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Assessment of Heavy Metal Contamination in Soil and Plants Around Bhutan's Main Landfill

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Abstract

Heavy metal contamination in landfill environments poses significant risks to public health and the environment. This study assessed heavy metal concentrations of iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) in soil and selected plants across three sites in Bhutan's main landfill using Flame Atomic Absorption Spectrophotometry. Results showed heavy metal concentrations in soil followed the order Fe > Mn > Zn > Cu, with Fe levels in some samples exceeding the WHO/FAO limit of 10 mg/kg set in 2001. This exceedance, observed in soil collected directly from the landfill environment, indicates contamination likely caused by leachate migration. At a depth of 15–30 cm, a significant difference ($P = 0.05$) in heavy metal concentrations was detected between areas above and below the landfill. Correlation analysis revealed the intricate interplay between heavy metal concentrations and soil attributes, with soil organic matter playing a pivotal role in binding heavy metals. At 0-15 cm depth, Zn showed strong negative correlations ($r = -0.940$, $p < .01$) with soil organic carbon (SOC percent) and soil organic matter (SOM percentage) and a positive correlation ($r = 0.940$, $p < .01$) with electrical conductivity (EC). At 15–30 cm depth, Mn negatively correlated ($r = –0.836$, $p < .05$) with pH and positively correlated ($r = 0.878$, $p < .01$) with SOC percentage and SOM percentage. Heavy metal concentration varied among plant species, with Fe being the most abundant metal at the two sites. The bioaccumulation outcome indicated that Zn was the most accumulated in the selected plant. *Bidens pilosa* L. and *Poa annua* L. showed higher heavy metal accumulation in roots, suggesting their potential for phytoremediation. The Zn, Cu, and Mn levels exceeded the permissible limits set by WHO/FAO in the specified years, whereas Fe levels in plants remained within the WHO/FAO standards. This study highlights heavy metal distribution, soil-plant interactions, and environmental risks in landfill areas, offering strategies for contamination management. Longitudinal studies are recommended to monitor temporal trends in heavy metal distribution, assess the cumulative impact of leachate migration over time, and refine mitigation strategies. In addition, leachate dilution rate assessments are necessary to better understand contaminant dynamics and environmental impacts.

Keywords: Bioaccumulation, heavy metal, landfill, leachate, plant, soil

Introduction

Heavy metals are metallic elements characterized by high density or atomic weight (Tchounwou *et al*., 2012). While trace amounts of essential metals such as iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), molyb-

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denum (Mo), and zinc (Zn) for metabolic functions, their accumulation beyond physiological levels can be harmful (Rai *et al*., 2019).

Municipal solid waste components such as spent batteries, waste electrical and electronic equipment (WEEE), rubber fractions, dyes, paints, and various plastic additives are major sources of heavy metal pollution. Leachate is an aqueous phase generated from moisture percolating through waste forms at an estimated rate of approximately 0.2 m^3 per metric ton of waste (Talalaj, 2015). This leachate transports toxic heavy metals into surrounding soils, surface water, groundwater, and ecosystems (Essien *et al.,* 2022).

Elevated heavy metal concentrations exceeding permissible limits pose significant risks to human health, wildlife, and ecological integrity. These risks highlight the necessity for continuous monitoring and the implementation of remediation strategies to prevent long-term environmental damage (Allison, 2019). Conventional remediation techniques, such as physical and chemical methods, are often expensive, energy-intensive, and may introduce secondary environmental burdens. In contrast, bio-based approaches, particularly phytoremediation, offer a cost-effective and sustainable alternative for cleaning heavy metal-contaminated sites (Ullah *et al*., 2015; Abdullahi *et al*., 2018).

While heavy metals in groundwater have been widely studied, the effects on landfill soil remain underexplored, calling for more comprehensive assessments (Makuleke & Ngole-Jeme, 2020). In Bhutan, Thimphu city generates approximately 40.3 tonnes/day of mixed stream of municipal solid waste, including wet (organic kitchen/garden residues), dry recyclable materials (paper, plastics, glass, metals, WEEE), textiles, and other residuals., of which 95% (38.3 tonnes/day) is directed to the Memelakha landfill (Wangmo, 2019). The mixed waste that reaches the landfill fill is exposed to heavy rainfall, leading to leachate runoff into the surrounding environment. Efforts to monitor and remediate environmental issues remain minimal, highlighting the need for enhanced strategies and resource allocation to address these challenges effectively. This study aimed to assess heavy metal concentrations in soil and selected plants, evaluate bioaccumulation potential for phytoremediation, and compare findings against WHO/FAO permissible limits to assess contamination levels.

Materials and Methods

S*tudy Area*

The Memelakha dumpsite, located 9.3 km east of Thimphu City (27°26'33" N, 89°41'37" E), is Bhutan's largest waste disposal site. The area is situated at an altitude ranging from 2,248 to 2,648 metres above sea level and supports a rich ecosystem characterized by diverse flora and fauna. Initially constructed in 1994, the landfill covers an area of 6.89 to 14.79 acres and has been in operation for over 23 years, with its size continually expanding. According to the National Environment Commission (2018), the landfill receives approximately 40.3 tonnes of waste per day, with organic waste making up the largest portion (58.1%), followed by plastic (13%) and paper (9.2%). Even during dry-waste collection periods, organic waste remains the predominant component of the municipal waste stream. On average, 23.5 tonnes of dry waste and 16.8 tonnes of wet waste are dumped daily.

Soil and Plant Sampling

The study site was divided into three distinct zones based on their spatial relation to the landfill: 100 m above the landfill (upslope and unaffected by landfill activities), on the landfill (the main waste disposal site), and 100 meters below the landfill (downslope and potentially influenced by runoff and leachate), covering a total of 8.44 hectares. In each of the selected sites, seven quadrats of $10\,m \times 10\,m$ were marked. Using a systematic random composite sampling approach, soil samples were collected with a soil auger at depths of 0–15 cm and 15–30 cm from five core points within each quadrat to capture variations in soil properties at different layers (International Atomic Energy Agency [(IAEA)], 2004; Kulikov & Ukpong, 2013). The 0–15 cm depth represented the topsoil, where heavy metals were expected to most likely accumulate due to direct contact with surface runoff, leachates, and organic matter deposition. The soil samples from 15–30 cm depth were to assess the potential vertical movement of heavy metals and the extent of contamination at deeper soil levels. A total of 210 soil sub-samples were collected from the three sites, with 105 sub-samples each taken from the 0–15 cm depth and 15–30 cm depth. Seven composite samples were prepared for each site at each depth by combining subsamples collected from the centre and corners of a square, following the method described by Slaninka *et al*. (2013). This resulted in 42 composite soil samples in total (21 from each depth range across all three sites). The selection of 210 soil samples ensured sufficient spatial coverage and replication to capture variability in heavy metal distribution across the three zones and two depth layers. The systematic random composite sampling approach further enhanced representatives by combining subsamples from multiple points within each quadrant.

In the landfill site, mixed solid waste was cleared using excavators to access the sampling points. All samples were stored in zip-locked plastic bags, labelled, and transported to the laboratory for analysis. In addition, plant samples were collected using 1m x 1m quadrats (Baxter, 2014), focusing on herb species. A total of 21 quadrats were placed across the three sites, and the three plant species with the highest counts within the quadrats were selected for heavy metal concentration testing. Plant and soil samples were collected from the same location. Root, stalk, leaf, and soil samples were stored in pre-cleaned zipper bags properly labelled. The plant species and individual counts were recorded. Root, stalk, and leaf samples were separated and ground separately to prepare them for laboratory testing (Elbl *et al*., 2018; Radziemska *et al*., 2017). Composite samples were prepared by mixing samples from the same plant species, and the resulting samples were tested in the laboratory.

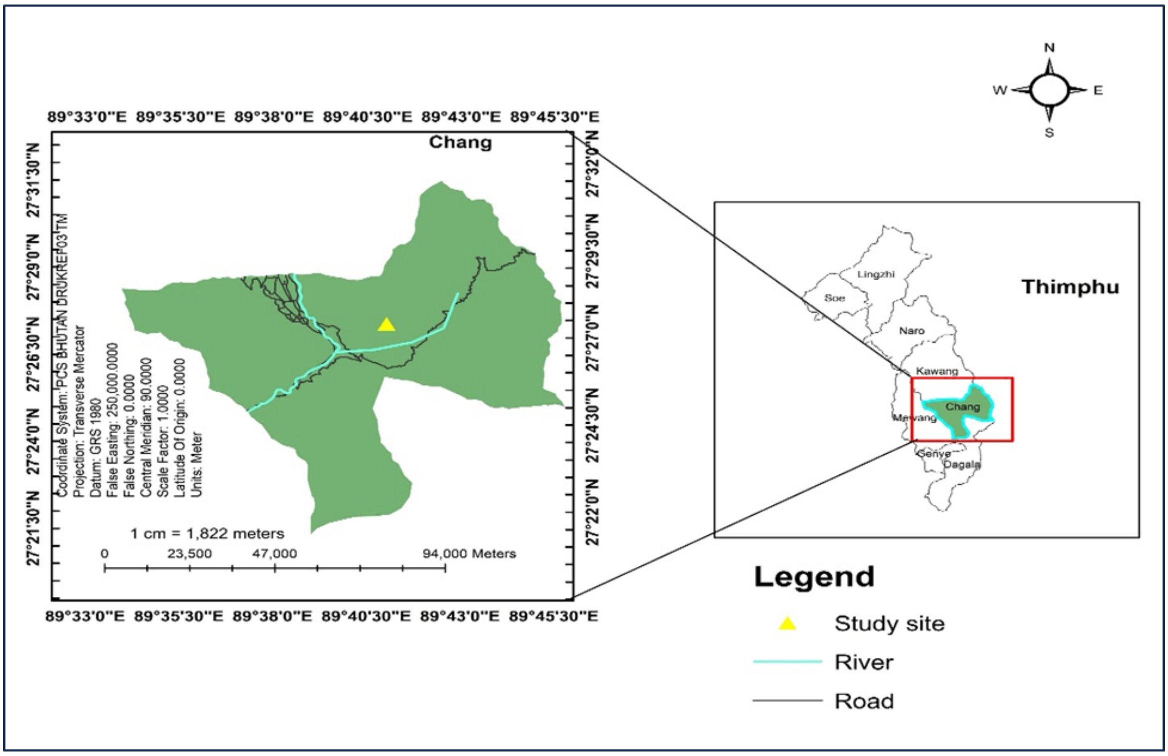


Figure 1: Depicting the assessed landfill site

Laboratory Sample Preparation

The dry ashing technique was employed for heavy metal concentration analysis in soil and plant samples (Akinyele & Shokunbi, 2015; Ranasinghe *et al*., 2016). After four days of air drying, soil samples were crushed into fine powder and sieved through a 2 mm mesh to separate coarse particles. Subsamples were prepared and analyzed for heavy metal concentrations and physicochemical properties.

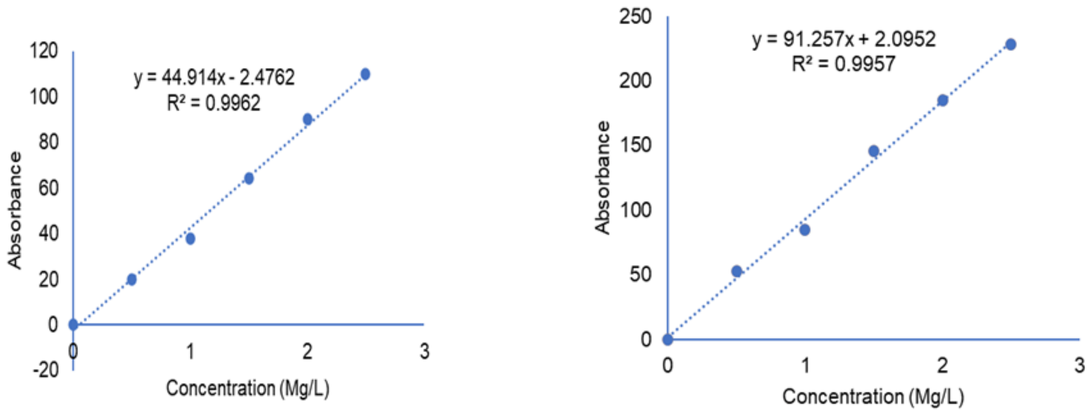
Plant samples were washed with distilled water, dried to constant weight, and ground into powder using an acid-washed mortar and pestle, then sieved through a 2 mm mesh screen (Nouri et al., 2009; Abdallah *et al*., 2012). Heavy metal concentrations were analyzed in three different parts of the plant: roots, stalks, and leaves.

One gram each of soil or plant sample was weighed, dried at 105°C to remove residual moisture, and ashed at 550°C in a muffle furnace over two hours. The ash was digested using a mixture of concentrated hydrochloric acid (HCl), nitric acid (HNO_3), and 27.5% hydrogen peroxide (H_2O_2.) following the method outlined by the USEPA (United States Environmental Protection Agency) as described by Abbruzzini *et al*. (2014). The digested samples were filtered and analyzed for heavy metal concentrations using a Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380) (Ali *et al*., 2020).

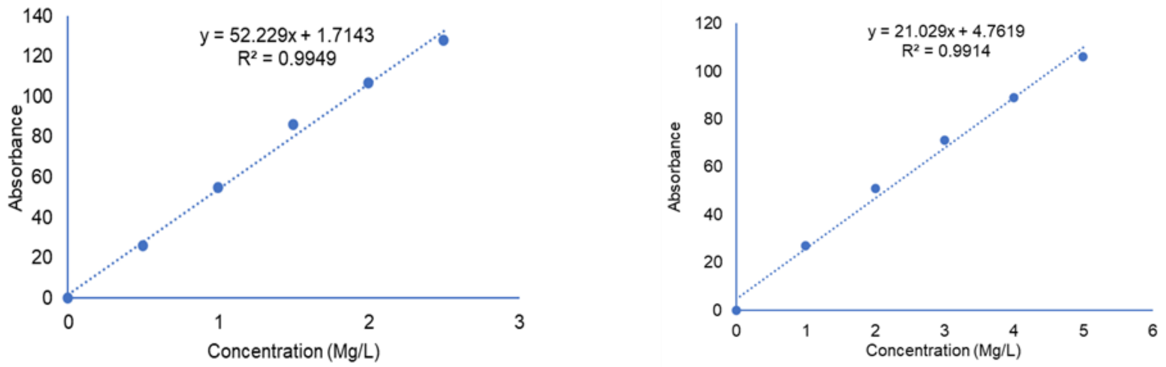
Blank samples, consisting of both soil and plant materials, were collected from an unpolluted site (200 m from the study site), within the same altitude range as the study site, and were prepared and analyzed using the same techniques and materials to ensure quality control. Quality control samples from each batch were stored under controlled conditions and analyzed using Flame Atomic Absorption Spectroscopy (FAAS), a well-established method for determining metal concentrations, to ensure analytical consistency and accuracy.

Standards and Calibration for Heavy Metal Analysis

Calibration linearity was verified as the first step in evaluating quality control samples, followed by assessing the recalibrated FAAS system's performance to ensure accuracy. Calibration standards were prepared and analyzed before, during, and after sample runs to monitor instrument precision and stability. Additionally, duplicate analyses of randomly selected samples were conducted to assess reproducibility. Specific standards, as outlined by Lindsay and Norvell (1978), were utilized to calibrate the FAAS and ensure precise heavy metal concentration analysis (Figure 2).



A **B**



C

D

Figure 2: Standard and Calibrations Displayed in FAAS. A: Cu; B: Mn; C: Zn; D: Fe

Bioaccumulation Factor (BAF) Calculation

The Bioaccumulation Factor (BAF) was calculated using the formula: BAF = Pi / Si, where BAF represents the accumulation of heavy metals in a specific plant species (Aladesanmi et al., 2019). Pi is the concentration of heavy metals in the plant (mg/kg). Si is the concentration of the same heavy metals in the surrounding soil (mg/kg). BAF was determined for each plant part, including the roots, stalks, and leaves.

The BAF is a critical indicator in assessing the phytoremediation potential of plants, as it quantifies the plant's ability to uptake and accumulate heavy metals from contaminated soils. A higher BAF value suggests a stronger capacity for metal uptake, which is crucial for identifying hyperaccumulator species suitable for phytoremediation (Shi *et al*., 2023). By evaluating BAF across different plant parts, the study provides insights into metal translocation and storage efficiency within plants, further supporting their potential use in soil remediation efforts.

Soil Physicochemical Properties Analysis

Soil physicochemical properties were analyzed using standard methods. pH was measured with a pH meter (Model: HANNA H19125), and EC was determined using an EC meter (Model: HANNA H18733). The EC values, reported in micro-Siemens per centimetre (µS/cm), were recorded after stable readings. Soil texture, which refers to the relative proportions of sand, silt, and clay particles in the soil, was assessed using the hand texture method (Ritchey *et al*., 2015; Vanek et al., 2018). Soil moisture content (% MC) was determined using the formula ([B_C) / (B_A]) × 100%, where A represents the weight of the empty crucible, B represents the weight of the crucible plus the fresh sample weight, and C represents the weight of the crucible plus the dry sample weight (Black *et al*., 2015).

The SOM percentage content was calculated using the loss of ignition method, comparing the weight of the sample before and after ignition. The formula used was SOM percentage = ([Wcs_Wf) / (Wcs_Wc]) × 100, where Wcs represents the weight of the oven-dried soil and crucible, Wf represents the weight of the furnace-fired sample and crucible, and Wc represents the weight of the crucible.

The SOC percentage content was calculated using the formula SOC percentage = (0.58 % × SOM percentage) (Black *et al.,* 2015; Konare *et al*., 2010; Hoogsteen *et al.,* 2015). All laboratory readings were replicated three times, and average readings were recorded for soil properties and FAAS.

Result analysis tools

The data were analyzed using Statistical Package for the Social Sciences (SPSS) v. 20 statistical software and R software v. 4.2.2. Descriptive statistics, such as standard deviation (SD) and mean, were employed to describe heavy metal concentrations and soil properties (Ali *et al.,* 2019). The average heavy metal concentration in the soil and the combined concentrations of heavy metals in selected plants (sum of concentrations in root, stalk, and leaf) were compared with the standard permissible limits set by the WHO/FAO (Fosu-Mensah *et al*., 2017*;* Liu *et al.,* 2023).

Pearson's correlation matrix was utilized to analyze the relationship between heavy metal contents and soil properties in three sites (Agbeshie *et al*., 2020). Before applying Pearson's correlation, data were checked for linearity using scatterplots and normality using the Shapiro-Wilk test, as these are critical assumptions for the validity of the test.

Pairwise comparisons of heavy metal concentrations were conducted between the three sites using the General Linear Model (GLM). Before applying the GLM, the normality of residuals was checked using the Shapiro-Wilk test, and the homogeneity of variance was assessed with Levene's test. Post-hoc tests (Tukey's HSD) were applied to explore significant differences in heavy metal concentrations among the three sites, providing a comprehensive understanding of the relationships. Statistical significance was considered was considered at $p < .05$.

Results and Discussion

Soil Physicochemical Properties Across Sites

The physicochemical properties measured at two depths in three sites are summarized in Table 1. The results showed that, at the 0–15 cm depth, the mean pH was higher below the landfill (7.09 ± 0.38) than above the landfill (5.67 ± 0.06). However, this trend was inconsistent at the 15–30 cm depth, where pH values varied irregularly (above the landfill: 5.85 ± 0.19, landfill: 7.35 ± 0.22, below the landfill: 7.16 ± 0.51). Overall, soil pH was acidic above the landfill and transitioned to alkaline at the landfill and below the landfill. These variations suggest an increase of 1.5 units in pH, especially at a depth of 15–30 cm, indicating potential changes in soil chemistry influenced by landfill materials. The findings align with El-Fadel *et al.* (2002), who reported that landfills commonly exhibit alkaline pH levels around a decade post-disposal due to reduced free volatile acids from anaerobic decomposition. Partially ionized fatty acids contribute to elevated pH, while leachate-containing mineral species in bicarbonate form may account for soil alkalinity (Seo *et al.*, 2007).

Table 1: Soil physicochemical properties across three sites (*n* = 7)

Depth	Test	Above landfill	Landfill	Below landfill
		(Mean ± *SD)*		
0-15 cm	pH	5.67 ± 0.06	7.01 ± 0.23	7.09 ± 0.38
	EC	47.96 ± 10.92	1153.71 ± 524.05	1609.49 ± 819.19
	% MC	2.29 ± 0.94	4.44 ± 2.56	3.00 ± 0.99
	% SOM	4.59 ± 1.52	3.29 ± 1.29	4.05 ± 1.44
	% SOC	2.66 ± 0.88	1.91 ± 0.75	2.35 ± 0.83
15-30 cm	pH	5.85 ± 0.19	7.35 ± 0.22	7.16 ± 0.51
	EC	29.31 ± 6.44	1248.19 ± 639.47	1500.46 ± 855.11
	% MC	2.80 ± 3.55	4.65 ± 2.72	2.95 ± 1.31
	% SOM	3.75 ± 0.84	2.51 ± 1.24	4.15 ± 1.64
	% SOC	2.18 ± 0.49	1.45 ± 0.72	2.41 ± 0.95

SD = Standard deviation

Electrical conductivity (EC) was noticeably higher from above the landfill to below the landfill. At the 0–15 cm depth, the average EC values were 47.96 ± 10.92 µS/cm for the soil samples above the landfill, 731.57 ± 431.67 µS/cm for the soil samples at the landfill, and 1609.49 ± 819.19 µS/cm for the soil samples below the landfill. Similarly, at the 15–30 cm depth, the average EC values were 29.31 ± 6.44 µS/cm for the soil samples above the landfill, 453.76 ± 284.21 µS/cm for the soil samples at the landfill, and 1500.46 ± 855.11 µS/cm for the soil samples below the landfill. These results indicate the potential leaching of contaminants into the surrounding soil and higher salinity levels in the soil profile. The higher the concentration of dissolved salts/ions, the greater the conductivity of the sample, resulting in a higher conductivity reading (Ismayilov *et al*., 2021). According to Sintorini *et al* (2021), higher pH means that the mobility of metals in the soil is less, creating a high availability of metals in the soil. Higher EC may enhance ionic strength and thus potentially increase the mobility of certain heavy metals; however, actual metal bioavailability is highly metal-specific and also depends on key soil parameters including pH, organic matter content, cation-exchange capacity, soil texture, and clay/mineral composition, which can significantly limit or promote mobility (Hassan & Umer, 2022).

Soil moisture content (MC percentage) varied across the study sites and depths. At the landfill site, MC ranged from 1.86 to 8.33 at the 0–15 cm depth and from 2.41 to 9.33 at the 15–30 cm depth. Below the landfill, MC ranged from 1.53 to 4.40 at the 0–15 cm depth and from 1.51 to 4.86 at the 15–30 cm depth. In contrast, above the landfill, MC values ranged from 1.00 to 3.46 at the 0–15 cm depth and from 0.86 to 10.73 at the 15–30 cm depth. Although variations were observed with depth, MC was consistently higher at the landfill and below it compared to above the landfill. These differences are likely attributed to increased water retention from liquid leachate flowing from the landfill.

The average SOM percentage varied across sites and depths but was lower at the landfill, ranging from 0.82–4.95 at 0–15 cm and 0.81–3.80 at 15–30 cm. These fluctuations indicate spatial variability in decomposition processes within the landfill. This aligns with findings from Kooch *et al*. (2024), who reported that landfill leachate reduces SOM and other soil health indicators, including porosity and nutrient availability. Similarly, SOC percentage exhibited a comparable trend to SOM percentage, with lower values recorded at the landfill site (0.47–2.87 at 0–15 cm and 0.50–2.20 at 15–30 cm). In contrast, higher SOC values were observed above the landfill (1.62–4.13 at 0–15 cm and 1.32–2.90 at 15–30 cm) and below the landfill (1.67–3.96 at 0–15 cm and 1.49–2.83 at 15–30 cm). These findings align with existing literature, which reported that SOM concentration in soils typically ranges from 1% to 6% of the total mass of topsoil (Kefyalew Girma *et al*., 2009). This indicates organic material enrichment above or below the landfill site. Soil characteristics such as climate, soil type, and management history influence SOM levels (Cotrufo & Lavallee, 2022; Hallman *et al*., 2024).

The higher % SOM and % SOC observed above the landfill are primarily due to natural inputs such as leaf litter, root turnover, and small animal residues typical of undisturbed vegetation, whereas below the landfill it result from both these natural inputs and additional organic matter delivered via landfill runoff, leachate deposition, and early-stage plant colonization of landfill-affected areas. Here, SOM encompasses all soil organic matter, with SOC constituting approximately 50–70 % of SOM (Cotrufo *et al*., 2019; Lal *et al*., 2007). The high proportion of food waste, garden debris, and paper in municipal waste likely contributes to SOM in the landfill. Organic matter influences soil structure, water retention, cation exchange, and complex formation (Lal, 2004).

The soil texture across all three sites (above the landfill, at the landfill, and below the landfill) was predominantly sandy clay loam, irrespective of site or depth (Figure 3). Below the landfill, the soil texture varied slightly, with loamy sand being the predominant texture at the 0–15 cm depth, while sandy clay loam became dominant at the 15–30 cm depth. These textural characteristics, particularly the high porosity and permeability associated with sandy loam and sandy clay loam soils, may facilitate leachate migration into adjacent environments. Furthermore, the lower clay concentration observed in these soils could reduce their sorption capacity, as noted by Makuleke and Ngole-Jeme (2020).

Heavy Metal Concentration in Soil

The mean concentrations of the studied heavy metals at two depths of 0–15 cm and 15–30 cm across three distinct study sites are displayed in Table 2. At both depths, the concentrations generally followed the order Fe > Mn > Zn > Cu. The highest Fe concentration (37.51 mg/kg) at the 0–15 cm depth was observed at the landfill site, while the highest concentration (30.44 mg/kg) at the 15–30 cm depth was found below the landfill. Heavy metal concentrations were significantly in and below the landfill than in the control site above the landfill. Heavy metals at both depths, with maximum concentrations in the lower regions of the landfill, indicate significant soil contamination due to leachate migration from the open dumping site. This contamination is likely exacerbated by the soil's sandy clay loam texture, high porosity, and permeability, which facilitate leachate movement. These findings underscore the landfill's clear impact on the distribution of heavy metals in the soil.

A study by Hosseini Beinabaj *et al.* (2023) reported iron as the most abundant metal, with concentrations of 17.01 mg/L and 22.94 mg/L in two landfill leachate samples collected from the Aradkoh landfill in southern Tehran, consistent with the current study's findings. The trend of Fe > Mn > Zn > Cu in heavy metal concentrations, with higher levels in landfill sites and areas below landfills compared to control sites, has been reported in other studies. For instance, Gebre and Debelie (2015) observed this trend in soils near solid waste dumping sites in Ethiopia, while Nyiramigisha *et al.* (2021) reported that heavy metal concentrations in Indonesian soils decreased with distance from landfills, underscoring the anthropogenic contributions to soil contamination from waste discharge. Similarly, Islam *et al.* (2022) reported of abandoned tannery waste disposal sites in Dhaka, Bangladesh, that soil

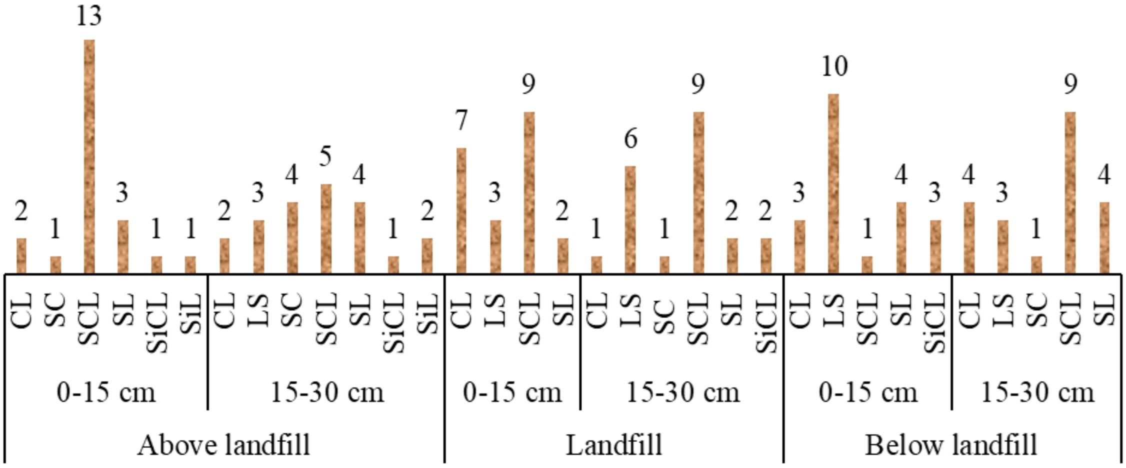


Figure 3: Count of average soil texture within three sites in two depths. (CL; Clay loam, SC; Sandy clay, SCL; Sandy clay loam, SL; sandy loam, SiCL; Silk clay loam, SiL; Silk loam, LS; loamy sand)

heavy metal concentrations followed the order Fe > Mn > Zn > Cu, with contaminated zones exhibiting significantly higher levels than adjacent control sites. The higher concentration of Fe in landfill soils can be attributed to the composition of landfill leachate and the chemical processes occurring within the landfill environment (Jagasri *et al*., 2024). Leachate, formed as water percolates through waste, carries high levels of soluble salts, organic acids, and heavy metals (Costa *et al.,* 2019; Lindamulla *et al*., 2022). This infiltration elevates heavy metal concentrations in the surrounding soil. Studies have identified leachate and waste particles as primary sources of heavy metals, including Fe, Mn, Cu, and Zn, in landfill environments (Jaradat *et al*., 2021).

Mn found at the current study site was likely came from the disposal of various items such as blades, bottle tops, medications, galvanized materials, paints, dyes, pesticides, cosmetics, and general waste (Hosseini Beinabaj *et al*., 2023). Mn is a constituent in iron alloys, employed to enhance iron's rigidity, toughness, and potency for fabricating automotive components. Consequently, the Mn concentration increases in the presence of high iron levels (Abagale *et al*., 2013). Zn in the soil across various locations may be attributed to the disposal and degradation of household battery cells, such as alkaline and zinc-carbon batteries, commonly referred to as 'dry cells' in municipal waste, as noted by Thorpe and Harrison (2008), as well as the incineration of electronic waste. Zn, an essential microelement that catalyzes enzyme reactions, varies in concentration depending on soil type (Noulas *et al*., 2018). However, elevated Zn levels in the soil can lead to bioaccumulation in plants and water systems, posing potential health risks to humans through the food chain and groundwater contamination (Ahmed *et al*., 2021; Hussain *et al*., 2022). The higher

Table 2: Average Heavy metal concentrations in soil from two depths and WHO/FAO permissible limits (mg/kg), (*n* = 7)

Depths		**Above landfill**	**Landfill**	**Below landfill**	**WHO/ FAO**	**FAO/ WHO**	**WHO**
			(Mean ± *SD)*				
0-15 cm	Zn	0.79 ± 0.43	1.96 ± 0.27	1.81 ± 0.19	200	300	50
	Cu	0.68 ± 0.26	1.63 ± 0.65	1.83 ± 0.63	100	100	36
	Fe	3.58 ± 1.11	10.19 ± 12.34	12.34 ± 9.71	10	-	-
	Mn	0.75 ± 0.37	1.60 ± 0.55	2.78 ± 1.41	-	-	-
15-30 cm	Zn	0.58 ± 0.45	2.16 ± 0.16	1.47 ± 0.66	-	-	-
	Cu	0.66 ± 0.32	1.71 ± 0.63	1.52 ± 0.84	-	-	-
	Fe	3.34 ± 0.98	7.62 ± 5.17	11.73±12.00	-	-	-
	Mn	0.46 ± 0.22	1.47 ± 0.45	2.39 ± 2.06	-	-	-

SD: Standard deviation; FAO: Food and Agriculture Organization; WHO: World Health Organization; WHO/FAO 2001 (Atta *et al*., 2023), FAO/WHO.1984 (Fosu-Mensah *et al*., 2017), WHO (1996) (Kocaman, 2021) concentration of Cu in landfill soils may result from burning electrical devices.

A post hoc pairwise comparison of total heavy metal concentrations across the study sites was conducted (Table 3). At the 0–15 cm depth, a mean difference (MD) of -3.08 in heavy metal concentration was observed between the above-landfill and below-landfill sites; however, this difference was not statistically significant (p = 0.15). Similarly, no significant differences were observed in heavy metal concentrations between the above-landfill and landfill sites (MD = -2.39, p = 0.38) or between the landfill and below-landfill sites (MD = -1.02, p = 1.00).

At the 15–30 cm depth, a significant difference in heavy metal concentration was noted between the above-landfill and below-landfill sites (MD = -3.01, p = 0.05). In contrast, no significant differences were observed between the above-landfill and landfill sites (MD = -1.97, p = 0.36) or between the below-landfill and landfill sites (MD = 1.04, p = 1.00). The significant difference at the 15–30 cm depth suggests notable variations in heavy metal concentrations with depth and location, highlighting the influence of landfill leachate on soil at deeper layers. This may be attributed to leachate migration, facilitated by the landfill's sloped geographic location in Bhutan's mountainous terrain. Variations in heavy metal concentrations may result from factors such as landfill location, national waste management practices, environmental conditions, seasonal changes, soil physicochemical properties, climatic variations, and matrix-to-matrix transfers (Vongdala *et al.*, 2019; Xaypanya *et al.*, 2018).

Table 3: Comparison of Combined Heavy Metal Concentrations between three sites, n = (28)

Depth	**Zones**		**MD**	***p value***
0-15 cm	Above landfill	Below landfill	-3.08	0.15
		Landfill	-2.39	0.38
	Below landfill	Above landfill	3.08	0.15
		landfill	0.69	1
	Landfill	Above landfill	2.39	0.38
		Below landfill	-0.69	1
15-30 cm	Above landfill	Below landfill	-3.01	0.05
		Landfill	-1.97	0.36
	Below landfill	Above landfill	3.01	0.05
		Landfill	1.04	1
	Landfill	Above landfill	1.97	0.35
		Below landfill	-1.04	1

p value = 0.05, MD = *mean difference*

Comparison of Heavy Metals Concentration in Soil with WHO/FAO Limits

The average concentrations of heavy metals were compared with permissible limits established by WHO/FAO standards for agricultural soils in 1984, 1996, and 2001 (Table 2). Cu concentrations were consistently below the permissible limits of 100 mg/kg and 36 mg/kg. Similarly, Zn concentrations remained below the permissible limits of 300 mg/kg, 200 mg/kg, and 50 mg/kg. In contrast, Fe concentrations ranged from 3.94–37.51 mg/kg at the 0–15 cm depth and 4.51–16.29 mg/kg at the 15–30 cm depth within the landfill site, and from 4.55–25.72 mg/kg at 0–15 cm and 4.65–30.44 mg/kg at 15–30 cm below the landfill. These values exceeded the WHO/FAO permissible limit of 10 mg/kg specified in 2001, indicating potential Fe contamination. Notably, no permissible limits for Fe were listed in the 1984 or 1996 standards, and specific limits for Mn in soil were not provided in any of the referenced standards.

Elevated Fe concentrations in landfill soils have been reported in other studies. For example, Agbeshie *et al.* (2020) observed Fe levels exceeding the 2001 WHO/FAO limit at solid waste sites in Ghana, while Nyiramigisha et al. (2021) reported similar exceedances at the Putri Campo landfill in Indonesia. These findings highlight Fe contamination as a consistent concern across different landfill environments. However, Zn and Cu concentrations in this study were consistently below the permissible limits established by WHO/FAO in 2001, 1996, and 1984. For comparison, Li *et al.* (2014) reported Cu concentrations ranging from 2.1–89.7 mg/kg in Chinese copper mining areas, which exceeded the levels observed in this study but remained below the 100 mg/kg WHO/FAO limit. Similarly, Essien et al. (2022) found heavy metals, including Zn and Cu, in landfill leachates, surface water, and sediments. Yet, their concentrations did not surpass WHO/FAO permissible limits. Ahmad *et al.* (2021) also noted elevated levels of Cu and Mn near landfills but within acceptable thresholds. These findings suggest that while Zn and Cu levels in this study do not pose immediate risks compared to the WHO/FAO guidelines, continuous monitoring is essential, as the accumulation of heavy metals in soil over time could potentially lead to contamination and associated environmental concerns.

Heavy metals such as Fe, Mn, Cu, and Zn often accumulate in soils and water bodies, particularly near contaminated effluent sources. This accumulation leads to soil pollution, reducing microbial activity and disrupting nutrient cycling, while also posing risks of groundwater contamination (Kumar *et al.*, 2019). In plants and crops, these metals are essential micronutrients involved in vital physiological and biochemical processes, but their effects are concentration-dependent. Elevated concentrations can cause toxicity symptoms such as chlorosis (leaf yellowing), inhibited seed germination, reduced root and stem elongation, disrupted photosynthesis, and impaired nutrient uptake, cellular respiration, and nitrogen metabolism. For instance, Zn toxicity has been observed to suppress biomass production, disturb enzyme activity and cell division, compromise structural integrity, and significantly reduce yield (Ulhassan *et al.*, 2025). While Fe is crucial for electron transport and photosynthesis, at high levels it can decrease photosynthetic rate, alter gas exchange patterns, and damage photosystem II, thereby impairing carbon fixation and energy synthesis in plants (Zahra *et al.*, 2021).

Relationship Between Heavy Metal Concentration and Soil Physicochemical Properties

At the above-landfill site (0–15 cm depth), Zn concentration showed a significant negative correlation with %SOC (r = -0.940, $p < .01$) and %SOM (r = -0.940, $p < .01$) (Table 4). This suggests that higher soil organic matter levels are associated with reduced Zn concentrations, likely due to SOM's ability to bind heavy metals and decrease their bioavailability and mobility (Li *et al.*, 2018; Saboor *et al.*, 2021).

Table 4: Relationship between soil physicochemical properties and heavy metals

Sites	Depths	Parameters	1	2	3	4	5	6	7	8
AL	0-15 cm	pH								
		EC	0.421							
		%MC	-0.515	0.454						
		%SOM	-0.084	0.491	0.38					
		%SOC	-0.084	0.491	0.38	1.000**				
		Cu	0.576	0.477	-0.086	0.568	0.568			
		Zn	0.21	-0.598	-0.573	-.940**	-.940**	-0.344		
		Mn	-0.058	0.252	0.439	-0.2	-0.2	-0.526	-0.039	
		Fe	-0.026	-0.28	-0.116	0.066	0.066	0.396	0.187	-0.529
	15-30cm	pH								
		EC	-0.532							
		%MC	-0.387	0.63						
		%SOM	-0.357	0.117	0.381					
		%SOC	-0.357	0.117	0.381	1.000**				
		Cu	-0.164	0.477	0.733	0.499	0.499			
		Zn	-0.222	-0.491	-0.451	-0.448	-0.448	-0.66		
		Mn	0.137	-0.196	-0.202	-0.298	-0.298	-0.534	0.177	
		Fe	0.361	0.375	-0.076	-0.158	-0.158	0.157	-0.521	-0.5
L	0-15 cm	pH								
		EC	0.532							
		%MC	0.575	.895**						
		%SOM	0.226	0.322	0.265					
		%SOC	0.226	0.322	0.265	1.000**				
		Cu	-0.407	-0.513	-0.751	0.016	0.016			
		Zn	-0.072	0.146	-0.013	-0.236	-0.236	-0.128		
		Mn	-0.646	-0.737	-0.521	-0.704	-0.704	0.113	-0.113	
		Fe	-0.716	-0.095	-0.046	-0.142	-0.142	-0.253	-0.026	0.468
	15-30cm	pH								
		EC	0.236							
		%MC	-0.07	.811*						
		%SOM	-0.142	0.276	0.302					
		%SOC	-0.142	0.276	0.302	1.000**				
		Cu	0.279	0.341	0.056	0.651	0.651			
		Zn	0.241	-0.031	-0.409	-0.273	-0.273	0.2		
		Mn	-.836*	-0.422	0.111	-0.021	-0.021	-0.499	-0.381	
		Fe	-0.733	-0.206	-0.084	0.199	0.199	0.205	-0.274	0.483
BL	0-15cm	pH								
		EC	0.436							
		%MC	-0.161	0.084						
		%SOM	-0.167	0.331	0.599					
		%SOC	-0.167	0.331	0.599	1.000**				
		Cu	-0.079	-0.001	0.434	0.586	0.586			
		Zn	0.413	.904**	-0.095	0.319	0.319	-0.147		
		Mn	0.366	-0.031	0.115	0.371	0.371	0.263	0.252	
		Fe	-0.364	-0.043	-0.096	0.313	0.313	-0.473	0.194	0.088
	15-30cm	pH								
		EC	0.731							
		%MC	0.023	-0.289						
		%SOM	.762*	0.583	0.276					
		%SOC	.762*	0.583	0.276	1.000**				
		Cu	-.784*	-0.37	0.056	-0.266	-0.266			
		Zn	0.245	0.213	-0.276	0.616	0.616	0.16		
		Mn	0.652	0.194	0.518	.878**	.878**	-0.364	0.494	
		Fe	-0.277	-0.408	0.287	0.173	0.173	0.301	0.339	0.387

*. Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed), (AL=Above landfill; L=Landfill; BL= Below landfill)

At the landfill site, Fe and Mn concentrations at the 0–15 cm depth were negatively correlated with pH, EC, SOM percentage, and SOC percentage, though these correlations were not statistically significant. At the 15–30 cm depth, Mn exhibited a significant negative correlation with pH (r = -0.836, $p < .05$), indicating that acidic conditions may enhance Mn mobility.

Below the landfill site (0–15 cm depth), Zn concentration showed a significant positive correlation with EC (r = 0.940, $p < .01$), suggesting that higher ionic strength, represented by EC, may influence Zn mobility (Holzle, 2019). At the 15–30 cm depth, Cu displayed a negative correlation with pH (r = -0.784, p = .05), while Mn showed a significant positive correlation with SOC percentage (r = 0.878, $p < .01$) and SOM percentage (r = 0.878, $p < .01$). This indicates that soil organic matter can play a crucial role in retaining Mn in the soil matrix. Furthermore, a positive correlation (r = 1.00, $p < .01$) was observed between SOC percentage and SOM percentage, reflecting the interconnected nature of these soil properties in influencing heavy metal dynamics.

These results underscore the influence of soil properties such as %SOC, %SOM, pH, and EC on heavy metal concentrations and their interactions. For example, the negative correlation between Mn and pH suggests that soil acidification could mobilize Mn, while the positive correlation between Zn and EC highlights the role of soluble salts in enhancing Zn mobility. Such interactions provide important insights into the mechanisms governing heavy metal behavior in landfill-impacted soils, contributing to a better understanding of environmental contamination dynamics in the study area.

Heavy Metal Concentration in Selected Plant Species

Among 21 sampling points across three sites, plants were found in 11 sampling points, while no plants were recorded at the Landfill site. The three plant species with the highest counts within the quadrats were *Artemisia moorcroftiana* Wall. ex DC., *Bidens pilosa* L., and *Poa annua* L. (Figure 4). The concentration of heavy metals in the selected plants is presented in Table 5. Across all the selected plant species, Fe exhibited the highest concentration, followed by Zn, Cu, and Mn, at both above and below the landfill sites. The dominance of Fe aligns with its high concentration in soil from these sites. Similar findings have been reported in studies by Hosseini Beinabaj *et al.* (2023) and Nawab *et al.* (2016), though with different plant species.

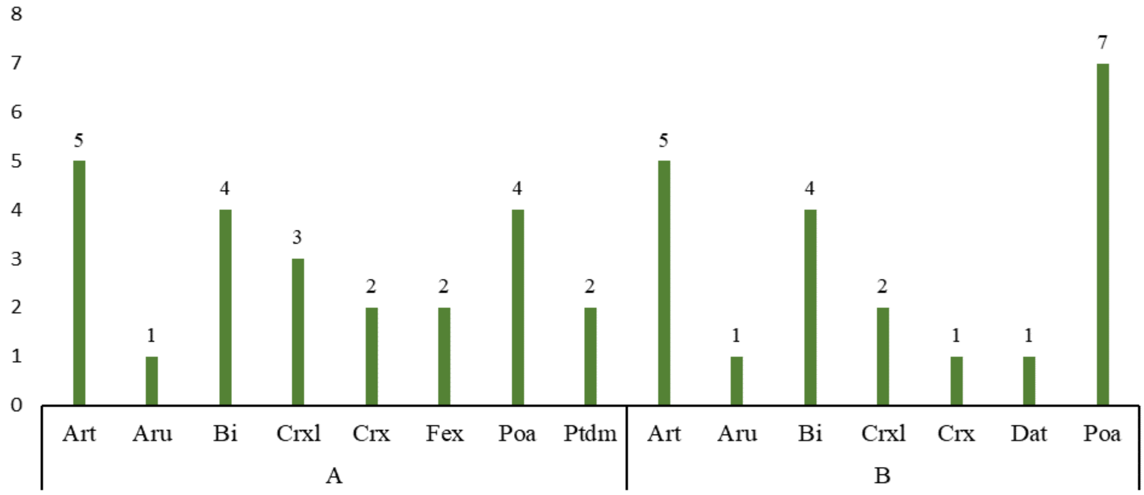


Figure 4: Count of each plant recorded using 100 cm² quadrat in two sites (A: above landfill; B: below landfill), (Art: *Artemisia moorcroftiana;* Aru: *Arudinella bengalensis;* Bi: *Bidens pilosa;* Crxl: *Carex longicrusis;* Crx: *Carex* sp., Fex: *Festuca* sp.*,* Poa: *Poa annua*; Ptdm: *Pteridium aquilinum;* Dat: *Datura* sp.

In the above landfill site, the total heavy metal concentration of Fe, Mn, Zn, and Cu for each plant (sum of root, stalk, and leaf) was as follows: *B. pilosa* (19.80 mg/kg) > *A. moorcroftiana* (19.52 mg/kg) > *P. annua* (10.36 mg/kg). Similarly, in the below landfill site, the concentration was highest in *P. annua* (32.50 mg/kg), followed by *A. moorcroftiana* (27.13 mg/kg) and *B. pilosa* (24.49 mg/kg). The highest concentrations of heavy metals were consistently found in the root parts of *P. annua* and *B. pilosa* at both sites. However, in *A. moorcroftiana*, the highest concentration of heavy metals was observed in the leaf parts rather than the roots, highlighting a variation compared to the other two species. Heavy metal concentrations were higher below the landfill than above, likely due to contamination from landfill leachate, which introduces metals into surrounding soils and water sources (Hosseini Beinabaj *et al.*, 2023).

Heavy metal concentrations showed distinct patterns between plant species and areas above and below the landfill, reflecting variations in species-specific metal uptake mechanisms, tolerance levels, and soil physicochemical properties. Soil pH, for instance, is a critical factor influencing metal bioavailability, as higher pH levels increase the formation of metal-organic matter complexes, enhancing bioaccumulation potential (Adamczyk-Szabela & Wolf, 2022).

Table 5: Heavy metal concentration in plants from two sites and WHO/FAO limits (mg/kg)

Sites	Organs	Cu	Zn	Mn	Fe
Above landfill	*Artemesia moorcroftiana*				
	Leaf	1.745	1.974	1.15	4.37
	Stalk	2.012	0.64	0.703	1.588
	Root	0.525	1.326	0.539	2.956
	Bidens pilosa				
	Leaf	1.368	0.869	0.681	3.05
	Stalk	1.324	1.231	1.063	2.579
	Root	1.768	1.707	1.488	2.673
	Poa annua				
	Leaf	0.104	0.526	0.212	1.164
	Stalk	0.148	0.793	0.321	1.353
	Root	0.259	1.326	0.703	3.474
Below landfill	*Artemesia moorcroftiana*				
	Leaf	1.634	2.355	1.15	4.559
	Stalk	1.324	2.24	0.877	4.04
	Root	1.524	1.936	1.03	4.464
	Bidens pilosa				
	Leaf	1.324	2.05	1.041	2.579
	Stalk	1.413	2.145	1.15	3.003
	Root	1.878	2.145	1.161	4.606
	Poa annua				
	Leaf	2.233	2.298	1.292	4.7
	Stalk	2.389	2.374	1.27	4.464
	Root	2.278	2.24	1.215	5.747
WHO/FAO 2001 (Atta et al., 2023)		73	99	0.2	425
FAO/WHO.1984 (Fosu-Mensah et al., 2017)		3	27.3	-	-
WHO permissible limits (Mg/kg) (1996)		10	0.6	-	-

FAO: Food and Agriculture Organization; WHO: World Health Organization

Table 6: Concentration of heavy metals in soil from where plants were collected

Sites	Plants	Cu	Zn	Mn	Fe
Above landfill	*A. moorcroftiana*	1.346	1.079	1.008	4.464
	B. pilosa	1.39	1.479	0.812	4.087
	P. annua	1.59	1.002	0.757	2.909
Below landfill	*A. moorcroftiana*	2.477	2.26	3.801	11.584
	B. pilosa	2.278	2.221	4.893	4.559
	P. annua	2.344	2.336	1.259	9.226

Bioaccumulation (Metals Uptake from Soil to Plant)

The heavy metal content and bioaccumulation in the selected plants exhibited notable variations. While bioaccumulation primarily involves the movement of heavy metals from soil to plant (Uddin *et al.,* 2021), studies have revealed another route, foliar transfer. Plant's aerial organs, such as leaves, can absorb heavy metals through foliar uptake following air particle accumulation on leaf surfaces (Shahid *et al*., 2017). Bioaccumulation of heavy metals in each selected plant species, illustrating species -specific uptake patterns, is presented in Table 7. Above the landfill, *B. pilosa* demonstrated the highest accumulation (11.79 mg/kg), followed by *A. moorcroftiana* (11.20 mg/kg) and *P. annua* (6.65 mg/kg). The aggregated metal accumulation across all species was in the order of Zn (8.86 mg/kg) > Mn (7.98 mg/kg) > Cu (6.71 mg/kg) > Fe (6.08 mg/kg). Below the landfill, *P. annua* accumulated the most metals (10.41 mg/kg), followed by *B. pilosa* (7.80 mg/kg) and *A. moorcroftiana* (6.60 mg/kg). The metal accumulation order remained similar: Zn (8.70 mg/kg) > Cu (6.77 mg/kg) > Fe (4.87 mg/kg) > Mn (4.48 mg/kg). Zn was consistently the most bioaccumulated metal, likely because it is an essential nutrient that only becomes hazardous at elevated concentrations (Fosu-Mensah *et al*., 2017).

Analysis of metal accumulation across plant sections revealed distinct patterns. Both *P. annua* and *B. pilosa* had higher metal concentrations in their roots, suggesting their potential as hyperaccumulators in polluted environments. Specifically, *B. pilosa* accumulated 5.43 mg/kg in leaves, 6.20 mg/kg in stalks, and 7.06 mg/kg in roots, while *P. annua* recorded 4.74 mg/kg, 5.30 mg/kg, and 7.01 mg/kg in leaves, stalks, and roots, respectively. These results were obtained by aggregating the metal accumulation values from all sections (root, stalk and leaf) of the plants.

Metal tolerance plays a crucial role in phytoremediation (Tong *et al*., 2004). Plant species from the Poaceae family, including *P. annua*, are particularly effective in remediation efforts due to their fast growth, high biomass production, and ability to withstand toxic conditions (Patra *et al*., 2021). Additionally, other high-biomass plants, such as *Helianthus annuus* L., *Cannabis sativa* L., and *Zea mays* L., have proven to be effective in removing heavy metals through phytoextraction (Herzig *et al*., 2014; Vangronsveld et al., 2009). These species excel in accumulating metals in the rhizosphere while limiting translocation to aerial parts.

Grasses are also considered promising due to their rapid life cycle, fast growth rate, and ability to withstand abiotic stresses (Malik *et al*., 2010). For instance, *Trifolium alexandrinum* L. is suitable for extracting Cd, Pb, Cu, and Zn because of its fast growth and multiple harvests in a single growing period (Ali et al., 2020). These findings are consistent with previous research. For instance, Wang *et al.* (2023) and Wei and Zhou (2008) demonstrated the hyperaccumulative characteristics of *B. pilosa* for various heavy metals. Similarly, this study found that both *B. pilosa* and *P. annua* exhibited strong bioaccumulation potential, confirming their suitability for phytoremediation in metal-contaminated environments.

Heavy Metals in Plants with WHO/FAO Limits

The average concentrations of heavy metals were compared with permissible limits established by WHO/FAO standards for agricultural soils in 1984, 1996, and 2001 (Table 5). The results indicate that Cu concentrations in the studied plant species exceeded the permissible limit of 3 mg/kg. Zn concentrations in all plant species from both sites were within the permissible limits of 27.3 mg/kg and 99 mg/kg but exceeded the lower permissible limit of 0.60 mg/kg. Mn concentrations in all studied plant species surpassed the permissible limit of 0.2 mg/kg, whereas Fe concentrations remained below the permissible limit of 425 mg/kg.

Although Zn, Cu, and Mn concentrations were within safe limits in the soil, they exceeded the permissible limits in plants, highlighting the differences between thresholds set for soil and plant materials. This can be attributed to the ability of plants to bioaccumulate heavy metals even when soil concentrations are low, as they actively absorb and concentrate these metals in their tissues (Nyiramigisha *et al*., 2021). For instance, in a related investigation, Pradhan and Kumar (2014) reported Cu concentrations of 11.08–23.07 mg/kg in plants from waste dump sites in India, while Nawab *et al*. (2016) observed values ranging from 0.21 mg/kg to 95.56 mg/kg in plants from waste and mining-impacted site in Pakistan. These concentrations exceed the FAO/WHO limit of 3.0 mg/kg, which could be attributed to the ongoing dumping of copper-containing electrical gadgets (Olafisoye *et al*., 2013).

Table 7: Bioaccumulation of heavy metals by selected plant species (mg/kg)

Sites	**Organs**	**Cu**	**Zn**	**Mn**	**Fe**
Above landfill	*Artemesia moorcroftiana*				
	Leaf	1.297	1.83	1.141	0.979
	Stalk	1.494	0.594	0.697	0.356
	Root	0.39	1.23	0.535	0.662
	Bidens pilosa				
	Leaf	0.984	0.588	0.839	0.746
	Stalk	0.952	0.833	1.309	0.631
	Root	1.271	1.155	1.833	0.654
	Poa annua				
	Leaf	0.065	0.525	0.279	0.4
	Stalk	0.093	0.791	0.424	0.465
	Root	0.163	1.323	0.928	1.195
Below landfill	*Artemesia moorcroftiana*				
	Leaf	0.66	1.042	0.303	0.394
	Stalk	0.534	0.992	0.231	0.349
	Root	0.615	0.857	0.271	0.385
	Bidens pilosa				
	Leaf	0.581	0.923	0.213	0.566
	Stalk	0.62	0.966	0.235	0.659
	Root	0.825	0.966	0.237	1.01
	Poa annua				
	Leaf	0.953	0.984	1.026	0.509
	Stalk	1.019	1.016	1.009	0.484
	Root	0.972	0.959	0.965	0.515

While Cu serves as a micronutrient essential for various biological processes, elevated concentrations can pose toxicity risks to plants (Abagale *et al*., 2013). Similarly, though critical in trace amounts for vital plant processes, Mn can become toxic when present in excessive concentrations. These findings underscore the importance of monitoring heavy metal concentrations in both soil and plants to mitigate potential environmental risks.

Conclusions and Recommendations

The study revealed relatively higher heavy metal concentrations in both soil and plants around the Memelakha landfill site. While average Zn, Cu, and Mn levels in soil and Fe in plants were generally within safe limits, certain metals, such as Fe in soil and Zn, Cu, and Mn in plants, exceeded permissible thresholds. The disparity in heavy metal concentrations, particularly the elevated levels below the landfill compared to the control site, highlights ongoing accumulation and identifies the area below the landfill as highly hazardous. This growing concern calls for immediate attentionfrom municipal authorities and the National Environment Commission (NEC) of Thimphu.The mobility of metals in the soil is influenced by physicochemical properties such as slightly alkaline soil, higher EC, soil organic matter binding, and the sandy clay loam texture ture with high porosity and permeability. These factors enhance heavy metal leaching, potentially polluting groundwater if unmanaged. For Bhutan, where incineration is costly and environmentally unviable, managing landfill sites to reduce heavy metal levels before disposal is critical. This study highlights the importance of implementing targeted mitigation strategies, including waste segregations at the source to minimize heavy metal-bearing waste, and implementing phytoremediation with hyperaccumulator plants such as *Poa annua* L., *Helianthus thus annuus* L., *Cannabis sativa* L., and *Zea mays* L., to restore soil health. Further, longitudinal studies and investigations into leachate dilution rates are needed to better understand contaminant dynamics and environmental impact. Additionally, certain metals like Fe and Mn lacked comparisons with some years' WHO/FAO limits due to the fact that they were unavailable for specific years. Future research could address this by utilizing updated standards to provide a more comprehensive assessment.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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