-ray crystallography and NMR spectroscopy. The database provide biologists with a broad information on biological functions of proteins, on mechanisms related to their functions and on relationship between biomolecules and their evolutionary history. Additionally this database provide biologists with comparative analysis of 3D structure of proteins. NCBI also called as MMDB (molecular modeling database) and includes 3D structure of macromolecules and visualization tools for comparative analysis of proteins.[8]

Database and tools for protein structure visualization:  
Cn3-D : "see in 3-D" is a viewer of structural sequence alignment for MMDB database. It facilitates viewing of 3-D structure and alignment of sequence –structure of structure-structure. It serves as a helper application for the browser. Files can be downloaded to the pc and the application can be launched.[9]

### SWISS PDB Viewer:

It facilitates and network for analysis of several proteins simultaneously. The proteins lay over each other in order to analyze structural alignment and provide comparison of their active sites, their amino acid mutations angles, distances and H bonds between their atoms. This viewer is joined to Swiss-Model server. [10] Chemscape Chime, Rasmol and protein explorer:

This tool is one of the usual tools for visualization of protein structure. It can read molecular structure files from PDB. Chemscape chime serves as a plug in to permit structure visualization with browser. Protein explorer serves as a plug in to permit viewing of protein structure with our browser. Both of these application namely Chemscape chime and protein explorer are primary derivation of Rasmol.[11]

Mage and Kinemages:

It is another tool for protein structure visualization. It is able for rotation of entire image in real time, displaying of parts by turning off and on them, selection of points for their identification and animation of change between different forms.[6]

PDBsum :

It is a database that facilitates a large illustrated graphic summary of the main information on each biomolecular structure from the protein data bank. It consists of images of structure, detailed structural analysis derived from PROMOTIF program, schematic graphs of interactions, summary PROCHEK results [12]

Protein structure alignment tools:

VAST (vector alignment sequence tool): it is a tool produced by NCBI and provides identification of similar proteins with 3D structure. So it is structure similarity and search service. [13].

DALI : It is an computational protein structure alignment tool used for comparison of protein structure in 3D.[14]

### **B: Domain architecture Database:**

Conserved Domain Database :(CDD) : is a database contain sequence alignment and profiles, showing

protein domain conserved during molecular evolution course.[15]

CDART: (Conserved Domain Architecture Retrieval Tool) used for searching protein having similar domain architectures.[16]

### **C. Bioinformatics tools for plotting protein –ligand interactions:**

Ligplot : It is used to find out interaction between protein and ligand also hydrogen and hydrophobic contacts can be represented in this tool.[17].

### **D. Approaches for classification of proteins:**

Classification of proteins b several databases usually is on the basis of their structural similarities. Both structural and evolutionary relationship is factors of their classification. In hierarchy of proteins several levels exist but the main level considered are such as Family, superfamily and fold

Family: In this level proteins are grouped together into family having clear and known evolutionary relatedness so called as clear evolutionarily relationship level.

Superfamily: In this level proteins are with low sequence identities but their structural and functional characters suggest a common evolutionary origin so the level called as probable common evolutionary origin. This proteins positioned in superfamily level.

Fold: In this level the proteins are not having evolutionary origin but structural similarities derived from physics and chemistry of proteins facilitating certain chain topologies and packing arrangements. So this level also called as major structural similarity level.

SCOP: It is a database for structural classification of proteins. It provides comprehensive classification of structural and evolutionary relationships between those proteins with known structures.[18].

CATH: (Class, Architecture, Topology and Homologous superfamily): This database facilitates a hierarchical classification for domain structures of proteins, which cause clustering of proteins at four different levels: C, A, T, H means Class, Architecture, Topology and Homologous superfamily, respectively.[19].



### III. RESULT AND DISCUSSION

Understanding and analysis of proteins structure and function is one of most important goal of bioinformatics as proteins are important key in biological science research and they are directly and indirectly related to development of diseases, evolution. Mutations and drug discovery. Knowing their structure and function as well as structure-function relationship are very important and helpful to biologists as experimental tools and technologies are not fully support research studies on proteins and their role in disease and other issues like evolution, mutation an drug discovery, investigators help bioinformatics tools for solving and u deep understanding of the biological problems.

Experimental technologies possess disadvantages of time and cost so bioinformatics tools as an alternative method support biologists to process their research studies for prediction of protein structures and their functions.

For example research studies on many proteins like CDKs, Aquaporins, Ion channels, G -protein coupled receptors, Biomarkers and so on for drug discovery, cancer treatment, classification of cancer and so on depend on prediction of structure or structure-function relationship of such biomolecules so for such new and emerging research studies these bioinformatics tools as accessory or alternative tools are very helpful. These tools in combination of many other bioinformatics tools can extent scope of research studies in many aspects for example tools for gene finding, biological pathways, SNP detection, bioinformatics tool of microarray technology and biotechnology tools. [20-29].

### IV. CONCLUSION

In this paper, the fundamentals of different structures of protein discussed as basic information. Protein visualization and classification tools help biologists to predict protein structure from its amino acid sequence. So for conducting the projects related to prediction of protein structure or understanding protein function structure, bioinformatics tools can be used in order to provide precise, fast, low cost results. This paper helps biologists to understand and learn basic information of

protein structure and bioinformatics tools for identification, classification and visualization of proteins.

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# Bioinformatics Analysis of Evolution of Secondary Structures of Protein Trypsin Beta

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## ABSTRACT

Impact of divergence of amino acid sequences of protein during evolution on the secondary structures of polypeptide and phylogenetic significance of different secondary structures such as alpha helix, beta sheet and random coil in evolution are not known. Due to conservation of conformation ally identifiable regions through evolution, in closely related species, the amino acid sequence will show close similarity, thereby giving rise to similar structural motifs after folding the proteins. To understand the most conserved secondary structure element of a protein, we have conducted a bioinformatics work for molecular evolution of protein trypsin beta as a sample in order to analysis the phylogeny of secondary structures (alpha helix, beta sheet and random coil) of proteins Trypsin Beta among 25 species. In this method we retrieved amino acid sequences of these proteins from 25 species from protein data bank then folded each individually into 3-D structure using the software J-Pred. From the folded sequence it was possible to identify sequences in regions forming alpha helix, Beta sheet, random coil, which we retrieved and individually ligated end-end to obtain peptides made up of sequence in the random coil, alpha helix and beta sheet conformations ( final functional shape). Then examined the phylogenetic trees built after aligning the sequence using four different multiple alignment protocols. The result has assumed that random coil of Trypsin beta was phylogenetically most conserved. This project plays significant role in understanding the role of molecular evolution of proteins and their phylogenetic significance.

**Keywords:** Bioinformatics, Molecular Evolution, Secondary Structures, Trypsin Beta, Multiple Sequence Alignment, Phylogeny.

## I. INTRODUCTION

Proteins are sequences of amino acids which form primary structure of the poly peptides. [1]. To become active , a protein should be folded , this folding initially started in the form of secondary structures which finally led into three dimensional form of the protein. The secondary structure elements of the proteins are called, Alpha helix, Beta sheet and random coil. [1]. Description of Protein secondary structure focused on the pattern of the hydrogen-bonding of the peptide backbone of the protein. [1], [2], The alpha helix ( $\alpha$ -helix) is the most common, regular segment of the secondary structure of proteins. [1, 2]This right-handed coil shows donation of a hydrogen bone from backbone of N-H group to the backbone C=O group of the amino acid four residue earlier. It is also called as 3. 613-helix , denoted the number of residues per turn, and

involvement of 13 atoms in the ring formed by the hydrogen bond. [1, 2]The  $\beta$  sheet (also  $\beta$ -pleated sheet) is the second element of secondary structures in proteins. [1, 2]. As compared to alpha helix, it is less common. Beta sheets formed of beta strands joined laterally by minimum two or three backbone hydrogen bonds, which led in formation of twisted pleated sheets. A beta strand is typically 3 to 10 amino acids long which formed a stretch of polypeptide. The beta sheet exhibits in two forms parallel and anti-parallel. [1, 2] The third element contributed in secondary structure of protein is called random coil, which is a polymer and it's monomer unit's orientation take place randomly. It is a major portion of the protein which lies at the surface of the protein. In respect to the significance in function, alpha helix plays the most important role and beta sheet plays scaffold role. [1, 2]Secondary structures serve an important role in stabilizing of the overall folding of protein by

providing the high amount of the enthalpy of stabilization of folding which led in existence of polar backbone groups in the hydrophobic of a folded polypeptide. [3] Divergence of protein sequences occurred during evolution, [4] but it is not known how this divergence impact secondary structures segments, nor significance of phylogenetic status of the secondary structure members in evolution. For instance, some of conformationally detectable regions shows conservation throughout evolution, so that , in closely related species , the amino acid sequences will show high degree of similarity thereby giving rise to similar structural motifs after folding the proteins. [4]

To find out the degree of conservation of secondary structure elements and phylogenetics status of individual element in order to analysis their significance in evolution , we have selected the trypsin beta protein as material and performed it's bioinformatics analysis in order to introduce this novel method for further bioinformatics analysis of different secondary structures of other important proteins and their role in evolution and other evolution related issues relevant to the secondary structures of the proteins.

## II. METHODS AND MATERIAL

we have selected 25 species having Trypsin beta such as *Rarobacter faecitabidus*, *Boltenia villosa*, *Bos Taurus*, *Canis lupus familiaris* , *Rattus norvegicus*, *Mus musculus* , *Homospaiens*, *Macaca mulatta*, *Trimeresurus jerdonii*, *Bitis gabonica*, *Anplopoma fimbria*, *Gadus morhua*, *Salmo salar*, *Tryophagus putrscentiae*, *Lonomia obliqua* , *Mamestra configurata*, *Hypoderma Diana*, *Drosophila virillis*, *Drosophila melanogaster*, *Phlebotomus papatasi*, *Lutzomyia longipalpis*, *Anopheles stephensi*, *Cultex quinquefasciatus*, *Caligus rogercresseyi*, *Radix peregra* , *Loligo bleekeri* , from Protein data bank and downloaded amino acids sequences of them in FASTA format. Then using online primary structure to secondary structure conversion software j-pred , [5] by which the amino acid sequences of proteins of each species converted into potential secondary structures polypeptide. The converted folded polypeptide ( contain all elements such as alpha helix , beta sheet and random coil) of all 25 species cut off into the secondary structure elements serially and then individually ligated end-end to obtain peptides made up

of sequence in the random coil, alpha helix and beta sheet conformations. Then these insilco ligated polypeptide along with original one subjected to four multiple sequence alignments namely:

1. Clustal w: (<http://www.ch.embnet.org/software/ClustalW.html>) [6]
2. ProbCon, (<http://toolkit.tuebingen.mpg.de/probcons>) [7],
3. Tcoffee, ([http://toolkit.tuebingen.mpg.de/t\\_coffee](http://toolkit.tuebingen.mpg.de/t_coffee)) [8]
4. Mafft(<http://www.ebi.ac.uk/Tools/msa/mafft/>) [9]

The results of all multiple sequence alignment saved in separate notepads. , then the result of each multiple sequence alignment softwares were subjected in Protdist software, ( <http://caps.ncbs.res.in/iws/protdist.html> ) [10] then the out file result of protdist subjected in neighbor software, [11] then the neighbor outtree 's result saved in a separate notepad, then the outtree's result opened with MEGA, [12] then species clustered using tree view X software (<http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/>) [13]. and saving in separate notepad and rename it as cluster file. txt, then outtree result of each software saved in separate notepad and rename as outtree. txt, then , then we opened python cluster software and enter in first line cluster. txt and in second line outtree. txt and click on enter the result which will appear automatically in a notepad and open it with Excel software, then calculation of the percentage of fidelity of each secondary structure obtained and saved in Excel file.

## III. RESULT AND DISCUSSION

For each MSA methods, different percentages of secondary structures conservation obtained, based on their specific performances. **Table 1.** Molecular evolution analysis of secondary structure (random coil, alpha helix Beta sheet) of Trypsin Beta protein by 4 different MSA is discussed as below: According to the result obtained from clustal w: The most conserved secondary structure is alpha helix and the second one is random and the least conserved( The most varied) is Beta sheet , which indicates that the most mutation occurred in Beta sheet during course of molecular evolution. The conservation percentages obtained from clustalw for Trypsin protein's secondary structure is as below: Alpha helix >Random coil> Beta sheet (61. 78%>59. 17%>55. 47% respectively). On the basis of the result obtained from Mafft method: The most conserved part of secondary structure for Trypsin Beta is Random

coil and the second one is Beta sheet and the least one is alpha helix as below: Random coil >Beta sheet> Alpha helix (57. 17%>55. 73%>53. 78%respectively ). The highest percentage is random coil which shows the most conservation. On the basis of the result obtained from ProbCons method: The most conserved part of secondary structure is random coil and the second one is beta sheet and the least one is alpha helix. The conservation percentages obtained from probcons for Trypsin protein 's secondary structure is as below:Random coil > Beta sheet>Alpha helix (61. 08 %>55. 04% >54. 6 %respectively ). The highest percentage is random coil which shows the most conservation. On the basis of the result obtained from Tcoffee Method: The most conserved part of secondary structure is Random coil and the second is Alpha helix and the least is Beat sheet. The conservation percentages obtained from Tcoffee A for protein TIFIIA's secondary structure is as below: Random coil >Alpha helix > Beta sheet 60. 95%>56. 47%>55. 82% respectively). The highest percentage is random coil which show the most conservation. Overall results of these finding is considered carefully and due to negotiable difference between percentages in different MSA, the result has shown that random coil phylogenically is the most conserved part of the protein

**Table 1:** Percentage of conservation of secondary structures of Trypsin beta by four different multiple sequence alignment protocol:

<b>Multiple sequence alignment</b>	<b><u>Random coil</u> %</b>	<b><u>Alpha Helix</u> %</b>	<b><u>Beta sheet</u> %</b>
<b>Clustal w</b>	59. 17	61. 78	55. 47
<b>Mafft</b>	57. 17	53. 78	55. 73
<b>Probcons</b>	61. 08	54. 6	55. 04
<b>Tcoffee</b>	60. 95	56. 47	55. 82

Phylogentic trees of secondary structures of trypsin beta using four different multiple sequence alignments constructed and compared with benchmark tree of classification of 25 species and visual analysis of the trees showed that in the tree constructed using clustal w , beta sheet element of mammalian underwent mutation

and human's beta sheet of trypsin beta is diverged from other four mammalian speices. For the nine studied fly species , the beta sheet is scattered and three of the fly species come together and remaining six are evolved together, also in this tree, tunicate and crustacean species evolved together , bacteria specie come near by two mollusca species but two snake species and three bony fishes species evolved tightly together. For random coil flies species are scattered in four regions and mammalian species split into two places and two mollusca underwent divergence but still snake and bony fishes are evolved together tightly which showed high degree of conservation of the element among these species. and for alpha helix the conservation degree showed maximum amount as there is no divergence of this segment among mammalian species , snake and bony fishes species but still divergence of one flies species is shown , so visually alpha helix element showed the maximum degree of conservation and beta sheet most mutated one and random coil takes place an intermediate place.

## Discussion

Conversion of linear polymer of polypeptide into a stable three dimensional functional protein is a thermodynamically dependent process [13]. secondary structures formation occurred during initial stages of protein folding so their role in protein folding is crucial as they provide much of enthalpy for stabilization of protein during folding. [14] As the secondary structures play significant role in protein folding, their conservation, evolution and phylogenetic status are significantly considered for understanding the entire protein evolution and mutation and other phylogenetic ally related issues. Here we have conducted an original work to find out the molecular evolution of secondary structure of trypsin beta. In this work, we have examined performance of four different multiple sequence alignments and for each MSA different percentages are obtained. Due to negotiable difference between percentages obtained from different multiple sequence alignment, the result has shown that random coil phylogenically is the most conserved. On the basis of phylogentic analysis of trees constructed based on secondary structures and compared with benchmark trees in which the species clustered in accordance with their taxonomy, it visualizes scattered distribution of

species and their genus, and evidences based on the trees shows that evolution of secondary structures occurred many times, although the exact reason of evolution is not known but occurrence of evolution within the secondary structures are well observed by this method.

#### IV. CONCLUSION

This work is helpful in understanding how secondary structures of a protein diverge or evolve among closely related species and the most conserved part of secondary structures of a protein is calculated by multiple sequence alignment protocol. Although still the reasons for evolution of secondary structures are open for discussion but this work is a starting point to study the reason of the molecular evolution and their significant role in the evolution and function of the appropriate protein. This project can be helpful for understanding protein structure and its evolution in drug discovery projects as many targets are protein. For more information about protein structure of drug targets please referee to the following publications.[15-20].

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# Inversion of Time in the Classical Kinematics of Material Point

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## ABSTRACT

As a result of the fact that the time in classical physics manifests itself only through the movement of the bodies, in the kinematics of material point the question of reversing the course of time (from the future to the past) is examined theoretically. The method is defined for inversion of time and its impact on the kinematic quantities and principles describing the motion. Three main types of motion in the kinematics have been discussed compared to reversal the course of time.

- a) Fully reversible motions - these are all constant motions. They can occur in the same way both in right and reverse direction of passage of time but they only indicate that the time is running not determining its direction;
- b) Semi reversible movements - these are all movements, in which the acceleration is an even function in case of inversion of time. They amend their nature temporary only in right and reverse direction of passage of time for a certain period. After that the semi-reversible movements run identically. They also show that the time is running but not determining its direction;
- c) Completely irreversible movements - these are movements in which the tangential acceleration is odd function compared to the inversion of time. These can occur only in one direction of the time course; in the opposite direction they are impossible. The irreversible movements can be used to determine the direction of time.

The reasons for these three types of movements have been examined and the relevant laws have been received: of motion, velocity and acceleration in right and reverse direction of passage of time. Their relationship with some basic quantities and laws of classical physics has been shown as well.

**Keywords:** Time, Inversion of Time, Fully Reversible Motions, Semi Reversible Movements , Completely Irreversible Movements Kinetic Energy

## I. INTRODUCTION

The major nature of time is that it is manifested through the movement of the bodies. Let we imagine for a moment that time flows in the opposite direction - from the future to the past. How this affects the movements in the kinematics?

## II. METHODS AND MATERIAL

### A. Definition

To reverse the direction of time of one randomly moving material point it means the same to go through the same

spatial positions in its movement, but in reverse order, with the same size of velocity but in the opposite direction [1]. The same definition is used also by the other authors [2-12].

### B. Description of the inversion of time

Suppose we examine a material point moving along the axis Ox (Figure 1). During the interval of time  $\Delta t = t_2 - t_1$  it passes distance  $\Delta x = x_2 - x_1$ . Projection of its velocity  $\vec{v}$  along Ox in the initial and final point is:  $v_{x1} \neq v_{x2}$ . According to the definition, the inversion of time means a point to go back the distance  $x_2 \rightarrow x_1$  with the same size of velocity but in the opposite

direction. To reverse the course of time we shall use the method described in [1], as in every moment of time  $t$  of the movement of point a negative value is attributed:

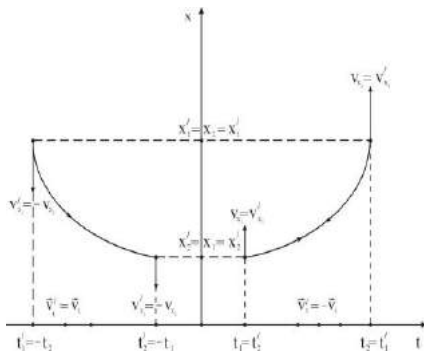


Figure 1: Description of the inversion of time

$$t' = -t$$

This is equivalent to a reflection on the time axis compared to its beginning (Figure 1 – the left part), but without changing the direction of the velocity of time:  $\vec{v}'_t = \vec{v}_t$ , although formally it now flows from the future to the past. At the same time, the time moments change their signs:  $t'_1 = -t_2 < 0$ ,  $t'_2 = -t_1 < 0$  as the final moment becomes initial one and vice versa, and the time intervals preserve their sign.

$$\Delta t' = t'_2 - t'_1 = -t_1 - (-t_2) = t_2 - t_1 = \Delta t > 0$$

The attitude positions preserve their sign, but follow in reverse order:

$$x'_1 = x_2; x'_2 = x_1$$

and spatial intervals change their sign:

$$\Delta x' = x'_2 - x'_1 = x_1 - x_2 = -(x_2 - x_1) = -\Delta x$$

The velocity of the point also changes its direction:

$$v'_x = \frac{\Delta x'}{\Delta t'} = \frac{-\Delta x}{\Delta t} = -v_x$$

thus satisfying the definition for inversion the course of time.

### C. Inversion of time in kinematic parameters describing the motion.

We shall examine at how the inversion of time affects the kinematic parameters describing the motion of a material point. The change of time direction we shall reflect as we shall substitute time moments  $t'$  with  $(-t)$  as the initial moment becomes final one and vice versa,

and we shall change the directions of the velocities:  $\vec{v}' = -\vec{v}$ , according to the mentioned above.

#### 1. Positions and moments:

$\vec{r}' = \vec{r}$  - radius vector;

$\vec{\varphi}' = \vec{\varphi}$  - positional angle at motion in a circle;

$t' = -t$  - instant of time.

#### 2. Changes in the position and time:

$$\Delta \vec{r}' = \vec{r}'_2 - \vec{r}'_1 = \vec{r}_1 - \vec{r}_2 = -(\vec{r}_2 - \vec{r}_1) = -\Delta \vec{r}$$

- displacement;

$\Delta s' = \Delta s$  - distance;

$$\Delta \vec{\varphi}' = \vec{\varphi}'_2 - \vec{\varphi}'_1 = \vec{\varphi}_1 - \vec{\varphi}_2 = -(\vec{\varphi}_2 - \vec{\varphi}_1) = -\Delta \vec{\varphi}$$

- angle of rotation

$$\Delta t' = t'_2 - t'_1 = -t_1 - (-t_2) = t_2 - t_1 = \Delta t$$

- time interval.

(The same is relevant to the differential changes)

$T' = T$  - period;

$\nu' = \frac{1}{T'} = \frac{1}{T} = \nu$  - frequency;

$\omega' = \frac{\Delta \varphi'}{\Delta t'} = \frac{-\Delta \varphi}{\Delta t} = -\omega$  - circular frequency

$$\vec{v}' = \frac{d\vec{r}'}{dt'} = \frac{-d\vec{r}}{dt} = -\vec{v}$$

#### 3. Velocities:

$$\vec{\omega}' = \frac{d\vec{\varphi}'}{dt'} = \frac{-d\vec{\varphi}}{dt} = -\vec{\omega}$$

- odd

functions compared to inversion of time;

$\vec{v}' = \vec{\omega}' \times \vec{r}' = -\vec{\omega} \times \vec{r} = -\vec{v}$  - right-hand screw rule preserves.

#### 4. Velocity changes:

$$\Delta \vec{v}' = \vec{v}'_2 - \vec{v}'_1 = -\vec{v}_1 - (-\vec{v}_2) = \vec{v}_2 - \vec{v}_1 = \Delta \vec{v}$$

$$\Delta \vec{\omega}' = \vec{\omega}'_2 - \vec{\omega}'_1 = -\vec{\omega}_1 - (-\vec{\omega}_2) = \vec{\omega}_2 - \vec{\omega}_1 = \Delta \vec{\omega}$$

(The same is relevant to the differential changes.)

#### 5. Accelerations:

First we shall believe that the acceleration is not proportional to the velocity.

$$\vec{a}' = \frac{d\vec{v}'}{dt'} = \frac{d\vec{v}}{dt} = \vec{a}$$

$$\vec{a}'_\tau = \frac{dv'}{dt'} \vec{e}'_\nu = \frac{-dv}{dt} (-\vec{e}_\nu) = \frac{dv}{dt} \vec{e}_\nu = \vec{a}_\tau$$

(Here the unit vector indicating the direction of the velocity, changes its direction:  $\vec{e}'_\nu = -\vec{e}_\nu$ ).

$$\vec{a}'_n = \omega'^2 \rho' \vec{n}' = (-\omega)^2 \rho \vec{n} = \vec{a}_n$$



(Here the radius of curvature of the trajectory and the normal vector to it does not change in case of inversion of time.)

$$\begin{aligned}\vec{a} &= \vec{a}_\tau + \vec{a}_n = \vec{a}'_\tau + \vec{a}'_n = \vec{a}' \\ \vec{\alpha}' &= \frac{d\vec{\omega}'}{dt'} = \frac{d\vec{\omega}}{dt} = \vec{\alpha} \\ \vec{a}'_\tau &= \vec{\alpha}' \times \vec{r}' = \vec{\alpha} \times \vec{r} = \vec{a}_\tau\end{aligned}$$

All accelerations are even functions in case of inversion of time.

The time derivatives of higher order can be considered similarly as well.

### 6. Law of motion, velocity and acceleration.

According to the first part of the definition of the inversion of time - the material point to pass through the same spatial positions, but in reverse order - it follows that the law of motion has to be even function in case of the inversion of time:

$$\vec{r}'(t') = \vec{r}(-t)$$

According to the second part of the definition of the inversion of time - the velocity of the material point to preserve its size, but to alter its direction backwards - it follows that the law of velocity has to be odd function in case of the inversion of time:

$$\vec{v}'(t') = -\vec{v}(-t)$$

The above two laws require in case of the inversion of time the law of acceleration to be even function as well:

$$\vec{a}'(t') = \vec{a}(-t)$$

But this condition is only necessary, but not sufficient, because the acceleration is determined not by law of movement but by the force acting on the point, and it can be not an even function (see below).

### 7. Equations of motion:

We shall examine how the inversion of time affects the fundamental equation of motion in classical mechanics - the second law of Newton [11]:

$$\begin{aligned}\vec{F} &= \frac{d\vec{p}}{dt} = \frac{d(m\vec{v})}{dt} = \frac{dm}{dt}\vec{v} + m\frac{d\vec{v}}{dt} = \\ &= -\frac{dm'}{dt'}(-\vec{v}') + m'\frac{d\vec{v}'}{dt'} = \\ &= \frac{dm'}{dt'}\vec{v}' + m'\frac{d\vec{v}'}{dt'} = \frac{d(m'\vec{v}')}{dt'} = \frac{d\vec{p}'}{dt'} = \vec{F}'\end{aligned}$$

(Here  $\vec{p} = m\vec{v}$  - momentum of the body;  $m = m'$  - body mass, which does not change in case of inversion of time;  $\frac{dm}{dt} = -\frac{dm'}{dt'}$  - rate of change of mass in reactive movements, which is odd function compared to the inversion of time.)

It is seen that the equation of motion (the second principle of dynamics) preserves in case of the inversion of time i.e. it is reversible over time. But and here this is only necessary but not sufficient condition.

### D. Examples of movements inverted in time.

The question placed - can we use a particular motion in order to determine the direction of course of time - from the past to the future or vice versa? At first sight, the answer is negative, because the equations of motion are symmetric toward the inversion of time - movements themselves can run in forward or reverse direction. But, as we shall see below, this is not exactly so.

#### 1. Movements fully reversible in time:

##### Rest

When the material point is at rest compared to the frame of reference, the kinematic variables are:  $\vec{r} = \text{const}$ ;  $\Delta\vec{r} = \mathbf{0}$ ;  $\Delta s = 0$ ;  $\vec{a} = \mathbf{0}$ . Only the time interval  $\Delta t$  is uncertain quantity. Reversing the direction of time does not affect all these quantities - they will not change. This is because the rest is a condition but not motion. We can put the question - whether in this case the time is running at all ( $\Delta t$  grows indefinitely) and if it is running - in what direction? Kinematics cannot answer the question in this case: the time may not run ( $\Delta t = 0$ ,  $t = \text{const}$ ), and we cannot determine its direction as well (if it is running) - from the past to the future, or vice versa. The reason for this is that we define the time through the movement, but in this case it is absent.

#### Constant straight-line motion (Fig. 2)

Let design the vector laws for acceleration, velocity and movement along the axis Ox of the selected coordinate frame of reference:

$$a_x(t) = 0$$

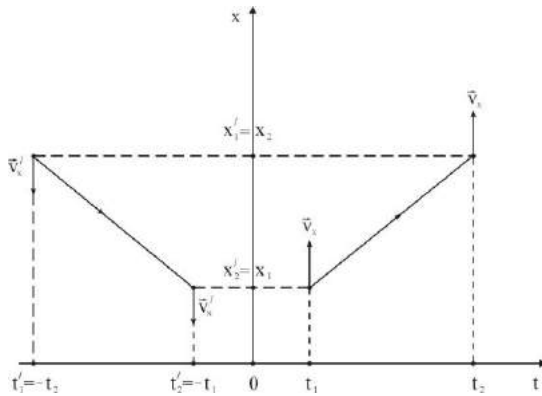
$$v_x(t) = v_{0x} = \text{const}$$

$$x(t) = x_0 + v_x(t - t_0), \quad t \in (-\infty, +\infty)$$

and to replace in them  $v'_x$  with  $-v_x$ ,  $v'_{0x}$  with  $-v_{0x}$  and moments of time  $t$  with:  $t' = -t$ ,  $t'_0 = -t_0$ , which

corresponds to the reversal the course of time. We shall receive:

$$\begin{aligned}
 a'_x(t') &= a_x(-t) = 0 \\
 v'_x(t') &= -v_x(-t) = -v_{0x} = -const \\
 x'(t') &= x'_0 + v'_x(t' - t'_0) = x_0 - v_x(-t + t_0) = \\
 &= x_0 + v_x(t - t_0) = x(t) \\
 t' &\in (-\infty, +\infty)
 \end{aligned}$$



**Figure 2 :** Inversion of time at Constant straight-line motion

The resulting laws for acceleration, velocity and movement show that the point moves constantly in a straight line in the opposite direction of Ox, as it passes through the same spatial positions with the same size of velocity, but in reverse order. (This is also true when  $t_0 = 0$ ).

It turns out that the law of velocity is odd, and the law for the acceleration and movement - even function toward the reversal of time t.

It is apparent that the inverted movement is completely analogical to the initial, but in reverse direction. Such a movement we shall call **fully reversible in time**. Moreover, the direction of movement shall be determined by the selection of coordinate frame of reference. Please note that if we invert the spatial axis Ox compared to its starting position accurate within an additive constant the movement does not differ from the initial one. What is more, if we consider two identical material points, moving constantly in a straight line with equal velocities against each other and if we turn the direction of time, we shall receive again two material points, moving against each other with the same velocities. The only difference is that two points have exchanged their places.

That is why we can draw a fantastic at first sight conclusion that we not able a priori to define for which bodies moving constantly in straight-line the time flows in one direction or in the other direction, i. e. the constant straight-line movements shows that the time is running out, but not determine its direction.

Another important note: the process of constant straight-line movement can be considered reversible, as there is no reason for this movement.

There are many other movements fully reversible in time, such as constant movement in a circle, ellipse, spread of a flat sine wave in environment non-swelling energy, etc., which will not be considered here by us.

### General case of fully reversible movements.

We shall prove the following theorem: any unspecified constant motion is completely reversible in time. Indeed, for any unspecified constant movement (in a curve or in straight-line) we have:

$$\begin{aligned}
 \vec{a}'_r(t') &= \vec{a}_r(-t) = 0 \\
 \vec{a}'_n(t') &= a'_n \vec{n}'(t') = (-\omega)^2 \rho' \vec{n}'(t') = \omega^2 \rho \vec{n}(-t) = \\
 &= \vec{a}_n(-t); a'_n = a_n = \omega^2 \rho = const \\
 \vec{\alpha}'(t') &= \vec{\alpha}(-t) = 0 \\
 \vec{a}'(t') &= \vec{a}'_r + \vec{a}'_n = \vec{a}_r + \vec{a}_n = \vec{a}(-t)
 \end{aligned}$$

It is apparent that the law of acceleration is an even function compared to the inversion of time, and this, together with the requirements for odd function of the law of velocity and for even function of the law of the movement leads to the fully reversible movement. The reason for this is that the force acting on the point in this case is only normal (perpendicular to velocity) and such force shall not modify the kinetic energy of the moving body.

And here is in effect the claiming that all fully reversible movements (whether they are periodical or not) do not determine the direction of the arrow of time.

### 2. Movements semi - reversible in time:

#### Uniformly variable straight-line motion (Figure 3)

We shall consider the particular case of constant accelerated straight-line motion. If we design the vector laws for acceleration, velocity and movement along of the coordinate axis Ox, we shall receive:

$$a_x(t) = a_{0x} = const$$

$$v_x(t) = v_{0x} + a_x(t-t_0)$$

$$x(t) = x_0 + v_{0x}(t-t_0) + \frac{1}{2}a_x(t-t_0)^2$$

The inversion of time leads to the following laws for acceleration, velocity and movement:

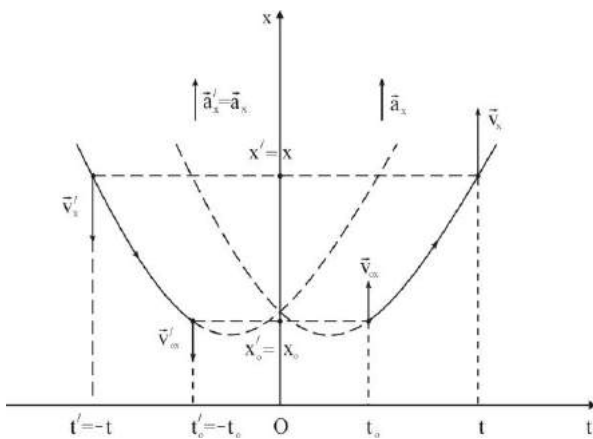
$$a'_x(t') = a'_{0x} = a_{0x} = a_{0x} = const = a_x(-t)$$

$$v'_x(t') = v'_{0x} + a'_x(t'-t'_0) = -v_{0x} + a_x(-t+t_0) = -v_{0x} - a_x(t-t_0) = -v_x(t)$$

$$x'(t') = x'_0 + v'_{0x}(t'-t'_0) + \frac{1}{2}a'_x(t'-t'_0)^2 =$$

$$= x_0 - v_{0x}(-t+t_0) + \frac{1}{2}a_x(-t+t_0)^2 =$$

$$= x_0 + v_{0x}(t-t_0) + \frac{1}{2}a_x(t-t_0)^2 = x(t)$$



**Figure 3.** Inversion of time at uniformly accelerated straight-line motion

It is apparent that the law of velocity is an odd function and the laws of acceleration and movement are even functions compared to the inversion of time **t**. The reversed movement represents a uniformly decelerated motion conversely to Ox, as the point goes through the same spatial positions with the same size of velocity, but in reverse order. Or the nature of the motion will be amended temporarily – from uniformly accelerated motion it will become uniformly decelerated motion. After the interval of time  $\Delta t = 2v/a$  inverted movement again becomes uniformly accelerated without any difference from the initial one. We shall name such movement semi - reversible in time because it amends

its nature only temporarily - for a certain time interval, which we shall name a period of "whirlwind of time."

Here the fact is essential that the reversal of time flow does not change the direction of acceleration. We shall note something more: each uniformly decelerated straight-line motion becomes uniformly accelerated in the opposite direction after a certain interval of time after the velocity passes through 0 and reverses its direction. The opposite is impossible – any uniformly accelerated straight-line motion will always remain the same as the velocity will increase to infinity. Even in case of reverse the time flow the uniformly accelerated motion becomes uniformly decelerated in the opposite direction only for a fixed time interval, only until the velocity passes through 0, then the motion is again uniformly accelerated in straight line direction as the velocity increases to infinity.

**Note:** Formally, we can consider that in case of uniformly decelerated straight-line motion when the velocity becomes 0 and reverses its direction (respectively its move), the time reverse occurs certainly!

The same consideration also applies when a non-inverting movement is uniformly decelerated straight-line motion.

Can such a movement to be used to determine the direction of the "arrow" of time? Unfortunately it is not possible. Here it only shows, as well, that the time is running out, but its direction is not determined.

Many other examples could be given of semi reversible movements - harmonic oscillation of the material point, parabolic motion, hyperbolic motion, elliptic motion in the field of gravitational force, etc., which will not be considered here by us.

### General case of semi - reversible movements

All results received above in the case of considered cases of movement in kinematics are mainly due to fact that the laws of motion and acceleration are even functions compared to the inversion of time, which in reflection (mirror image) in the course of time do not alter their shape. Law of velocity is odd function compared to **t** and in case of reversal in the course of time the sign of the velocity changes. The requirement

the Law for acceleration to be even function is significant as it does not follow from the definition of inversion of time as at the other two laws. We shall prove the following theorem: if tangential acceleration at random movement of a material point is even function compared to the inversion of time, the movement is completely or semi - reversible in time.

We have

$$\vec{a}(t) = \vec{a}_\tau(t) + \vec{a}_n(t)$$

For normal acceleration we have already proven that it is always an even function at the inversion of time:

$$\vec{a}'_n(t') = \vec{a}_n(-t)$$

Then, from the condition that:  $\vec{a}'_\tau(t') = \vec{a}_\tau(-t)$  is an even function, it follows that the full acceleration is an even function:  $\vec{a}'(t') = \vec{a}(-t)$ .

Then the movement meets the conditions of reversibility in time and is completely or semi - reversible.

The requirement  $\vec{a}_\tau(t)$  to be an even function is fulfilled when the acceleration depends only on the position in space and time, but does not depend on the velocity of the body:

$$\vec{a} = \vec{a}(\vec{r}(t), t)$$

This is true for homogeneous stationary field of the acceleration (respectively the force) - completely reversible movements - or for central stationary field - semi - reversible movements. Non-stationary fields require further consideration.

In all these cases the conclusion is in force that completely or semi - reversible movements cannot be used to determine the direction of the course of time.

### 3. Irreversible movements:

What will happen to the reversibility of movements when acceleration is odd function compared to the inversion of time? We shall consider that the acceleration of the material point is proportional to a power of velocity of the body:

$$\vec{a} = \pm k |\vec{v}|^n \vec{e}_v$$

where k is a coefficient of proportionality, and n = 0, 1, 2, 3, ... is an integer.

The mark (-) corresponds to the acceleration obtained under the action of so-called dissipative forces of friction and resistance at movement of the body in a given environment.

Examples of such forces:

TABLE 1

n = 0	-	Forces of dry friction at sliding of the body on some surface [11];
n = 1	-	the resistance forces in case of movement of the body with minor velocity into a viscous fluid - so-called resistance as a result of friction [11];
n = 2	-	vortex resistance on the body, moving at high velocity in a fluid [11];
n = 3	-	resistance due to shock wave at movement of the body with a supersonic velocity in a fluid [11], etc.

All of these forces tend to reduce the kinetic energy of the moving body, which is dissipated to the environment [11]. The work of these forces is negative.

The sign (+) corresponds to the accelerations, obtained by the action of so-called cumulative forces that act on the body, when the environment is moving along with it. These forces increase the kinetic energy of the body at the expense of energy from the environment and the work they do is positive.

In both cases, the acceleration of the body is parallel to the velocity and the movement is delayed or accelerating.

### Movement with dry friction at sliding (Figure 4)

Lets a body moves in a straight line along the axis Ox (glides loosely) on a support. Its movement will be decelerated under the action of the friction force. According to the first law of Coulomb for a dry friction at sliding [11], the resistance force acting on the body is:

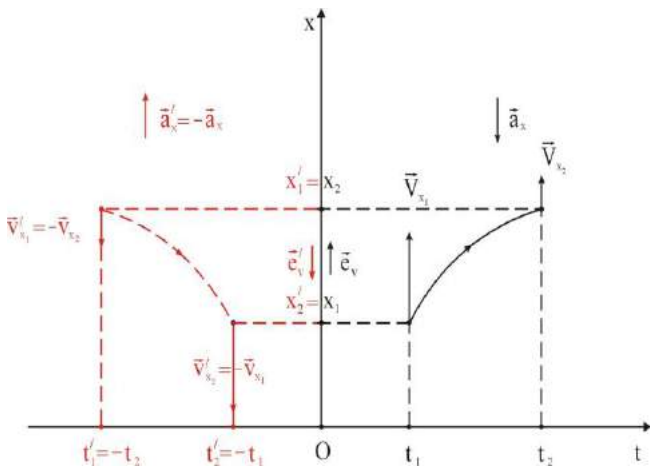
$$\vec{F}_T = -fN \frac{\vec{v}}{v} = -fN \vec{e}_v = const$$

where  $\mathbf{f}$  is the coefficient of friction at sliding,  $\mathbf{N}$  - the pressure of the body on the support, and  $\vec{\mathbf{e}}_v$  is the unit vector having always the direction of the velocity. From the second principle of the dynamics for the acceleration of the body we shall receive:

$$\vec{\mathbf{a}} = -k|\vec{\mathbf{v}}|^0 \vec{\mathbf{e}}_v = -\frac{fN}{m} \vec{\mathbf{e}}_v = \text{const}$$

where  $\mathbf{m}$  is the mass of the body. What is important here is that the direction of acceleration is always opposite to the direction of the velocity. From the projection of the above law of acceleration along the axis Ox and integration, we get the law of the velocity.

$$v_x(t) = v_{0x} - \frac{fN}{m} e_v t$$



**Figure 4:** Inversion of time in case of movement with dry friction at sliding

The movement is uniformly decelerated. Through re-integrating in  $\mathbf{t}$ , we receive the Law of movement as well:

$$x(t) = x_0 + v_{0x}t - \frac{1}{2} \frac{fN}{m} e_v t^2$$

Let us now reverse to the direction of time:

$$a'(t') = -\frac{fN'}{m'} e'_v = -\frac{fN}{m} (-e_v) = -a(-t)$$

which is an odd function of time;

$$\begin{aligned} v'(t') &= v'_{0x} - \frac{fN'}{m'} e'_v t' = -v_{0x} - \frac{fN}{m} (-e_v)(-t) = \\ &= -v_{0x} - \frac{fN}{m} e_v t \end{aligned}$$

which is neither even nor odd function. In the particular case when  $v_0 = 0$ , the law of velocity appears even function, but such movement (decelerated motion with zero initial speed) is impossible;

$$\begin{aligned} x'(t') &= x'_0 + v'_{0x} t' - \frac{1}{2} \frac{fN'}{m'} e'_v t'^2 = \\ &= x_0 + (-v_{0x})(-t) - \frac{1}{2} \frac{fN}{m} (-e_v)(-t)^2 = \\ &= x_0 + v_{0x} t + \frac{1}{2} \frac{fN}{m} e_v t^2 \end{aligned}$$

which also is neither even nor odd function. The law becomes an odd function only in the particular case when  $x_0 = 0$  and  $v_0 = 0$ , but such movement is impossible.

Inverted movement should be uniformly accelerated in the opposite direction of the Ox with acceleration  $\vec{\mathbf{a}}'$ , opposite of the velocity  $\vec{\mathbf{v}}'$ ! But such movement is impossible!

Or the general conclusion at considered movement is that it is absolutely irreversible in time. This is illustrated in Figure 4. It is seen that the body cannot go through the same spatial positions with the same size of the velocity, but in reverse order. The reason for this is that the acceleration changes its direction in case of inversion of time - it is always reverse to the velocity, and in this case it is an odd function of time.

### Flow of body by fluid with a moderate velocity (Figure 5)

Consider a body initially at rest, flown by fluid evenly moving along it with small velocity  $\vec{\mathbf{v}}_f = \text{const}$ . The fluid will act on the body with cumulative force:

$$\vec{\mathbf{F}} = r(\vec{\mathbf{v}}_f - \vec{\mathbf{v}}(t))$$

where  $\vec{\mathbf{v}}(t)$  is the velocity of the body in a subsequent moment of time, and  $r$  is a coefficient of proportionality. The body will start to move accelerating in the direction of the force while its velocity becomes equal to that of the fluid. Accordingly, the acceleration of the body will be:

$$\vec{\mathbf{a}}(t) = k|\vec{\mathbf{v}}_f - \vec{\mathbf{v}}(t)|^1 \vec{\mathbf{e}}_v = \frac{r}{m} |\vec{\mathbf{v}}_f - \vec{\mathbf{v}}(t)|^1 \vec{\mathbf{e}}_v$$

From the projection of the vector law along the axis Ox by integrating we receive the law on velocity:

$$v_x(t) = v_{fx} - v_{fx} e^{-\frac{r}{m}t}$$

By reintegrating we receive the law on movement:

$$x(t) = x_0 - \frac{m}{r} v_{fx} + v_{fx} t + \frac{m}{r} v_{fx} e^{-\frac{r}{m}t}$$

Let us now reverse to the direction of time. The above laws become as follows:

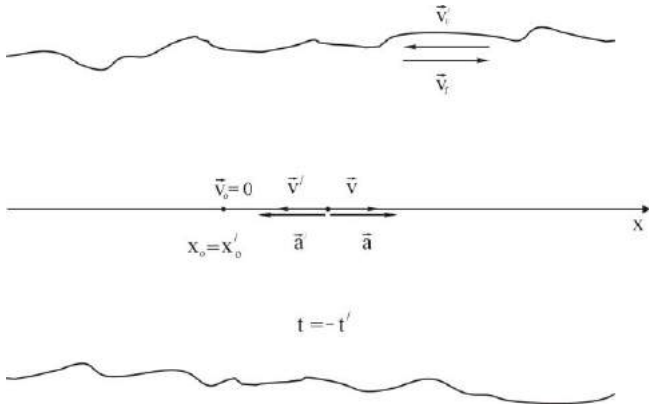


Figure 4: Inversion of time in the case when the body flowing by fluid

$$\begin{aligned} \bar{a}'(t') &= \frac{r'}{m'} |\bar{v}'_f - \bar{v}'_v(t')| \bar{e}_v = \frac{r}{m} |-\bar{v}_f - (-\bar{v}(-t))| (-\bar{e}_v) = \\ &= -\frac{r}{m} |\bar{v}(-t) - \bar{v}_f| \bar{e}_v = -\frac{r}{m} |\bar{v}_f - \bar{v}(-t)| \bar{e}_v = -\bar{a}(-t) \end{aligned}$$

which represents an odd function compared to the inversion of time;

$$\begin{aligned} v'_x(t') &= v'_{fx} - v'_{fx} e^{-\frac{r'}{m'}t'} = \\ &= -v_{fx} - (-v_{fx}) e^{-\frac{r}{m}(-t)} = -v_{fx} + v_{fx} e^{\frac{r}{m}t} \end{aligned}$$

which is neither even nor odd function (it is odd only in the initial moment ( $t = 0$ ));

$$\begin{aligned} x'(t') &= x'_0 - \frac{m'}{r'} v'_{fx} + v'_{fx} t' + \frac{m'}{r'} v'_{fx} e^{-\frac{r'}{m'}t'} = \\ &= x_0 - \frac{m}{r} (-v_{fx}) + (-v_{fx})(-t) + \frac{m}{r} (-v_{fx}) e^{-\frac{r}{m}(-t)} = \\ &= x_0 + \frac{m}{r} v_{fx} + v_{fx} t - \frac{m}{r} v_{fx} e^{\frac{r}{m}t} \end{aligned}$$

which also is neither even nor odd function compared to the inversion of time (it becomes even only at  $v_{fx} = 0$  at the moment of time  $t = 0$ ).

Inverted movement should be decelerated in the opposite direction to the axis Ox with acceleration in the same direction as the velocity, but such movement is impossible. Or this movement is also fully irreversible over time.

Other examples can be given of fully irreversible movements with law of the motion:

$$x(t) = \sqrt[n]{At}$$

where  $n = 2, 3, \dots$ , and  $A$  is a constant, connecting with the measure units:  $A = [m^p/s]$ , but because of the unknown physical character of the forces leading to such movements, they will be not considered here by us.

### General case of completely irreversible movements

The main conclusion that can be made from these examples is that, when the acceleration is an odd function compared to the inversion of time ( $\bar{a}$  has always had a direction parallel to the direction of the velocity), then the respective movement is fully irreversible over time.

All that remains in force when the movement is curvilinear as well, as in contrast to the normal acceleration (which is always even function compared to the inversion of time), the tangential acceleration remains odd function.

The reason for the irreversibility of such movements is that they become under the influence of dissipative or cumulative forces which always dissipate the energy of the moving object in the environment or vice versa. The source of these forces is any point of the environment in which the body moves, in contrast to all other forces, the source of which is localized in space and does not change in the case of inversion of time. Moreover, the presence of such forces at presence of fully reversible or semi-reversible movements radically alter their nature - they become completely irreversible. For example, to consider the constant straight line motion (which otherwise is completely reversible) with dry friction at sliding. Such movement accomplishes through the action of force  $\vec{F}_0$  and force of friction  $\vec{F}_T$ , whose amount is always:  $\vec{F}_0 + \vec{F}_T = 0$ . Acceleration of the point in this movement is:  $\bar{a}(t) = \bar{a}_0(t) + \bar{a}_T(t) = 0$ . Inversion in time changes acceleration as follows:

$\vec{a}'(t') = \vec{a}'_0(t') + \vec{a}'_T(t') = \vec{a}'_0(-t) - \vec{a}'_T(-t) \neq 0$  , which leads to irregular motion - movement becomes irreversible in time.

We shall note the following fact: the irreversible movements can still be carried out at the inversion of time, but this requires the presence of additional force  $\vec{F}_{ad}$  at inverted movement to compensate the irreversibility of the movement. For example, in the above case the inverted movement must be done under the action of forces:

$$\vec{F}'_{ad} + \vec{F}'_0 + \vec{F}'_T = \vec{F}_{ad} + \vec{F}_0 - \vec{F}_T = \vec{F}_{ad} - 2\vec{F}_T = 0$$

i.e. it cannot take place without external influence.

The question arises: can irreversible movements completely to be used to determine the direction of the arrow of time? Yes, because of their irreversibility they can take place only in a certain direction of the course of time. The question is what is this direction - from the past to the future or vice versa? Classical kinematics cannot answer this question. A priori here should be postulated that completely irreversible movements occur always in the direction of the arrow of time "past → future."

### III. RESULTS AND DISCUSSION

We shall summarize the conclusions made here.

1. The time and movement in kinematics are inextricably linked. The very flow of time can only be described by the movement of the bodies. The rest does not allow determining whether time flows at all.
2. Reversal of course of time in kinematics means moving material point to go in reverse order through the same spatial positions with the velocity with the same size but opposite direction.  
Upon inversion of time the spatial positions of the body, covered distance and acceleration (when it does not depend on speed) preserve but direction of motion and velocity change.
3. Equations in kinematics (law of motion, velocity, acceleration, etc.) are reversible when changing direction of time in case of absence of dissipative or cumulative forces. Similar results are obtained also in the papers [6-10].

4. There are three types of movements concerning the inversion of time:
  - a) Fully reversible movements - these are all constant movements. They display that the time is running out, but not determine its direction.
  - b) Semi reversible movements – they are all movements, in which the acceleration is an even function at the inversion of time. They also indicate that time is running out, but not determine its direction.
  - c) Completely irreversible movements - these are movements in which the tangential acceleration is odd function compared to the inversion of time. They can be used to determine the direction of the arrow of time.
5. Reversible and irreversible processes introduced in the thermodynamics ([11], [2], [7], [8], [9], [10]), connecting to the concept of entropy and its growth in a closed thermodynamic system. As can be shown, these processes and the entropy are associated with reversibility and irreversibility of the movement in the kinematics.
6. None movement in kinematics does not allow individual moments of time to be measured directly, but only to be determined time intervals (finite or infinitesimal). This is because the time continuum is assumed to be continuous, i.e. "quantum-time" does not exist. The same applies to the space - we can determine only the lengths (the difference between the positions of two points in it), but we are not able to determine directly the positions themselves of the points in the space. The spatial continuum is continuous and "quantum-space" does not exist as well. The above mentioned is reflected in the main measuring units of time and distance in the system SI - second and meter that are interval values, but not absolute ones.

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# Appraising Use of Mobile Messaging Application as an Additional Tool for Undergraduate Teaching

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## ABSTRACT

This article highlights how undergraduate medical students can use mobile messaging applications for academic purposes with their peers and faculty. Few suggestions along with potential advantages of mobile messaging tools are put forth. The major challenges that could be encountered are also encompassed in the paper.

**Keywords:** Mobile, Messaging, Students, Applications

## I. INTRODUCTION

The profound influence of mobile in our lives today is no news. It has permeated almost all spheres of our lives allowing us to be better, faster and save time and money. It is no different for the education sector as well. Over the last decade, teaching learning modalities have seen drastic change. Use of internet among students has increased manifold and medical students form an integral portion of its users. Moreover the uses of mobile applications are also growing leaps and bounds among the students and they are using it quite pervasively. They are familiar with social networks and pretty comfortable with them.

## II. CONCEPT OF MOBILE LEARNING

It is one of the hot educational trends for the coming years. Apps, and smartphones are storming into classrooms around the world. Mid level and higher education in developed and developing countries are now trying to adopt the use of smartphones in the learning process from different perspectives and teaching methods.[1]

Mobile phones are becoming popular as many people can afford them. Currently the use of mobile communication devices has gone beyond the traditional

communication role that it is now used in supporting teaching and learning. In education, mobile phones have led to the evolution of new paradigm known as mobile learning [2].

Mobile apps represent a potential breakthrough in the way students can learn and the amount they can learn while doing it. These apps can improve information accessibility; facilitate communication enhancing synchronous learning and increase cooperation among students.

Mobile phones can be used for sharing information resources through mobile applications such as *WhatsApp*, *hike*, *viber*, *wechat* etc. which can be used very well for sharing academic information resources. *WhatsApp* and other messaging services could become the next platforms to reach 1 billion users within a year or two [3].

According to Common Wealth of learning [4], teachers can share with students' movies, audio files and other learning materials through their mobile phones.

### A. Suggestions feasible for undergraduate teaching:

Considering these facts we intend to recommend use of mobile applications such as *WhatsApp*, *hike*, *viber* etc.

amongst medical students as a valuable adjunct and an important tool to be utilized for teaching, learning and communicating in medical education.

- We suggest that mini groups of students and mentors on such applications can be made.
- Students should be free to become group members of the same.
- It will create a good platform where they can express their views without any fear.
- Small quizzes, informative pictures, short surveys pertaining to subjects can be posted.
- Even a teacher can periodically upload educational material in the form of videos, illustrations and diagrams.

Visual impressions definitely have several advantages like generating interest in class and improving attitude of students towards content. It fosters in depth learning and leaves indelible visual impact in their mind.

Medical Professionals have started appreciating the use of intelligent, open source lectures available online to supplement their classroom lessons (on myriad topics) and even learn a few things they themselves didn't know before.

It could serve as the best method to obtain feedback from students. They can express difficulties which they are facing in their academic as well as non-academic life. Posts from students should be heartily accepted and need to be encouraged from time to time.

The "new generation" will find something close to their heart.

Though economic difference amongst students is prevailing but smart mobiles are within the reach of everyone. We agree that not all students are using such applications and it may be its biggest limitation but it's always good to start something innovative. Implementation of change in teaching learning methodology has been welcomed from time to time.

Every new idea fetches criticism and our suggestion might encounter or may have to face some contradictory or nasty comments. Nevertheless its better late than never to start something new and if we from faculty community won't change, our students might consider us as "Obsolete". There is enormous potential of cloud-based technology, which would allow students to log in to specific mobile apps in order to access their curricular assignments, thereby creating the scaffold for paperless submission. According to Huang et al [5] mobile

learning applications can facilitate students not only learning contents conveniently but also interacting with others collaboratively anytime and anywhere. Hence, the development of m- learning as a new strategy for education has implications for the way students and tutors in educational institutions interact.

According to Cui and Wang [6], SMS System can be utilized to help students learn many a things and teachers can use SMS to communicate with one student or even one group of students. For instance teachers in higher education in UK have implicated SMS as prompt for course requirements, polling classes, pop quizzes to students and sending information about time table and reminding students about dates for examination [7].

## **B. Benefits of mobile messaging tools:**

### **1. Instantaneous**

It has been found that students check for text messages on their mobile phones frequently and always respond to the arrival tone [8]. Therefore, a significant feature of text messaging is the immediate capture of the recipient's attention. Such attention-getting may lead to an improvement in students' focus and motivation as per Martinez-Torres et al [9] and result in an enhanced learning experience. Allen, Witt, and Wheelless [10] found that an immediate response from the instructor increased students' motivation and the cognitive mastery of material.

### **2. Pervasive**

Text messaging is also an advantage because mobile devices are nearly always turned on and owned by the majority of students [11]

### **3. More fitting than email**

It is notable that text messaging is perceived by students to be more "instant" than email and is the dominant mode of e-communication among students Harley et al [12]. Given its role as a primary communication channel, text messaging has been identified as preferable to email in building both social and academic relationships.

#### 4. Time management

Because of its immediacy and ubiquity, text messaging is particularly well suited to providing time-management assistance to students. Text messaging has been suggested as a means of reminding students of assignment or application due dates, and timetable or procedural changes.

### C. Key Challenging Issues

Despite the massive advantages that mobile phones do have in the teaching and learning process, there are some challenges of m-learning among tutors and students.

Some of the mobile phones do not have programs that have direct compatibility with the academic programs such as pdf, words, excel and PowerPoint. Screen size is another limiting factor for mobile learning.

#### 1. Short messages

Given that a single-text messages is limited to 140 characters, faculty and students may be challenged by the short message length and brevity of language required.

#### 2. Divided attention

When students respond to the arrival tone of new text messages, their attention is drawn away from the current task in which they are engaged. This can be a significant distraction when text messaging is used in class (Markett et al [13] Similar problems exist for instant messaging conversations. Junco and Cotton [14] observed that instant messaging is detrimental to learning because it requires students to split their attention in an academic setting.

#### 3. User fees

Until free services to the education market are provided, cost could be a factor in adopting text messaging for academic purposes [13]. The same challenge does not exist for instant messaging, as nearly all services are available for free. However, the unique challenge to instant messaging is that the user has to be signed in to a computer in order to participate.

#### 4. Personal space

While text and instant messaging can serve to increase familiarity between students and faculty, students were seen to resent such communication when it was used for academic purposes. Students consider their mobile phones a personal technology, and in some cases, disliked receiving text messages from faculty or tutors because it encroached into their personal space as quoted by Brett [15]. Instant messaging is also considered a private, peer-to-peer communication tool, and students do not always want to appear visible to their instructor or to other students.

The teaching staff in many colleges makes calls and sends text messages for alerting students and communicating with colleagues on academic issues.

This article highlights how medical students can use mobile messaging applications for academic purposes with their peers and faculty. Specifically, comfort level, frequency of use, usefulness, reasons for messaging and differences between peer-to-peer and peer-to-instructor interactions are key factors why the students feel that they are very comfortable with using messaging. Students consider mobile messaging as very useful for academic purposes. Key reasons cited for using messaging included saving time, resolving administrative issues, convenience and ease of use. Text messaging appears to be the preferred mode of communication for students with respect to communicating with both peers and instructors.

Since many institutes are offering free Wi-Fi to students, therefore it should be channelized somehow for constructive methods. Students will feel free to come out with their expression on this media as compared to directly expressing their view in front of their mentors or teachers. It can be a reasonably good educational tool despite the presence of certain distractions. Students would agree upon the fact that social networking time can be put to good educational use.

### III. CONCLUSION

To conclude, the use of mobile applications can be envisaged fundamentally as an adjunct to the conventional teaching learning aids to improve students' learning ability. It can be a useful and viable tool for

augmenting student's communication among peers and faculty in medical education.

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# Measurement of Uranium Concentration in the Soil Samples by Using Solid State Nuclear Track Detectors (SSNTDs)

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## ABSTRACT

The research aims to measure the uranium concentration of soil samples collected from different regions are located around the nuclear research center at Tuwaitha to a contamination ratio statement of these regions, which include agricultural residential regions as well as being the regions for livestock of all kinds. The nuclear track detector (CR-39) was used as a method to detect uranium of through calculations depended on comparison with standard samples. Results indicate that the uranium concentration ranged between  $(1.07 \pm 0.46 \text{ ppm})$  and  $(4.20 \pm 0.50 \text{ ppm})$  and with average weighted equal to  $(2.40 \pm 0.22 \text{ ppm})$ , these results are within allowed limit that equals (11.7 ppm).

**Keywords:** Soil, Uranium Concentration, Contamination, CR-39 Detector, Tuwaitha, SSNTD

## I. INTRODUCTION

Natural radiation has always been part of the human environment. Its main components are cosmic and cosmogenic radiation, terrestrial gamma radiation from natural radionuclides in rocks and soil, and natural radioactive substances in our diet and in the air we breathe[1].

Radionuclides are found in the environment as naturally occurring elements and as products or by-products of nuclear technologies, one of the most common radionuclides is uranium (U), all isotopes of uranium are radioactive, so it is very important their quantity to be under control [2].

Technique of the tracks count of the fission fragments was used for find the concentration of uranium in soil, because of its ease and accuracy in determining the emitting elements of the alpha particles even if the concentration is very small, the CR-39 detector is considered of the best detectors to record the tracks of alpha particles and nuclear fission fragments, that is because of the advantage of its high sensitivity and the efficiency[3, 4].

The research aims to determine the concentration of uranium in surface soil in the surrounding regions nuclear research center Al-Tuwaitha, and identify contaminated regions with uranium by using the solid state nuclear track detection technique.

### A. Radiation in Soil

Soil is the upper part of the earth's crust and is formed as a result of rock deformation by complex physicochemical processes, which include weathering, decomposition and water movement, so the soil is the result of the action of weather and human activities on the crust rocks of the earth. The soil is naturally radioactive, because of the mineral content. The natural radioactivity may vary considerably from one type of soil to another[5].

Soil radionuclide activity concentration is one of the main determinants of the natural background radiation. Volcanic geographic structures as well as rocks that are rich in phosphate, granite and salt contain natural radionuclides like uranium-238, thorium-232 and potassium-40. When rocks are disintegrated through natural processes, radionuclides are carried in soil by rain and flows. In addition to the natural sources, soil

radioactivity is also affected from man-made activities[6].

## B. Natural Uranium

The natural uranium includes numerous isotopes, but the most important are three isotopes:  $^{238}\text{U}$ ,  $^{235}\text{U}$ ,  $^{234}\text{U}$ . All uranium isotopes are radioactive, as it is shown in Table (1).

**Table 1.** shown the Natural uranium isotopes [7, 8]

Natural Uranium Isotopes (Alpha emission)			
Isotope	Natural abundance (%)	Half-life (in years)	Decay Energy (Mev)
$^{238}_{92}\text{U}_{146}$	99.284	4.46 billion	4.270
$^{235}_{92}\text{U}_{143}$	0.72	704 million	4.679
$^{234}_{92}\text{U}_{142}$	0.0055	245000	4.859

The nuclei of radioactive elements are unstable, meaning they are transformed into other elements, typically by emitting particles (and sometimes by absorbing particles)[7].

Uranium is toxic for both humans and animals for two basic reasons: as a heavy metal, it has toxic chemical effects, and as an alpha-emitter, it also has radioactive effects.

Exposure to uranium and its compounds can cause adverse health effects due to radiological hazard caused by absorption of radiation emitted from uranium and its decay products. Radiation exposure can originate either from external sources, e.g. an area contaminated with uranium from projectiles which missed their targets, or deposited uranium oxides released from damaged tanks as well as from internal sources, like uranium taken up by inhalation of  $\text{UO}_x$  aerosols or ingestion of contaminated food or water [9].

## C. Solid State Nuclear Track Detectors (SSNTDs)

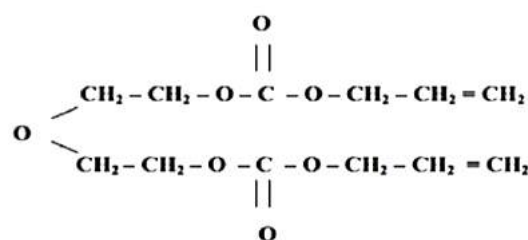
Solid state nuclear track detector (SSNTD) is a widely used technique among the various known methods for

detection of nuclear radiation. This method owes its popularity mainly to its simplicity, cost effectiveness and capacity to store permanent records. Although minerals and glasses have been tested as SSNTDs, the higher sensitivity of the plastic materials to many charged particles and ease of track development and evaluation has made plastic materials more popular as SSNTDs [10].

CR-39 (Polyallyl diglycol carbonate) is a commonly used solid state nuclear track detector (SSNTD). CR-39 detectors are widely used in different branches of sciences such as nuclear physics, radon dosimeter and radiobiological experiments [11]. It was discovered in 1978 by Cartwright, Shirk and Price. Its density is  $1.32 \text{ g.cm}^{-3}$ . The monomer is containing two of Allyl groups:



The chemical form for CR-39 may be written as  $(\text{C}_{12}\text{H}_{18}\text{O}_7)$ . It is illustrated in Fig. (1) [12].



**Figure 1.** The chemical form of CR-39 detector [12].

The detector (CR-39) has a high efficiency to record the tracks in comparison with other detectors and it has some specifications as [13]:

- 1) Optically transparent
- 2) Very sensitive to radiation
- 3) Highly isotropic and homogeneous
- 4) Not cross-linking after radiation damage has broken the chemical bonds
- 5) Having a non-solvent chemical etchant
- 6) This polymer is resistant almost to all solvents, and to the heating Weak links of Carbon increase the sensitivity of the detector to radiation because it breaks easily when exposed to radiation. The lowest rate of charge can be detected in CR-39 [12].

## II. METHODS AND MATERIAL

Soil surface samples were taken from different locations around the Tuwaitha nuclear research center in Iraq, as shown in Table (2). After collecting the samples, The samples were cleaned, placing each soil sample in an oven for drying at a temperature of 80°C for 2h until a constant weight was reached, The dried samples were grinded into a fine powder and passed through a standard mesh with size 75  $\mu\text{m}$ . 0.5gm of soil samples was pressed into a pellet of 1cm diameter and 2mm thickness.

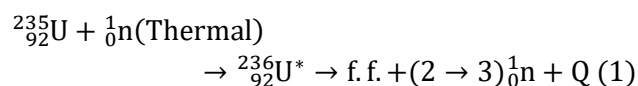
**Table 2.** shows the location and the coordinates (GPS) of the study samples

No.	code	Locations	Coordinates
1	S1	Taha mosque near square Salman	33°12'59.0"N
	S2		44°32'59.3"E
2	S3	Stores of the Center for Research AL-Tuwaitha	33°13'15.9"N
	S4		44°32'22.7"E
3	S5	Municipal Council from front of AL-Tuwaitha N.R.C.	33°13'12.3"N
	S6		44°31'31.3"E
4	S7	Ibn Zahr hospital near AL-Tuwaitha Research Center	33°13'25.4"N
	S8		44°30'39.4"E
5	S9	Beginning AL-Tuwaitha N.R.C.	33°13'19.6"N
	S10		44°30'40.7"E
6	S11	End AL-Tuwaitha N.R.C.	33°12'14.3"N
	S12		44°29'42.3"E
7	S13	Towers high-pressure behind the AL-Tuwaitha N.R.C.	33°10'55.7"N
	S14		44°30'01.5"E
	S15		
8	S16	An agricultural area near to the high-pressure Towers	33°10'40.6"N
	S17		44°29'49.8"E
9	S18	Al Bustan near the end of the AL-Tuwaitha N.R.C.	33°12'05.3"N
	S19		44°29'39.4"E
	S20		
10	S21	Department liquefaction water near AL-Tuwaitha N.R.C.	33°12'54.5"N
	S22		44°30'17.5"E
11	S23	Near a large mound of dirt AL-Tuwaitha N.R.C.	33°12'37.0"N
	S24		44°30'36.2"E
	S25		
	S26		

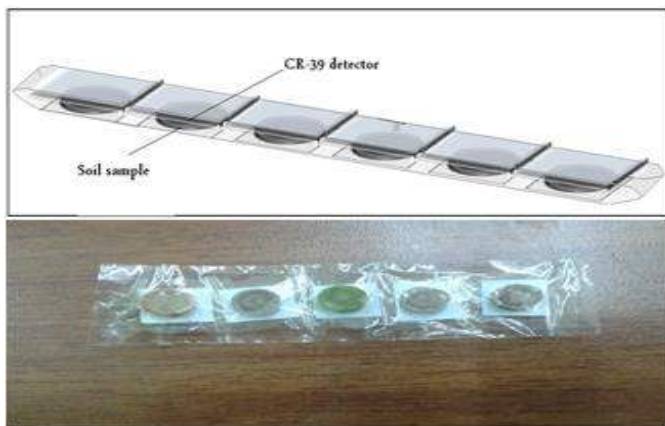
12	S27	Jabir Ibn Abdullah	33°11'46.8"N
	S28	Ansari neighborhood (farm A)	44°32'45.5"E
13	S29	Jabir Ibn Abdullah	33°11'50.5"N
	S30	Ansari neighborhood (farm B)	44°32'39.0"E
14	S31	Jabir Ibn Abdullah	33°11'43.8"N
	S32	Ansari neighborhood (farm C)	44°32'41.7"E
15	S33	Jabir Ibn Abdullah	33°11'39.9"N
	S34	Ansari neighborhood (farm D)	44°32'34.1"E
16	S35	Ishtar region near AL-Tuwaitha N.R.C.	33°11'32.9"N
	S36		44°31'49.3"E
17	S37	Al -Waredya region near AL-Tuwaitha N.R.C.	33°11'16.3"N 44°32'22.5"E

\* (N.R.C) = Nuclear Research Center

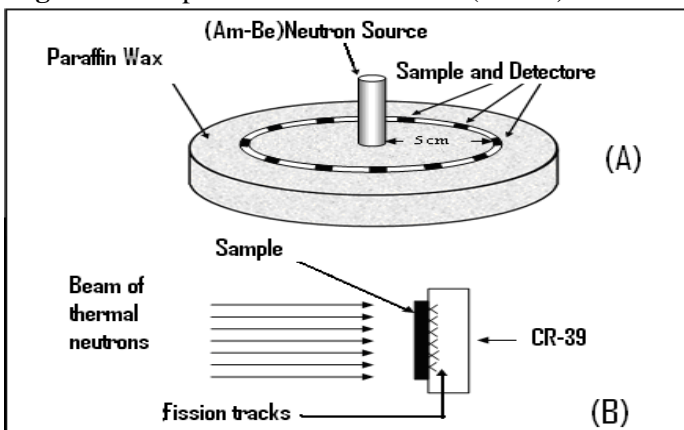
The pellets were covered with (CR-39) detector as shown in Fig (2) and put in a plate of paraffin wax at a distance of (5cm) from the neutron source ( $^{241}\text{Am-}^9\text{Be}$ ) as shown Fig. (3), with flux ( $10^5 \text{ n.cm}^{-2}.\text{s}^{-1}$ ) and the fluence of thermal neutron ( $6.048 \times 10^{10} \text{ n.cm}^{-2}$ ) for (7 days), to obtain induced fission fragments according to the equation (1) :



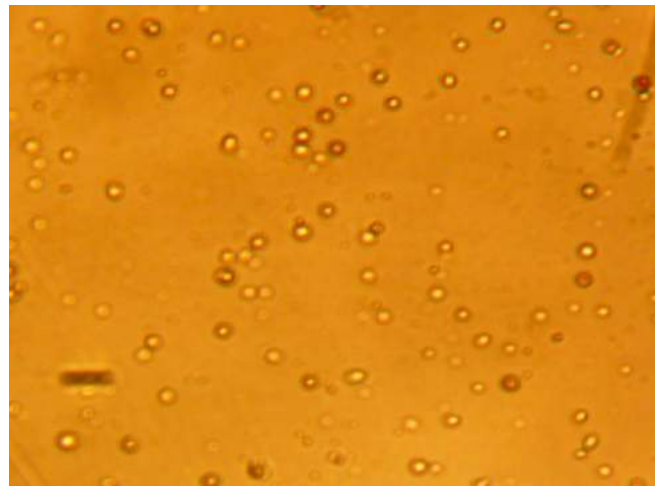
After irradiation CR-39 track detector and the samples, the chemical etching process to the CR-39 detectors has been done in order to show the fission tracks. This operation is for the use of sodium hydroxide solution NaOH as an etchant solution, with normality (6.25N) and temperature (60°C) for (3.5 hours) as the most favorable conditions. After the etching time (3.5h), the detectors have been removed from the etchant solution by forceps and washed with distilled water and then dried.



**Figure 2.** The pellets are covered with (CR-39) detector



**Figure 3.** The irradiation of the detectors and samples by the neutron source.



**Figure 4.** photograph of tracks in a soil sample corresponding to one location.

The uranium concentration in the soil samples was measured by comparing between track densities registered on the detectors of the sample pellet and that of the standard geological sample pellets according to the relation [14]:

$$C_x = C_s (\rho_x / \rho_s) \quad (3)$$

$$C_x = \rho_x / \text{slope} \quad (4)$$

Where:

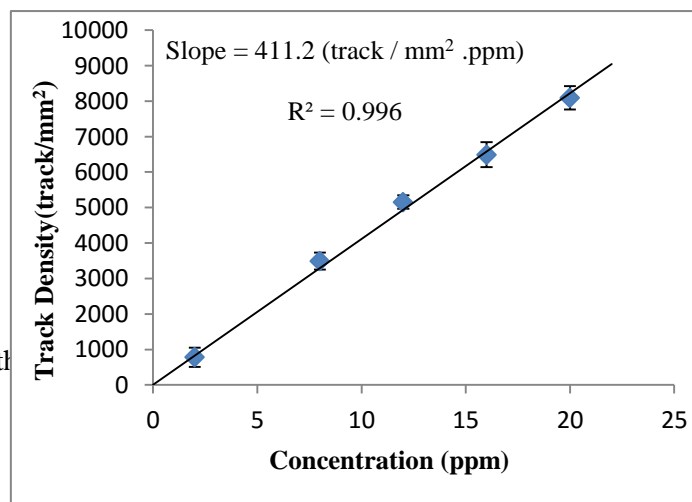
$C_x$  : Uranium concentration in unknown sample (ppm).

$C_s$  : Uranium concentration in standard sample (ppm).

$\rho_x$  : Track density of unknown sample (tracks/mm<sup>2</sup>).

$\rho_s$  : Track density of standard sample (tracks/mm<sup>2</sup>).

The fig. (5) Shows of the relation between uranium concentration and track density in standard sample of the soil.



**Figure 5.** The relation between track density and uranium concentration (ppm) for standard soil samples.

### I. Calculations

After etching chemical, begin the process of the track observation by optical microscope: (Novel) made in China: It is capable of giving magnifications by an objective (4x, 10x, 40x and 100x) and two eyepieces (10x) to measure the number of nuclear tracks . After counting the track of nuclear fission fragments and alpha particles on the surface of the detector, the track density was calculated by using the following equation [14]:

$$\text{Tracks density } (\rho) = \frac{N_{\text{ave}}}{A} \quad (2)$$

Where:

$N_{\text{ave}}$  : Average number of total pits(track).

$A$  : Area of field view.

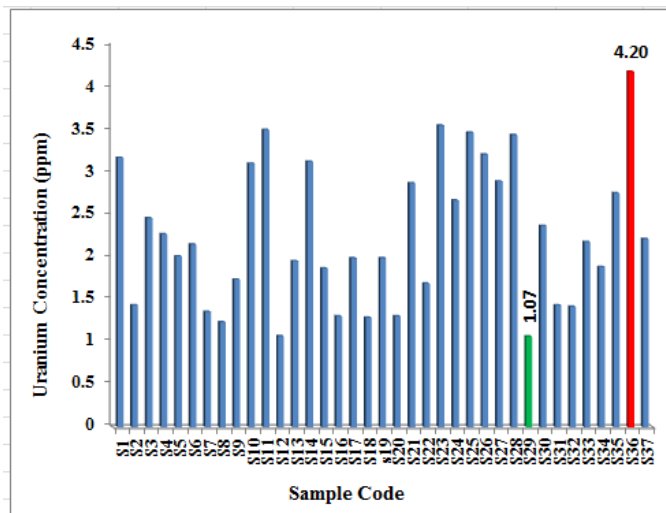
An example of the photograph of observed tracks with the samples is shown in Figure (4).



### III. RESULT AND DISCUSSION

In this research, the soil samples were used from surrounding regions of the nuclear research center at Tuwaitha to know the extent of contamination of these regions with uranium, uranium concentration has been calculated by using technique of counting the tracks of nuclear fission fragments using nuclear track detector (CR-39), and the results were arranged in the Table (3). The selection of these regions to measure the ratio of uranium, depending on several factors, the most important are:

- 1) Because this regions are surrounding the Tuwaitha nuclear research center, which contained several nuclear reactors.
- 2) Because of the nuclear research center at Tuwaitha was looted by some population during the war in Iraq.



**Figure 6.** the concentration of uranium in the soil samples.

Therefore, it was necessary to monitor the level of pollution in those regions for estimating the dangers arising from pollution and processed as quickly and the best ways to ensure the safety of the population. Through the table (3), we find that the concentrations of uranium in soil samples ranging from (1.07 ± 0.46 ppm) in the sample (S29), which located in the region ( Jabir Ibn Abdullah Ansari neighborhood (farm B) ) and (4.20 ± 0.50 ppm) in the sample (S36), which located in the region (Ishtar region near AL-Tuwaitha N.R.C.) ,

and a weighted average equal to (2.40 ± 0.22 ppm). These results are within allowed limit, which is equal to (11.7 ppm)[15], the figure (6) shows the relationship between the uranium concentration and the sample code.

**Table 3.** Results of the uranium concentration in the soil samples.

No. Locations	Sample code	Tracks density (track/mm <sup>2</sup> )	Uranium concentration (ppm)
1	S1	1308.9 ± 322.8	3.18 ± 0.79
	S2	593.0 ± 179.1	1.44 ± 0.44
2	S3	1016.1 ± 454.7	2.47 ± 1.11
	S4	937.5 ± 118.8	2.28 ± 0.29
3	S5	830.4 ± 448.2	2.02 ± 1.09
	S6	887.5 ± 385.4	2.16 ± 0.94
4	S7	560.7 ± 510.8	1.36 ± 1.24
	S8	510.7 ± 180.1	1.24 ± 0.44
5	S9	717.9 ± 265.4	1.75 ± 0.65
	S10	1280.4 ± 175.0	3.11 ± 0.43
6	S11	1444.6 ± 274.1	3.51 ± 0.67
	S12	442.9 ± 163.0	1.08 ± 0.40
7	S13	807.1 ± 339.7	1.96 ± 0.83
	S14	1291.1 ± 179.6	3.14 ± 0.44
	S15	771.4 ± 332.0	1.88 ± 0.81
8	S16	539.3 ± 258.5	1.31 ± 0.63
	S17	821.4 ± 399.8	2.00 ± 0.97
9	S18	532.1 ± 326.3	1.29 ± 0.79
	S19	821.4 ± 229.8	2.00 ± 0.56
	S20	539.1 ± 261.9	1.31 ± 0.64
10	S21	1185.7 ± 340.7	2.88 ± 0.83
	S22	698.2 ± 380.0	1.70 ± 0.92
11	S23	1466.1 ± 120.1	3.57 ± 0.29
	S24	1101.8 ± 191.8	2.68 ± 0.47
	S25	1432.1 ± 302.4	3.48 ± 0.74
	S26	1325.0 ± 157.4	3.22 ± 0.38
12	S27	1194.6 ± 363.7	2.91 ± 0.88
	S28	1419.6 ± 180.4	3.45 ± 0.44
13	S29	440.8 ± 189.2	1.07 ± 0.46
	S30	978.6 ± 132.2	2.38 ± 0.32
14	S31	592.9 ± 356.0	1.44 ± 0.87
	S32	585.7 ± 171.2	1.42 ± 0.42
15	S33	900.0 ± 485.4	2.19 ± 1.18
	S34	778.6 ± 507.3	1.89 ± 1.23
16	S35	1137.5 ± 359.7	2.77 ± 0.87
	S36	1726.8 ± 204.1	4.20 ± 0.50
17	S37	914.3 ± 330.0	2.22 ± 0.80
<b>Weighted average</b>			<b>2.4 ± 0.22</b>
<b>Allowed limit [15]</b>			<b>11.7</b>

#### IV. CONCLUSION

- 1) The highest concentration of uranium in the soil samples was in the sample (S 36) which is equal to  $(4.20 \pm 0.50 \text{ ppm})$ , this value is less than the allowed limit, which is equal to  $(11.7 \text{ ppm})$ .
- 2) The pollution ratio in the region (Ishtar region near AL-Tuwaitha N.R.C.) with uranium is the highest in the comparison with other regions, and this means that the people of this region are the most vulnerable to uranium from the other regions.
- 3) The uranium contamination ratio in (Ishtar region near AL-Tuwaitha N.R.C.) and the region (Near a large mound of dirt AL-Tuwaitha N.R.C.) despite being within allowed limit, but it is the ratio cannot be underestimated, the uranium ratio is relatively high, so it is advisable to processed with all means to ensure the safety of the population from continuous exposure to uranium, while the rest of the proportions of the other regions are reasonable proportions.

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# Design and Fabrication of Wind Mill for Power Generation

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## ABSTRACT

The year 1973 has awakened the entire world from the energy crisis. Since then the entire focus of all developing countries turned towards non-conventional sources of energy. Of all the non-conventional energy sources wind energy is an inexhaustible, non-polluting, freely available energy. Enormous potential is associated with the kinetic energy of the wind. Previously the KE of the wind was utilized to drive wind turbines mostly for pumping water and grinding corns. The first wind turbine was employed for generation electricity in Denmark in 1890. India has started its power generation through (WEGs) from 1986. Later on number of wind electric generators (WEGs) has been installed every year. The paper deals with the technical details involved in the generation of power through wind technology. It discusses the factors responsible for generation of wind power and the limitations of the generator. While the emphasis is given on the various schemes used for production of electricity using wind power, the paper also gives insight into energy storage methods, safety precautions and site selection criteria. Wind power gave rise to public debate as the acceptance of wind turbines decreased during the expansion phase. These challenges were countered by policies enacted by state actors at the regional and local levels. Decisive factors were the amount, duration and reliability of the feed-in compensation, funding policy and the zoning and building laws. The successful establishment of wind power has been possible in spite the fact that it has been difficult to integrate wind power into the energy supply system due to wind power's intermittent nature, and despite resistance from actors of the fossil-nuclear energy supply system. This has been possible as a result of continually adjusting the policy approaches at various governance levels and reflecting various requirements in the different phases of the innovation process. Wind Power Generation in Germany the timing of policies demands a flexible design that is both relevant to a number of different public policy levels, yet tailored to the process in question.

**Keywords:** Power Generation, Wind Mill, Wind Speed, Aerodynamics, Local Ecology

## I. INTRODUCTION

Wind result from air in motion. Air in motion arises from a pressure gradient. On a global basis one primary forcing function causing surface winds from the poles toward the equator is convective circulation. Solar radiation heats the air near the equator, and this low density heated air is buoyed up. At the surface it is displaced by cooler more dense higher pressure air flowing from the poles. In the upper atmosphere near the equator the air thus tend to flow back toward the poles and away from the equator. The net result is a global convective circulation with surface wins from north to south in the northern hemisphere. It is clear from the above over simplified model that the wind is basically

caused by the solar energy irradiating the earth. This is why wind utilization is considered a part of solar technology. It actuality the wind is much more complex. The above model ignores the earth's rotation which causes a carioles force resulting in an easterly wind velocity component in the northern hemisphere. There is the further complication of boundary layer frictional effects between the moving air and the earth's rough surface. Mountains, trees, buildings, and similar obstructions impair stream line air flow. Turbulence results and the wind velocity in a horizontal direction markedly increase with altitude near the surface.

Local winds are caused by two mechanisms. The first is differential hating of land and water. Solar isolation

during the day is readily converted to sensible energy of the land surface but is partly absorbed in layers below the water surface and partly consumed in evaporating some of that water. The land mass becomes hotter than the water, which causes the air above the land to heat up and become warmer than the air above water. The warmer lighter air above the land rises and the cooler heavier air above the water moves in to replace it. This is the mechanism of shore breezes. At night, the direction of the breezes is reversed because the land mass cools to the sky more rapidly than the water, assuming a sky. The second mechanism of local winds is caused by hills and mountain sides. The air above the slopes heats up during the day and cools down at night, more rapidly than the air above the low lands. This causes heated air the day to rise along the slopes and relatively cool heavy air to flow down at night. Wind turbines produce rotational motion; wind energy is readily converted into electrical energy by connecting the turbine to an electric generator. The combination of wind turbine and generator is sometimes referred to as an aero generator. A step-up transmission is usually required to match the relatively slow speed of the wind rotor to the higher speed of an electric generator.

In India the interest in the windmills was shown in the last fifties and early sixties. Apart from importing a few from outside, new designs were also developed, but it was not sustained. It is only in the last few years that development work is going on in many institutions. An important reason for this lack of interest in wind energy must be that wind, in India, is relatively low and varies appreciably with the seasons. Data quoted by some scientists that for India wind speed values lie between 5 km/hr to 15-20 km/hr. These low and seasonal winds imply a high cost of exploitation of wind energy. Calculations based on the performance of a typical windmill have indicated that a unit of energy derived from a windmill will be at least several times more expensive than energy derivable for electric distribution lines at the standard rates, provided such electrical energy is at all available at the windmill site.

The above argument is not fully applicable in rural areas for several reasons. First, electric power is not and will not be available in many such areas due to the high cost of generation and distribution to small dispersed users. Secondly, there is a possibility of reducing the cost of the windmills by suitable design. Lastly, on small scales, the total first cost for serving a felt need and low

maintenance costs are more important than the unit cost of energy. The last point is illustrated easily: dry cells provide energy at the astronomical cost of about Rs.300 per kWh and yet they are in common use in both rural and urban areas.

Wind energy offers another source for pumping as well as electric power generation. India has a potential of over 20,000 MW for power generation and ranks as one of the promising countries for tapping this source. The cost of power generation from wind farms has now become lower than diesel power and comparable to thermal power in several areas of our country especially near the coasts. Wind power projects of aggregate capacity of 8 MW including 7 wind farms projects of capacity 6.85 MW have been established in different parts of the country of which 3 MW capacities have been completed in 1989 by DNES. Wind farms are operating successfully and have already fed over 150 lakh units of electricity to the respective state grids. Over 25 MW of additional power capacity from wind is under implementation. Under demonstration programme 271 wind pumps have been installed up to February 1989. Sixty small wind battery charges of capacities 300 watts to 4 kW are under installation. Likewise, stand-alone wind electric generators of 10 to 25 kW are under installation.

### **A. Utilization of Wind Energy**

The utilization of wind energy can be dated back to 5000 B.C. when sail boats were propelled across the river Nile. It was recorded that from 200 B.C. onwards wind was used as an energy source to pump water, grind grain, and drive vehicles and ships in ancient China and Middle East. The first documented windmill was in a book *Pneumatics* written by Hero of Alexandria around the first century B.C. or the first century A.D. Effectively, these wind mills are used to convert kinetic energy into mechanical energy.

The use of wind energy to generate electricity first appeared in the late 19th century but did not gain ground owing to the then dominance of steam turbines in electricity generation. The interest in wind energy was renewed in the mid-1970s following the oil crises and increased concerns over resource conservation. Initially, wind energy started to gain popularity in electricity

generation to charge batteries in remote power systems, residential scale power systems, isolated or island power systems, and utility networks. These wind turbines themselves are generally small (rated less than 100kW) but could be made up to a large wind farm (rated 5MW or so).

## II. BASIC PRINCIPLES OF WIND ENERGY CONVERSION

The circulation of air in the atmosphere is caused by the non-uniform heating of the earth's surface by the sun. The air immediately above a warm area expands; it is forced upwards by cool, denser air which flows in from surrounding areas causing a wind. The nature of the terrain, the degree of cloud cover and the angle of the sun in the sky are all factors which influence this process. In general, during the day the air above the land mass tends to heat up more rapidly than the air over water. In coastal regions this manifests itself in a strong onshore wind. At night the process is reversed because the air cools down more rapidly over the land and the breeze therefore blows off shore. The main planetary winds are caused in much the same way: Cool surface air sweeps down from the poles forcing the warm air over the tropics to rise. But the direction of these massive air movements is affected by the rotation of the earth and the net pressure areas in the countries-clockwise circulation of air around low pressure areas in the northern hemisphere, and clockwise circulation in the southern hemisphere. The strength and direction of these planetary winds change with the seasons as the solar input varies.

Despite the wind's intermittent nature, wind patterns at any particular site remains remarkably constant year by year. Average wind speeds are greater in hilly and coastal areas than they are well inland. The winds also tend to blow more consistently and with greater strength over the surface of the water where there is a less surface drag. Wind speeds increase with height. They have traditionally been measured at a standard height of ten meters where they are found to be 20-25% greater than close to the surface. At a height of 60 m they may be 30-60% higher because of the reduction in the drag effect of the earth's surface.

### A. The Power in the Wind

Wind possesses energy by virtue of its motion. Any device capable of slowing down the mass of moving air, like a sail or propeller, can extract part of the energy and convert it into useful work. Three factors determine the output from a wind energy converter:

- The wind speed;
- The cross-section of wind swept by rotor; and
- The overall conversion efficiency of the rotor, transmission system and generator or pump.

No device, however well designed, can extract all of the wind's energy because the wind would have to be brought to a halt and this would prevent the passage of more air through the rotor. The most that is possible is for the rotor to decelerate the whole horizontal column of intercepted air to about one-third of its free velocity. A 100% efficient aero generator would therefore only be able to convert up to a maximum of around 60% of the available energy in wind into mechanical energy. Well-designed blades will typically extract 70% of the theoretical maximum, but losses incurred in the gearbox, transmission system and generator or pump could decrease overall wind turbine efficiency to 35% or less. The schematic diagram of turbine wheel is shown in figure 1. The power in the wind can be computed by using the concept of kinetics. The wind works on the principle of converting kinetic energy of the wind to mechanical energy. We know that power is equal to energy per unit time. The energy available is the kinetic energy of the wind. The kinetic energy of any particle is equal to one half its mass times the square of its velocity, or  $\frac{1}{2}m V^2$ . The amount of air passing in unit time, through an area A, with velocity V, is AV, and its mass m is equal to its volume multiplied by its density  $\rho$  of air, or

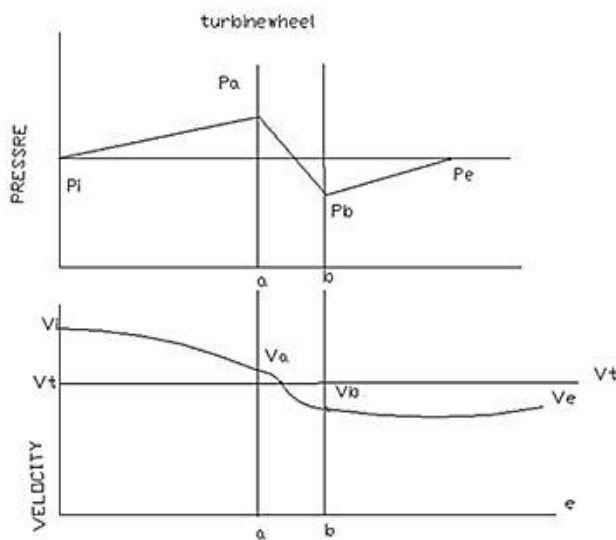
$$m = \rho AV$$

(m is the mass of air transverse the area A swept by the rotating blades of a wind mill type generator).

Substituting this value of the mass in the expression for the kinetic energy, we obtain, kinetic energy =  $\frac{1}{2} \rho AV \cdot V^2$  watts.

$$= \frac{1}{2} \rho AV^3 \text{ watts}$$

Equation tells us that the maximum wind available the actual amount will be somewhat less because all the available energy is not extractable-is proportional to the cube of the wind speed. It is thus evident that small increase in wind speed can have a marked effect on the power in the wind. Equation also tells us that the power available is proportional to air density  $1.225 \text{ kg/m}^3$  at sea level). It may vary 10-15 percent during the year because of pressure and temperature change. It changes negligibly with water content. Equation also tells us that the wind power is proportional to the intercept area. Thus an aero turbine with a large swept area has higher power than a smaller area machine; but there are added implications. Since the area is normally circular of diameter  $D$  in horizontal axis aero turbines, then  $A = \pi/4 D^2$ , (sq.m), which when put in equation gives.



**Figure 1:** Line diagram of Turbine wheel

$$\begin{aligned} \text{Available wind power } P\alpha &= \frac{1}{2} \rho \pi/4 D^2 V^3 \text{ watts} \\ &= 1/8 \rho \pi D^2 V^3 \end{aligned}$$

The power extracted by the rotor is equal to the product of the wind speed as it passes through the rotor (i.e.  $V_t$ ) and the pressure drop  $\Delta p$ . In order to maximize the rotor power it would therefore be desirable to have both wind speed and pressure drop as large as possible. However, as  $V$  is increased for a given value of the free wind speed (and air density), increases at first, passes through a maximum, and then decreases. Hence for the specified free-wind speed, there is a maximum value of the rotor power. Where power available is calculated from the air density, rotor diameter, and free wind speed as shown above. The maximum theoretical power coefficient is

equal to  $16/27$  or  $0.593$ . This value cannot be exceeded by a rotor in a free-flow wind-stream.

## B. Maximum Power

The total power cannot be converted to mechanical power. Consider a horizontal-axis, propeller-type windmill, henceforth to be called a wind turbine, which is the most common type used today. Assume that the wheel of such a turbine has thickness  $\alpha b$ . Let  $p_i$  and  $V_i$  are the wind pressure and velocity at the upstream of the turbine.  $V_e$  is less than  $V_i$  because the turbine extracts kinetic energy. Considering the incoming air between  $I$  and  $a$  as a thermodynamic system, and assuming that the air density remains constant (since changes in pressure and temperature are very small compared to ambient), that the potential energy is zero, and no heat or work are added or removed between  $i$  and  $a$ , the general energy equation reduces to the kinetic and flow energy-terms only:

## C. Wind Energy Conversion

Traditional windmills were used extensively in the middle Ages to mill grain and lift water for land drainage and watering cattle. Wind energy converters are still used for these purposes today in some parts of the world, but the main focus of attention now lies with their use to generate electricity. There is also growing interest in generating heat from the wind for space and water heating and for glass-houses but the potential market is much smaller than for electricity generation.

The term “wind mill” is still widely used to describe wind energy conversion systems, however it is hardly adopt. Description any more. Modern wind energy conversion systems are more correctly referred to as ‘WECS’, ‘aero generators’, ‘wind turbine generators’, or simply ‘wind turbines’. The fact that the wind is variable and intermittent source of energy is immaterial of some applications such as pumping water for land drainage – provided, of course, that there is a broad match between the energy supplied over any critical period and the energy required. If the wind blows, the job gets done; if it does not, the job waits. However, for many of the uses to which electricity is put, the interruption of supply may be highly inconvenient. Operators or users of wind turbines must ensure that there is some form of back-up to cover periods when there is insufficient (or too much) wind available. For small producers, back-up can take the form of :

- Battery storage,
- Connection with the local electricity distribution system; or

For utilities responsible for public supply, the integration of medium – sized and large wind turbines into their distribution network could require some additional plant which is capable of responding quickly to meet fluctuating demand.

### III. WIND DATA AND ENERGY ESTIMATION

The seasonal as well as instantaneous changes in wind both with regard to magnitude and direction need to be well understood to make the best use of them in windmill designs. Winds are known to fluctuate by a factor of 2 or more within seconds (and thus causing the power to fluctuate by a factor of 8 or more). This calls for a proper recording and analysis of the wind characteristics. There are various ways the data on wind behaviour is collected depending on the use it is intended to be put into. The hourly mean wind velocity as collected by the meteorological observations is the basic data used in a windmill designs. The holy means is the one averaged over a particular hour of the day, over the day, month, year and years. The factors, which affect the nature of the wind close to the surface of the earth, they are:

- a) Latitude of the place,
- b) Altitude of the place,
- c) Topography of the place,
- d) Scale of the ours, month or year.

Winds being an unsteady phenomenon, the scale of the periods considered are an important set of date required in the design. The hourly mean velocity (for many years) provides the data for establishing the potential of the place for tapping the wind energy. The scale of the month is useful to indicate whether it is going to be useful during particular periods of the year and what storage if necessary is to be provided for. Since the winds near the surface of the earth are derived from large scale movements of atmospheric winds, the location height above ground level at which the wind is measured and the nature of the surface on earth have an influence on the velocity of wind at any given time. The winds near the surface of the earth are interpreted in

terms of boundary layer concept, keeping in mind the factors that influence its development. The wind velocity at a given height can be represented in terms of gradient height and velocity.

In as much as the height of the windmill rotor depends on the design wind velocity and cost of supporting structure. The above factors have a bearing on the design. Similarly, winds being an unsteady phenomenon, the scale of periods considered for this the temporal parameters (scale of our, month and year) is an important set of data required in the design. While the hourly mean velocity (for many years) provides the data for establishing the potential of the place for tapping the wind energy. The scale of the month is useful to indicate whether it is going to be useful during particular periods of the year and what storage if necessary is to be provided for as already mentioned above. The data based on scale of the hour is useful for mechanical aspects of design. In addition to the data on the hourly mean velocity, two other information's required are:

- Spells of low wind speeds, and
- Gusts

The site choice for a single or a spatial array of WECS (wind energy conversion system) is an important matter when wind electric is looked at from the systems points of view of aero turbine generators feeding power into a conventional electric grid. If the WECS sites are wrongly or poorly chosen the net wind electric generated energy per year may be sub optimal with resulting high capital cost for the WECS apparatus, high cost for wind generated electrical energy, and no returns on investment. Even if the WECS is to the small generator not tied to the electric grid, the sitting must be carefully chosen if inordinately long break even times to the avoided. Technical, economic environmental, social, and other factors are examined before a decision is made to erect a generating plant on a specific site. Some of the main considerations are discussed below.

1. High annual average wind speed. A fundamental requirement of the successful use of WECS, obviously, is an adequate supply of wind has stated above. The wind velocity is the critical parameter. The power in the wind  $P_w$ , through a given cross sectional area for a uniform wind velocity  $V$  is

$$P_w = KV^3$$

Where K is a constant. It is evident; because of the cubic dependence on wind velocity that small increases in V markedly affect the power in the wind, EX. Doubling V, increases P<sub>w</sub> by a factor of 8. It is obviously desirable to select a site for WECS with high wind velocity. Thus a high average wind velocity is the principal fundamental parameter of concern in initially appraising a WECS site. For a more detailed estimate value, one would like to have the average of the velocity cubed. Anemometer data is normally based on wind speed measurements from a height of 10m. For the most accurate assessment of wind power potential it is absolutely essential that anemometer data be obtained at the precise site and hub height for any proposed WECS.

Strategy for sifting is generally recognized to consist of

- Survey of historical wind data.
  - Contour maps of terrain and wind are consulted.
  - Potential sites are visited.
  - Best sites are instrumental for approximately one year.
  - Choose optimal site.
2. Availability of anemometry data. It is another important sitting factor. The principal object is to measure the wind speed, which basically determines the WECS output power, but there are many practical difficulties with the instrumentation and measurement methods. The anemometer height above ground, accuracy, linearity, location on the support tower, shadowing and inaccurate readings there from, icing inertia of rotor whether it measures the horizontal velocity component or vertical, and temperature effects are a few of the many difficulties encountered
  3. Availability of wind V<sub>(t)</sub> curve at the proposed site. This important curve determines the maximum energy in the wind and hence is the principal initially controlling factor in predicting the electrical output and hence revenue returns of the WECS machines.
  4. Wind structure at the proposed site. The ideal case for the WECS would be a site such that the V<sub>(t)</sub> curve was flat, i.e. a smooth steady wind that blows all the time; but a typical site is always less than ideal. Wind especially near the ground is turbulent and gusty, and changes rapidly in direction and in velocity. This departure from homogeneous flow is collectively referred to as “the structure of the wind”.
  5. Altitude of the proposed site. It affects the air density and thus the power in the wind and hence the useful WECS electric power output. Also, as is well known, the winds tend to have higher velocities at higher altitudes. One must be careful to distinguish altitude from height above ground. They are not the same except for a sea level WECS site.
  6. Terrain and its aerodynamic. One should know about terrain of the site to be chosen. If the WECS is to be placed near the top but not on the top of a not too blunt hill facing the prevailing wind, then it may be possible to obtain a ‘speed up’ of the wind velocity over what it would otherwise be. Also the wind here may not flow horizontal making it necessary to tip the axis of the rotor so that the aero turbine is always perpendicular to the actual wind flow.
  7. Terrain and its aerodynamic. One should know about terrain of the site to be chosen. If the WECS is to be placed near the top but not on the top of a not too blunt hill facing the prevailing wind, then it may be possible to obtain a ‘speed up’ of the wind velocity over what it would otherwise be. Also the wind here may not flow horizontal making it necessary to tip the axis of the rotor so that the aero turbine is always perpendicular to the actual wind flow. It may be possible to make use of hills or mountains, which channel the prevailing winds into a pass region, thereby obtaining higher wind power.
  8. Local Ecology. If the surface is bare rock it may mean lower hub heights hence lower structure cost. If trees or grass or vegetation are present, all of which tend to restructure the wind, then higher hub heights will be needed resulting in large system costs than the bare ground case.
  9. Distance to Roads or Railways. This is another factor the system engineer must consider for heavy machinery, structures, materials, blades and other apparatus will have to be moved into any chosen WECS site.
  10. Nearness of site to local center/users. This obvious criterion minimizes transmission line length and hence losses and costs. After applying all the



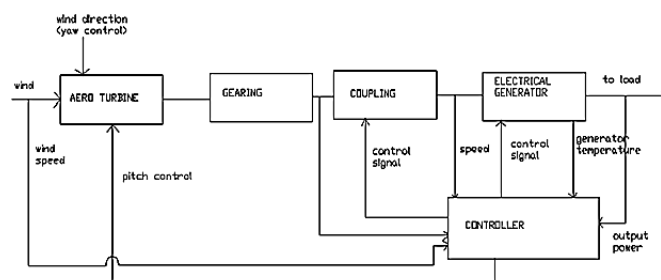
previous sitting criteria, hope fully as one narrows the proposed WECS sites to one or two they would be relatively near to the users of the generated electric energy.

11. Nature of ground. Ground condition should be such that the foundations for a WECS, destroying the foundations for a WECS are secured. Ground surface should be stable. Erosion problem should not be there, as it could possibly later wash out the foundations of a WECS, destroying the whole system.
12. Favourable land cost. Land cost should be favorable as this along with other sitting costs, enters into the total WECS system cost.
13. Other conditions such as icing problem, salt spray or blowing dust should not present at the site, as they may affect aero turbine blades, or environmental is generally adverse to machinery and electrical apparatus.

If the area contains buildings, trees, wind machines, or other obstacles, the variation of the wind speed with altitude above ground level is usually greater for these obstructed areas than for the case of open water and flat plains. The characteristics of a good wind power site may be summarized as follows:

- A site should have a high annual wind speed.
- There should be no tall obstructions for a radius of 3 km.
- An open plain or an open shore line may be good location.
- The top of a smooth, well rounded hill with gentle slopes lying on a flat plain or located on an island in a lake or sea is a good site.
- A mountain gap that produces to wind funnelling is good.

The main components of a WECS are shown in Figure 2 in block diagram form. Summary of the system operation is as follows :



**Figure 2.** The main components of a WECS

Aero turbines convert energy in moving air to rotary mechanical energy. In general, they require pitch control and yaw control (only in the case of horizontal or wind axis machines) for proper operation. A mechanical interface consisting of a step up gear and a suitable coupling transmits the rotary mechanical energy to an electrical generator. The output of this generator is connected to the load or power grid as the application warrants. For localities with the prevailing wind in one direction, the design of the turbine can be greatly simplified. The rotor can be in a fixed orientation with the swept area perpendicular to the predominant wind direction. Such a machine is said to be yaw fixed. Most wind turbine, however, are yaw active that is to say, as the wind direction changes, a motor rotates the turbine slowly about the vertical (or yaw) axis so as to face the blades in to the wind. The purpose of the controller is to sense wind speed, wind direction, shafts speeds and torques at one or more points, output power and generator temperature as necessary and appropriate control signals for matching the electrical output to the wind energy input and project the system from extreme conditions brought upon by strong winds electrical faults, and the like.

The physical embodiment for such an aero-generator is shown in a generalized form. The sub-components of the windmill are:

- Wind turbine or rotor
- Wind mill head
- Transmission and control, and
- Supporting structure

Such a machine typically is a large impressive structure.

## A. Rotors

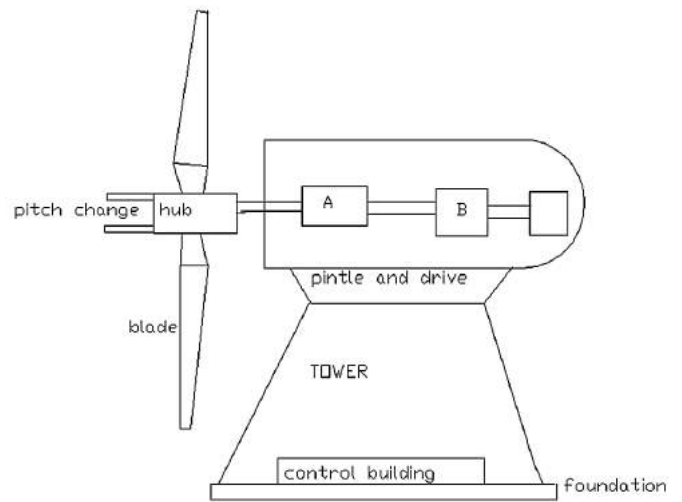
The Rotors are mainly classified into two types:

- (i) Horizontal axis rotor and
- (ii) Vertical axis rotor.

One advantage of vertical – axis machines is that they operate in all wind directions and thus need no yaw adjustment. The rotor is only one of the important components. For an effective utilization, all the components need to be properly designed and matched with the rest of the components. The windmill head supports the rotor, housing the rotor bearings. It also houses any control mechanism incorporated like changing the pitch of the blades for safety devices and tail vane to orient the rotor to face the wind. The latter is facilitated by mounting it on the top of the supporting structure on suitable bearings.

## B. Transmission

Varying the pitch of the rotor blades, conveniently controls the rate of rotation of large wind turbine generators operating at rated capacity or below, but it is low, about 40 to 50 revolutions per minute (rpm). Because optimum generator output requires much greater rates of rotation, such as 1800 rpm, it is necessary to increase greatly the low rotor of turning. Among the transmission options are mechanical systems involving fixed ratio gears, belts, and chains, singly or in combination or hydraulic systems involving fluid pumps and motors. For bottom mounted equipment which requires a right-angle drive, transmission costs might be reduced substantially by using large diameter bearings with ring gears mounted on the hub to serve as a transmission to increase rotor speed to generator speed. Such a combination offers a high degree of design flexibility as well as large potential savings. The transmission system of WEC is shown in figure 3.



**Figure 3:** The transmission system of WEC

## C. Generator

Either constant or variable speed generators are a possibility, but variable speed units are expensive and/or unproved. Among the constant speed generator candidates for use are synchronous induction and permanent magnet types. The generator of choice is the synchronous unit for large aero generator systems because it is very versatile and has an expensive data base. Other electrical components and systems are, however, under development.

## D. Controls

The modern large wind turbine generator requires a versatile and reliable control system to perform the following functions:

- The orientation of the rotor into the wind (azimuth of yaw);
- Start up and cut-in of the equipment;
- Power control of the rotor by varying the pitch of the blades;
- Generator output monitoring – status, data computation, and storage;
- Shutdown and cut out owing to malfunction or very high winds;
- Protection for the generator, the utility accepting the power and the prime mover;
- Auxiliary and / or emergency power; and
- Maintenance mode.

## E. Towers

Four types of supporting towers deserve consideration, these are:

- The reinforced concrete tower,
- The pole tower,
- The built up steel- tube tower, and
- The truss tower.

Among these, the truss tower is favoured because it is proved and widely adaptable, cost is low, parts are readily available, it is readily transported, and it is potentially stiff. Shell-tube towers also have attractive features and may prove to be competitive with truss towers. The type of the supporting structure and its height is related to cost and the transmission system incorporated. It is designed to withstand the wind load during gusts (even if they occur frequently and for very short periods). Horizontal axis wind turbines are mounted on towers so as to be above the level of turbulence and other ground – related effects. The minimum tower height for a small WECS is about 10m, and the maximum practical height is estimated to be roughly 60m. The turbine may be located either upwind or downwind of the tower.

## IV. CLASSIFICATION OF WEC SYSTEMS

The WEC are generally classified into two broad classifications:

- Horizontal Axis Machines.** The axis of rotation is horizontal and the aero turbine plane is vertical facing the wind.
- Vertical Axis Machines.** The axis of rotation is vertical. The sails or blades may also be vertical, as on the ancient Persian windmills, or nearly so, as on the modern Darrieus rotor machine.

Then, they are classified according to size as determined by their useful electrical power output.

- Small Scale (up to 2 kW). These might be used on farms, remote applications, and other places requiring relatively low power.
- Medium Size Machines (2-100kW). These wind turbines may be used to supply less than

100 kW rated capacity, to several residences or local use.

- Large Scale or Large Size Machines (100 kW and up). Large wind turbines are those of 100 kW rated capacity or greater. They are used to generate power for distribution in central power grids. As per the type of output power, wind energy generators are classified as:
  - DC output
  - DC generator
  - Alternator rectifier
  - AC output
  - Variable frequency, variable or constant voltage AC.
  - Constant frequency, variable or constant voltage AC.

As per the rotational speed of the aero turbines, these are classified as:

- Constant Speed with variable pitch blades. This mode implies use of a synchronous generator with its constant frequency output.
- Nearly Constant Speed with fixed pitch blades. This mode implies an induction generator.

Variable Speed with fixed pitch blades. This mode could imply, for constant frequency output:

- Field modulated system
- AC-DC-AC link
- Double output induction generator
- AC commutation generator
- Other variable speed constant frequency generating systems.

Wind turbines are also classified as per how the utilization of output is made:

- Battery storage
- Direct connection to an electromagnetic energy converter
- Other forms (thermal potential etc.) of storage.
- Interconnection with conventional electric utility grids.

The system engineer seeking to integrate WECS will, naturally be most interested in the latter case but should be aware that WECS will, naturally be most interested in the latter case but should be aware that WECS offer other options as well.

### A. Advantage and Disadvantage of WECS

- It is a renewable source of energy
- Like all forms of solar energy, wind power systems are non-polluting, so it has no adverse influence on the environment.
- Wind energy systems avoid fuel provision and transport.
- On a small-scale up to a few kilowatt system is less costly. On a large-scale costs can be competitive with conventional electricity and lower costs could be achieved by mass production.

### B. Disadvantage of wind energy are:

- Wind energy available in dilute and fluctuating in nature.
- Unlike water energy wind energy needs storage capacity because of its irregularity.
- Wind energy systems are noisy in operation; a large unit can be heard may kilometres away.
- Wind power systems have a relatively high overall weight, because they involve the construction of a high tower and include also a gearbox, a hub and pitch changer, a generator coupling shaft etc. for large systems a weight of 110 kg/kW (rated) has been estimated.
- Large areas are needed, typically, propellers 1 to 3 m in diameter, deliver power in the 30 to 300 W ranges.
- Present systems are neither maintenance free not-practically reliable. However, the fact that highly reliable propeller engines are built for aircraft suggest that the present troubles could be overcome by industrial development work.

### C. Horizontal – Axial Machines

The common wind turbine with a horizontal (or almost horizontal) axis, is simple in principle, but the design of a complete system, especially a large one that will produce electric power economically, is complex. Not only must be individual components, such as the rotor, transmission, generator, and tower, be as efficient as possible, but these components must function effectively in combination as shown in figure 4. Some of the horizontal axis type wind machines are below.

- Horizontal axis using two aerodynamic blades. In this type of design, rotor derives generator through a step up gear box. The blade rotor is usually designed to be oriented downwind of the tower. The components are mounted on a bed plate which is attached on a pentle at the top of the tower.
- The rotors blades are continuously flexed by unsteady aerodynamic, gravitational and inertia loads, when the machine is in operation. If the blades are made of metal, flexing reduces their fatigue life with rotor the tower is also subjected to above loads, which may cause serious damage.
- To reduce rotor cost, use of low cost counter weight is recommended which balance long blade centrifugally.

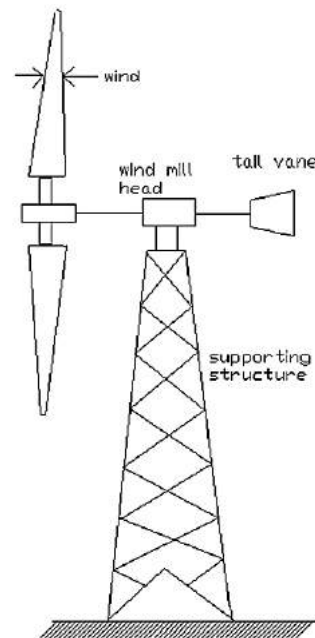
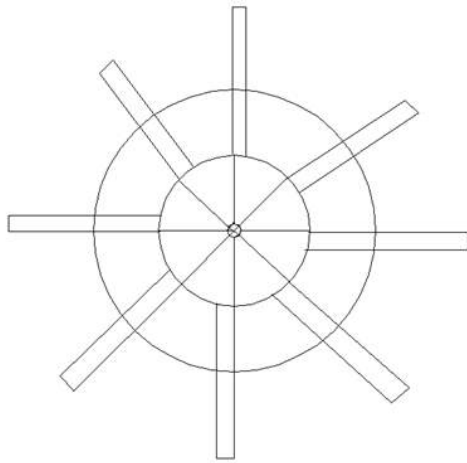


Figure 4 : Horizontal – Axial Machines

### D. Horizontal axis Multibladed Type

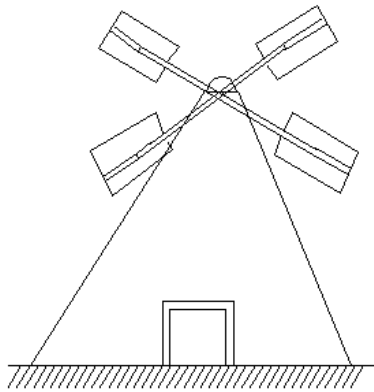
This type of design for multiblades, made from sheet metal or aluminium as shown in figure 5. The rotors have high strength to weight ratios and have been known to service hours of freewheeling operation in 60 km/hr winds. They have good power coefficient, high starting torque and added advantage of simplicity and low cost.



**Figure 5:** Horizontal axis multibladed type

**E. Horizontal axis wind mill-Dutch type.**

The blade surfaces are made from an array of wooden slats which ‘feather’ at high wind speeds as shown in figure



**Figure 6 :** Horizontal axis wind mill-Dutch type

**F. Sail Type**

It is of recent origin. The blade surfaces are made from cloth, nylon or plastics arranged as meet and pole or sail wings. There is also variation in the number of sails used.

**V. POWER GENERATION**

**A. Generating Systems**

Aero turbines convert wind energy into rotary mechanical energy. A mechanical interface, consisting of a step-up gear and a suitable coupling transmits the energy to an electrical generator. The output of this generator is connected to the load or system grid. The controller senses the wind direction, wind speed, power output of the generator and other necessary performance quantities of the system and initiates appropriate control signals to take suitable corrective actions. The system

should be protected from excessive temperature raise of the generator, electrical faults and extra wind conditions as shown in figures 7(top view) and 8. The choice of an electrical generator and control method to be employed (if any) can be decided by consideration of the following three factors:

- The basis of operation i.e. either constant tip speed or constant tip speed ratio.
- The wind-power rating of the turbine and
- The type of load demand e.g. battery connection.

Wind power ratings can be divided into three convenient grouping, small to 1kW, medium to 50 kW and large 200 kW to megawatt frame size.



**Figure 7:** The schematic view WEC



**Figure 8:** The schematic view WEC

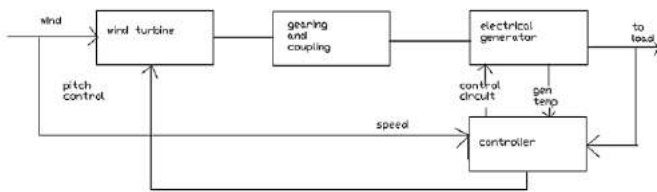


Figure 9: The electrical components of WEC

## B. Schemes for electric generation

Several schemes for electric generation have been developed. These schemes can be broadly classified under three categories:

- Constant – speed constant frequency systems (CSCF)
- Variable speed constant frequency systems (VSCF)
- Variable speed variable frequency systems (VSVF)

## C. Constant speed constant frequency system (CSCF).

Constant speed drive has been used for large generators connected directly to the grid where constant frequency operation is essential.

- Synchronous Generator.

For such machines the requirement of constant speed is very rigid and only minor fluctuations about 1% for short durations (fraction of second) could be allowed. Synchronization of wind driven generator with power grid also will pose problems with gusty winds.

- Induction Generator.

If the stator of an induction machine is connected to the power grid and if the rotor is driven above synchronous speed  $N_s$  ( $N_s = 120f/p$ ), the machine becomes a generator and delivers constant line frequency power to the grid. ( $f$  = line frequency – and  $p$  = number of poles for which the stator winding is made). Per unit slip is 0 and 0.05. The output power of wind driven induction generator is uniquely determined by the operating speed. The pull out torque  $T^m$  condition should not be exceeded. When this happens the speed continues to increase and the system may ‘run away’.

## D Variable speed constant frequency scheme. (VSCF scheme).

Variable-speed drive is typical for most small wind generators used in autonomous applications, generally producing variable frequency and variable voltage output. The variable speed operation of wind-electric system yield higher outputs for both low and high wind

speeds. This results in higher annual energy yields per rated installed kW capacity. Both horizontal axis and vertical axis turbines will exhibit this gain under variable speed operation. The popular schemes to obtain constant frequency output are as follows:

- AC-DC-AC link.

With the advent of high powered thyristor and high voltage D.C. transmission systems, A.C. output of the 3-phase alternator is rectified using a bridge rectifier and then converted back to A.C. using line commutated inverters.

- Double Output Induction Generator.

In this system a slip-ring induction motor is used. Rotor power output at slip frequency is converted to line frequency power by rectification and inversion output power is obtained both from stator and rotor and hence this device is called double output induction generator. Rotor output power has the electrical equivalence of additional impedance in the rotor circuit.

- A.C. Communication generator

This system is also known as Scherbius system employs two polyphase windings in the stator and a commutator winding on the rotor. Basic problems in employing this device for wind energy conversion are the cost and care required by the commutator and the brush gear.

## E. Site selection

Following factors are to be considered for selection of good site for wind power generation:

- High annual wind speed.
- No tall obstructions for a radius of 3 Km.
- Open plain or open shore
- Top of a smooth, well rounded hill with gentle slopes
- Mountain gap which produces wind funneling.

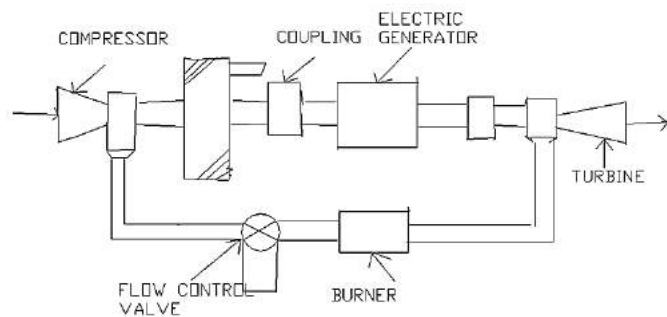
## F. Generating system

Wind - electric conversion system consists of the following components

- Wind Turbine (WT) - Converts wind energy into rotational (mechanical) energy
- Gear system and coupling (G/C) - It steps up the speed and transmits it to the generator rotor
- Generator (G) - Converts rotational energy into electrical energy.

## VI. APPLICATIONS OF WIND ENERGY

Wind power can also be used to compress air for use in various applications, including the operation of gas turbines for generating electricity during the peak-demand periods of a public utility system. For this type of application, conventional gas turbines can be modified to separate the compressor, generator, and power stages by clutches. In one mode of operation, the motor generator operating as a motor and powered by a wind machine drives the air compressor as shown in figure 10. The compressed air is fed into a storage tank or into a large cavern, aquifer, or depleted natural gas well. Under this mode, the power turbine is inoperative, and no fuel is consumed. In a second mode of operation, when the demand for power exceeds the supply of the base-load utility system, the compressor is disengaged, and the power turbine is connected to the generator. The burner that drives the power turbine is fed fuel and compressed air from storage to generate power for the utility system. Wind powered pumps can be used to desalinate water, using reverse osmosis units. Wind powered pumps can also be used to save fuel and electricity by compressing the working fluids used in heat pumps for space heating applications.



**Figure 10 :** The mode of operation

### A. Direct heat applications

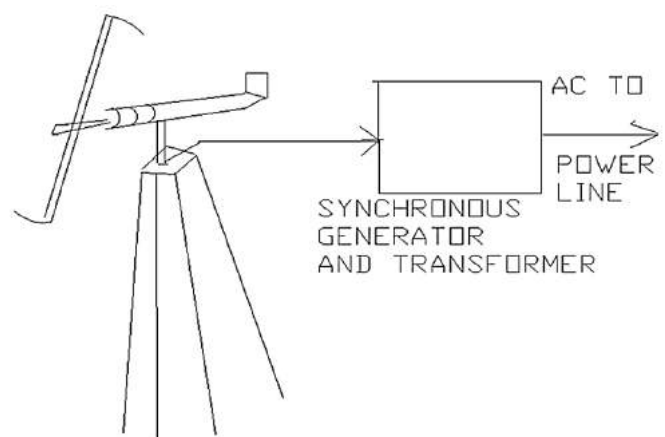
Mechanical motion derived from wind power can be used to drive heat pumps or to produce heat from the friction of solid materials, or by the churning of water or other fluids, or in other cases, by the use of centrifugal or other types of pumps in combination with restrictive orifices that produces heat from friction and turbulence when the working fluid flows through them. This heat may then be stored in materials having a high heat capacity, such as water, stones, eutectic salts, etc.

## B. Electric Generation Applications

Wind power can be used in centralized utility applications to drive synchronous A.C. electrical generators. In such applications, the energy is fed directly into power networks through voltage step-up transformers. WECS units can be integrated with existing hydro electrical networks and used in a "water-saver" mode of operation. When the wind is blowing, electrical energy equal to the amount being produced can reduce generation at the hydroelectric plants in the network. Thus, the wind turbines supply part of the network load that is ordinarily produced by the hydroelectric generators. Under these conditions some of the water that would have been used by the hydroelectric plant to supply the load is saved in the reservoir and made available for later use when the wind is not blowing.

### C. Battery charger

The hydrogen and oxygen can be stored in liquid form in tanks, or in gaseous form in tanks, caverns, aquifers, depleted natural gas wells, etc. The stored hydrogen can be used either as a fuel or direct space heating or industrial process heat, or it can be reconverted to electricity through the use of fuel cells, gas turbine generators that burn hydrogen, or by other means as shown in figure 11.



**Figure 11:** The battery charging unit of WEC

## VII. SAFETY SYSTEMS

The Safety systems of the wind turbines comprise the following features :

- a) The computer. The wind turbine is controlled by a computer which monitors the most important

gauging instruments and compares the results. If errors are found the wind turbine is stopped.

- b) **Emergency stop.** If a situation arises which calls for the wind turbine to be stopped immediately, the emergency stop is used. The wind turbine will stop in few seconds by feathering the blades directly into the wind. It cannot be stated again before what caused the emergency stop has been rectified.
- c) **Revolution Counters.** To prevent the rotor from racing, two revolution counters have been mounted on the shaft. These operate quiet independently and activate the emergency stop if the revolutions of the turbine exceed 24 rpm which is maximum.
- d) **Wind Velocity.** This is measured and controlled by the computer in two ways. First gusts of wind are registered and if they are too strong the turbine is stopped. Then average wind speeds are measured over periods of 10 minutes, and the wind turbine is also stopped if there are too high.
- e) **The Parachutes.** Each blade tip has a parachute, which is activated if the rpm exceeds 28. An iron plumb bob, otherwise held in place by a magnet, is released from the blade trip, the centrifugal force exceeding the force of the magnet pulling out the parachute. This decrease the speed of the wind turbine con
- f) **Siderable enough to stop it from racing.** The parachute is an extra safety device should other fail. Till now they never had been used.
- g) **Lightning Rods.** The tree blades and the mill or wind turbine cap are protected from lighting by these rods going from the tip of each blade to the ground.

#### **A. Environmental Aspects**

Wind turbines are not without environmental impact and their operation is not entirely risk-free. Following are the main effects due to a wind turbine.

- a) **Electromagnetic interference.** Interference with TV and other electromagnetic communication systems is a possibility with wind turbines as it is with other tall structures. TV interference is most likely in areas where there is a weak signal because of the distance from the transmitter, where existing reception is none too good due to the surrounding hills and where the wind turbine is exposed in good position to receive and scatter the signals. Dispensing with aerials and sending TV signals by

cable in areas that would otherwise be affected can overcome interference.

- b) **Noise.** The noise produced by wind farms falls into two categories. The first type is a mechanical noise from the gearbox, generating equipment and linkages and the second type of aerodynamic in nature produced by the movement of the turbine blades. One component of the latter is the broad band noise which ranges up to several kilo hertz and the other is a low frequency noise of 15-20 Hz. Revolving blades generate noise which can be heard in the immediate vicinity of the installation, but noise does not travel too far.
- c) **Visual Effects.** Megawatts power generating wind turbines are massive structures which would be quite visible over a wide area in some locations. Variety characteristics such as colour pattern, shape, rotational speed and reflectance of blade materials can be adjusted to modify the visual effects of wind turbines including the land scape in which they are installed.

#### **B. Advantages of wind energy conversion system**

- The major advantage of this design is that the rotor blades can accept the wind from any compass.
- Another added advantage is that the machine can be mounted on the ground eliminating tower structures and lifting of huge weight of machine assembly, i.e. it can be operated close to the ground level.
- Since this machine has vertical axis symmetry, it eliminates yaw control requirement for its rotor to capture wind energy
- Airfoil rotor fabrication costs are expected to be reduced over conventional rotor blade costs.
- The absence of pitch control requirements for synchronous operation may yield additional cost savings.
- The tip speed ratio and power coefficient are considerably better than those of the S-rotor but are still below the values for a modern horizontal-axis, two-bladed propeller rotor.

#### **C. Disadvantages of wind energy conversion system**

- Rotor power output efficiency of a Darrieus wind energy conversion system is also somewhat lower than that of a conventional horizontal rotor.



- Because a Darrieus rotor is generally situated near ground proximity, it may also experience lower velocity wind compared to a tower mounted conventional wind energy conversion system of comparable projected rotor disc area. This may yield less energy output.

### III. CONCLUSION

This work has provided us an excellent opportunity and experience, to use our limited knowledge. We gained a lot of practical knowledge regarding, planning, purchasing, assembling and machining while doing this project work. We feel that the project work is a good solution to bridge the gates between institution and industries.

- We are proud that we have completed the work with the limited time successfully. The **Domestic Wind Mill Power Generation** is working with satisfactory conditions. We are able to understand the difficulties in maintaining the tolerances and also quality. We have done to our ability and skill making maximum use of available facilities.
- In conclusion remarks of our project work, let us add a few more lines about our impression project work.
- Thus we have developed a “*wind mill power generation*” which helps to know how to achieve non-conventional power generation. The application of pneumatics produces smooth operation.
- By using more techniques, they can be modified and developed according to the applications.

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# Extirpation of Traumatically Ruptured and Maggoted eyeball in a Sambar Deer (*Cervus unicolor*)

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## ABSTRACT

A 10 years old male Sambar deer (*Cervus unicolor*) of Nandankanan Zoo was presented with traumatically ruptured and maggot infested right eyeball in conjunction with clinical signs like severe ptosis, epiphora, orbital inflammation, periorbital swelling, marked pain upon retropulsion, abnormal ipsilateral nasal odour and discharge. In order to correct the thwarted condition, extirpation of eye ball and tarsorrhaphy was performed. Obligation of proper surgical techniques and maintenance of adequate postoperative measures rewarded with prevention of infection into the brain.

**Keywords :** Extirpation, Eyeball, Trauma, Maggot and Sambar Deer

## I. INTRODUCTION

Orbital injuries may be inflicted due to direct or indirect trauma. The degree of injury may vary from a simple contusion or an abrasion of skin in orbital region to the fracture of orbit (Irby, 2004; Das *et al.*, 2008; Khan *et al.*, 2014). The sambar (*Cervus unicolor*) is a large deer native to Indian subcontinent and the state animal of Odisha, characterised by presence of large, rugged antlers. Traumatic injuries courtesy of antlers to the head, orbit or globe are very common in these animals. The present communication deals with successful extirpation of eyeball which had been ruptured due to infight between two sambar deer and subsequent infestation with maggots.

## History

A 10 years old male Sambar deer weighing 220 kg, of Nandankan Zoo was presented with traumatically ruptured and maggot infested right eyeball. Examination and manipulation of ocular and periocular tissues were done after attainment of adequate physical restraint. History of anorexia, depression and physical examination revealed severe ptosis with exposed keratitis, epiphora, orbital inflammation, cellulitis,

periorbital swelling, loss of vision and reflexes, abnormal ipsilateral nasal odour and discharge, marked pain when the affected eye is retropulsed into the orbit (Fig. 1). On the basis of clinical signs, it was decided for extirpation of eye ball and tarsorrhaphy on an emergency basis.



**Figure 1.** Showing traumatically ruptured and maggot infested right eyeball (inset) of Sambar deer (*Cervus unicolor*) in conjunction with clinical signs like severe proptosis, epiphora, orbital inflammation, periorbital swelling, marked pain upon retropulsion, abnormal ipsilateral nasal odour and discharge.

## II. METHODS AND MATERIAL

### Surgical Management

Animal was chemically restrained with a mixture of 330 mg of xylazine hydrochloride (Xylazil®; Troy laboratories Pvt. Ltd.) and 660 mg of ketamine hydrochloride (Ketamil®; Troy laboratories Pvt. Ltd.) darted intramuscularly. After attainment of sedation, the external jugular vein was catheterised and approximately 2 litres of dextrose and normal saline (DNS 5%) was administered intra-operatively. In order to achieve complete analgesia of eyeball four points retrobulbar nerve block was performed by depositing 40 ml of 2% lignocaine hydrochloride solution in the retrobulbar cone using a slightly curved 8.75cm (31/ 2in) 18 gauge needle. Surgical site was cleaned with 1% boric acid solution.

A circumferential approach around the ruptured, swollen eyeball was made by blunt dissection around the orbit until the optic stalk and blood supply were reached. After cruciate ligation of the optic stalk with strong absorbable suture (Catgut #2, Ethicon Inc.), extirpation was performed (Fig. 2). Following surgery, a large dead space was obliterated with sterilised gauze soaked with povidone iodine and Vasocon. Then tarsorrhaphy was done (Fig. 3). After surgery, 33 mg of yohimbine hydrochloride (Antagozil®; Troy laboratories Pvt. Ltd.) was injected intravenously. The deer regained consciousness within two-three minutes. Post-operative antibiotic with ceftriaxone sodium and tazobactam™ 3375 mg ( Intacef Tazo®, Intas Pharmaceuticals Pvt. Ltd.) and analgesic (40 mg of Meloxicam; Melonex®, Intas Pharmaceuticals Pvt. Ltd.) was continued for five days with regular dressing of extirpated orbit and the animal recovered.



**Figure 2.** Showing extirpation of thwarted eyeball after cruciate ligation of the optic stalk with the help of strong absorbable suture threads (Catgut #2).



**Figure 3.** Showing obliterated orbital space followed by tarsorrhaphy.

## III. RESULT AND DISCUSSION

Acquired ocular conditions used to occur at a higher incidence than congenital conditions. Among those acquired conditions serious head, ocular and orbital trauma are always emergencies. Lacerations, ruptured and protrusion of eye ball are the most common traumatic injury in horned animals. Extirpation technique is indicated following ocular trauma leading to panophthalmia, painful glaucomatous eyes, extensive ocular tumours, irreparable injury, orbital abscess, severe trauma with loss of globe. Complete and permanent tarsorrhaphy usually followed to enucleation and extenteration is the best mean of treatment with no postsurgical complications and successful recovery (Irby, 2004; Das *et al.*, 2008; Khan *et al.*, 2014). Maggot wounds are usually present in areas where animals cannot lick and unable to repel the flies. Because of feral nature of the animal, aforementioned condition was lately detected which exacerbated to maggot infestation. Local dressing with magacidal agents like oil of turpentine is used to remove the maggots from the wound, while treating maggot wounds on the face near eyes, the oil may accidentally seep into the eyes causing conjunctivitis (Chawla *et al.*, 2010). Therefore it was decided to go for extirpation instead of daily wound management for better prognosis. In many instances,

these procedures can be done in standing sedated animal. However, general anaesthesia is certainly advantageous when the animal is fractious or has a severe lesion or for humane concerns (Irby, 2004).

#### **IV. ACKNOWLEDGEMENT**

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, Bhubaneswar and Doctor in charge, Veterinary Hospital, Nadankanan Zoo, Bhubaneswar, Odisha for their support and cooperation in carrying out the surgical intervention.

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# Treatment of Haemophilia A by Replacement Therapy using Factor VIII Inhibitors

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## ABSTRACT

Haemophilia A is a very severe disease which is caused due to deficiency of a protein clotting factor VIII. Haemophilia A is X-linked recessive disorder which can be treated by the replacement therapy or by development of an inhibitors or antibodies against the FVIII. Hemarthroses is a disease in which bleeding in the joint spaces occur. This disease occurs approximately 70 to 80% in haemophilic patients. Then here we review about the epidimicity that deals with the transformation of haemophilia A. Then the process of inhibition stated, in which under the influence of some chemical substances the specific chemical process may inhibit. So, different replacement therapy may be ineffective when an inhibitor affect the factor VIII gene. In the end here stated different treatments for the management & control of disease, so among them the counselling between physician & patient is very effective.

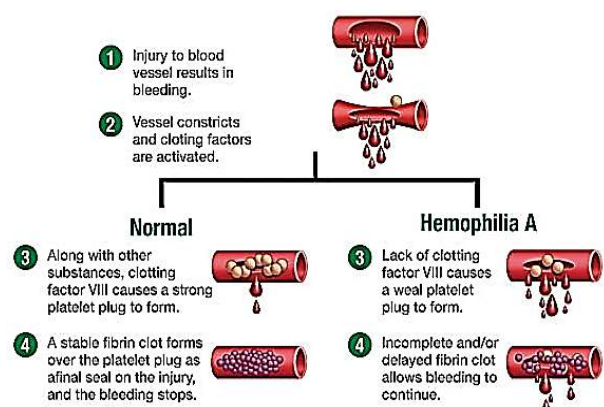
**Keywords :** Hemarthroses, Haemophilia, Epidimicity, Epidemiology, DNA, AVD, IgG, TCR, Arthropathy, Prophylaxis

## I. INTRODUCTION

There are many different types of bleeding disorders in which haemophilia A is known as a result of the deficiency of the protein clotting factor VIII and is genetically severe bleeding disorder. The deficiency of factor VIII in 1 in every 5000 male births without racial majority as X-linked recessive abnormality (Soucie *et al.*, 1998). Haemophilia A, as the most common disorder in patients, is intra-articular hemorrhages. The cure of haemophilia started from the last 40 years. In history, patients of haemophilia, including adults and children, had no ability of survival but now days this disease can be controlled and helps the patients to do their work activities in efficient way (DiMichele & Neufeld, 1998). Proper treatment of these patients requires deeply understanding of AHA path physiology by anaesthesiologists.

There are different therapeutic treatments for other bleeding disorders so that haemophilia A also cured by specific medication and blood products. At present the best treatment of haemophilia A is to replace the factor VIII by using the plasma or recombinant FVIII in order

to stable its homeostasis. When a patient is develop an inhibitor against the factor VIII then the replacement therapy of haemophilia A becomes hopeless. Now a day's development of the inhibitor is the most important treatment of haemophilic patient (Fig. 1). Although there is decrease in death rate due to improvement in homeostatic agent but still there is another complication that is high rate of bleeding (Gringeri *et al.*, 2003; Darby *et al.*, 2004; Morfini *et al.*, 2007; Brown *et al.*, 2009; Di Minno *et al.*, 2010).



**Figure 1.** Difference between a normal and haemophilic blood vessel

## II. METHODS AND MATERIAL

### Epidemiology

The branch of medicines which deals with the transmission and control of diseases in population is known as Epidemiology. Haemophilia A is an x-linked medical disease which is triggered due to deficiency or deprivation of clotting FVIII. If we Talk about its epidemicity then we can see that Europe and some parts of North America is affected by Haemophilia A with the approximate ratio of 1:5000 male births. In other words, it prevails in 20.6 cases per 100,000 males, out of which 60% are severely affects by this disease. In other 40% of the cases, mutation occurs which includes transformation of Haemophilia A with FVIII gene. Other cases involve mutation in the DNA like deletions and insertion of genes (Tuddenham *et al.*, 1994; Ghosh & Shetty, 2009; Astermark 2010). Genetically haemophilia is usually identified during or soon after birth, may be possible during AVD (Assisted vaginal deliveries) and circumcisions (Chorba *et al.*, 1994).

### Inhibitors

A substance capable of stopping or slowing a specific chemical or biological process is known as inhibitor. Antibodies are also known as inhibitors for example immunoglobulin G (IgG) (Scandella *et al.*, 1989). Immune system utilize antibody to determine and neutralize toxin objects for example viruses and bacteria. As we know that antibody is a large Y-shaped protein which is produced by the plasma cells (Fig. 2). There is an increase in risk of inhibitors developing in those chronic haemophilic patients which exposes about 10 to 20 days to FVIII concentrate. The treatment of haemophilic patients with inhibitions is difficult as that of prevention of orthopaedic complication. There is need of improvement in clinical outcomes in order to get a better result. With small exposure, chronic haemophilic patients in childhood about 20 to 30% will develop inhibitions to FVIII. Researchers tell us that there is a mutual relation between the inhibitions and low quality of life. When haemophilic patient is exposing to FVIII again & again then it develops in those patients and binds to inactivate FVIII. There are 2 types of inhibitors which are reversible inhibitors and irreversible inhibitors.



**Figure 2.** The antibodies working as inhibitors

### Mechanism of the development of factor VIII inhibitors

This mechanism is indicated by various studies that FVIII is actually responsible for the activation of immune response. As we know that FVIII is mainly T helper cell-mediated and always present on the antigen presenting cells for example dendritic cells, B cells and macrophages (André *et al.*, 2009; Astermark 2010). Whenever antibodies combines against FVIII then CAAs should incorporate FVIII and then after degeneration it is important that it should introduces to major histocompatibility complex molecules of Class II. After the attachment of FVIII peptides with MHC molecules this complex will accessible for the determination by the CD4+ T cells then in result CAAs will develop a plasma layer complex. Moreover, peptides located or occurring within the cells particles of FVIII, which incorporates in minute quantities in the patient, are introduced via MHC Class I molecular particles to CD8+ T cells which is accepted by antigen T cells receptors (TCRs) (Chaves & Rodrigues, 2009). For the arrangement of antigens to the TCRs in order to be well organized and systemic, a second indication takes place between CAAs and T cells which is the co-triggered CD80/86 molecules declared in CAAs attached to CD28 stated in T cells (Hoyer 1995; Wight & Paisley, 2003). T cells gets activated when both signals are there which can be the type-1 helper T cell (Th1) activation, in charge for the discharge of cytokines as that we know about the work of interferon gamma tumour necrosis factor alpha and interleukin 10 (IL-10), amid others that are significant in humeral resilience or immunity.

In addition to this, the declaration of CD2, CD30, CD40 and CD28 expands on the CD4+ T cell surface (Oldenburg & Pavlova, 2006; André *et al.*, 2009; Ghosh & Shetty, 2009; Pratt & Thompson, 2009). When excreted by the Th1 or Th2 then these all cytokines induces the distinction or divergence of B cells, which alters the isotype of the immunoglobulin and yield

particular antibodies opposed to plasma VIII. Moreover, the B cells discharge the cytokine interleukin-12 (IL-12) which quicken the Th1-mediated growth of Interferon. The cytokines from Th1 evoke the progress of immunoglobulin G1 and immunoglobulin G4 (IgG4). The titers of highly responsive inhibitors are coordinated to IgG4 levels as shown in patients with haemophilia A, which proposes that the Th2-mediated immune reaction is strongly affiliated to the combination of anti-FVIII antibodies (Pratt & Thompson, 2009).

### **Factors that help to prompt patients for the development of factor VIII inhibitors**

#### **Ethnicity and family history of factor VIII inhibitors**

Ethnicity and genealogy is well known with the respect to inclination for your real advancement connected with FVIII inhibitors. One study revealed that the Afro-Americans had very high danger of production of inhibitors (Gill 1999). In the Malmo international brother study the affiliation and genealogy of the advancement of inhibitors were seen. The results of this study show the rate of inhibitors is a brilliant well spring of the sub group of individuals of African drops in correlation to Caucasians (55.6% contrasted with 27.4%). It's accepted that this real racial part will be based upon hereditary variations with all due respect system reaction determinants, essentially on the grounds that FVIII transformation range won't contrast in the middle of races (Astermark *et al.*, 2001). Moreover, it was observed that the danger for the influencing of inhibitors increases specially in those patients which already had a family history of the improvement in inhibitors was 48% (95% certainty interim 95% CI :11-12%).

An alternate study by the same gathering surveyed the likelihood of hereditary changes affecting the arrangement of inhibitors relating to haemophilia in people of the same group. One hundred and thirteen primarily Caucasian families had two or more siblings with serious haemophilia were broke down. All siblings present in the 59 of the families created inhibitors from which 25 (42.4%) of the persons contained a family history of inhibitor improvement. It was discovered that the most widely recognized kind of transformation in the FVIII quality was reversal of intron. Inhibitors were

distinguished in the 45 out of the 74 families (60.8%) with the help of transformation and in 18 (40%) out of these 45 different families all the siblings created inhibitors. On the behalf of this information, the impact of hereditary variables in the advancement of inhibitors is evident. Non hereditary components likewise seem to impact the invulnerable reaction and therefore change the danger of creating inhibitors in every crew. In any case it is impossible that these variables alone can clarify the similitudes reported. These perceptions propose that there is necessary need of changes in the invulnerable reaction that may be focused around both hereditary markers and non-hereditary factors.

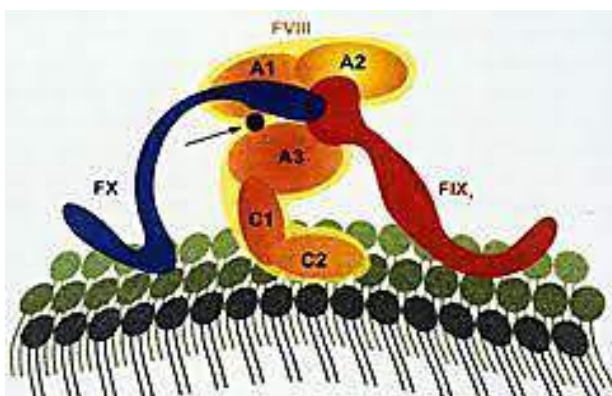
#### **Mutation occurs in the FVIII gene**

The quality of factor VIII gene is spotted by the presence on the end of the lengthy arm of chromosomes X. It includes 186,000 base sets disseminated between the 26 exons and the 25 introns. The result of this quality of the factor VIII is a polypeptide containing 2332 amino acids (inactivated flowing master cofactor) and the enacted polypeptide is framed of six masterminded domains (Shen *et al.*, 2008; Gitschier *et al.*, 1984; Castaldo *et al.*, 2007). The spaces of A2, A3 and C2 are the areas against which FVIII antibodies can respond and hinder the coagulation cascade (Scandella *et al.*, 1989). It was demonstrated by complete study that the patients with the missense transformations are grouped in the areas of A2 and C2 then the danger of inhibitor arrangement is fourfold more noteworthy than in the patients having changes outside this area. This demonstrates that any progressions in the 3 dimensional structures then a piece of the FVIII atom may influence its immunogenicity. The opposition to A2 antibodies and at same time against C2 antibodies can connect with the A2 space and C2 area individually and kill the procoagulant action of FVIII (fig 3). The C2 space however influenced by against C2 antibodies counteract FVIII tying to phospholipids and von will brand factor and then hostile to A3 antibodies which then focus on the A3 area to keep the collaboration of the important FIX with the activation of FVIII gene which is responsible for the treatment of haemophilia (Lenting *et al.*, 1996).

In the year 2012, 5243 sorts of transformations connected with this disease had been accounted for as



per the hamsters (test of mutation in haemophilia A and also site of resource search) electronic database. The gathering with the most astounding danger of delivering hostile to FVIII antibodies is unified with the finest variations in gene. One Meta investigation watched that the danger of inhibitor development in the patients with vast cancellations and babble changes is always greater than in those patients which have intron 22 reversals (pooled OR=3.6 as well as= 1.4, separately). The danger of patients with intron 1 reversals and join site changes is practically equivalent (pooled OR= 0.9 as well as=1.0 separately) and the danger of patients having small amount of cancellations and insertions and missense transformations are lower (pooled OR= 0.5 as well as= 0.3, respectively) (Gouw *et al.*, 2012). Reversals in intron 22 (30 half) and intron 1 (0-5%) are generally connected with extreme haemophilia A phenotype and a middle person hazard for structuring inhibitors. In Brazil, an investigation of 86 Caucasian patients, examined the event of reversal transformations present in the 47 patients from which 33 (70%) had serious haemophilia and 14 (30%) with moderate or mellow haemophilia. An increment in the recurrence of reversal transformations (13/13; 39.4%) was seen in the subgroup named serious with the larger part (11/13; 86.4%) being changes in intron 22 (Soares *et al.*, 2001).



**Figure 3:** Mutation in factor VIII gene

### Arthropathy

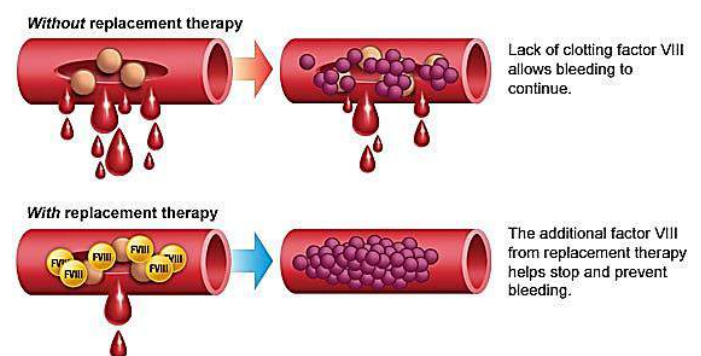
Arthropathy is the term which is used for the diseases and abnormalities of joints (ex. hemarthroses). Hemarthroses is a condition in which there is bleeding in the joint spaces and this condition occurs in haemophilic patients about 70 to 80% (Parameswaran *et al.*, 2005). There are different causes of this disease like injury,

knee joint arthroplasty and inflammation in the joints (DiMichele & Neufeld, 1998). This disease can be treated by the administration of clotting factor. Arthropathy is most commonly occurs in haemophilic patients. This condition occurs during the twenty to thirty series of life. The condition can be quite painful and they usually require treatment to inhibit further degeneration of the joint. Uncontrolled bleeding is the cause of severe arthropathy in haemophilic patients usually in young patients. The symptoms of arthropathy depend on the root cause. The risk of joint diseases and other sicknesses can be reduced by staying active and eating a balanced.

## III. RESULT AND DISCUSSION

### Treatment

Concentrated FVIII was first produced using a cry precipitation method in 1965 that was simply duplicated by blood banks to make use of it for routine analysis and treating joint and muscle haemorrhage (Tullis *et al.*, 1965; DiMichele & Neufeld, 1998). In spite of the fact that this method provided immense clinical advantages to patients of haemophilia, it also caused rise in risk of blood borne aggressive viral infectious disease. Aggressive transmission of HIV and HCV happened in the haemophilia group via contaminated human plasma products (Tencer *et al.*, 2007). It was estimated in 2004 that minimum one third of victims who got affected by haemophilia were also affected with HIV and 80% with HCV (Fig. 4).



**Figure 4.** Replacement therapy through additional factor VIII

This calamity for the haemophilia group not only caused the application of screening but also viral inactivation procedures by using heat methodologies. In spite of the fact that products obtained from plasma are more

secured it normally faces infectious instruments that might be against the current procedures for viral, chemical or even physical inactivation (non-lipid enveloped diseases). There may not have been announced cases of HIV or HCV transferrable congealing factor since 1986 and 1997 (National Haemophilia Foundation 2012). Recently, a HIV transferred case from the exchange of fresh frozen plasma in USA highlights the possibility of transferable wrapped up diseases from human derived blood products is not zero (Centers for disease control and prevention, 2010).

Substantial developments have been witnessed in haemophilic victims without inhibitors. Patients with serious haemophilia had an expected life of 11 years in 1960 and it grew to 50 years in 1980s (Strine et al., 1994; Chorba *et al.*, 2001). Latest advancement in treatment of haemophilia focuses to lessen the mortality rate and upgrade the life standard of haemophilic patients. Patients with inhibitors have less medical treatment options compared with those without one. The focus is to stop critical bleeding spells in patients with inhibitors and maintain homeostasis. In these days, there are two bypassing agents accessible ways for nursing haemophilia A patients with inhibitors. Recombinant factor 7a is approved to sustain homeostasis in haemophilic patients with inhibitors. ITT is composed by managing repeat high doses of F7. This method later enables substituting F7 to restarting usage effectively. ITT was victorious in more than 80% of the patients with inhibitors according to international immune tolerance registry. By comparing high dosage and low dosage, only 69% patients were successful but bleeding spells were considerably reduced in high dosage community but unfortunately, these findings didn't affect the outcome significantly.

### **Prophylaxis and Episodic Treatment of Bleeding Disorders**

Patients of Chronic haemophilia suffer from continuous bleeding in joints. The result of chronic haemophilia is severe pain and weak joints. Prophylaxis is the prevention of disease and control of its possible spread over the society. Prophylactic treatment is the preventive treatment of hemarthrosis and musculoskeletal disorders which are common in haemophilic patients. If we talk

about the levels of prevention so in primary prevention we can see that it is the method to avoid occurrence of disease by removing disease agents and increases the resistance to disease. For example by immunization, by maintaining healthy diet, exercise and by avoid smoking. The secondary level of prevention is the method which is used to detect an existing disease before its symptoms will appear. The treatment of hypertension by cancer screening is one of the examples. Then the tertiary prevention is the method which is used to reduce all the negative impacts of disease, whose symptoms are known, such as disability or death by the rehabilitation and treatment e.g., by surgical procedures. When prophylactic treatment begins before the age of three years, best results are achieved.

Episodic treatment of haemophilic patients is also an effective method. Episodic treatment is a pattern of medical & nursing in which services are provided to a particular patient, without an ongoing relationship being established between person and health professionals. For example in emergency department there are many advantages of this type of treatment like orthopaedic function becomes better and it also improves overall quality of life. It also helps in rapid bleed control (Lusher 1998). In USA 251u/kg to 40u/kg of FVIII three times weak of haemophilia.

### **Primary Care and Haemophilia Treatment Centres**

In the treatment of haemophilia A, interaction with a physician is very important for patients and their families. The relationship of patient with physician is very critical for the long time treatment with the respect to prophylaxis. In some cases, patients are responsible for their own care (Geraghty *et al.*, 2006). Recently, by the studies of treatment centres it proves that increase in only one physician per 10,000 individual populations was associated with an average reduced in death rate of 49 per 100,000 people per year. These studies also showed that a patient which is treated at HTCS has death rate only 40% but in comparison with a patient which is treated by local hospitals carries towards more severe sickness (Soucie *et al.*, 2000).

#### IV. CONCLUSION

Haemophilia A can be treated by the replacement of FVIII and it may also be treated by introducing an inhibitor. This disease can be treated by complete contact with consultant and by proper care. This may also be treated by giving people awareness at each level starting from schools. Episodic treatment is also very affective for haemophilic patient.

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# Electron Transfer Reaction of Pyridine with 12-Tungstocobaltate (III) in Acidic Buffer Medium

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## ABSTRACT

The electron transfer reaction of pyridine with 12-tungstocobaltate (III) was studied spectrophotometrically over the range  $3.8 \leq 10^4[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}} \leq 4.2$ ,  $4.2 \leq 10^2 [\text{PyN}]_{\text{T}} \leq 12.6$ ,  $3.8 \leq \text{pH} \leq 5.4$  and  $313\text{K} \leq T \leq 328\text{K}$ . The reaction shows zero order dependence in  $[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}}$  and first order in  $[\text{PyN}]_{\text{T}}$  and inverse dependence in  $[\text{H}^+]_{\text{T}}$ .  $k_{\text{obs}}$  and second order rate constant,  $k_2$  were found to increase with the increase in temperature. The activation parameters,  $\Delta H^\ddagger$  (kJ/mol) and  $\Delta S^\ddagger$  ( $\text{JK}^{-1}\text{mol}^{-1}$ ) were found to be 53.16 and -34.22. Negative value of  $\Delta S^\ddagger$  indicates ordered transition state for the electron transfer reaction.

**Keywords :** Pyridine, Tungstocobaltate(III), Spectrophotometer, Kinetics, Product Analysis, Activation Parameters.

## I. INTRODUCTION

The oxidant Keggin type 12-tungsto cobaltate (III), ( $\text{Co}^{\text{III}}\text{W}_{12}\text{O}_{40}^{5-}$  or  $\text{Co}^{\text{III}}\text{W}^{5-}$ ) Cluster has a reduction potential of  $1.0\text{V}^1$ . Hence it can accept electrons from pyridine. The above cluster is known for photochemical hydrogen generation and is involved in multielectron reduction process<sup>2-4</sup>. The redox behaviour of above cobalt cluster with few biomolecules like ascorbic acid<sup>5</sup>, glutathione<sup>6</sup>, citric acid<sup>7</sup>, DL-methionine<sup>8</sup> and cystine<sup>9</sup> have been investigated., In order to examine the redox behavior of 12-tungstocobaltate(III), its reaction with pyridine has been undertaken.

## II. METHODS AND MATERIAL

### A. Experimental

$[\text{Co}^{\text{III}}\text{W}_{12}\text{O}_{40}]^{5-}$  was prepared by the reported method<sup>10</sup> and was characterized spectro photometrically<sup>11</sup> and by elemental analysis. All chemicals used were of AnalaR grade. Pyridine (Merck) was used as such. Ionic strength was maintained with  $\text{NaClO}_4$  (Merck). Electron transfer reaction was studied at  $3.8 \leq \text{pH} \leq 5.4$  using a pre-standardised Elico (India) digital pH meter equipped with glass electrode. The pH of solution was maintained by using potassium hydrogen phthalate, sodium acetate and acetic acid buffer solution. The solutions were

prepared in freshly prepared doubly distilled water using an all quartz distillation apparatus containing little amount of  $\text{KMnO}_4$  crystals.

### Kinetics Measurement

Kinetics measurements were performed using systronic 2202 UV-vis spectrophotometer. The kinetics was followed under pseudo first order conditions keeping large excess of [pyridine] over [oxidant]. The reaction was initiated by mixing previously thermostated solution of [pyridine] and  $[\text{CoW}_{12}\text{O}_{40}]^{5-}$  which contained required amount of acidic buffer and double distilled water. The progress of the reaction was followed spectrophotometrically at 388 nm (spectral scan) by monitoring decrease of absorbance of oxidant with time. The pseudo-first order rate constant ( $k_{\text{obs}}$ ) were determined from the slope of linear plot of  $\ln(A_t - A_\infty)$  vs  $t$  (s) using EXCEL programme.

$$\ln(A_t - A_\infty) = \ln(A_o - A_\infty) - k_{\text{obs}}t \quad (1)$$

where  $A_t$  and  $A_\infty$  are the absorbance of the reaction mixture at time 't' and at equilibrium respectively. The reported data represents an average of duplicate runs. The rate constant were reproducible within  $\pm 5\%$  and the reaction was studied upto 80% completion. The co-

relation coefficients of plots used to determine  $k_{obs}$  were found to be 0.99 in most of the cases.

### Stoichiometry and Identification of the product

The stoichiometry of the electron transfer reaction was determined in the following way. The pH of the mixture containing tungstocobaltate (III) and pyridine in a mole ratio (1:5) was maintained at pH = 5.4 and the temperature of mixture was maintained at 50°C and kept about 6 hours. After the reaction was completed, the solution was analysed by determining the unreacted tungstocobaltate spectrophotometrically. It was found that 2 moles of oxidant reacted with 1 mole of pyridine. Therefore the stoichiometry of reaction can written as

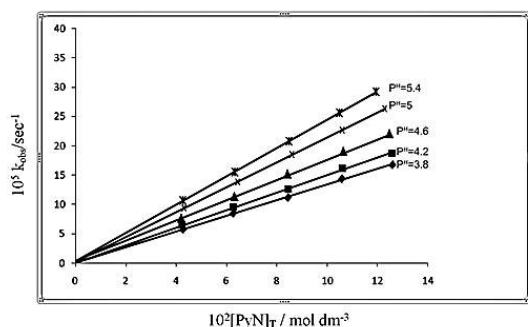
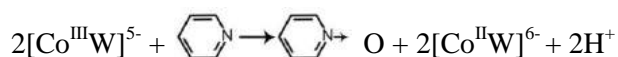


Figure - 1 The Plot of  $10^2[\text{PyN}]_T / \text{mol dm}^{-3}$  vs  $10^5 k_{obs}/\text{sec}^{-1}$

Conditions :  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T = 4.2 \times 10^{-4}\text{M}$

Temp = 50°C

$\lambda = 388.5 \text{ nm}$

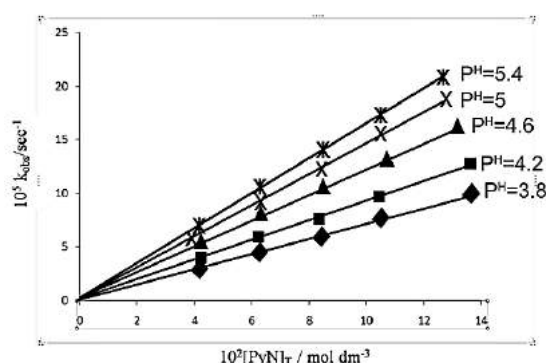


Figure - 2 The Plot of  $10^2[\text{PyN}]_T / \text{mol dm}^{-3}$  vs  $10^5 k_{obs}/\text{sec}^{-1}$

Conditions :  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T = 4.2 \times 10^{-4}\text{M}$

Temp = 40°C

$\lambda = 388.5 \text{ nm}$

At the end of the redox reaction, the solution was treated with Dowex 50 x 8 ( $\text{Na}^+$  form) resin to remove metal ion. A part of the solution shows the presence of Cobalt(II)

by Kitson method. The remaining solution was evaporated at low temperature till the volume was reduced to 1/3rd of the initial volume of the solution. It was kept in refrigerator overnight. The crystalline product was isolated and it was filtered and dried in desiccator. FTIR spectra of the product were determined. Fig. 2.(a) is the FTIR spectra of the reactant which is compared with the FTIR spectra of the product Fig.2(b). Most of the peaks showing very slight shift and one new peak appeared at  $1315\text{cm}^{-1}$  in Fig.2(b), due to  $\text{N}=\text{O}$  stretching frequency without disturbing the molecular frame of pyridine. Hence the isolated product was identified as pyridine N-oxide.

### III. RESULT AND DISCUSSION

#### Rate dependence on $[\text{Co}^{\text{III}}\text{W}^{5-}]_T$

Keeping all conditions constant,  $[\text{Pyridine}] = 12.6 \times 10^{-2} \text{ mol dm}^{-3}$ , pH= 5.4, temperature = 50°C,  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T$  was varied from  $3.8 \times 10^{-4}$  to  $4.2 \times 10^{-4} \text{ mol dm}^{-3}$ . The average  $k_{obs}$  value did not change appreciably and was found to be  $(3.48 \pm 0.5)10^{-5} \text{ sec}^{-1}$ . This fact indicates that the reaction is showing zero order dependence in  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T$ .

#### Rate dependence on pH

Keeping all the conditions constant,  $[\text{Pyridine}] = 12.6 \times 10^{-2} \text{ mol dm}^{-3}$  and  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T = 4.2 \times 10^{-4} \text{ mol dm}^{-3}$ , temperature = 50°C, when pH changes from 3.8 to 5.4, rate constant increases from  $16.8 \times 10^{-5} \text{ sec}^{-1}$  to  $34.8 \times 10^{-5} \text{ sec}^{-1}$ . The rate of reaction was found to increase with the increase in pH.  $\text{P}K_b$  of pyridine = 8.77 at 25°C. So it indicates that at pH= 8.77, there will be 50% protonated form of pyridine. As pH increased from 3.8 to 5.4, the percentage of neutral form was increased. Hence, the rate of reaction was increased, because the protonated form of pyridine could not participate in electron transfer reaction.

#### Rate dependence on [pyridine]

With increasing  $[\text{pyridine}]$  at a given pH and at a given temperature with constant  $[\text{oxidant}]$ , the rate of the reaction is found to increase (Table-1&2). Keeping  $[\text{Co}^{\text{III}}\text{W}^{5-}]_T = 4.2 \times 10^{-4} \text{ mol dm}^{-3}$ , temp = 50°C when

[Pyridine] were varied from  $4.2 \times 10^{-2} \text{ mol dm}^{-3}$  to  $12.6 \times 10^{-2} \text{ mol dm}^{-3}$ ,  $10^5 k_{\text{obs}}$  increased from 12.5 to  $34.8 \text{ sec}^{-1}$  at  $\text{pH} = 5.4$ . This shows that as the [pyridine] is increased at a given pH, the concentration of neutral form was increased and hence the rate of reaction was increased.

**Table 1.** Pseudo- first order rate constants for electron transfer reaction of 12-tungstocobaltate(III) with pyridine  
 $[\text{Co}^{\text{III}}\text{W}^5]_{\text{T}} = 4.2 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $\text{temp} = 50^{\circ}\text{C}$ ,  $\lambda = 388\text{nm}$

$10^2[\text{PyN}]_{\text{T}} \text{ mold m}^{-3}$	pH	$10^5 k_{\text{obs}}$
12.6	5.4	34.8
	5.0	24.8
	4.6	22.0
	4.2	18.5
	3.8	16.8
10.5	5.4	25.7
	5.0	23.5
	4.6	21.8
	4.2	19.2
	3.8	16.2
8.4	5.4	21.0
	5.0	19.2
	4.6	18.2
	4.2	15.7
	3.8	14.5
6.3	5.4	16.3
	5	14.7
	4.6	10.3
	7.2	9.83
	3.8	7.66
4.2	5.4	12.5
	5.0	10.66
	4.6	8.66
	4.2	7.50
	3.8	6.83

$10^2[\text{PyN}]_{\text{T}} \text{ mold m}^{-3}$	pH	$10^5 k_{\text{obs}}$
12.6	5.4	19.5
	5.0	15.2
	4.6	11.5
	4.2	9.66
	3.8	7.83
10.5	5.4	17.0
	5.0	13.3
	4.6	10.7
	4.2	8.50
	3.8	6.85
8.4	5.4	14.2
	5.0	12.4
	4.6	9.4
	4.2	7.6
	3.8	5.9
6.3	5.4	10.5
	5.0	9.4
	4.6	8.6
	7.2	6.3
	3.8	5.8
4.2	5.4	5.0
	5.0	4.3
	4.6	3.8
	4.2	3.2
	3.8	2.98

**Table -2.** Pseudo- first order rate constants for electron transfer reaction of 12-tungstocobaltate(III) with pyridine

$[\text{Co}^{\text{III}}\text{W}^5]_{\text{T}} = 4.2 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $\text{temp} = 40^{\circ}\text{C}$ ,  $\lambda = 388\text{nm}$

**Table 3.** Electron transfer rate constant, activation parameters and thermodynamic parameters for redox reaction of 12-tungstocobaltate (III) with pyridine.

Condition : pH=5.4, [PyN] = 12.6 x 10<sup>-2</sup> mol dm<sup>-3</sup>,  
[Co(III)] = 4.2 x 10<sup>-3</sup> mol dm<sup>-3</sup>

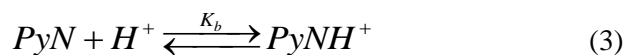
Tem p (t) (°C)	10 <sup>3</sup> k <sub>ob</sub> s	10 <sup>5</sup> k <sub>ob</sub> s (sec <sup>-1</sup> ) 1)	k <sub>2</sub> = $\frac{k_{obs}}{[PyN]}$ (mol <sup>-1</sup> lit sec <sup>-1</sup> )	k <sub>2</sub> /T	Log (k <sub>2</sub> /T)
55	3.0	45.3	3.6 x 10 <sup>-3</sup>	1.179	0.0715
50	3.1	34.8	2.8 x 10 <sup>-3</sup>	0.892	7
45	3.1	22.5	1.8 x 10 <sup>-3</sup>	0.568	-
40	3.2	19.5	1.5 x 10 <sup>-3</sup>	0.484	0.0495
				5	8
					-
					0.2456
					-
					0.3147

$\Delta H^\ddagger = 53.16 \text{ kJ mol}^{-1}$        $\Delta S^\ddagger = -34.22 \text{ JK}^{-1} \text{ mol}^{-1}$   
 $\Delta G^\ddagger (298\text{K}) = 63.3 \text{ kJ mol}^{-1}$

### Rate dependence on temperature

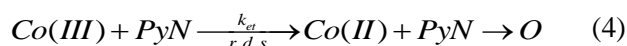
Keeping all the conditions constant, the rate of the redox reaction was increased when temperature was increased. This can be explained on the basis of Arrhenius equation. Basing on the above facts, the mechanism of reaction can be delineated and rate law can be derived.

### Mechanism



$$K_b = \frac{[PyNH^+]}{[PyN][H^+]}$$

$$[PyNH^+] = K_b [PyN][H^+]$$



$$Rate = k_{et} [PyN]_e [Co(III)]$$

$$[PyN]_T = [PyN]_e + [PyNH^+]_e$$

$$= [PyN]_e + K_b [PyN][H^+]$$

$$= [PyN]_e \{1 + K_b [H^+]\}$$

$$[PyN]_e = [PyN]_T / \{1 + K_b [H^+]\}$$

$$Rate = \frac{k_{et} [PyN]_T [Co(III)]}{1 + K_b [H^+]} \quad (5)$$

$$Rate = k_{obs} [Co(III)] \quad (6)$$

$$k_{obs} = \frac{k_{et} [PyN]_T}{1 + K_b [H^+]} \quad (7)$$

$$k_{obs} (1 + K_b [H^+]) = k_{et} [PyN]_T \quad (8)$$

The plot of L.H.S. = k<sub>obs</sub> (1+K<sub>b</sub>[H<sup>+</sup>]) vs [PyN]<sub>T</sub>, is linear. Slope of the plot is equal to k<sub>et</sub>. The value of k<sub>et</sub> at temperature range 313K to 328K are collected in Table 3. The ΔH<sup>‡</sup> (kJ/mol) = 53.16 and ΔS<sup>‡</sup> (JK<sup>-1</sup> mole<sup>-1</sup>) = -34.22 has been calculated using Eyring equation. since K<sub>b</sub>(PyN) = 1.7 x 10<sup>-9</sup> (25°C), the product K<sub>b</sub>[H<sup>+</sup>] (in the pH range 3.8 to 5.4) will be small in comparison to 1.0. Hence plot of k<sub>obs</sub> vs [PyN]<sub>T</sub> is linear (fig 4 & 5). Negative activation entropy indicates that the reaction is passing through an ordered transition state.

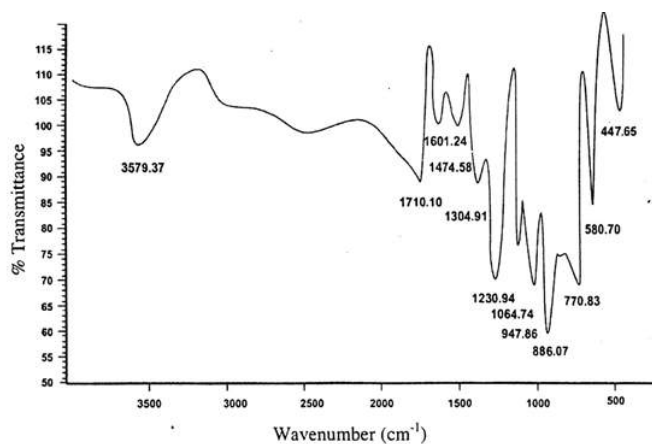


Figure-3 FTIR spectra of 12- tungstocobaltate



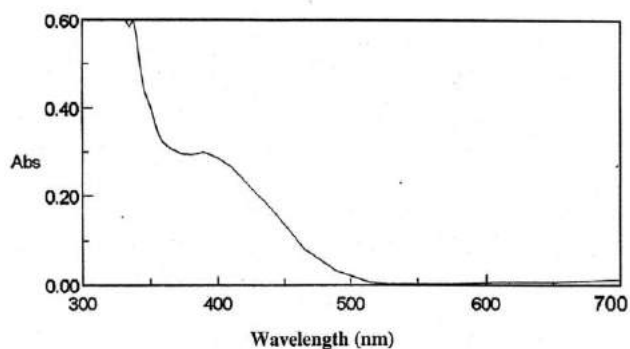


Figure-4 UV-Vis spectra of the 12- tungstocobaltate

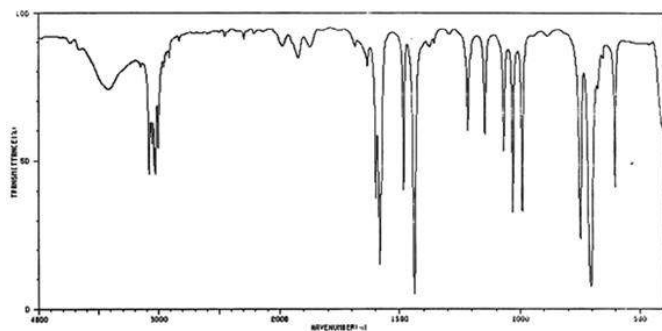


Figure 5. FTIR spectra of Pyridine Oxide

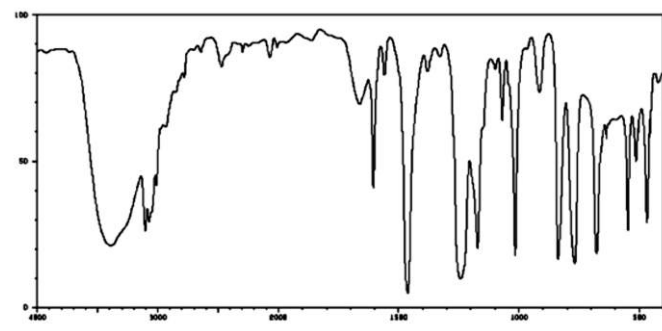


Figure 6. FTIR spectra of Pyridine Oxide

#### IV. CONCLUSION

The Kinetics of redox reaction between 12-tungstocobaltate (III) and pyridine under varying pH, temperature, concentration of pyridine shows first order dependence in [PyN], inverse dependence in  $[H^+]$ , Zero order in 12-tungstocobaltate (III). The overall stoichiometry found to be 2:1 corresponding to oxidant and reductant. Second order electron transfer rate was found to increase with increase in temperature. Moderate activation parameters favour the electron transfer process.

#### V. ACKNOWLEDGEMENT

One of the author B. Dash is thankful to HOD, Chemistry, Department of Chemistry Utkal University, Bhubaneswar for providing necessary facilities and also thankful to principal, Dhamnagar College, Dhamnagar, Bhadrak for allowing to complete research work for Ph.D degree.

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# Modelling and Structural Analysis of a Pressure Hull under Dynamic Load

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## ABSTRACT

Pressure hulls are the main load bearing structures of naval submarines, and autonomous underwater vehicles (AUVs). A pressure hull is a structure that is designed to withstand the compressive forces associated with hydrostatic pressure. In this thesis, the assembly of the pressure hull including Navigation compartment shell, Battery compartment shell which is connected with bolts is designed which is used at present. Around 16 M12 bolts are used to connect the Navigation compartment shell and Battery compartment shell. These shells are to be disassembled very frequently for battery charging and other maintenance purpose. It is observed that the disassembling and assembling process is a very time consuming because it requires unbolting and bolting of all the 16 bolts every time. A new design of pressure hull is proposed where the bolts are removed and the navigation compartment and battery compartment are welded. In the present paper, design calculations are done as per ASME codes to withstand the pressure of 65 Bar. The design constraints considered for the analysis were stresses and deflections. The pressure hulls have been modeled considering the elliptical cross section. The modeling of the pressure hull has been carried out by pro-5.0 and analysis software is ANSYS 14.0 software packages

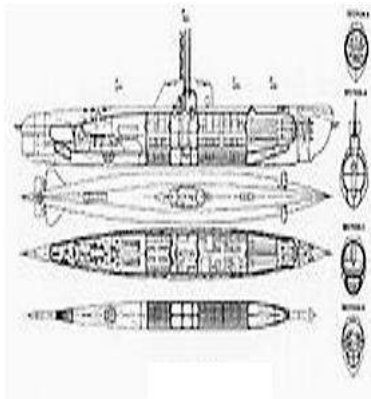
**Keywords :** Pressure hulls, steel, Static analysis, dynamic analysis.

## I. INTRODUCTION

A light hull (casing in British usage) of a submarine is the outer non-watertight hull which provides a hydrodynamic ally efficient shape. The pressure hull is the inner hull of a submarine; this holds the difference between outside and inside pressure.

Type XXI U-Boat, late WWII, with pressure hull almost fully enclosed inside the light hull Modern submarines is usually cigar-shaped. This design, already visible on very early submarines is called a "teardrop hull", and was patterned after the bodies of whales. It significantly reduces the hydrodynamic drag on the sub when submerged, but decreases the sea-keeping capabilities and increases the drag while surfaced.

## SUBMARINE HULL



## TYPES

All small modern submarines and submersibles, as well as the oldest ones, have a single hull. However, for large submarines, the approaches have separated. All Soviet heavy submarines are built with a double hull structure, but American submarines usually are single-hulled. They still have light hull sections in bow and stern, which house main ballast tanks and provide hydro dynamically optimized shape, but the main, usually cylindrical, hull section has only a single plating layer.

## LIGHT HULL

The double hull of a submarine is different from a ship's double hull. The external hull, which actually forms the shape of submarine, is called the outer hull, casing or light hull. This term is especially appropriate for Russian submarine construction, where the light hull is usually made of steel that is only 2 to 4 millimetres thick, as it has the same pressure on both sides. The light hull can be used to mount equipment, which if attached directly to the pressure hull could cause unnecessary stress.

## PRESSURE HULL

Inside the outer hull there is a strong hull, or pressure hull, which actually withstands the outside pressure and has normal atmospheric pressure inside. The pressure hull is generally constructed of thick high-strength steel with a complex structure and high strength reserve, and is separated with watertight bulkheads into several compartments. The pressure and light hulls aren't separated, and form a three-dimensional structure with increased strength. The interhull space is used for some of the equipment which doesn't require constant pressure to operate. The list significantly differs between submarines, and generally includes different water/air tanks. In case of a single-hull submarine, the light hull and the pressure hull are the same.

## II. METHODS AND MATERIAL

### A. Specification of the Problem

The objective of the present work is to design and analyses, of steel pressure hull including Navigation compartment shell; Battery compartment shell which is connected with bolts is designed. These shells are to be disassembled very frequently for battery charging and other maintenance purpose. It is observed that the disassembling and assembling process is a very time consuming because it requires unbolting and bolting of all the 16 bolts every time. A new design of pressure hull is proposed where the bolts are removed and the navigation compartment and battery compartment are welded. Pressure hull is created in Pro-E 5.0. Model is imported in ANSYS 14.0 for analysis by applying normal load and dynamic load conditions. After analysis

a comparison is made between existing in terms of deflections and stresses, to choose the best one.

### B. Structural Analysis of Pressure Hull

Dimensions of the structural and dynamic analysis the design of pressure hull without bolts and pressure hull with bolts. Pressure hull consists of 5 layers (thickness of each layer, 0.6mm). Diameter of the pressure hull is 125mm. Since the properties of pressure hull vary with directions of a 3-D model of pressure hull is used for analysis in ANSYS 14.0. The loading conditions are assumed to be static and dynamic. The element chosen is SHELL LINEAR LAYER 99, which is a layered version of the 8-node structural shell model. The element has six degrees of freedom at each node: translations in the nodal x, y, and z directions and rotations about the nodal x, y, and z-axes. The finite element analysis is carried out on pressure hull with bolts as well as pressure hull without bolts. From the analysis the equivalent stress (Von-mises stress) and displacements were determined and are shown in figure 2-15. Table 2 - 4 shows the Comparative structural and dynamic analysis of a pressure hull with bolts and without bolts.

### C. Specifications of Existing Pressure Hull

Table 1 shows the specifications of a steel pressure hull of alister 3000. The typical chemical composition of the material is 0.565C, 1.8% Si, 0.7%Mn, 0.045%P and 0.045% S.

Sr.no	parameters	value
1	Total length of pressure hull(Eye to Eye)	245mm
2	Diameter of pressure Hull	125 mm
3	Thickness of pressure Hull	3 mm
4	Applying the pressure	6.326 N/mm <sup>2</sup>
5	Young's modulus of steel	2.1e5 N/mm <sup>2</sup>
6	Density of pressure Hull	7860 kg/m <sup>3</sup>
7	Yield strength	500 N/mm <sup>2</sup>
8	Tensile strength	620 N/mm <sup>2</sup>
9	Passion ratio	0.3

## D. Structural and Dynamic Analysis of Pressure Hull

### A. Pressure Hull with Bolts

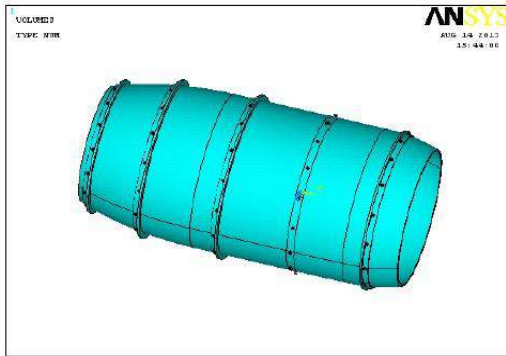


Figure 2. Analysis of pressure hulls without bolts

### 1. Structural Analysis

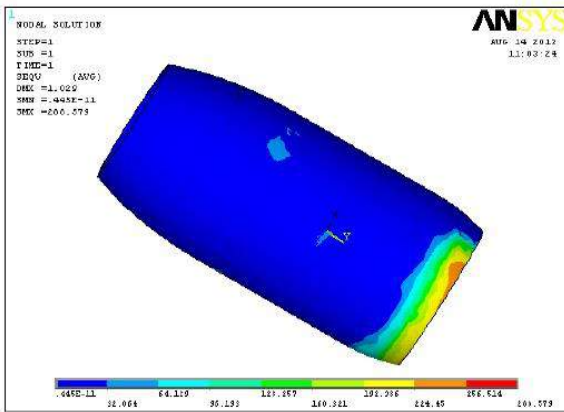


Figure 3. stress distribution for pressure hulls

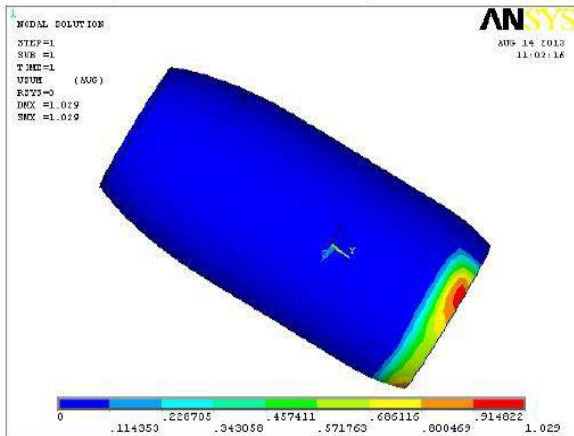


Figure 4. Displacement pattern for pressure hulls

### 2. Dynamic Analysis : 10 Secs

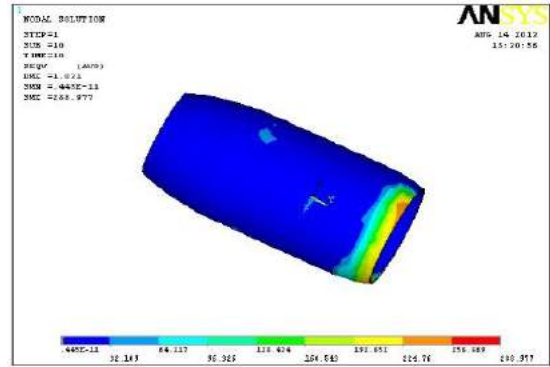


Figure 5. Stress distribution for pressure hulls

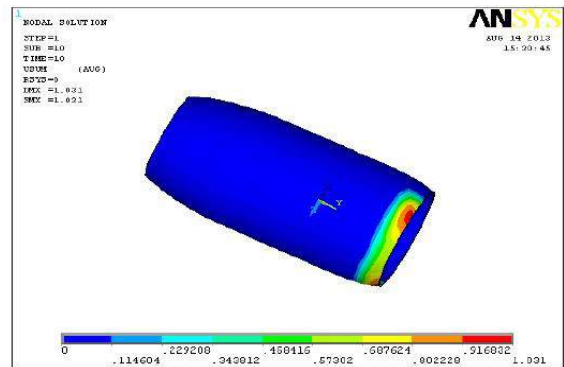


Figure 6. Displacement pattern for pressure hulls

### 3. Dynamic Analysis : 20 Secs

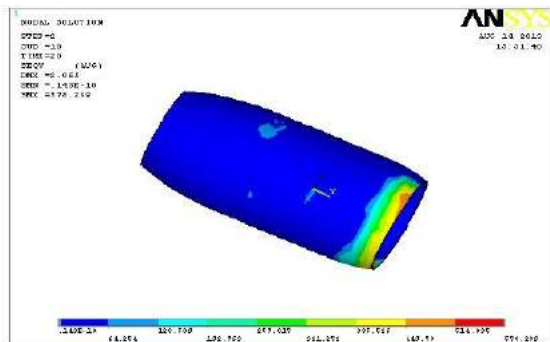


Figure 7. Stress distribution for pressure hulls

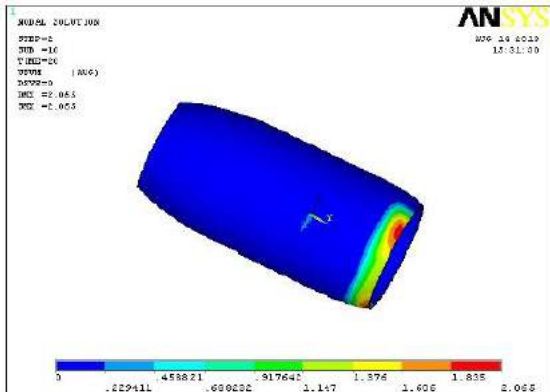
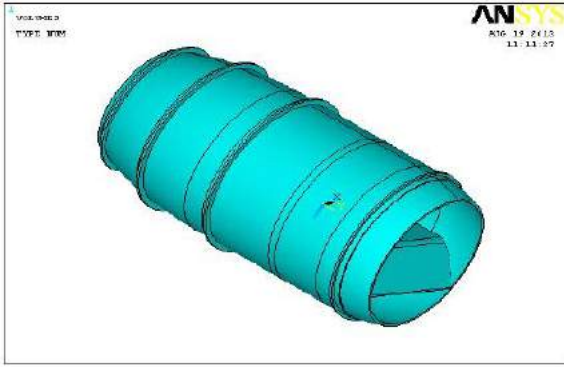


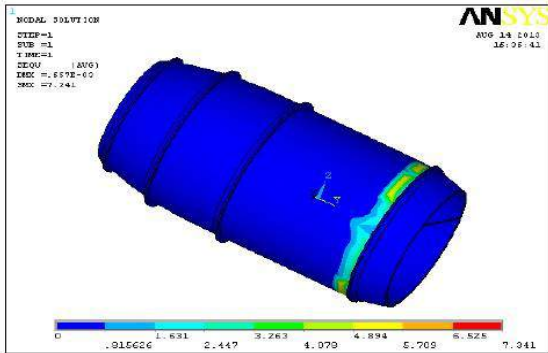
Figure 8. Displacement pattern for pressure hulls

**B. Pressure Hull with Out Bolts**

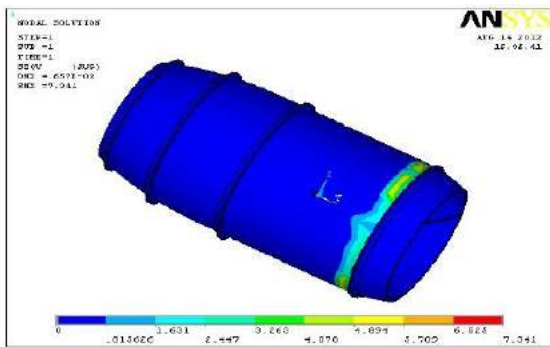


**Figure 9.** Analysis of pressure hulls without bolts

**1. Structural Analysis**

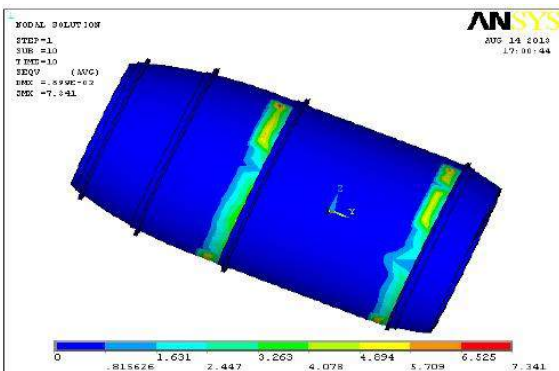


**Figure 10.** Stress distribution for pressure hulls

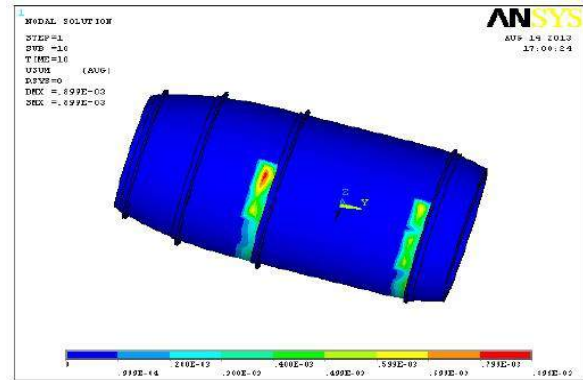


**Figure 11.** Displacement pattern for pressure hull

**2. Dynamic Analysis : 10 Secs**

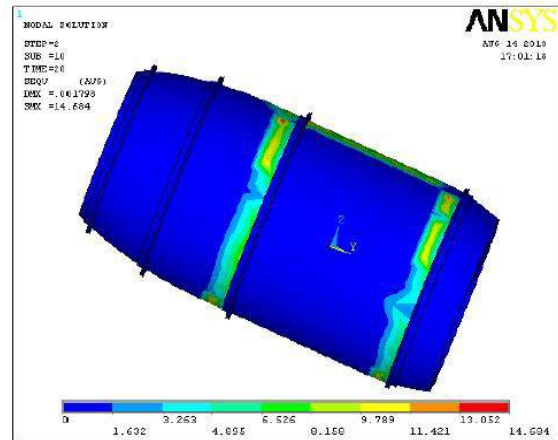


**Figure 12.** Stress distribution for pressure hulls

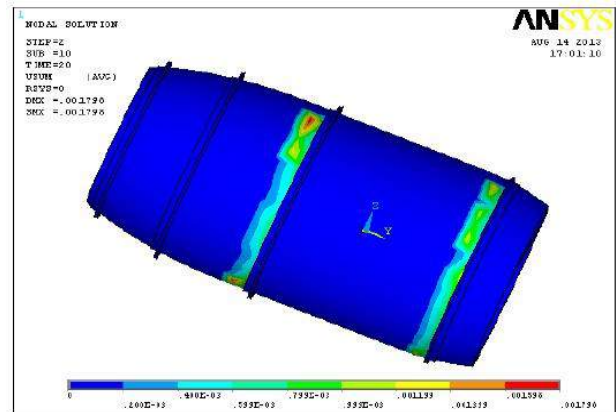


**Figure 13.** Displacement pattern for pressure hulls

**3. Dynamic Analysis : 20 Secs**



**Figure 14.** Stress distribution for pressure hulls



**Figure 15.** Displacement pattern for pressure hulls

**III. RESULT AND DISCUSSION**

**A. Structural Analysis Results**

**Table 2 :** Comparative structural analysis of a pressure hull with bolts and without bolts

Sr. No	PH	Stress (N/mm <sup>2</sup> )	Displacement mm	Strain
1	WB	288.579	1.029	0.001861
2	WOB	7.341	0.000657	0.000046

Notes: 1. PH = pressure hull  
 2. WB = with bolts  
 3. WOB = without bolts.

### 1. Dynamic Analysis Results

**Table 3 :** Comparative dynamic analysis of a pressure hull with bolts and without bolts.

Sr. No	parameters	Time	PHWB	PHWOB
1	STRESS (N/mm <sup>2</sup> )	10 sec	288.97	7.341
		20 sec	578.289	14.684
		30 sec	867.749	22.06
2	Displacement (mm)	10 sec	1.031	0.000899
		20 sec	2.065	0.001798
		30 sec	3.099	0.002697
3	Strain	10 sec	0.001863	0.000046
		20 sec	0.003729	0.000094
4	STRESS (N/mm <sup>2</sup> )	10 sec	288.97	7.341
		30 sec	0.005595	0.000141

Notes: 1. PHWB = pressure hull with bolts,  
 2. PHWOB = pressure hull without bolts.

### 2. Modal Analysis Results

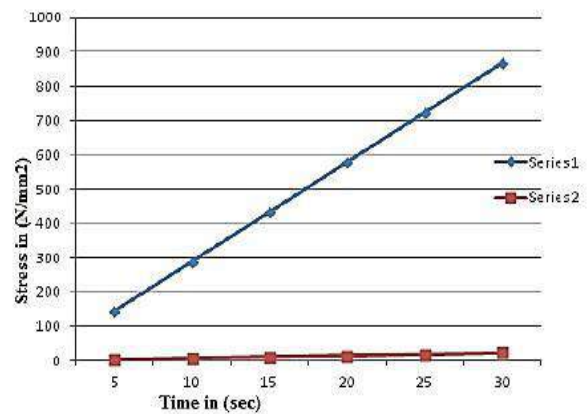
**Table 4 :** Comparative modal analysis of a pressure hull with bolts and without bolts

Sr.No	PH with bolts		PH Without bolts	
	Displacement (mm)	Frequency (Hz)	Displacement (mm)	Frequency (Hz)
1	1.0029	0	0.218416	0
2	0.32194	21.646	0.19668	0
3	0.23168	25.742	0.10598	0.000007
4	0.276541	26.2929	0.228769	0.0000081

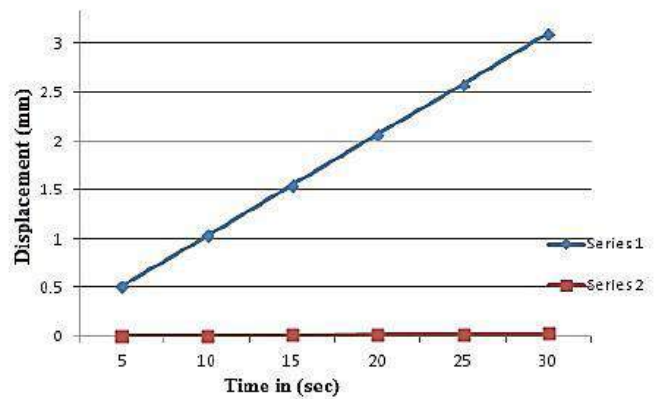
Notes: 1. PHWB = pressure hull with bolts,  
 2. PHWOB = pressure hull without bolts

## B. Graphs

### Pressure Hull with bolts and without bolts for dynamic analysis



**Figure 16.** Time – Displacement curves for PHWB AND PHWOB



**Figure 17.** Time – Displacement curves for PHWB AND PHWOB

Notes: 1. PHWB = pressure hull with bolts,  
 2. PH WOB = pressure hull without bolts,  
 3. Series 1 = PHWB curve  
 4. Series 2 = PHWOB curve

## IV. CONCLUSION

To observe the all results and to compare the pressure hull with bolts and without bolts with respect to weight, stiffness and strength.

By employing the pressure hull without bolts for the same load carrying capacity, there is reduction in are 32%- 41% higher than pressure hull with bolts and 52%- 63% stiffer than pressure hull with bolt.

Present used material for pressure hull with bolts is Stainless steel. I have considered pressure hull with bolts

and pressure hull without bolts. Based on the results, it was inferred that pressure hull without bolts has superior strength and stiffness and lesser in weight compared to pressure hull with bolts.

From the results, it is observed that the pressure hull without bolts is lighter and more economical than the conventional pressure hull with bolts with similar design specifications

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# Responsiveness of Unemployment to Inflation: Empirical Evidence from Nigeria

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## ABSTRACT

The debate on the link between inflation and unemployment cuts across various economic interests. Researchers and economists pick on the subject since unemployment remains one of the greatest challenges facing the developing and emerging economies of the world. Other things being equal, achieving price stability is widely acknowledged to have positive effect on unemployment and growth especially if the ideal threshold can be marked. However, finding the perfect trade-off seems quite elusive and Central Banks across the globe contend to achieve high level of employment without sacrificing price level stability. Hence in this study we attempt to determine the response of unemployment to variation in price level in Nigeria using annualized data from 1989 to 2014. The error correction Model (ECM) and the Johansen analytical tools were employed to process the data set. The results of the findings show that inflation impact negatively on unemployment while money supply and exchange rate were found to have positive influence on unemployment. Although unemployment and inflation have long-run association, there is divergence along the equilibrium part which is corrected at 65 percent speed of adjustment each year. We recommend rather than relying solely on monetary targeting as means balance between unemployment and inflation level, output targeting through economic deepening can play a supporting role in maintaining optimal inflation rate and minimal unemployment level.

**Keywords:** Inflation, Unemployment, Phillips Curve, Error Correction Model.

## I. INTRODUCTION

Price stability remains the central objectives of Central Bank monetary policy. To achieve this very objective, the Central Bank employs forecasts of inflation. These forecasts are useful to policymakers as they help to determine the appropriate stance of monetary policy; either expansionary or contractionary stance. Doh (2011) writes that slack in economic activities is one of the most commonly used short-term predictors for inflation. Economic slack reflects the underutilization of resources (such as labour and capital) in the economy. Economists use the Phillips curve to describe the short-term relationship between slow-moving economy and inflation. Using unemployment as an indicator of economic slack, the Phillips curve describes the cyclical components of the unemployment rate and inflation. The

Phillips curve demonstrates that an increase in the cyclical component of the unemployment rate predictably exerts downward pressure on the cyclical component of inflation (Doh, 2011).

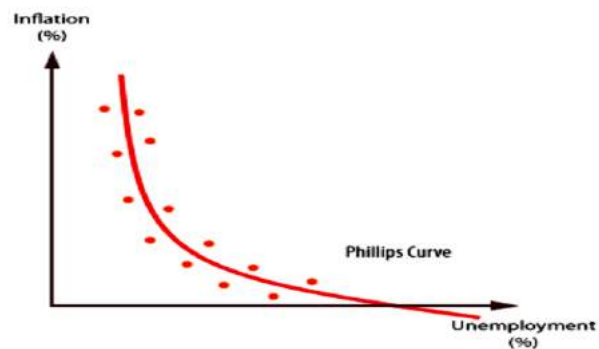
Unemployment has become a global problem, and poses serious challenge to both developed and developing countries. The proportion of the active population that is out of employment has grown to alarming rate and both fiscal and monetary policy strategies have struggled to plug this disturbing reality. Unemployment is a condition where the active population of a country who are willing and able to work stay out of job because there is no opportunity for them to work. Economists have for some time held the assumption that there is a trade-off between unemployment and inflation, which would be of great interest to policymakers. It then can be



inferred that lower unemployment could be achieved by bearing a higher rate of inflation. But then, as regards the long-run, that perception is no longer widely held. While it is a desirable policy goal to keep unemployment at a minimal level, some economists argue that full employment is a situation where everyone who wants a job automatically gets employed. However, some would contend that full employment is realized when unemployment rate is the lowest and inflation rate is at the same time stable; a situation economists refer to as the natural rate of inflation. Determining the natural rate of unemployment will no doubt be very useful to policymakers. One of the characteristics of inflation is that it tends to respond slowly to monetary policy changes designed to control it. For instance, the effects of expansionary monetary policy stance on inflation might not be immediately evident. Similarly, sometimes when rate of inflation is rather high, there is the likelihood that it will respond slowly to contractionary monetary policy strategies designed to reduce it. As a result of such attribute, and given the fact that policy measures aimed at curtailing inflation possibly will have short-term economic costs, it is generally believed that it would be a better option to keep inflation rate as low as possible (Cashell, 2004).

Phillips (1958) graphically represented a remarkable inverse relationship between the unemployment rate and the rate of inflation. The Phillips curve demonstrated that decreases in unemployment rate (or increase in employment level) correlate with increase in the rate of inflation. In other words, at a lower rate of inflation, unemployment level will increase. While this link is widely acknowledged to subsist in the short-run, it may however not be applicable in the long-run when inflationary policies are unlikely to decrease unemployment. Samuelson and Solow (1960) adopted the Phillips' hypothesis in their empirical study on the unemployment-inflation connection in U.S. They made explicit the fact that there is inverse relationship between unemployment level and that rate of inflation. They state that when unemployment was high, inflation was low, and vice versa. In essences, it was adduced that pursuing monetary or fiscal expansion, which might trigger inflation, would effectively exchange for lower rate of unemployment. In this case there is a trade-off, which entails that it is difficult for governments to achieve full high employment and at the same time maintain price

stability within the economy. While government deficit spending stimulate the economy, increasing the gross domestic product and lowering unemployment rate, inflation rate would increase, which is the cost of enjoying minimal unemployment. However, if such policy measure is effectively executed, it could push inflation rate to a level capable of undermining the nation's macroeconomic stability (Qin and Wang, 2013). According to Cashell (2004), any indicator that helps policymakers prevent higher Inflation rate in the first place would be exceedingly useful. An improvement over the natural rate of unemployment concept is the non-accelerating inflation rate of unemployment (NAIRU). NAIRU is typically the level of unemployment below which inflation rises. The assumption is that while gradual increases in prices are allowed, unemployment is tolerated and managed at a certain level that would ensure stable rate of inflation



**Figure 1.** Phillips Curve

The figure above which illustrates the Phillips curve explains that as the demand for labour increases, unemployment rate decreases and workers might well demand for wage increase. Conversely, when demand for labour decreases, unemployment level increases and workers are unlikely to have the opportunity to demand for higher wages (Hussein, 2014). Wage and inflation expectations could also have some effects on the formation of the current wage as well as inflation. A study by Zilli (2015) analyzed the ability of the Phillips curve to explain the dynamics of Brazilian inflation, utilizing aggregate data from 2002 to 2014. It was discovered that the expected inflation had greater impact on the formation of the current inflation than the past inflation; a confirmation of the theory of rational expectation.

## II. METHODS AND MATERIAL

### A. Empirical Review

The link between unemployment and inflation has long been attracting attention not only from labour economists but from analysts and researchers. We will explore related works on this subject in order to embrace dynamic nature of the two variables in different economies of the world. For instance, on the South African economy, Vermeulen (2015) applied the Engle-Granger Error-Correction approach as he investigated the effect of inflation on employment in South Africa. The main objective of the study was to the sole aim of find out whether higher inflation could lead to employment creation. The findings reveal that a positive long run relationship between employment and output, which supports the claim that anything that negatively affects output (such as high inflation), will by extension impair employment creation. In the short-run, it was found that the relationship between inflation and employment creation was not significant.

Qin and Wang (2013) argue that the Phillips curve is ineffective in determining the causal relationship between unemployment and inflation in China. This was revealed in the empirical study which investigated the correlation of coefficient and causal link between unemployment and inflation rate in China between 1978 and 2011. It stresses that the non-applicability of the Phillips curve on the communist state was as a result of the complexity of the Chinese economy.

Similarly, the findings of Umoru and Anyiwe (2013) opposed the Phillips curve position on inflation-unemployment relation. The study assessed the dynamics of inflation and unemployment in Nigeria over a twenty seven years period, using the Vector Error Correction Technique. Evidence of stagflation was found in the Nigerian economy within the period covered by the study. But Nitzan (1990) in his paper, macroeconomic perspective of unemployment and inflation stresses that though some factors like stagflation may have posed serious challenge to the Phillips curve, such contention only modifies and do not nullify the hypotheses. He stated that in inverse relation between inflation and unemployment is too significant

to be discarded hence auxiliary factor may come in simply to augment.

In contrast to the inverse relation represented in the Phillips curve, it was revealed a positive relationship between inflation and unemployment in Nigeria thereby invalidating the Phillips curve on the case of Nigeria. However, the outcome of the study conducted by Mirza and Mujahid (2015) on Pakistani economy supports the Phillips Curve postulation by disclosing the existence of indirect relationship between unemployment rate and inflation rate in Pakistan within the period 1973 and 2014.

Using the Johansen cointegration test and the Granger causality test, Hussein (2014) investigated a trade-off relationship exists between unemployment and inflation in the Jordan between 1984 and 2011. The result showed there was no causal relationship between inflation rate and unemployment in Jordan within the period covered by the study, indicating that no trade-off relationship existed between the two variables.

Gur (2015) used panel data analysis in examining the factors that affect unemployment in the BRIC countries (Brazil, Russia, India and China) from 2001 to 2012. The results indicated that inflation and population growth are the causes of rising unemployment among the BRIC countries, whereas growth in GDP and industrial product growth are among the factors that lead to reduction in unemployment level.

Thayaparan (2014) contends that for the case of Sri Lanka, only inflation has a significant effect in reducing unemployment while the gross domestic product exerts positive but non-significant positively influences on unemployment. The study which examined the effect of inflation and economic growth on unemployment in Sri Lanka within the period 1990 and 2012 also established a unidirectional causality between inflation and unemployment, and bidirectional causal relationships between unemployment and gross domestic product; and between inflation and gross domestic product in Sri Lanka.

Orji, Orji and Okafor (2015) examined the inflation and unemployment connection in Nigeria with the aim of finding out if the original Phillips curve proposition

holds for Nigeria. The result revealed that unemployment is a major determinant of inflation and that there is a positive relationship between inflation and unemployment rate in Nigeria. This is in line with the findings of Umoru and Anyiwe (2013) which invalidated the Phillips curve hypothesis in Nigeria. On the contrary, Resurreccion (2014) evaluated the linkage between unemployment, inflation and economic growth in Phillipines for the period 1980 to 2009. It was shown that unemployment has indirect relationship with inflation and economic growth. The finding confirms Okun's Law and Philips Curve in the Philippines.

Similarly, Furuoka and Munir (2014) argue that the Phillips curve hypothesis holds in the Malaysian economy. In the study, the Error Correction model (ECM) to analyse the relation between unemployment and inflation in Malaysia. The results indicate there is long-run equilibrium relationship between the two variables, and also found an inverse relationship between unemployment and inflation in Malaysia thereby supporting the validity of the original proposition of the Phillips curve.

Umaru, Donga and Musa (2013) employed the Johansen cointegration and the Granger causality test to investigate the effect of unemployment and inflation on economic growth in Nigeria from 1986 to 2010. The results of Causality suggest that unemployment and inflation are causal for real GDP and not real GDP is not causal for unemployment and inflation. In other words it is a one-way causality running from unemployment and inflation to real GDP. The Johansen cointegration tests confirm the existence of long run relationship between economic growth, unemployment and inflation. The results also showed that unemployment and inflation exert a positive impact on economic growth.

Aurangzeb and Asif (2013) examined the macroeconomic determinants of the unemployment for India, China and Pakistan for the period 1980 to 2009, using the Johansen cointegration, Granger causality test and regression analysis. The result of granger causality indicates that bidirectional causality does not exist between unemployment, inflation and economic growth for all three countries. Cointegration result revealed that long term relationship does exist among the three variables for all the models.

Kogid, *et al* (2013) examined the trade-off relationship between unemployment and inflation in Malaysia using ARDL bounds testing to cointegration, ECM based ARDL and Toda-Yamamoto techniques for the period of 1975-2007 in Malaysia. The study showed the existence of the long-run relationship between inflation and unemployment, and revealed unidirectional causal relationship running from inflation to unemployment, which points to the fact that inflation influenced unemployment. This study eventually found that there is inflation-unemployment trade-off relationship in Malaysia.

Umaru and Zubairu (2012) investigated the relationship between unemployment and inflation in the Nigerian economy within the period 1977 and 2009. The results indicate that inflation has negative impact on unemployment. The Granger causality test revealed that there is no causal link between unemployment and inflation in Nigeria during the period of study. Johansen cointegration test showed that a long-run relationship exists between the two variables.

## **B. Data and Methodology**

Research design for this study is *ex post-facto* which entails that the event we are studying indeed taken place already. We are therefore using secondary data sourced from World Bank National account data files and the OECD National accounts data files. Variables of interest include the unemployment rate, inflation rate, money stock and exchange rate. This study covers the period 1960 to 2014. Because our study period is long-term and we are using time series data, there is therefore the need to ensure that our data is stationary before we subject them to higher econometric estimations. This measure will ensure that in the end our result will not be spurious hence reliable. The Augmented Dickey-Fuller (ADF) unit root test will be used to ascertain if our variables are stationary or not. Further techniques of analysis employed to evaluate the linkage between unemployment and inflation are the Johansen cointegration test, Error Correction Model (ECM).

## **Model Specification**

This study is patterned after Furuoka and Munir (2014) which examine inflation-unemployment relation in

Malaysia. The model adopted by the authors is of the form:

$$IFR_t = \alpha_0 + \gamma_1 \Delta UER_{t-1} + \varepsilon_t \text{ --- (1)}$$

Where  $\alpha_0$  = constant,  $\gamma_1$  = slope coefficient,  $IFR_t$  = inflation rate in the year  $t$ ,  $UER_t$  = unemployment rate in the year  $t$ , and  $\varepsilon_t$  is the error term. We modified the above model to arrive at our baseline equation, which accommodates our peculiar variables. We represent our distinct model thus:

$$UER_t = \beta_0 + \beta_1 INFL_t + \beta_2 MS_t + \beta_3 EXR_t + \varepsilon_t \text{ --- (2)}$$

Where  $UER$  = unemployment rate,  $INFL$  = inflation,  $\beta_0$  = constant,  $\beta_1 - \beta_4$  = parameter estimates,  $MS$  = broad money stock relative to GDP,  $EXR$  = exchange rate, and  $\varepsilon$  = error term.

There are three stages to our estimation. First we test our variables for stationarity using the Phillip-Perron unit root test. Time-series data is stationary if its mean and variance are constant over time (Gujarati, 2003). The Phillip-Perron is based on the following model:

$$\Delta y_t = \mu + \alpha_{t-1} t + \sum_{i=1}^n \gamma_i \Delta y_{t-1} + \varepsilon_t \text{ --- (3)}$$

where  $t$  = linear time trend,  $\mu$  = constant,  $\Delta$  = differencing operator, and  $\varepsilon_t$  is the error term. If the variables are stationary or integrated at order zero,  $I(0)$ , we can employ the Ordinary least square (OLS). However, if our variables are integrated of order one,  $I(1)$ , we will have the justification to run the Johansen co-integration test.

This second stage enables us find out if long run relationship exist among the variables. If at this point our variables are co-integrated, we then move on to the third stage where we the run the Error Correction model (ECM) by modifying our baseline equation thus:

$$\begin{aligned} \Delta UER_t = & \beta_0 + \sum_{i=0}^n \beta_1 \Delta UER_{t-1} \\ & + \sum_{i=0}^n \beta_2 \Delta INFL_{t-1} + \sum_{i=0}^n \beta_3 \Delta MS_{t-1} \\ & + \sum_{i=0}^n \beta_4 \Delta EXR_{t-1} + \beta_5 ECT_{t-1} + \varepsilon_t \text{ --- (4)} \end{aligned}$$

Where  $ECT_{t-1}$  = lagged value of the error correction term.

### III. RESULT AND DISCUSSION

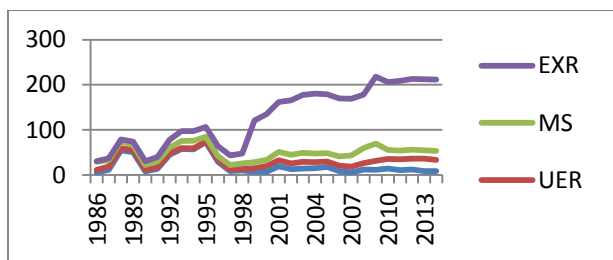


Figure 2. Graphical representations of proxied variables

Table 1. Descriptive Statistics

	UER	INFL	MS	EXR
Mean	10.94138	20.82897	17.36207	79.44724
Median	11.90000	12.22000	17.70000	101.7000
Maximum	27.40000	72.84000	38.00000	158.4300
Minimum	1.800000	5.380000	8.600000	1.750000
Std. Dev.	7.937232	19.34240	6.399186	61.97985
Skewness	0.601332	1.427411	1.493655	-0.062328
Kurtosis	2.162715	3.579714	5.758168	1.225971
Jarque-Bera	2.594831	10.25401	19.97557	3.821616
Probability	0.273237	0.005934	0.000046	0.147961
Sum	317.3000	604.0400	503.5000	2303.970
Sum Sq. Dev.	1763.990	10475.60	1146.588	107562.0
Observations	29	29	29	29

Source : Authors

Table 2. Unit root test at level

VARIABLES	ADF Statistic	CRITICAL VALUE			REMARK
		1%	5%	10%	
UER	-0.357574	-3.653730	-2.957110	-2.617434	Non-Stationary
INFL	-2.330254	-3.661661	-2.960411	-2.619160	Non-Stationary
MS	-2.521482	-3.653730	-2.957110	-2.617434	Non-Stationary
EXR	-2.463775	-3.653730	-2.957110	-2.617434	Non-Stationary

Source: Authors Views result

The result of the unit test as presented in the table 4.1 above shows that all the variables included in the model are all not stationary at level. This however will take us to the next step in ADF approach which requires differencing of the variables to see if it will be stationary at first difference.

Table 3. Unit root test at first difference

VARIABLES	ADF Statistic	CRITICAL VALUE			REMARK
		1%	5%	10%	
UER	-3.848396	-3.653730	-2.976263	-2.617434	Stationary
INFL	-3.484005	-3.484005	-2.998064	-2.619160	Stationary
MS	-4.871434	-3.653730	-2.976263	-2.617434	Stationary
EXR	-5.012615	-3.653730	-2.976263	-2.617434	Stationary

Source: Authors Views result

Table 4.2 presents the results of unit root test at first difference which indicates that all the variables are stationary at 5% level of significance. The stationarities so attained are all at order one,  $I(1)$ , and are therefore integrated at same order. This result now allows us to run the Johansen cointegration test to establish if the variables move along together in the long-run.

### TEST FOR LONG-RUN ASSOCIATION

#### Johansen Cointegration Test

Table 4 : Result of Johansen Co-Integration Analysis

Hypothesized No. of CE(s)	Eigenvalue	Trace Statistic	5% Critical Value	Prob.**
None *	0.704655	66.86688	47.85613	0.0003
At most 1 *	0.541628	33.93735	29.79707	0.0158
At most 2	0.333186	12.87535	15.49471	0.1195
At most 3	0.069116	1.933746	3.841466	0.1643

Source: Authors' Eview Result

The results of Johansen Cointegration test in table 4.3 show that the variables are cointegrated, which means they have long-run relationship. The trace statistic revealed this long run association and is confirmed by the max-Eigen statistic. The implication is that unemployment, inflation, money stock and exchange rate move together in the long-run.

## REGRESSION RESULTS

**Table 5.** Error Correction Model (ECM) Results

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	0.267161	0.362941	0.736101	0.4698
D(INFL-1)	-0.014075	0.022204	-0.633885	0.5330
D(MS-1)	0.053677	0.078569	0.683182	0.5020
D(EXR-1)	0.076563	0.023012	3.327028	0.0032
ECT(-1)	-0.650515	0.004450	-3.066066	0.0014

Source: Eview result

The result of the short run dynamics model as represented in table 4.4 above indicates that MS, has a positive and insignificant impact on UER. EXR has a positive and significant effect on the UER in the short run. In other words it also shows that in the short run INFRT has a negative and statistically insignificant impact on the UER in Nigeria. The error correction term (ECT) demonstrates the speed of adjustment to long-run equilibrium. ECT is negative and significant, and the coefficient value of -0.650515 indicates that 65% of deviations from long-run equilibrium is corrected each year. Generally, table 4.4 shows that the overall regression is significant and the Durbin-Watson statistic indicate that our model estimation has no autocorrelation problem.

**Table 6.** Result of The Long-Run Regression Model

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	2.326896	2.280445	1.020369	0.3173
INFL	-0.025186	0.038035	-0.662185	0.5139
MS	0.012021	0.130102	0.092394	0.9271
EXR	0.112406	0.014640	7.678210	0.0000
R-squared	0.840219	F-statistic	43.82125	
Adjusted R-squared	0.821045	Prob(F-statistic)	0.000000	
Durbin-Watson stat	1.698344			

Source: Authors Eview result

Table 4.5 is the estimation of the long run equation. The regression results reveal that inflation has negative effect on unemployment in the long run but not significant in explaining it. This finding is in line with theoretical expectation which explains that inflation impacts negatively on unemployment level. The results also show that money stock has positive and insignificant impact on unemployment while exchange rate exerts positive and significant effects on unemployment. The result of the long run analysis is in line with that of the short run dynamic model which suggest that

the effect of the variables included in the model exert the same level of impact on the explained variable in both the long and the short run.

## IV. CONCLUSION AND RECOMMENDATION

The debate on the link between inflation and unemployment cuts across various economic interests. Researchers and economists pick on the subject since unemployment remains one of the greatest challenges facing the developing and emerging economies of the world. Other things being equal, achieving price stability is widely acknowledged to have positive effect on unemployment and growth especially if the ideal threshold can be marked. However, finding the perfect trade-off seems quite elusive and Central Banks across the globe contend to achieve high level of employment without sacrificing price level stability. Hence in this study we attempt to determine the response of unemployment to variation in price level in Nigeria using annualized data from 1989 to 2014. The results of the findings show that inflation impact negatively on unemployment while money supply and exchange rate were found to have positive influence on unemployment. Although unemployment and inflation have long-run association, there is divergence along the equilibrium part which is corrected at 65 percent speed of adjustment each year. We recommend rather than relying solely on monetary targeting as means balance between unemployment and inflation level, output targeting through economic deepening can play a supporting role in maintaining optimal inflation rate and minimal unemployment level.

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# Querying Unintelligible Data on Geospatial Trajectory Database

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## ABSTRACT

Current GPS technologies collect objects and its movement and store the trajectories periodically in the MOD (Moving Object Database). In such environment, some location errors may arise and some models are unable to capture the changes in trajectories dynamically. Especially, the uncertainty capturing is a challenging one. In order to handle these issues in spatial database, the proposed system develops a new trajectory model to handle the uncertainty and querying on uncertain spatial queries. Initially this develops an adaptable trajectory approach to provide actual positions and temporal changes in uncertainty along with improbable uncertainty ranges. The next part of ongoing implementation provides effective spatial query processing with successful indexing process. This presents the temporal R+ tree indexing with inverted list. This provides an efficient mechanism to evaluate improbable range objects and its spatial queries using Rife-density trajectories.

**Keywords:** RFID sensors, R+Tree, GSM, Trajectory Model, Road Network, Dynamic Route Map.

## I. INTRODUCTION

The spatial database is defines special data types for geometric objects and allows to store geometric data (usually of a geographic nature) in regular database tables. It provides special functions and indexes for querying and manipulating that data using something like Structured Query Language (SQL). While it is often used as just a storage container for spatial data, it can do much more than that. Although a spatial database need not be relational in nature, most of the well-known ones are [1]. Uncertainty management is a central issue in trajectory databases. The research interests, optimization goals, and methodologies in this domain are indeed rich and diverse. Despite this diversity, these studies are generally established upon a common principle location uncertainty is captured by a certain range centered on the position recorded in the database. This principle was initially discussed by Poser and Jensen in the database literature, which is longer than a decade ago [2]. As of today, GPS is no longer the only primary means for positioning, yet a wide spectrum of technologies are being used to produce trajectory data, including RFID sensors, location estimation with 802.11, smart-phone

sensors, infrared and ultrasonic systems, GSM beacons, and even vision sensors. These positioning systems typically yield different characteristics of trajectory data, which also exhibit various properties as well as degrees of uncertainty.

Uncertain Spatiotemporal Data: Query processing in trajectory databases has received significant interest over the last ten years. Initially, trajectories have been assumed to be certain, by employing linear or more complex types of interpolation to handle missing measurements [12]. These interpolation techniques, however can lead to impossible patterns of movement as, for example, a car might be assumed to drive through a lake. Other solutions such as computing the shortest path between consecutive locations can produce valid results, but do not provide probabilities for quantifying the result quality. As a result, a variety of uncertainty models and query evaluation techniques has been developed for moving object trajectories [28].

## A. Uncertain Trajectory Models

Uncertain Trajectory Models: There are two major reasons why uncertainty occurs in trajectory data. One is known as measurement error which is caused by limited accuracy of positioning technology, e.g., GPS error. The other is sampling error that originates from discrete sampling of continuous movements of an object, the locations of the object between two sampled positions are unknown [8]. To deal with these uncertainty factors in trajectory data management, a rich body of studies have proposed various uncertainty models. These models commonly represent a trajectory using a sequence of uncertainty areas, so called uncertain trajectory. Each of the uncertainty areas captures the measurement and sampling errors. The main aim of this paper is to establish core foundations for uncertain trajectory management, based on the new modelling method. This requires a wide variety of re-innovations; particularly we focus on three important problems [15]. That are, for time-dependent constrains problem, we introducing a new uncertain trajectory model that represents a trajectory using Pearson distributions, capturing the dynamicity of location uncertainty without any unrealistic assumption and facilitating efficient query processing that infer time-varying densities of location data. For a multivariate positional data, we develop [27] effective methods for estimating time-dependent probability distributions. And finally we used an effective mechanism that indexes evolving-density trajectories, and efficiently evaluates probabilistic range queries using the indexes. As the uncertainty ranges of evolving-density trajectories are unbounded, and vary over time.

## II. RELATED WORKS

In this section, we review existing works in the domains related with the current works Brakatsoulas, et al [1] present three novel algorithms that exploit the trajectory nature of the tracking data, i.e., considering the entire path of a vehicle as opposed to its current position. The incremental map-matching algorithm employs a greedy strategy of sequentially matching portions of the trajectory to the road network.

Li, Xu, et al [2]. Work carried on vehicular ad hoc networks (VANET) by using these vehicles if we imagine that vehicles can communicate with each other.

The taxi companies, though, often set sensor with a long sampling period, such as 1-2 minutes, which is very diverse from ordinary sampling rate of GPS device, such as 1Hz. This is because taxi companies want to reduce communication cost of sending sensor data to the data centre and are only interested in the general location of their vehicles. On the other hand, sensors with a short sampling interval would produce enormous data traffic, resultant in potential network jamming. In our work, we utilize 4000 taxis and 1000 buses equipped with GPS-based mobile sensors. Currently, vehicular ad hoc networks (VANET) have been paid much concentration. Also, we utilize 4000 taxis and 1000 buses prepared with GPS-based mobile sensors in Shanghai city, which constitute GPS-based vehicular networks.

Kuijpers, et al [3] focus on the trajectories that are produced by moving objects and on organizing and querying them in a database. Hence, they think it is more suitable to talk about trajectory databases, rather than to refer to the moving objects that produce these trajectories. It can review our outcome as follows: they give a data model for trajectory data; an capable way of modeling uncertainty; we study transformations for which significant physical properties of trajectories are invariant and we give first-order complete and computationally complete query languages for queries invariant under these transformations. They propose two types of trajectory data. Firstly, we have trajectories, which are curves in the real plane  $R^2$  that are rationally parameterized by time ( $R$  denotes the set of real numbers).

Liu, Hechen et al [4] study the moving objects has aroused a lot of interest in many fields such as mobile networking, transportation management, weather report and forecasting, etc. Moving objects describe the continuous evolution of spatial objects over time. The feature that their locations change continuously with time makes them more complicated than static spatial objects in some aspects; one aspect refers to the topological relationships. Hajari, Hadi, et al [5] present OGC-based models consisting of relations for modeling spatial networks and a number of User Defined Types (UDTs) and operations for optimal storage, representation, and querying of static and moving features on the extensibility interface of Object-Relational database systems. They address some



implementation issues of the proposed framework on top of the OGC-compliant Oracle spatial data model.

Karthikeyani, V. [6], focus the moving object in the spatial temporal concept, the method for acquire and in place of the movements in which the positions of moving objects are sampled at selected points in time. The algorithmic discussion and equation describe object movement focusing accurately. The trajectory based queries are classified in ‘topological’ queries, which involve the whole information of the movement of an object and ‘navigational’ queries which involve derived information. Trajcewski, Goce, et al [7], deal in a systematic way with the issue of uncertainty of the trajectory of a moving object. Uncertainty is an inherent aspect in databases which store information about the location of moving objects. They introduce a set of novel but natural spatio-temporal operators which capture uncertainty, and are used to express spatio-temporal range queries. We also devise and analyze algorithms to process the operators. The operators have been implemented as a part of our DOMINO project.

### III. METHODOLOGY

The goal of this research is to establish core foundations for uncertain trajectory management, based on the new modeling approach. This requires a wide variety of re-innovations; particularly, this focuses on three important problems and makes the following significant contributions: Moving objects produces trajectories, where Uncertainty those trajectories produce improper results. This section describes a set of data model for trajectories and trajectory samples. This provides an efficient way of modeling uncertainty via network for trajectory samples. This work introduces a new uncertain trajectory model which is named as Rife Density trajectory (RDT) model. The RDT model evolves the popularity of the navigation, query and other aspects of objects. So this can able to easily handle the uncertainty in moving objects.

#### Contributions

- The study proposes a new model for Uncertain Trajectory named as Rife Density Trajectory (RDT) representing the motion along with a road network, and provides an incorporated density clustering for

the possible locations of a moving object at a given time-instant.

- This formulates both spatial and Continuous Probabilistic Range Query whose semantics captures the uncertainty model.
- This also designs an effective indexing structure as well as efficient processing algorithms for spatial continuous range queries.
- This also facilitates the Temporal R+-Tree technique to evaluate the query much faster on uncertain environment.

#### A. Rife Density Trajectory Model

Covering the pitfalls of the existing uncertain trajectory models, this proposes a new model for capturing and representing the uncertainty of trajectory, termed Rife-density trajectory.

The first contribution of this paper is to introduce a new uncertain trajectory model that represents a trajectory as time-dependent Pearson distributions. In each such distribution, skewness and kurtosis values are acquired with the mean value to represent an actual location, while the standard deviation reflects the degree of an uncertainty range. This model can effectively capture the dynamicity of location uncertainty without any unrealistic assumption, while facilitating efficient query processing. This also provides a flexible framework that allows various approaches including domain-specific models to precisely infer such evolving normal distributions. In the consequence, this introduces a set of key principles that establish the new uncertainty model, and then describe the system framework that supports the model.

#### Process of RD

The Valuable information like location and uncertain event reports must be converted into raw trajectories for decision making purpose. While scrutinizing the object mobility and uncertainty, a good indication of behavior is vector and motion. Here vector is the direction of the movement and the motion is captured by trajectories which indicate the spatio-temporal characteristics of objects and encode behavior.

A key examination for uncertainty analysis on trajectories is that typical actions are repetitive while the

unusual do not occur often. In this process the objects and trajectories were stored in the dataset. From the dataset the trajectory status can be monitored.

### Actual Position

In order to reduce the noisy data there are several filtering schemes are applied in literature. Such schemes are Kalman filtering for GPS data, particle filtering for RFID and Map matching for network constrained object locations. These approaches provide means that can infer more reliable positions where an object was actually located by eliminating the noisy and redundant data's. The Rife-density trajectory model supports such an inferred location as an actual location of the object and helps to predict the future move based on the vector, which serves as the center point of an uncertainty range.

### Pearson distribution

The approach develops object's location prediction using Pearson distributions. The main objective of using Pearson distribution is as follows

1. This is used to describe the distribution of the sample mean, which is a collection of samples from normal population's. To Describe the distribution of the sample mean, which is the collection of samples from a population that is not normal.

The normal distribution of a statistic is a probability distribution for all possible values of the statistic computed from a sample of size n. Here the sampling distribution of the sample mean is the probability distribution of all possible values of the random variable, which are computed from a sample of size n from a population with mean  $\mu$  and standard deviation  $\sigma$ .

The followings are the steps involved in the density calculation function to handle uncertainty.

#### Steps:

- Step 1:** Obtain a random sample of size n.
- Step 2:** Compute the mean.
- Step 3:** Assuming that the sample from a finite population, repeat Steps 1 and 2 until all simple random samples of size n have been obtained.

Approximate the sampling distribution of the sample mean by obtaining 200 simple random samples of size  $n = 20$  from the population of weights of pennies minted after 1982 ( $\mu=2.46$  grams and  $\sigma=0.02$  grams)

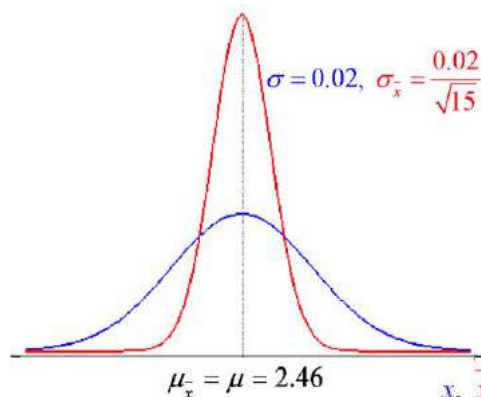


Figure 1. Probability distribution using Pearson

The mean of the 200 sample means for  $n=20$  is still 2.46, but the standard deviation is now 0.0045 (0.0086 for  $n=5$ ). As expected, there is less variability in the distribution of the sample mean with  $n=20$  than with  $n=5$ . Suppose that a simple random sample of size n is drawn from a large population with mean  $\mu$  and standard deviation  $\sigma$ .

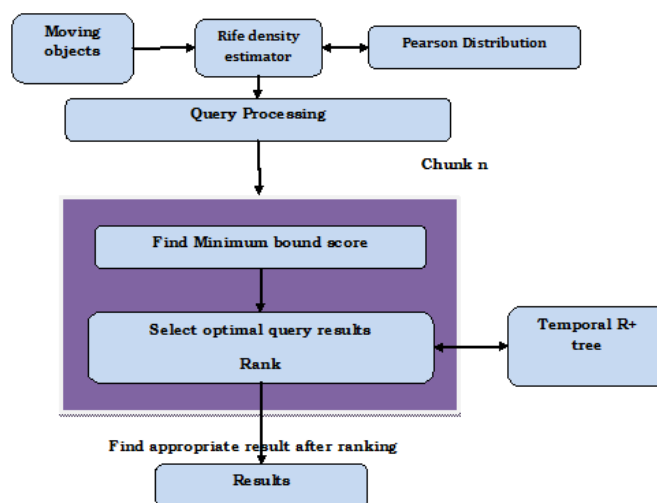


Figure 2. Proposed density trajectory model

### B. Spatial Query Processing

The dynamic nature of the spatial attribute makes dynamic location based queries in Location Based Searches unique and challenging. Spatial queries at different locations will be more challenging in uncertain trajectory management. As a result the spatial query

results differ for different locations based on the spatial property. To efficiently compute location-based results with the trajectory model there are several number of algorithms used in literature. The proposed Rife Density trajectory model along with KNN query helps to process the spatial query processing.. These algorithms assume that the query location is an exact location point. Spatial query processing which takes a spatial range as the input of user location and performs the dynamic clustering algorithm to a point or a line in existing location based systems. The distance and mobility based spatial queries are more practical when compared with static skyline environments.

Query Processor supports efficient processing of probabilistic range queries on the Rife-density trajectories managed in the system. Specifically the processor implements the well-known filter-and-refinement paradigm. At the filter step, both a temporal R+-tree and a hash table are used to quickly prune those trajectory records whose time or position attributes are irrelevant to a given query condition. At the refinement step, the query processor evaluates whether each candidate resulted from the filtering step actually satisfies the given query (probability) condition. This process is performed by calling probability computation functions that are built-on Pearson densities in a precise manner.

Spatial probabilistic range queries are possibly the most common query type on uncertain trajectories, as they can effectively retrieve uncertain objects or trajectories using solid mathematical foundations in probability theory. Algorithm 1 presents the overall mechanism for processing spatial range queries, consisting of the filtering phase in Lines 1, 2 and 3 and the ranking phase in Lines 7–12.

The proposal introduces an efficient mechanism for probabilistic range queries on Rife-density trajectory databases. The framework first extends the definition of probabilistic range queries on Rife -density trajectories. Then it presents access methods to index Rife -density trajectories, as well as an algorithm for assessing the optimal queries based the indexes. Note that other probabilistic query types (e.g., probabilistic nearest neighbor queries) can also be evaluated over rife-density trajectories using existing KNN query-processing methods, as the uncertain trajectory model offers full

information in terms of probability distribution at each position, which is sufficient for the probabilistic distance measures used in previous works.

```

Algorithm: spatial Query processing algorithm
1:  $\epsilon_p \leftarrow LocationPoint(\rho)$ 
2:  $\epsilon_t \leftarrow Time(t)$ 
3:  $\epsilon_q \leftarrow query(q)$ 
4:  $\epsilon_{p_t} \leftarrow services(s)$ 
   - extract services based on the location point and temporal details
5: for each service(s) in Tree  $\in$  node do
      5: if (Ti is non leaf in T)
6: check Ti  $\in$   $\epsilon_t$  - temporal verification
7:   spatialQuery( $q, r_q, t_q, Ti$ )
   - spatial query passing based on the parameters
8: for each node entry T  $\in$  Ti do
      9: if  $A_{qt} \leq 1$  then - get a single record
10:   result  $\leftarrow TrajHash[A_{qt}]$  - store in the hash table
      11: else calculate bscore()
  
```

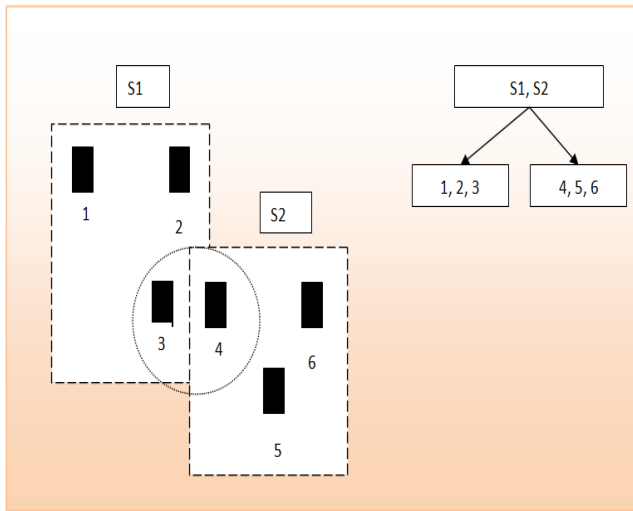
### C. Indexing Method

The third contribution is to present an effective mechanism that indexes evolving-rife density trajectories, and efficiently evaluates probabilistic range queries using the indexes. The proposal introduces a Indexing method to address the query processing in uncertain environment problem. To address this problem, the study utilizes a **temporal R+-tree** as well as a reverse key index and hash table for quickly identifying a candidate set of uncertain trajectories, by dynamically computing the rank for each point and service. This helps to satisfy a given query condition. This process does not affect by any uncertainty object queries and results. The process does not a part of information loss during probabilistic query processing.

### SR Index

The spatial Reverse list (SR-index) is essentially a compressed version of an I-index with embedded coordinates. Query processing with an SR-index can be done either by merging or together with R+-trees in a distance searching method. In addition the compression eliminates the defect of a conventional I-index such that an SR-index consumes much less space. R+-Trees can

organize any-dimensional data by representing the data by a minimum bounding box. R+-tree is an extension of the R-tree. In contrast to R-tree bounding rectangles of the nodes at one level don't overlap in this structure.



**Figure 3.** Temporal R+-Tree

This feature decreases the number of searched branches of the tree and reduces the time consumption for searching. In the R+-tree it is allowed to split data objects so that different parts of one object can be stored in more nodes of one tree level. If a rectangle overlaps another one, this decomposes it into a group of non overlapping rectangles which cover the same data objects. This process increases space consumption but allows zero overlap of the nodes and therefore reduces the time consumption for service retrieval.

Every node bounds with its children and a node can have many objects in it. The leaves point in the tree represents the actual objects and the height is always  $\log n$  (it is height balanced). The main advantage of using temporal R+ trees in the RDT model is follows.

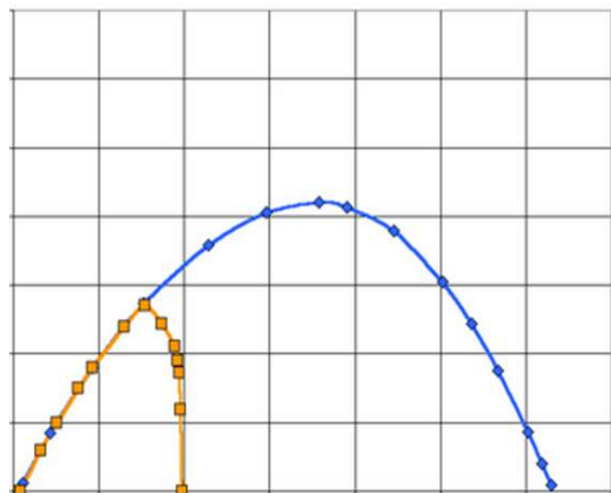
- ❖ Nodes are not overlapped with each other in R+ trees, Point of Interest based query results are covered by at most one node.
- ❖ A acyclic path is followed and appropriate nodes are visited than with the R-tree

The spatial query framework with uncertainty consists of five steps. Given a query, this first find all sub graphs that intersect with the query range. Next, this uses the Filter Trees of these sub graphs to retrieve the points whose strings are potentially similar to the query string.

In the third step, this prunes away some of these candidate points by calculating the lower and upper bounds of their distances to the query point, using VR. The fourth step is to further prune away some candidate points using the exact edit distance between the query string and strings of remaining candidates. After this step, the string predicate has been fully explored. In the final step, for the remaining candidate points, this checks their exact distances to the query point and return those with distances within  $r$ .

### Objects in R+-Trees:

The location point tree saves the location points along with the temporal detail. The system identifies the service by using the spatial index key which will be stored on the Temporal R+-Tree. To speed up query processing in uncertain trajectory database the reverse indexes has applied. This finds all the objects' which is related to the temporal data and finds the subspace scopes by the Temporal R+ tree where the subspace spatial scopes are stored in the leaf nodes as data entries. Moreover to support query processing this follows similar ideas of existing R-tree and R\*-tree to maintain a series of digests for all index nodes in the tree structure. And this also uses the reverse index to handle the direction in trajectories.



**Figure 4.** Location and service point

In the above diagram 4 shows  $p_1, p_2, p_3, p_4, p_5 \dots p_7$  are the service points. In the proposed system every service point will be stored with a temporal data for query processing. The proposed system stores the above service point in the tree style.

**Table 1.** Location point and available services in the location

Trajectory points	Services (S)
P1	S1,S3
P2	S4,S6
P3	S5,S4
P4	S4,S7
P5	S2,S8

The above table 4.1 represents the location point and available services in the location. The system can able provide appropriate services for the query based on the location point and temporal based service list. This helps in two ways. One is the retrieval time has reduced. And the next is managing uncertainty in trajectories in the tree helps to avoid wrong results.

**Algorithm 1 :** Temporal R+- tree

**Steps:**

1. Initial location source with respect point  $p_1, p_2, \dots, p_n$  and time  $T_1, T_2, \dots, T_n$
2. For every point in the source P do
  - a. Initialize service  $S_1 \dots S_n$  for respective points.
  - b. Set index for every service
3. Get rank for each service and store into the ascending order.
4. Store the node in the top level based on the rank
5. Prune the other items from the TR+tree.

The above algorithm represents the data extraction from temporal R+ tree based on the user query. This initially collects location points and its respective time. After that this will enable the service extraction from repository using TR+ tree.

**Spatial Reverse index (SR-index)**

The location based reverse index helps to track all the service related to the user query. For fast search SR index method has been proposed to provide appropriate service, the point selection also helps to avoid the uncertainty in service selection by location based servers. The proposed system designed a variant of skyline reverse index that is optimized for multidimensional temporal points with reference keys and is thus named the spatial reverse index (SR-index). This access method successfully incorporates point coordinates into a conventional reverse index with small extra space which is owing to a delicate compact storage scheme for fast accessibility. Meanwhile an SR-index preserves the spatial locality of data points and temporal details, and comes with an R+- Tree built on every reverse list at little space overhead. Finally it offers two competing ways for skyline query processing in location based search. This can consecutively merge multiple lists very much like merging traditional reverse lists by unique id of each object. This can also leverage the LP trees to browse the points of all relevant lists in ascending order of their distances to the query point by CSP.

**IV. CONCLUSION**

This work re-modulates the existing trajectory models to handle the uncertainty in trajectory database. The system proposed a new and effective approach to modelling the uncertainty of trajectories, as their modeling powers are insufficient to capture several important properties of trajectory data. To complement this, this proposed the Rife-density trajectory model that represents a trajectory as time-dependent Pearson distributions. This then introduced Rife density trajectory model along with effective indexing technique. The usage of temporal R+ Tree with inverted list improved the searching efficiency, which is suitable for vector based data searching that effectively infer time-varying densities of location data. This also developed an efficient mechanism to process spatial range queries on indexed Rife-density trajectories.

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# Surveillance of Helminthes and Molecular Phylogeny of *Fasciola Gigantica* Infecting Goats in Sadat District, Egypt

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## ABSTRACT

Helminthes cause health problems in ruminants as anemia, anorexia, weight loss, diarrhea, decreased production and deaths therefore this study was aimed to identify helminthes of goats, their incidence and genotyping of *Fasciola gigantica* recovered from Sadat district (Kafr Dawoud, Wadi Elnatron and ElKhatatba abattoirs) Egypt. The study was applied on 790 animals from March 2014 to March 2015. The overall infection rate with helminthes was 23.54% among slaughtered goats at Sadat district, Egypt. Prevalence in different localities was recorded. The overall prevalence rate of trematode helminth was 9.66% and the recovered trematode species and their associated infection rates were *Paramphistomum cervi* (1.43%), *Fasciola hepatica* (0.41%), *Fasciola gigantica* (5.83%), *Caromyerius gregarius* (1.99%). While the prevalence rate of cestodes was 9% and the recovered species and their correlated infection rates were *Moniezia expansa* (4.28%), *Moniezia benedeni* (0.71%) and *Avitellina centripunctata* (4%). Genotyping of *Fasciola gigantica* of the current study was done by amplifying 18S ribosomal RNA gene (656 bp of nucleic acids), there are no Single Nucleotides Polymorphisms (SNPs) within the sequence of 18S-rRNA gene and when aligned with isolates of the previous studies; the homology was very high (99.9%) and the divergence was very low (0.1).

**Keywords:** Goat, Helminth, Surveillance, *Fasciola Gigantica*, Molecular, Phylogeny Egypt

## I. INTRODUCTION

In Egypt, the incidence of parasitic infection among farm animals varied according to many factors including irrigation, season and frequency of exposure of animal to infection, immune condition of the animal, the geographic location and climatic conditions [1]. Undoubtedly the most pathogenic and economically important helminthes are the liver flukes (*Fasciola gigantica*) where they cause traumatic hepatitis, peritonitis and sudden death in acute fascioliasis.

DNA studies and using of molecular tools aid in the exact identification of helminthes parasites. In ribosomal DNA (rDNA) the Internal Transcribed Spacer 2 (ITS2) gene have proven useful for diagnostic purposes at the level of species [2,3]. ITS2 sequences have also been used to characterize and identify different *Fasciola* spp., [4,5]. While,

mitochondrial DNA (mtDNA) genes proves to be excellent markers to differentiate in-between closely-related species and testing the polymorphism of *Fasciola* species [5]. In Egypt, *F. gigantica* and *Paramphistomum* spp are prevalent among livestock in the Nile delta [6], the aim of the present work is to identify helminthes infecting goats, their prevalence, morphology and molecular characterization of *Fasciola gigantica* infecting goats in Sadat district, Egypt.

## II. METHODS AND MATERIAL

### A. Samples collection and Morphological identification

Samples were collected from 790 goats at Sadat district from local abattoirs in 3 different localities; Kafr Dawoud, Wadi Elnatron and ElKhatatba. Worms were recovered from slaughtered goats from

different organs and during evacuation of gastrointestinal tract. Worms were washed, relaxed, fixed, stained and mounted [7]. Morphological description and identification of the collected worms were done on mounted specimens according to [8].

### B. DNA extraction from *Fasciola gigantica* and PCR

Adults of *F. gigantica* were collected from the liver of slaughtered goats and were identified according to morphometric parameters and all other unclear or doubtful samples were rejected [9,10]. These worms were washed several times with PBS and then preserved in tubes that stored at -20°C till DNA extraction [11].

Genomic DNA was extracted using 12 individual worms using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. All DNA concentrations were determined using an Epoch spectrophotometer (Biotek, Winooski, VT).

ITS2 region was amplified by polymerase chain reaction (PCR) using the forward primer (3S) 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and the reverse (A28) 5'-TAAAGAAAG AACATAATGAAAATAATC-3', [12,13].

The PCR conditions were as follows: 94°C for 3 min, 50°C for 1 min, and 72°C for 3 min for 30 cycles. Amplification reactions were performed in a final volume of 25 µl containing primers (3.2 pmol), deoxynucleoside triphosphates (dNTPs, 0.2 mM), and Taq polymerase (2.5 U/reaction). PCR products were purified using GFX PCR DNA and Gel Band Purification Kit (GE) according to manufacturer's protocol.

### C. Sequencing and phylogenetic analysis

PCR products were sequenced using Big Dye Terminator v 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems) in a 3100 Automated DNA Sequencer (Applied Biosystems) as recommended by the supplier. Amplicons were sequenced in each direction using the NC5 and NC2 primers, in separate reactions. Bio Edit v7.0.4.1 and DNASTAR Lasergene SeqMan v7.0.0 programs

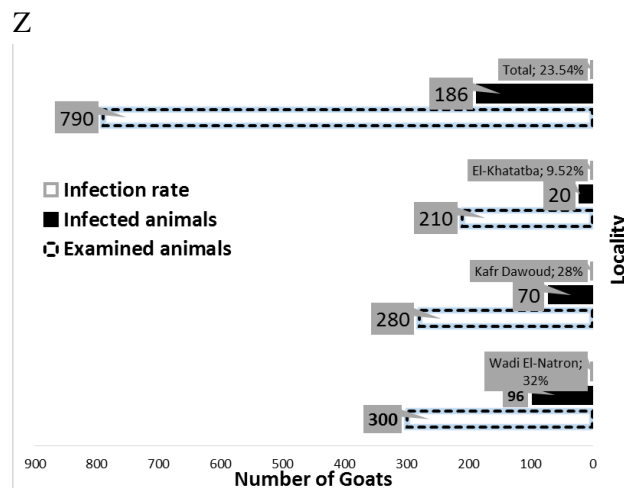
were used for analyzing and editing the DNA sequences.

Sequences of ITS2 regions from different isolates of previous studies were aligned against the isolate of the current study using ClustalW [14] multiple sequences alignment and the Phylogenetic trees were carried out using MEGA5 software [15].

## III. RESULTS AND DISCUSSION

### A. Incidence of the recovered helminthes

The overall incidence of different helminth species among examined slaughtered goats was 23.54% (186 out of 790) with variable infection rates in different localities where it was at Wadi El-Natron locality 32% (96 out of 300), at Kafr Dawoud was 6.25 % (70 out of 280) and at El-Khatatba locality was 9.52% (20 out of 210) (see Fig. 1).



**Figure 1:** Prevalence of helminth infections of slaughtered goats in different localities (Wadi El-Natron, Kafr Dawoud and El-Khatatba) at Sadat District, Egypt

The overall prevalence rate of trematode infection was 9.66% and the recovered trematode species and their associated infection rates were *Paramphistomum cervi* (1.43%), *F. hepatica* (0.41%), *F. gigantica* (5.83%), *Carmyerius gregarious* (1.99%). While the prevalence rate of cestodes was 9% and the recovered species and their correlated infection rates were *Moniezia expansa* (4.28%), *Moniezia benedeni* (0.71%) and *Avitellina centripunctata* (4%) (See Table 1).



**Table 1 :** Prevalence of different helminth infections in slaughtered goats (N=790) from different localities at Sadat District, Egypt.

	Infection rate %
Trematodes	9.66
<i>Fasciola gigantica</i>	5.83
<i>Fasciola hepatica</i>	0.41
<i>Carmyerius gregarius</i>	1.99
<i>Paramphistomum cervi</i>	1.43
Cestodes	9
<i>Moniezia expansa</i>	4.28
<i>Moniezia benedeni</i>	0.71
<i>Avitellina centripunctata</i>	4

The helminthes recovered from slaughtered goats were *F. gigantica*, *F. hepatica*, *Paramphistomum cervi*, *Carmyerius gregarius*, *Moniezia expansa*, *Moniezia benedeni*, *Moniezia denticulata* and *Avitellina centripunctata*. The infection rate of *Moniezia expansa*, was agreed with the results recorded in Rudolphi [16] and in Menofia governorate, Egypt [17], but it was disagreed with that recorded by [18] in north Sinai, Egypt.

**B. Genotyping of *Fasciola gigantica***

The genotyping of *F. gigantica* which recovered from goat in the current study is based on amplification of 18S ribosomal RNA gene sequence and its characteristics and sequence alignments (Fig. 2).

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NNGCNNCNCNCNTCGCTACTACCGATTGATGGTTTAGCAAGGTCCTCGGATTGG
TGCCATTTGCAAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAACTT
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GTCATCGGATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCAT
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TCCGACGGGTGGTGTACACAGAGGGTGGGTGCTATCCAGCCCAAAACCAA
    
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**Figure 2.** Complete sequences of the 18S-rRNA gene from *Fasciola gigantica* isolate Y15 of the current study.

For comparative purposes, pairwise and multiple alignments of the gene sequences was done with 18S-rRNA gene sequence of Fasciolides from different hosts and geographical regions obtained

from Gene Bank to evaluate sequence homology and diversity (Fig.3).

Main variable regions were identified in the 18S-rRNA gene sequences which were characterized by a considerable number of indels (insertion and deletion of nucleotides) as Single Nucleotide Polymorphism (SNPs) with 18S-rRNA gene of *F. gigantica* isolate BDF-Ct.109 (Accession number: KC476170) and *F. gigantica* isolate ADC53 (Accession number: KJ728737). By using pairwise and multiple alignment of 18S-rRNA gene of *F. gigantica* isolate 124 (FJ756397) with that of our strain, both sequences have revealed a high sequence homology, due to only a few indels and SNPs and shared a high number of identical nucleotides with *F. gigantica* isolate 124 (FJ756397) which was the most similar sequences (Fig.3).

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KC476170 -----CTCTACCGATTGAATGGTTTAGCAAGGTCCT 31
KC424484 -----TACTACCGATATGATGGTTTAGCAAGGTCCT 31
AB207141 -----TACTACCGATGAATGGTTTAGCAAGGTCCT 31
GQ925431 CCCTGCCCTTTGTACAGACCCCGCTCGACTACCCGATTGAATGGTTTAGCAAGGTCCT 68
KF982045 -----GATTA-ATACATCG-----ACTACTGATGAATGGTTTAGCAAGGTCCT 43
KJ728738 -----GATTA-ATACATCG-----ACTACTGATGAATGGTTTAGCAAGGTCCT 43
KJ728737 -----GATTA-ATACATCG-----ACTACTGATGAATGGTTTAGCAAGGTCCT 43
FJ756397 -----GATTA-ATACATCG-----ACTACTGATGAATGGTTTAGCAAGGTCCT 43
Y15 -----GATTA-ATACATCG-----ACTACTGATGAATGGTTTAGCAAGGTCCT 43
-----NNGCNNCNCNCNTCGCTACTACCGATTG-ATGGTTTAGCAAGGTCCT 46

KC476170 CCGATTGGTGCATT-GCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 98
KC424484 CCGATTGGTGCATT-GCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 98
AB207141 CCGATTGGTGCATT-GTAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 98
GQ925431 CCGATTGGTGCATT-GTAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 119
KF982045 CCGATTGGTGCATT-GTAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 182
KJ728738 CCGATTGGTGCATT-GCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 61
KJ728737 CCGATTGGTGCATT-GCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 61
FJ756397 CCGATTGGTGCATT-GCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 13
Y15 CCGATTGGTGCATTTGCAGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAA 186
*****

KC476170 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 158
KC424484 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 158
AB207141 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 158
GQ925431 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 179
KF982045 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 162
KJ728738 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 121
KJ728737 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 121
FJ756397 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 73
Y15 CTTGATCATTTAGAGGAAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACTGCGGAA 166
*****

KC476170 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 218
KC424484 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 218
AB207141 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 218
GQ925431 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 239
KF982045 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 222
KJ728738 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 181
KJ728737 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 181
FJ756397 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 133
Y15 GATCATTACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCACTG 226
*****

KC476170 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 270
KC424484 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 270
AB207141 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 270
GQ925431 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 299
KF982045 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 282
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KJ728737 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 241
FJ756397 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 193
Y15 GATAAAAAATTTGCGGACGGCTATGCTGGCTCATTGAGGTACAGCATATCCGATCACTG 286
*****

KC476170 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 330
KC424484 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 330
AB207141 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 330
GQ925431 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 359
KF982045 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 342
KJ728738 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 301
KJ728737 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 301
FJ756397 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 253
Y15 ATGGGGTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCGGGGCGCTTGTG 346
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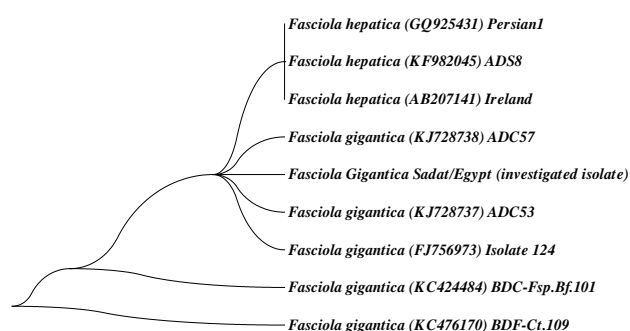
**Figure 3 :** Sequence characteristics and Sequence comparisons of the 18S-rRNA gene from *Fasciola gigantica* isolate Y15 represents the 18S-rRNA gene from *Fasciola gigantica* isolate of the current study)

Our isolate in the present study was very high homologous (99.9%) with other *F. gigantica* isolates, and the divergence was very low (0.1%), they were the closest inter specific pair, but the homology was much lower among *F. hepatica* and *F. gigantica* isolate ADC53 (KJ728737) which indicates that the ITS2 region allows discrimination between species of Fasciolidae [19].

Inter specific variation (between species) in the regions exceeded that within species. The variation between species ranged between 0.1 and 1.4%, and so, the alignments of the 18S rRNA gene sequence of the different isolates showed that the species differed from each other by single base substitutions and indels, and the first 250 and last 190 nucleotides of the gene were identical in all isolates.

### C. The phylogenetic analysis

By reading the phylogenetic tree (Fig.4), *F. gigantica* isolate in the present study was clustered with *F. gigantica* isolate 124 (FJ756397) as they were very identical to each other, but somewhat differ from *F. gigantica* isolate BDC-Fsp.Bf.101 (KC424484), isolate BDF-Ct.109 (KC476170) and isolate ADC57 (KJ728738) but it lying faraway from *F. hepatica* isolate Persian 1 (GQ925431).



**Figure 4.** Neighbor-Joining phylogenetic tree [20]. The phylogenetic distances were computed using the Maximum Composite Likelihood method [21] and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 404 positions in the final dataset. Phylogenetic analyses were conducted in MEGA5 [15].

The phylogenetic tree based on ITS2 region displayed a close relationship between investigated *F. gigantica* isolate from Egypt and other parts of the world (Fig.4).

In Egypt, *F. hepatica* and *F. gigantica* are prevalent among livestock in the Nile delta [7] results showed that identification of Egyptian *Fasciola* based only on morphometric criteria is not a countable as the presence of *F. hepatica*, *F. gigantica* besides the hybrid form in Egypt was confirmed [22]. In a study was done in Egypt [13], nucleotide sequences of the mitochondrial DNA, cytochrome oxidase subunit 1 (CO1) and Internal Transcribed Spacer 2 (ITS2) of the ribosomal RNA gene were used to identify *Fasciola* species that infects cattle in Qena province, Upper Egypt, and they concluded that, to sequencing, amino acids analysis and studying the phylogenetic relationship are precise tools to identify *Fasciola* species.

## IV. CONCLUSION

The results displayed herein indicated that the using of ITS2 region is a powerful tool for identification and discrimination between the Fasciolidae species.

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# Bio-activity of Cabbage (*Brassica oleracea var capitata*) Plant Product as Insecticides to control mustard aphid, *Lipaphis erysimi* Kaltenbach (Aphididae: Homoptera)

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## ABSTRACT

Three botanical pesticides, *Azadirachta indica* leaves extract, *Acacia catechu* leaf and bark extract, *Carica papaya* seed extract and three chemical pesticides Indocarb 15 SC, 0.006%, @30 a.i./ha, 200 ml / g / ha, Fipronil 5EC 0.005%, 25-50 g ai / ha, 500 ml / g / ha and Endosulfan 35 EC, 0.05-0.07 %, 250-500 a.i. / ha 700-1004 ml / g / h were tested against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the *L. erysimi* on field cabbage under both laboratory and field conditions. In square dip experiment a highly significant difference was recorded amongst the different treatments for mean mortality of *L. erysimi*. The maximum mean mortality was obtained at NLE2.5% >NLE5%> NSE 10% >AcLE 2.5%> AcSE 2.5%. The order was found to be descending. Repellency test through square dip experiments showed that the significant difference was recorded amongst the different treatments for mean mortality of larvae. During larval immersion method NLE2.5% was proved to be most significant followed by NLE 5%> AcLE 2.5% > ASE (2.5-5%) > Fipronil 0.0005%. However, NLE was found superior than botanicals in both square dip and larval immersion methods. The field spray schedule showed the significant results with the spray of NSE (10%) > ALE (10%) >ASE10% CpLE5% > CpLE-10% while, the second spray was significantly effective against CpLE 10% > Endosulfan 35EC@0.05-0.07% > NLE5% while the spray by CpLE10% and Endosulfan 35EC@0.05-0.07 were found significant and most promising.

**Keywords:** *Lipaphis erysimi*, Carica seed, Carica leaf, Neem leaf, Acacia bark, Acacia leaf, Extract, Repellent.

## I. INTRODUCTION

Today the rapid increase in population and demand of food materials has initiated the large use of insecticides and pesticides. These toxic chemical insecticides and pesticides are resulting in harmful effects and biomagnifications which is continuously polluting fertile lands and acquiring infertility. No doubt they provide results in eradication of insects, pests and diseases but are also killing useful organism the soil and affects soil fertility. The conventional farming practices based on chemical methods broadly kill arthropods, resulting in the malfunctioning of food chain and food web.

Bio-control is the best method to cope with the losses done by the chemicals. In these method insects, pests and pathogens are removed using biological methods without harming the environment and other organism. This is based on natural predation rather than introduced chemicals. The use of bio-insecticides and pesticides also comes under this category. Today due to awareness about the harmful effects of the chemical insecticides and pesticides, most of the farmers are diverting towards the organic farming. In our local area many such plants, waste matter etc. are available from which these bio-insecticides and pesticides can be prepared by using natural means only. Conventional pesticides

are generally synthetic materials that directly kill or inactivate the pest. Being single chemical entity, chemical pesticides have resulted in increased resistance in pests.

Biological Pesticides are pesticides derived from natural materials as animals, plants, bacteria, and certain minerals. Bio pesticides are less toxic and also reduce the pollution problems caused by conventional pesticides. The use of bio-insecticides and bio-pesticides also fall under this category only. Organic agriculture is a unique production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity, and this is accomplished by using on-farm agronomic, biological and mechanical methods in exclusion of all synthetic off-farm inputs. Organic farming generally produces somewhat lower yields but sustains better yields during drought years, allowing it to reap higher yields in some cases. Studies thus far have shown that organic farming requires less water, uses few and always natural pesticides, prevents soil erosion, leaches dramatically fewer nitrates, and has been shown to have improved nutrient qualities including as much as double the flavonoids, an important antioxidant. "Biopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs." Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields. With the advent of chemical pesticides, this crisis was resolved to a great extent. But the over dependence on chemical pesticides and eventual uninhibited use of them has necessitated for alternatives mainly for environmental concerns. Degraded soils and groundwater pollution has resulted in nutritionally imbalanced and unproductive lands. Violative pesticide residues also sometimes raise food

safety concerns among domestic consumers and pose trade impediments for export crops. Therefore, an ecofriendly alternative is the need of the hour. Biopesticides or biological pesticides based on plant extracts specific to a target pest offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health. The potential benefits to agriculture and public health programmes through the use of biopesticides are considerable. The interest in biopesticides is based on the advantages associated with such products which are: (i) inherently less harmful and less environmental load, (ii) designed to affect only one specific pest or, in some cases, a few target organisms, (iii) often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems and (iv) when used as a component of Integrated Pest Management (IPM) programs, biopesticides can contribute greatly.

*Lipaphis erysimi* is regarded as the most important pest of cruciferous crops worldwide (Prasad and Phadke 1988; Bonnemaïson 1965). It causes considerable yield loss to late season cabbage crop. Several insecticides have been recommended for its control (Murthy et al., 1982; Yadava et al., 1988., Dhura and Hameed., 1990; Zhu et al., 1996; Lal et al., 1999). In addition to the damage it does as a sapsucker, it is also a vector of several viral diseases (Guan and Wang 1980; Ahlawat and Chenulu 1982; Castle et al. 1992; Kennedy and Abou-Ghandir 1987; Liu et al. 1997; Liu and Yue 2001; Bridge et al. 2001). Mohan et al; (1981) found that *L. erysimi* and *Crociodolomia binotalis*, are major pest of cabbage, Methamidophos at 0.25 or 0.5 kg/h gave an excellent control of both pests. Pandey et al (1987) evaluated the 3 concentrations (0.5, 1.0 and 1.5%) of Neem seed kernel extract against *L. erysimi* under laboratory conditions and found that 80 percent was given by 1.5% concentration. 1.0% concentration was also effective.

Insect-pests are known to cause significant

damage to crops and affect agricultural productivity. The environmental hazards posed by synthetic pesticides. Due to high cost of protecting crops from these pests with chemical pesticides and the increasing resistance and resurgence to many chemical pesticides (Armes et al. 1992; Brewer & Trumble 1994) there is growing interest in the use of biological products such as bacterial and viral-based insecticides, and parasitoids (Nagarkatti 1982), predators (King et al., 1982) and botanical pesticides (Rao et al. 1990). These groups have different mode of action from conventional products (Thompson et al. 1999) and their properties may differ considerably from the conventional chemicals with which growers are familiar. It is therefore important to generate information on the likely differences in the performances of these products to educate growers and facilitate adoption.

The objective of this study was to evaluate commercially available biological and botanical pesticides both individually and partly in combination against mustard aphid species on cabbage to determine their effects under laboratory and field conditions. So, this additional information would make existing IPM programmes more effective and sustainable, while decreasing the reliance on synthetic insecticides.

## II. METHODS AND MATERIAL

### Extraction of Plant Materials

#### (A) **Azadirchata Indica**

The shade dried leaves of different neem plants were ground in an electrical grinder to make a fine powder. For extraction, 10gm powder of each plant leaves were weighed for extraction through petroleum ether (40-600C) and then another sample of 10 gm each was taken for obtaining alcoholic extracts with the help of soxhlet apparatus. The extraction was completed within 4 hours. The extracts obtained in the reservoir of the soxhlet apparatus evaporated on a water bath till they remained about 15 ml and then transferred to

pre-weighed 50 ml beakers, through filtration from a thick layer of anhydrous sodium sulphate made on silica jel on glass wool plugged funnels. The extracts were again dried over water bath so as to obtain a semi-solid extractive of each plant. The extractives were used to make the stock solution. One percent stock solutions of all the fractions in methanol were prepared from the residues obtained at each stage of the purification process and the fractions were tested at different concentrations.

(B) **Acacia catechu:** One kg of the dried leaves and bark Acacia catechu was taken in an aluminium pot to which ten litres of water were added so that the chips completely immersed under water. It was boiled over an open fire for four hours and allowed to stand for 24 hours so that more catechu might diffuse into the water. The extract was decanted off in a pot and was filtered through a fine muslin cloth to remove wood chips and other suspended materials. The filtrate was evaporated and the residue obtained was air dried and weighed (180g). Yield of catechu was 18%. Isolated catechu (150g) was taken in a five-litre stainless steel beaker containing one litre distilled water. It was boiled with constant stirring for complete dissolution and filtered through a filter paper. Then it was evaporated to 500 ml and allowed to stand for 24 hours. The obtained precipitate was filtered using a filter paper. The aqueous filtrate was rejected. The residue was dissolve in ethanol and filtered. The ethanolic solution was evaporated to dryness and the residue was dissolved into hot water (500 ml). It was allowed to stand for 24 hours. The precipitate was filtered and dried in air (m.p. 95-6°C, yield 37.5g, 25%).

(C) **Carica papaya :** papaya fruit was obtained from market. Seeds were shade-dried for a minimum of 15 days. Powdered seeds (1 kg) were extracted with chloroform (3.0 L), under reflux, for 4 h; the extract was cooled to room temperature and filtered. Solvent was removed under reduced pressure by rotatory evaporator and the extract was dried in a vacuum oven at room temperature for 12 h (yield, 7.2% by weight). Fatty acid methyl esters were prepared according to the AOAC-IUPAC Method 969.33 [18]. Chloroform extract (90 mg) and 1 N solution of NaOH in methanol (4 mL) were placed in a round-bottomed flask, and the mixture was heated at boiling point with stirring for 15 min. Next, BF<sub>3</sub>-MeOH (5 mL, 15% w/w) were added and heating continued for 5 min. Iso-octane (2 mL)

was added; the mixture was stirred for 5 min, more and extracted with hexane (2 mL). The organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The fatty acid methyl esters were analyzed on an Agilent Technologies 6890N GC equipped with an HP-5MS column (30 m in length; 25 mm internal diameter; 0.25 µm film thickness) equipped with an Agilent EM 5973 detector, at 150 °C. The carrier gas was helium, at a flow rate of 1 mL/min; the split ratio was 2:1. The column temperature was initially 60 °C (for 3 min) and was gradually increased to 170 °C, at 3 °C/min; this temperature was held for 1 min. Next, the temperature was raised to 330 °C, at a rate of 10 °C/min; this temperature was held for 10 min. The injector temperature was 330 °C and 1 µL of organic phase were injected by duplicate.

### **Insects**

The larvae used for the study were collected from the host plants of different vegetables in the fields and brought to lab, under laboratory conditions. The culture of *L.erysimi* was maintained in the laboratory on semi synthetic diet as suggested by Nagarkatti and Prakash (1974) with some modifications at a temperature of 27± 1°C and relative humidity 60 ± 1 percent. They were reared on artificial diet in small round plastic vials (3.5x2.0Cm) till pupation under laboratory conditions. Studies were carried out using I-VI instar larvae of *L. erysimi* against the leaf extract of *A.indica*. The percentage mortality was calculated after a period of 24h. Generally, second and fourth-stage larvae were used in various experiments and they were starved for 12 h before all experiments.

### **Bioefficacy Evaluation**

The various botanical and synthetic preparations used in laboratory and field are listed in Table 1 (Figure 1). The host plants (*Brassica oleracea* var. *capitata*) used for the spraying tests in the laboratory and field were 3 to 5 weeks old and with 7-8 branches. Under laboratory conditions, the tests were carried out in petri dishes (8.5 cm diameter).

In Square Dip Experiment, design was CRD with three replications. The medium sized test leaves were collected from unsprayed fields. A total of 30 equal sized squares were dipped into each treatment for 20 seconds as shown in Table 1, and then air dried for 60 minutes. Weight of each larva was recorded before

treatment application using sensitive balance. The treated leaves were placed into the Petri dishes on moistened filter paper (one larvae per petri dish) with the adaxial surface uppermost. *L. erysimi* larvae were then placed onto the leaf disc and then a cover was put onto the dish. For control treatments the leaves were dipped in water only.

In larval immersion experiment, the larvae were immersed into the respective treatments for 20 seconds and then transferred to paper padded tray in order to remove excessive liquid from the body of the larvae. The purpose of this experiment is to evaluate the contact effect of pesticides on insects. The design in this experiment is CRD with three replications. Like in the square dip experiment, a total of 30 larvae were tested in each treatment. Third instar larvae were weighed before treatment application.

The experiments were conducted in the laboratory with a temperature of 25 ± 1 °C light regime of 14 h light 10 h dark and relative humidity of 65 ± 1 %. Mortality was assessed every 24 h, 48 h, and 72 h in all the experiments.

For the experiments under field conditions, the plants of *Brassica oleracea* var. *capitata* were grown 3-5 weeks prior to conducting the experiments in plots. The planting distances were 70 cm x30cm on plots that measured 4.2mx4.0m. When the plants attained about 7-8 branches, the solutions of various treatments were applied with a trigger sprayer, misting to run-off level. Water was used as a control. The spray equipment was drained and triple rinsed after each treatment to avoid any contamination. Second and third instars of *L. erysimi* were placed on each plant and ten plants were used in each treatment (30 larvae per treatment) and observations were recorded before and after 4 hrs, 8 hrs, 24 hrs and 32 hrs from the time of spray. In the experimental field trial three replication for each treatment were performed.

### **Statistical Analysis**

For statistical analysis of efficacy of insecticides to *L. erysimi* mortality due to the different insecticides was analysed using the Tukey's Studentized Rang (HSD) Test.

### III. RESULT AND DISCUSSION

#### Toxicity of insecticides to *L. erysimi*

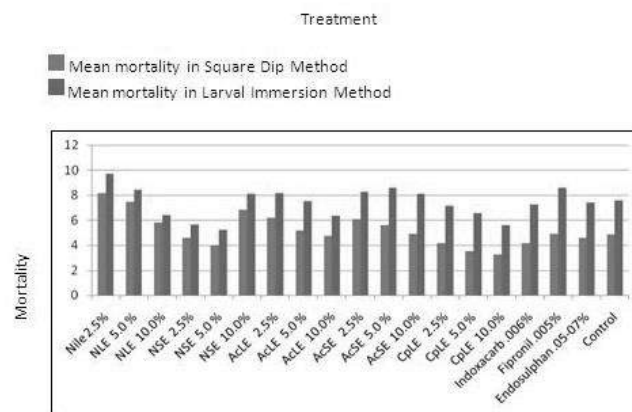
Our results show significant differences in the mortality recorded from the different treatments under laboratory and field conditions. The lowest mean mortality was recorded by CpLE-10% and control water treatment as shown in Table1(figure1). Significantly higher mortality was detected in all the treatments compared with the untreated controls as shown in Table 2 (Figure 2 and Figure 2 continued) and Table 3 (Figure 3). The effect of feeding on larvae found highly significant at 72 hours after treatment in *Carica papaya* leaf extract 2.5% followed to 48 hours after treatment in Neem Leaf extract 2.5%, 48 hours after treatment in Neem Seed Extract2.5%,24 hours after treatment in Neem Seed extract 5%, 24 hours after treatment in *Carica papaya* leaf extract 2.5%, 24-72 hours after treatment in *Carica papaya* leaf extract 5% while, other treatment were not found significant. Bhatal et al.,(1993) studied the effects of AZT-VR-K(an Azadirachtin rich acetone extract of Neem seed kernel extract)and commercial Neem products and reported that development, reproduction and mortality of mustard aphid (*L. erysimi*) reduced. Sontakke and Das (1996) used Neem formulations for the control of *L. erysimi* infesting mustard and found that quinolphos was the most effective followed by Chlorpyrifos and Endosulfan and result in the highest seed yield. The results obtained on the effect of repellency, feeding larvae, their weight loss through square dip and larval immersion methods were found in conformity of Klocke (1987). Azadirachtin- rich diets lead to decreased feeding and weight gain, as well as biomass conversion rates.

The field spray schedule for I, II, III spray showed the significant results on I spray by Neem Seed Extract 10% followed to Acacia leaf extract 10%,Acacia Seed Extract10%,*Carica papaya* leaf extract (5-10%) while, the II spray was significantly effective against CpLE10%, Endosulfan35@ 0.05-0.07% ,NLE5% while the spray by CpLE 10% and Endosulfan 35 EC@0.05-0.07 were found significant and most promising. These findings were found in conformity of Kabir and Mia (1987), who found Neem reduced the infestation and increased the yield.

**Table 1 :** Repellency Test, Mean number of *Lipaphis erysimi* larvae died (Square Dip and Larval Immersion Method)

S.N.	Botanicals Treatment	Square Dip Method Mean $\pm$ SE	Larval Immersion Method Mean $\pm$ SE
01	NLE 2.5%	8.183 $\pm$ 0.093 <sup>a</sup>	9.700 $\pm$ 0.050 <sup>b</sup>
02	NLE 5.0 %	7.467 $\pm$ 0.174 <sup>b</sup>	8.417 $\pm$ 0.167 <sup>c</sup>
03	NLE 10.0%	5.833 $\pm$ 0.083 <sup>de</sup>	6.417 $\pm$ 0.167 <sup>g</sup>
04	NSE 2.5%	4.583 $\pm$ 0.220 <sup>g</sup>	5.667 $\pm$ 0.220 <sup>h</sup>
05	NSE 5.0 %	4.000 $\pm$ 0.144 <sup>h</sup>	5.250 $\pm$ 0.144 <sup>h</sup>
06	NSE 10.0%	6.833 $\pm$ 0.083 <sup>c</sup>	8.083 $\pm$ 0.083 <sup>cd</sup>
07	AcLE 2.5%	6.167 $\pm$ 0.083 <sup>d</sup>	8.183 $\pm$ 0.093 <sup>c</sup>
08	AcLE 5.0 %	5.167 $\pm$ 0.083 <sup>f</sup>	7.500 $\pm$ 0.144 <sup>f</sup>
09	AcLE 10.0%	4.750 $\pm$ 0.144 <sup>g</sup>	6.333 $\pm$ 0.220 <sup>g</sup>
10	AcSE 2.5%	6.083 $\pm$ 0.083 <sup>d</sup>	8.250 $\pm$ 0.144 <sup>c</sup>
11	AcSE 5.0 %	5.583 $\pm$ 0.083 <sup>e</sup>	8.583 $\pm$ 0.083 <sup>c</sup>
12	AcSE 10.0%	4.917 $\pm$ 0.083 <sup>fg</sup>	8.083 $\pm$ 0.083 <sup>cd</sup>
13	CpLE 2.5%	4.167 $\pm$ 0.083 <sup>h</sup>	7.167 $\pm$ 0.083 <sup>def</sup>
14	CpLE 5.0 %	3.500 $\pm$ 0.144 <sup>i</sup>	6.583 $\pm$ 0.300 <sup>g</sup>
15	CpLE 10.0%	3.233 $\pm$ 0.145 <sup>i</sup>	5.583 $\pm$ 0.083 <sup>h</sup>
16	Indoxacarb .006%	4.167 $\pm$ 0.083 <sup>h</sup>	7.250 $\pm$ 0.144 <sup>f</sup>
17	Fipronil .005%	4.917 $\pm$ 0.083 <sup>fg</sup>	8.583 $\pm$ 0.300 <sup>c</sup>
18	Endosulphan .05-07%	4.567 $\pm$ 0.067 <sup>g</sup>	7.417 $\pm$ 0.300 <sup>f</sup>
19	Control	4.833 $\pm$ 0.083 <sup>fg</sup>	7.583 $\pm$ 0.167 <sup>df</sup>

Mean followed by the same letter within the column are not significantly different from each other at P < 0.05, Tukey 's Studentized Rang (HSD) Test. NLE = Neem Leaf Extract,; AcLE = Acacia leaf Extract; CpLE =Carica Papaya leaf Extract



**Figure 1:** Repellency Test, Mean number of *Lipaphis erysimi* larvae died (Square Dip and Larval Immersion Method)

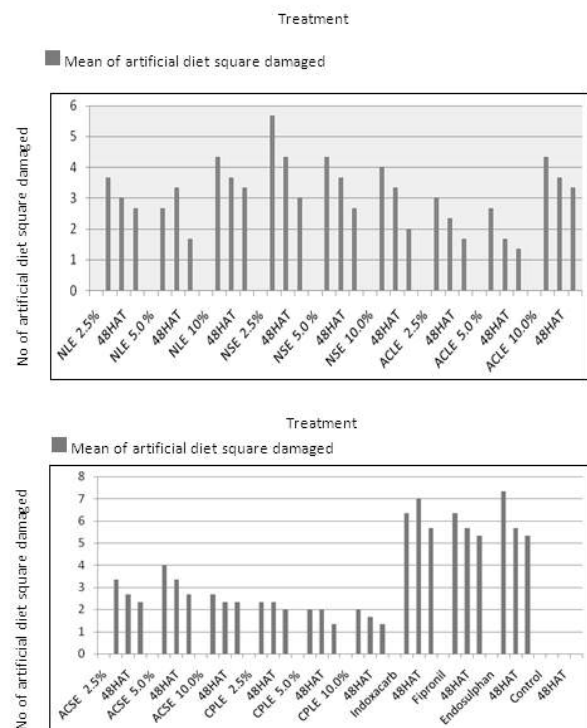
**Table 2:** Effect on Feeding: Mean number of *Lipaphis erysimi* damaged square within 24, 48, 72 hrs after treatment in square dip method



S.N.	Hours After Treatments	Mean of artificial Diet Square Damaged Mean ± SE
01	NLE 2.5%	
	24HAT	3.667± 0.333 <sup>def</sup>
	48HAT	3.000± 0.577 <sup>efgh</sup>
	72HAT	2.667± 0.333 <sup>fghi</sup>
02	NLE 5.0 %	
	24HAT	2.667± 0.333 <sup>fghi</sup>
	48HAT	3.333± 0.333 <sup>defg</sup>
	72HAT	1.667± 0.333 <sup>ij</sup>
03	NLE 10%	
	24HAT	4.333± 0.333 <sup>d</sup>
	48HAT	3.667± 0.333 <sup>def</sup>
	72HAT	3.333± 0.333 <sup>defg</sup>
04	NSE 2.5%	
	24HAT	5.667± 0.333 <sup>c</sup>
	48HAT	4.333± 0.333 <sup>d</sup>
	72HAT	3.000± 0.000 <sup>efgh</sup>
05	NSE 5.0 %	
	24HAT	4.333± 0.333 <sup>d</sup>
	48HAT	3.667± 0.333 <sup>def</sup>
	72HAT	2.667± 0.333 <sup>fghi</sup>
06	NSE 10.0%	
	24HAT	4.000± 0.000 <sup>de</sup>
	48HAT	3.333± 0.333 <sup>defg</sup>
	72HAT	2.000± 0.000 <sup>hij</sup>
07	ACLE 2.5%	
	24HAT	3.000± 0.000 <sup>efgh</sup>
	48HAT	2.333±0.333 <sup>ghij</sup>
	72HAT	1.667±0.333 <sup>ij</sup>
08	ACLE 5.0 %	
	24HAT	2.667±0.333 <sup>fghi</sup>
	48HAT	1.667±0.333 <sup>ij</sup>
	72HAT	1.333±0.333 <sup>j</sup>
09	ACLE 10.0%	
	24HAT	4.333±0.333 <sup>d</sup>
	48HAT	3.667±0.333 <sup>def</sup>
	72HAT	3.333±0.333 <sup>defg</sup>
10	ACSE 2.5%	
	24HAT	3.333±0.333 <sup>defg</sup>
	48HAT	2.667±0.667 <sup>fghi</sup>
	72HAT	2.333±0.333 <sup>ghij</sup>
11	ACSE 5.0 %	
	24HAT	4.000±0.000 <sup>de</sup>
	48HAT	3.333±0.333 <sup>defg</sup>
	72HAT	2.667±0.333 <sup>fghi</sup>
12	ACSE 10.0%	
	24HAT	2.667±0.333 <sup>fghi</sup>
	48HAT	2.333±0.333 <sup>ghij</sup>
	72HAT	2.333±0.333 <sup>ghij</sup>
13	CPLE 2.5%	
	24HAT	2.333±0.333 <sup>ghij</sup>

S.N.	Hours After Treatments	Mean of artificial Diet Square Damaged Mean ± SE
14	CPL 5.0 %	
	24HAT	2.000±0.577 <sup>hij</sup>
	48HAT	2.000±0.000 <sup>hij</sup>
	72HAT	1.333±0.333 <sup>j</sup>
15	CPL 10.0%	
	24HAT	2.000±0.000 <sup>hij</sup>
	48HAT	1.667±0.333 <sup>ij</sup>
	72HAT	1.333±0.333 <sup>j</sup>
16	Indoxacarb	
	24HAT	6.333±0.333 <sup>bc</sup>
	48HAT	7.000±0.000 <sup>ab</sup>
	72HAT	5.667±0.333 <sup>c</sup>
17	Fipronil	
	24HAT	6.333±0.333 <sup>bc</sup>
	48HAT	5.667±0.333 <sup>c</sup>
	72HAT	5.333±0.333 <sup>c</sup>
18	Endosulphan	
	24HAT	7.333±0.333 <sup>a</sup>
	48HAT	5.667±0.333 <sup>c</sup>
	72HAT	5.333±0.333 <sup>c</sup>
19	Control	
	24HAT	0.000±0.000 <sup>k</sup>
	48HAT	0.000±0.000 <sup>k</sup>
	72HAT	0.000±0.000 <sup>k</sup>

Mean followed by the same letter within the column are not significantly different from each other at P < 0.05, Tukey's Studentized Range (HSD) Test. NLE = Neem Leaf Extract,; AcLE = Acacia leaf Extract; CPL = Carica Papaya leaf Extract

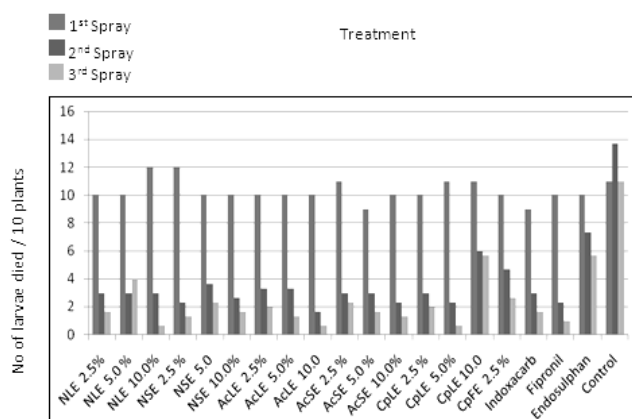


**Figure 2 :** Effect on Feeding: Mean number of *Lipaphis erysimi* damaged square within 24, 48, 72 hrs after treatment in square dip method

**Table 3 :** Effects of Different field treatment on *Lipaphis erysimi* at *Brassicae* crop

S.N.	Treatments	Pre-count	I spray larvae died/ 10 Plants	II Spray larvae died/ 10 Plants	III Spray larvae died/ 10 Plants	Fruit damage (%)
01	NLE 2.5%	10.	3.000±0.577 <sup>de</sup>	1.667±0.333 <sup>def</sup>	0.333±0.333 <sup>f</sup>	2.50%
02	NLE 5.0 %	10.	3.000±0.577 <sup>de</sup>	04.000±0.577 <sup>c</sup>	1.667±0.333 <sup>bde</sup>	3.50%
03	NLE 10.0%	12.	3.000±1.528 <sup>de</sup>	00.667±0.333 <sup>f</sup>	0.333±0.333 <sup>f</sup>	2.50%
04	NSE 2.5 %	12.	2.333±0.333 <sup>e</sup>	1.333±0.333 <sup>def</sup>	0.333±0.333 <sup>f</sup>	2.50%
05	NSE 5.0	10.	3.667±0.333 <sup>de</sup>	2.333±0.333 <sup>de</sup>	0.333±0.333 <sup>f</sup>	2.50%
06	NSE 10.0%	10.	2.667±0.667 <sup>de</sup>	1.667±0.333 <sup>def</sup>	0.333±0.333 <sup>f</sup>	2.50%
07	AcLE 2.5%	10	3.333±0.333 <sup>de</sup>	2.000±0.577 <sup>def</sup>	0.667±0.333 <sup>ef</sup>	2.50%
08	AcLE 5.0%	10	3.333±0.882 <sup>de</sup>	1.333±0.333 <sup>def</sup>	0.333±0.333 <sup>f</sup>	2.50%
09	AcLE 10.0	10	1.667±0.667 <sup>e</sup>	00.667±0.333 <sup>f</sup>	0.333±0.333 <sup>f</sup>	2.50%
10	AcSE 2.5 %	11	3.000±0.577 <sup>de</sup>	2.333±0.333 <sup>de</sup>	1.333±0.333 <sup>def</sup>	3.75%
11	AcSE 5.0 %	9.	3.000±0.577 <sup>de</sup>	1.667±0.333 <sup>def</sup>	0.667±0.333 <sup>ef</sup>	3.00 %
12	AcSE 10.0%	10	2.333±0.333 <sup>e</sup>	1.333±0.333 <sup>def</sup>	0.333±0.333 <sup>f</sup>	2.50%
13	CpLE 2.5 %	10	3.000±0.577 <sup>de</sup>	2.000±0.000 <sup>def</sup>	1.000±0.000 <sup>def</sup>	3.25%
14	CpLE 5.0%	11	02.333±0.882 <sup>e</sup>	00.667±0.333 <sup>f</sup>	0.333±0.333 <sup>def</sup>	2.50%
15	CpLE 10.0	11	6.000±0.577 <sup>bc</sup>	05.667±0.333 <sup>b</sup>	2.667±0.333 <sup>b</sup>	3.00%
16	CpFE 2.5 %	10	4.667±0.882 <sup>cd</sup>	02.667±0.667 <sup>d</sup>	2.000±0.577 <sup>bcd</sup>	2.75%
17	Indoxacarb	09	3.000±0.577 <sup>de</sup>	1.667±0.333 <sup>def</sup>	1.000±0.000 <sup>def</sup>	3.25%
18	Fipronil	10	2.333±0.882 <sup>e</sup>	01.000±0.577 <sup>ef</sup>	0.333±0.333 <sup>def</sup>	2.50%
19	Endosulphan	10	7.333±0.333 <sup>b</sup>	05.667±0.333 <sup>b</sup>	2.667±0.333 <sup>b</sup>	3.00%
20	Control	11	13.67±0.333 <sup>a</sup>	11.00±0.577 <sup>a</sup>	6.330±0.333 <sup>a</sup>	8.00%

Mean followed by the same letter within the column are not significantly different from each other at P < 0.05, Tukey 's Studentized Rang (HSD) Test. NLE = Neem Leaf Extract,; AcLE = Acacia leaf Extract; AcSE = Acacia Seed Extract; CpLE =Carica Papaya leaf Extract



**Figure 3.** Effects of Different field treatment on *Lipaphis erysimi* at *Brassicae* crop

#### IV. CONCLUSION

A chemical pesticide is used to protect crops and to kill pests. Use of synthetic pesticides causes some unfortunate consequences like environmental pollution, pest resistance and toxicity to other non-target organisms. To allay the fear of the hazardous effect of chemical residues to human and animal health, several studies were conducted to determine the most effective control methods without using insecticides. In this research work we have used various botanicals against one of the most notorious pest *Lipaphis erysimi*, KALT (Mustard: Aphid) belonging to order Homoptera: Aphididae) is a serious pest on several cruciferae and other economically important crops and is widely distributed worldwide.

The results of our study indicate that the plant products could be the best alternatives for the sustainable management of *L.erysimi* on cabbage with less impact on the naturally occurring predatory arthropods. Few botanicals have been reported as effective managers of insect-pests and commercialized. Much knowledge and experience of using these is treasured in farmer's traditional knowledge. Derived from the Neem tree (*Azadirachta indica*), this contains several chemicals, including 'Azadirachtin', which affects the reproductive and digestive process of a number of important pests. Recent research carried out in India and abroad has led to the development of effective formulations of Neem, which are being commercially produced. As Neem is non-toxic to birds and mammals and is non-carcinogenic, its demand is likely to increase.

Our view has been supported by Lal (1996) Nimbicidine and NeemMark caused 20-26% mortality of winged adult aphid after 2 days application while NeemGold and Jawan gave about 15% mortality. Patel et al., (1996) Endosulfan (0.035%, Chlorpyrifos (0.02%) and Neem Seed Kernal Suspension (NSKS 0.3%) were most effective in controlling the pest.

It is widely recognized that we face a major challenge continuing to increase agricultural productivity to keep pace with a population racing toward 9 billion within the next few decades. Agricultural practices developed and honed in the 20th century, from the development of synthetic nitrogen fertilizers by Fritz Haber in the early nineteenth century (Smil 2004) to the invention of synthetic pesticides in the decades following, (Casida & Ousted 1998, Knight et al. 1997) have greatly improved crop productivity which has helped cope with an ever-increasing global population to date. While crop production has certainly benefited, technological improvements have unfortunately also led to unexpected consequences for non-target organisms, soil and water quality. The development of synthetic pesticides has additionally resulted in challenges related to pest resistance which further complicates the drive towards improving yields. Growers struggle against a variety of pests during the crop season. Plant pathogens, for example, are responsible for dramatic yield losses. The Crop Life Foundation's 2005 study reviewed and endorsed by 38 commodity groups (including the National Cotton Council and United Soybean Board) says if left untreated, yields of most fruit and vegetable crops

would plunge 50 to 95 percent (Gianissi 2005). Weeds and insect damage contribute to substantial impact on crop losses. In early agricultural practices, fungicides such as sulfur and copper were used to cope with plant diseases. These products have been used for centuries and are still heavily relied upon today. However, a step change in approach was experienced with the discovery of single site mode of action fungicides, often with systemic properties. These highly potent molecules provided exceptional disease control with much lower use rates.

Unfortunately, the ever-evolving pathogen population has been able to adapt to these new chemical classes quickly because of their selective modes of action. It is found that more recently developed chemical fungicides also correlate with more rapid reports of resistance in the field (adapted from Thind, 2011). One of the greatest challenges to agriculture today is the paucity of new active ingredients with new modes of action unrelated to previously introduced chemistries. Since the use of agrochemicals with single site modes of action became widespread in the last fifty years, this has become of greater and greater concern. In recent years, interest in the use of biopesticides in conventional agricultural practices, both by growers and the agrichemical companies, has grown (Reiter 2011). Biopesticides are appealing for a number of reasons. According to the EPA, biopesticides are usually less toxic than conventional pesticides, generally affect only the target pest and closely related organisms, often are effective in very small quantities and decompose quickly, and can greatly decrease the use of conventional pesticides while crop yields remain high. Growers and agrichemical companies also see biopesticides as potentially important tools in their efforts to stave off the development of pesticide resistance. Biopesticides are often complex in their activities and modes of action, offering new tools in the quest to develop programs that can manage resistance. For example, products based on the *Bacillus Subtilis* strain QST 713, including Serenade ASO® fungicide, Serenade Max® fungicide and Serenade Soil® fungicide have been demonstrated to have several modes of activity. These include complex secondary metabolite profiles responsible for both anti-fungal and anti-bacterial activity. Detailed studies of the biophysical interaction of the lipopeptide class of compounds produced by this strain have shown complex membrane interactions (Patel et al. 2011).

These require somewhat greater application rates (as high as 1% active ingredient) and may require frequent reapplication when used out-of-doors. It is known that these extracts contain Azadirachtin in Neem, Catechin in Acacia catechu and Palmitic acid in Carica papaya. The management efficacy of these compounds in comparison to the chemical pesticides was also remarkable and cost effective. Neem pesticides do not leave any residue on the crop. They also work as systemic pesticide; absorbed into the plant, transported to all the tissues and are ingested by plant feeding insects. Azadirachtin is considered nontoxic to mammals, fish and pollinators, having low mammalian toxicity with LD50 of >5000 mg/kg for rat. It is classified by Environment Protection Agency (EPA) as class IV. It is felt that none of the synthetic pesticides developed so far has the excellent virtues of Neem in pest management. Thus, it opens opportunity for their commercialization on large scale without any adverse effects on crop and soil.

## V. ACKNOWLEDGMENT

We thank Dr. P.N. Chowdhary, Former Principal Scientist, IARI, New Delhi, India and Dr. Seema Bhadauria, Reader, Department of Botany, Raja Balwant Singh College, Dr.B.R.A.University, Agra, India for providing all related guidance for field, lab/experiment work and all required support for certification of statistics work related for this research work.

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# Classification of Epileptic & Non Epileptic EEG Signal Using Matlab

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## ABSTRACT

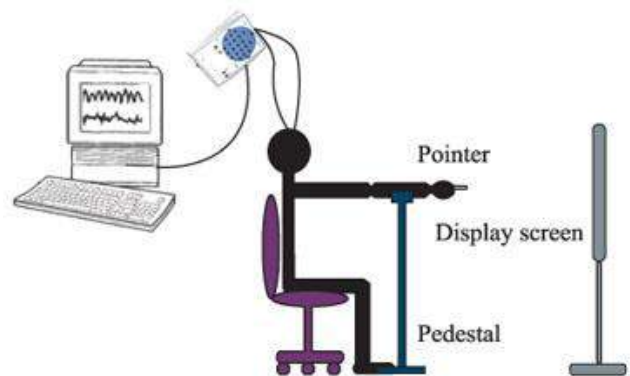
Epilepsy is a typical incessant neurological issue. Epilepsy seizures are the consequence of the transient and surprising electrical aggravation of the cerebrum. Around 50 million individuals worldwide have epilepsy, and about two out of each three new cases are found in creating nations. Epilepsy will probably happen in youthful youngsters or individuals beyond 65 years old years; nonetheless, it can happen at any age. The identification of epilepsy is conceivable by investigating EEG signals. In this paper we are using a technique to classify normal & epileptic EEG signal using k-means clustering algorithm in MATLAB. Further the SVM & Discriminant classifier in MATLAB Machine learning toolbox is used to classify the epileptic and normal EEG signal & wavelet transform is used to process the EEG signal. After the implementation of the signals there is ~70% accuracy with SVM classifier and ~93% accuracy with discriminant classifier.

**Keywords:** Epilepsy seizures, SVM Classifier, MATLAB, k-means clustering, wavelet transform, Discriminant Classifier.

## I. INTRODUCTION

The electroencephalogram (EEG) consists of a time series data of evoked potentials resulting from the systematic neural activities in a brain. The recording data of the human EEGs are carried out by placing the electrodes on the scalp, and plotted as voltage magnitude against time. The voltage of the EEG signal corresponds to its amplitude. The general voltage range of the scalp EEG lie between 10 and 100  $\mu$ V, and in adults more frequently in the range of 10 and 50 $\mu$ V. In the frequency spectrum range of the EEG, the frequency range extends from ultraslow to ultra-fast frequency components. The extreme frequency ranges play no significant role in the clinical EEG. The general frequency range of interest lies between 0.1Hz and 100Hz for the classification purpose. The frequency range is generally classified into several frequency components, or delta rhythm (0.5 - 4Hz), theta rhythm (4 - 8Hz), alpha rhythm (8 - 13Hz) and beta

rhythm (13- 30Hz). For normal adults, the slow ranges (0.3 -7Hz) and the very fast range (>30Hz) are sparsely represented, and medium (8 - 13Hz) and fast (14 - 30Hz) components predominate.



**Fig 1:** Position of the subject during data acquisition

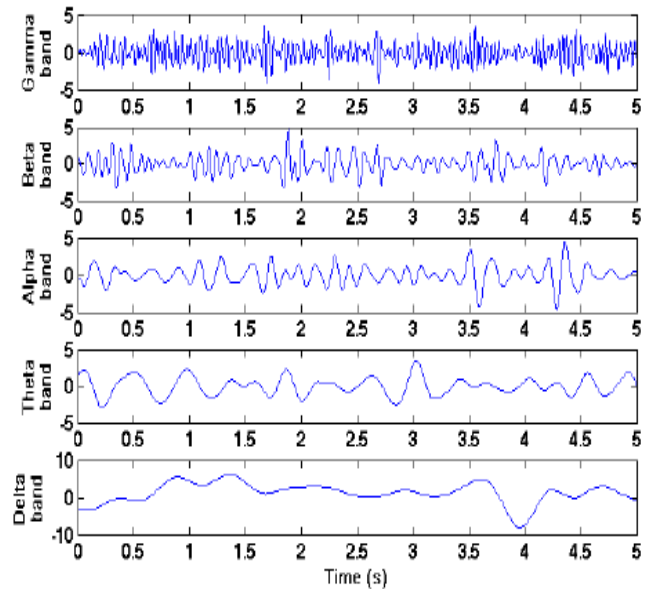
One of the most common neurological diseases is Epilepsy which is very common in patients having epileptic seizures which are unpredictable and

recurrent. To diagnose such disorder, EEGs or Scalp are used clinically, also this can be detected by using blood test. These signals can appear in many forms like, spikes, poly-spikes and waves. Ictal (i.e. state during epileptic seizure) EEG recordings are more reliable in diagnosing epilepsy than interictal (i.e. state between two epileptic seizures) recordings but they are expensive and difficult to obtain in patients with infrequent epileptic seizures.

### Standard Frequency bands of the EEG signal

Most of EEG waves range from 0.5-500Hz, however the following four frequency bands are clinically relevant:

1. **Delta waves:** Delta waves frequency is up to 3 Hz. It is slowest wave having highest amplitude. It is dominant in infants up to one year and adults in deep sleep.
2. **Theta waves:** It is a slow wave with frequency range from 4 Hz to 7 Hz. It emerges with closing of the eyes and with relaxation. It is normally seen in young children and in adults.
3. **Alpha waves:** Alpha has frequency range from 7 Hz to 12 Hz. It is most commonly seen in adults. Alpha activity occurs rhythmically on both sides of the head. Alpha wave appears with closing eyes (relaxation state) and disappears normally with opening eyes/stress. It is treated as a normal waveform.
4. **Beta waves:** Beta activity is fast with small amplitude. It has frequency range from 14 Hz to 30 Hz. It is dominant in patients who are alert or anxious or who have their eyes open. Beta waves usually seen on both sides in symmetrical distribution and is most evident frontally. It is a normal rhythm and observed in all age groups. These mostly appear in frontal and central portion of the brain. The amplitude of the beta wave is less than  $30\mu\text{V}$ .



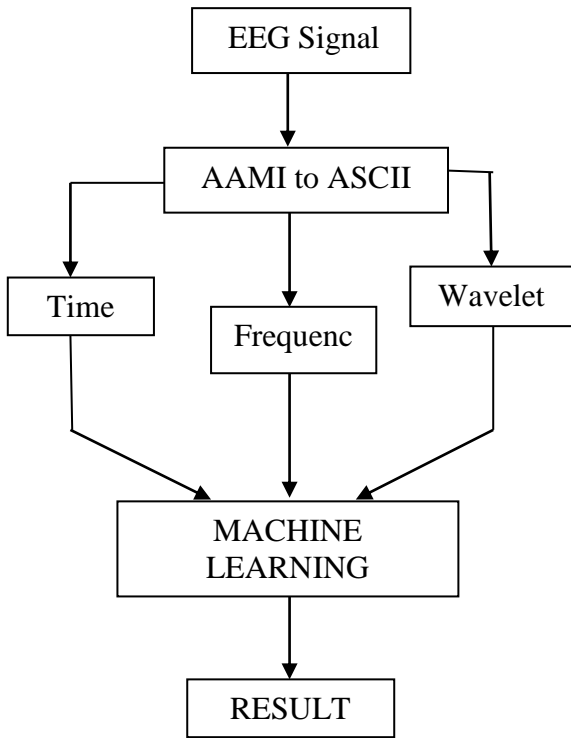
**Figure 2.** The five Frequency bands of normal EEG Signal

## II. METHODOLOGY

The main aim of this work is to analyse EEG signals as epileptic or non-epileptic for the diagnosis of Epilepsy by using machine learning algorithms. In this, the signals which are epileptic in nature are focused on ictal discharge and non-epileptic signals are made up of normal and abnormal interictal discharges. The algorithm used to achieve this is:

1. Extraction of EEG Signals and normalization of the signal.
2. Extract statistical features to create a feature set.
3. Decompose the signal using wavelet decomposition.
4. Reduce the number of features of the feature set by using k-means Clustering to decrease runtime.
5. Use the reduced feature set to train the Support Vector Machine.
6. Compare the performance of the SVM trained on original and reduced feature set to separate epileptic from non-epileptic signals on a test data set.

The proposed strategy utilizes MWT and Time and frequency domain parameters and Machine learning to characterize the EEG signal for epilepsy seizure discovery. The underneath square graph demonstrates stream of proposed procedure.



**Figure 3.** Flow chart for the Process

The EEG database is collected from Klinik for Epileptologie, which provides free access to web to large collections of recorded physiologic signals. EEG signals are recorded with machines having sampling frequency of 4090 Hz with 1000 samples/sec. This data set contains the signals of 10 users of both patients having epileptic seizures or non-epileptic seizures. Each user contains 4090 sample values.

The EEG sign is changed over into ASCII arrange and put away in the temp.txt record utilizing MAT to ASCII converter. The yield of the converter is given as a data to MWT, the mind sign is disintegrated and the anomalies of the sign are dictated by utilizing the T&F process. At that point the T&F yield is prepared by utilizing Machine learning. The multi-wavelet transform thought begins from the speculation of scalar wavelets. Rather than one scaling capacity and one wavelet, various scaling capacities and wavelets are utilized. This prompts more level of opportunity in developing wavelets. In this way contradicted to scalar wavelets, properties, for example, smaller backing, orthogonally, symmetry, vanishing minutes, short backing can be accumulated all the while in multi-wavelets.

In this T&F, MWT is utilized to separate the components of EEG sign. The MWT utilizes

numerous scaling capacities and various wavelet capacities. The vector documentation of scaling capacity and wavelet capacity is as per the following.

$$\Phi(x) = [\Phi_1(x), \Phi_2(x), \dots, \Phi_n(x)]^T$$

$$\Psi(x) = [\Psi_1(x), \Psi_2(x), \dots, \Psi_n(x)]^T$$

Where, T is denoted as the vector response and  $n > 1$  is an integer. The wavelet relation of low pass filter and high pass filter is as follows.

$$\Phi(x) = \sqrt{2} \sum_{k \in \mathbb{Z}} H_k \Phi(2x - k)$$

$$\Psi(x) = \sqrt{2} \sum_{k \in \mathbb{Z}} G_k \Psi(2x - k)$$

Where,  $H_k$  is the low pass filter coefficient and  $G_k$  is the high pass filter coefficient. The initial basis condition of scaling function and wavelet function is given below.

$$\Phi(x) = \begin{cases} 1 & x = 0 \\ 0 & \text{otherwise} \end{cases}$$

$$\Psi(x) = \begin{cases} 1 & x = 1/2 \\ 0 & \text{otherwise} \end{cases}$$

These are the initial basis condition of scaling and wavelet function of MWT.

The decomposition of MWT is calculated by using the following formulas. The decomposition of low frequency component is calculated as,

$$A_{i-1} = \sum_k H_k A_{i,2k+n}$$

The decomposition of high frequency component is calculated as,

$$D_{i-1} = \sum_k G_k D_{i,2k+n}$$

Using the above two formulas, the decomposition of MWT is calculated.

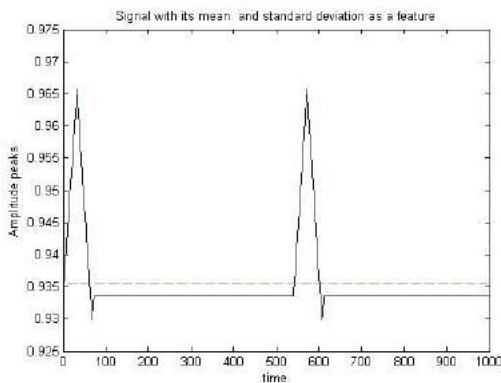
Model was created for identifying the most suitable combination of dimensionality reduction technique paired with SVM that gave the highest sensitivity and specificity in classifying epileptic and non-epileptic data.



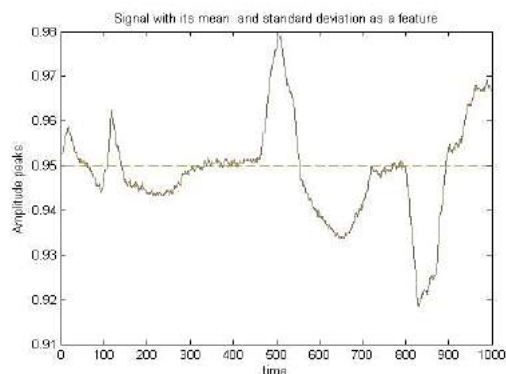
### III. RESULTS AND DISCUSSIONS

In this section a great amount of heed has been paid as the entire research work deals with active results for different EEG signals.

Ten different patients are taken for our existing research work. In this research work a single patient recording length is approximately 4090 samples, recorded at a rate 1000 samples per second, for duration of 4.9 seconds. In the advent of such recordings it is important to analyze the time domain representation of the signal in such cases. Figure 3 and 4 shows results of time domain waveforms as given in the database for visualizing it with respect to its mean.

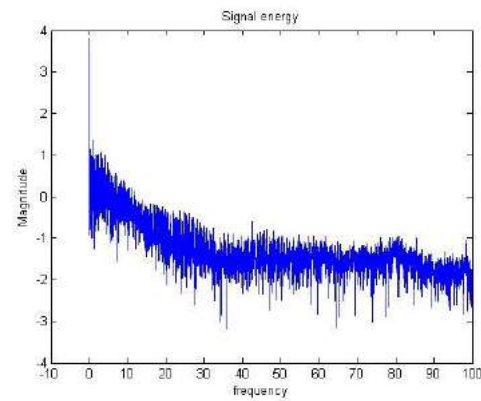


**Figure 4.** Original non epileptic waveform showing amplitude ( $\mu\text{V}$ ) on y-axis and time(sec) on x-axis.

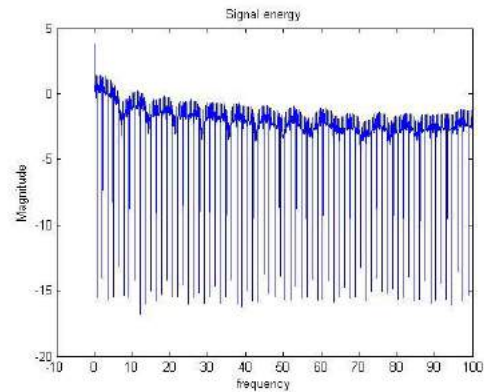


**Figure 5.** Epileptic waveforms measured for 1000 samples showing amplitude ( $\mu\text{V}$ ) on y-axis and time(sec) on x-axis.

Similarly visualizing waveforms evaluation will be generated for both epileptic and non-epileptic signal by applying fast fourier transform. Figure 6 and 7 shows a similar type of distinguished waveforms for both signals.



**Figure 6.** EEG epileptic signal for frequency domain visualization.



**Figure 7:** EEG non epileptic signal for frequency domain

Now it is most important for the current work to introduce the classification technique which is of utmost importance, when any arbitrary signal is given in this then how much will be recognition score, so most importantly there will be two different techniques for complete work analysis, i.e. support vector machine and discriminant analysis.

**Table 1:** Matching Score for two classification techniques.

S.No	Feature names	Feature classifier	Score
1.	T&F , wavlet	SVM	70%
2.	T&F , wavelet	Discriminant	93%

### IV. CONCLUSION

As per the work conducted in this paper the number of observation taken was 10. Each observation as EEG signal is composite signal of multiple frequencies comprising 7200 samples. Similarly in a similar manner the second important point is complete analysis has been done in time and frequency domain as well as wavelet transform has been included as features. For individual signal three time domain features were calculated one frequency

domain energy, and one level four decomposition has been found. Later stage is a verification stage where two different classifier has been used where first is support vector machine the other is linear discriminant analysis.

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# Modular Neural Networks Chronicles in Biological Aspects

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## ABSTRACT

Modular Neural Network Is A One of The Model Of Artificial Neural Networks. This Manuscript Describes The Urge Of Modular Neural Network (MNN) And How It Can Be Applied In The Biological Aspects, Since All The Cell Functions Are Modular In Nature And Can Be Applied In All Most All Cell Structures And Function Through The Connectionist Approach And The Weightless Logical Approach For Best Optimized Error.

**Keywords :** *Modular Neural Network, Architecture, Evolutionary Approach, Connectionist Approach, Weightless Logical Approach.*

## I. INTRODUCTION

As an artificial neural network is an information-processing system that performs convinced features on biological neural networks. Mathematical models of cell functions can be accomplished by one of the model of ANN i.e Modular neural networks. Modular neural networking is the learning of networks of flexible nodes interacting with other nodes /neurons which are developed for generalization of neural biology.

Our human body contains near about 100 trillion cells, as cell is the basic structural, functional and biological units with the help of this cell n number of system runs in our body that is digestive system, endocrine system, immune system lymphatic system nervous system muscular system reproductive system skeletal system respiratory system urinary system integument system all this above system are computerized based on the concept modularity.

## II. METHODS AND MATERIAL

### A. Modularity

Modularity is motivated by human body processes, which involves a combination of serial and parallel processing. Neuropsychologists study revealed that

the information processing system of humans is focused particularly on a partly damaged brain. The damage region of the brain has stopped implications on the cognitive abilities which are situated in this particular area. The non-damaged parts are still working and its performance is improved to compensate the loss in the other parts. These results that the brain has a highly modular and parallel structure

Modularity concept in the brain functioning results in optimization of the information capacity in the brain neural pathways. Since the brain consists of many modules and some f the modules are repeating using the feature of replication.

Modularity is the building block of Modular neural network, this network is based on reverse engineering<sup>2,3,4,5,6</sup> Classification<sup>7</sup> function approximation, Iteration , Unsupervised clustering and mathematical modeling. The basic concept depends on replication and decomposition ,since replication provide reusing phenomenon through which once a module is created and can replicates many modules in architecture. We observe in living organisms, our hands legs and even hairs can be replicated by module created once and decomposition feature provided will rearrange the modules depending of its functionality as cells function depends on integration of this

modules. Modular systems allow for the reuse of modules in different activities, without having to re-implement the function represented on each different task (De Jong et al., 2004)(Garibay, 2004). Modularity has found robust feature depending on structural and functional activity of networks <sup>10</sup>.which is an extension of the principle of divide and conquer leads subdivision of task into simpler subtask into modular neural systems Chiang and fu <sup>11</sup>proposed Divide-and-Conquer methodology which leads sub networks with their respective data for managing networks individually or serially and then integrated in a whole architecture which enables the decision on integrating sub networks for the appropriate outputs of individual subnets into final output of the system.

## B. Modular Neural Networks

The Urge for Modular neural network : The brain functions are been processes into individual functions, which are broken up into sub-processes to facilitate to execute in separate modules without mutual interference. <sup>12</sup> This information was first given by Happle and Murre which sequentially lead to the development in a long evolutionary process

The evolutions of modules in modular neural networks have high reflection of any living organisms, which raises the curiosity of research how the modularity is merged in natural phenomena .Wagner<sup>13,14</sup> have review many researchers proposing evolvability –stating the theories behind modular encodings. Recently a paper specifying theoretical the biology, give emphasize on the role of selection – pressure, explained in the modular organization. Lipson, kashtan and Alon<sup>15</sup> had brought numerical models indicating a rapid changes in evolution of modular systems<sup>14</sup> by designing modular encodings with different approaches as Gruau <sup>16</sup> Hornby <sup>17</sup> and Pollack <sup>18</sup> and Mouret and Doncieux <sup>19</sup> did through evolutionary programming to develop the natural modularity through tree-based representations. Doncieux and Meyer <sup>[20]</sup> and Reisinger.<sup>21]</sup> represented based on a number of modules, directly encoded, and outlined that how modules are connected. ”The executable pathway to biological networks “(Jasmin Fisher and Nir Piterman .Describes ‘Executable Biology” that dynamicity and ultimately explain how molecular function generates cellular function using modular neural networks

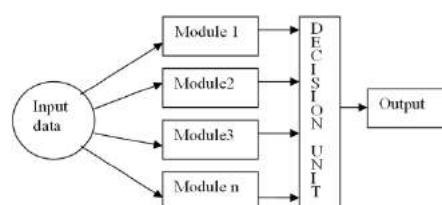
They suggestions appraised by developing a structure additional to modular artificial neural networks which are similar to the modular structure of the brain. This architecture can be applied in many real scenarios including day to day activities also. An inspiration of modular neural network architecture is to build a hug network by using modules as building blocks. All modules are neural networks. The architecture of a single module is simpler as that off sub-networks which are smaller than a monolithic network. Due to the structural modifications the task -module has to learn in general becomes much easier than the whole task of the network. This formulates easier to train a single module. In a additional the modules are connected to a network of modules moderately to a network of neurons. The modules are independent to work in parallel.

Many researchers have being researching how to generalized the large networks into smaller networks , the result of these is Modular neural networks .Which divide the hug network into the simple smaller networks into modules which exhibits the architecture of neural networks <sup>22</sup> , the major task to be accomplished is how to interconnect the modules in the Modular neural networks

The major aspect of modular neural networks revolute around the how to form modules , simple interconnection of modules, which is based on the communication of modules between them, the task performing is parallel processing , is it fault tolerant and learning ,has the ability to generalized to perform complex tasks to whole architecture .

## C. Modular neural network Designing

The major contents of designing Modular neural networks are forming of modules, followed by learning of modules and then combining different modules for complete networks. More investigation has been done on module formation, module communication (interaction) and module reuse during evolution for a variety of classification and prediction tasks <sup>23</sup>.



**Figure 1.** Modular Neural Network (MNN) architecture

## D. Modular Neural Network Designing Requirement

Modular neural network design starts with the analysis of problem to be solved, according to the approaches. Basically there are two major 1. The Connectionist Approach and 2.The Weightless Logical Approach

### Connectionist Approach

Connectionist approach is applicable to neural computing where a simple processors, known as neurons are interconnected to form biological nervous structure .Here the training is provided to solve the problem rather than programming .This approach was inspired by psychologist and biologist .A hug contribution was provided by MC Culloch<sup>49</sup> and Pitts<sup>50</sup>, Heb<sup>51</sup>and Rosenblatt<sup>52</sup> for parallel and interconnection of neural networks. Training and learning is applied to this architecture

### Weightless Approach

These approach provides logic to neural computing, here the data is stored as part of patterns .In this approach the neural network has no weights , due to which the training is done fast and learning algorithm is extremely simple but this approach doesn't support biological and psychological model.

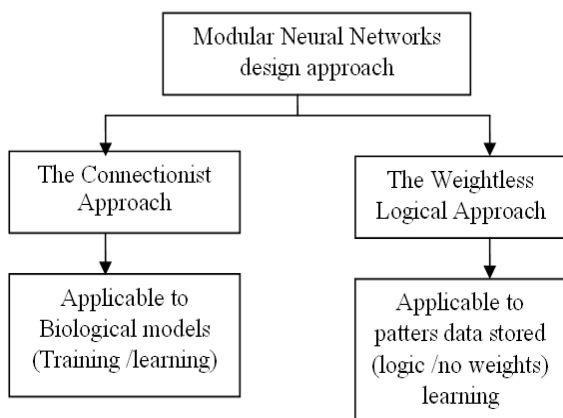


Figure 2. Modular Neural Network (MNN) design

## III. RESULT AND DISCUSSION

### A. Modular Neural Network Designing Requirement

Modular neural network first proposed by Jacobs, Jordan, Nowlan and Hinton<sup>24,26,27,28</sup>, It mainly deals with what type of architecture should be used for formation of module ( i.e **Task decomposition** ) depending on the input/output relation of a particular problem , it does have any specific technique for it.

Naturally it can be attained, mostly used architecture are (MLP,LNN,SOM,ART1,ART2) describes that the complex problems is solved by dividing it into simpler problems through “Divide and Conquer” algorithms by Chiang and Fu<sup>25</sup> is applicable to the architecture built out of sub networks able to manages a subsets of the data and depending on its **Modularizing learning** ,the relevance task is decomposed and distributed over sub-task separated in modules deals with determination of the number and size of individual module in the Modular neural network(MNN). Decomposing of classification task entails clustering (grouping)and then a separate classifier can be applied to all groups. Finally the integration engine that determines which is According this algorithms it states that the connections between nodes, called weights, carry activation levels from one neuron to another or to itself. According to the interconnection scheme used for a neural network, it can be categorized as (feed-forward, recurrent) and the integration Engine is trained to determine to which module should be considered.

Modular neural networks –Modules are connected to a network of modules rather than to a network of neurons. The modules are independent to work in parallel. For this modular approach it is always necessary to have a control system to enable the modules to work together in a useful way through **Training algorithm** for increasing ability to generalize the network.

Define abbreviations and acronyms the first time they are used in the text, even after they have been defined in the abstract. Abbreviations such as IEEE, SI, MKS, CGS, sc, dc, and rms do not have to be defined. Do not use abbreviations in the title or heads unless they are unavoidable.

### B. Design Structure

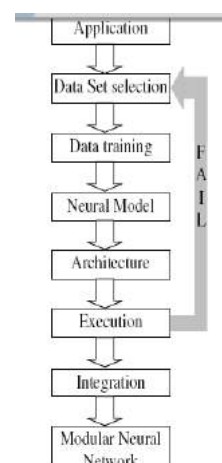


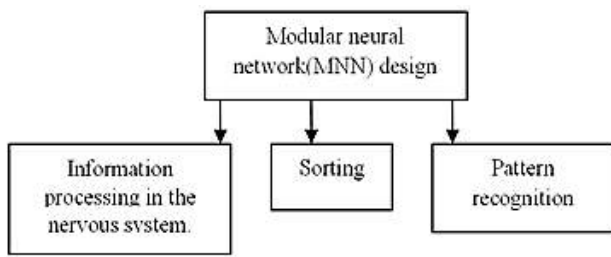
Figure 3. Modular Neural Network (MNN) design structure

### Formation of Modules

The first step in designing MNN is formation of modules or Task decomposition, here the input data is naturally defined in a module .As it has to undergo the feasibility by training and learning phenomena to reduce error. Ishi describes the learning for modular structured networks ,that overcomes the problem of training hug networks

### Combining of modules

Integration of module is very important task to be performed

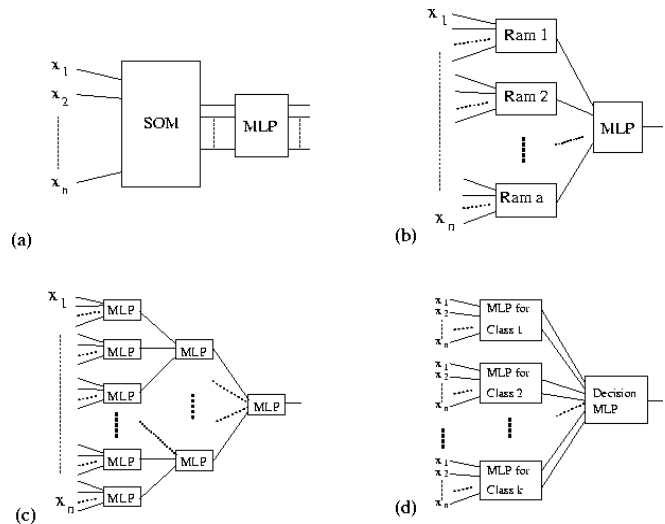


**Figure 4.** Modular Neural Network (MNN) design structure

Modular neural networks are inspired by biological concepts, which is simulates through defined architectures. Basically to solve complex problems ensemble-based and modular 29, 30 was introduced .In ensemble architecture, the neural networks can be formed with arbitrarily unstable set of initial weights, with different topologies followed by training 31, 32, this architecture is applicable in the field of classification, function approximate and learning mechanism 33, 34, 35, 36 through boosting algorithm is been used 37, 38 more enforces is given to initial weights varying with varying topology of training data set of neural network .The main advantage of using this architecture was linear combination of the outputs of the particular sub-neural network(39, 40, 41, 42, 43, 44, 45, 46, 47, 48)the result of these combination is distribution .Another architecture, which uses both supervised and unsupervised learning in sequential order is Decoupled modules architecture 53.In this architecture first stage, an adaptive resonant theory (ART) network was proposed by G. Bartfei 59, here the input data is decomposed into its inherent clusters in an unsupervised way as once the input data is classified in a each module .Individual modules is learned and then trained in parallel using supervised learning .There is no communication between modules results in input –output model

### VII. EVOLUTIONARY APPROACH TO THE DESIGN OF MODULAR ARCHITECTURES:

This approach is applicable to biological fields ,give rise to diversity at every level of biological functions .Here are some of the architecture inspire by evolutionary approach for Modular neural network(MNN)



**Figure 5.** Some Examples of Modular Architectures

### IV. CONCLUSION

The proposed MNN model has inconsistent advanced performance when the network topology is chosen optimally. However, the proposed MNN model has a drawback at some point of time. The connections between the modules depend upon the architecture, although it initializes to positive values at the beginning of the training phase, can change the output considering the desired output of the whole network. This problem can be resolved by an introduction of a more robust learning algorithm which ensures that the training algorithm [54, 55, 56, 57, 58]can reduce the error of particular model execution without respect of working parallel or sequentially.

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# Using Concept Mapping to Remediate Chemistry Teacher Trainees' Understanding of Chemical Phenomena – Before and After

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## ABSTRACT

Researchers have shown interest in how beginning chemistry teacher trainees can improve on their technological pedagogical content knowledge so as to be able to engage their students in concept-based and reflective activities. This is a task that trainees have to build up on their own by first mastering their content knowledge. This article focuses on 29 first year teacher trainees' understanding of chemical phenomena and how their understanding could be improved through concept mapping. An intervention was designed to enhance their basic conceptions upon which other concepts could be built. We analysed concept maps on linkages of periodic properties and how they affect chemical bonding. Their submissions were analysed based on three categories of conceptual understanding. The results showed that their levels of conception improved after the concept mapping intervention.

**Keywords:** Alternative Conceptions, Chemical Phenomena, Periodicity, Teacher Trainees.

## I. INTRODUCTION

Chemistry is a discipline that enables students to develop acceptable ideas about the world in which they live. However, naïve, alternative, or wrong ideas, often called misconceptions could also be developed instead in a variety of ways. Wesson (2001) explained that when new information arrives in the cerebral cortex for analysis, the brain attempts to match each component with previously stored memory elements on the existing neural network and then files the new data by making connections to the old. If new information does not fit a learner's established pattern of thinking, they unconsciously or consciously refashion it to somehow fit their existing mental model. Thus, misconceptions are unknowingly created and reinforced upon faulty reasoning. Such misconceptions could be compounded when linkages are formed to yet other misconstrued or inaccurate ideas and further carried on from one generation to another- thus, creating a vicious cycle of misconceptions. The longer a misconception remains unchallenged, the more likely it is to become entrenched. Misconceptions are of considerable importance and cannot be ignored in the learning process since prior conceptions are foundations upon which knowledge is built (Pine, Messer & John, 2001).

Students' unscientific beliefs can influence them to have misconceptions in their learning processes. These beliefs can persist as lingering suspicions in their minds and hinder further learning. Students must therefore confront their own beliefs along with their associated paradoxes and limitations and then attempt to reconstruct the knowledge necessary to understand the scientific model being presented (Hanson, 2015). According to Gooding and Metz (2011), this process requires that teachers:

- Identify students' misconceptions
- Provide a forum for students to confront their misconceptions, and
- Help students reconstruct and internalize their knowledge, based on scientific models.

If meaningful learning does not occur, rote learning takes precedence. According to Clark, Nguyen and Sweller (2006) cognitive load could also lead to rote learning and subsequently, the formation of erroneous concepts. A consequence of rote learning is that new material is merely memorized, easily forgotten and not transferred (Bretz, 2001). Science educators must therefore improve their skills in such a way that students' misconceptions can be identified and addressed during teaching. Gooding and Metz (2011) stated that teachers

should focus not just on repairing that which is broken, but also on preventive measures to help students avoid misconceptions from occurring.

Chemistry is one of the most important branches of science, but has been regarded as a difficult discipline by young students and some educators (Haider & Naqabi, 2008). The reasons for the said difficulties vary from the abstract nature of many chemical concepts to the difficulty level of the language of chemistry (Ozem, 2004). Besides the identified abstract nature, words from everyday language such as force, pressure and energy, with different meanings in the world of science, pose relational and interpretative problems for students during lessons (Taber, 2002). Such misconceptions constitute a major problem of concern to science educators, scientists, researchers, and students (Taber & Tan, 2011). Thus, this makes the identification of students' understandings and misconceptions an important area of research. Some of the conceptual areas in which most studies have been conducted have been on elements, compounds, and mixtures (Papageorgiou & Sakka, 2000), acids and bases (Boz, 2009), chemical stoichiometry (Haider & Al Naqabi, 2008) and chemical bonding (Ozmen, Demircioglu, & Demircioglu, 2009).

Although vernacular and factual misconceptions can often be easily corrected, even by the students themselves, it is not advised that teachers insist that learners dismiss preconceived notions and ingrained non-scientific beliefs without attending to the type or source of misconception. This must be done systematically and professionally. Recent research on students' conceptual misunderstandings of natural phenomena indicate that new concepts cannot be learned if alternative models that explain a phenomenon already exist in their minds (Hanson, Twumasi, & Antwi, 2015; Supasorn, 2015). Although scientists commonly view such erroneous models with disdain, they are often preferred by the learner because they seem more reasonable and perhaps more useful for the learner's purpose. Too often, teachers' zeal to get the right answers from students overshadows the efforts and implications behind students' own analysis to answers, especially if the student is wholly wrong. Incorrect answers are simply dismissed, instead of identifying the type of conception and seeing it as an opportunity for reflection, conceptual teaching and change (Gooding &

Metz, 2011). Prior assessment of what students already know is thus a critical component of curriculum change and design which cannot be underestimated (Holme, et al., 2010; Singer, Nielsen, & Scweingruber, 2012).

### **Tools for students' knowledge structure**

In order to assess what students know, which is a critical component of curriculum change, chemistry education researchers use a variety of diagnostic tools to uncover students' original, naïve, alternative, or prior conceptual understanding. A few of the diagnostic methods include think-aloud interviews (Cheung, 2009), concept inventories (Barbera, 2013; McClary & Bretz, 2012), conceptual text (Sevgi, Nurdane, Yezdan, Ayla, & Oktay, 2009) and concept mapping (Lopez, Shavelson, Nandagopal, Szu, & Penn, 2014; Greene, Lubin, & Walden, 2013). Two-tiered and four-tiered test items have also been used to identify students' own conceptions (Taber & Tan, 2011). This study will employ concept mapping to remediate and enhance teacher trainees' understanding about chemical phenomenon.

According to Nikita, Burrows and Suazette (2015), concept maps give teachers insight into students' 'whole' knowledge structures and allow them to pinpoint specific gaps in their mental structures. Studies conducted by Lopez, Shavelson, Nandagopal, Szu, and Penn (2014) and Szu, Nandagopal, Shavelson, Lopez, Penn, Scharberg and Hill (2011), have demonstrated that concept maps can be used to represent students' mental models or knowledge structures in organic chemistry. Specifically, their studies showed that concept map scores were correlated with scores on problem sets and final course grades. They also found that students' knowledge structures measured by concept maps were indicators of success in organic chemistry. Hanson, Twumasi and Antwi (2015), found that teacher trainees who could not provide appropriate links between science concepts had low assessment scores. They thus developed a concept test to scientifically assess their understanding of chemical phenomenon. Twelve (12) major misconceptions were identified in that study. They recommended that teachers assess the validity of their students' prior understanding of basic science concepts before teaching advanced topics that would require the application of those prior basic concepts. This suggests

that educators of the sciences could improve teaching and learning of science by ensuring that misconceptions identified during teaching and evaluation exercises are addressed. In this current study, an in-depth study into trainees' conceptions about further work on chemical phenomena was carried out. This was because the level of the trainees' mastery of elementary concepts such as electronegativity, ionization energy, shielding effect, polarizability, effective nuclear charge and their linkages required for advanced studies on chemical phenomena was low.

Concept mapping is an ideal tool for assessing the depth and breadth of students' knowledge structures. It can indicate how students organize information into logical frameworks and allows teachers to visualise how students relate concepts to each other (Wheeldon & Faubert, 2009). Several studies have established the validity and utility of concept maps as an evaluation tool (Lopez et al., 2011).

Basically, concept maps are graphical tools used to organise and represent an individual's mental model or knowledge structure as they create relationships between a single concept and others in the same category in the form of propositions (Novak & Canas, 2006). It is a two-dimensional schema that illustrates mutual correlation or inter-conceptual relationships in graphics and help students to learn in a more meaningful way by relating old ideas to new ones in their minds. This kind of organisation prevents the formation of misconceptions and results in the identification of relationships. In this way, learning becomes more meaningful to students. Concept maps consist of three components – concept terms, linking arrows, and linking phrases. The linking arrows provide a directional relationship between two concepts while the linking phrases (words linking concepts) represent the specific relationships between at least two concepts. In chemistry studies, this could lead to the improvement of deductive, critical, analytical and reflective thinking skills, which are paramount to enhance gains in cognition.

### **Aim of Study**

This study was a follow up to an earlier one on chemical phenomena (Hanson, Twumasi, & Antwi, 2015) in which teacher trainees' understanding about some basic chemical principles were assessed. A number of

alternative conceptions about chemical phenomena were identified. The purpose of this current study was to use concept mapping to determine the trainees' conceptions after a treatment process related to bonding and applications of some periodic trends through concept mapping.

### **Research Questions**

- i. What conceptions do students have about the applications of periodic parameters?
- ii. What improvements are evident in students' conceptions about chemical phenomena after using concept maps?
- iii. How useful are concept tools as an innovative conceptual change strategy?

## **II. METHODS AND MATERIAL**

### **Research Design**

This was a case study which employed an intervention to solve an identified problem in three phases. Thus, concept mapping was used as a conceptual change tool to re-uncover the knowledge structure of teacher trainees before (pre-map) and after they had gone through a treatment period (post-map). Twenty one (21) chemistry undergraduate teacher trainees from the University of Education participated in the study. Pre- and post-intervention concept maps were constructed by trainees to enable the researchers identify new conceptions gained by them after the treatment period.

To begin with, the trainees were encouraged to use concept mapping or flow diagrams to illustrate how 14 terms on periodic properties (given to them) connected with each other to explain the concept of reactivity among group one elements. These terms were taken from their course text (Tro, 2010; McMurry, 2007). A 20-minute hands-on tutorial on how to construct concept maps was given to the trainees, prior to the construction of their mapping, so that they could all begin work with the innovative strategy from a common work level. They were encouraged to use extra terms which had not been supplied, if required. They were then given 60 minutes to complete their concept mapping, after which their constructed maps were assessed and scored by two trained chemistry senior lecturers of the Department of

Chemistry, University of Education, Winneba. Adjustments were made to the treatment designs (lessons) based on a review of the trainees' submissions. After the analysis of the pre-concept maps three lessons on periodicity were taught in which the researchers employed concept mapping. These treatment lessons became necessary as they were meant to address trainees' identified misconceptions from their pre-concept maps. After this treatment period, they were given an exercise akin to the pre-concept mapping assessment. These were also assessed using the same scoring mode as for the pre-concept maps.

## Data Analysis

### Concept Maps

The concept maps were scored by the trained researchers using the following four-level scale proposed by Lopez, Shavelson, Nandagopal, Szu and Penn (2014) and Szu

(2011). They proposed the following scoring codes: 0 – incorrect or scientifically irrelevant, 1 – partially incorrect, 2 – correct but scientifically 'thin' (i.e. technically correct but answers are too general and/or vague), and 3 – scientifically correct and precisely stated. Each proposition in the concept map was therefore given a score between 0 and 3, according to the grading scale. The scores of pre- and post-map were compared and the percentages of trainees who scored between 0 – 1.4, 1.5 – 2.4 and 2.5 – 3.0 were grouped under descriptions of alternate conception (AC), partial conception (PC) and correct conception (CC) respectively. This was done to comparatively assess trainees' revised conceptual frameworks and gains after the treatment. According to Wang and Barrow (2013) identification of levels of conceptions for different group of students clarifies how they explain and reason when asked to work with targeted concepts. A typical concept map is presented as Appendix A.

## III. RESULT AND DISCUSSION

The results of the pre- and post-maps have been presented as Table 1.

**Table 1 :** Level of trainees' performance in the pre-and post-concept mapping

CONCEPT	PRE-MAPS (%)			POST-MAPS (%)			Overall gains (Themes)		
	AC	PC	CC	AC	PC	CC	AC	PC	CC
Bond strength & temperature	51.3	32.1	16.6	20.1	48.0	31.9	0.64	0.23	0.18
Strength of hydrogen bond	25.5	51.0	23.5	21.0	35.5	43.5	0.06	0.32	0.26
Effect of nuclear charge on size	31.4	38.0	30.6	21.5	30.3	51.2	0.14	0.12	0.30
Electronegativity within groups	28.0	60.3	11.7	4.9	21.9	85.1	0.32	0.97	0.83
Strengths of bases within groups	20.2	51.4	28.4	15.0	36.4	48.6	0.07	0.31	0.28
Factors that affect lattice energy	53.5	26.3	20.2	31.5	36.0	32.5	0.47	0.13	0.15
Heat of hydration	42.1	30.5	27.4	34.0	33.2	32.8	0.14	0.04	0.07
Concept of electronegativity	36.7	55.6	7.7	15	37	48.0	0.34	0.42	0.44
Type of Bond	35.4	52.5	12.1	18	26.3	55.7	0.27	0.55	0.50

From Table 1, it is observed that moderate and high gains were made in the concepts about relating bond strength and temperature, the concept of

electronegativity and how it affects elements within groups, and types of bonds. Not much gains were made in students' understanding about hydrogen bonding, the

effect of nuclear charge on sizes of atoms, and heat of hydration. In all there were six (6) thematic conceptual gains and five (5) partial conceptual gains, out of a total of nine (9) themes.

#### *Bond strength and temperature*

After treatment, there was an increase in the percentage of trainees (16.6% to 31.9%) who were able to give conceptual explanation to bond strength and temperature. The percentage of trainees who demonstrated alternate and partial responses in the post-map was 20.1% and 48.0% respectively. They had difficulty in arranging types of bonds in terms of their strength and could not predict bonds that might have high boiling or melting point.

#### *Strength of hydrogen bond*

The percentage of trainees who made logical constructions increased from 23.5% to 43.5%. Most of the partial answers did not show linkages of hydrogen bonds to electronegative elements like nitrogen and oxygen. Such concept maps indicated that trainees had difficulty in identifying compounds which contained hydrogen bonds. Most of them perceived that all compounds containing hydrogen have hydrogen bond, such that a H<sub>2</sub> molecule was associated with hydrogen bonding.

#### *The effect of nuclear charge on size*

An increase in percentage of correct linkages rose from 30.6% to 51.2% due to trainees' ability to use more correct linking words. Majority were able to relate atomic size to nuclear effect. A few had difficulty in comparing ionic radii and so failed to identify and arrange isoelectronic species. Some also demonstrated alternative conceptions as they linked nuclear charge to the charge on atoms. All trainees correctly mapped that nuclear charge increased across the period.

#### *Electronegativity within groups*

The percentage of trainees who had correct propositions and links rose from 11.7% to 85.1% after treatment. This indicates that most of the trainees who had alternate and partial conceptions in their pre-maps reached correct conceptions through the treatment and were able to link correct propositions to explain the trend of electronegativity within groups. Few trainees with

partial conceptions linked the trend of electronegativity to either nuclear charge or screening and shielding effect but not both.

#### *Strengths of bases within groups*

Only 48.6% constructed good maps about the trend of basicity of elements within a group and across a period with 36.4% and 15.0% showing partial and alternate conception respectively. The trainees had difficulties in comparing the basic strengths of elements in a period and within groups. Most of them intimated that basicity increased with electronegativity across the group and from bottom to the top within a group.

#### *Factors that affect lattice energy*

The percentage of trainees with correct conception increased marginally, from 31.2 to 32.5%. Results indicated that they had difficulties in stating and explaining factors that affect lattice energy. About 36% of trainees who were able to associate lattice energy to ionic charge and size of an ion could not give proper reasons for their decisions. The remaining 31.5% were those who had either wrong linkages or could not provide any at all.

#### *Concept of electronegativity*

The percentages of trainees who demonstrated alternate, partial and correct conceptions were about 15%, 37% and 48% respectively. It appears that majority of trainees could not reach full conception even after treatment. The concept-maps showed that they had difficulty on the concept of electronegativity which would require further treatment through other strategies. Trainees who gave alternate conceptions considered polarity as a type of bond but not an additional characteristic of bonding. Most of those who demonstrated partial conception could not give specific examples of polar and non-polar covalent bonds. A few however, were able to relate the essence of electronegativity values to bond polarity.

#### *Type of bond*

It was found that 55.7% of the trainees gained scientific understanding after treatment while 18% and 26.3% indicated alternative and partial conceptions respectively. Most of them could state, explain and give examples of types of bonds. For instance, a trainee linked ionic bond

to *bonds that occur in opposite charges* but later indicated that the octet rule was the sole reason for bond formation. Metallic bonding was the least used term. Some major findings were that:

1. Analysis of constructed maps revealed that, trainees used a lot of invalid prepositions and unlinked conceptions in the pre-map than in the post-map.
2. Some trainees stuck to their old beliefs and wouldn't let go, but majority acquired scientific conceptions as the naïve conceptions disappeared from their post-maps
3. There was very little demonstration about knowledge of metallic bonding.
4. Participants were able to use more scientific terms and constructed more logical linkages with prepositions given to them than in their pre-maps.
5. Most trainees who demonstrated partial conception lacked the knowledge to expand responses to phenomena.

The purpose of the study was for students to use concept mapping to express their conception of chemical phenomenon after they had gone through a 21 day treatment period. Results after treatment indicated that concept mapping was useful and revealed differences in conceptual frameworks between trainees with high and low levels of content knowledge, and pinpointed some key ideas lacking in their frameworks. There were increases in the percentages of trainees who gave correct responses in the post mapping. This happened because their content knowledge and skills for constructing concept maps were heightened during the treatment period. This enabled them to use more understandable concepts to form valid propositions. The use of concept maps enabled the researchers to evaluate trainees' own mental models and identified essential concepts that needed to be stressed to bridge learners' understandings to advanced levels (Nikita & Suazette, 2015). Findings from the study are consistent with that by Mustafa and Murset (2013) that concept mapping addresses different forms of learning and individual differences between students. The validity and utility of concept maps were verified in this study as intimated by (Lopez, Shavelson, Nandagopal, Szu, & Penn, 2014; Greene, Lubin, & Walden, 2013). There was however, an apparent lack of knowledge about metallic bonding which was quite strange and will require further remediation.

## IV. CONCLUSION

The depth and breadth of trainees' knowledge structures were easy to diagnose through concept mapping. Findings showed the importance of using diagnostic tools, especially concept mapping, to enable trainees to relate current concept to explain chemical phenomenon. Nahum, Mamlok-Naaman and Taber (2010) confirm that learning about chemical bonding allows the learner to make predictions, and give explanations, about physical and chemical properties of substances. Science educators must ensure that misconceptions of students are addressed to enhance effective learning of science (Greene, Lubin, & Walden, 2013).

## V. ACKNOWLEDGEMENT

The authors wish to acknowledge the immense contributions of trainees who participated in this study.

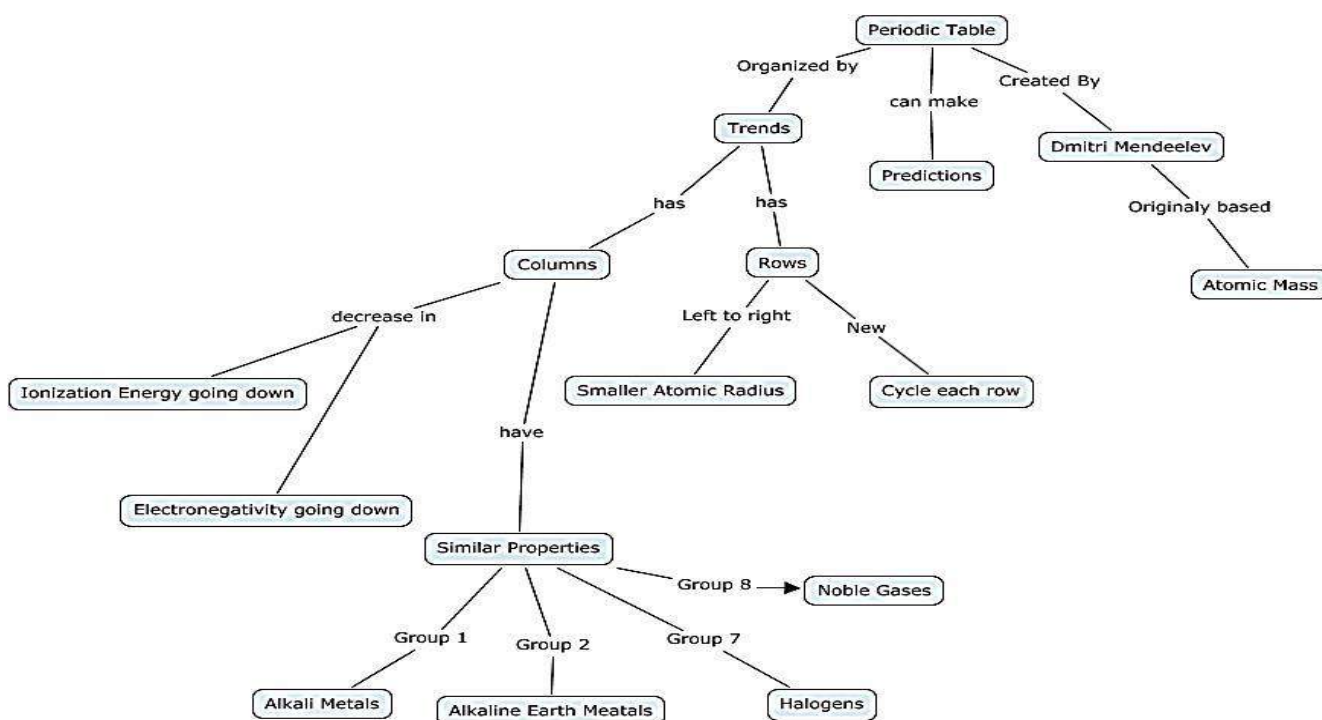
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### Appendix A : Example of Concept Map on Periodic Trends





# Forest Fires Model and SIR Model Used in Spread of Ebola Virus in Prediction and Prevention

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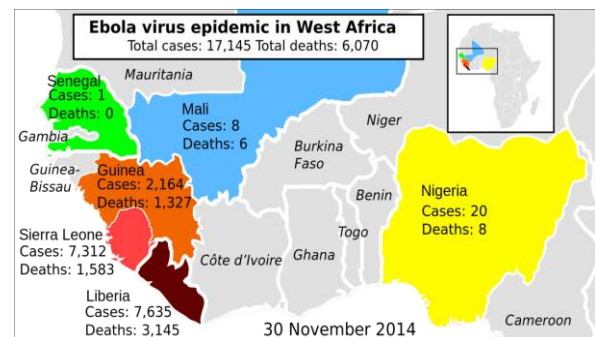
## ABSTRACT

Ebola has been repeatedly ravaged Earth. Since the end of 2013, Ebola swept West Africa, has caused tens of thousands of people infected or death. Based on this, it is an effective way to establish an effective, workable, practical significance of the mathematical model for health care workers and researchers to help in the fight against the Ebola epidemic. We base on assumptions of population stability in West Africa, the epidemic is no longer continue to expand, the drug can effectively reaches the hands of the patient once it put in; By SIR model, the most classic model in infectious disease, and published data, we realize the epidemic prediction. And base on them, by using forest fires model of cellular automata and two-compartment model of pharmacokinetic, we analyze and forecast three aspects for Vaccine/drug Efficacy, Epidemic Area, Vaccine/drug Production and R&D Speed. We propose drug distribution points in West Africa should be in accordance with changes by the strength of the Epidemic area, and drug development should begin at the growth of the epidemic stage 1/3, and 1/2 of the growth of the epidemic stage clinical trials in order to achieve the best results. Although limited ability, we analyze factors with a single control and then propose solution. But we built the best model out.

**Keywords:** *Ebola Virus Disease, Eradicating Ebola Epidemic, SIR Model, Forest-Fire Model, Two-Compartment Model, Epidemic Prevention*

## I. INTRODUCTION

Ebola Virus Disease (EVD) is a human disease, which is caused by the Ebola Virus (EBOV). EVD first appeared in Central Africa Zaire (which is Democratic Republic of Congo now) and South Sudan in 1976. After that, EBOV has outbreak around 24 times in Central Africa, all of these happened in remote areas away from the local city. Ebola mortality rate of 90%, the incubation period of infection ranging from 2 to 21 days. In the early stages of the disease, Ebola virus may not be highly contagious, so people don't be infected even they touch patient in this period. As the disease progresses, the body fluid from the patient will be excluded with a high degree of biological risk. Also, Ebola Virus is usually transmitted through blood and other body fluids. Fortunately, there is no confirmation that it can spread by air to anyone. [1, 2, 3, 4]



**Figure 1:** Ebola virus epidemic in West Africa (Since 30 Nov 2014) [6]

The spread of Ebola virus is an infectious disease problem. In the field of mathematical modelling has been a lot of research about it. The SIR model is more complete one. SIR model by establishing a set of differential equations, as a general propagation mechanism establish a centralized model. We will select the number of confirmed cases which published by the World Health Organization (WHO) for the

establishment of SIR model. We choose West Africa as the main research district of Ebola virus infection. It is well known cases of Ebola virus infection in Liberia, Sierra Leone and Guinea, such as the three countries are becoming the focus of attention. [1, 2, 5, 6, 8]

In addition, we choose Forest Fires model to research about any of possible factors which can affect the Ebola outbreak. By using Forest Fires model of Cellular Automata, we can analogy the patient to the ignition point. And effective cure meaning Firemen extinguishing, and the optimal solution is what we want.

First of all, we need to conduct a diagnostic population in Ebola epidemic area, determine the epidemic stricken area and the epidemic situation by SIR model. Then, we discuss the theory on three aspects of Vaccine/Drug Efficacy, Epidemic Area, Vaccine/Drug Production and R&D Speed. Finally, through the establishment of Forest Fires model to put forward a reasonable and effective solution for Ebola epidemic.

## II. ASSUMPTIONS

- For an effective cure model, we require if a district spent 42 days and found no new cases, we can declare the end of Ebola virus outbreak, which is an effective cure program. Here, 42 days showing the twice than Ebola maximum incubation period (21 days). And this 42 days period from anyone in the country with confirmed or probable cases of Ebola appeared last contact date since the beginning.
- For Ebola epidemic control has many factors, here, we select the greatest impact in three factors: Vaccine/Drug Efficacy, Epidemic Area, Vaccine/Drug Production and R&D Speed, as considerations for the relevant modeling and design.
- Cure patients have immunity, after that no longer suffer Ebola anymore.
- The incubation period is obtained from virus infection to the onset of symptoms in the time interval, it lasts 2 to 21 days. When the patient began showing symptoms, he/she is contagious.
- In each model, is not considered drug loss during transport.
- Because Ebola epidemic has been controlled effectively, so we will not discuss uncontrolled, no latency infection model.

## III. SYMBOL DESCRIPTION

In the section, we use some symbols for constructing the model as follows.

**Table 1:** The Description and Explanation of Symbol in this Paper

Symbol	Description
$S(t)$	Susceptible group people per unit time
$I(t)$	Incidence group people per unit time
$R(t)$	Exit group people per unit time
$E(t)$	Latent group people per unit time
$\theta$	Week Contact Rate Constant
$\lambda$	Rescue Rate/ The speed of each ambulance personnel
$f_0(t)$	The rate of administration
$\lambda_1$	The speed of production of medicines
$M, N$	Constant coefficient

Ps: Other symbols instructions will be given in the text.

## IV. PREPARED MODEL ABOUT EBOLA OUTBREAK

### 4.1 Model in Non-considering Latency

#### 4.1.1 Assumption

- 1) In the period of Ebola Virus spreads, the total number of people within West Africa for  $N$  unchanged, Neither consider life and death, nor migration, the crowd divided into four groups: Susceptible  $S$ , Incidence  $I$  and Exit  $R$  (including those who died and recovery). In the time  $t$ , These three types of people in the proportion of the total number of people are  $S(t), I(t), R(t)$ .
- 2) The growth rate with time of  $I(t)$  and  $S(t)$  are proportional. The constant of proportionality is  $\theta$ . The speed of reduce the number of patients is directly proportional to the total number of patients. The constant of proportionality is  $\nu$ . Cure patients have immunity, after that no longer suffer Ebola anymore.
- 3) The sum of  $S(t), I(t), R(t)$  is a constant 1.

### 4.1.2 Constitution

Here is the West African Ebola epidemic WHO confirmed the number of cases; we pick up some days in the whole chart:

**Table 2:** West African Ebola epidemic WHO confirmed the number of cases [2]

DATE	CASES	DEATHS
13-May	245	164
20-May	270	181
27-May	309	200
4-Jun	354	208
11-Jun	438	231
18-Jun	528	337
25-Jun	599	338
2-Jul	759	467
9-Jul	888	539
16-Jul	1048	632
21-Jul	1201	672
28-Jul	1440	826
4-Aug	1711	932
11-Aug	1975	1069
18-Aug	2473	1350
25-Aug	3069	1552
1-Sep	3707	1848
8-Sep	4366	2177
15-Sep	5339	2586
22-Sep	6574	3043
29-Sep	7192	3286
6-Oct	8386	3988

Susceptible people become patient after effective contact with other patient. Set  $\theta S(t)$  as the number of susceptible people contact effective to patient per day. The patients with number of  $NI(t)$  can make  $\theta S(t)NI(t)$  susceptible people become latent virus people. So we can conclusion as:

$$\frac{dS(t)}{dt} = -\theta S(t)I(t) \quad (1)$$

And the change of Exit in unit of time is equal to reducing the incidence as follows:

$$\frac{dR(t)}{dt} = \nu I(t) \quad (2)$$

The change of incidence is equal to the part of susceptible-in, we can express as:

$$\frac{dI(t)}{dt} = \theta S(t)I(t) - \nu I(t) \quad (3)$$

We define the healthy people are  $S_0$  and the patients are  $R_0$  at the start time. And set  $R_0 = 0$ .

### 4.1.3 Solution

The equations (1), (2), (3) cannot find out the analytical solution, so we set a new variable  $\sigma$ , which is equal  $\theta/\nu$ .

So, we can solute the equation as follows:

$$i = (s_0 + i_0) - s + \frac{1}{\sigma} \ln \frac{s}{s_0} \quad (4)$$

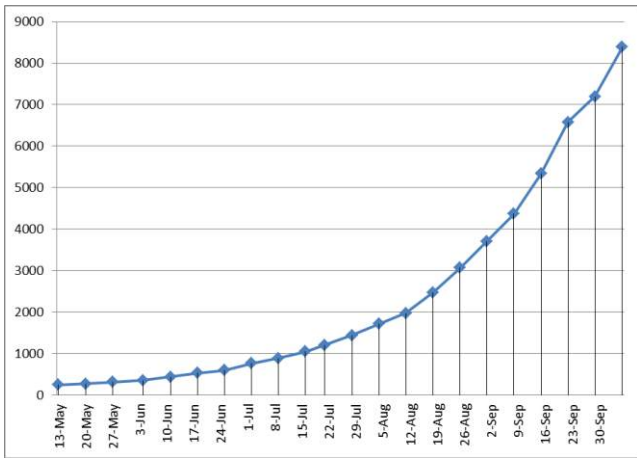
Now, we are analysis of the change of the  $S(t), I(t), R(t)$  as follow:

- 1) Whatever  $S_0, R_0$ , patients will be disappear, its  $i_\infty = 0$ .
- 2) The proportion of heathy people who don't be infected at the last time is  $S_\infty$ , and it's  $(s_0 + i_0) - s + \frac{1}{\sigma} \ln \frac{s}{s_0} = 0$  root in  $(0, 1/\sigma)$ .
- 3) If  $s_0 > 1/\sigma$ , then at first beginning:  $i(t)$  will be increase first. When  $s_0 = 1/\sigma$ ,  $i(t)$  reach the maximum, then  $i(t)$  decrease and tends to zero, and  $s(t)$  monotone decreasing to  $S_\infty$ .
- 4) If  $s_0 \leq 1/\sigma$ , then  $i(t)$  monotone decreasing to five, and  $s(t)$  monotone decreasing to  $S_\infty$ .

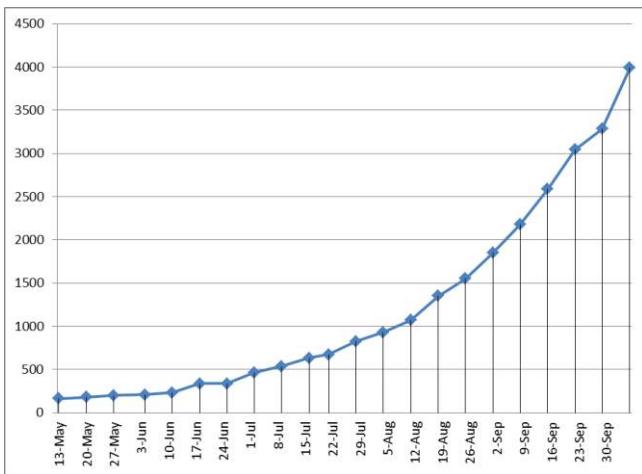
We found that the people health level is higher, the smaller weekly contact rate gets; and medical level is higher, on the higher cure rate, and the is smaller. So improve the level of health and medical level can prevent infectious diseases from spreading.

We can combine with the specific situation of West Africa and assumptions analysis as follows:

According to the obtained data to draw a West African Ebola prevalence curves and cure number change curve:



**Figure 2:** The number of patients change chart



**Figure 3:** The number of cure (including death) people change chart

According to the figure, Ebola virus shows the form of spread in West Africa, which is now, belongs to the case of  $\sigma = \theta / \nu > 1 / s_0$ . From the range of assumptions, we can know  $\theta$  between 1400~1600. Now we take  $\theta = 1600$ , it shows  $\nu < \theta / (1 / s_0) = 1600$ . In other words, the average number of West African cure for a maximum is 1600 people per week; this is different from the data released by World Health Organization. If that, the number of cure people in 30-Sep is  $1600 * 23 = 36,800$ ; this is far away from the actual situation. The reason of this problem has the following several aspects:

Firstly, the average number of each patient's weekly effective contact estimate is smaller. It's not a direct relationship simply; it should be a multiple party relationship, even exponential relationship.

Secondly, West African Ebola epidemic data from WHO has hysteresis.

Thirdly,  $s_0 \approx 0$  may not be set up in West Africa. We can put those strong, pay attention to their personal health people are excluded.

## 4.2 Model in Considering Latency

### 4.2.1 Assumption

- 1) In the period of Ebola Virus spreads, the total number of people within West Africa for  $N$  unchanged, Neither consider life and death, nor migration, the crowd divided into four groups: Susceptible  $S$ , Latent  $E$ , Incidence  $I$  and Exit  $R$  (including those who died and recovery). In the time  $t$ , These four types of people in the proportion of the total number of people are  $S(t), E(t), I(t), R(t)$ .
- 2) The average number of effective contacts for each patient weekly is  $\theta$ , we can call Weekly Contact Rate. When infected people and susceptible people make effective contact, the susceptible one will change into the latent group. And the latent crowd converted into patient group later again, patient group be cured.

### 4.2.2 Constitution

Susceptible contact patients effectively and be incubation. Let  $\theta(t)S(t)$  be a weekly average number of effective contact susceptible of each patient. The number of  $NI(t)$  patients can make number of  $\theta(t)S(t)NI(t)$  susceptible become latent group per week. So we have an equation as follow:

$$N \frac{dS(t)}{dt} = -\theta(t)S(t)NI(t) \quad (5)$$

The simplification as follow:

$$\frac{dS(t)}{dt} = -\theta(t)S(t)I(t) \quad (6)$$

The latent group change is equal to the transfer number of susceptible group minus a transfer number of patients. So the equation is

$$\frac{dE(t)}{dt} = \theta(t)S(t)I(t) - \alpha(t)E(t) \quad (7)$$

Among them,  $\alpha(t)$  says the latency weekly morbidity, each latent average effective number of patients.

The change for Exit group in unit time is equal to patients decrease. The equation is

$$\frac{dR(t)}{dt} = v(t)I(t) \quad (8)$$

Wherein  $v(t)$  represent Week Withdrawal Rate, the number of average effective condition of each patient recover or death.

Changes in incidence of people are equal to the number of people into the latent. The equation is

$$\frac{dI(t)}{dt} = \alpha(t)E(t) - v(t)I(t) \quad (9)$$

And

$$S(t) + I(t) + R(t) = 1 \quad (10)$$

The initial time susceptible group, infected group and recover immune group are  $s_0 (s_0 > 0), i_0 (i_0 > 0), r_0 = 0$ .

### 4.2.3 Solution

The incubation period is obtained from virus infection to the onset of symptoms in the time interval; it lasts 2 to 21 days. When the patient began showing symptoms, he/she is contagious. Because the incubation period of group cannot be determined, so they can be regarded as part of a susceptible group. Therefore, the solving process is same as non-considering latency.

## V. MODEL BASED ON FACTORS & PREPARED MODEL AS ABOVE

### 5.1 Vaccine/Drug Efficacy

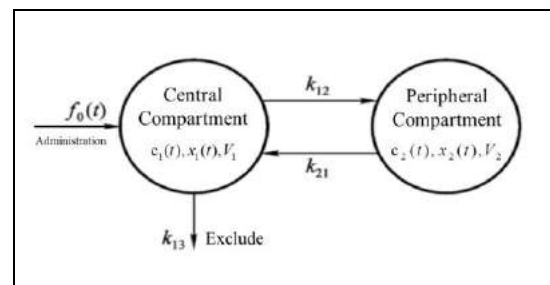
#### 5.1.1 Assumption

For the efficacy of the drug in the human body, we will be distributed in the human body which to build a two-compartment model. Use differential equation model describing the dynamic characteristics. In order to further simplify the problem, to obtain linear equations with constant coefficients, the following assumptions are:

- 1) The body is divided into the central compartment (Compartment I) and peripheral compartment (Compartment II), the volume of the two chambers (i.e. blood volume or volume of drug distribution) remains unchanged in the process.
- 2) Drugs transfer rate from a compartment to another and vitro rate to the exclusion, is proportional to the plasma concentration of the chamber.
- 3) Only the central compartment exchange drug with vitro that is drug from the vitro into the central, and finally excreted from the central compartment. Compared with the number of metastatic and exclusion, absorption of the drug can be ignored.

#### 5.1.2 Constitution

According to the model assumptions, and make a schematic diagram of a two-compartment model:



**Figure 4:** A schematic diagram of a two-compartment model

Where  $c_i(t), x_i(t), V_i(t)$  denote compartment of  $i$  ( $i=1,2$ ) plasma concentration, dose and volume.  $k_{12}$  and  $k_{21}$  is the rate coefficient of drug transfer between the two compartments.  $k_{13}$  is the excluded rate coefficient of the drug from the Compartment I to the vitro.  $f_0(t)$  is the rate of administration.

According to the assumptions and Figure 4 can write the dosage volume of two compartments  $x_1(t), x_2(t)$ .  $x_1(t)$  satisfies the differential equation of the rate of change is combine with transfer Compartment I to II  $-k_{12}x_1$ ,

Compartment I exclude to vitro  $-k_{13}x_1$ , transfer Compartment II to I  $k_{21}x_2$  and Administration  $f_0(t)$ .  $x_2(t)$  satisfies the differential equation of the rate of change is combine with transfer Compartment I to II  $k_{12}x_1$  and transfer Compartment II to I  $-k_{21}x_2$ . So here we give equations as follow:

$$\begin{aligned}x_1(t) &= -k_{12}x_1 - k_{13}x_1 + k_{21}x_2 + f_0(t) \\x_2(t) &= k_{12}x_1 - k_{21}x_2\end{aligned}\quad (11)$$

$x_i(t)$  and plasma concentration  $c_i(t)$ , is clearly the relationship between the atrioventricular volume  $V_i(t)$ . So, here is the equation:

$$x_i(t) = V_i c_i(t), i = 1, 2 \quad (12)$$

Equation (12) assignment equation (11) can be given:

$$\begin{aligned}c_1(t) &= -(k_{12} + k_{13})c_1 + \frac{V_2}{V_1}k_{21}c_2 + \frac{f_0(t)}{V_1} \\c_2(t) &= \frac{V_1}{V_2}k_{12}c_1 - k_{21}c_2\end{aligned}\quad (13)$$

We can get the general solutions as follow:

$$\begin{aligned}\overline{c_1(t)} &= M_1 e^{-\alpha t} + N_1 e^{-\beta t} \\ \overline{c_2(t)} &= M_2 e^{-\alpha t} + N_2 e^{-\beta t}\end{aligned}\quad (14)$$

Among them, the equations are established as follow:

$$\begin{aligned}\alpha + \beta &= k_{12} + k_{21} + k_{13} \\ \alpha\beta &= k_{21}k_{13}\end{aligned}\quad (15)$$

### 5.1.3 Solution

Taking into account the Ebola virus infection commonly used method of administration; here we give two methods as the following:

#### 1) Rapid intravenous injection

Setting the rate of administration  $f_0(t)$ , in this condition, we can make the model simply at  $t = 0$ , the instantaneous drug dose  $D_0$  input central compartment, immediately rose to  $D_0 V_1$ . So we can get equations:

$$f_0(t) = 0, c_1(0) = \frac{D_0}{V_1}, c_2(0) = 0 \quad (16)$$

Solution of equations (13) (16) can be obtained:

$$\begin{aligned}c_1(t) &= M e^{-\alpha t} + N e^{-\beta t}, M = \frac{D_0(k_{21} - \alpha)}{V_1(\beta - \alpha)}, N = \frac{D_0(\beta - k_{21})}{V_1(\beta - \alpha)} \\ c_2(t) &= \frac{D_0 k_{12}}{V_2(\beta - \alpha)}(e^{-\alpha t} - e^{-\beta t})\end{aligned}\quad (17)$$

So, as above, we can conclude:

Equation shows a rapid intravenous injection model. While in clinical trials, the people who infected with Ebola virus immune responses were damaged. And currently developed drugs for human use, either ZMapp, TKM-Ebola or JK-05, requires repeated dosing in order to maintain effective plasma concentrations during treatment. Based on this, we fixed a dosing interval, an interval of every treatment administered, modeling impulsive differential equations. But this is beyond our ability, and then we will fight with Ebola by other modeling tools.

#### 2) Constant rate infusion

When intravenous infusion at a rate of constant  $k_0$ , set dosing rate  $f_0(t)$ , the initial condition is

$$f_0(t) = k_0, c_1(0) = 0, c_2(0) = 0 \quad (18)$$

Solution of equations (13) (18) can be obtained:

$$\begin{aligned}c_1(t) &= M_1 e^{-\alpha t} + N_1 e^{-\beta t} + \frac{k_0}{k_{13}V_1}, c_2(t) = M_2 e^{-\alpha t} + N_2 e^{-\beta t} + \frac{k_{12}k_0}{k_{21}k_{13}V_2} \\ M_2 &= \frac{V_1(k_{12} + k_{13} - \alpha)}{k_{21}V_2}, M_1, N_2 = \frac{V_1(k_{12} + k_{13} - \beta)}{k_{21}V_2} N_1\end{aligned}\quad (19)$$

So, as above, we can conclude:

When  $t$  is large enough,  $c_1(t), c_2(t)$  will tend to 3<sup>rd</sup> of equations (19), the right side represents a constant value.

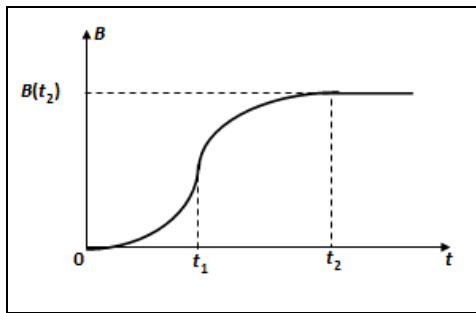
In fact, after the infusion is stopped if  $t = T$  (T is the time when stopping injection or infusion), then  $c_1(t), c_2(t)$  exponentially decays to zero after  $t > T$ .

Derived from human infections EBOV valid data obtained, was acquired immunity in fatal and non-fatal cases, significantly different. This implies that the acquired immunity in EBOV infection important role. Study of survivors showed the body to produce specific IgM antibody was 2 days after the attack, after 5 ~ 8 days to produce specific IgG antibodies. In contrast, in fatal cases, only 30% of patients were detected low levels of specific IgM, and specific IgG is not detected. In the early days after the onset of symptoms appearing acquired immune response can have a major impact on patient survival; survivor after EBOV infection occurs early inflammatory response supported this conclusion.[10,11,12]

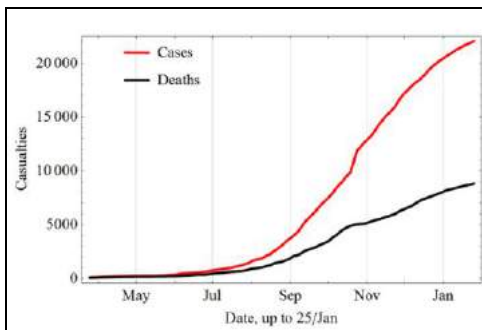
## 5.2 Epidemic Area

### 5.2.1 Analysis

Rescue progress generally proportional to the range of the virus infection, but the infection scope of the outbreak of Ebola virus, and treatment time are required to cure, and treatment time depends on the number and speed of drug delivery, the drug in the shortest possible time delivery of more treatment sooner. Rescue costs and the number of delivery of both drug-related, but also with the duration of drug delivery related. Setting time  $t = 0$  for Ebola outbreak start, begin rescue work time for  $t = t_1$ , the rescue is completed in time for  $t = t_2$ . Set time  $t$  with affected by the Ebola virus area range is  $B(t_1)$ , After the affected areas by Ebola is  $B(t_2)$ . To do rough statistics for the area affected by the Ebola virus:



**Figure 5:** Rough statistics for the area affected by the Ebola virus [6]



**Figure 6:** Ebola outbreak in 2014 and the cumulative infections and deaths has evolved [6]

(It is easy to see that it's similar with the first half of the evolution trend of our model)

Because analysis of  $x$  is more difficult, we explore, the spread of the Ebola virus in the region per unit time  $\frac{dB}{dt}$ , represents the propagation velocity of Ebola virus. In the ambulance before that  $0 \leq t \leq t_1$ , expand the scope of the Ebola virus is growing, that  $\frac{dB}{dt}$  increases with increasing

$t$ ; when begin a large-scale rescue work, that  $t_1 \leq t \leq t_2$ . If the rescue work fully enough, the Ebola virus will be

contained, that  $\frac{dB}{dt}$  should be reduced, and when the equation is zero when  $t = t_2$ .

### 5.2.2 Assumption

We need make assumptions about form of degree  $\frac{dB}{dt}$  of the transport rescue in speed and spread of fire:

- 1) Ebola virus outbreak in the original point as the center, spread evenly rounded to four weeks, the radius  $r$  is proportional to  $t$ .
- 2) The number of people infected with Ebola virus is proportional to the area of spreading Ebola virus is  $B(t_2)$ , scale factor  $c_1$  is the number of rescue medicines for unit area.
- 3) From the outbreak of the Ebola virus to begin rescue operations  $0 \leq t \leq t_1$ , Ebola virus degree of diffusion is directly proportional to the time  $t$ , scale factor  $\beta$  (Ebola virus from spreading rate).
- 4) Ambulance personnel were dispatched  $x$ , after start of rescue work ( $t \geq t_1$ ), Ebola virus from spreading rate dropped  $\beta - \lambda x$ , Where  $\lambda$  can be regarded as the speed of each ambulance personnel. Obviously  $\beta < \lambda x$ .
- 5) Each rescue ambulance unit time required for the number of drug  $c_2$ , so every ambulance crew rescue medication is  $c_2(t_2 - t_1)$ , and the total number of drug required for each patient is  $c_3$ .

### 5.2.3 Constitution

According to 3) 4) of assumptions as above, the extent of the spread of the Ebola virus  $\frac{dB}{dt}$ , increased linearly at  $0 \leq t \leq t_1$ , decreases linearly at  $t_1 \leq t \leq t_2$ .  $\frac{dB}{dt} \sim t$  pattern as shown in **Figure 6**.

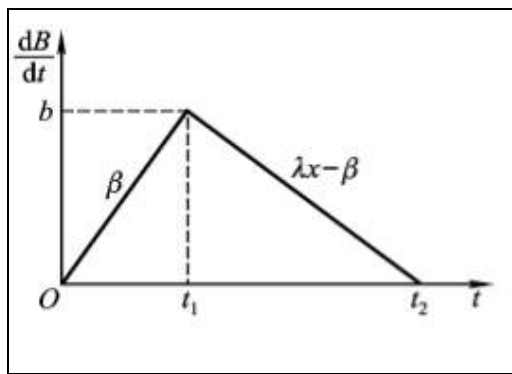


Figure 7:  $\frac{dB}{dt} \sim t$  linear graph

Set  $t = t_1$ , and then  $\frac{dB}{dt} = b$ . The number of Ebola virus infection  $B(t_2) = \int_0^{t_2} \frac{dB}{dt} dt$  is precisely the area of the triangle diagram, clearly  $B(t_2) = \frac{1}{2} b t_2$ .

And  $t_2$  satisfy:

$$t_2 - t_1 = \frac{b}{\lambda x - \beta} = \frac{\beta t_1}{\lambda x - \beta} \quad (20)$$

So

$$B(t_2) = \frac{\beta t_1^2}{2} + \frac{\beta^2 t_1^2}{2(\lambda x - \beta)} \quad (21)$$

#### 5.2.4 Solution

According to 2) 5) of assumptions, the number of assumptions for Ebola virus is  $c_1 B(t_2)$ , rescue medication need  $c_2 x(t_2 - t_1) + c_3 x$ . Substituting into equation (20), (21), to obtain the desired drug to total relief is

$$C(x) = \frac{c_1 \beta t_1^2}{2} + \frac{c_1 \beta^2 t_1^2}{2(\lambda x - \beta)} + \frac{c_2 \beta t_1 x}{\lambda x - \beta} + c_3 x \quad (22)$$

For the sake of  $x$ , so that  $C(x)$  to a minimum, we can get the number of rescue workers should be sent is

$$x = \frac{\beta}{\lambda} + \beta \frac{c_1 \lambda t_1^2 + 2c_2 t_1}{2c_3 \lambda^2} \quad (23)$$

Based on this, we propose we should set drug distribution points in West Africa should be in accordance with changes by the strength of the Epidemic area. Based on this model of computing and serious epidemic situation, each radiation locale should set 2 to 5 points. At the same time, we want to deploy second "mobile lab" in guinea and mali border line at the midpoint, in order to carry out the epidemic diagnosis faster and medical issuance.

### 5.3 Vaccine/Drug Production and R&D Speed

#### 5.3.1 Assumption

We assume that the speed and degree of development of vaccine production is analogous to the infection distance and disaster area. So we can still use the Forest-Fire model to building a specifically, intuitive, and effective model to solve the problem. So, the assumptions as above section will be reuse. So, the assumptions are rewritten as follows:

- 1) The number of people infected with Ebola virus is proportional to the area of spreading Ebola virus is  $B(t_2)$ , scale factor  $d_1$  is the number of production of drugs for unit area.
- 2) From the outbreak of the Ebola virus to end of the first drug production operations  $0 \leq t \leq t_1$ , Ebola virus degree of diffusion is directly proportional to the time  $t$ , scale factor  $\beta$  (Ebola virus from spreading rate).
- 3) The number of each production for drug  $x$ , after production of drug ( $t \geq t_1$ ), Ebola virus from spreading rate dropped  $\beta - \lambda_1 x$ , Where  $\lambda_1$  can be regarded as the speed of production of medicines. Obviously  $\beta < \lambda_1 x$ .
- 4) In order to ensure efficient use of time speed, the speed of the production of drugs and transportation of drugs should be the same. That is  $\lambda_1 = \lambda$ .

#### 5.3.2 Constitution & Solution

As the above section mentioned, we can deduce

$$b = \beta t_1, t_2 - t_1 = \frac{b}{\lambda_1 x - \beta}, \text{ in other words, } t_2 = t_1 + \frac{\beta t_1}{\lambda_1 x - \beta}.$$

So, the number of Ebola virus infection is  $B(t_2) = \int_0^{t_2} \frac{dB}{dt} dt$ , and the total number of drugs needed to cure is

$$C(x) = \frac{c_1 \beta t_1^2}{2} + \frac{c_1 \beta^2 t_1^2}{2(\lambda_1 x - \beta)} + \frac{c_2 \beta t_1 x}{\lambda_1 x - \beta} + c_3 x \quad (24)$$

According to equation solving, Ebola virus from spreading area is

$$B(t_2) = \int_0^{t_2} \frac{dB}{dt} dt = \frac{b t_2}{2} = \frac{\beta t_1^2}{2} + \frac{\beta^2 t_1^2}{2(\lambda_1 x - \beta)} \quad (25)$$



Find  $x$  so that  $C(x)$  minimum:  $\frac{dC}{dx}=0$ , the result is

$$x = \frac{\beta}{\lambda_1} + \beta \sqrt{\frac{c_1 \lambda_1 t_1^2 + 2c_2 t_1}{2c_3 \lambda_1^2}} \quad (26)$$

Based on this, we propose drug development should begin at the growth of the epidemic stage 1/3, and 1/2 of the growth of the epidemic stage clinical trials in order to achieve the best results. Now the main reason for the slow progress in Ebola drug development is the outbreak stabilizes. But we still want to the company which is in the development as soon as possible to develop drugs or vaccines to deal with Ebola outbreak.

## VI. MODEL RESULTS AND CONCLUSION

### Advantage and improve:

How to determine the Week Contact Rate value of  $\theta$ . How to determine the week contact rate can be improved, according to the epidemic cure rate before this Ebola outbreak, we can calculate the weighted average value, rather than simply a proportional relationship. The spread of the virus in the crowd is stage with a burst phase at first, Week Contact Rate of  $\theta$  will be greatly at this period; we can set it to an impulse variable.

For Epidemic Area of Forest-Fire model, this model assumes that the Ebola virus outbreak in the original for the center, a radial spread around, the actual situation may be due to the terrain, lifestyle and other reasons for the differences. For the treatment of patients with an average speed of rescue workers  $\lambda$ , may be related to the rescue start time  $t$ . As  $t$  grows, the rescue rate  $\lambda$  is also growing.

### Disadvantage:

For Vaccine/Drug Production and R&D Speed of Forest-Fire model, because the trial drug itself has some limitations and latency, total drug production scheme of arrangement exist error. And we choose the averaging method to use in the drugs used at the treatment of the amount recovered; such simple treatment will affect the calculation of the total demand for drugs.

Overall, for the Eradicating Ebola, we should be a comprehensive analysis of a model by normalized all of models as above, the results will be an optimal solution. But limited ability, we did not do so. But we have the optimal solution for a single model was a good deal. So the result of us compare with the integrated optimal solution will not be much apart.

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# Effect of Al Dopants on the Optical and Dispersion Parameters of Iron Oxide thin Films

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## ABSTRACT

Uniform and adherent  $\text{Fe}_2\text{O}_3:\text{Al}$  thin films were deposited on glass substrate using spray pyrolysis technique. The optical properties and dispersion parameters of iron oxide thin films have been studied as a function of doping concentration with Aluminum (Al). It has to be mentioned that changed in direct optical energy band gap of iron oxide was recorded, as expected, after doping. The data show that the optical energy gap  $E_g$  decreased from 2.52 eV for the undoped  $\text{Fe}_2\text{O}_3$  to 2.46 eV with the increasing of doping concentration of Al to 5%. Therefore, the changes in dispersion parameters and Urbach tails were investigated. An increase in the doping concentration causes a decrease in the average oscillator strength. The single-oscillator parameter has been reported.

**Keywords :** Dispersion parameters, spray pyrolysis, iron oxide,  $\text{Fe}_2\text{O}_3:\text{Al}$

## I. INTRODUCTION

There are about 15 phases formed by Fe and O, as oxides of iron <sup>[1]</sup>. They can be synthesized in pure, mixed oxides as well as doped structures. Iron oxide is used as an electrode in non-aqueous and alkaline batteries <sup>[2,3]</sup> and as a cathode in brine electrolysis <sup>[4]</sup>. As is known,  $\text{Fe}_2\text{O}_3$  belongs to wideband gap semiconductor group. Iron oxide is widely used for direct water splitting under solar illumination due to their band gap, high resistivity toward corrosion, stability in solution, ease of manufacturing and material availability, and low cost <sup>[5]</sup>. Furthermore, the iron oxide is essential material for humidity and gas sensors, catalyst, magnetic recording and medical fields <sup>[6-8]</sup>. Many methods have been adopted to grow  $\text{Fe}_2\text{O}_3$  film such as sol gel <sup>[9]</sup>, metal organic <sup>[10]</sup>, pulsed laser deposition <sup>[11]</sup>, spray pyrolysis <sup>[12]</sup>, filtered arc deposition <sup>[13]</sup> and MBE <sup>[14]</sup>. In this work, the spray pyrolysis was adopted to prepare this film.

Generally, selective elements as dopant materials in  $\text{Fe}_2\text{O}_3$  can be classified into two groups of materials. One group can substitute for Iron and the other can substitute for Oxide. These different types of doping materials can exhibit different optical properties for  $\text{Fe}_2\text{O}_3$  due to the different treatments of Fe and O in the

$\text{Fe}_2\text{O}_3$  structure. Each exhibits very different behavior as dopant material in  $\text{Fe}_2\text{O}_3$  nanostructures. Aluminum can be applied as an impurity that changes the band-gap of  $\text{Fe}_2\text{O}_3$ . By alloying  $\text{Fe}_2\text{O}_3$ , with other material of a different band-gap, the band-gap of  $\text{Fe}_2\text{O}_3$  can be fine-tuned. In this work, un-doped and Al-doped  $\text{Fe}_2\text{O}_3$  films have been prepared by using the spray pyrolysis technique.

This paper reports the influence of doping with Aluminum on the preparation and properties of Iron Oxide  $\text{Fe}_2\text{O}_3$  thin films by spray pyrolysis technique (SPT). The optical features are significant to be determined accurately, not only to know the basic mechanisms underlying these phenomena, but also to exploit and develop their interesting technological applications

## II. METHODS AND MATERIAL

### Experimental Procedure

Iron chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), 0.1 M, as matrix material and aluminum chloride ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), 0.1, as a doping agent with a concentration of 3% and 7% have been dissolved in de-ionized water in order to form the final spray solution. Few drops of HCl were added to make

the solution clear, the total volume of 50 ml was used in each deposition, these two starting solutions were used for deposition of Fe<sub>2</sub>O<sub>3</sub>:Al thin films. The spray pyrolysis was done by using a laboratory designed glass atomizer, which has an output nozzle of 1 mm. The films were deposited on preheated glass substrates at a temperature of 400°C. The preparation conditions, however, have been optimized such as spray time was 8 seconds and the stopping period was two minutes. The latter period is enough to avoid excessive cooling of glass substrate. The carrier gas (filtered compressed air) was maintained at a pressure of 10<sup>5</sup> Pascal, distance between nozzle and substrate was about 28 cm, solution flow rate 5 ml/min. Thickness of the sample was measured using the weighting method and was found to be around 350 nm., Optical transmittance and absorbance were recorded in the wavelength range (300-900nm) using UV-visible spectrophotometer (Shimadzu Company Japan). Optical transmittance and absorbance were reported in order to find the effect of doping on the parameters under investigation.

### III. RESULT AND DISCUSSION

It is quite important to get information about the optical transmittance in order to assess the optical performance of a conductive oxide film. Fig.1 shows the transmittance spectra in UV and visible wavelength regions of the films. The optical transmission in the visible wavelength region of un-doped (pure) and Al-doped Fe<sub>2</sub>O<sub>3</sub> (with different percentages) films increases with increasing the wavelength. Furthermore, the average values of the optical transmission in the visible range (400-800 nm) were estimated. It is found that the average transmittance values for different films were 40% 45% and 50% in the near infrared region for Fe<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>:Al 3%, and Fe<sub>2</sub>O<sub>3</sub>:Al 7% thin films, respectively. It is observed in Fig. 2 that in the visible region, the reflectance value ranges from 0.38 % to 0.41% passing through a maximum value of about 0.64 %.

It has to be mentioned that this maximum value of reflectance is valid to all doping percentages at a wavelength of about 540 nm. It is also noticed that all different doping percentage films have the same behavior in which the reflectance increases with the increase of wavelength, however, they reach a maximum value (aforementioned) and then decreases with

increasing the wavelength. This feature is actually quite interested and could be used in duality-based applications.

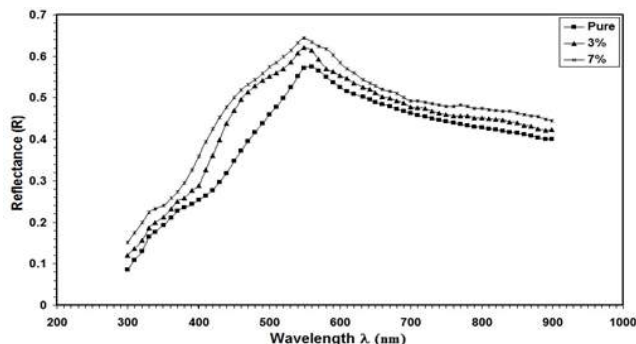


Figure 1. Transmittance versus wavelength

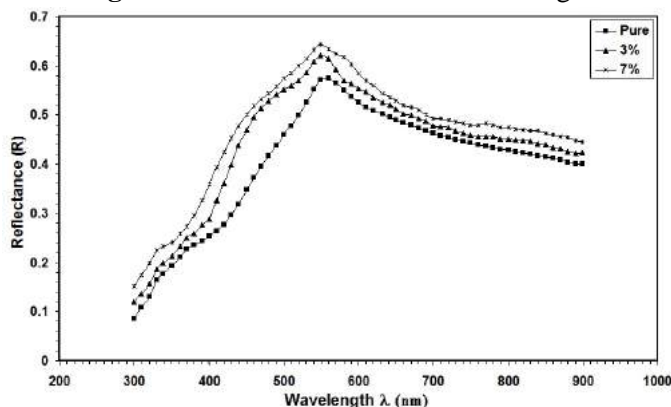


Figure 2. Reflectance versus wavelength

The incorporation of impurity into semiconductors often results in the formation of band tailing in the band gap. The tail of the absorption edge is exponential, which indicates the presence of localized states in the energy band gap. The amount of tailing can be predicted by means of plotting the absorption edge data in terms of an equation originally given by Urbach<sup>[15]</sup>. The absorption edge gives a measure of the energy band gap and the exponential dependence of the absorption coefficient, in the exponential edge region Urbach rule is expressed as<sup>[16, 17]</sup>

$$\dots\dots\dots (1)$$

{ EMBED Equation.3 }

where  $\alpha^0$  is a constant,  $E_U$  is the Urbach energy which characterizes the slope of the exponential edge. Figure 3 shows Urbach plots of the different films. In order to obtain the value  $E_U$ , the inverse of the slope of  $\ln \alpha$  versus  $h\nu$  has to be calculated and is given in Table 1. The dopants would result in change the width of the localized states in the optical band. The Urbach energy value changes inversely with the optical band gap. The  $E_U$  values of pure Fe<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>:Al 3%, and Fe<sub>2</sub>O<sub>3</sub>:Al 7%

thin films were measured to be 724,735 and 793 meV respectively. The decrease in  $E_U$  can be attributed to the atomic structural disorder of  $Fe_2O_3$  films increase by Aluminum doping. This behavior comes as a result of increasing the concentration of point defects induced by the dissolution of Al atoms in  $Fe_2O_3$  crystals and formation of solid solutions. Therefore, this decreasing leads to a redistribution of states, from band to tail. Consequently, an increase in the optical gap and a narrowing in the Urbach tail have taken place.

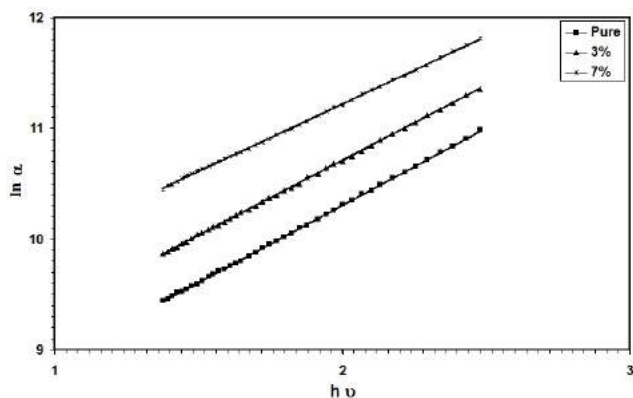


Figure 3.  $\ln \alpha$  versus photon energy for  $Fe_2O_3$  and  $Fe_2O_3:Al$  films.

The refractive index dispersion plays an important role in optical communication as well as designing the optical devices. Therefore, dispersion parameters have to be determined. The dispersion parameters of the films were evaluated according to the single-effective-oscillator model using the following relation [18, 19].

$$\dots\dots\dots (2)$$

{ EMBED Equation.3 }

where  $E_o$  stand for single-oscillator energy which has a physical meaning. Its physical meaning is simulates all the electronic excitation involved and  $E_d$  is the dispersion energy related to the average strength of the optical transitions [20]. The latter is defined as a measure of the intensity of the entire band optical. This model describes the dielectric response to transitions below the optical gap. The quantity of  $(n^2 - 1)^{-1}$  has been plotted vs.  $(h\nu)^2$  as shown in Fig. 4.  $E_o$  and  $E_d$  values were determined from the slope,  $(E_o E_d)^{-1}$  and intercept  $(E_o/E_d)$ , on the vertical axis and are given in Table 1.  $E_o$  values increased with the dopants as the optical band gap increase. According to the single-oscillator model, the single oscillator parameters  $E_o$  and  $E_d$  are related to the imaginary part of the complex dielectric constant, the moments of the imaginary part of the optical spectrum

$M_1$  and  $M_3$  moments can be derived from the following relations [21].

$$\dots\dots\dots (3)$$

{ EMBED Equation.3 }

$$\dots\dots\dots (4)$$

{ EMBED Equation.3 }

The values obtained for the dispersion parameters  $E_o$ ,  $E_d$ ,  $M_1$ , and  $M_3$  are listed in Table 1, Table2 The obtained  $M_1$  and  $M_3$  moments changes with the dopants. For the definition of the dependence of the refractive index ( $n$ ) on the light wavelength ( $\lambda$ ), the single-term Sellmeier relation can be used [18].

$$\dots\dots\dots (5)$$

{ EMBED Equation.3 }

where  $\lambda_o$  is the average oscillator position and  $S_o$  is the average oscillator strength. The other parameters in the above equation, namely,  $S_o$  and  $\lambda_o$  can be obtained experimentally by plotting  $(n^2 - 1)^{-1}$  versus  $\lambda^{-2}$  as shown in Fig. 5. The slope of the resulting straight line gives  $1/S_o$ , while the infinite interception of wavelength yields  $1/S_o \{ \text{QUOTE } \lambda_o^2 \}$ . The results reveal an increase in the band gap which can be attributed to the presence of unstructured defects. The latter in turn decrease the density of localized states and cause a narrowing in the Urbach tail and hence increasing the energy gap.

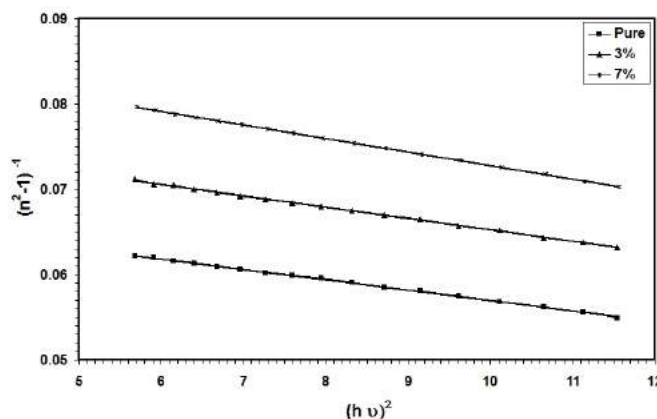
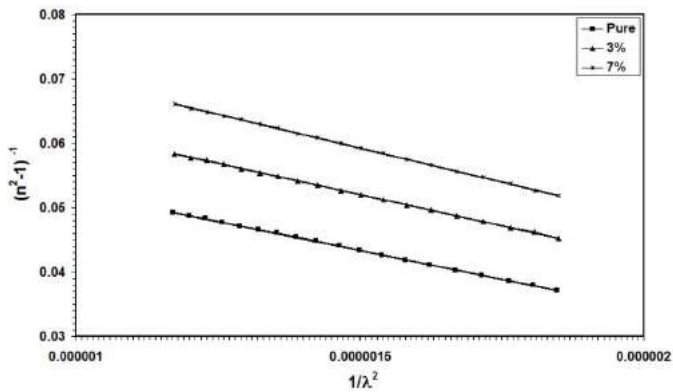


Figure 4. Variation  $(n^2 - 1)^{-1}$  as a function of  $(h\nu)^2$  for  $Fe_2O_3$ ,  $Fe_2O_3:Al$  films.



**Figure 5.** Variation  $(n^2 - 1)^{-1}$  as a function of  $(\lambda)^{-2}$  for  $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3:\text{Al}$  films.

**Table 1.** The optical parameters

Sample	$E_d$ (eV)	$E_o$ (eV)	$E_g$ (eV)	{ EMB ED Equati on.3 }	$n(o)$
Pure	79.05	5.05	2.52	16.62	4.07
3%	71.42	5.00	2.50	15.28	3.90
5%	61.54	4.92	2.46	13.50	3.67

**Table 2.** The optical parameters

Sample	$M_1$ $\text{eV}^{-2}$	$M_3$ $\text{eV}^{-2}$	$S_o$ $\times 10^{13}$ $\text{m}^{-2}$	$\lambda_o$ nm	$E_U$ meV
Pure	15.62	0.610	5.64	595	724
3%	14.28	0.571	5.26	562	735
5%	12.50	0.515	4.96	536	793

#### IV. CONCLUSION

$\text{Fe}_2\text{O}_3:\text{Al}$  thin films were prepared by means of spray pyrolysis technique. In this work, the doped and undoped samples were characterized. Due to doping, the optical band gap was increased. Likewise, optical transmittance was affected for moderate doping.

Furthermore, the single-oscillator parameters were determined. In this paper, it is shown that the dispersion

parameters of thin films obeyed the single oscillator model, the change in dispersion energy was investigated and its value decreased from 79.05 to 61.54 eV for  $\text{Fe}_2\text{O}_3:\text{Al}$  films with increasing doping concentration .

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# A Review on Anticancer Drug from Marine

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## ABSTRACT

The marine environment is a rich source of both biological and chemical diversity. It is very much likely that marine organisms would be wonderful source of biologically active molecules. The collection of the marine therapeutics includes molecules with antibiotic, antiviral, antiprastic, analgesic and anticancer agent from bacteria, cyanobacteria, *tunica*, fungi, sponge. This review focuses on the latest studies and critical research in this field and evidences the immense potential of marine organisms as sources of bioactive peptides and other anticancer biomolecules. Various anticancer compounds like Aplidine, Bryostatin-1, Didemin B, Dolastatin, Ecteinascidine with diverse modes of action, such as, anti-proliferative, antioxidant, anti-microtubule have been isolated from marine sources. Traditional chemotherapeutic agents have a range of side effects like fatigue, gastrointestinal distress and depression of immune system which introduces the these sources have been shown to have antioxidant activity and cytotoxic effect on several human cancers such as leukemia, lymphoma, ovarian, melanoma, breast, bladder, neuroendocrine, prostatic, colon and non-small cell lung cancer very potently.

**Keywords:** Marine Organisms, Bryostatin-1, Dolastatin, Human Cancers

## I. INTRODUCTION

Over the past few years, about 3000 new compounds like anti-tumor, anti-microtubule, anti-proliferative, photoprotective, antibiotic and anti-infective discover from various marine sources some have entered clinical trials. This activity has been largely due to improvements in the technologies involved in deep-sea sample collection and large-scale drug production through aquaculture and drug synthesis<sup>7</sup> which took place in the 1980s. These developments suggest that, in the future, the oceans will become an important source of novel chemical classes not found in the terrestrial environment.

## II. METHODS AND MATERIAL

Therapeutic Agents from Marine Sources

### Bacteria

Marine microorganisms are a rich source of new genes, exploitation of which is likely to lead to the discovery of new drugs and therapeutic approaches. Only a few marine bacteria can be isolated under laboratory conditions and there is an urgent need to develop new

culture techniques to isolate slow-growing bacteria and also to isolate the bacteria that are unique in production of novel natural products. (e.g. *Seudoverticillium*, *Topsentins*, *Scytonemin* and *Manoalide*), anticancer agents (e.g., *Bryostatins*, *Discodermolide*, *Eleutherobin* and *Sarcodictyin*) and antibiotics (e.g. *Marinone*). Anti-Parasitic compound *Valinomycin* isolated from *Streptomyces sp.* strains of Mediterranean

### Cyanobacteria

The cyanobacteria population comprises 150 genera and about 2000 species of considerable diversity. The potency of marine cyanobacteria as anticancer agents is the most explored among all marine derived chemicals. Besides cytotoxicity in tumor cell lines, several compounds have emerged as templates for the development of new anticancer drugs. Well studied species of marine cyanobacteria includes *Nostoc*, *Calothrix*, *Lyngbya*, *Symploca*

### Fungi

Marine derived fungi provide plenty of structurally unique and biologically active secondary metabolites. The Anthracenedione derivatives acting as the potent



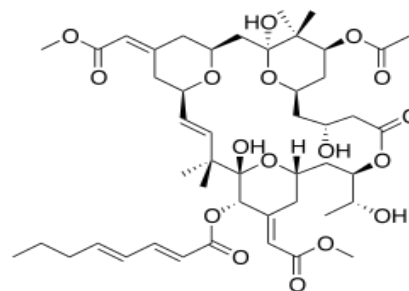
anticancer agents screened from the mangrove endophytic fungus *Halorosellinia* sp. and *Guignardia* sp. For example, Cytarabine, an antileukemic drug and Trabectedin, an agent for treating soft tissue sarcoma are developed from marine fungi sources [8]. Besides, marine-derived fungi are known to be a source of antioxidative natural products such as Acremonin A from *Acremonium* sp. and Xanthone derivative from *Wardomyces anomalus* [9].

### Sponge

Approximately 10,000 sponges have been found worldwide [11] and most of them live in marine environments [12]. Marine sponges have yielded over 70 novel compounds to date that exhibit significant inhibitory activity towards a range of protein kinases. A range of bioactive compounds has been found in about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce influential anticancer and anti-inflammatory agents [13].

### Source organism: *Bugula neritina* (Bryozon)

Bryostatin-1 is a macrocyclic natural lactone isolated from the marine Bryozoan, *Bugulaneritina* (Fig.1). It has shown both antitumour as well as immunomodulatory effects [1,2]. It is a potent activator of the protein kinase C (PKC) family, lacking tumour-promoting activity and with antagonistic effects on tumour-promoting phorbol esters. This effect is probably related to down-regulation of PKC or by specific isoform activation. It also stimulates cytokine production, bone marrow progenitor cells and neutrophils [3,4]. In vitro, bryostatin-1 has cytotoxic activity against various leukaemia and solid tumour cell lines. It has also in vivo antitumour activity in various murine models, including leukemia, lymphoma, ovarian cancer and melanoma. It was shown to enhance the antitumour effects of various anticancer agents, such as vincristine, cytosine arabinoside, cisplatin, melphalan, paclitaxel and others.



Bryostatin-1

These effects may be schedule-dependent [1,2,5]. This agent was studied in phase I trials at different infusion schedules. The recommended doses for phase II trials were 25-35 mg/m<sup>2</sup> when administered over one hour for three of every four weeks; 25 mg/m<sup>2</sup> given as a weekly 24 hour infusion. Myalgia was the DLT in all trials. Other toxicities were joint aches and a transient decrease in platelet counts [6,7]. Notably, partial responses were reported in patients with melanoma, ovarian cancer and. Phase II trials of bryostatin-1 are being conducted at various infusion regimens in a large number of tumour types in both solid and haematological malignancies. In addition, phase I trials of bryostatin-1 in combination with other agents, such as cisplatin, paclitaxel, fludarabine, vincristine, cytosine arabinoside and 2-CDA are also being conducted.

SOURCE	COMPOUND	BIOACTIVITY
Bacteria	Valinomycin	Anti-Parasitic
Cyanobacteria	Calothrixin A and B	Antimalarial
Cyanobacteria	Curacao extracts	Antiproliferative
Fungi	Cephalosporins	Antibiotic
Fungi	Antioxidants	Atherosclerosis, dementia
Soft coral	Methanol extracts	Anticancer
Sponge	Kuanoniamines	Growth inhibitor
Sponge	Steroid	Inflammation, asthma
Sponge	Ara-c	Antiviral

### BRYOSTATIN-1



Figure 1. The Bryozoan *Bugula neritina*

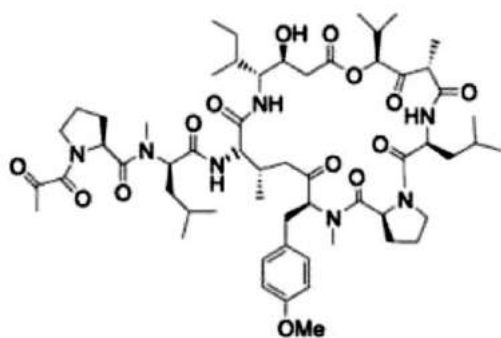
## APLIDINE



**Figure 2.** The tunicate *Aplidium albicans*

Source Organism: *Aplidium albicans* (tunicate)

Aplidine was obtained from a Mediterranean colonial tunicate, *Aplidium albicans*. It has a pyruvyl group replacing the lactyl group in DB and its synthesis has been achieved. It appears more active than DB in preclinical models and apparently not cardiotoxic. Aplidine entered clinical trials in 1999 both in Europe and in the US under the sponsorship of the Spanish company Pharma Mar. It is appeared that these cancer cells are sensitive to low concentrations of this compound. *Aplidine's* mode of action involves several pathways; that's why *Aplidine* is described as multifactorial apoptosis inducer. The compound induces rapid cell cycle arrest at G1-G2 and inhibition of protein synthesis, thus introduce apoptosis of cancer cells [9] *Aplidine* also inhibits the expression of the vascular endothelial growth factor gene, having antiangiogenic effects [10]. However, *Aplidine* is appeared as more active than *Didemnin* in preclinical models and so far has not shown evidence of life threatening neuromuscular toxicity [8,9,4].



Aplidine

The dose limiting toxicity with the protracted schedule is muscular with a remarkable lack of haematological toxicity in spite of the cytotoxicity noted at low

concentrations in leukemic blasts explanted from patients [11,12]. Consistent evidence of activity has been noted in pretreated neuroendocrine tumors [13] and other tumor types. Phase II studies are now ongoing with an every other week schedule giving APL as a protracted or 3 hours intravenous infusion at a dose of 5mg/m<sup>2</sup>.

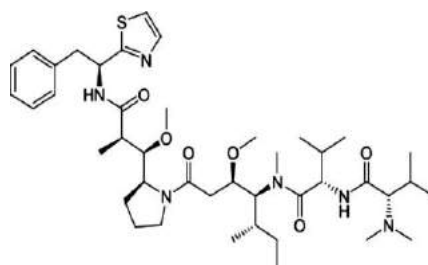
## DOLASTATINS



**Figure 3.** *Dolabella auricularia*

Source Organism: *Dolabella auricularia*

The dolastatins are cytotoxic peptides, which can be cyclic or linear, derived from the sea hare, *Dolabella auricularia*, a mollusc from the Indian Ocean. Dolastatin 10 and 15 are small peptides that were shown to interact with tubulin. Dolastatin was selected for initial clinical development because of its more favourable preclinical profile. It is extremely potent in vitro and it was shown to inhibit microtubule assembly, tubulin-dependent guanosinetriphosphate (GTP) binding and inhibit vincristine and vinblastine binding to tubulin. It causes cells to accumulate in metaphase arrest and is modulated by the MDR gene product [14,15]. Dolastatin has in vitro activity against several human leukaemia, lymphoma and solid tumour cell lines.



Dolastatin

It has documented antitumour activity in various human solid tumour models, such as LOX#IMVI melanoma,

OVCAR-3 ovarian carcinoma and NCI-H522 NSCLC cell lines. In animal toxicology studies, myelo suppression was the dose-limiting toxicity. This agent is highly bound to plasma proteins and pharmacokinetic studies in animals showed a rapid degradation probably by hepatic metabolism [16,17]. This agent entered phase I trials as an i.v. bolus injection every 3 weeks. The maximum tolerated dose (MTD) was 300 mg/m<sup>2</sup> for heavily pretreated patients, while 400 mg/m<sup>2</sup> appears to be the MTD for minimally pre-treated patients. The dose-limiting toxicity (DLT) was myelo suppression, and local irritation and phlebitis, and mild peripheral neuropathy were also observed. Phase II trials are being initiated in breast, colon, lung, ovarian and prostate cancer, as well as lymphomas and leukaemias [18, 19]. side effects observed were peripheral sensory neuropathies, pain, swelling, and erythematic at the injection site. Complexity and low yield of chemical synthesis of *Dolastatins* together with low water solubility

#### ❖ ECTEINASCIDINS(ETS)



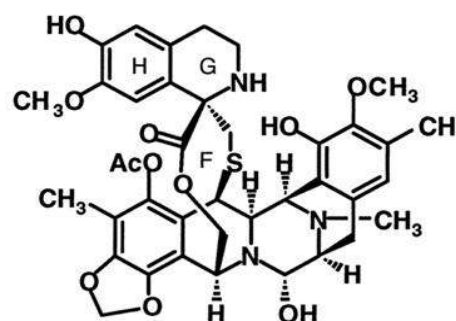
**Figure 4.** The tunicate *Ecteinascidiaturbinata*

#### Source Organism: *Ecteinascidiaturbinata*

The ecteinascidins (Ets) are derived from the Caribbean tunicate *Ecteinascidiaturbinata*. Following a period of supply problems, enough amounts of this compound could be obtained from aquaculture and synthesis. The derivative Ecteinascidin 743 (ET 743) showed promising activity in murine and human tumour models, and is currently in early clinical development. It is a tetrahydro isoquinoline alkaloid that alkylates selectively guanine N2 from the DNA minor groove, and this alkylation is reversed by DNA denaturation.

Therefore, it differs from other DNA alkylating agents so far used in the clinic.

Recent mechanistic data demonstrates that ET-743 induces a broad inhibition of activated transcription with no effect on the constitutive transcription [20,21,22]: ET-743 inhibits the activation of the multidrug resistant pathway [23] that is considered to be the main mechanism of primary and acquired resistance of cancer cells to natural drugs such doxorubicin and taxanes. ET-743 is the only known anticancer entity for which there is an inverse correlation between the DNA repair efficiency and the sensitivity/resistance pattern [24,25]; such evidence offered a rationale to Marine Drugs **2004**, 2 18implement combinations studies with platin salts [26] and to seek for correlations in patients between the DNA efficiency and the response to ET-743 [27] An extensive phase I program assessing different schedules of administration was completed[28,29,30]: The dose limiting toxicities were bone marrow toxicity and fatigue. As predicted in the preclinical toxicology, transaminitis is noted in the majority of the patients but such a drug induced effect is transient/reversible and non cumulative and therefore does not represent a limiting factor for long-term therapy.



Ecteinascidins

Consistent evidence of antitumor activity in patients bearing resistant disease was reported in the phase I program. In fact, objective remissions in breast cancer, melanoma and mesothelioma were observed together with a consistent evidence of antitumor activity in patients with advanced resistant sarcoma. Such evidence was the starting point for a fast track pivotal phase II program in patients with advanced soft tissue sarcoma resistant or relapsed to conventional therapies. Long-term results from such studies have clearly confirmed a significant therapeutic impact in this disease setting [36,

37, 38, 39, and 40]. In these studies ET-743 has been given as a 24 hours intravenous infusion every 3 weeks at a dose of 1.5 mg/m<sup>2</sup>.

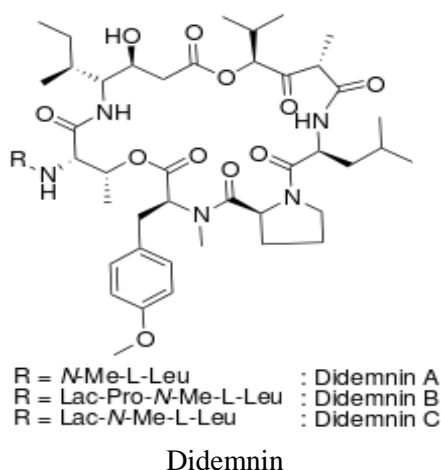
## DIDEMNIN B



**Figure 5.** Trididemnum Solidum

Source Organism: Trididemnum Solidum

Different types of tunicates and ascidia are inhabitants of sea floor. They produce complex antitumor compounds that are estimated as more effective than any other cancer medicine now in use. One of these potent compounds is Didemnin which was isolated first from Caribbean tunicate Trididemnum solidum[41]. But later has also been extracted from other species of the same genus [42]. Among various Didemnin, Didemnin B has the most potent antitumor and antiproliferative activity against human prostatic cancer cell lines [41]. Didemnin B is the first marine peptide to enter into clinical trial as a potent anticancer drug [43]. This is acyclic depsipeptide which exerts antitumor activity via protein synthesis inhibition [44]. However, high toxicity, poor solubility and short life span led to the discontinuation of clinical trials of Didemnin B [43].



It has shown impressive antitumor activity in human tumour models in vitro as well as in tumours growing in athymic mice.<sup>10</sup> In initial clinical trials, patients with various solid tumours or non-Hodgkin lymphoma were given a short intravenous infusion of didemnin B every 3 weeks, and antitumor effects were observed. However, severe neuromuscular and cardiotoxic effects led to the discontinuation of clinical trials.<sup>11,12</sup>

## III. CONCLUSION

The marine environment is a rich source of both biological and chemical diversity. It is very much likely that marine organisms would be a wonderful source of biologically active molecules. The collection of the marine therapeutics includes molecules with antibiotic, antiviral, antiprastic, analgesic and anticancer agent from bacteria, cyanobacteria, *tunica*, fungi, sponge. This review focuses on the latest studies and critical research in this field and evidences the immense potential of marine organisms as sources of bioactive peptides and other anticancer biomolecules. Various anticancer compounds like Aplidine, Bryostatin-1, Didemin B, Dolastatin, Ecteinascidine with diverse modes of action, such as, anti-proliferative, antioxidant, anti-microtubule have been isolated from marine sources. Traditional chemotherapeutic agents have a range of side effects like fatigue, gastrointestinal distress and depression of immune system which introduces the these sources have been shown to have antioxidant activity and cytotoxic effect on several human cancers such as leukemia, lymphoma, ovarian, melanoma, breast, bladder, neuroendocrine, prostatic, colon and non-smallcell lung cancer very potently..

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# Microwave Assisted Synthesis, Characterization and Antibacterial Activity of 2-Chloromethyl Benz Imidazole Derivatives

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## ABSTRACT

Objective-the objective of present research work to synthesize and screen novel 2-chloromethyl-1-h-benzimidazole derivative for antibacterial activity. Method-2-chloromethyl-1-H-benzimidazole was prepared by condensing 2-chloromethyl-1-h-benzimidazole with different aromatic amines and heterocyclic. The synthesized compounds were screened for their antibacterial activity against stap. Aurious by well plate method. 2-chloromethyl benzimidazole can be synthesized by the reaction of o-phenylenediamine with chloroacetic acid. This on reaction with substituted anilines in presence of ethanolic KOH gives corresponding benzimidazole derivatives. The synthesized compounds were characterized by TLC & IR data.

**Keywords :** 2-Chloromethyl Benzimidazole, O-Phenylenediamine, Chloroacetic Acid Aniline.

## I. INTRODUCTION

In the field of science of technology, medicinal chemistry has been a fascinating subject. The rapid development in the last 7 decades has been truly a challenging and very exciting. Medical chemistry according to Burger, tries to be based on the ever increasing hope that biochemical rationals for drug discovery may be found.

Medicinal chemistry is the branch of science, which has remarkable value for synthesis of novel drugs with intense therapeutic activity. It concern with discovery, development, identification and interpretation of mode of action of biologically active compounds at molecular level.

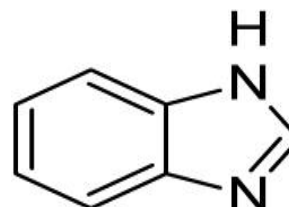
These developments have provided new challenges and opportunities for drug research in general and drug desings in particular. Pure organic compounds, natural or synthetic products are the chief source of agents for the cure, the mitigation or the prevention of disease today. The major objectives of the medicinal chemists are transformation of path biochemical and physiological data into a 'chemical language' with the

aim of designing molecules interacting specifically with the derailed or degenerating processes in the diseased organisms.

The development of chemotherapy during past 60 years constitute one of most important therapeutic advances in history of medicine and antibacterial drugs are the greatest contribution of present century to therapeutics. Potential therapeutic targets are being disclosed with increasing frequency and the exponential growth will continue during the next decates.

### A. Benzimidazole

The benzimidazole contain a phenyl ring fused to an imidazole ring, was shown in structure (1).



**Figure 1.** Benzimidazole

Compounds bearing Benz imidazole nucleus have been of great interest to synthetic and medicinal chemists for a long time due to their unique chemical and biological properties. Historically the first Benz imidazole was prepared in 1872 by Hoebrecker who obtained 2,5 or 2,6-dimethyl Benz imidazole by the reduction of 2-nitro-4-methylacetanilide. Several years later Ladenburg obtained the same compound by refluxing 3,4-diaminotoluene with acetic acid. The Benz imidazole are known also as Benz imidazole or benzoglyoxalines. Thus, Benz imidazole according to this nomenclature would be called methyl-o-phenylenediamine and 2-methyl Benz imidazole.

This tautomerism is analogous to that found in the imidazole and amidines. In fact, the Benz imidazole may be considered as cyclic analogs of the amidines.

Benz imidazole is a aromatic heterocyclic compound having imidazole ring fused to benzene. The most prominent Benz imidazole compound in nature is N-ribosyl –dimethyl Benz imidazole, which serves as an axial ligand for cobalt in vitamin B12. The nucleus is present in some drugs such as proton pump inhibitors and anthelmintic agents.

Mebendazole, thiabendazole which have anthelmintic and antifungal properties are Benz imidazole class of compounds. Benz imidazole and its derivatives are widely used as intermediate in synthesis of organic target compound including pharmaceuticals, agrochemicals, dyes, photographic chemicals, corrosion inhibitors, epoxy curing agents, adhesives and plastic modifiers. Benz imidazole is a white to slightly being solid; melting at 145-150 °C, boils at 360 °C, slightly soluble in water, soluble in ethanol. Benz imidazole and its derivatives are used in organic synthesis and vermicides and fungicides.

Melting points of the synthesized compounds were determined by open capillary method and were uncorrected. IR spectral analysis was carried out using FTIR-410, Jasco at Rajarambapu college of pharmacy, Kasegaon.

Physical properties of Benzimidazole:

- 1) Benzimidazole having high melting points. The introduction of substituents at 1-position lowers the melting point.
- 2) Benzimidazoles are usually soluble in polar solvents and sparingly soluble in non-polar solvents.
- 3) Benzimidazoles are weakly basic, being somewhat less basic than imidazole.
- 4) Benzimidazoles are also sufficiently acidic to be generally soluble in aq. alkali and form N-metallic compounds. The acidic properties of benzimidazole, like those of imidazole, seem to be due to stabilisation of the ion by resonance.
- 5) The pKa value of Benzimidazole is pKa=5.30 for 2-methyl Benzimidazoles and pKa=12.33 for 2-amino Benzimidazoles.

## B. Role of Pharmaceutical Chemistry in Drug Discover

Pharmaceutical chemistry plays important role in identification of lead compound it is also known as Hit. So 1) Identification of lead, 2) Optimization of lead, 3) Lead Development these are most important steps in discovery.

Further chemistry and analysis is necessary, first to identify and “triage” compounds that do not provide series displaying suitable SAR and chemical characteristics associated with long-term potential for development, then to improve remaining hit series with regard to the desired primary activity, as well as secondary activities and physicochemical properties such that agents will be useful when administered in real patients.

The next through final synthetic chemical stages involve production of lead compound in suitable quantity and quality to allow large scale animal and eventual, extensive human clinical trials. This involves the optimization of the synthetic route for bulk industrial production, and discovery of the most suitable drug formulation.



## Review of Literature:

- 1) Z. Kazimirczuk, M. Anderzejewska ET; al. Are evaluated the synthesized compound for their activity against 4 mycobacterium strains.
- 2) A. Idhaya Dhullaet. Al.(2011) reported synthesis of Benzimidazole derivative and their antimicrobial activity.
- 3) R. K. Bansal (2005) Heterocyclic chemistry reported I.R and NMR interpretation.

### C. Need of Investigation

Many important biochemical compounds and drugs of natural origin contain heterocyclic ring structure. Among these, Carbohydrate, essential amino acid, vitamins, alkaloids, glycosides, etc the presence of heterocyclic structures in diverse type of compounds is strongly indicative type of the pharmacological activity and recognition of this is reflected in efforts to find useful synthetic drugs.

The development of resistant to current antibacterial therapy continuous to stimulate search for more agents, the increasing clinical importance of drug resistant and bacterial pathogens has lent additional urgency to microbiological research and development of novel biologically active compounds. Hence the aim of this work is to synthesize some novel 2-chloromethyl 1-H-Benzimidazole derivative and carry out antibacterial potentials with good activity and less toxic effects. The biological activity of the compounds containing basic moiety have been well documented.

- (1) The present work describes the 2-chloromethyl 1-H-Benzimidazole and their derivatives in search of bioactive molecules.
- (2) Work also emphasized on the structural elucidation and pharmacological screening for antibacterial activity of synthesized compounds.

### D. Objectives

The discovery and development of pharmacologically active molecules has been guided not only by classical medicinal chemistry but also by the use of sophisticated mechanistic approaches and biochemical assay.

The reviews clearly emphasize the importance of heterocyclic in naturally occurring as well as synthetic agents and does an important class itself possess diversified pharmacological actions such as antimicrobial, antiprotozoal, antimalarial and antiallergic etc. This point encourages further investigation in the field. The logic supporting the work presented in this dissertation was formulated, bearing in mind that the biological activities of known moieties and attempting certain structural modification or adaptation in light of the recent trends in drug research incorporating newly emerged pharmacophores on existing moiety.

The development of resistance to current anti-bacterial therapy continues to stimulate the search for more effective agents. The increasing clinical importance of drug resistant and bacterial pathogens has lent additional urgency to microbiological research and development of novel biologically active compounds. Hence in the present study we plan synthesized some novel benzimidazoles with good activity and less toxic effect.

### E. Microwave Technique

Some derivatives were synthesized by using Microwave technique. This technique also refers as Green chemistry. By this technique required time was less and yield was higher as compare to conventional technique. Melting points were taken by using Thiele's tube apparatus and were uncorrected. Thin layer chromatography was used to assess the course of reaction and the purity of intermediate and final compounds, giving single spot on TLC plate (silica gel), using various solvent systems. Visualization of spot on plate was done by exposure to iodine vapours.

Infrared(IR) spectra were recorded in KBR disc on a Jasco FT-IR-410 spectrometer.

### F. Chemicals

All chemicals and solvents were produced from commercial sources and purified and dried using standard procedures from literature whenever required. Chemicals used for the synthesis were enlisted below with their manufacturer mentioned in parentheses.

O-phenylenediamine - Research laboratory, Islampur  
 Hydrochloric acid - Research laboratory, Islampur  
 Chloroacetic acid - Research laboratory, Islampur  
 Ethanol - Research laboratory, Islampur  
 Potassium hydroxide - Research laboratory, Islampur.  
 Ammonium hydroxide - Research laboratory, Islampur  
 Dimethyl sulfoxide (DMSO).- Research laboratory, Islampur

### G. Preparation of TLC Reagent

For the identification of benzimidazoles using thin layer chromatographic technique the reagent used is a mixture of Chloroform and methanol was taken in a ratio of 9:1 as shown in fig no. 2



Figure 2. TLC plate of intermediate

Mobile phase: Chloroform:Methanol-9:1

R<sub>f</sub> value= Distance travelled by solute/ Distance travelled by solvent

$$= 6/9$$

$$= 0.66$$

### H. Methodology- Scheme of The Experiment

O-Phenylenediamine was condensed in microwave by using chloroacetic acid in the presence of 5N NaCl to give 2-chloromethyl-1-h-benzimidazoles using different aniline derivatives.

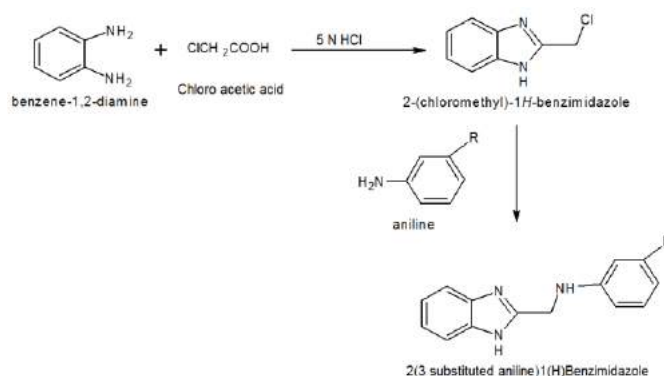


Figure 3. Scheme of the Experiment

### 1<sup>st</sup> step-Procedure-

In a 250ml three necked flask a solution containing 3gm of chloroacetic acid and 3gm of O-phenylenediamine dissolved in a 30ml of 5N HCL. The mixture was heated for 35 min. on 7<sup>th</sup> power with constant stirring in microwave. The reaction mixture is cooled to about 5°c. It was neutralized with aq. Ammonium hydroxide or dil. NaOH. The product was filtered and washed with water to remove traces of chloromethyl-1 H-benzimidazole derivatives by using different aromatic amines and heterocyclic and to evaluate them for antibacterial activity.

### Scheme-1

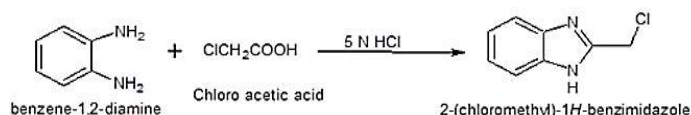


Figure 4. 1<sup>st</sup> step

### Scheme-2

#### 2<sup>nd</sup> step- procedure

General procedure for synthesis of 1-H-benzimidazole 2-yl-methyl-amine derivatives.

In the ethanolic KOH solution 2-chloromethyl benzimidazole and substituted anilines were added and it was heated for 35 min. on 7<sup>th</sup> power in microwave. Hot mixture was poured in crushed ice with constant stirring. Separated solid was filtered, dried and recrystallized from ethanol. The yields ranged from 30-45%.

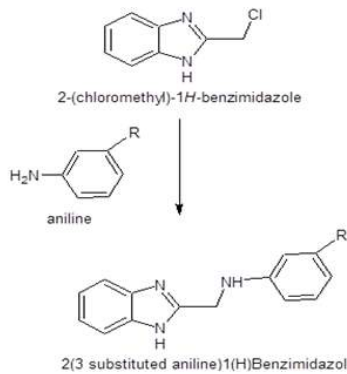


Figure 5. 2<sup>nd</sup> step- procedure

Physiochemical data of intermediate and derivatives (1A, 1B, 1C):

Sr. no.	Comp. code/name	R	Theoretical yield	Practical yield	% yield	Melting point	RF value	Mol. wt
1	Intermediate	R	3.54gm	2.2gm	62.14%	153-155°C	0.66	166
2	1A)	-N=N-C <sub>6</sub> H <sub>5</sub>	3.93gm	2.80gm	71.24%	148-150°C	0.54	327
3	1B)	-Br	1.81gm	1.42gm	77.34%	152-154°C	0.76	302
4	1C)	-O-CH <sub>3</sub>	1.46gm	1.10gm	75.34%	150-154°C	0.61	122

Table 1. Physiochemical data of intermediate and derivatives

I.R of compound 1A N(1-H-benzimidazole-2ylmethyl)3-phenyldiazonyl aniline:

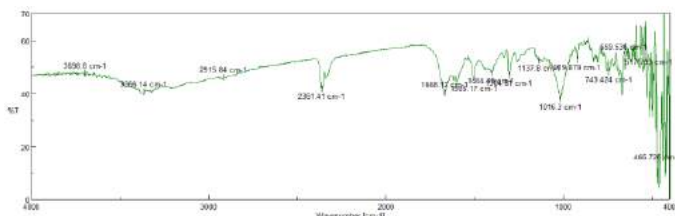


Figure 6. I. R. spectrum of comp. 1A

Sr.no.	Functional Group	Peak
1	N=N	1505
2	C=C	1668
3	NH	3366
4	C=N	1668
5	Phenol	1404

Table 2. I.R spectral data of comp. 1 A

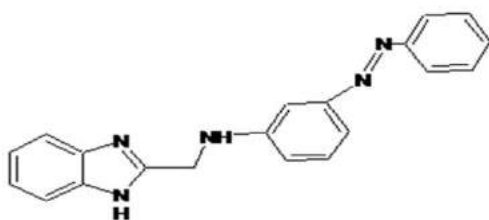


Figure 7. Structure of comp. 1A

I.R of comp. N(1-H-benzimidazole 2ylmethyl) 3-bromo aniline.

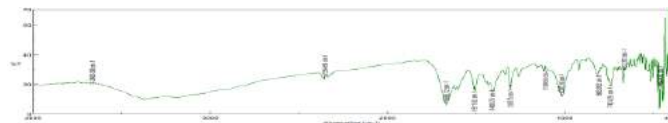


Figure 8. I.R. spectrum data of compound 1B

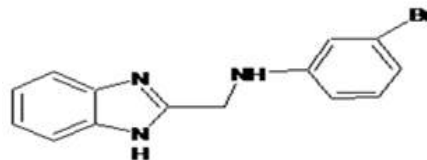


Figure 9. Structure of compound 1B

Sr.no.	Functional Group	Peak
1	C-Br	600-500
2	CH <sub>2</sub>	1408
3	C=C	1511
4	C=N	1408
5	C-N	1109

Table 3. I. R. spectral data of compound 1B

I. R. of comp. N(1-H-benzimidazole-2ylmethyl) 3-methoxy aniline:

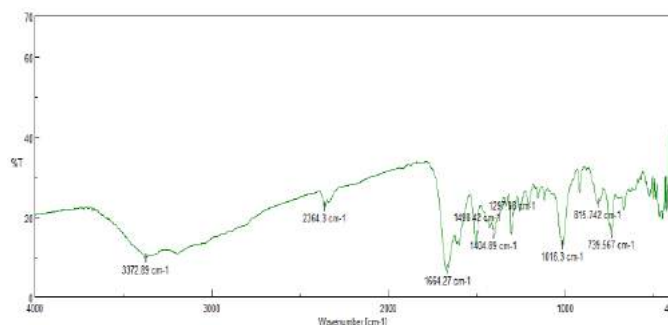


Figure 10. I. R. spectrum data of comp. 1C

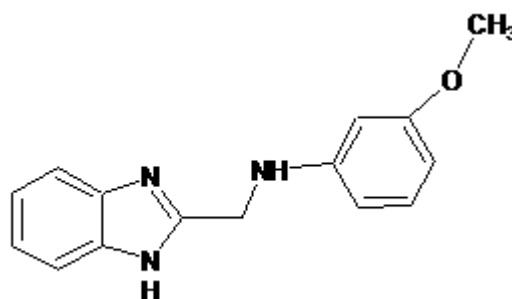


Figure 11. Structure of compound 1C

Sr.no.	Functional Group	Ranges
1	C=C	1498
2	N-H	3372
3	CH <sub>2</sub> str.	1498
4	C=N	1664

## II. METHODS AND MATERIAL

### A. Chemicals

All chemicals and solvents were procured from commercial sources, purified and sterilized using standard procedures from literature whenever required. Nutrient agar medium (Research lab, Mumbai)

### B. Dilution of the compounds

All the synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) so as to get concentration of 200 µg/ml and standard drugs Ciprofloxacin in DMSO as a concentration of 10 mg/ml.

### C. Preparation of nutrient agar medium slant:

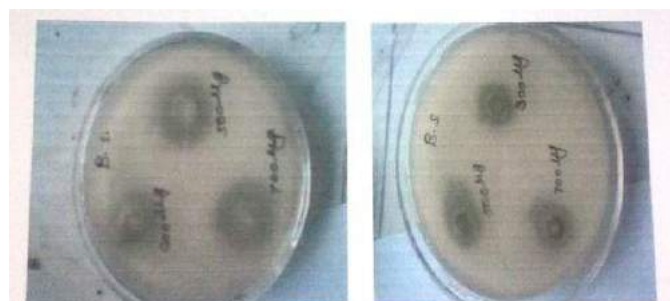
Nutrient agar medium 112 mg and agar powder 100 mg was dissolved in 4 ml distilled water, boiled and then poured in the test tube then plugged with cotton and sterilized in autoclave at 15 lbs pressure (121 °C) for 15 min. after sterilization the tubes containing the nutrient agar medium were kept in inclined position for 30 min. then on the surface of slants pure culture of bacillus Substiles, Escherichia coli were streaked in aseptic condition and incubated at 37 °C for 24 hrs.

Antimicrobial Drug sensitivity Tests : Antimicrobial sensitivity test have been carried out by using disc-diffusion method, performed in nutrient agar for bacterial and saboraud's agar for fungi. Inoculation of suspension of bacteria and fungi on culture media: Sterile, non-toxic cotton swab were dipped in to the standardized inoculums (turbidity as adjusted as to obtained confluent growth on the Petri plate) and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between streaking. Then the streaked inoculums were allowed to dry for 5-15 mins with lid in place.



**Figure 12.** Inoculation of suspension of bacteria and fungi on culture media

Sterile paper disc made by punching whatman (No.41) paper were dipped separately in to the solutions containing synthesized drug (300 µg/ml of DMSO) and standard drug ciprofloxacin (10 mg/ml of DMSO.) & Flucanazole (10 mg/ml of DMSO) in aseptic condition with help of sterile forceps and were then placed on the surface of inoculated culture media after which the plates were kept in refrigeration for 30 mins. For the diffusion of the drug from the paper disc in to the culture media. After 30 mins the plates were incubated at 37 °C.



**Figure 13.** Standard (ciprofloxacin) derivative (1A)

SR. NO.	COMP. NO	NAME OF ORGANISM	
		E. Coli	B.Subtilis
1	1A	++	++
2	Ciprofloxacin	+++	+++

**Table 5.** Zone of inhibition

Below 6 mm it shows negative activity (-)  
 Between 6 mm -9 mm (and) sign. (Slight activity)  
 In between 9 mm – 12 mm (++) sign. (Moderate activity)  
 In between 12 mm-16mm (+++) sign. (Higher activity)

### III. RESULT AND DISCUSSION

The antibacterial activities of synthesized (1A) was carried out by using disc diffusion method and screened against *Bacillus subtilis*, *E. Coli* microorganism using standard ciprofloxacin (300µg/ml) and derivative compound 300,500,700 per ml.

#### Discussion

Novel compounds 1A, 1B and 1C were found to show antibacterial activity when checked with Ciprofloxacin as standard. Compound 1A showed moderate antibacterial activity against Gram positive, *Bacillus subtilis*, while higher activity against Gram negative (*Escherichia-coli*).

### IV. CONCLUSION

Evaluation of the novel compounds established that some of the synthesized comp. N (1-H-benzimidazole-2ylmethyl) 3-phenyldiazonyl aniline, N (1-H-benzimidazole-2ylmethyl)3-bromoaniline, N (1-H-benzimidazol-2ylmethyl) 3-methoxy aniline. Showed antibacterial activity which was not found to be less than that of ciprofloxacin in case of Gram positive (*Bacillus subtilis*) while moderate activity against Gram negative (*E.coli*).

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# Opinion Classification from Online Reviews based on Support Vector Machine

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## ABSTRACT

With the rapid growth of internet, a huge number of product reviews are evolved up on the Web. From these reviews, customers can get direct assessments of product information and direct supervision of their purchase actions. Meanwhile, manufacturers can obtain immediate advice and opportunities to improve the quality of their products in a timely fashion. Any customers who buy a product can place their opinion about product features. New customers who want to buy a product he reads the reviews of the previous customers. So reviews are helps to new customers to decide to buy a product or not. Product manufacturer are also use the reviews of customer for product development. Opinions of customers are sentiments about product. Opinions given by customers are not in specific format and it does not have any syntax to define it. Reviews may be in single word or may be in one sentence or it may be in multiline format. And every customer has different opinions about product so that new customer need to read all the reviews this process is difficult and time consuming process. Here we have implement a system for online reviews classification based on polarity by using support vector machine and provide a review based rating system. From these online reviews we also find opinion target and opinion word using word alignment model and shows the topical relation.

**Keywords:** Feature Extraction, Opinion target and word, Text Classification

## I. INTRODUCTION

Now a day's due to the rapid growth of ecommerce and increase in online merchants online shopping dramatically increases. Due to the number of online merchant sites large number of user's increases. This number of customers which purchase online products increases rapidly. To improve the customer fulfillment, product manufacturers and merchants allow customers to review or express their feelings or opinion about the product or services. The customers can now place a review for products at merchant sites. These online customer reviews, thereafter, become an important source of information which can be useful for both potential customers and product manufacturers. New customers will use this information to support their decision on whether to purchase the product or not. For product manufacturer customers feedback is more important and

valuable for product development and marketing. Since feedbacks of customer affects decision of other customer's decision, the review documents have become an important source of information for business organizations and useful to take development plans. Opinion Mining deals with the broad area of natural language processing and text mining involving the computational study of opinions, sentiments and emotions expressed in text. Hence, an alternate term for Opinion Mining is also called as Sentiment Analysis. Opinion mining has many application including science and technology, entertainment, education, politics, marketing, accounting, law, research and development. But with the tremendous growth of the World Wide Web, has large number of text opinions in the form of blogs, reviews, discussion groups are available for analysis making the World Wide Web the fastest, most broad and easily accessible medium for sentiment analysis.

## II. METHODS AND MATERIAL

### A. Literature Review

1. According to **Hu and Liu, Ding et al., Li et al**[1],[2], opinion target extraction can be divided into two main categories: supervised and unsupervised methods. In supervised approaches, the opinion target extraction task was usually regarded as a sequence labeling task where several classical methods are used such as CRFs.
2. According to **Li, Jin, Huang, Ma and Wan Wu et al**[3], [4], et al., the main limitation of supervised and unsupervised methods is that labeling training data for each domain is time consuming and impracticable. In unsupervised methods, approaches regarded opinion words as the important indicators for opinion targets.
3. **Hu, Liu et al** [5], exploited an association (Nearest Neighbor rule) mining rule algorithm and frequency information to extract relation among words.
4. **Qiu et al** [6], proposed a Double Propagation method to expand sentiment words and opinion targets iteratively, where they also exploited syntactic relations between words. The main limitation of Qiu's method is that the patterns based on dependency parsing tree may introduce many noises for the large corpora. Besides the patterns used in Qiu's method, they adopted some other special designed patterns to increase recall.
5. **Lui et al**[5], employed word alignment model to capture opinion relations rather than syntactic parsing. These alignment methods are more effective than syntax based approaches for online informal texts.
6. The Naive Bayes algorithm [8] is generally used algorithm for document classification. Naive Bayes is a simple and effective classification algorithm. The basic idea is to find probabilities of categories for given a test document by using the joint probabilities of words and categories. The naïve part of model is the assumption of word independence. The simplicity makes the computation of Naive Bayes classifier far more efficient.
7. The K-nearest neighbor (KNN) [9] is example based classifier that does not build an explicit, declarative representation of the category. For given a test document  $d$ , then among training

documents system finds the  $k$  nearest neighbors. The resemblance score of each nearest neighbor document to the test document is used as the weight of the classes of the neighbor document (**Songho tan**, 2008). Besides this classifier other classifiers like ID3 and C5 are also investigated (**Rudy Prabowo**, 2009).

8. **Rui Xia, Ziqiong, Songho tan, Rudy Prabowo**, [7] [8] et.al. proposed a Support vector machines (SVM). SVM is a discriminative classifier is considered the best text classification method. The support vector machine is a statistical classification method. SVM seeks a decision surface to separate the training data points into two classes and makes decisions based on the support vectors that are selected as the only effective elements in the training set. Multiple variants of SVM have been developed in which Multi class SVM is used for Sentiment.

### B. Proposed Work

Opinion mining is viewpoint of text mining. To conclude some decision from the reviews or the feedbacks retrieved from the user customers opinions are generally taken to collect the thoughts and various aspects of the product that also include the technical details. Once the opinions are collected the filtration over reviews are performed to identify most relevant information in the form of opinion class or sentiment class.

The proposed work has following objectives

- ✓ To design a system to perform feature extraction from user reviews and relevant feature selection from multi sentence reviews.
- ✓ To implement opinion classification based on polarity.
- ✓ To implement rating system based on data which is classified in different polarities.
- ✓ To design and implement a system which construct topical relation between sentences of reviews

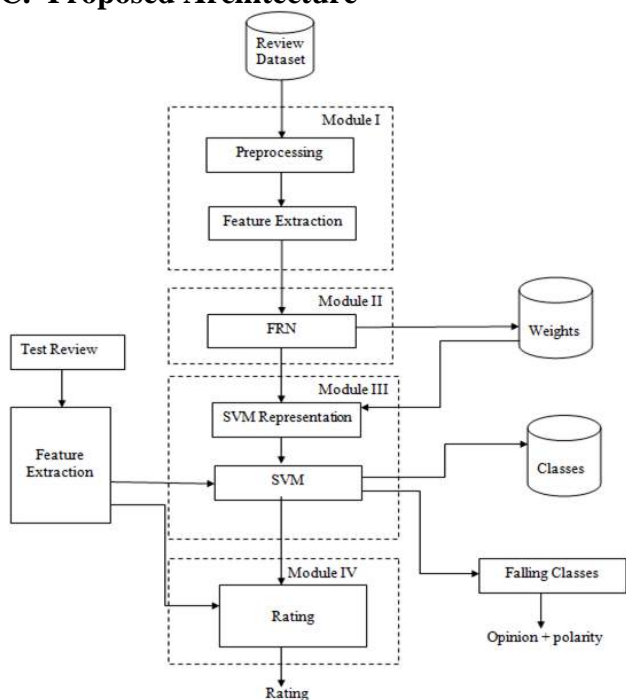
Following techniques are used for proposed work

- ✓ Feature Relational Network (FRN) algorithm can be used to for feature extraction. FRN is based on N-gram technique which is used for separating

text in different grams such as N-char or N-words. These N-grams are used for feature extraction and to find association that will be used in classification further.

- ✓ The Support Vector Machine is discriminative (SVM) classifier used for text classification. SVM is based on decision planes that define decision boundaries. Decision plane separates set of objects having different class membership. When SVM is used with FRN it gives good performance
- ✓ Calculating the rating for product based on the reviews.
- ✓ The Word alignment model used to find the opinion target and words in the reviews. We use here phrase based technique which use noun verb phrases in the review.

### C. Proposed Architecture



**Figure 1:** System Architecture

The proposed architecture consists of four modules.

#### 1. Feature Extraction Module

Feature Extraction Module take review dataset as input. The dataset contains the product reviews which are given by customer on merchant’s site. This review dataset is used for preprocessing to remove noisy, erroneous, and inconsistent data. This preprocessed data is then used for feature extraction. All text features are extracted using n-gram feature set. The n-gram feature set using character level n-gram, word level n-gram, and part of speech tag n-grams and so

on. Character N-gram based on set of characters. Word level N-gram is based on group of words. It extracts all the feature text from reviews. Suppose the sentence is like “I love this smart phone”

N-gram Category	Examples
1-char	I, l, o, v, e, t, h, i, s, s, m, a, r, t, p, h, o, n, e
2-char	Il, ov, et, ,hi, ss ma, rt, ph
1-word	I, love, this, smart, phone
2-word	I love, this smart

**Table 1 :** N-gram Feature set

#### 2. Feature Selection Module

Text features selected by Feature Extraction Module are used by Feature Relational Network (FRN). FRN algorithm is used to select text feature considering relevance between features and redundancy factors. It uses a rule based multivariate text feature selection method that considers semantic information and syntactic relationship between n-grams features in order to efficiently remove redundant and irrelevant features. FRN utilizes subsumption relation which enables intelligent comparison between features to facilitate removal of redundant and irrelevant features. FRN also assigns numeric values called as weight to the features.

Feature group	Subsumption Relation
N-Char	1-char -> 2-char, 1-char -> 3-char, 2-char -> 3-char,
N-Word	1-word->2-word, 1-word->3-word, 2-word->3-word

**Table 2 :** Subsumption relation

#### 3. Feature Classification Module

Features are classified by using Support Vector Machine (SVM) classifier SVM is discriminative classifier and it is the best text classifier. It is a statistical classification method. SVM separates decisions surface into separate training data points into classes and perform classification based on Support vector. Reviews are in the form of text so

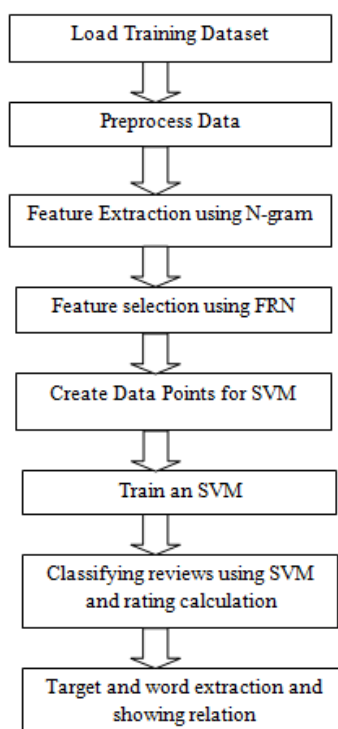


reviews need to be converting in SVM representation to perform classification. Each review is then converted into numeric value names as data points. Each review is considered as four dimensional points. Using numeric representation of review text SVM perform classification on input data. Features are placed in different classes depends on polarity.

#### 4. Rating Module

Rating module used to calculate the rate for test review and its following class of review. This module also used to calculate the overall rating of the product from all reviews.

#### D. Implementaion Steps



**Figure 2:** Implementation Steps

The implementation steps are as follows:

Step 1: In first step we load the dataset which is used for training. The data set is in the json format we first extract and then transform it mysql database for easy access and then extract only reviews in single file.

Step 2: In step 2 preprocessing is done on the reviews from file to remove stopwords.

Step 3: Feature extraction is done using n-gram in step 3.

Step 4: Feature Selection is performed by using feature relational network.

Step 5: In step 5 SVM data points are calculated which are used for SVM training. These data points are numeric values on which SVM is works.

Step 6: By using SVM data points SVM is trained in step 6.

Step 7: Test on single and multiple reviews are performed and ratings are calculated.

Step 8: In last step opinion words and targets are calculated and we show the relation between them graphically.

#### E. Scope

Proposed work is based on opinion mining and text classification. Classification is done on based on polarity.

Using N-gram feature set we find the features from text review. N is any positive number used in feature extraction. Here we use value of N is 3 because to find feature relational network it requires the same value.

Feature relational network (FRN) finds the relation between features and association between them.

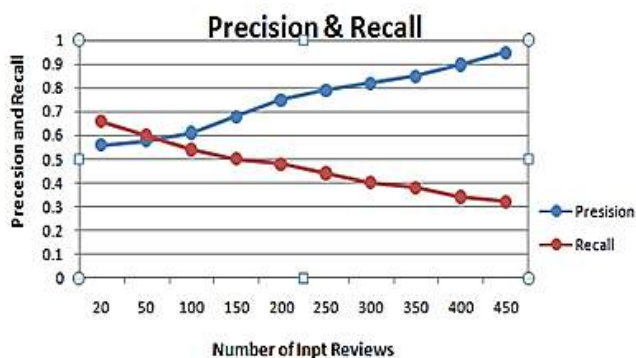
Support Vector Machine is classifier which classifies text in classes. Classes need to be predefined. It works on numeric data so here needs to convert text into numeric value and based on these values SVM is trained.

Word alignment model finds the opinion words and opinion targets from reviews. Target is entity on which opinion is given and opinion word is opinion about the product or target.

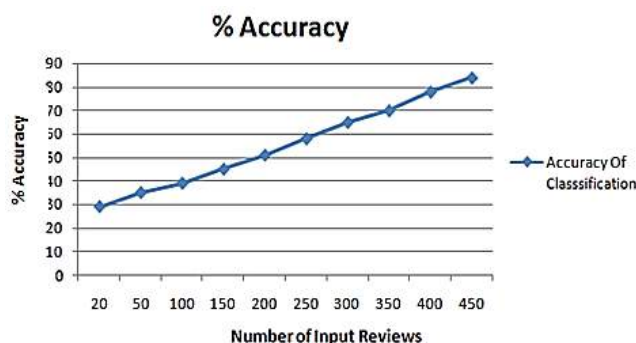
### III. RESULTS AND DISCUSSION

The experiment for the classification on data is performed by SVM algorithm carried out on review dataset. The approach proposed for text review data and classifies the review in positive, negative and neutral classes. The result analysis is done on the

classification for accuracy factor. The accuracy is calculated by calculating precision and recall for the input data. In graph following graphs horizontal axis represent number of input reviews to system and vertical axis represent precision and recall of review classification for first graph and percentage accuracy of classification for second graph. The graph 1 shows that for less number of reviews precision is less than the recall. After gradually increasing the number of input reviews precision gets increases and recall decreases. Graph 2 is for accuracy which is based on precision. Accuracy increases as the number of records increases.



**Graph 1:** Precision and recall for proposed system (Classification)



**Graph 1:** Accuracy of Classification

#### IV.CONCLUSION

In the proposed work classification of online reviews are and rating calculation is performed. In proposed framework is combination of feature extraction from reviews by feature relational network and classification of these reviews based on support vector machine. Feature relational network allows finding relevant feature by using N-gram technique and selecting the features by using FRN. Support vector machine takes review text as input and classify them to the different classes like positive, negative or neutral depending on the training pattern. Here we

also perform a rating of the product based on the review. The proposed system gives classes of reviews with rating which is useful for the user for to take decision about purchasing product and they can easily supervise the product.

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# Correlation Analysis of Irrigation Water Quality parameter from the major rivers in the Amansie West District, Ashanti Region, Ghana

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## ABSTRACT

A research was conducted at Amansie West District in the Ashanti Region of Ghana to establish the correlation between irrigation water quality parameters from the major rivers and their tributaries. Data collected were subjected to statistical analysis SPSS version 20. Pearson correlation coefficient was estimated to test the degree of relationship between irrigation water quality parameters. There was positive correlation between SAR and Ca, Mg, K, Na, EC<sub>w</sub>, and TDS except the ratio of Ca: Mg which showed negative correlation. Analysis of the correlation between SAR and HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> is highly recommended.

**Keywords:** Irrigation, Water Quality, Correlation, Rivers

## I. INTRODUCTION

The mining industry is the backbone of many economics in the developing countries. Its recovery in Ghana since 1989 was compelled by the global paradigm which emphasizes private sector-led development as the engine of economic growth in developing countries. The historical importance of mining in the economic development of Ghana is evident in the country's colonial name, Gold Coast (Akabzaa T. and Darimani A., 2001).

The mining industry of Ghana accounts for 5% of the country's GDP and minerals make up 37% of total exports, of which gold contributes over 90% of the total mineral exports. Export earnings from minerals averaged 35%, and the sector is one of the largest contributors to Government revenues through the payment of mineral royalties, employee income taxes, and corporate taxes and employment generation. The mining industry therefore remains critical to Ghana's socio-economic growth and development. (Ghana Chamber of Mines, 2008).

Small and large-scale mining operations are inherently affecting the environment, producing enormous quantities of waste that can have negative impacts for decades (UNEP, 1997). Mining activities that affect water quality include the disposal of waste rock, tailings deposition, and effluent discharges from different stages of mineral processing (Dock, 2005). According to Ripley (1996) effluent released from gold mines is made up of heavy metals mainly from pyrite (FeS<sub>2</sub>) and chalcopyrite (CuFeS<sub>2</sub>). Koryak (1997) argues that the effluent produced from waste rock dumps has a potential of causing acid mine drainage (AMD) in stream and river waters.

An important chemical parameter for assessing the degree of suitability of water for irrigation is sodium content, which is expressed as the sodium adsorption ratio (SAR) measures the potential dangers posed by excessive sodium in irrigation water (Alagbe, 2006). When residual sodium carbonate (RSC) is positive, calcium is lost from the soil solution and increases SAR in the soil solution, thereby increasing the sodium hazard (Hopkins et al, 2007).

The quality of freshwater is mainly affected by natural processes such as weathering and soil erosion as well as anthropogenic activities. The anthropogenic activity represents a constant polluting source whereas surface runoff is a seasonal phenomenon, mainly affected by climatic conditions (Singh et al., 2004). Water quality monitoring has a high priority for the determination of current conditions and long term trends for effective management.

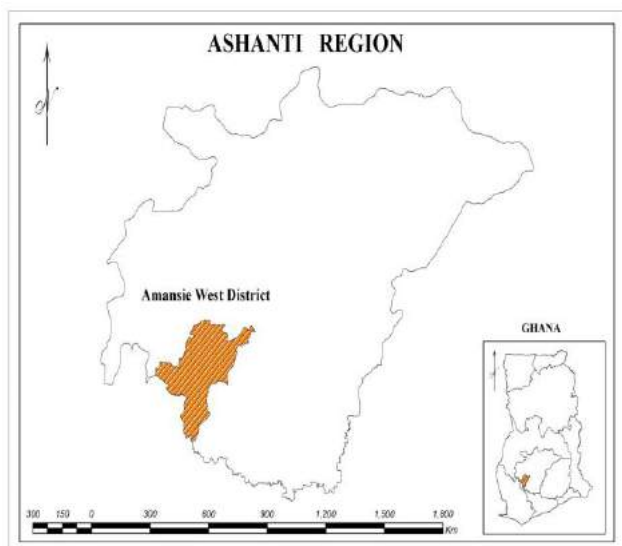
The purpose of the study was to use correlation analysis in assessing irrigation water quality parameters of water from the major rivers and their tributaries in the Amansie west district.

## II. METHODS AND MATERIAL

### A. Description of the study area

Amansie West District is located in the Ashanti Region of Ghana, and it is an area where Galamsey activities (illegal mining) are very rampant (Figure 1).

Rivers served as a source of drinking water for the local people living in the area and currently serves as irrigation water for the farmers in the area especially during dry season and erratic weather condition.



**Figure 1:** Map of Ashanti Region showing the location of the study area

The climate is characterized by a dry season between December and February, highest rainfall in June (major

season), and a cooler and drier period in August with smaller rainfall in October (minor season).

The average monthly temperature ranges from 23.2° C in August to 26.8° C in February, and average monthly humidity range from 84.7% in August to 75.3% in January (Quansah et al., 2016).

The data used for the correlation analysis was obtained from the research previously done by Quansah et al, 2016. These data were used to estimate the Sodium Adsorption Ratio (SAR) and the ratio of calcium to magnesium.

### B. Statistical analysis

Data collected were exposed to statistical analysis SPSS version 20. Pearson correlation coefficient was evaluated to test the degree of relationship between irrigation water quality parameters. This was calculated using the relation:

$$r = \frac{n \sum xy - \sum x \sum y}{\sqrt{[n \sum x^2 - (\sum x)^2] * [n \sum y^2 - (\sum y)^2]}}$$

According to Pallant (2011, p. 134), a correlation coefficient can be described as: small correlation  $-0.10 \leq r \leq 0.29$ , medium correlation  $-0.30 \leq r \leq 0.49$  and large correlation  $-0.50 \leq r \leq 1.0$ . The positive and the negative point to the direction of the relationship, where the positive indicates an increase in one variable associated with an increase in the other, whilst the negative correlation means an increase in one variable related to a decrease in the other. The coefficient of determination which explains the changes in one variable as explained by the changes in the other variable ( $r^2$ ) was calculated.

## III. RESULTS AND DISCUSSION

Tables 1 and 2 show average values of the irrigation water quality parameters and the correlation between these parameters from major rivers and their tributaries in the Amansie West District in the Ashanti Region of Ghana.

The range of pH in the samples is from 5.8 to 6.9 with mean of 6.5 which is moderately acidic and suitable for irrigation purpose according to Chiroma et al., 2014. (Table1). Temperature, electrical conductivity ( $EC_w$ ) and

Total dissolve solids recode maximum of 24.30°C, 184.70µscm<sup>-1</sup> and 92.50mg/l with mean of 24.09°C, 121.1 µscm<sup>-1</sup> and 60.67mg/l.

### A. Correlation analysis

From the Pearson correlation coefficient SAR showed positive correlation with Ca (+0.126), EC<sub>w</sub> (+0.025), K (+0.771), Mg (+0.100) and Na (+0.872), but the correlation is very strong in K<sup>+</sup> and Na<sup>+</sup> indication that an increase in Na<sup>+</sup> and K<sup>+</sup> lead to increase in SAR. This is so because SAR is determined using Na<sup>+</sup>. This is expected for Na<sup>+</sup> since an increase in SAR indicate high availability of Na<sup>+</sup> in the irrigation water and hence in soils. A high SAR value implies a hazard of sodium (alkali) replacing Ca<sup>2+</sup> and Mg<sup>2+</sup> in the soil through a cation exchange process that damages soil structure, mainly permeability, and which ultimately affects the fertility status of the soil and reduces crop yield (Gupta, 2005).

SAR also showed weak negative correlation with Ca:Mg ratio and this could be explained by the fact that according to Michael, 1992, the bonding energy of Mg<sup>2+</sup> is less than that of Ca<sup>2+</sup>, allowing more Na<sup>+</sup> adsorption and this can be seen clearly when the ratio exceeds 4.0. The presence of high Na<sup>+</sup> in irrigation water promotes soil dispersion and structure breakdown when Na<sup>+</sup> to Ca<sup>2+</sup> ratio exceeds 3.1(Michael, 1992). This also results in severe water infiltration problems, mainly due to insufficient Ca<sup>2+</sup> to suppress the dispersing effect of Na<sup>+</sup> (Ayers and Westcot, 1985). Excessive Na<sup>+</sup> also creates problems in crop water uptake, poor seedling emergence, lack of aeration, plant and root disease (Ayers and Westcot, 1985).

TABLE I  
MEAN VALUES OF THE IRRIGATION WATER QUALITY PARAMETERS

Sample	pH	Temp	EC <sub>w</sub>	TDS	Ca
		°C	µscm <sup>-1</sup>	mg/l	
Mean	6.451	24.09	121.1	60.67	6.77
Range	5.8-6.9	23.90-24.30	72.60-184.7	36.30-92.50	3.20-12.82

TABLE II  
MEAN VALUES OF THE IRRIGATION WATER QUALITY PARAMETERS (CONTINUATION)

Sample	Mg	Na	K	Ca:Mg	SAR
	mg/l				
Mean	4.75	0.04	0.06	0.75	0.002
Range	1.46-7.78	0.01-0.07	0.010-0.013	0.30-1.33	0.001-0.004

TABLE III  
CORRELATION COEFFICIENTS AMONG SELECTED WATER QUALITY PARAMETERS

Variates	Ca	Ca:Mg	EC <sub>w</sub>	K
Ca	-			
Ca:Mg	-0.3796	-		
EC	0.7152	0.1275	-	
K	0.5157	0.0814	0.5172	-
Mg	0.5047	0.5538	0.8011	0.5779
Na	0.3955	0.0457	0.4214	0.9414
SAR	0.1256	-0.0682	0.0248	0.7705
TDS	0.7134	0.1286	1.0000	0.5160

TABLE IV  
CORRELATION COEFFICIENTS AMONG SELECTED WATER QUALITY PARAMETERS (CONTINUATION)

Variates	Mg	Na	SAR	TDS
Ca				
Ca:Mg				
EC				
K				
Mg	-			
Na	0.4754	-		
SAR	0.0998	0.8716	-	
TDS	0.8013	0.4205	0.0233	
	4.75	0.04	0.06	0.75
	1.46-7.78	0.01-0.07	0.010-0.013	0.30-1.33
				0.001-0.004

### IV. CONCLUSION

The quality of irrigation water available to farmers and other irrigators has considerable impact on what plant can be successfully grown, the productivity of these plants, water infiltration and other soil physical

conditions. It is obvious that the quality are within the permissible limits for irrigation water and therefore suitable for irrigation purpose. SAR showed positive correlation with all the parameters except the ratio of Ca to Mg.

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# The Technical Teacher, Teaching and Technology: Grappling with the Internationalization of Education in Nigeria

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## ABSTRACT

This paper discussed the challenges faced by the technical teacher in the light of heightened societal expectations. The identity and functions of the technical teacher is changing with respect to new trade areas and skills occasioned by new technologists, objectives of education, new policy thrust tilting towards entrepreneurship as well as the issues of internalization of education. These issues are discussed in details using relevant subheadings. It is concluded that the technical teacher, then, needs to be knowledgeable himself, improving on his skills, knowledge and experience for him to be able to impart new and relevant skills to the students. Until the education of the teacher is taken as paramount as that of the student, no student will ever be better than his/her teacher. It was recommended amongst others that the ministry of education should engage in periodic training of technical teachers on new trends in their chosen fields, to improve their knowledge of their chosen professions and the ministry of education should consider credit transfer system for the west African sub region, where a student can start a technical course in Nigeria and finish in any other participating country in west Africa.

**Keywords :** Education; Quality of teaching; Technical Teacher; Technology; Globalization

## I. INTRODUCTION

The question of what knowledge, attitudes, behaviours and skills teachers should possess has been a longstanding issue of debate. This is simply because teachers are saddled with the responsibility of developing students through the provision of information, advice, wisdom and facilitating learners' acquisition of the key knowledge, attitudes and behaviours that they will need to be active participants in society. However, with heightened societal expectations from education and the challenge of internalization of education, upon the dynamic role of technology in shaping educational outcomes, the identity and functions of the Technical teacher as well as the environment in which they function, needs to be reviewed. Nonetheless, such reviews can only be effective only when an appraisal of current trends in the technical teaching profession is carried out. This study explores the roles of technical teachers; Issues in

teaching technology education/industrial programmes as well as the challenges of internalization of education with respect to the Nigerian context.

## II. METHODS AND MATERIAL

### A. The Changing Roles of the Technical Teacher

The technical teacher is an essential factor in the delivery of instruction to students, regardless of the mode of instruction. A teacher has professional knowledge and skills gained through formal preparation and experience. Teachers provide personal, caring service to students by diagnosing their needs and by planning, selecting and using methods and evaluation procedures designed to promote learning. The educational interests of students are best served by teachers who practice under conditions that enable them to exercise professional judgment.

Teachers have been portrayed in a number of ways in media, more often than not, with detrimental images and some, they take the face of the hero of societies, as the one who masters his/her craft and helps students excel. The effective teacher can be seen, heard and sensed. Teachers are the front-line service providers in education. Good teachers do more than teach curriculum content: they inspire and enthuse their pupils while also serving as role models, in terms of attitudes and social relationships as they model behaviours. The role of technical teachers is to promote the acquisition of technical skills as well as scientific knowledge that will help the students develop employability skills as well as become participative and responsible citizens.

Mahmood (2013) observed that teachers performance consists of teachers academic qualification, quality of teacher training, teaching experiences, pedagogical practices, professional development, mentoring, coaching, subject knowledge, dedication, commitment level and classroom management abilities. The demands made on teachers, school leaders, and teacher educators are increasing and changing. They are called on to play a key role in modernising education. The challenges facing vocational education and technical training systems in developing countries and transition economies like Nigeria and indeed, Africa are immense. They must equip learners with new skills and competencies and educational institutions must do all of this for more learners with different backgrounds, experiences, levels of motivation and preferences. According to Harden and Crosby(2000), implicit in the widely accepted and far reaching changes in education is the changing role for the teacher. twelve roles for the teacher have been identified in this regard and these can be grouped into six areas in the model presented; The Information Provider; the role model on the job and in more formal teaching settings; the facilitator as a mentor and learning facilitator; the student assessor and curriculum evaluator; the curriculum and course planner; the resource material creator and study guide producer. The teacher is the yardstick that measures all the achievements and aspirations of the society. The worth and potentials of country get evaluated in and through the work of the teacher. The people of a country are enlarged versions of their teacher and they are the real nation builders. There should therefore be adequate quality provision for effective learning to occur. This

requires taking measures to including increase in teacher employment and improvement in the quality and status of the teaching force.

A key element of education in Nigeria is the Technical Education, aimed at developing manpower for the nation's economy. This is done through occupational training in specific trades by mostly Technical colleges and Polytechnics across Nigeria. Education for employability and self-reliance has been gaining momentum amongst policy makers and the policy thrust in recent times has been towards entrepreneurship education through vocational and technical education training. The burden of transformation rests on the training institutions and the teachers, to turn policies into practice, implement educational reforms while also bringing learning closer to the students of today, by introducing technology and student centred approaches to instruction.

## **B. The Technical Teacher and the Quality of Teaching in the Knowledge Economy**

In its broadest sense, teaching is a process that facilitates learning. Teaching is the specialized application of knowledge, skills and attributes designed to provide unique service to meet the educational needs of the individual and of society. The choice of learning activities whereby the goals of education are realized in the school is the responsibility of the teaching profession. In addition to providing students with learning opportunities to meet curriculum outcomes, teaching emphasizes the development of values and guides students in their social relationships. Teachers employ practices that develop positive self-concept in students. Although the work of teachers typically takes place in a classroom setting, the direct interaction between teacher and student is the single most important element in teaching.

What society expects of teachers depends in large part on what it wants from education per time. National educational policies have been marked by an increasing concern for quality and relevance, especially in practical skills acquisition. Education systems in Nigeria, it is widely felt, are not performing effectively, not doing what they should be doing to ensure that the young



people passing through them learn well what they are supposed to learn and are well prepared to assume their future adult roles and responsibilities in the family, the workplace and the wider community and society. In many countries in Africa, Nigeria inclusive, there is a growing sense that education is the key to the future and that the challenges and jobs of tomorrow will require an education of better quality than that which most students receive today.

Increasingly, educational quality and relevance are defined by reference to students' learning outcomes. Yet if learning is to improve, teaching will need to contribute to that improvement. The importance of the quality of teaching, and therefore of teachers, cannot be overemphasized (Asia-Pacific Centre of Educational Innovation for Development, 1996).

Traditional educational systems, in which the teacher is the sole source of knowledge, are ill suited to equip people to work and live in a knowledge economy. Some of the competencies such a society demands includes teamwork, problem solving, motivation for lifelong learning cannot be acquired in a learning setting in which teachers dictate facts to learners who seek to learn them only in order to be able to repeat them. A lifelong learning system must reach larger segments of the population, including people with diverse learning needs. It must be competency driven rather than age related. Within traditional institutional settings, new curricula and new teaching methods are needed. At the same time, efforts need to be made to reach learners who cannot enroll in programmes at traditional institutions. According to the World Education report of 2008, corroborated by National reports presented at the 45th session of the International Conference on Education (1996), providing people with the tools they need to function in the knowledge economy requires adoption of a new pedagogical model. This model differs from the traditional model in many ways. Teachers and trainers serve as facilitators rather than transmitters of knowledge, and more emphasis is placed on learning by doing, working on teams, and thinking creatively. According to Kirimi and Wycliffe (2013), the increased emphasis on student autonomy in education has shifted the centre of gravity away from the teacher and closer to the student. Indeed, it has become the trend to focus on the learning and the learner than on teaching and the

teacher. However, it has to be recognized that teaching and learning are closely linked and that the purpose of teaching is to enhance learning. It is important to state that the changing role of the teacher is not neglected. The role of the teacher becomes that of facilitating learning rather than primary sources of information, instruction becomes interaction in the classroom and the students assume a more active role in the teaching and learning process. The students become increasingly responsible for their learning, giving them more motivation and setting the pace for them to become successful life-long learners. The teacher in turn becomes a resource, tutor and evaluator, guiding the students in their problem solving efforts. Though the teacher instructs the students, the teacher gradually removes the support offered to the learners as instruction and interaction continues and as the learners gradually internalizes and understands the content and are able to do more on their own.

### **C. Teaching Technology Education: Integration and Professional Use of Technology**

From investigating instructor attitudes toward technology and the utilization of computers in schools, researchers concluded that the use of technology for curricular purposes is almost exclusively a function of teachers' access to that technology (Norris, 2003). Questions concerning teachers and teaching have emerged at the centre of debate over how education can make best use of the new information and communication technologies. This is a new key feature of professionalism in teaching, requiring an understanding of the pedagogical potential of technology and the ability to integrate it into teaching strategies.

The use of technology has long been the strength of Vocational/Technical education, but changing technologies and automation continue to alter the nature of work tasks in industries for engineers and technicians (Thach & Woodman, 1994), requiring employees to learn new ways to perform their jobs. The technical teachers as Educators, increasingly recognize that in order to teach creatively, teachers need to shift from traditional teaching toward approaches that help students incorporate technology into their learning. However, such a transformation of curriculum and pedagogy is a complex task for teachers who must develop new

knowledge, skills and attitudes themselves (Wiske, Sick, and Wirsig, 2001). To bring learning closer to the technology savvy students of the information society, Technical teachers need to develop technological competencies. The Teacher Technology Competencies are a set of technology standards that define proficiency in using computer technology in the classroom. The competencies consist of computer-related skills grouped into personal and professional use of technology tools and application of technology in instruction. The competencies are grouped into: basic technology operations; personal/professional use of technology; social and ethical issues and application of technology to instruction. In Basic Technology Operation, the Technical teacher must be able to demonstrate the use of a multimedia computer system with related devices in order to run programs; to access, generate, and manipulate data; and to communicate results. At this level, technical teachers need to be conversant with technological tools and their operations.

In the Personal/Professional Use of Technology Tools, Instructional staff will apply tools for enhancing their own professional growth and productivity. Technical teachers will use technology in communicating, collaborating, conducting research, and problem solving. Activities here include: specialized technical packages such as Auto CAD, electrical design softwares, building design softwares; database management skills; Word processing; Database; Spreadsheet; Graphics; Multimedia applications; Telecomputing and Teacher/administrative Applications.

The Social, Ethical, and Human Issues- the technical teacher is expected to demonstrate knowledge of equity, ethics, legal, and human issues concerning the use of computers and technology. Issues of national policy on education, technology guidelines - ethics / Acceptable Use Policy.

In the Application of Technology in Instruction - the technical teacher is expected to apply computers and related technologies to support instruction in their grade level and subject areas. Technical teachers must plan and deliver instructional units that integrate a variety of software, applications, and learning tools. Lessons developed must reflect effective grouping and assessment strategies for diverse populations.

The single most important factor in determining the success of technology in the classroom is a teacher who is comfortable with and knowledgeable about computers. Yet many teachers, especially those who entered the teaching profession before technology assumed such a pervasive role in society, have had little or no special training in computers. Even teachers who can demonstrate basic computer literacy are unlikely to be familiar with the full range of tools that technology can offer, from spreadsheets to digital graphics to instructional software.

Just as technology pervades all walks of life and almost every field of human endeavor, technology skills are becoming essential in all subject areas because the computer is now the universal vehicle for the acquisition and dissemination of information in all fields. Technology belongs throughout the entire curriculum because of its extraordinary potential for enhancing learning. The learning process becomes active instead of passive because students control their own learning: students must think about and interact with what is on the computer screen. Since computers can radically expand information access and communication, they especially benefit industrial technology students by increasing their participation in the learning process. Just as other professionals utilize specific technologies as tools to enhance their work, teachers must likewise become adept in putting technology to use as the field of educational software evolves with the various academic disciplines. Regardless of grade level or subject, technology can support teachers in numerous professional activities — first and foremost in stimulating learning in the classroom but also in simplifying their administrative duties, improving personal productivity, and advancing professional growth.

According to Muir-Herzig, (2004), Technology itself is an interactive medium for manipulating our world, so the process of acquiring the technology competencies must necessarily involve ample hands-on practice, access to a wide range of tools, and, most important, an opportunity to discover the impressive possibilities of technology. Technology integration refers to the use of a wide range of technology tools across a broad spectrum of relevant and meaningful contexts. Although it must begin with fundamental computer skills, it includes any

technology application that enhances creativity, decision making, problem solving, collaboration, and overall productivity in the learning process.

In a learning environment where technology is truly integrated and not an adjunct, students and teachers use technology tools to enhance all areas of the teaching and learning process. For example, with computers students can access, organize, and analyze a vast world of rich resources — whether downloading original source documents from the Library of Congress or taking a virtual tour of a museum. Students on different campuses can collaborate on projects, sharing and accommodating their diverse perspectives. They can participate in original research projects that put them in touch with actual researchers in the field, and they can receive electronic mentoring from noted experts around the world. Technology gives students powerful tools for communicating what they have learned, motivating them to learn more.

Effectively managing a classroom where students engage in these activities in a manner that improves academic achievement requires a teacher with expertise in the sophisticated and increasingly complex field of instructional technology. The Teacher Technology Competencies are designed to give teachers this expertise to select and use the technological resources that not only meet students' learning needs but also equip them with appropriate skills for the future.

### III. RESULT AND DISCUSSION

#### **The Technical Teacher and the Challenge of the Internationalization of Education**

According to Eugeniusz (2012), the globalisation process is a process in which the markets and economies of individual countries become more and more dependent on one another as a result of the continuous increase in the dynamics of goods, services, capital and people exchange. As the priorities of education have changed, so also have the training systems. Education, especially, vocational training systems is directly affected by the trend towards globalization. It has to be said that globalisation has contributed immensely to the growth of African economies. However, it has also brought about changes to educational goals. As a follow

up to internationalization, education has become global and national systems are becoming obsolete. Globalisation has opened the door to competition, not just for market share, but job competition, one that favours occupationally mobile workers from anywhere in the world. For technical teachers in Nigeria, whose responsibility is to train skilled personnel for industries and multinational companies operating in Nigeria, the job is made more daunting, because the teacher has to develop skills in the student that will give the student-worker the level playing field to compete for jobs with other job seekers from other countries. A technologist trained in Nigeria should be able to compete with other technologists trained elsewhere in the world for jobs in his home country and abroad. This is only possible if the technical training received in his home country is world class and relevant. Furthermore, the goals of education in the knowledge economy have changed. In the knowledge economy, the labour force is called knowledge workers, engaged in handling information as opposed to more tangible factors of production. As the world economy transforms itself from the industrial era to the knowledge economy, it is the challenge of educators and training institutions to develop workers that can produce knowledge by learning how to source and utilize information.

In traditional post-secondary institutions like Technical colleges, internationalization initiatives include creating a more international curriculum, fostering opportunities for students to study abroad, encouraging faculty (technical teachers) and student exchanges, increasing international student recruitment efforts, and exporting or importing programmes and most importantly, creating a credit transfer system for sub-Saharan region.

The globalisation process requires the school to prepare students to play future social roles, and in particular for effective functioning in a modern democratic society, with special emphasis on effective functioning on the labour market. The point is to make them able to compete for a position with other potential candidates, not only from their own country, but from any country. According to Yang (2004) the school should equip students with suitable knowledge and skills that must be perceived as understanding the reality through personal experience and emotional reactions with the surrounding world of objects and specific situations. Such knowledge

should be preceded by appropriately designed and organised learning process – learning that brings about substantial changes in the perspective of individual vision and perception of the world (O’Sullivan, 2008). Referring to and adapting the approach of Graham and Phelps (2003) to the issue of teacher’s role in the modern world, the teacher’s most important tasks include: (1) Understanding and working in a defined school system; (2) Developing skills as well as the applied strategies and methods; (3) Teaching in the context of extended existing programme; (4) Integrating theory with practice, (5) Responding to social demands and problems; (6) Creating an atmosphere that facilitates learning; (7) Working in a group as a team member; (8) Assessing and forming lifelong learning habits.

#### IV. CONCLUSION

The teacher is made to teach in environments as provided by the government. Governments on the other hand are setting lofty standards and policies, expecting teachers to implement them. While the pressure from governments and the need to reform education is being grappled with by the teacher, changing roles of work occasioned by new occupational areas created by technology as well as new frontiers created by internationalization are opening new challenges for the technical teacher. The technical teacher, then, needs to be knowledgeable in him, improving on his skills, knowledge and experience for him to be able to impart new and relevant skills to the students. Until the education of the teacher is taken as paramount as that of the student, no student will ever be better than his/her teacher. Then, it is possible, the educational system in Nigeria may just be going round in circles, without having to break free into the mainstream and join the elite educational systems in Africa.

#### V. RECOMMENDATIONS

Based on the issues discussed, the following recommendations were made

1. The ministry of education should engage in periodic training of technical teachers on new technologies and applications to enhance their technical skills.
2. The ministry of education should engage in periodic training of technical teachers on new trends in their

chosen fields, to improve their knowledge of their chosen professions.

3. The ministry of education should consider credit transfer system for the West African sub region, where a student can start a technical course in Nigeria and finish in any other participating country in West Africa.
4. The introduction of technology both as a professional tool for teachers and as an instructional tool should be promoted and supported by the ministry of education.
5. The federal ministry of education should consider a review of technical college training programmes in the light of internationalization of education.
6. The ministry of education should consider employment of new technical teachers to meet the shortfall in technical college teachers.

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# A Paper on Multisite Framework for Web page Recommendation Using Incremental Mining

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## ABSTRACT

Large amounts of information present on web due to that web user finds difficulty when surfing the web. Useful and Effective suggestions provided by Recommendation systems which help web users. User's future interest evaluate by using the learning and prediction models which learn user's behavior. Web page recommendation improves web user satisfaction and website usage. Semantic enhanced "Multisite framework for Web page Recommendation using Incremental Mining" improves web page recommendation. In which web page recommendation will be done by using multiple website in same domain. Will be give effective recommendation results given by Different sites. To identify and collect more appropriate web usage data Dynamic web page recommendations tool is generated. Related terms identified which help to generate more appropriate data. Incremental mining update web usage knowledge and updates the knowledge base.

**Keywords:** Incremental Mining, IPNAR, Semantic Network Model, Ontology, Semantic Knowledge, FWAP

## I. INTRODUCTION

Web users make poor decisions because of enormous amounts of information. Recommendation systems have proved in recent years to be a valuable means of helping web users by providing useful and effective suggestions. The core techniques in recommendation systems are the learning and prediction models which learn user's behaviour and evaluate what users would like to view in the future.

Web page will be recommended to particular user based on his current web navigation behaviour. It will be improved web user satisfaction and website usage. Knowledge bases used in web page recommendation system includes web usage knowledge domain and website knowledge domain. Web page recommendation system extracts the semantic of web pages from web pages title. It generates domain term of web pages from single site only. This can be improved by Semantic enhanced multisite framework for Web page Recommendation using Incremental Mining.

## II. METHODS AND MATERIAL

### A. Literature Review

1. Thi Thanh Sang Nguyen, Hai Yan Lu, and Jie Lu et al proposed a novel method to efficiently provide better web page recommendation through semantic enhancement by integrating the domain and Web usage knowledge of a website.
2. M.Venu Gopalacharii, Po Sammulal et al presented an effective method to integrate the domain knowledge and web usage knowledge of a website through semantics. A new model is framed to construct a semantic hierarchy of the web log data and the domain contents, which represents the integrated usage knowledge and domain knowledge.
3. Nazneen Tarannum, S.H.Rizvi, Prof. R.R. Keole et al proposed a desktop search utility which uses web usage mining process for finding term patterns in web query data which can be used for predicting the possible next pages in browsing sessions.
4. Mohammad Amir Sharif, Vijay V. Raghavan et al presented a novel hybrid recommendation system based on clustering of items using co-occurrence

information of pages and content information of pages. These two different types of clusters are used in a parametric form to get aggregated recommendations based on the available preference information of users.

5. Anuradha Veleti, T.Nagalakshmi et al proposed an incremental algorithm (IPNAR) that mines positive and negative association rules in web usage data. The incremental based algorithm incrementally update web log association rules by utilizing the metadata of old database transactions as well as old mined rules, performs single scan over the dataset.
6. Archana Godbole, Mahendra Kumar Rai et al explores the deployment of clicking pattern algorithms in a distributed grid computing environment and demonstrates its effectiveness by empirical cases. The basic task is a weblog analysis for huge, widely distributed, hypertext information repository of World Wide Web.

### B. Why Incremental Mining

Websites and the knowledge bases have been evolving with respect to time, Therefore domain and Web usage knowledge bases need to be updated with respect to time. Web usage data source is the Web log file. So for the updating domain and Web usage knowledge bases the system can take a limited segment of the log file. Because to build the Web usage knowledge base the size of the log file can be huge. So we can use incremental mining method for discovered Web usage knowledge and keeping it updated, incremental mining method can dynamically update the knowledge bases.

### C. Proposed Work

In proposed system, web page recommendation will be done by using multiple website in same domain. The system will take the log files from the websites as the input. The semantic analysis of domain terms in different sites will be give effective recommendation results. The existing system works with static Web pages. For dynamic web page recommendations advanced tools will be generated to identify and collect more appropriate web usage data than web logs. Dynamic web click stream analysis will be conducted in the data preparation stage, in which the web page will be identified as dynamic contents rather than static pages. Synonyms of the domain

terms will be identified and clustered into common topics. This would help to cluster web page in to relevant topics and there by optimize the semantic network model of web page. Websites have been evolving over time therefore the knowledge bases, including the domain and Web usage knowledge bases need to update accordingly. To keep discovered web usage knowledge up to date, incremental mining will be developed for dynamically update the knowledge base.

The proposed work has following objectives,

1. To build multisite framework for the web page recommendation.
2. To develop tools for identify and collect appropriate web usage data from dynamic web page.
3. To enhance domain knowledge discovery by using synonyms of domain terms.
4. To update web usage knowledge base by using incremental mining method.

### D. Proposed Architecture

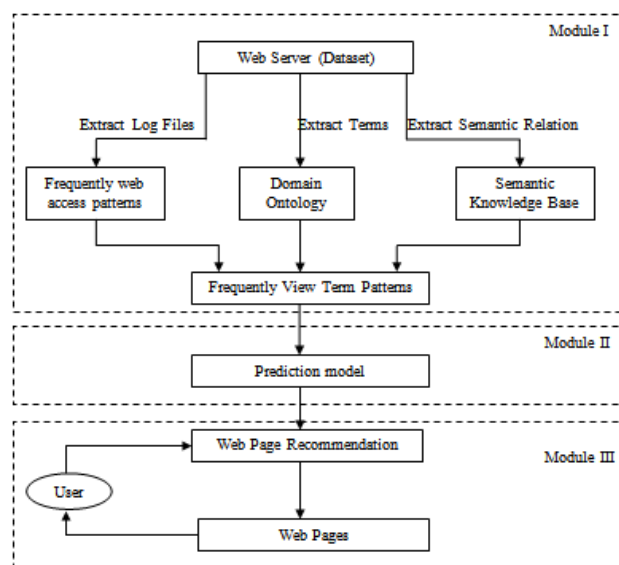


Figure 1: Architecture of Multisite Framework for Web page Recommendation Using Incremental Mining

Following terms will be used for proposed work.

#### 1. Data Collection

Frequent web access pattern will generated from web usage data by using crawler from dataset. It will give the count of term. Domain Ontology will be generated from web usage data. It will give domain knowledge

of extracted term. Symantec knowledge will be generated from web structure data. It will give association between domain term and web pages. In the dataset, users will be identified by id and web pages will be identified by their titles and URLs. The dataset is divided into two subsets, one for training and one for testing. Pre-processing step will be performed on log data to analyze the web log files and produce a list of URLs of web pages that were accessed by users. Crawler will access the current web page browsed by user and to extract the titles from web pages. Similar domain term data will be collected from multisite. Dynamic web click stream will be used to collect data dynamically

## **2. Generation of Frequent View Term Pattern**

Frequent view term pattern will be generated by extracting frequent web access pattern, domain ontology and semantic knowledge from web usage data and web structure data. Frequent web access pattern will give the count of term which is used by user frequently. It will be extracted from web usage data. Domain ontology represents domain knowledge of extracted term. Taxonomic and non-taxonomic relation of term will be found in domain ontology. Semantic Knowledge is kind of knowledge map which represents domain terms, web pages and relation of domain term. Semantic Knowledge also represents association between domain term and web pages. Symantec knowledge will be updated using clustering method where similar domain term will be used for multisite framework.

## **3. Generation of Prediction Model**

Prediction model will develop for generating weighted semantic knowledge of frequently viewed terms pattern. Synonyms of domain term will be used for enhancing domain knowledge discovery. Also to update knowledge bases dynamically incremental mining method will be used. This model will use frequent view term pattern for giving the weight to frequently viewed terms pattern. Weight is probability of the transition between two adjacent terms based on frequently viewed term patterns. Also synonyms of users domain term will be found out and weight is applied to it. Weighted semantic knowledge of frequently viewed terms will be used for semantic enhanced web page recommendation.

## **4. Web Page Recommendation**

This model will develop for web page recommendation to web user by using weighted semantic knowledge. User query will take and terms will be compared with weighted semantic knowledge of frequently viewed terms pattern. Term having high probability will be compared with users query. Web page recommendation will use high probability term for web page prediction. Frequently viewed term related with user query will be found out and most probable web page will be recommended to web user.

First part of this module will work in order to collect data. Data will be collected from data set of web server. Collection of data consists of web log files and web structure data from multisite. Pre-processing unit will analyze web log file and produce list of URLs of web pages that were accessed by user. Crawler will be used for pre-processing unit to extract the titles. Advanced tool will develop to collect more appropriate web usage data like Click stream data.

In second part, frequent web access pattern, domain ontology and semantic knowledge will be generated from collected data. Frequent web access pattern will give the count of term which is used by user frequently. Domain ontology represents domain knowledge of extracted term. Semantic Knowledge is kind of knowledge map which represents domain terms, web pages and relation of domain term. Semantic Knowledge also represents association between domain term and web pages. This module will generate frequent view term pattern by using frequent web access pattern, domain ontology and semantic knowledge.

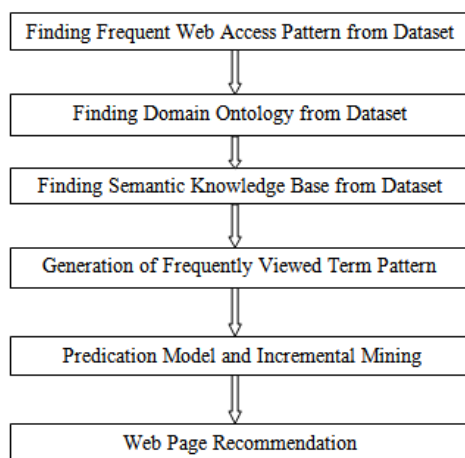
Prediction model will develop for generating weighted semantic knowledge of frequently viewed terms pattern. This model will use frequent view term pattern for giving the weight to frequently viewed terms pattern. Weight is probability of the transition between two adjacent terms based on frequently viewed term patterns. Also synonyms of users domain term will be found out and weight is applied to it. Weighted semantic knowledge of frequently viewed terms will be used for semantic enhanced web page recommendation.

In web page recommendation, users query will take as input and compare this query to frequently viewed



term. The term having most probability will be used for recommend of web page. Finally predicted web page will recommend given to user.

### E. Implementation Steps



The implementation steps are as follows:

Step I: In step I Frequent web access pattern will generated from web usage data by using crawler from dataset. It will give the count of term which is used by user frequently. Further frequent web access pattern will used in Generation of Frequently Viewed Term Pattern.

Step II: In step II Domain Ontology will be generated from web usage data. It will give domain knowledge of extracted term. Further this domain knowledge will used in Generation of Frequently Viewed Term Pattern.

Step III: In step IV Symantec knowledge will generated from web structure data. It will give association between domain term and web pages. Further this semantic will used in Generation of Frequently Viewed Term Pattern.

Step IV: In step V term generated in above step i.e. Symantec knowledge, Domain Ontology and frequent web access pattern will used to generate frequently viewed terms pattern.

Step V: In this step Prediction model will develop for generating weighted semantic knowledge of frequently viewed terms pattern. Also to update knowledge bases dynamically incremental mining method will be used.

Step VI: In this step using weighted semantic knowledge, web page will recommend to the user by web page recommendation module.

### F. SCOPE

Web page recommendation system generates domain term of web pages from single site only. The proposed framework makes web page recommendations for multiple websites in the same domain. The system should take the log files from these websites as the input.

With the advancement in Web technology, pages have been evolving in to dynamic structures. The system will works with dynamic web pages and develop advanced tools to identify and collect more appropriate web usage data than web logs such as click stream data. In proposed system dynamic web click stream analysis will be conducted in the data preparation stage.

System will make usage of synonyms of the domain terms; need to be clustered into common topics. This would help to cluster web pages into relevant topics and thereby optimize the semantic network model of web page.

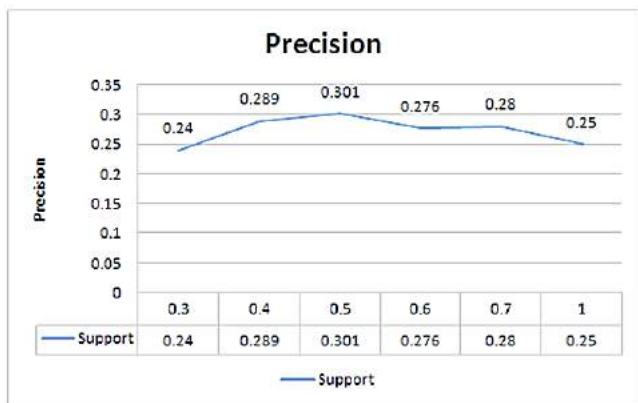
In the traditional web usage data source like web log file, the system can only take a limited segment of the log file to build the web usage knowledge base due to the fact that the size of the log file can be huge. Incremental mining method will keep the discovered web usage knowledge up to date and dynamically update the web usage knowledge bases.

## III. RESULT AND DISCUSSION

For Result and analysis we have divided our data set in to training and testing set .we use minsup as threshold value for analysis. It is minimum support value for web access sequence on Frequent Web Access Patten (FWAP).Minsup ranges from 0.3 to 1.Measure value foe evolution decided. They are Precision and Satisfaction.

**PRECISION:** -LetRc be the sub-set of R, which consists of all correct recommendation rules. The Web-page recommendation precision is defined as: Precision =|Rc|/|R| .

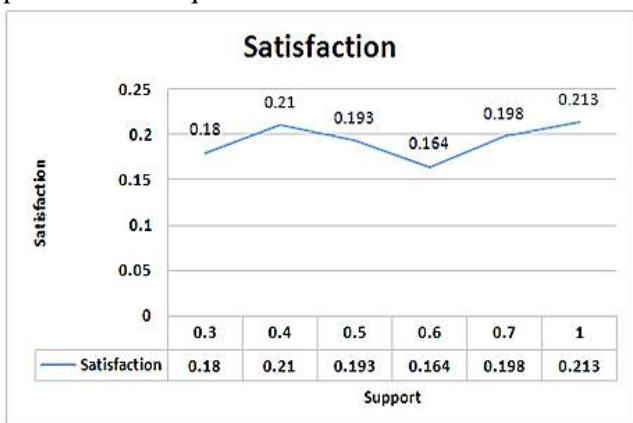
Precision specify ratio of correct recommendation against all recommendation.



**SATISFACTION:** - Let  $R_s$  be the sub-set of  $R$ , which consists of all satisfied recommendation rules. The Satisfaction for Web-page recommendation is defined as:

$$\text{Satisfaction} = \frac{|R_s|}{|R|}$$

Satisfaction specifies ratio of satisfactory recommendation against all recommendation. Result proves that with our approach results are better than previous technique.



#### IV. CONCLUSION

In proposed work three modules are proposed. Three modules has been completed, namely

1. Generation of frequent view term pattern
2. Generation of prediction model
3. Web page Recommendation

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# Discovering Frequent Item Set Mining Using Transaction Splitting

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## ABSTRACT

Frequent Item sets Mining (FIM) is the most well-known techniques to extract knowledge from dataset. Private Frequent pattern growth algorithm is proposed to gain high time efficiency using transaction splitting. It consist of two phases pre-processing phase and mining phase. In pre-processing phase long transaction are split into multiple subset and transformed database is created. In mining phase, actual support of original database and transformed database is computed.

**Keywords :** Frequent Item set Mining, Apriori Algorithm, FP-Growth Algorithm, and Private Frequent Pattern Growth Algorithm.

## I. INTRODUCTION

Frequent Item set Mining (FIM) is the most well-known techniques to extract knowledge from dataset. Frequent item set occur in a data set with frequency greater than a user-specified threshold. For example, a set of items, such as mobile phone and memory card that appear frequently together in a transaction data set is a frequent item set. The existing system has problem of large time complexity and privacy threats. The situation become worse when there are lots of long transactions in database.

So, to solve this problem, there is need to develop a time efficient FIM algorithm. In case of frequent item set mining (FIM) when database of transaction is given and each transaction contains a set of items, it tries to find item set that occur most frequently than given threshold. The concept of transaction splitting (for long transaction) is used in Private-FP growth (PFP) algorithm. In transformed database long transactions are split into sub-transactions and splitting process is performed only once for given database. After that actual support is calculated in both original and transformed database. Finally Frequent Item set will mine from transformed database. Here algorithm takes a dataset consisting of the transactions by a group of individuals as an input and produces the frequent item sets as output. The goal is to improve the time efficiency. As compared to Apriori algorithm and FP-Growth algorithm, the private Frequent Pattern algorithm achieve high time efficiency and high privacy.

## II. METHODS AND MATERIAL

### A. Related Work

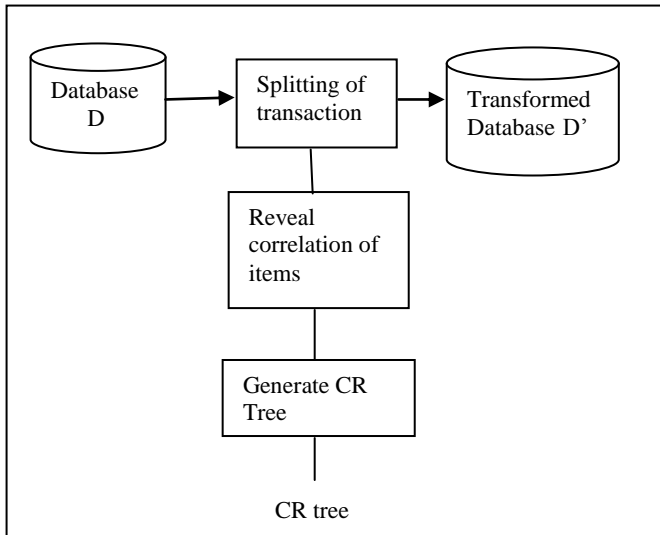
Recent research involves vertically partitioned database and use secure computation of scalar products to find global frequent item sets [2].

To meet the challenge of high dimensionality of transaction database, Li et al. [5] propose the PrivBasis (PB) algorithm to solve challenge of high dimensionality of transaction database. This algorithm construct a graph using frequent items and finds the maximal cliques in the graph to select dimensions.

Sequential Generative model describe the social correlation matrix and Unified Generative Model are two generative models. Efficient parameter estimation solution based on the expectation maximization is proposed. This work is focused on item adoption predication based on the social links Differential private transaction splitting methods [10].

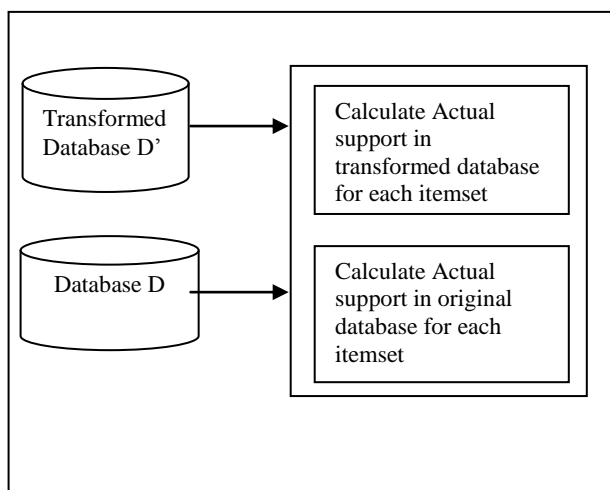
### B. Proposed Work

The PFP-growth (Private Frequent Pattern) algorithm consists of two phases. In the pre-processing phase, some statistical information is extracted from the original database and long transaction are split into multiple subset. Here long transactions are split into multiple subsets each of which meets the maximal length constraint. Fig.1 shows pre-processing phase below,



**Figure 1:** Pre-Processing phase

The run-time estimation method is proposed in mining phase. The method consists of two steps: 1) actual support in the transformed database is estimated, and 2) then further actual support in the original database is computed. For each item set, “average” support is estimated to determine whether it is frequent. Below figure shows mining phase,



**Algorithm 1: GetCount**

**Input:** Query File (QF)

**Output:** List of Attributes with count [LAC]

- 1: For every query Q in QF
- 2: Separate attributes [AS] from Q
- 3: For every attribute A in AS
- 4: increment count of A by 1
- 5: End For
- 6: End For
- 7: return

In this algorithm all the attributes in query are separated and Count is maintained.

**Algorithm 2 : GetAttributeCount**

**Input:** Attribute Name [ANS]

**Output:** List of Attributes with count [LAC]

- 1: For each query Q in File Query
- 2: Get attribute list (AL) from Q
- 3: For each attribute A in AL
- 4: If ANS=A Then
- 5: For each other attribute AO in AL
- 6: If AO in LAC Then increment count of AO
- 7: Else
  - Add attribute AO in LAC with count 0
- 8: End If
- 9: End If
- 10: End For
- 11: End For
- 12: return LAC

In this algorithm, attribute list from query in query file is collected and input attribute is checked with attribute in attribute list (AL). If same then count of other attribute AO in AL is incremented else that attribute (AO) count is set to 0. And list of attribute count (LAC) is return.

**Algorithm 3: Split Query**

**Input:** List of attributes [LA] to display threshold

**Output:** List of Queries [LQ]

- 1: call GetAttributeCount ()
- 2: For every attribute A in LA
- 3: Increment count of A in LAC
- 4: End for
- 5: Sort LAC in descending order
- 6: For each A in LAC
- 7: Add A in LQ [var]
- 8: increment count by 1
- 9: If count>threshold then
  - A: Increment var
  - B: Set count to 1
- 10: End If
- 11: End For
- 12: return

In split query algorithm, query is split into sub queries depending on threshold. Every attribute A in LAC is added to list query array LQ [var] and count is incremented by 1. If count is greater than

defined threshold then var is incremented and count is set to 0.

### III. RESULT AND DISCUSSION

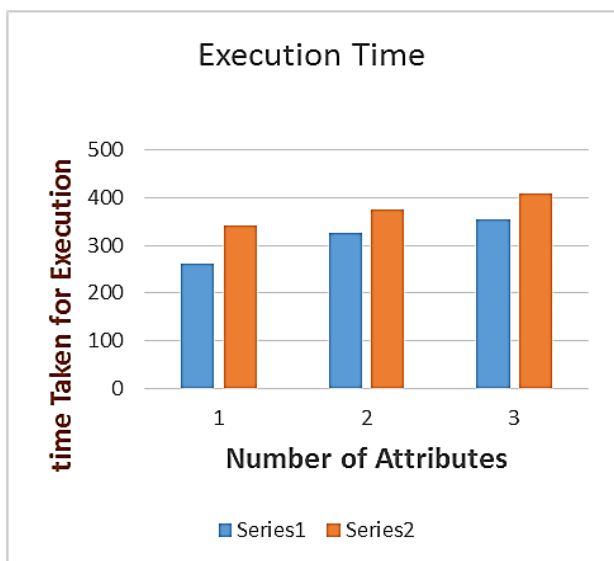
#### Experimental Results

We tried our program for different number of attribute. We executed our program for multithreading and for single thread. The execution time is noted (maximum thread execution time for Multithreading) and graphs are as shown below.

First time we considered 8 and 13 attributes. Series1 is for Multithreading Execution and Series2 Single threading Execution.



In Second time we considered 16, 19, and 22 attributes Series1 is for multithreading execution and series2 is for single thread execution.



Above graphs clearly indicates that if we break the query into sub parts and if we execute then on multithreading, it takes less time than executing single query for all attribute.

### IV. CONCLUSION

Frequent item set is very important to find out from the large data set. Previously some algorithms contributes for achieving efficiency of frequent item sets mining, Private FP-growth (PFP-growth) algorithm tries to achieve better time efficiency. This algorithm performs well in terms of execution time, especially when the database contains lots of long transactions.

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# Evaluation of Resource Needs and Service Delivery of Performance Contracting in Public Primary Teachers Training Colleges in Kenya

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## ABSTRACT

Performance contract as a branch of management science refers to a management control systems which is a contractual agreement to execute a service according to an agreed upon terms, within an established time period and with a stipulated use of resources and performance standards. The government of Kenya introduced performance contract signing in 2004. The aim was to improve service delivery and resource utilization in public institutions among other objectives. The introduction of performance contracting in public primary teachers training colleges was an effort to institute a framework of accountability and enhance service delivery in Kenya. This study sought to establish the availability of resources for implementing Performance contracting with an aim of improving service delivery to the public. It employed descriptive survey design. The target population was 2270 employees and 783 members of the students' councils of Public Primary Teachers Training Colleges. Purposive sampling, simple random sampling and stratified random sampling were used to identify the sample population. A total of 429 respondents formed the sample size representing 30% of the study population. The study revealed several challenges in the implementation process which included lack of funds, lack of training and capacity building of employees regarding performance contracting, ambiguity in the process and resistance by personnel to participate in the process hence these challenges had hindered the successful implementation of Performance contracts in PPTTCs. It is recommended that performance contracting strategy should be strengthened with the availing of more financial resources to ensure that the required learning and teaching resources are all made available.

**Keywords :** Ranking, Resources, Targets, Tutors, Service Delivery, Training.

## I. INTRODUCTION

The term performance contracting can be traced from France in the late 1960s and in other countries including India, Pakistan and Korea (OECD, 1997). Kenya Sensitization Training Manual (2004), defines performance contract as a freely negotiated performance agreement between Government Organizations and individuals on one hand and the Agencies on the other hand. Gathai (2012), looks at performance contract as a branch of management science referred to as management control systems which is a contractual agreement to execute a service according to an agreed upon terms, within an established time period and with a stipulated use of resources and performance standards. From the above definitions, it can be concluded that performance contracting is all about

mutual agreement between two or more groups where one party acts as the owner or representative of the Public Agency and the management of the Agency on the other hand agreeing to perform well in a contractual activity at hand.

In Latin America for example, PC has been used at different times in Argentina, Brazil, Bolivia, Chile, Colombia, Mexico, Uruguay and Venezuela with success in improving the performance of their own public sector. This was done after carefully examining an adapting to their particular needs and learning from the lessons of the vast international experience with regards to performance contracts. It has also been adopted by developing countries in Africa including Nigeria, Gambia, Ghana, Rwanda and now Kenya (Kobia and Muhamed, 2006). In Nigeria for example,

performance contracts are based on the premise that: “what gets measured gets done; if you cannot see and measure success you cannot reward it, if you cannot recognize failure, you cannot correct it and if you can demonstrate results, you can win public support” (Darma, 2013).

Darma looks at performance contract as a method of establishing defined expectations, accountability and consequences for meeting or not meeting a set standard of execution excellence. Two or more parties agree on the actions the performer will execute and the expected results from implementing those actions. He continues to explain that in the absence of a national development intervention within the context of a result based framework, performance cannot be defined and measured hence tracking performance and measuring results is next to impossible. The introduction of performance contracts in Nigeria was aimed at enabling the federal government of Nigeria to measure performance of Ministries, Department and Agencies (MDAs) in a fair, objective and comprehensive manner and to create a results-oriented public service delivery mechanism. Many other African countries have adopted the practice of performance contract and they include South Africa, Gambia, Mali and Botswana among others. Performance contract has been found to be quite successful in these countries.

Performance contract was introduced in Kenya on 1<sup>st</sup> October, 2004, in 16 largely commercial state corporations. This would see institutions freely negotiate performance agreement with the Government every year. Regular monitoring of the implementation of the contract agreed upon has been a vital aspect for its success. The institution provides feedback by forwarding quarterly reports and taking corrective action where necessary. Kenya is still at the first stage of development and was ranked number 93 in the Global competitive index with a score of 3.84, in 2009, compared with countries such as Singapore which was ranked number 5 with a score of 1.53 according to the world Economic Forum (2008-2009). In 2007, the Government of Kenya published Kenya vision 2030 as its long term strategy for achieving global competitiveness and prosperity. The country also aims at linking reforms and economic growth to actual quality

of life of Kenyans, increase customer satisfaction with government services and to build trust in the government. Thus, one of the foundations of the Kenya vision 2030 is an efficient, motivated and well trained public service. Among the key strategies should be to link result based management and performance contracting to the implementation of the visions’ goals making it easier to reward public servants on merit and performance.

In order to be effective in conducting business or rendering services, organizations need to implement management procedures and practices that will yield the desired results. Under PCs, targets are set and although the areas of concern are the outcomes rather than the processes, the processes do determine the outcomes. A performance contract is anchored on two items, the strategic plan and service delivery charter. The development of strategic plans is one of the things that state corporations are required to do before entering into performance contracts. A strategic plan enables an organization to be better focused on its core business. It also helps the organization to clearly set out its objectives and action plans that will enable it achieve the objectives. In effect what the institutions are called to do is to plan performance, thus the development of strategic plans (Republic of Kenya 2005).

Operational plans are a critical component for any organization thus the planning performance stage is important in setting targets against available resources. The resources then provide the much needed support whether in terms of skilled personnel, facilities and equipment or even much needed funds. Regular monitoring and evaluation of the set targets and their achievement set basis for review of performance and drawing up of relevant objectives for the planning stage (Republic of Kenya 2007). One of the advantages of the performance contract system is that it makes the strategy of the department clear to all its operational units and, above all, spells out the resources that will be made available for achieving the targets that they have formally undertaken to meet. Since performance contracting has been seen to yield positive results in terms of better service delivery in countries which have implemented it, it was the expectation of the researcher that the introduction of PC in PPTTCs must have improve service delivery to

clients. From previous evaluation results, it had been observed that most tertiary institutions were performing poorly in annual evaluation results. Whereas the general public and even some high ranking public servants may very much welcome the idea of performance contracting and measuring performance, it might not have been readily accepted by everybody. In state corporations and tertiary institutions, there is a general feeling that it is unfair to grade state corporations operating in different sectors of the economy together with tertiary institutions which function below the expected amount of resources supposed to be allocated to them annually. Another challenge is the applications of the composite score which allows for comparisons of different ministries and state corporations. Some ministries and even state corporations prefer to be grouped and ranked differently citing their uniqueness emerging from the areas of service delivery, size in terms of turnovers, number of employees and even their mandate (Obong'o, 2009).

Performance contracting is a system that operates on the premise that resources for development would be available in good time. However, due to a myriad of reasons, these resources are not always available as envisaged. The affected institutions therefore feel disadvantaged and would not comfortably identify with the outcome of PC. Government services are meant to be available to all citizens throughout the country as it imparts a sense of national belonging in that citizen. The critical argument from several quarters is the disparities in allocation of the available resources and yet the evaluation measurement is the same for all institutions. The study therefore attempted to find out from college employees whether resource reasons could have contributed to their low ranking in the National performance evaluation results of the first seven years after the signing of Performance contracting took effect in PPTTCs in Kenya.

## **II. METHODS AND MATERIAL**

### **A. Statement of the problem**

The survival of an organization, its increased productivity and high profit margins are dependent on resources and in particular human resource. Without proper resources, even the best designed organization

that is guided by well made plans with necessary equipment cannot achieve its performance potentials. It is the people who supply the organization with their human efforts and work. Workers use their talent to creatively combine and utilize the equipment, machines, tools and other non-human resources which include facilities, structures, time, technology, land, transportation, the organization climate, geographical orientation and information (Umunadi, 2012). The introduction of performance contracting in Kenya was aimed at among other things to bring about a radical transformation in resource utilization in public institutions.

However, dissatisfaction with the way some of these institutions approach the subject of resource utilization is ripe. Public Primary Teachers Training Colleges for example have expressed dissatisfaction with the way financial resources are managed as it has sometimes resulted in public outcry over poor performance in service delivery leading to satisfactory results in PTE examinations, their core function. Stakeholders have complained of inability of Government institutions to meet their set targets in PC evaluation areas among them Teacher Training Colleges. Tutors have complained of lack of motivation and support by administrators especially in the process of acquiring teaching and learning resources for their departments. This might have contributed to reduced efforts in attainment of the required standards in service delivery. Another group of complainants are the suppliers of goods and services. They claim that they are not paid on time and sometimes are paid at a period exceeding one year which has seen some of them close their businesses. Students have also expressed their dissatisfactions in the way complains are handled especially when it comes to students affairs. This study aimed at finding out why targets are not met even when set using all the agreed performance indicators and a surety of availability of resources by Government and the institution concern. Majority of stakeholders who are affected by this low ranking feel disadvantaged and would not comfortably identify with the outcome of PC. They are not in a position to own up the results and yet they are the target setters. This therefore means that there is something these institutions are not getting right. It is against this background that this study was carried out to establish the amount of resources allocated to colleges for the implementation of



performance contracts for the sole purpose of improving service delivery in Teacher Training Colleges in Kenya.

## **B. Objective of the Study**

The objective guiding the study was:

To assess the adequacy of resources for implementing performance contract to improve service delivery in Public Primary Teachers Training Colleges in Kenya.

## **C. Research Design and Methodology**

This study adopted a descriptive survey design. Surveys are used in preliminary and exploratory studies to allow researchers to gather information, summarize, present and interpret data for the purpose of clarifications (Orodho, 2002). This approach was also to enable the researcher to establish the feelings, opinions and perceptions of tutors teachers training colleges about performance contracting and its impact on teaching/learning resources' utilization. The design was used by the researcher because it enabled him collect information on the subject of the study in its current state. It was also to enable the researcher to receive detailed information from respondents who are either directly or indirectly involved in issues of performance contracting in Public Primary Teachers Training Colleges. It helped in getting their opinions about other components like performance assessment, performance targets and performance rewards. That enabled the researcher to find out the impact performance contracts had on resource utilization.

## **D. The Study Location**

The study was carried out in Public Primary Teachers Training Colleges in Kenya. These institutions were found to have been ranked way below other tertiary institutions in most annual PC evaluation by the Government. Ranking depends on evaluation matrix where marks are awarded depending on the level of achievements of targets. There are 21 PPTTCs and these are Igoji, Kamwenja, Machakos, Kilimambogo, Thogoto, Kigari, Bondo, Mosoriot, Migori, Asumbi, Tambach, Kaimosi, Shanzu, Garissa, Murang'a, Baringo, Meru, Kitui, Narok, Eregi, and Kericho.

## **E. The Target Population**

In this study, the target population consisted of teacher trainees, Tutors (Principals, Deputy Principals, Deans, PC committee members and the rest of the teaching staff) and members of the Non-teaching staff of PPTTCs in Kenya. The respondents were targeted because of the structure of college administrations where there are service providers and service recipients. PC committees who are part of the teaching staff oversee the implementation process of Performance contracts and the College Principals who are the chief executive officers of their institutions and chairs PC meetings were in a position to explain the success of PC implementation in their colleges. The respondents were 1414 tutors (Principals, Deputy Principals, Deans, Heads of Departments and the rest of the teaching staff) and 856 Non-teaching staff together with all Heads of sections and a total of 783 members of the student councils.

## **F. Sample Size and Sampling Procedures**

The researcher used probability sampling techniques namely cluster sampling, simple random sampling and stratified random sampling. It also used non-probability sampling techniques of purposive sampling to select a sample from the populations. These were appropriate to the research objectives. Stratified random sampling was used to ensure that the sample represents key subgroups of the population. It was also used to determine the sample size in the study because it gave equal opportunities to all subjects in the target population who were to be selected for the study. Colleges were clustered in to six Zones. From the 21 colleges, 12 were selected using stratified technique to capture all colleges representing all regions of Kenya. The entire sampling matrix comprised of 12 Principals, 65 PC Committee members, 129 teaching staff members, who are not in PC committees, 85 NTS and 116 members of the student council; yielding a total sample of 429 respondents for the study.

**Table 1:** Sample Size Distribution for The Study

Respondents	Population (N)	Sample population	Percentage (%)
Principals	12	12	100
PC Committee	216	65	30
Tutors	430	129	30
NTS.	354	107	30
Student council	386	116	30
<b>Total</b>	<b>1398</b>	<b>429</b>	<b>30.7</b>

**Source: Field Data (2014)**

Stratified random sampling technique was used to group the teaching staff and student councils according to their departments and areas of operation respectively. Each college has 8 departments but the population of the teaching staff in each department varies from institution to institution depending on the population of the students. A sample of 129 members of the teaching staff was selected to participate from a population of 430 and a sample of 116 members of the student council was also selected from a population of 386. Simple random sampling was used to select members of the Non-teaching staff were 107 participants were expected to respond to the questionnaires. A total of 41 questionnaires were returned while 44 participate through interviews making a total of 85 respondents representing 80% of the expected responses from this group which was a representative sample enough to give findings that can be generalized. O'Connor (2011) argued that there are formulas for determining sample size, but the main thing is to be practical and for a small population of interest, you would most likely need to sample about 30% of the population. The study considered utilizing this formula to obtain the sample population. The rationale for this study was informed by poor evaluation results posted by PPTTCs in all PC evaluation results since its signing in 2007.

### III. RESULT AND DISCUSSION

#### A. Findings

##### Resource allocation for performance contract activities

The objective of this study was to establish whether the level of availability of resources was a contributor to poor performance in annual performance contacting evaluation in Public Primary Teachers Training Colleges

in Kenya. Previous annual evaluation result show that PPTTCs were not at bar with other tertiary institutions under the same category when it comes to PC annual evaluation of all tertiary institutions. The Principals pointed out that proper utilization of learning and teaching resources were hindered by scarcity of financial resources. The lack of finances made some of the resources not to be availed in time or not to be procured at all hence hindering the impact of PC on the ground. Some tutors claimed that there was no much change on the ground as far as the availability of teaching and learning resources were concerned. They claimed PC as a strategy was hampered by too much paper work and lack of enough training in resource utilization for its impact to be strongly felt on the ground. Heads of Departments however indicated that the utilization of resources was hindered by the fact that the teaching staffs were not properly prepared to implement the planned performance strategy that had in turn made them not to appreciate the strategy positively. Performance contract system makes the strategy of a department clear to all its operational units and above all spells out the resources that will be made available for achieving the targets they have formally undertaken to meet. Teachers training colleges suffer from inadequate, old and poorly maintained transport facilities which affect some of the PC target areas such as teaching practices for teacher trainees. The study intended to find out from the groups of respondents whether the resources available in their institution could help meet the set PC targets to enable them get a good ranking. Table 2 indicates the responses of employees on this matter.

**Table 2 :** Employees' responses on adequacy of resources for PC implementation

Respondents	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Principals	3(25%)	1(8.3%) 0	0	5(41.7%)	3(25%)
PC Committee	2(3.1%)	28(43.1%)	12(18.5%)	14(21.5%)	8(12.3%)
Non- teaching staff	5(12.2%)	14(34.1%)	12(29.3%)	6(14.6%)	2(4.9%)

Source : field data 2014

As to whether colleges have adequate resources for the implementation of PC the table above indicates the results from all participants where 4(33.3%) of the Principals agreed that resources were adequate but 8(66.7%) disagreed meaning this is one of the challenges affecting the success of PC in PPTTCs in Kenya. On the same question, 46.2% of PC Committees were in agreement while 46.3% of the Non-teaching staffs agreed that PC had adequate resource for PC implementation in their institutions meaning there was a likelihood that lack of clarity existed in the use of resources. Interviews conducted revealed that NTS were not aware of what was being contracted as they claimed that their routine work had remained the same. According to Kobia and Mohammed (2006), stability of resources enhances the Motivating effect of the contract and yet organizations still experience challenges such as the lack of adequate resources, resources not being released on time; some performance targets being highly ambitious and unplanned transfer of staff. When resources are not available or availed late, the staff involved gets frustrated.

Hooker (2011) and Umunadi (2012) argue that the survival of an organization, its increased productivity and high profit margins are dependent on resources and in particular human resource. The researchers' interviews and observations revealed that PPTTCs suffer from low funding especially at their learning resource centers. It was also found that Teacher trainees are trained on the use of modern technology in lesson presentation. There was inadequate ICT infrastructure equipment and materials which affected ICT programs in the training of teachers. Currently computer lessons and the use of computers by both pupils and teachers seem to be the way forward in copying with changes in technology. Respondents' suggestions were that one way to mitigate the problem of resource scarcity was to encourage creativity and innovative practices internally within an institution. Utilization of allocated funds ensures that funds are applied to programs, projects and activities for which they were appropriated and were planned for. Allocated funds include financial resources from outside the exchequer, including donor funds. This links the process of budgeting to performance target setting. The PC committees explained that variance analysis was always carried out on all budget items to establish conformity and absorption capacity. The study

wanted to find out if compliance to the set budget, 100% collection of levies, proper utilization of funds and cost savings and reduction was being adhered to as expected. The most difficult of all these is fee collection from students as majority of them are believed to be coming from humble backgrounds. This makes 100% collection of fees as a target next to impossible hence affecting most target areas which depend on money paid in terms of fees. The findings had mixed reactions from employees of different colleges. The table below provides this information.

**Table 3 :** Responses of Principals and PC Committees on finance

Respondents	Strongly agree	Agree	Neutral	disagree	Strongly disagree
<b>Comply with college budget</b>					
Principals	7(58.3%)	5(41.7%)	0	0	0
PC Committee	10(15.4%)	40(61.5%)	5(7.7%)	6(9.2%)	1(1.5%)
<b>College collect 100% levies</b>					
Principals	1(8.3%)	5(41.7%)	3(25%)	3(25%)	0
PC Committee	3(4.6%)	11(16.9%)	14(21.5%)	20(30.8%)	15(23.1%)
<b>Proper utilization of funds</b>					
Principals	3(25%)	7(58.3%)	2(16.6%)	0	0
PC Committee	7(10.8%)	36(55.4%)	10(15.4%)	9(13.8%)	2(3.1%)
<b>Progress in cost reduction</b>					
Principals	2(16.7%)	7(58.3%)	2(16.7%)	0	0

Source: field data 2014

From Table 3 above 100% of the principals and 76.9% of PC committee agreed that college administrators do comply with college budgets. Both the Government and PC guidelines require all college principals to strictly adhere to the planned budget and spend 100% of all collected levies. Colleges are expected to collect 100% levies from various sources. This is shown through audited financial reports or accounts, showing all monies collected from fees, house rent and seminars among others during any given financial year. For example, from Table 3 above, 100% of the principals agreed that it was possible to comply with the set targets in PC. This is a confirmation that setting of targets was within the means and the ability of any institution. The findings also indicate that 50% of the principals and 21.5% of PC committees were able to collect 100% of their levies.

The same table also reveals that 83.3% of the principals and 66.2% of the PC committees agreed that there was proper utilization of funds in PPTTCs. Proper utilization of funds can be obtained through the audited accounts, receipts supporting purchases, payment vouchers and other relevant documentary evidence. All institutions must show convincing vote by vote compliance and

provide authenticated proof of reasons for partial and/or non-compliance and the authorization so as to be evaluated using the standard criteria for PC. That is if they have to be said they complied with the set budget. Majority of the institutions had evidence showing their expenditure as was found in the document analysis. The study also found out that 75% of the principals agreed that PC had made them experience reduction in cost of goods and services. In this case of cost savings and reduction, institutions must show by way of evidence the savings such as costs reduced through bulk purchases, discount given by suppliers and the official communication between the Agency and the suppliers, comparative cost for purchases made at different times during the financial year in question. Table 4 below is a practical example of one of the institutions which had documented the way they planned to spend their expected income 2014/2015 financial year.

**Table 4 :** A Colleges’ expected income for 2014/2015 financial year in on PPTTC

s/n	ITEMS	COST (Kshs)
1	Fees from students	66,122,650.00
2	House rent	1,350,000.00
3	Grants	13,376,568.00
4	The farm	1,302,850.00
5	Seminars	4,200,000.00
	Total	86,200,000.00

Source: Field Data 2015

The table indicates a classic example of the sources of income for most PPTTCs in Kenya. The guidelines expected the institution concern to collect 100% of all the indicated monies which in normal cases may be not possible within the period in question. Colleges do not allocate any money to PC activities as a vote head. However, the PC activities are footed under the general college vote. It should be noted that all activities to be undertaken by the college are evaluated under performance contracting. Information on PC activities can be obtained in commitments’ manual of the board of management in every institution under PC. The BoM is accountable to the membership base to adopt the best possible practices of Governance, management and operations in order to ensure and provide the best possible experience for service users (GoK 2011). It is therefore necessary for college administrators to fully involve all employees in the activities of performance

contracting. This will make them understand well the content of agreement, what they are expected of, and what their clients expects from them and to strictly adhere to the planned institutional budget of the year. According to U.S Department of education retrieved on 7<sup>th</sup> April 2015, a major element of financial data activity rests in the act of budgeting. Budgeting is the process of allocating finite resources to the prioritized needs of an institution. In most cases, for a Governmental entity, the budget represents the legal authority to spend money. Adoption of a budget in the public sector implies that a set of decisions has been made by the Governing board and administrators that culminates in matching a Government's resources with the entity's needs. As such, the budget is a product of the planning process. The budget also provides an important tool for the control, evaluation and the uses of resources.

Using the accounting system to enact the will of the Governing body, administrators are able to execute and control activities that have been authorized by the budget and to evaluate financial performance on the basis of comparisons between budgeted and actual operations. Thus, the budget is implicitly linked to financial accountability and relates directly to the financial reporting objectives. Client visiting the institution face some challenges at some point and therefore they need to be assisted. This brings about the necessity of a customer care desk and complains handling department.

Table 5 indicates the responses of employees on how problems relating to customers are handled. Service delivery begins at the entrance of any institution and clients are given direction at a reception room or desk. From the reception area, clients are able to access the service delivery charters and gauge how long they are likely to be served in that particular institution.

**Table 5 :** Availability of customer desk and a complains handling department

Respondents	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Teaching staff	9(7%)	38(29.5%)	33(25.6%)	19(14.7%)	19(14.7%)
Non-teaching staff	3(7.3%)	12(29.3%)	7(17.1%)	12(29.3%)	5(12.2%)
Student council	16(13.8%)	25(21.6%)	9(16.4%)	19(16.4%)	45(38.8%)
Principals	2(16.7%)	9(75%)	1(8.3%)	0	0

Source: field data 2014

The results in Table 5 above shows that 91.7% of the principals were in agreement that customer desk and a department for handling complain was available in their institutions. On the contrary only 36.5% of the teaching staff, 36.6% of the Non-teaching staff and 35.4% of the student council were in agreement of the same indicating that majority of the employees do agree that the resource was not available. The observation schedule showed that majority of the institutions visited had a customer desk only. None of them had complains handling department. From this result, the important aspect of quality service delivery at a point of service level is the existence of easily accessible, acceptable and transparent complains handling mechanisms to which customers can have recourse to if they are dissatisfied with the services received. This can only happen if the resources mentioned above are available. It is therefore prudent for institutions to use friendly customer complains handling mechanisms which are consistent with the promises contained in the service delivery charters in every institution. The study sought to find out whether the respondents had been trained on Performance Contract or not. Human resource is a very important element in any organization. This kind of resource should be well equipped with the right kind of skills needed in that particular organization.

Table 6 below indicate the responses of college employees on the training of staff in PC activities from the period PC was signed by the college administration as part of a college program to the time this research was being undertaken.

**Table 6 :** PPTTCs Staff have been train on PC activities

Respondents	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Principals	1(8.3%)	0	3(25%)	6(50%)	2(16.7%)
PC committee	3(4.6%)	18(27.7%)	3(4.6%)	31(47.7%)	9(13.8%)
Teaching staff	13(10.1%)	26(20.2%)	23(17.8%)	43(33.3%)	20(15.5%)
Non-teaching staff	4(9.8%)	17.1%)	4(9.8%)	16(39.6%)	7(17.1%)

Source; field data 2014

According to the findings, 8.3% of the principals indicated that they had trained staff on Performance Contract while 66.7% of these respondents indicated that they had not trained their staff. Majority 61.5% of PC Committee in answering the question on training of staff

disagreed that there is any training given to the staff while 30.3% were in agreement. From this results it is possible to conclude that this could be one of the reasons why PPTTCs are tailing in PC annual evaluation. The study findings revealed also that 48.8% of the Teaching staff disagreed that there was any kind of PC training in their institution and only 30.3% agreed while 17.8% remain uncommitted. This could be another cause of poor achievements as lack of training is a thread to implementation process. At least all PC committee should have been trained on PC activities. On the same item, 56.7% of the NTS disagreed that staff had been trained on PC while 26.9% agreed. This is an indication that training is necessary in PPTTCs if at all there is need for proper PC implementation. This is an indication that majority of the respondents have no idea on how to go about PC activities. It is therefore necessary for PPTTCs to improve on their human resource. The research findings gave a clear indication that most employees were not trained and therefore BoM should intensify the training of all employees on PC. OECD (1999) identifies training as a key determinant in Performance Contracting related activities. Training improves management capabilities and abilities to take advantage of the opportunities that can add value to the institutions' day to day operations. Training on PC is of great importance because it would deepen the understanding of PC concept. The fact that training had not been done for the majority of the employees was something to get worried of the researcher tested the objective of the study to find out whether there was any relationship between resource allocation and service delivery in PPTTCs in Kenya. Resource allocation here includes both human and material resource necessary for the implementation of PC in PPTTCs. The objective stated as:  $H_{03}$ : There is no significant relationship between availability of adequate resources for implementing performance contract and service delivery in Public Primary Teachers Training Colleges in Kenya.

**Table 7:**  $X^2$  results of adequacy of resources and service delivery in PPTTCs

Characteristics: Availability of resources ^ Service delivery	Pearson chi-square value	Df	Asymp. Sig. (2 sided)
Principals	49.333	35	.045
PC Committee	281.979	210	.001
Teaching staff	651.267	504	.000
Non-teaching staff	346.564	256	.000

Source: field data 2014

From table 7 above, it is evident that chi-square test has all values of  $P < .05$  hence the hypothesis above was rejected in favor of an alternative hypothesis. The results indicate that there is a significant relationship between availability of adequate resources for PC implementation and service delivery. The implication of these results is that for service delivery to improve there must be adequate resources for the program. Availability of resources will also eliminate excuses for non-performance. We can conclude that resource availability will improve service delivery in PPTTCs.

## **B. Discussions of the Findings**

Performance Contract is a challenging program full of complex activities. In some cases, it may be difficult to identify concrete outcomes or results for a service. For example, training and education services might be provided with the goal of disseminating information and modifying people's behavior. However, it may be difficult or impossible to track participants and determine whether the training helped them to think and act differently. In these cases, the development of / or output measures such as the number of people served or the number of training sessions or outcome measures to evaluate the impact of the training effort such as pre or post test scores should be developed. This requires well trained personnel to undertake the program. If a department is unable to identify performance outcomes for a specific service type, a meeting with head of the section and other departments may be useful to stimulate new ideas and share best practices (Korir, 2005). The Government exaggerates its expectations especially in relation to funding projects within a contract period. Government funds are not released on time while at the same time Students pay fees when they are about to do exams. This delays the achievement of set targets in the contract period as PC is evaluated quarterly. In giving their general views on PC, employees agreed that signing of Performance contract should be done in conjunction with provision of all the resources; finances, facilities and staff motivation. Professor Guest of United Kingdom College concurs with the above argument in that good performance management involves ensuring that people have the resources for accomplishing set goals in a contract period. Republic of Kenya (2010) asserts that Government officers are being publicly challenged to account for public resources entrusted to them on a day-to-day basis as the bar of achievement is

raised each year. This implies that the Government dictates the goals in the contract period and also raising the bar of achievement every year and does not provide funding on time as claimed by majority of the administrators who participated in the research.

Opondo (2004) claim that an institution with employees who are specialized will achieve targets more than those with little training. This implies that staffs that do not have the necessary knowledge regarding PC are likely to miss out of their target and therefore there is need for training so that they get the necessary knowledge and skills on PC. Effective training and sensitization on PC to all employees of PPTTCs is therefore a critical aspect that needs to be urgently addressed so that there is synergy in the implementation of PC (Gatere, 2013). The principal factor which is clearly seen to be impacting negatively on quality of services offered by various institutions are inadequate preparation, lack of proper supervision at both department or section and institution level and sensitization of employees on the role and importance of PC and the need to participate fully by enabling them to cope up with the changes in service delivery systems and information technology. Another factor is unavailability of adequate resources and physical facilities, inadequate mechanisms for assessing customer satisfaction and limited professional commitment to quality service provision by some employees. For customers to be served well, a customer desk must be available to address the challenges facing those who are seeking services in various sections of an institution. Complain handling department must also be set so as to address challenges facing clients at all times.

According to the interviews conducted on the availability of resources for implementation of PC in teacher training colleges, it was found out that performance contracts had little impact on adequacy of teaching and learning materials as well as the use of ICT. The teaching staff had challenges in procuring teaching and learning materials as they had no direct means of acquiring the funds. According to Abdulkaram, Fassas, and Akinubi (2001), all educational resources are vital to the achievement of Institutional and a National objective hence availing resources is mandatory. The research continue to report that most PPTTCs had not trained their human resource on PC implementation process; therefore, most of them end up working so hard but

without targeting PC indicator areas simply because they are not aware. Afolabi (2005) observes that no matter how beautiful the programs and assets of an institution are, without a well-trained academic staff and availability of other resources, attainment of institutional goals and objectives would prove abortive. This is because human resources constitute a workforce in an institution.

Güven and Gulbahar (2008), observes that the integration of ICT as a resource has the potential to help revitalize teachers and students. This could help to improve and develop the quality of education by providing curricular support in difficult subject areas. The study findings also found out that there is inadequate ICT infrastructure equipment and materials to incorporate ICT programs in the training of teachers. The use of ICT in all areas of teaching and learning and other service areas in Colleges should be made mandatory as this can be the only sure way of improving service delivery and improving transparency. The research findings indicated that all Public Primary Teachers Training Colleges operate on vote-head based budgets and therefore faces serious constraints at the resource level to implement the various commitments in the contract. Moreover, fees chargeable to Students are seldom collected 100% since students cannot be send home for fees as it is also the ministry of education's policy to ensure that there is a 100% completion rates by all the students enrolled in public learning institutions was achieved. In general the research findings revealed that 8(66.7%) of the principals disagreed that resource were available to implement PC activities. Resources here mean other support mechanisms other than monetary support. At the same time 46.3% of the Non-teaching staff agreed that PC had improved utilization of the available resources in their institutions. It was also found that it is possible to comply with set budgetary levels according to 76.9% of the PC Committees. If this is to be true then college administrators have to comply and reduce wastage so as to improve their service provision and raise their ranking. Majority 83.3% of the principals accepted that utilization of funds from their institutions were of the required standards. This means most of the principals were accountable for the funds they receive in their institutions. Clients expect quick solutions to their problem. Solutions to problems are one of the best indicators of quality service delivery. On the

part of the teaching staff, the study revealed that 36.5% of them agreed that they had both a customer care desk and complains department.

Efficiency and transparency in management of public resources has been the desire of every citizen all over the world and especially in Kenya, (AAPAM, 2005). Principals of Colleges have failed to complete the automation program as expected in PC manual. Automation has been proven to lead to a more competitive and customer oriented employees' Service delivery. It also improves employees' accountability and transparency in an institution. The findings of this study through observation check list indicated that service charters were available in most PPTTCs but what was lacking in all of them was an office or department where users would seek redress in case of dissatisfaction. Service charter is therefore not an option for an institution. Performance contracting is anchored on strategic plans and service charters. Less than 50% of the employees think that service charters and strategic plans are adhered to which is a worrying trend.

Another PC indicator which proved difficult to achieve was the collection of levies. The Government expects institutions to collect 100% levies from various sources under their mandate. This is difficult because a number of Students enrolled in PPTTCs cannot afford to pay fees because of their humble background. They expect assistance from the Government through bursaries and donations from friends. As a result the bursaries given are not enough hence they continue to study with huge arrears of college fees making the indicator difficult to be achieved. Institutions are expected to comply with set budgetary levels. Majority of the respondents agreed that the indicator is achievable if resources are well managed to avoid wastage. Colleges have innovated ways of improving their resource base through the introduction of self-sponsored programs where students who were not selected to join PPTTCs through the Government selection program are selected and integrated into the system but have to pay a higher fee to the institution. Programs like school based have also been introduced in some colleges as a way of boosting their resource base.

#### IV. CONCLUSION

The study reveals that most PPTTCs have not trained their human resource on PC implementation process; therefore, most of them end up working so hard but without targeting PC indicator areas simply because they are not aware. Opondo (2004) claim that an institution with employees who are specialized will achieve targets more than those with little training. This implies that staffs that do not have the necessary knowledge regarding PC are likely to miss out of their target and therefore there is need for training so that they get the necessary knowledge and skills on PC. The research findings gave a clear indication that most employees were not trained and therefore BoM should intensify the training of all employees on PC. The research findings also indicates that all Public Primary Teachers Training Colleges operate on vote-head based budgets and therefore faces serious constraints at the resource level to implement the various commitments in the contract. The study found out that Principals of Colleges have failed to complete the automation program as expected in PC manual. Automation has been proven to lead to a more competitive and customer oriented employees' Service delivery. It also improves employees' accountability and transparency in an institution. It is because of this reason that none of the 21 PPTTCs qualifies to be ISO certified. The findings of this study through observation check list reveals that service charters were available in most PPTTCs but what was lacking in all of them was an office or department where users would seek redress in case of dissatisfaction. Colleges were not able to collect levies as expected. The Government expects institutions to collect 100% levies from various sources under their mandate. This is difficult because a number of students enrolled in PPTTCs cannot afford to pay fees because of their humble background. Government funds are not also released on time which includes bursaries for students while at the same time students pay fees when they are about to do exams. This delays the achievement of set targets as PC is evaluated quarterly. Colleges do not allocate any money to PC activities as a vote head. However, the PC activities are footed under the general college vote. It should be noted that all activities to be undertaken by the college are evaluated under performance contracting. This means the colleges must have enough funds to run all activities under PC

evaluation otherwise the PC activity area without resources will score the least mark. HoDs, however indicated that the utilization of resources was hindered by the fact that the teaching staffs were not properly prepared to implement the planned performance strategy that had in turn made them not to appreciate the strategy positively.

#### V. RECOMMENDATIONS

1. Colleges should partner with the Government in providing adequate resources.
2. A vote head should be designed for PC program.
3. Board of Managements should intensify the training of all employees on PC

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# Diversity of Phytoplankton in Ayyanakere Lake, Chikmagalur District, Karnataka

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## ABSTRACT

In the present study, phytoplankton belonging to 40 species under 27 genera was recorded from Ayyanakere lake, Chikmagalur, Karnataka during the period from April 2008 to March 2009. Results revealed that, Zygnemophyceae was found to be the dominant group of phytoplankton (25.0 %) followed by Chlorophyceae and Bacillariophyceae with 22.5 % and 20.0 % respectively and Euglenophyceae with 15%. Ayyanakere lake is found to be rich in phytoplankton diversity and hence productive. Physico-chemical factors were found to be the important factors influencing the growth of phytoplankton. The lake is said to be moderately oligotrophic.

**Keywords :** Diversity, Phytoplankton, Physico-chemical parameters, Ayyanakere lake.

## I. INTRODUCTION

Phytoplankton is a functional aquatic community based upon which the aquatic food web is culminating (Reynolds, 1984). Studies on physicochemical factors and phytoplankton standing crop of its habitat are essential for the proper management of water resources and for the prediction of the potential changes in the aquatic ecosystem (Kobbia, 1982; Descy, 1987). Sheehan (1984) reported that phytoplankton populations are mostly structured by the physical and chemical variables of their environment. These factors have also been reported to be responsible for the heterogeneity in phytoplankton composition and biomass (El-Ayouty *et al.*, 1994, 1999; Ahmad *et al.*, 2001; Ibrahim *et al.*, 2003).

The present study has been carried out to know the diversity and distribution of phytoplankton in Ayyanakere Lake of Karnataka. This study is relevant since the present water body forms the source of water for irrigation, drinking and fisheries.

## II. METHODS AND MATERIAL

### Study Area

Ayyanakere is an Anicut lake (Figure 1) constructed by Rukumanda Raya, chief of Sakharayapatna and renovated later in 1156 A.D. during the Hoysalas period. The large lake situated at the eastern base of Dattapeetha (Baba Budan) range, 18 kms northeast of Chikmagalur town provides irrigation facilities to more than 1574 hectares of registered land on a hillock adjacent to the lake.



**Figure 1:** A View of Ayyanakere lake

Ayyanakere area possess evergreen to deciduous forest type. It is one of the most coffee and tea growing regions in India. The climate of the region is cool and dominated by many hillocks. The water body is completely surrounded by the small to larger hillocks with perennial streams.

Figure 2 shows location of the study area. The geographical location of this lake is 13°41'42" north latitude and 75°04'46" eastern longitude. This lake constructed to the upper Veda river. The water from this lake along with some other small tributaries forms river Veda and joins the river Avathi at Yagatipura to form Vedavathi. The Vedavathi joins to Krishna river which ultimately joins Bay of Bengal.



**Figure 2:** Location of the Ayyanakere Lake

Many hillocks surrounded to the lake which forms the natural reservoirs. It is a shallow lake has an area of 15 sq. kms. The catchment area of 116.59 sq. kms water spread area 118.54 ha. The bund forms from the natural hills and stones with length of 450 m and height 4.80 m. The maximum depth of the lake is 30 m and an average depth is 20 m.

### Water Quality Parameters

The sampling was carried out during morning between 8.00 AM to 9.00 AM. For physico-chemical analysis samples were collected weekly during April 2008 to March 2009. Water samples were collected in 2 litre capacity plastic cans. The water and air temperature were recorded at the sampling site itself by mercury

thermometer. Dissolved oxygen was fixed on the spot itself in BOD bottles. The parameters like free CO<sub>2</sub>, alkalinity, total hardness, total dissolved solids, Calcium, magnesium, phosphates and chlorides were estimated as per the standard methods ( APHA ,1995; Trivedy and Goel, 1986).

### Phytoplankton Diversity

For the estimation of phytoplankton, the water samples were transferred to cleaned 500 ml polyethylene bottles. Then, 5 ml of lugols iodine solution and 10-15 ml of 4% formaldehyde were added to it for fixation and preservation of planktonic cells. Plankton were enumerated using Sedgewick-Rafter cell (Welch, 1948) and expressed as numbers per litre. Qualitative identification of phytoplankton organisms was done with the help of monographs and they are identified upto species level (Edmondson,1959; Prasad and Mehrotra,1977; Needham & Needham,1978; Bharathi and Hegde, 1982; Digamber and Vidyavathi, 1985; Adoni et al. 1985; Hegde and Bharathi, 1985 and Coesel, 1996).

## III. RESULT AND DISCUSSION

### Water Quality

Seasonal variations in the physico-chemical parameters of water of the present lake is summarized in Table 1. Seasonal analysis of water temperature showed that it was highest in summer and relatively lower in monsoon and winter (Table 1). pH is considered as important chemical parameter in waterbody since most of the aquatic organisms are adapted to an average pH and do not withstand abrupt changes. The maximum pH was recorded in pre-monsoon it may due to leaching of soil followed by the decomposition of plankton (Swarnalatha and NarasingaRao, 1993) and minimum in monsoon. The present study shows the acceptable range of pH for fish culture.

Maximum CO<sub>2</sub> recorded during post-monsoon and low during monsoon season (Table 1). It has been found that CO<sub>2</sub> content is inversely proportional to the oxygen content due to photosynthesis and respiratory activities (Deshmukhet *al.*, 1964; Wetzel, 2001).

The present investigation showed that maximum dissolved oxygen recorded during monsoon because of increased phytoplankton high primary productivity and low metabolic activity of organisms, DO is high due to photosynthetic activity, minimum in pre-monsoon. These observations are in agreement with earlier work of Kumar and Singh (2000), Munwar (1970), Honneshappa (2008) and Rajashekar and Vijaykumar (2008). The present study also reveals that the total DO is suitable for fish growth.

The seasonal variation of calcium concentration is maximum in monsoon, but less in pre-monsoon. Similar observation was recorded by Kamran Tasaduque *et al.* (2003). The seasonal variation of magnesium concentration is maximum value in post-monsoon and slightly less in monsoon. The minimum value recorded during pre-monsoon. This is in conformity with the findings of Janardhana Rao (1982), Zutshi and Khan (1988), Shardendu and Ambasht (1991) which have concluded that normally natural water contains magnesium concentration lower than the concentration of calcium.

Regarding TDS seasonal analysis states that low in pre-monsoon and maximum value was recorded in post-monsoon (Table 1). The higher values may be due to lower water level and perhaps various kinds of ions present in the waterbody. Similar observations were found by Rajashekar *et al.* (2003) in the river Umshyripi as Shillong Meghalaya. In the present study also reveals that the total dissolved solids is suitable for fish growth.

The seasonal values of chloride was recorded maximum in post-monsoon and low in pre-monsoon (Table 1). This is in close agreement with that of the observation made by Patilet *et al.* (1986), Swarnalatha and Narasinga Rao (1993) and Sinha (1995). The reduced concentration of chloride content recorded during monsoon season when compared to pre-monsoon season and may be due to dilution, caused by rain water. Similar opinion has been given by Orborne *et al.* (1980) and Lowe (1980).

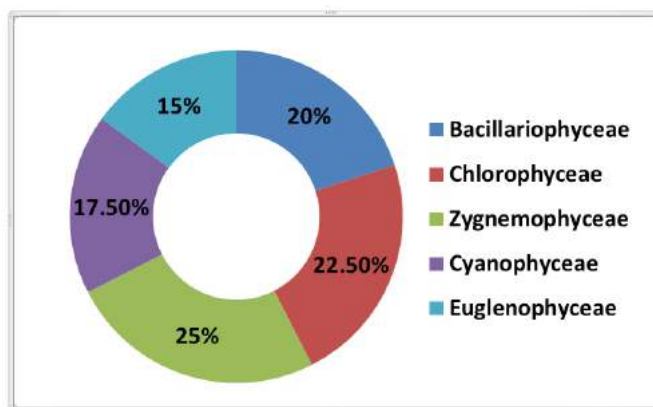
Highest total alkalinity recorded during post-monsoon and lowest in the monsoon (Table 1). The present study the total hardness is 92, 96 and 108 mg/l during the period of pre-monsoon, monsoon and post-monsoon

respectively. Highest total hardness recorded during post-monsoon and lowest in the pre-monsoon (Table 1). Values for the total hardness in the present investigation falls in the range of moderately hard category (Mitra, 1982; Birsal *et al.*, 1985).

The phosphate level minimum during monsoon and maximum in post-monsoon period (Table 1). Many researchers such as Venkateshwarlu (1969a), Sampath Kumar (1977) and Nirmal Kumari (1984) have observed an increase in phosphate concentration in such water bodies they may receive domestic waste. This was contributed by the surface runoff draining the agricultural fields and mixing with the influent water of the pond.

### Phytoplankton diversity

Phytoplankton population in the Ayyanakere was summarized in Table 2. The percentage composition of phytoplankton was shown in Table 3 and Fig. 3. The 5 classes of phytoplankton were Bacillariophyceae, Chlorophyceae, Zygnemophyceae, Cyanophyceae and Euglenophyceae formed 20%, 22.5%, 25%, 17.5% and 15% respectively.



**Figure 3.** Percentage of the phytoplankton diversity of different classes

Among these, Zygnemophyceae dominated the other groups in the lake. Phytoplankton diversity was found in the order of Zygnemophyceae > Chlorophyceae > Bacillariophyceae > Cyanophyceae > Euglenophyceae. The Zygnemophyceae, formed the dominant group of phytoplankton community and its numerical abundance was relatively high in all three seasons. Water temperature plays an important role in controlling the occurrence and abundance of phytoplankton (Nazneen, 1980). The pH

has got its effects on phytoplankton population abundance (Nandan and Patel, 1992). Observed that high pH values promote the growth of algae and results in blooms.

**Table 1.** Seasonal variations in Physico-chemical parameters of water of Ayyanakere lake during 2008-09

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Air temp.	32	27	22
Water temp.	29.5	25	21
pH	7.5	7	7.2
DO	6.4	12.2	9.75
Free CO <sub>2</sub>	6.83	5.06	7.05
Total hardness	92	96	108
Calcium	16.03	19.63	18.83
Magnesium	12.67	13.20	15.10
TDS	100.31	127.13	134.70
Chlorides	24	23.90	28.4
Total alkalinity	63.18	50.65	66.25
Phosphates	0.45	0.28	0.59

All the parameters are in mg/l except air and water temperature (°C), pH

Among phytoplankton community, diatoms play a very important role ecologically as they comprise of major components of producers in wetland ecosystem (Zalewski et al., 1997; Murulidhar and Yogananda Murthy, 2015). Diatoms are ubiquitous, unicellular microorganisms form the basic bulk of planktonic population in freshwaters characterized by siliceous cell wall (Round et al., 1990). Blue-greens exhibited heavy growth in polluted water bodies and dominated over Chlorophyceae and Bacillariophyceae (Paramasivam and Srinivasan, 1981; Murulidhar and Yogananda Murthy, 2015). Desmids are sensitive organisms, act as indicators of water pollution. Abundance of desmids

clearly indicates the unpolluted condition of the wetland (Sabir et al., 2007; Murulidhar and Yogananda Murthy, 2015). AsheshTiwari and Chauhan (2006) reported that, in Kithamlake, Agra, euglenoids density was maximum during summer followed by rainy and minimum during winter months. Euglenoids occur in greater number in polluted water bodies. Tripathi and Pandey (1995) have recorded maximum euglenoids during monsoon and low during post-monsoon. In the present study, Zygnemophyceae is dominant followed by Chlorophyceae.

**Table 2.** Distribution of phytoplankton in Ayyanakere lake

CHLOROPHYCEAE - Chlorococcales		CYANOPHYCEAE - Blue-greens
1	<i>Coelastrum microporum</i>	1. <i>Anabena spiroides</i>
2	<i>Pediastrum simplex</i>	2. <i>Merismopedia glauca</i>
3	<i>Scenedesmus platydiscus</i>	3. <i>Arthrospira platensis</i>
4	<i>Scenedesmus quadricauda</i>	4. <i>Oscillatoria subrevis</i>
5	<i>Tetraedon trigonum</i>	5. <i>Phormidium fragile</i>
6	<i>Tetraedon minimum</i>	6. <i>Microcystis aeruginosa</i>
7	<i>Korshikoviella limnetica</i>	7. <i>Oscillatoria auguina</i>
8	<i>Ankistrodesmus falcatus</i>	
9	<i>Micractinium pussillum</i>	
ZYGNEPHYCEAE - Desmids		BACILLARIOPHYCEAE - Diatoms
1	<i>Micrasterias inciser</i>	1. <i>Synedra ulna</i>

2	<i>Cosmarium tumidum</i>	2. <i>Pinnularia major</i>
3	<i>Closterium lunula</i>	3. <i>Navicula hustedtii</i>
4	<i>Cosmarium constactum</i>	4. <i>N. palea</i>
5	<i>Closterium accutum</i>	5. <i>N. radiosa</i>
6	<i>Euastrum rublobatum</i>	6. <i>N. rhomboides</i>
7	<i>Cosmarium constactum</i>	7. <i>Epithemia turgida</i>
8	<i>C. pseudoconnatum</i>	8. <i>Melosira granulata</i>
9	<i>C. retusiformii</i>	<b>EUGLENOPHYCEAE - Euglenoids</b>
10	<i>Desmidium baileyii</i>	1. <i>Euglena acus</i>
		2. <i>E. elatica</i>
		3. <i>E. polymorpha</i>
		4. <i>E. minuta</i>
		5. <i>Phacus tortus</i>
		6. <i>Trachelomonas robusta</i>

**Table 3.**Percentage composition of Phytoplankton during the study period

Class	Nos.	Percentage (%)
Bacillariophyceae	8	20.0
Chlorophyceae	9	22.5
Zygnemophyceae	10	25.0
Cyanophyceae	7	17.5
Euglenophyceae	6	15.0

#### IV. CONCLUSION

Plankton are important food sources of fishes specially the commercially culturing fish species. The present study shows the rich in phyto plankton diversity they are chlorococcales, diatoms, desmids, euglenoids and blue-greens .The commercially culturing fishes are almost

plankton feeders. So the lake is suitable for fish culture. Based on the present investigations the water is soft water, it is dominating phytoplankton diversity.

Ayyanakere lake harboured 40 species belonging to 27 genera is rich in phytoplankton diversity and hence productive. Based on the data on physico-chemical parameters in relation to phytoplankton distribution and abundance forms a useful tool for further ecological assessment and monitoring of water bodies. They are the risk of pollution by human anthropogenic and agricultural activities. It is suggested that continuous assessing of water body and make a plan to protect it from pollution.

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