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Assessing the Biochemical Responses of *Cicer arietinum* L. to Organic Pollutants in Agricultural Soil

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ABSTRACT

The present research investigates the biochemical changes in *Cicer arietinum* L. (commonly known as chickpea) in response to organic pollutants present in agricultural soil. Conducted in the vicinity of Veer Bahadur Singh Purvanchal University, Jaunpur, the study employs a combination of field experiments and biochemical assays to assess the impact of pollutants on enzyme activities, lipid profiles, and other metabolites in chickpea plants. Preliminary findings reveal significant alterations in these biochemical parameters, highlighting the plant's stress responses to environmental contaminants. The results underscore the importance of adopting eco-friendly agricultural practices to mitigate the adverse effects of soil pollution on essential crops like chickpea.

Keywords: *Cicer arietinum* L., Organic Pollutants, Biochemical Responses, Agricultural Soil, Enzyme Activities, Lipid Profiles, Sustainable Agriculture.

I. INTRODUCTION

Soil pollution, primarily from organic pollutants, poses a significant threat to agricultural sustainability and food security. Among the crops most affected by such pollution is *Cicer arietinum* L., commonly known as chickpea, which is a crucial source of protein in many diets and a vital legume in rotational cropping systems for soil fertility management. Despite its economic and nutritional significance, there is limited research that adequately addresses the biochemical responses of chickpea plants to soil pollution. Conducted in the academic context of Veer Bahadur Singh Purvanchal University, Jaunpur, this study aims to fill this gap by evaluating how different types of organic pollutants commonly found in agricultural soils affect the biochemical parameters of chickpea plants.

The escalating issue of soil pollution, especially from organic pollutants, has become a significant concern for agricultural sustainability and food security. One of the crops severely affected by such pollution is *Cicer arietinum* L., commonly known as chickpea, a critical source of protein in various diets and an essential legume in soil fertility management through rotational cropping. Despite its economic and nutritional importance, there is a scarcity of research focused on understanding how chickpea plants biochemically respond to soil pollution. Conducted at Veer Bahadur Singh Purvanchal University in Jaunpur, this study aims to fill this research gap. The study focuses on identifying the biochemical changes, particularly in enzyme activities, lipid profiles, and other metabolites, that occur in chickpea plants when exposed to organic pollutants in agricultural soils. The findings of this research are crucial for devising mitigation strategies to counteract the adverse effects of soil pollution and thereby protect the yield and quality of one of the world's most consumed legumes. This study also aims to offer empirical evidence to support the urgent need for more sustainable agricultural practices and soil pollution control measures.

Building on this foundation, the research aims to offer a more nuanced understanding of plant-pollutant interactions, particularly within economically important crops like chickpea. This becomes even more pertinent in the current era, where rapid industrialization and the extensive use of agrochemicals are constantly changing the composition of the soil, often to the detriment of crop health and yield. The study goes beyond mere identification of biochemical changes, seeking to understand how these alterations might impact the long-term viability of chickpea crops, including nutritional quality and resistance to diseases. In addition, by situating this study within the academic context of Veer Bahadur Singh Purvanchal University, we aim to foster academic-industry partnerships that can lead to practical, implementable solutions for farmers and policymakers alike. Overall, this research aspires to be a pivotal step in promoting more sustainable, eco-friendly agricultural practices that not only preserve the integrity of our soil but also ensure the long-term sustainability of vital crops like chickpea.

II. METHODS AND MATERIAL

The objective of this study is to assess the biochemical and physiological responses of three different species of chickpea (Cicer arietinum) to soil pollutants, namely heavy metals (Lead and Cobalt), industrial effluents, and pesticide treatments.

Plant Material and Preparation

Source: Three different chickpea species will be procured from the Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India.

Preparation:

- Seed surfaces will be sterilized using a dilute solution of Sodium hypochlorite (NaOCl) to prevent fungal contamination.
- The sterilized seeds will then be rinsed thrice with distilled water.

Soil Preparation and Treatment

Control Group

- Seeds will be sown in earthen pots containing equal quantities of washed, acid-treated, loamy sand soil.
- Soil will be treated with Evans and Nason nutrient solution.

Treatment Groups

- Heavy Metal Treatment: Soil will be treated with solutions of cobalt chloride and lead acetate at concentrations of 50, 100, and 150 µm/L.
- Industrial Effluent Treatment: 50 liters of industrial sewage water collected from SIDA Satharia, Jaunpur will be used for soil contamination.
- Pesticide Treatment: Standard pesticide solutions prepared at concentrations of 5, 10, and 20 µm/L.

Experimentation Procedure

- All sets of experiments will be conducted under controlled day-night cycles.
- Treatment and nutrient solutions will be applied twice a week.

Sampling

Samples will be taken from two-week-old seedlings for chemical and biochemical analyses.

Soil Analysis

Both pre- and post-germination soil will be analyzed for:

Particle size Plant Analysis

- pH value Seed germination
- Acid content Seedling growth
- Inorganic materials Growth and development
- Organic materials Seedling growth
- Microbial analysis

Biochemical Analysis

The following parameters will be examined:

- pH value of homogenized materials
- Protein content
- Soluble sugar content
- Chlorophyll assay (Chl-a and Chl-b)
- Proline content
- Ascorbic acid content
- Nitrate reductase activity
- Carbonic anhydrase activity

Growth Parameters

Parameters such as germination rate, plumule length, radicle length, and the number of lateral roots will be observed 14 and 21 days after seedling emergence.

III. RESULTS AND DISCUSSION

According to the findings shown in Table 1 and displayed in Plate VI, a 1% dosage of oxygenated peptone was most effective in increasing seed germination percentage, root length, shoot length, biomass, and vigor index when compared to a control and other oxygenated peptone doses (0.5%, 1.5%, and 2%). At a dosage of 1% oxygenated peptone, all the findings are statistically significant. The majority of the other findings lack significance. Therefore, for the current study, 1% oxygenated peptone dosage was chosen for further tests.

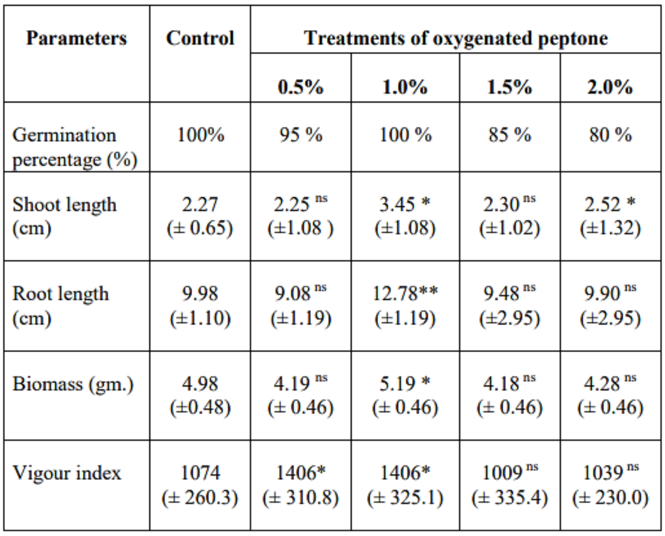
Seed germination and seedling growth :

It is generally recognized that GA has an impact on germination. Additionally, it is well documented how oxygen and nitrogen affect seed germination. The research on peptone-enriched oxygen, however, is scant. Furthermore, oxygenated peptone can be utilized in organic agricultural situations, whereas GA cannot. Consequently, a comparison of the effects of GA and oxygenated peptone was conducted.

A. Morphological parameters:

Consuming water and a waking or activation of protoplasm are the two components of the initial stage of germination. Protein parts of the cells that were created as the seeds grew became dormant as they matured. The mechanism is reactivated and protein synthesis starts up again after a water absorption. Enzymes and hormones emerge, start to break down reserve materials in the storage tissues, and deliver the byproducts to the embryo's growth sites. The timing of the activation of certain enzymes and the control of their activity play a role in the metabolic pattern that takes place during germination. Four groups of plant hormones work together to exert control: cytokinins, which govern organ differentiation, auxins, which regulate root development and growth, gibberellins, which regulate protein synthesis, and inhibitors like abscisic acid, which prevent germination. Some plants are thought to use ethylene for a control function. In certain cases, the following three restrictions are combined to force difficult-to-germinate seeds out of dormancy.

Table 1 : Effect of pre-sowing soaking treatment of different doses of oxygenated peptone on seeds of chickpea (*Cicer arietinum* L. cv. Vijay) during germination (6 DAS).



Parameters	Control	Treatments of oxygenated peptone			
		0.5%	1.0%	1.5%	2.0%
Germination percentage (%)	100%	95 %	100 %	85 %	80 %
Shoot length (cm)	2.27 (± 0.65)	2.25 ^{ns} (±1.08)	3.45 * (±1.08)	2.30 ^{ns} (±1.02)	2.52 * (±1.32)
Root length (cm)	9.98 (±1.10)	9.08 ^{ns} (±1.19)	12.78** (±1.19)	9.48 ^{ns} (±2.95)	9.90 ^{ns} (±2.95)
Biomass (gm.)	4.98 (±0.48)	4.19 ^{ns} (± 0.46)	5.19 * (± 0.46)	4.18 ^{ns} (± 0.46)	4.28 ^{ns} (± 0.46)
Vigour index	1074 (± 260.3)	1406* (± 310.8)	1406* (± 325.1)	1009 ^{ns} (± 335.4)	1039 ^{ns} (± 230.0)

PHOTOPLATE – VI :

The influence of a pre-sowing soaking treatment with varying concentrations of oxygenated peptone (0.5 percent, 1 percent, 1.5 percent, and 2 percent) on the germination of chickpea (*Cicer arietinum* L. cv. Vijay) on the sixth day after sowing was investigated.

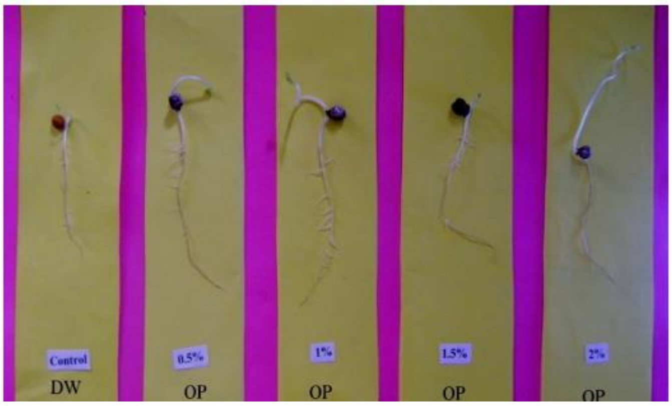


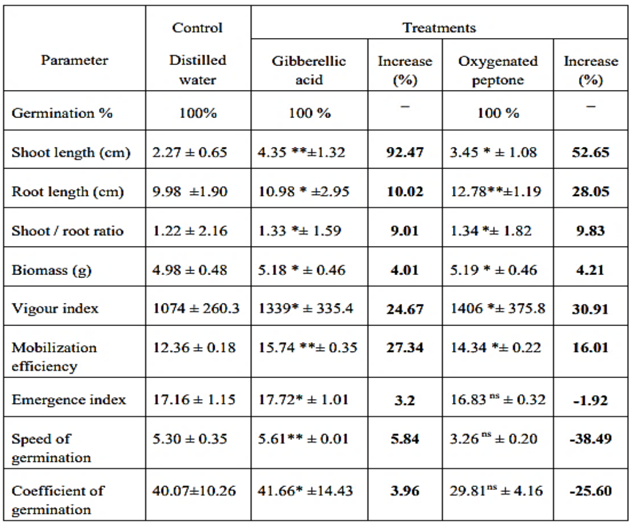
Figure 1: Effect of pre-sowing soaking treatment of different doses of oxygenated peptone

Pre-treatment of seeds, often known as "seed priming," has an osmoconditioning effect. It is a physiological technique that enhances seed functionality and promotes quicker and more coordinated germination. It has been discovered that this treatment enhances seedling establishment and germination. It has been shown that seed priming enhances germination and emergence in seeds of several crops. For the germination and subsequent growth of maize, rice, and chickpea, hydro-priming is beneficial. The best technique for enhancing onion seed germination was hydro-priming. Pre-sowing therapy improves vigor and germination. Compared to unprimed seeds, primed seeds showed increased germination rates. He continued by saying that priming may be utilized to boost germination efficiency.

It has been shown that plant growth regulators (PGRs) play a pivotal role in the coordination of plant responses. PGRs also aid in the germination process. The significance of gibberellic acid in the germination of seeds is well-documented. There is evidence that gibberellic acid improves germination rates and the growth of seedlings. Exogenous GA increases amylase production in the barley endosperm.

Abundant oxygen is necessary during germination for the quick breakdown of complex reserve food into simpler chemicals, which in turn demands a high rate of respiration to release more ATPs essential for cellular metabolism. Activation and hydration of mitochondrial enzymes in the citric acid cycle and the electron transport chain cause an initial surge in oxygen use during germination.

Table - 2 : Effect of soaking chickpea (*Cicer arietinum* L. cv. Vijay) seeds in gibberellic acid and oxygenated peptone before to planting on morphological parameters as of the sixth day



Parameter	Control	Treatments			
	Distilled water	Gibberellic acid	Increase (%)	Oxygenated peptone	Increase (%)
Germination %	100%	100 %	–	100 %	–
Shoot length (cm)	2.27 ± 0.65	4.35 **±1.32	**92.47**	3.45 * ± 1.08	**52.65**
Root length (cm)	9.98 ±1.90	10.98 * ±2.95	**10.02**	12.78**±1.19	**28.05**
Shoot / root ratio	1.22 ± 2.16	1.33 *± 1.59	**9.01**	1.34 *± 1.82	**9.83**
Biomass (g)	4.98 ± 0.48	5.18 * ± 0.46	**4.01**	5.19 * ± 0.46	**4.21**
Vigour index	1074 ± 260.3	1339* ± 335.4	**24.67**	1406 *± 375.8	**30.91**
Mobilization efficiency	12.36 ± 0.18	15.74 **± 0.35	**27.34**	14.34 *± 0.22	**16.01**
Emergence index	17.16 ± 1.15	17.72* ± 1.01	**3.2**	16.83 ^{ns} ± 0.32	**-1.92**
Speed of germination	5.30 ± 0.35	5.61** ± 0.01	**5.84**	3.26 ^{ns} ± 0.20	**-38.49**
Coefficient of germination	40.07±10.26	41.66* ±14.43	**3.96**	29.81^{ns} ± 4.16	**-25.60**

The germination reactions of seed to GA and oxygen have not been compared in any studies. Therefore, priming of seed is done in the current experiment producing germination 6 days after treatment. Overall, it was evident that germination occurred six days following treatment. The overall picture demonstrated that both treatments improved biomass, shoot/root ratio, root length, and shoot/shoot ratio. According to Riley (1987), GA directly affects stem elongation. Accordingly, treatment with GA produced higher improvements in shoot length (92.47%) than did treatment with oxygenated peptone (52.65%). This is corroborated by Jamil and Rha's (2007) finding, which showed that under GA treatment, shoot length and root length increased to their highest levels. This suggests that shoot growth is more favorable than root growth, as seen by the 92.47% rise in shoot length and the 10.02% increase in root length. However, the oxygenated peptone treatment (28.08%) causes a greater increase in root length than the GA therapy (10.02%). Evidently, GA had a lower shoot/root ratio (9.01%) than oxygenated peptone (9.83%). By providing soluble nitrogen and oxygen, it seems that oxygenated peptone helps the growth of the root system rather than the shoot system during germination, resulting in a seedling that is well-established with an expanded root system. The biomass also outperformed GA (4.01%) with an increase of 4.21% in oxygenated peptone treatment.

Due to the fact that the Vigour Index (VI), which is based on germination percentage, root length, and shoot length, was higher in oxygenated peptone at the same time. However, GA (27.34%) demonstrated a higher degree of mobilization efficiency (ME) than oxygenated peptone (16.01%). Interestingly, seeds treated with GA exhibited positive values for the Emergence Index (EI), Speed of Germination (SG), and Coefficient of Velocity of germination (CVG), but seeds treated with oxygenated peptone showed negative values.

Sharma et al. (1984) suggested that an increase in root and apical meristem tissue cell division and proliferation may be the cause of the improved seedling vigor. According to Poljakoff-Mayber (1978), greater seedling vigor and biomass always result in the creation of a robust and healthy crop stand, which in turn results in an improvement in crop yield. From this perspective, it is extremely important to note the improved seedling vigor and biomass shown under the oxygenated peptone treatment condition.

According to Photoplate VII, oxygenated peptone responded to pre-sowing soaking treatment more favorably than GA. The plate makes it abundantly evident that, despite the fact that the oxygenated peptone treatment condition resulted in shorter shoots, these shoots seemed more robust and strong with dark brown testa than the fragile, elongated shoots with light-colored testa under GA treatment. Testa's dark brown color is the consequence of enzymatic browning brought on by increased

PHOTOPLATE – VII

Chickpea (*Cicer arietinum* L. cv. Vijay) seed germination and seedling development under control, gibberellic acid treatment, and oxygenated peptone treatment as of day six.

A : Seed germination

Figure 2: Seed Germination

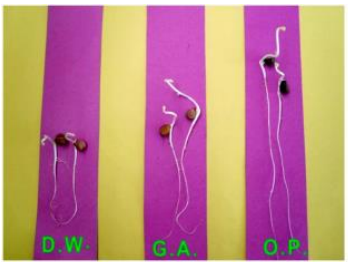


Figure 3: Seedling growth

Activity of polyphenol oxidase (PPO) and oxygen supply from oxygenated peptone. PPO is said to catalyze the hydroxylation of monophenols to diphenols, which are then transformed into quinines by molecular oxygen, according to Mathew and Parpia (1971). These quinines are exceedingly reactive and insecure. They interact with proteins, amino acids, and each other to form brown, black, or red heterogenous polymers that change color. The enzymatic browning of the testa of oxygenated peptone treated seeds may be caused by an increase in polyphenol oxidase activity during germination caused by oxygenated peptone treatment. But more evidence is required to support this.

According to Taiz and Zeiger (2002), the oxygen availability affects how quickly seeds germinate. Lack of oxygen impairs electron transport during respiration and reduces the production of ATP (Crawford and Brandle, 1996). By seed priming, oxygenated peptone has been discovered to increase the germination processes in solanaceous fruit and vegetable species such as tomato, brinjal, and chilli (Patil et al., 2008). Wijte and Gallagher (1996) discovered that during the first phases of salt marsh plant development, neither plumule or root growth took place under anoxia. In addition to oxygen, the oxygenated peptone employed in the current study's pre-soaking treatment of seeds also includes peptone, a soluble form of nitrogen. Peptone gives seedlings soluble nitrogen that is necessary for germination and improved development. Bose and Pandey's (2003) findings that soaking seeds in a variety of nitrate salts before planting okra had a good effect on germination and growth lend credence to this.

A. Biochemical constituents

The impact of pre-soaking chickpea seeds (8 DAS) in GA and oxygenated peptone on biochemical components is shown in Figure 4. Total carbs increased as a result of both treatments, but oxygenated peptone therapy had a 20.43% advantage. Because oxygenated peptone also included soluble nitrogen in the form of peptone in addition to oxygen, the number of soluble proteins rose dramatically (68.11%).

The chickpea's nutritional value is improved by proteins. According to Fait et al. (2006), the germination of seeds and the use of storage reserves are controlled separately. In the current study, soluble proteins and total carbs increased coupled with a rise in amylase and protease enzyme activity. Cerium's impacts on aubergine seed germination and seedling development under cold stress were researched by Qiu et al. in 2005. They discovered that cerium improved seed vigor and seedling development, reducing the negative impacts of cold stress. They observed that these effects could be strongly related to an increase in the quantity of sugar and soluble proteins. When chickpea seeds germinated, Fernandez and Berry (1988) saw a significant improvement in the nutritional quality thanks to higher protein and ascorbic acid levels. Under the impact of both therapies, nucleic acids such as DNA and RNA also increased, with GA having the advantage.

B. Enzyme activity

The effects of pre-sowing treatment with GA and oxygenated peptone on the enzyme activity of chickpea seeds (8 DAS) are shown in Table 3. When compared to the control, amylase, catalase, and protease activity increased in both treatments, with oxygenated peptone exceeding the other two by 80.39%, 106%, and 90%, respectively. Catalase is an oxidative enzyme. The increased amylase and protease activity is strongly related to the greater amounts of proteins and carbs. On the other hand, it was observed by Misra and Kar (1990) and Ashton (1976) that a rise in protease activity was followed by a decline in storage and enzymatic protein.

GA derived from outside sources promotes amylase activity. The aleurone layer of the endosperm is vulnerable to GA. GA causes the release of amylase and protease. These enzymes help food that has been stored to be reduced to its most basic forms. These are then sent to the growing embryo where they act as a source of energy for development. So both GA and oxygen aid in the germination of seeds. Gibberellic acid, according to Chen and Chang (1972), facilitates germination by boosting amylase synthesis. Seed germination and plant stress tolerance may both benefit from PGRs. The reserve materials are mobilized together with the enzyme activation, according to Muhyaddin and Wiebe (1989), and are subsequently delivered to the embryo through osmotic conditioning. The seedlings get stronger as a consequence of the embryo's growth. Super-oxygenation could be advantageous in the early stages of the germination process. According to Agarwal and Kharlukhi (1987), it has been shown that seeds that have been matured, either naturally or artificially, have increased.

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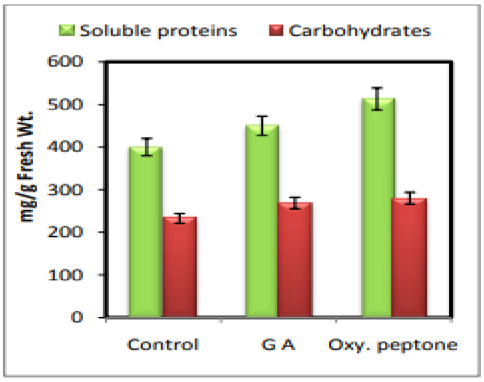

Figure 4: Values are mean of five determinations.

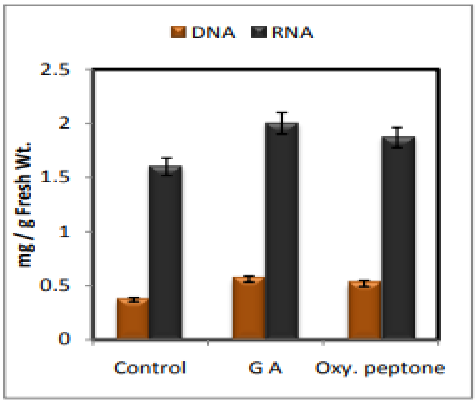

Figure 5: The error bars represent percent error.

Table 3 : Effect of soaking chickpea (*Cicer arietinum* L. cv. Vijay) seeds in GA and oxygenated peptone before to planting on enzyme activity as of day eight of germination.

Parameters	Control Distilled water	Treatments			
		Gibberellic acid	Increase (%)	Oxygenated peptone	Increase (%)
Amylase (mg maltose/ 5 min/g fr. wt.)	10.1 (± 0.1)	16.1* (±1.9)	**59.40**	18.4* (± 1.50)	**80.39**
Catalase (µ mole H_2O_2/ min/g fr. wt.)	1.86 (±0.51)	3.54* (±0.3)	**0.90**	3.84* (±0.27)	**1.06**
Protease (µg tyrosin/hr/ mg protein)	0.10 (±0.005)	0.17* (±0.01)	**70**	0.19* (±0.001)	**90**

Protease activity in Vigna radiata and Cicer arietinum. Nitrogen metabolism is altered under hypoxic circumstances. The present experimental setup comprises a supply of nitrogen in the form of soluble organic nitrogen (peptone) as well as super-oxygenation. Such a booster dose may have accelerated the enzymes' synthesis and raised their activity. With diverse responses for different parameters, it can be said that the pre-sowing soaking treatment with oxygenated peptone and gibberellic acid is beneficial in boosting chickpea germination potential. However, oxygenated peptone can be used in organic farming even if gibberellic acid cannot. Therefore, for sustainable agriculture, the pre-sowing soaking treatment with oxygenated peptone is more advantageous and cost-effective.

IV. CONCLUSION

The research concludes with strong evidence that soil contaminants, such as pesticides, industrial effluents, and heavy metals (Lead and Cobalt), have a significant negative influence on the development and biochemical characteristics of several species of chickpea (Cicer arietinum). Reduced seed germination, slowed development, changed branching patterns, and major changes in biochemical indicators including protein content, soluble sugars, and stress-related substances like proline are some of the detrimental impacts. Additionally, the study shows that these contaminants affect important soil properties including pH, microbial activity, and nutrient content, hinting to possible long-term environmental effects. The results highlight the urgent need for stricter laws and efficient corrective actions to reduce soil contamination and promote sustainable agriculture practices. The effectiveness of different soil remediation procedures and genotypic alterations brought on by pollution might be the main topics of future study.

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