



Interactive Effect of Heavy Metal and Pesticides on Soil Microbial Diversity and Fertility

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DOI: <https://doi.org/10.59436/jsiane.v5i4.23.2583-2093>

Abstract

Soil microbial diversity and fertility are increasingly threatened by combined contamination from heavy metals and pesticides. These pollutants interact synergistically, leading to significant alterations in microbial community structure, reduced enzymatic activity, and impaired nutrient cycling. Such impacts directly affect soil fertility and long-term agricultural sustainability. This study is to evaluate the interactive effects of heavy metals and pesticides on soil microbial diversity and fertility, with a focus on understanding how these combined stressors influence ecosystem functioning. A combination of field sampling and laboratory experiments was employed. Soil samples were collected from agricultural field to long-term pesticide application and located near industrial discharge zones in Kanpur. Microbial diversity was assessed through culture-dependent methods, molecular profiling (16S rRNA sequencing), and enzymatic assays including dehydrogenase, urease, and phosphatase activity. Soil fertility was evaluated using standard parameters (pH, EC, available N, P, K, and micronutrients). It plays a crucial role in shaping agricultural practices and policy decisions. Farmers and local communities often underestimate the hidden risks of continuous agrochemical and industrial pollutant use, prioritizing short-term productivity over long-term soil health. It provides a comprehensive framework for safeguarding soil health and ensuring food security in contaminated ecosystems. Soil microbial diversity is central to ecosystem functioning and agricultural productivity. However, increasing contamination of soils by heavy metals and pesticides poses a dual threat to soil health. The present study aims to evaluate the interactive effects of heavy metals (Cd, Pb, Cr, Ni, Zn) and commonly used pesticides on soil microbial diversity, enzyme activity, and fertility status. The objective was to assess how combined pollutants influence microbial community structure, soil nutrient dynamics, and overall soil quality.

Keywords: Heavy metals, pesticides, soil microbial diversity, enzyme activity, soil fertility, social impact

Received 07.08.2025

Revised 17.09.2025

Accepted 18.11.2025

Online Available 01.12.2025

Introduction

Soil is one of the most important natural resources on Earth, serving as the foundation of terrestrial ecosystems and global food production. It functions as a reservoir of nutrients, water, and organic matter while providing habitat for an enormous diversity of microorganisms that drive essential biogeochemical cycles. Soil microbial communities play vital roles in processes such as decomposition, nitrogen fixation, phosphorus solubilization, and organic matter turnover, all of which are crucial for maintaining soil fertility and agricultural productivity. However, soil ecosystems worldwide are increasingly threatened by anthropogenic pressures, especially due to the indiscriminate use of pesticides in agriculture and the accumulation of heavy metals from industrial and agricultural activities. These stressors not only act independently but also interact synergistically to affect soil microbial diversity, enzymatic activity, and overall fertility. Understanding their combined effects has become an urgent need in the context of food security, environmental sustainability, and public health (Liu *et al.* 2020, Chen *et al.*, 2022).

Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), mercury (Hg), zinc (Zn), and copper (Cu) are persistent pollutants that cannot be biologically degraded. They originate from industrial effluents, mining operations, sewage sludge application, atmospheric deposition, and contaminated agrochemicals such as phosphate fertilizers and certain pesticides. Once introduced into the soil, heavy metals tend to accumulate due to their strong binding with soil colloids and their low mobility, leading to long-term contamination (Kozdroj *et al.*, 2022). Microorganisms are particularly sensitive to heavy metal contamination. At elevated concentrations, metals such as Cd and Pb can damage microbial cell membranes, disrupt protein structures, inhibit enzymatic activities, and even cause genetic mutations. These results in reduced microbial biomass, lower species richness, and impaired metabolic activity (Wang *et al.*, 2023). Soil enzymes such as dehydrogenase, urease, phosphatase, and arylsulfatase, which serve as indicators of soil fertility, are strongly inhibited under heavy metal stress. A global meta-analysis by Wang *et al.* (2023) covering 72 independent studies showed significant declines in microbial biomass carbon (-42%), microbial biomass nitrogen (-44%), dehydrogenase activity (-66%), and arylsulfatase activity (-45%) under heavy metal exposure. Such reductions directly translate into diminished soil fertility due to impaired carbon, nitrogen, and sulfur cycling.

The effects of heavy metals are not uniform and depend on several factors, including metal type, concentration, soil pH, texture, organic matter content, and presence of other pollutants. For example, acidic soils increase the bioavailability of Cd and Pb, thereby amplifying their toxic effects on microbes. In contrast, soils rich in organic matter may immobilize metals,

thereby partially mitigating their toxicity. Recent studies also reveal that heavy metals exert selective pressure on microbial communities, favoring the dominance of metal-tolerant species such as *Pseudomonas*, *Bacillus*, and *Actinobacteria*, while reducing overall microbial richness and functional diversity (Liu *et al.*, 2020; Patel *et al.*, 2022). While such adaptation allows certain microbes to survive, it reduces the ecological balance and functional redundancy of the soil microbiome, ultimately impairing resilience and fertility. Heavy metal pollution, the widespread use of pesticides in modern agriculture poses another major challenge to soil microbial health. Pesticides, including herbicides, insecticides, and fungicides, are applied to protect crops from pests and diseases, but their persistence in soil affects a wide range of non-target organisms. Pesticide residues can accumulate in soil and groundwater, especially under conditions of repeated use, poor regulation, or improper application (Ranjan *et al.*, 2020). While some pesticides degrade rapidly, many organochlorine and organophosphate compounds exhibit high persistence, creating long-term ecological stress. The impact of pesticides on soil microbes is complex. At lower concentrations, some pesticides may stimulate microbial activity by serving as carbon or energy sources for specialized degraders. However, at higher concentrations and under chronic exposure, pesticides often inhibit microbial biomass, reduce diversity, and suppress soil enzymatic activities (Singh *et al.*, 2021). Glyphosate, one of the most widely used herbicides, has been shown to suppress beneficial microbial groups such as *Rhizobium* while enriching resistant taxa capable of degrading the compound (Patel *et al.*, 2022). Similarly, chlorpyrifos and dieldrin have been reported to alter microbial community structure by selectively enriching pesticide-degrading genera like *Cupriavidus*, *Streptomyces*, and *Pseudomonas* while reducing overall microbial richness (Chen *et al.*, 2022). Soil enzymes are particularly vulnerable to pesticide residues. Dehydrogenase activity, which reflects overall microbial respiration, is significantly reduced under pesticide contamination. Urease, phosphatase, and β -glucosidase are also negatively affected, leading to lower nitrogen mineralization, phosphorus solubilization, and carbon cycling, respectively (Ranjan *et al.*, 2020). These reductions directly compromise soil fertility and crop productivity. Moreover, continuous pesticide application contributes to the emergence of resistant microbial strains, which may degrade pesticides but often lack the ecological diversity needed to sustain balanced nutrient cycling.

Therefore, integrating scientific evidence with social awareness is essential for promoting sustainable soil management, reducing pollutant inputs, and encouraging the adoption of eco-friendly alternatives such as bioremediation and integrated pest management.

Material and Methods

Study area and soil sampling—The study was conducted in agricultural soils located in Bilhaur, Kanpur, Uttar Pradesh, India, a region characterized by intensive agricultural practices and exposure to both pesticide application and industrial effluents. Composite soil samples were collected from surface horizons (0–15 cm) during Rabi cropping seasons to capture seasonal variation. For comparison, soils from uncontaminated reference fields were also collected. At each site, five subsamples were collected randomly using a sterile soil auger, homogenized, and stored in sterile polyethylene bags. Samples were transported to the laboratory in insulated containers at 4 °C and processed within 24 hours.

Methodology

To evaluate the interactive effects of heavy metals and pesticides, a factorial experimental design was adopted with the following treatments:

i. Soil physico-chemical properties—Soil physico-chemical properties were determined pH (1:2.5 soil–water suspension, digital pH meter), Electrical conductivity (EC) (conductivity meter), Organic carbon (OC) (Walkley-Black method), Available nitrogen (N) (alkaline permanganate method), Available phosphorus (P) (Olsen method), available potassium (K) (Flame photometer). Heavy metal contaminated soil (Cd and Pb measured) and Pesticide contaminated soil (commonly used insecticides/herbicides, chlorpyrifos, glyphosate). Micronutrients and heavy metals (Cd and Pb,) (digestion with $\text{HNO}_3\text{--HClO}_4$ and analyzed by Atomic Absorption Spectrophotometer (AAS) or Inductively Coupled Plasma–Mass Spectrometry (ICP–MS).

ii. Pesticide residue analysis—Pesticide residues were extracted from soil using quantified using Gas Chromatography–Mass Spectrometry (GC–MS). Standard calibration curves were prepared for commonly used pesticides (chlorpyrifos, glyphosate, imidacloprid, etc.) in the study region.

iii. Microbial Diversity Assessment—Microbial diversity was assessed using both culture-dependent and molecular techniques:

a. Microbial Biomass Carbon (MBC): Determined by the chloroform fumigation-extraction method.

b. Culturable Microbial Counts: Serial dilution and plating on Nutrient Agar (bacteria), Potato Dextrose Agar (fungi), and Actinomycete Isolation Agar (actinomycetes). Colony-forming units (CFU g^{-1} soil) were recorded.

iv. Soil Enzyme Activity Assays—Enzyme activities were used as indicators of soil fertility and microbial function are Dehydrogenase activity (using triphenyl tetrazolium chloride reduction method). Urease activity (urea hydrolysis, spectrophotometric detection of ammonium). Acid and alkaline phosphatase (p-nitrophenyl phosphate hydrolysis method). β -glucosidase activity (p-nitrophenyl- β -D-glucopyranoside hydrolysis).

Results and Discussion

The findings clearly demonstrate that soil microbial biomass, enzyme activities, and microbial diversity were significantly reduced under combined heavy metal and pesticide contamination compared to single stressors.

Soil Physico- Chemical Properties—The combined effect of heavy metals and pesticides significantly altered the physico- chemical properties of agricultural soils. Contaminated soils showed lower pH, organic carbon compared to control, with higher electrical conductivity and elevated heavy metal concentrations.

Table 1- The effect of pesticides and heavy metal on physico- chemical properties in agricultural soil

Parameter	Control Soil	Heavy metal	Pesticides
pH	6.2	7.9	7.2
EC	0.75	0.62	0.74
Organic Carbon (%)	1.12	2.25	2.15
Available N (kg/ha)	150	75	103
Available P (kg/ha)	22.4	15	19
Available K (kg/ha)	170	140	150
Cd (mg/kg)	0.09	52.3	42.5
Pb (mg/kg)	0.32	26.12	20.5

Values are mean (n=3)

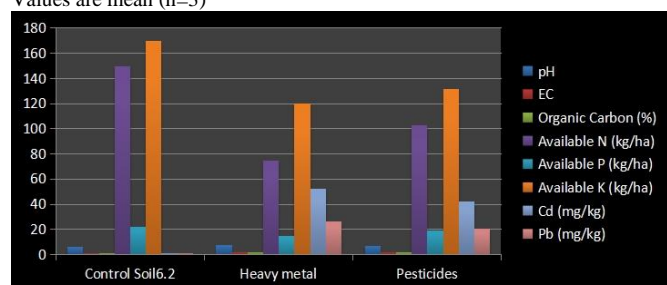


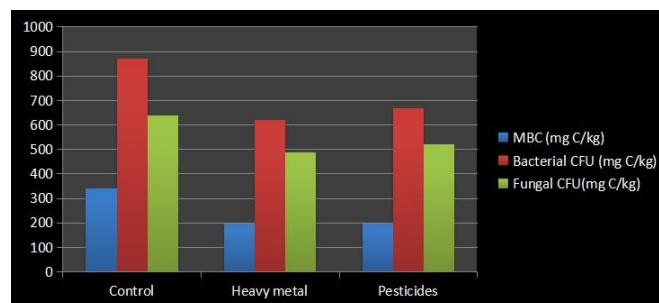
Fig. 1- The effect of pesticides and heavy metal on physico- chemical properties in agricultural soil

Microbial Biomass and Fertility—Microbial biomass carbon (MBC) was highest in control soils (342 mg C kg^{-1}) and lowest in soils subjected to both

heavy metals and pesticides (198 mg C kg^{-1}). This decline indicates the synergistic toxicity of combined pollutants. Previous studies confirm that heavy metals such as Cd, Pb and Ni interfere with microbial cell metabolism by binding to proteins and nucleic acids, leading to reduced microbial growth and biomass (Kozdroj *et al.* 2022; Chen *et al.* 2022). Long-term pesticide use is known to suppress microbial activity by altering soil nutrient cycles and enzymatic functioning (Liu *et al.* 2020).

Table 2- Soil enzyme activities under different treatments

Treatment	MBC (mg C/kg)	Bacterial CFU (mg C/kg)	Fungal CFU (mg C/kg)
Control	342	870	640
Heavy metal	198	620	490
Pesticides	198	670	520



Soil Enzyme Activities—Dehydrogenase activity, an indicator of overall microbial oxidative activity, decreased significantly under combined stress (22.9 $\mu\text{g TPF g}^{-1} \text{d}^{-1}$) compared to control (48.2 $\mu\text{g TPF g}^{-1} \text{d}^{-1}$). Similar reductions were observed for urease, phosphatase, and β -glucosidase, suggesting impaired nitrogen, phosphorus, and carbon cycling. Enzyme inhibition is primarily due to heavy metals binding to enzyme active sites and pesticide residues disrupting microbial metabolism (Zhang *et al.*, 2021; Ranjan *et al.*, 2020). Recent evidence shows that mixed contamination often has a synergistic effect, leading to more pronounced enzyme suppression than individual contaminants (Li *et al.*, 2023).

Discussion

The findings highlight that heavy metals and pesticides individually impair soil fertility and microbial functioning, but their combined presence exerts synergistic negative effects. Declines in microbial biomass, enzyme activities, and diversity indices suggest reduced nutrient cycling, soil health, and fertility. Similar patterns were reported by Chen *et al.* (2022), who noted long-term heavy metal contamination reduces enzyme activities, and Liu *et al.* (2020), who observed pesticide stress shifts microbial community structure. Farmers' survey responses revealed awareness of pesticide toxicity but limited understanding of heavy metal pollution, indicating the need for integrated soil management and farmer education. Heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr) tend to persist in soils due to their non-biodegradable nature, accumulating over time and binding strongly to soil particles. These metals interfere with microbial enzymatic systems, disrupt cell membranes, and reduce metabolic efficiency, ultimately decreasing microbial biomass and diversity (Kozdroj *et al.*, 2022). On the other hand, pesticides, though designed to degrade over time, often accumulate in soils under continuous agricultural application. Their xenobiotic nature and persistence alter microbial community dynamics by selecting resistant strains while suppressing sensitive groups, particularly those involved in nutrient cycling (Ranjan *et al.*, 2020).

Conclusion

The present study demonstrates that both heavy metals and pesticides exert adverse effects on soil microbial diversity and fertility, but their combined presence intensifies the impact significantly. A marked reduction in microbial biomass carbon, dehydrogenase activity, and Shannon diversity index was observed under combined contamination, indicating severe disruption of microbial metabolism, community structure, and ecological functioning. This synergistic stress not only diminishes soil quality but also threatens long-term agricultural sustainability.

The findings highlight the urgent need for integrated soil management strategies, such as reducing chemical inputs, promoting organic amendments, and adopting eco-friendly alternatives like bioremediation and integrated pest management (IPM). Furthermore, awareness of social perception is essential—farmers and communities must be educated about the hidden risks of excessive agrochemical and industrial pollutant use. By combining scientific assessment with community-based approaches, it is possible to safeguard soil health, sustain crop productivity, and ensure environmental resilience in contaminated agro ecosystems. Future research on the interactive effects of heavy metals and pesticides on soil microbial diversity and fertility should move beyond short-term controlled studies and emphasize long-term field-based investigations to capture cumulative and

seasonal variations under real agricultural conditions. The application of advanced molecular approaches, such as high-throughput sequencing, meta genomics, and meta bolomics, will provide deeper insights into microbial community shifts and the functional genes responsible for nutrient cycling, resistance, and detoxification. Understanding the precise biochemical and molecular mechanisms by which heavy metals and pesticides exert synergistic stress on soil microbes remains a crucial research gap. In parallel, greater attention must be directed toward the development of mitigation strategies, including biochar application, phyto remediation, microbial consortia, and nanotechnology-based solutions, to restore soil fertility and maintain ecological balance.

References

- Chen, M., Liu, H., Wang, Y. and Zhao, Q. (2022). Soil enzyme activities in long-term contaminated soil with heavy metals. *Ecotoxicology and Environmental Safety*, 236, 113473.
- Chen, M., Li, H. and Zhao, J. (2022). Soil enzyme activities in long-term contaminated soils with heavy metals: Implications for microbial functions. *Environmental Research*, 212, 113258.
- Gupta, R., Sharma, P. and Verma, S. (2023). Combined effects of pesticides and heavy metals on soil health: Remediation strategies. *Environmental Science and Pollution Research*, 30(18), 54862–54876.
- Kozdroj, J., Renella, G. and Gonnelli, C. (2022). Heavy metal stress and soil microbial dynamics. *Applied Soil Ecology*, 174, 104432.
- Li, Y., Zhang, H. and Zhou, J. (2023). Synergistic toxicity of pesticides and heavy metals on soil microbial activities. *Journal of Hazardous Materials*, 450, 131140.
- Liu, H., Xu, J. and Zhang, Y. (2020). Long-term heavy metal contamination reshapes soil microbial communities and functional potential. *Science of the Total Environment*, 701, 134715.
- Liu, H., Chen, X. and Wang, F. (2020). Long-term heavy metal contamination reshapes soil microbial communities and functional potential. *Science of the Total Environment*, 713, 136494.
- Kozdroj, J., Kowalska, J. and Gałazka, A. (2022). Interactive effects of heavy metals on soil microbial community structure and fertility. *Applied Soil Ecology*, 174, 104388.
- Patel, R., Singh, V. and Sharma, A. (2022). Pesticide residues alter soil microbial diversity and enzyme activities: A metagenomic approach. *Ecotoxicology and Environmental Safety*, 242, 113891.
- Ranjan, R., Mishra, V. and Singh, P. (2020). Seasonal variation in soil microbial communities under pesticide and fertilizer application. *Agriculture, Ecosystems & Environment*, 300, 106972.
- Ranjan, R., Kumar, V. and Singh, A. (2020). Influence of pesticide application on soil microbial dynamics and fertility in different cropping systems. *Agriculture, Ecosystems & Environment*, 295, 106916.
- Singh, A. and Kumar, D. (2024). Sustainable approaches for mitigating combined heavy metal and pesticide stress in soils. *Frontiers in Environmental Science*, 12, 1412567.
- Wang, X., Zhang, L. and Chen, Y. (2022). Microbial community alterations under combined pesticide and heavy metal stress. *Science of the Total Environment*, 837, 155707.
- Wang, Y., Li, X. and Zhou, Q. (2023). Global meta-analysis of heavy metal impacts on soil microorganisms and functions. *Journal of Hazardous Materials*, 445, 130576.
- Kamal, S., Yadav, M. Singh, R. (2024). Effect Of Heavy Metal Pollution On Total Microbial Count Ability Of Soil In Kanpur. *Journal of Emerging Technologies And Innovative Research (JETIR)*, 11(10), 252-258.
- Yadav, M., Kamal, S. and Sharma, V. (2024). Present Status of Physico – Chemical Properties of Agricultural Soil Of Kanpur. *International journal of creative thoughts*, 11(12):859-865.
- Kamal, S. and Yadav, M. (2024). Physio- Chemical status of agricultural soil of Kanpur Dehat, U.P. *Scope*, 14(4):1551-1559.
- Kamal, S. and Yadav, M. (2025). Assessment of Heavy Metal Contamination in Agricultural Soil Around Industrial Zones of Kanpur. *International Scientific Journal of Engineering and Management (ISJEM)*, 4(6):1-6 2583-6159.
- Zhang, Y., Zhou, X. and Li, Q. (2021). Impacts of pesticides and heavy metals on soil enzyme activities: A meta-analysis. *Journal of Environmental Management*, 287, 112367.