© 2017 IJSRST | Volume 3 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Assessment of Enzymatic Stress & Protein Content in *Cirrhinus mrigala* Exposed to Thermal Power Plant Effluent

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ABSTRACT

In recent year thermal power plants are emerges as a most polluted trade. These studies indicate the power plant trade also affects water bodies as much as air. In present study fish *Cirrhinus mrigala* exposed in different concentration of thermal power plant's effluent to assess the biochemical activities like Total protein and enzymes ACP (acid phosphatase), ALP (alkaline phosphatase), AST (aspartate aminotransferases), and ALT (alanine aminotransferase). Total protein and AST, ACP, ALP level increased in liver, kidney and muscle tissue indicate stress condition in fish. ALT level decreased in liver, kidney and muscle tissue. Data found in this study suggest stress condition in fish exposed to TPP's effluent.

Keyword: *Cirrhinus mrigala,* Fish, Enzyme, Phosphatase, Transferases, protein, Thermal Power Plant Effluent,

I. INTRODUCTION

In India, major part of electricity production based on the coal based power plant followed by other sources like gas, oil, hydro, etc. India has one of the largest reserves of coal, produced about 650 million tones. Coal found in India is poor quality and hence produce high amount of ash. During production of electricity in power plant waste materials like CO2, NOx, trace gases, fly ash, bottom ash, boiler slag etc. are generated. These waste materials cause environmental pollution in terms of land use, health hazards and air, soil and water leads to environmental dangers [1],[2]. From waste material about 55.69 % of fly ash utilize in cement industry, brick manufacture, road construction and fly ash based polymer production. Rest part of waste disposed of by either dry method or wet method. In wet method, ash disposed off in slurry form by pipes in ash pond and then to the nearest river. These effluent cause water pollution and affect the water flora and fauna. A review of the toxicological literature reveals that the exposure to the toxic chemicals can produce unexpected effects in non-target organisms [3]. Effluent from power plant containing heavy metals influenced the water quality and fish physiology [4].

Fish is one of the cheapest sources of high quality proteins, contains sufficient amount of all the essential amino acids required for growth and maintenance [5]. Protein is also one of the important parameter to access the health and biological mechanism under stress [6]. With protein, enzyme like AST and ALT are used as indicator to check the stress condition of fish. Both the enzymes are mainly found in liver, kidney and muscle. Alternation in enzymes indicates the damaging of tissue. Determinations of enzymes, such as AST & ALT are considered a useful biomarker to determine pollution levels during chronic exposure [7]. Fish *Lepidocephalus thermalis* exposed to sub lethal concentration of sugar factory effluent and observed decreasing protein level of fish liver, kidney, and muscle [8]. Result obtained in *Cyprinus carpio* found elevated level in serum total protein disclosed to Cu and Ni [9].

Cyprinus carpio exposed in electroplating effluent was found to decrease level of protein in various tissues

[10]. The protein level in fish *Labeo rohita* exposed to tannery effluent, electroplating effluent, and textile effluent found significantly alternation in muscle, liver, kidney, and gill [11]. *Cirrhinus mrigala* is one of the fresh water major carp fish normally inhabited in fresh water. Aim of present study was to evaluate the total protein and enzyme activities of fish exposed in power plant effluent.

II. MATERIAL AND METHOD

1. Experiment design

The fingerlings of fresh water fish *Cirrhinus mrigala* measuring 3-5 cm in length and about 2 -3 gram were collected from the Government fish rearing farm, Ratania, Gujarat. The fish were brought in oxygenated polythene bag and transferred to the rectangular glass aquarium (30x15x12inch) having 60 liters capacity, disinfected with 0.1% $KMnO_4$. Fish acclimatized for 15 days with continuous aeration and given artificial fish food twice a day as per APHA standard.

Effluent from power plant directly collected from waste water rivulet of thermal power station. 10 fishes were exposed in 40 L of normal water served as control. Three different concentrations (20%, 50%, and 100%) of effluent were prepared with normal water. Aeration and feeding at fixed time was done throughout the experiment.

2. Sample collection

Sample water was collected in pre-cleaned plastic container. Dissolved oxygen (D.O), total solids (T.S), total dissolved solids (T.D.S) and total suspended solids (T.S.S) were determined using standard methods [12]. The temperature and pH were recorded on the spot using thermometer and pH meter. For heavy metals analysis sample collected as per APHA guideline.

3. Biochemical assay

The biochemical assay for total protein and enzyme assay AST and ALT were carried out by standard technique. Each biochemical assay was performed in triplicate set. For each assay fresh tissue collected at 5, 10, 15 and 20 days interval and stored in 4^0 C till test performed.

a. **Total protein:** Homogenize prepared in sterilized normal saline (0.9 %) in centrifuged at 2000 rpm for 20 minute and supernatant used for assay. Total protein was estimated by Folin Lowry method [13]

b. **Enzyme assay:** For enzyme assay homogenize prepared in chilled normal sucrose (0.25M) in centrifuged at 5000 rpm at 4^0 C for 15 minute. Supernatant used for AST and ALT assay. Both test were estimated by Reitman and Frankle 's 2-4 DNPH method [14]. ALP and ACP activity measured by Mod. Kind & King's method [15]

III. STATISTICAL ANALYSIS

Statistical analysis was performed by mean ± standard deviation. Data has been analyzed by using Microsoft excel.

IV. RESULT & DISCUSSION

Result

a. Water analysis

Physicochemical of sample water temperature = 29.8^0 C, pH = 8.2, turbidity 1.9 = NTU, alkalinity = 28.7 mg/l, electric conductivity = 192µS/cm, total dissolve solid = 137.6 mg/l, total suspended solids = 15.0mg/l, BOD = 21.6 mg/l, and COD = 92 mg /l. As per ICP-AES heavy metals like Al, Li, Ni, Mn, Ba, V, Ti, Zn, Fe, Cu, and many other metals like Na, Ca, K, B, Mg, Mo, S, Si, P found in effluent.

Table 1. Metal present in thermal power plant effluent compare with WHO guideline and USA standards

Metals	Present study	WHO guideline (ppm)	USA standards (ppm)
Fe	1.161	-	0.3

K	3.337	-	
Li	0.029	-	
Mn	0.019	0.5	0.05
Zn	0.016	3	5
Al	6.045	-	
B	0.347	-	
Ba	0.064	-	
Cu	0.011	2	1.3

b. Biochemical activity

In *Cirrhinus mrigala* total protein, alkaline phosphatase, acid phosphatase, AST, and ALT level shown in following figure 1-3

Liver

In liver total protein level increased in all different concentration of power plant effluent. In enzyme activity, ALP, ACP and AST level significantly increased while ALT level found decrease.

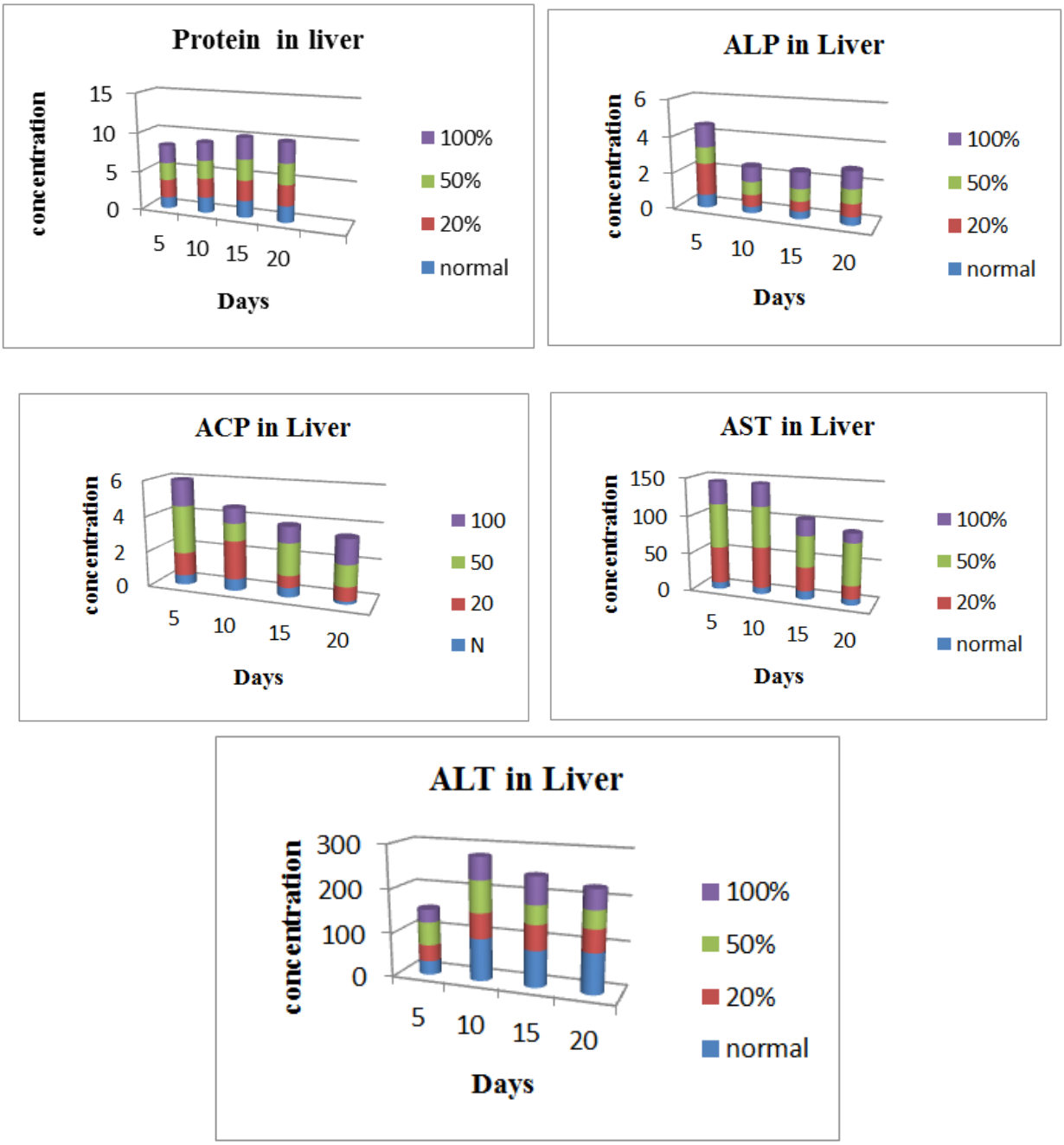

Figure 1. Protein and enzyme activity in liver tissue of *Cirrhinus mrigala* exposed to thermal power plant effluent for 4 different time period.

Kidney

In kidney protein level continuously increase in 20%, while in other concentration protein level fluctuated. In enzyme activity AST, ALT and ACP level increased and In ALP increasing level found in 50% and 100% and slightly found decreasing in 20% concentration.

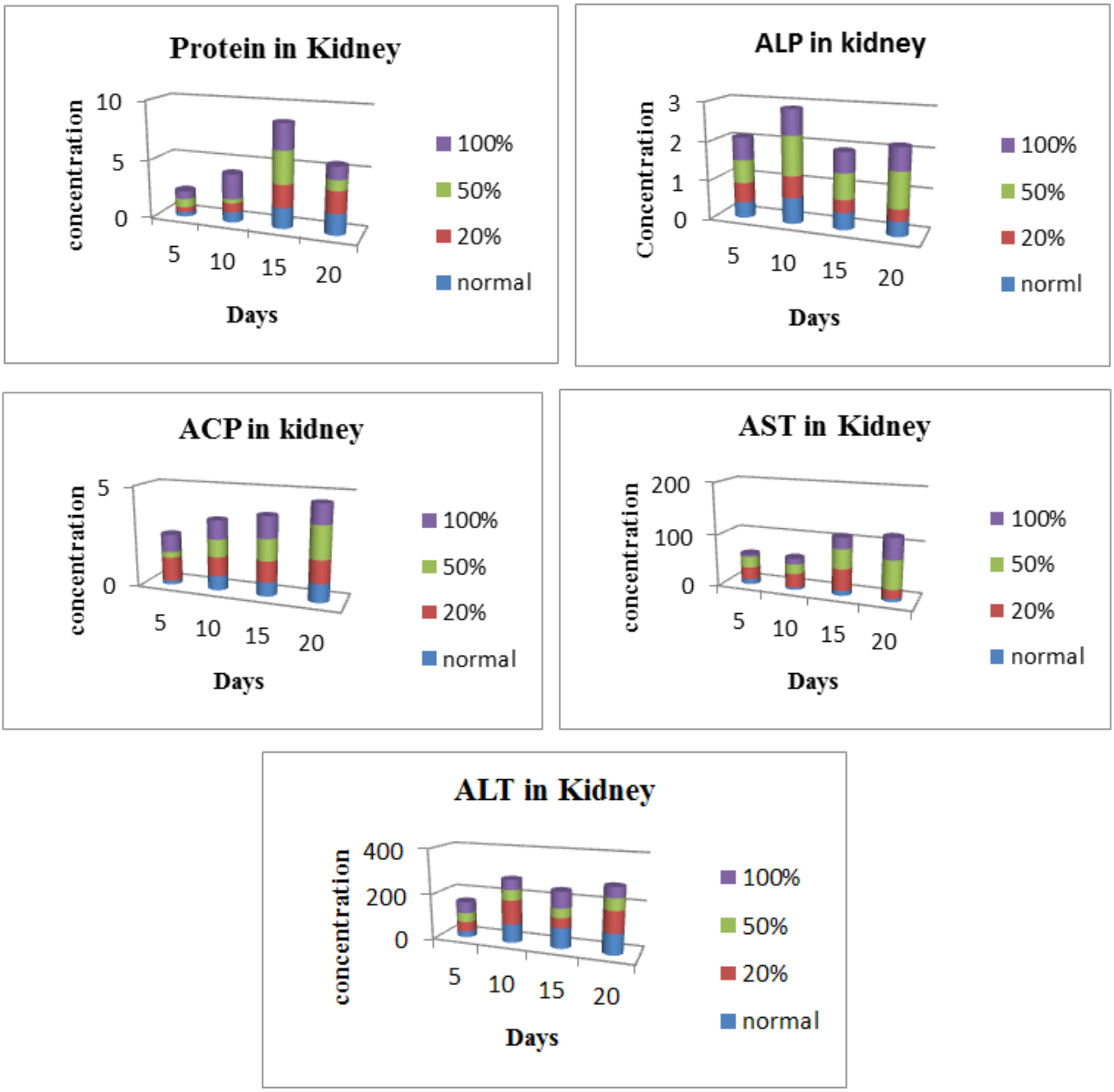

Figure 2. Protein and enzyme activity in kidney tissue of *Cirrhinus mrigala* exposed to thermal power plant effluent for 4 different time period.

Muscles

In muscles total protein, AST, and ALT level continuously increase in all concentration at different time interval. In normal condition ALP level continuously decreased and ACP level increased in muscle tissue but in exposure period both parameter shows drastically changes.

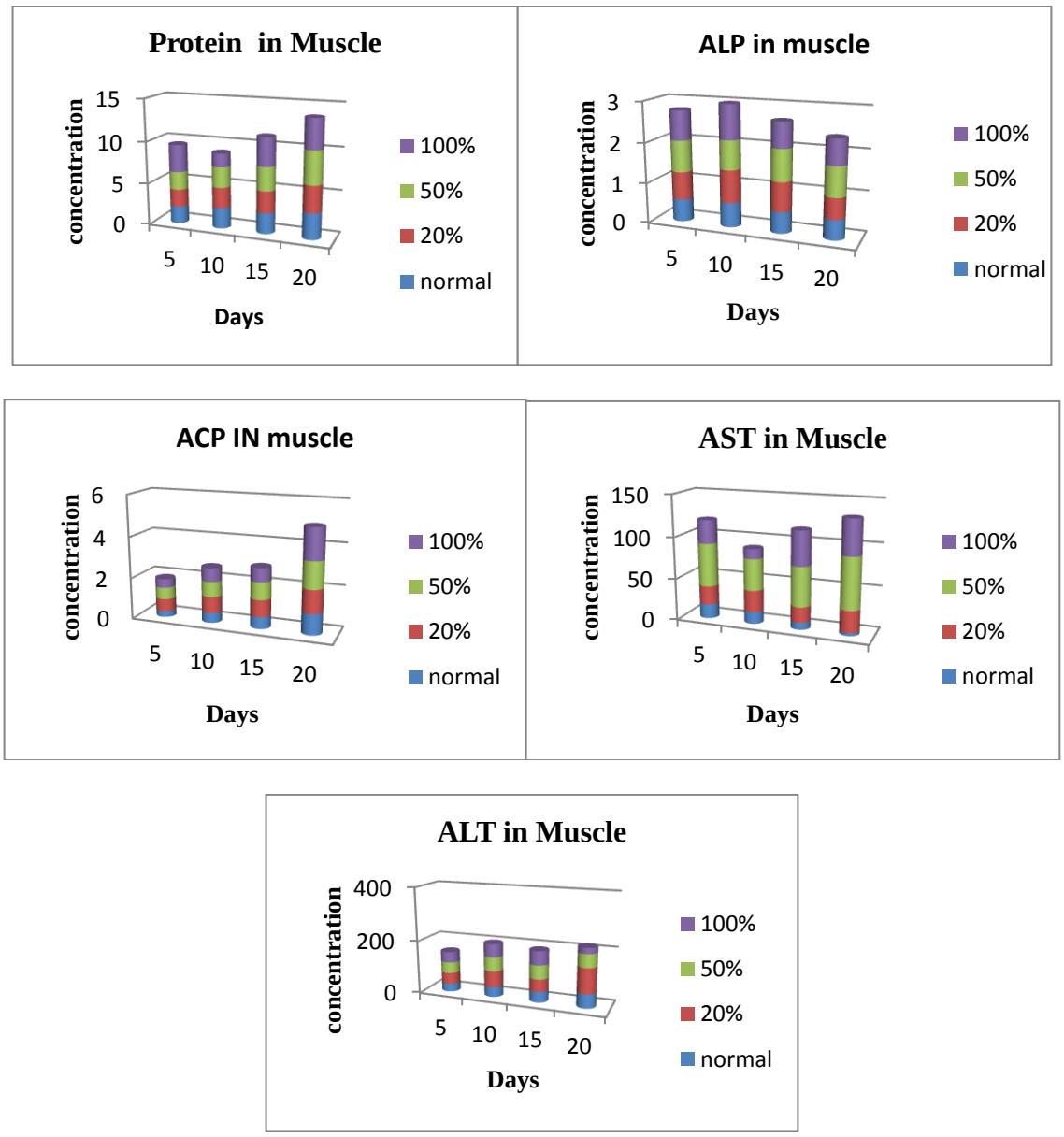

Figure 3. Protein and enzyme activity in muscle tissue of *Cirrhinus mrigala* exposed to thermal power plant effluent for 4 different time period.

V. DISCUSSION

As per the physicochemical analysis sample water is harmless to fish. But presence of the metals like Al, Cu, Mn and Fe in excess amount may harm the fish. May the physicochemical analysis prove the water is suitable for fishes but presence of heavy metals in water harmful for the fish inhabitant [16]. In present study we examined the total protein and enzyme assay on fish *Cirrhinus mrigala* at short time period. The presence of heavy metals in inlet water caused alternation in total protein and enzyme assay in experiment period. Total protein level continuously increased in fish liver, kidney, and muscle in study.

Elevation in protein level may because of prevent the stress level. The impact of heavy metals present in thermal power plant effluent and observed the elevated protein level in liver and muscle of *Channa punctatus* [16]. Freshwater fish *Channa punctatus* protein profile and reveals that increasing protein level may be help the organs for developing resistance to toxic stress [17]. In many studies researcher demonstrated the role of marker enzymes in initiating water pollution induced changes in fish species. The impact of composite tannery effluent on transaminase activity of *Poecilia reticulata* and suggested the use of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as potential marker enzymes for diagnosing liver, muscle, and gill damage in fish attributed to pollutants [18]. In present study AST level increased in liver, kidney and muscle tissue. ALT and AST are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathological changes [19]. ALT and AST catalyse the reactions of transamination of alanine, glutamine, and aspartic acids. They couple the protein, carbohydrate, and fat metabolism and tricarboxylic acid cycle under altered physiological pathological and induced environmental stress condition [20]. ACP and ALP enzymes are respectively lysosomal and brush border enzyme, alternation in these enzyme affect the metabolism system of animal. It breaks down the energy pathway from normal ATPase system, including phosphorylation. In present study acid phosphatase and alkaline phosphatase increased in liver, kidney and muscle indicate the tissue damaging. The effect of surfactants on fish and found the increased level of ALP, while in ACP they noted the initially increasing level later decline with increased surfactant concentration [21].

VI. CONCLUSION

In present study, biochemical changes are found in fish exposed to thermal power plant effluent. These changes indicate abnormal condition which further influences major changes in fish physiology.

VII. ACKNOWLEDGEMENT

The authors thankful to the Professor and Head, Department of Biosciences, Veer Narmad South Gujarat University for providing necessary laboratory facilities to carry out the work successfully.

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