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Assessment of Heavy Metals contamination in *Cannabis sativa*, soil and industrial wastewater from Mirpur (Azad Jammu and Kashmir)

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Abstract

Industrial effluent–derived heavy metal contamination poses serious ecological and human health risks due to persistence, toxicity, and bioaccumulation in soil–plant–water systems. This study assessed the distribution, bioaccumulation, and ecological risk of selected heavy metals in *Cannabis sativa*, soil and water from an industrial area of Mirpur, Azad Jammu and Kashmir (AJK). *Cannabis sativa* and soil samples were collected from three locations at increasing distances from the industrial zone, while water samples were obtained from steel, textile, pharmaceutical, and combined industrial effluents. After acid digestion, concentrations of zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co), nickel (Ni), cadmium (Cd), tin (Sn), and silver (Ag) were measured using atomic absorption spectrophotometry and compared with WHO permissible limits. Soil-to-plant transfer and water contamination patterns were evaluated to infer ecological and bioaccumulation risk. Results revealed elevated metal levels across all matrices. In *C. sativa*, mean concentrations of Zn (3.62 ppm), Mn (3.48 ppm), Cu (2.44 ppm), Co (2.33 ppm), Ni (1.23 ppm), Cd (2.16 ppm), Sn (1.21 ppm), and Ag (1.54 ppm) exceeded WHO limits. Soil concentrations were higher, e.g., Zn (13.09 ppm), Mn (16.34 ppm), Co (10.45 ppm), Ni (3.90 ppm), Cd (3.13 ppm). Water samples exhibited the highest concentrations in mixed effluents: Co (8.67 ppm), Ni (13.22 ppm), Mn (9.73 ppm), Zn (11.23 ppm), Cu (3.99 ppm), Cd (6.23 ppm), Ag (1.12 ppm), and Sn (2.02 ppm). Cadmium, cobalt, and zinc showed higher bioaccumulation potential in plants. Overall, contamination and bioaccumulation indices indicate moderate to high ecological risk, highlighting the need for continuous monitoring and stricter regulation of industrial effluent discharge in Mirpur AJK.

Keywords*: Heavy metals; Cannabis sativa; soil, waste water; Mirpur.*

Introduction

Industrial effluent–derived heavy metal contamination has emerged as a major environmental concern due to its persistence, bioaccumulation, and toxicity in soil, water, and living organisms. Rapid industrialization has resulted in the release of toxic metals, such as cadmium (Cd), lead (Pb), mercury (Hg), nickel (Ni), cobalt (Co), zinc (Zn), copper (Cu), tin (Sn), and silver (Ag), into the environment, which accumulate in soil and water and subsequently enter plants, posing significant ecological and human health risks (Ahmed et. Al., 2019; Miretzky et. al., 2004; Wang et. al., 2025). Soil acts as, both a reservoir and a transport medium for these metals, while plants serve as bioindicators and potential vectors of contamination in food chains (Brümmer, 1986; Sabir et. Al., 2022). Medicinal plants, including *Cannabis sativa*, are particularly vulnerable to heavy metal accumulation due to their ecological and pharmacological significance. *C. sativa* is widely cultivated in temperate regions, including Pakistan, Iran, and Afghanistan and has long been utilized for fibers, food products, and therapeutic derivatives such as marijuana and hemp seeds (Adams and Martin, 1996; Lynch and Campbell, 2011). Its bioactive compounds exhibit analgesic, anti-

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inflammatory and neuroprotective properties and are applied in treating conditions such as headaches, inflammation, asthma, multiple sclerosis, and alopecia (Chandra, 2017). However, the accumulation of heavy metals in this plant not only threatens its medicinal quality but also increases the risk of metal transfer to humans and animals via food chains. Industrial effluents from steel, textile, pharmaceutical and combined industries represent a major source of metal contamination in soil, water, and plants. The degree of accumulation depends on proximity to the effluent source, metal concentration, and plant species characteristics (Adeel et al., 2013; Asiminicesei, 2020). Assessing the distribution, bioaccumulation, and ecological risk of heavy metals in soils, water, and *C. sativa* is therefore critical to understanding environmental pollution, evaluating health risks, and ensuring the safe use of medicinal plants. This study focuses on the industrial area of Mirpur, Azad Jammu and Kashmir (AJK), where both soil and water contamination influence plant growth and quality, highlighting the urgent need for monitoring and regulation of industrial effluents.

Materials and Methods

Study area and site description

The study was conducted in the industrial region of Mirpur, the largest city of Azad Jammu and Kashmir (AJK), Pakistan, covering 1,010 km². Mirpur is located 125 km southeast of Islamabad at 33.11°–33.34° N latitude and 73.31°–73.55° E longitude, with an elevation of 459 m above sea level. The terrain comprises plains and foothills of the Himalayas, and the climate is moderately hot, with maximum temperatures reaching 40°C. The Mangla Dam, situated in the study area, supplies ~35% of Pakistan's electricity. The area includes mixed industrial activities, primarily steel, textile, and pharmaceutical units, which discharge untreated or particularly treated effluents into the surrounding environment. The sampling sites were selected along a distance based contamination gradient from a major effluent discharge point:

- effluent zone (0 Km): immediate proximity to discharge site;
- two-kilometer away: intermediate zone;
- four-kilometer away: relatively less impacted zone.

Importantly the area is not subjected to controlled wastewater irrigation system; rather, contamination arises from direct industrial discharge, surface runoff, and infiltration into surrounding soils and water bodies.

Materials

Plastic bottles, polyethylene bags, glassware, hot plates, centrifuge, oven, mortar and pestle, hydrogen peroxide (H_2O_2), nitric acid (HNO_3), perchloric acid ($HClO_4$), and distilled water were used. Heavy metals were quantified using a PerkinElmer Analyst 200 Atomic Absorption Spectrophotometer (AAS).

Sampling design and collection

Plant samples. Whole plants were collected from three locations (effluents zone, Two-kilometer away, Four-kilometer away). Samples were transported in polyethylene bags, washed with tap water followed by distilled water, and dried in air and subsequently in an oven at 60–70°C to constant weight.

Soil samples. Three soil samples were collected from the same locations. Topsoil (0–10 cm) was removed and subsurface soil was collected, air-dried for one week, ground, and passed through a 2 mm sieve. The soil of the study area was predominantly sandy clay, typical of semi-arid regions, with moderate permeability facilitating contaminant mobility.

Water samples. Water samples were collected from effluents of steel, textile, and pharmaceutical industries, and from the site of mixed effluents discharge. Samples were stored in pre-cleaned bottles prior to analysis.

Sample digestion

Plant samples: Dried plant powder (2 g) was digested with a 1:3 mixture of H_2O_2 and HNO_3 on a hot plate until the volume reduced to ~2 mL. The digest was diluted to 20 mL with distilled water.

Soil and water samples: Dried soil (2 g) and water aliquots were digested using a mixture of HNO_3 and $HClO_4$ following standard methods (Alloway, 2013; Kabata-Pendias, 2011).

Heavy metal analysis

Concentrations of Zn, Mn, Cu, Co, Ni, Cd, Sn, and Ag in plant, soil and water samples were measured using AAS. Calibration standards and blanks were run to ensure accuracy, and all analyses were performed in triplicate (Miretzky et al., 2004).

Quality control

All glassware was cleaned with 10% HNO_3 and rinsed with distilled water before use. Procedural blanks and standard reference materials (SRMs) were used to verify analytical precision and recovery. Digestion and measurement procedures were repeated to confirm reproducibility. Heavy metal concentrations were expressed in ppm (mg/kg for soil and plants; mg/L for water).

Data analysis and risk assessment

Two-way analysis of variance (ANOVA) without replication was applied to evaluate the effects of region and element type on heavy metal concentrations in plant, soil, and water samples. Statistical significance was set at $p < 0.05$.

Results

Heavy metals in plant samples

The concentrations of heavy metals in plant samples collected from the three selected regions are presented in Table 1. Overall, metal concentrations were highest in samples collected from the effluent area, followed by those from the two-kilometer site, while the lowest concentrations were generally observed at the four kilometer distance. This spatial pattern indicates a clear atenuation of metal accumulation in plants with increasing distance from the effluent discharge source.

Table 1. *Concentrations of heavy metals (ppm) in plant samples collected from three selected regions.*

Elements	Effluent area	Two-kilometer away	Four-kilometer away
Zn	3.62	3.16	2.98
Mn	3.48	2.85	2.27
Cu	2.44	2.03	0.63
Co	2.33	1.32	0.87
Ni	1.23	1.09	0.76
Cd	2.16	1.01	0.16
Sn	1.21	1.05	0.15
Ag	1.54	1.23	1.03

Two-way analysis of variance (ANOVA) without replication revealed a statistically significant effect of region on metal concentrations ($F = 23.97$, $p < 0.05$), demon-strating a significant decline in concentrations with increasing distance from the effluent source (Table 2). A significant effect of element type was also observed ($F = 21.95$, $p < 0.05$), indicating substantial variability in accumulation behavior among the analyzed metals.

Table 2. *Two way ANOVA (Region x Element) plant samples collected from three selected regions.*

Source	df	F-value	p-value
Region	2	23.97	0.00003
Element	7	21.95	0.000002
Error	14	—	—

Heavy metals in soil samples

The concentrations of heavy metals in soil samples from the three selected regions are summarized in Table 3. Similar to plant samples, soil collected from the effluent area exhibited markedly higher metal concentrations compared to samples obtained at two-kilometer and four-kilometer distances. This trend reflects the strong influence of effluent discharge on soil metal loading and subsequent spatial dilution away from the source.

Table 3. *Concentrations of heavy metals (ppm) in soil samples collected from three selected regions.*

Elements	Effluent area	Two-kilometer away	Four-kilometer away
Zn	13.09	10.98	2.33
Mn	16.34	11.33	10.74
Cu	1.06	0.89	0.42
Co	10.45	8.56	1.23
Ni	3.9	2.45	1.65
Cd	3.13	2.52	1.56
Sn	6.42	4.23	3.45
Ag	2.34	1.05	0.18

Two-way ANOVA without replication showed a significant effect of region on soil metal concentrations ($F = 8.45$, $p < 0.05$). In addition, a significant effect of element type was observed ($F = 11.42$, $p < 0.05$), confirming that different metals display distinct accumulation patterns in soil (Table 4). The observed spatial decline suggests progressive attenuation through dilution, adsorption, and stabilization processes as distance from the effluent source increases.

Table 4. *Two way ANOVA (Region x Element) for soil samples collected from three selected regions.*

Source	Df	F	p-value
Region	2	8.45	0.0039
Element	7	11.42	< 0.001
Error	14	—	—

Heavy metals in water samples

Concentrations of heavy metals in water samples collected from industrial effluent discharge points and the combined effluent site are presented in Table 5. Water samples from the combined effluent location generally showed higher metal concentrations compared to individual industrial discharge points, indicating cumulative contamination from multiple sources.

Elements	Steel	Textile	Pharmaceutical	Combined
Zn	10.02	5.98	6.33	11.23
Mn	9.34	1.33	6.74	9.73
Cu	3.06	3.89	3.42	3.99
Co	2.45	8.56	4.23	8.67
Ni	3.77	12.45	7.65	13.22
Cd	1.13	2.52	5.56	6.23
Sn	0.42	1.23	0.45	2.02
Ag	0.91	0.15	0.18	1.12

Table 5
Concentrations of heavy metals (ppm) in water samples collected from industrial effluent discharge points and the combined effluent site.

Two-way ANOVA without replication demonstrated a significant effect of sampling site on metal concentrations in water (F = 11.58, $p < 0.05$). Element type also had a significant effect (F = 5.69, $p < 0.05$), reflecting differential contributions of metals from the investigated industrial activities (Table 6). Elevated concentrations at the combined effluent site highlight the impact of mixed industrial wastewater inputs on receiving water bodies.

Table 6. *Two way ANOVA (Region × Element) for water samples collected from industrial effluent discharge points and the combined effluent site.*

Source	df	F	p-value
Site	3	11.58	< 0.001
Element	7	5.69	< 0.001
Error	24	—	—

Statistical note

Metal concentrations are expressed in parts per million (ppm). As the ANOVA was conducted without replication, interaction effects between factors could not be statistically evaluated, and the results should be interpreted accordingly.

Discussion

The present study demonstrates a consistent and statistically significant spatial gradient of heavy metal contamination across plant, soil, and water matrices in the investigated industrial area. In all three environmental compartments, concentrations were highest at locations closest to effluent discharge points and progressively decreased with increasing distance, indicating the dominant influence of industrial effluents as a primary source of metal inputs. Similar spatial trends associated with industrial discharge have been widely reported in contaminated agro-ecosystems and peri-urban environments (Alloway, 2013; Tchounwou et al., 2012). The significant regional effect observed in plant samples confirms that vegetation effectively reflects localized contamination levels and responds sensitively to changes in environmental metal availability. Higher concentrations in plants collected from the effluent area may be associated with effluent derived contamination and soil-plant transfer mechanism. Previous studies have shown that plants growing near industrial and wastewater-impacted sites tend to accumulate elevated levels of metals due to combined soil–water–air exposure pathways (Kabata-Pendias, 2010; Nagajyoti et al., 2010). The decline in plant metal concentrations with distance suggests reduced exposure and uptake as contamination pressure diminishes. Similarly, soil samples exhibited a clear decrease in metal concentrations away from the effluent source, highlighting the role of soil as a major sink for industrially derived metals. The significant effect of element type indicates differential mobility and retention beha-

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vior among metals, likely governed by factors such as ionic charge, affinity for soil particles, and interaction with organic matter. These findings are consistent with earlier reports emphasizing the strong influence of soil physicochemical properties on metal persistence and spatial distribution (Alloway, 2013; Bradl, 2004). Water samples showed significantly higher metal concentrations at the combined effluent site compared to individual industrial discharge points, emphasizing the cumulative impact of mixed industrial wastewater. The significant site effect reflects variability in industrial activities and effluent composition, while the element-specific differences suggest distinct industrial signatures for individual metals. Comparable observations have been reported in studies assessing metal contamination in surface waters receiving mixed industrial effluents (Akpor and Muchie, 2011; Fu and Wang, 2011). Such combined discharge zones may therefore represent critical hotspots for environmental and ecological risk. Overall, the concordant trends observed across plant, soil, and water matrices indicate strong interconnectivity within the local environment and support the use of an integrated, multi-compartment approach for assessing industrial pollution (Tóth et al., 2016). Although interaction effects could not be statistically evaluated due to the absence of replication, the consistency of spatial patterns across all matrices strengthens the reliability of the findings. These results underscore the need for effective effluent management and regular environmental monitoring to mitigate long-term metal accumulation and associated ecological risks.

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