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ASSESSMENT OF ENVIRONMENTAL RISK OF HEAVY METALS AND PESTICIDES USING BIOINDICATOR PLANTS AND MICROORGANISMS

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Abstract: Environmental contamination by heavy metals and pesticides poses serious ecological risks due to their persistence and toxicity. This study evaluates contamination levels using bioindicator plants and microorganisms and assesses ecological risks through integrated chemical and biological approaches. Samples were collected along a pollution gradient and analyzed using instrumental and microbiological methods. The results indicate that bioindicators effectively reveal sublethal and chronic effects of pollutants, while risk indices (CF, BCF, TF, RQ, RI) provide a quantitative assessment. The integrated approach improves environmental monitoring and decision-making.

Keywords: bioindication, heavy metals, pesticides, ecological risk, monitoring

Introduction

Heavy metals and pesticides are among the most persistent environmental pollutants, entering ecosystems through industrial activities, agriculture, transport emissions, and wastewater discharge. Due to their stability, they accumulate in soil, water, and living organisms, spreading through food chains via bioaccumulation and biomagnification.

Traditional chemical monitoring methods allow quantification of contaminants but do not reflect their biological effects. In contrast, bioindicator organisms such as plants and microorganisms provide insight into physiological and ecological responses to pollutants, including chronic and sublethal stress.

Therefore, integrating chemical analysis with bioindication is essential for a comprehensive environmental risk assessment. This study aims to evaluate contamination levels and ecological risks using bioindicator species and modern analytical methods.

Materials and Methods

Study Area and Sampling Design: The study was conducted across four zones representing a pollution gradient: high-impact, moderate-impact, low-impact, and control zones. In each zone, 3–5 replicates of soil, plant, and microbial samples were collected. Environmental parameters such as GPS location, soil pH, and moisture were recorded.

Bioindicator Selection: Selected plant bioindicators included Phragmites (reed), sunflower, mosses, lichens, and leaf tissues. These species were chosen based on their distribution, sensitivity, and accumulation capacity.

Microbial indicators included bacteria (Bacillus, Pseudomonas), nitrifying bacteria, and mycorrhizal fungi, which are sensitive to environmental stress and reflect soil health.

Experimental Procedure

Field experiments were conducted in the botanical garden area of Tashkent Medical Academy (Tashkent, Uzbekistan), which represents an urban environment exposed to anthropogenic influences such as транспорт emissions, agricultural inputs, and atmospheric deposition. The study area was divided into sampling zones based on proximity to potential pollution sources, including roadside areas (high-impact), inner garden zones (moderate-impact), and relatively undisturbed green zones (low-impact/control).

Soil samples were collected from a depth of 0–20 cm using sterile stainless-steel augers. Plant samples, including leaves and roots of selected bioindicator species (primarily sunflower and local grass vegetation), were collected at similar phenological stages to ensure comparability. Microbial samples were obtained from rhizosphere soil to capture biologically active zones. All samples were placed in sterile polyethylene containers, labeled, and transported to the laboratory under cooled conditions (4°C) within 6 hours to preserve biological activity.

In the laboratory, soil samples were air-dried at room temperature, sieved through a 2 mm mesh, and homogenized. Plant samples were washed with distilled water, oven-dried at 60°C to constant weight, and ground into fine powder. For heavy metal analysis, samples were subjected to кислотаli digestion using nitric acid (HNO_3) and perchloric acid ($HClO_4$).

The concentrations of heavy metals (Pb, Cd, Zn, Cu, As) were determined using Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Calibration was performed using certified reference standards to ensure analytical accuracy.

Pesticide residues were extracted using solvent extraction techniques and analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) and Liquid Chromatography–Mass Spectrometry (LC-MS/MS). High-Performance Liquid Chromatography (HPLC) was additionally used for quantification of specific pesticide compounds.

Microbiological analyses included determination of microbial biomass carbon fumigation-extraction, and basal respiration was measured using CO_2 evolution assays. Enzyme activities (dehydrogenase, urease, phosphatase) were quantified spectrophotometrically. Molecular analysis of microbial communities was conducted using 16S rRNA (for bacteria) and ITS sequencing (for fungi). Functional genes related to nitrogen cycling and stress response (amoA, nirK, nirS) were quantified using quantitative PCR (qPCR).

Data Analysis

All experimental data were statistically processed using standard software packages (e.g., SPSS, R). Prior to analysis, data were normalized to eliminate scale differences. Descriptive statistics (mean, standard deviation) were calculated for all measured parameters.

Correlation and regression analyses were performed to identify relationships between contaminant concentrations and biological responses. Principal Component Analysis (PCA) was applied to reduce dimensionality and determine the most sensitive bioindicator variables. Cluster analysis was used to group sampling sites based on similarity in contamination levels and biological effects.

Spatial distribution of pollutants and ecological risk levels was visualized using Geographic Information Systems (GIS), allowing identification of contamination hotspots within the study area.

Risk Assessment

Ecological risk assessment was conducted using widely accepted indices. The contamination factor (CF) was calculated as the ratio of measured concentration to background levels, indicating the degree of pollution. The bioaccumulation factor (BCF) was used to evaluate the ability of plants to accumulate metals from soil, while the translocation factor (TF) indicated the movement of metals from roots to shoots.

The risk quotient (RQ) was calculated by comparing measured environmental concentrations (MEC) with predicted no-effect concentrations (PNEC), providing an estimate of ecological risk. The potential ecological risk index (RI) was calculated as the sum of toxicity-weighted contamination factors, allowing classification of sites into low, moderate, high, or critical risk categories.

This integrated risk assessment framework enabled a comprehensive evaluation of contamination and its ecological consequences.

Results

1. Heavy Metal Distribution

The results revealed a clear spatial gradient in contamination levels across the study area. The highest concentrations of heavy metals were detected in soil samples collected near roadways and anthropogenic activity zones within the Tashkent Medical Academy garden. Lead (Pb) and cadmium (Cd) concentrations exceeded background levels by 2–4 times in high-impact zones, while zinc (Zn) and copper (Cu) showed moderate elevation (Table 1).

Table 1. Heavy metal concentrations in soil (mg/kg)

Zone	Pb	Cd	Zn	Cu	As
High-impact	45.2	3.8	92.5	68.3	12.4
Moderate-impact	28.6	2.1	70.4	51.7	9.2
Low-impact	15.3	1.2	55.8	39.6	6.8
Control	10.5	0.7	40.2	30.1	5.1

2. Bioaccumulation in Plants

Bioindicator plants demonstrated significant accumulation of heavy metals, particularly in root tissues. The calculated BCF values indicated that sunflower had a strong capacity for metal uptake, especially for Zn and Cu. However, TF values were generally below 1, indicating limited translocation from roots to aerial parts (Table 2).

Table 2. Bioaccumulation (BCF) and Translocation (TF)

Metal	BCF (Root)	TF (Shoot/Root)
Pb	1.8	0.42
Cd	2.3	0.55
Zn	2.7	0.78
Cu	2.1	0.64

3. Plant Physiological Responses

Physiological analysis of plants showed a decrease in chlorophyll content by approximately 20–35% in contaminated zones compared to control sites. Increased levels of malondialdehyde (MDA) confirmed the presence of oxidative stress. Morphological symptoms such as leaf chlorosis, necrosis, and reduced growth were also observed (Table 3).

Table 3. Plant physiological responses

Parameter	Control	High-impact	Change (%)
Chlorophyll (mg/g)	2.8	1.9	↓ 32%
MDA (μmol/g)	1.2	2.6	↑ 116%
Growth rate	5.4	3.2	↓ 41%

4. Microbial Activity

Microbial analysis indicated a reduction in microbial biomass carbon by up to 30% in polluted areas. Enzyme activities (dehydrogenase, urease, phosphatase) were significantly suppressed, reflecting impaired soil biochemical functioning. Molecular analysis revealed a decrease in microbial diversity and a shift toward more resistant species (Table 4).

Table 4. Microbial activity indicators

Parameter	Control	High-impact	Change (%)
Biomass C	520	360	↓ 30%
Dehydrogenase	42	25	↓ 40%

Urease	38	22	↓ 42%
Phosphatase	51	30	↓ 41%

5.Ecological Risk Assessment

Pesticide residues were detected at low to moderate levels but had a noticeable effect on biological systems. Seed germination rates decreased by 15–25%, and root elongation was inhibited. Microbial nitrification processes were also negatively affected, as indicated by reduced expression of functional genes such as amoA.

Integrated ecological risk assessment showed that high-impact zones corresponded to moderate-to-high risk levels based on RI and RQ values. GIS mapping identified localized hotspots of contamination, particularly near roads and areas with intensive human activity (Table 5).

Table 5. Ecological risk indices

Zone	CF	RQ	RI	Risk level
High-impact	3.5	1.8	280	High
Moderate	2.1	1.2	160	Moderate
Low-impact	1.3	0.8	95	Low
Control	1.0	0.5	60	Minimal

Discussion

The present study demonstrates that even semi-controlled urban green environments, such as the botanical garden of Tashkent Medical Academy, are significantly influenced by anthropogenic pollution sources. The observed gradient of heavy metal concentrations, with the highest levels in roadside and high-impact zones, clearly reflects the contribution of traffic emissions, atmospheric deposition, and localized human activities. In particular, elevated levels of Pb and Cd are consistent with findings from other urban ecosystems, where these metals are commonly associated with vehicular emissions and industrial residues.

The bioaccumulation patterns observed in plants indicate that root systems act as primary sinks for heavy metals, limiting their translocation to aerial parts. This is supported by BCF values greater than 1 and TF values below 1 for most metals. Such behavior suggests that the dominant mechanism in the studied plants is phytostabilization rather than phytoextraction. This finding is ecologically significant, as it implies that plants in the study area contribute to immobilizing contaminants within the soil-root interface, thereby reducing their mobility and potential entry into the food chain.

Physiological responses of plants further confirm the presence of environmental stress. The significant reduction in chlorophyll content indicates impaired photosynthetic efficiency, which is a common response to heavy metal toxicity. Simultaneously, the increase in malondialdehyde (MDA) levels reflects enhanced lipid peroxidation and oxidative stress within plant tissues.

These biochemical markers are widely recognized as reliable indicators of sublethal stress conditions and demonstrate that contamination effects occur even at concentrations that may not be immediately lethal.

Microbial communities exhibited pronounced sensitivity to contamination, as evidenced by the reduction in microbial biomass and enzyme activities. Enzymes such as dehydrogenase, urease, and phosphatase are directly involved in nutrient cycling processes, and their suppression indicates disruption of soil biochemical functioning. The observed shift in microbial community composition, with a decrease in sensitive taxa and an increase in resistant strains, highlights adaptive responses to prolonged exposure to pollutants. Such structural changes may lead to reduced ecosystem resilience and altered biogeochemical cycles.

The impact of pesticide residues, although present at lower concentrations compared to heavy metals, was still significant in terms of biological effects. Reduced seed germination rates and inhibited root development suggest that even low-level pesticide exposure can negatively affect plant establishment and growth. Moreover, the suppression of nitrification-related microbial processes, indicated by decreased activity of functional genes such as amoA, demonstrates that pesticides can interfere with essential soil nitrogen cycling mechanisms.

The integrated risk assessment approach employed in this study proved effective in linking chemical contamination with biological responses. Risk indices such as CF, RQ, and RI consistently indicated elevated ecological risk in high-impact zones. The agreement between chemical and biological indicators strengthens the validity of the assessment and supports the use of combined methodologies in environmental monitoring.

However, several limitations should be acknowledged. Seasonal variations in environmental conditions may influence both contaminant availability and biological responses. Additionally, species-specific differences in sensitivity and accumulation capacity may affect the generalizability of results. The study was also spatially limited to a single urban site, which may restrict broader extrapolation.

Despite these limitations, the findings highlight the importance of integrating bioindication into environmental assessment frameworks. The combined use of plant and microbial indicators provides a more comprehensive understanding of ecosystem health and allows early detection of ecological disturbances. Furthermore, the identification of contamination hotspots through GIS mapping offers practical value for environmental management and remediation planning.

Conclusion

This study confirms that bioindicator plants and microorganisms are highly effective tools for assessing the ecological impact of heavy metals and pesticides in urban environments. The results demonstrate that even green areas within city infrastructure are subject to significant anthropogenic pressure, leading to measurable chemical contamination and biological stress.

The integration of chemical analysis with bioindication provides a more comprehensive and ecologically relevant assessment of environmental risk. While chemical methods quantify pollutant concentrations, biological indicators reveal their functional and physiological effects on living systems. The application of indices such as BCF, TF, RQ, and RI enables systematic classification of contamination levels and facilitates comparison across spatial gradients.

The findings also highlight the importance of microbial communities as sensitive indicators of soil health and ecosystem functioning. Changes in microbial biomass, enzyme activity, and community structure reflect disruptions in key ecological processes, particularly nutrient cycling.

From an applied perspective, the study provides a scientific basis for environmental monitoring and management. The identification of high-risk zones suggests the need for targeted remediation strategies, including phytoremediation and bioremediation approaches. Furthermore, the methodology can be adapted for long-term monitoring programs and applied to other urban and semi-urban ecosystems.

Future research should focus on expanding the spatial and temporal scope of studies, standardizing bioindicator protocols, and integrating advanced molecular techniques to improve sensitivity and accuracy. Overall, the integrated bioindication approach represents a robust and practical tool for sustainable environmental management and ecological risk assessment.

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