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Leaf Accumulation Capacity of Herbaceous Plants Growing on Fields Contaminated With Anthropogenically Induced Potentially Toxic Elements Under Natural Soil Conditions

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ABSTRACT

Estimation and knowledge of potentially toxic elements (PTEs) in soils and herbaceous plants are vital for ecotoxicological reasons. This study explored PTE accumulation in the aerial organ (leaf) of herbaceous plants in PTE-contaminated soils in a linear transect of three localities close to the Litavka River in Pribram, Czech Republic. Leaf accumulation answers questions on PTE hyperaccumulation and removal during phytoremediation. The study adopted the pseudo-total extraction (aqua regia soluble) of PTE contents (Cd, Cu, Zn, Pb, and Ni) of soil and leaf samples determined by inductively coupled plasma optical emission spectroscopy. Except for Ni, the contents of Cd, Cu, Pb, and Zn were above the permissible limits in soil following WHO with contamination factors ranging from moderate to serious contamination in the sites. Of the 118 herbaceous species in the sites, 43% (52 species) accumulated Cd above the WHO limit in leaves with *Geranium pusillum* Burm. f. having the highest content (0.84 mg kg^{-1}) while only *Geum urbanum* L. (132 mg kg^{-1}) and *G. pusillum* (166 mg kg^{-1}) were above the limit that supports the principal option to clean the infested soils. There was a low bioaccumulation of all plants indicated by a bioaccumulation factor less < 1 ($BAF_{leaf} < 1$) with PTE content lower than reported hyperaccumulator values. The hyperaccumulation status of PTE of plants stated by previous authors in leaves can be unreliable, under natural soil conditions, accumulations can even be lower than the permissible limits. Further studies requiring different soils and other plants are needed to make a reliable inference on which organs of plants support hyperaccumulation status.

1 | Introduction

Studies on the spontaneous growth of herbaceous plants in contaminated sites like other higher plants demonstrate diverse mechanisms for removing potential toxic elements (PTE) from soils, for example, through below (roots and rhizomes) and aerial, for example, shoot, organs accumulation (Khan et al. 2021; Kafle et al. 2022; Jungová et al. 2022). For example, *Arabidopsis helleri* (L.) recorded shoot accumulation of 6151 mg Zn kg^{-1} in soil contaminated with 5988 mg Zn kg^{-1} (unpublished data for authors). The accumulation of PTE, for example, Cu, Zn, As, Cd, Pb, and Ni in plants above the permissible limits according to both world (e.g., WHO/FAO) and regional limits renders them appropriate for phytoremediation of several degrees of contaminated soils (Nworie, Qin, and Lin 2019; Ranjeev, Ram, and Vishal 2007).

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Mechanisms such as apoplastic restriction of PTEs, for example, Pb in the root cell, and vacuolar sequestration are major approaches to accumulating PTEs by many plant species (Asare, Száková, and Tlustoš 2023a, 2023b) while others exclude PTEs in soils (Jungová et al. 2022). Studies show the edibility of the Alpine dock, *Rumex alpinus* in the Alps of Austria and Italy together with the Krkonoše Mountains, in the Czech Republic is questionable due to the uptake and accumulation of As, Pb, Zn, and Cu above their permissible limits in plants (Jungová et al. 2022).

These submissions and other studies including Wang, Zhang, and Hu (2023) suggest that various herbaceous plants exhibit the possibility of being used in PTE removal in contaminated soils.

Considering the potential remediation options, using herbaceous plants producing a high quantity of secondary metabolites and essential oils could be valuable regarding one of the phyto-management objectives, that is, the restoration of economic activity. Several species of herbaceous plants have exhibited beneficial human needs, for example, *Molineria latifolia* (Lim 2012), and *Asystasia gangetica* L. (Bussmann, Paniagua-Zambrana, and Njoroge 2020) are very effective in asthma treatment. Furthermore, metabolites, phytoalexins, and their derivatives developed from these plants exhibit antioxidant, antidiabetic, and anticoagulant properties (Teoh 2016).

Similarly, *Artemisia annua* L. leaves contain a terpene, used to produce artemisinin, a remedy for fever, and similarly, lifesaving terpene and placitaxol (a diterpene), which are effective against ovarian, breast, colon, non-small cell lung cancer, and malignant melanoma (Harper 1998; MovahediYaghutiWei, Rutland, and SunMousaviLiZhuge 2021). In our recent publication by Asare et al. (2023), Asare and Száková (2023), and Asare et al. (2024), different herbaceous plant species demonstrated the ability to accumulate excess Cu, Zn, As, Cd, and Pb above their permissible limits, making them suitable contenders for phytoextraction of contaminated fields in the tropics and temperate climatic zones. Such studies also reflect the bioindication of crops for animal and human consumption.

Although a vast array of herbaceous plants can accumulate different contents of excess PTE, the type of soil, the climatic zones, the content of PTE, and the plant compartment for accumulation results in dynamism in the level of PTE in plants (Garau et al. 2023). Meanwhile, PTE accumulation in the aboveground biomass is considered a more effective form of soil cleaning—phytoextraction as metals are permanently removed. Here aerial organs of accumulated plants can be harvested, for example, *Salix* and *Populus* species (Zárubová et al. 2015).

PTEs, such as, As and Cd are relatively easily transported to aboveground biomass, for example, the leaf. According to studies by Yamaguchi, Park, and Inouye (2011), Yan et al. (2020), and Skuza et al. (2022), at the organ level leaves are well-considered in establishing plants' hyperaccumulating (aboveground organ accumulation) statuses. Meanwhile, studies on only leaf accumulation of PTEs are less pronounced. However, this part of the accumulation of PTE is vital not only for cleaning contaminated soils but also for health purposes. For instance, many secondary metabolites and their derivatives develop in the leaf even as used for fodder (Asare, Száková, and Tlustoš 2023a). Although extreme accumulation of PTE in the aboveground biomass indicates hyperaccumulation, authors such as Baker et al. (2000) and Baker et al. (2000) provided a guideline that Cd, Pb, and Zn-hyperaccumulator can accumulate (in $mg\,kg^{-1}$ dry weight) >100 (Baker et al. 2000), >1000, respectively, in any aboveground organ.

The study hypothesizes that several herbaceous plants may not reflect hyperaccumulating status in the leaf even though they undergo excess accumulative processes (i.e., PTE uptake–mobilization–translocation–detoxification by chelation and vacuolar sequestration) as in all other plants used in phytoremediation of PTEs but are not hyperaccumulators. We draw insight from the fact that most herbaceous plants are considered for their medicinal use, especially in the leaf, however, PTE content from contaminated soil can be higher than their permissible limits in the leaf and can affect the potency, raising an alarm for cautiousness.

Furthermore, due to health and ecotoxicological reasons, PTE contents in plants have assigned global and regional permissible limits for sustainability. Soil–plant translocation of PTE is a key step in entering the food chain (Palansooriya et al. 2020). Additionally, due to the tolerance of many herbaceous plants in contaminated sites, they have been used in the past decade to assist phytoremediation (Asare, Száková, and Tlustoš 2023a, 2023b; Asare and Száková 2023; Asare et al. 2024). In such cases, accumulation should be benchmarked according to expected values in most plants and fodder, for example, WHO/FAO, local limits, and other relevant limits, for example Kabata-Pendias (2011). The current study explores herbaceous plant species that grow in different post-mine and smelting sites in Příbram, Czech Republic to determine various tolerable spontaneous herbaceous species for their leaf accumulation abilities of PTEs.

Notably, metals including Cd are of no relevance to plants and cause more detrimental effects when they exceed a limit in plants and can cause cardiovascular effects on exposure to the human system, for example, via ingestion of herbaceous plants growing in such localities (Jaishankar et al. 2014). Although some work has been done on the uptake of PTE by plants in contaminated soils (Martínez-Sánchez et al. 2022), there is still a lack of information on the uptake and translocation of PTE by herbaceous plants in multi-PTE-contaminated soils.

Therefore, this study tried to ask the following research questions: (a) To what extent can PTEs accumulate in the leaves of herbaceous plants? (b) According to different permissible values, which plants are more susceptible to accumulating above the limit for (1) removing PTE in contaminated soils through the leaf? (2) greenfeed, especially from only the leaf? and (c) Can the leaf of herbaceous plants be used as a reliable indicator of hyperaccumulation?

2 | Materials and Methods

2.1 | Study Site Description

The study was conducted at three post-mine and smelting sites (A, B, and C) forming a linear transect with the Litavka River in Příbram, the Central Bohemian Region, 54–56 km southwest of Prague, Czech Republic.

Except for the control (C), the remaining sites are well-characterized physical relics of metallurgical activities (e.g., litharge and smelter) known to contain high Cd, Pb, and Zn contents in the soils (Borůvka and Vacha 2006; Zárubová et al. 2015). Historical evidence indicates that silver (Ag), lead (Pb), zinc (Zn), and iron (Pb) ore were extracted from the mines in Příbram, and also strategic uranium (U) after World War II.

The sites are characterized mainly by Modal Cambisol with fluvisol/alluvial sediments in control, with relatively similar elevations and geological substrates (Czech Geological Survey 2012; Table 1). Příbram was promoted to a royal town in 1579 due to precious metal mining in the mid-16th century. However, extensive mining activities did not come until the 19th century when almost all Ag and Pb production in the Austrian–Hungarian Empire came from the Příbram mines (Vojtěch et al. 2010).

2.2 | Soils and Plant Sampling Techniques and Collection

Soil sampling involved random collection within site A–C, where herbaceous plants were found in an area of exactly $30 \times 30\,m^2$ area in each site to avoid bias. Such soil sample collection avoids lateral mixing. Additionally, soil collection in this approach around studied plants enables the direct influence of herbaceous species with soil chemical properties. Each soil sample represented composite samples of five subsamples collected from the sampling square ($5\,m^2$). In total, 15 soil samples (six replicates × three sites) were collected.

The mature herbaceous plant species were randomly sampled to cover the locations chosen along the Litavka River near the creek. The choice of a collection of herbaceous species was dictated by their spread in each selected site and area.

The nomenclature and classification of plant species were performed in situ following the Angiosperm Phylogeny Group—APG IV (https://www.gbif.org/species/3106). Leaves of plant samples were collected with knives and forceps and kept in plastic bags in July 2023. The soil and plant samples were then put in appropriately labeled polyethylene bags and immediately transported to the laboratory on the same day for pretreatment (cleaning from environmental pollutants).

2.3 | Sample Preparation

The soil samples were air-dried for 72 h, homogenized through a 0.2 mm sieve, and analyzed for the pseudo-total content of metals. Similarly, leaf samples of plant species were air-dried at room temperature (20°C–22°C) for 5 days. Each sample was homogenized by milling with a Retsch friction mill of 0–1 mm particle size (Retsch, Haan, Germany).

2.4 | Analytical Methods

Strict quality-control procedures were followed to minimize external contamination during analysis. Laboratory contamination was routinely controlled using analytical process blanks. All solutions were vacuum-filtered before use in the extraction process. All laboratory glassware used during digesting and filtering was covered with aluminum (Al) foil to prevent airborne contamination, while all glassware was rinsed in triplicate with filtered, deionized water.

To determine the pseudo-total (aqua regia soluble) element contents of soil and leaf samples, an aliquot (~0.5 g of dry matter) of each material was weighed into a digestion vessel. Concentrated HNO_3 (8.0 mL) and 30% hydrogen peroxide (H_2O_2, 2.0 mL) were added. The digest was heated in an Ethos 1 (MLS GmbH, Germany-microwave-assisted wet digestion system) for 30 min at 220°C. After cooling, the digest was quantitatively transferred to a 20-ml glass tube, topped with deionized H_2O, and kept at laboratory temperature. The element contents of soils together with the biomass of plants were determined with inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 720, Agilent 159 Technologies Inc. USA).

For quality assurance of the analytical data for the leaf of herbaceous species, a certified reference material (NIST CRM tomato leaves 1573) for the determination of total contents of Cd, Cu, Ni, Cr, and Zn in plant biomass was used (http://www.nist.gov/srm) and contents of studied metals determined by same ICP-OES. Moreover, ICP-OES has been used to determine the total Pb content in dried-weight vascular and nonvascular plants. The results of triplicate control elements (Cd, Cu, Ni, and Zn) revealed a recovery (R) of 90% with an obtained CRM value of 0.061 ± 0.01 Cd, 11.2 ± 0.01 Cu, 2.31 ± 0.11 Ni, and 80.4 ± 3.1 Zn $mg\,kg^{-1}$ to original values—0.068 ± 0.012 Cd, 12.22 ± 0.4 Cu, 2.142 ± 0.058 Ni, and 82.3 ± 3.9 Zn $mg\,kg^{-1}$, respectively (Magnusson and Örnemark 2014).

2.5 | Operating Condition of ICP-OES

The ICP-OES was equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material (experimental 161 conditions. The power of 1.2 kW, plasma flow of $15.0\,L\,min^{-1}$, the auxiliary flow of $0.75\,L\,min^{-1}$, nebulizer flow of $0.9\,L\,min^{-1}$) was used for the determination of elements in the digests.

2.6 | Pollution Index

The pollution index was calculated and interpreted according to Chen et al. (2015).

$$Ii = Ci / Si$$

where Ci = content of measured RE; Si = permissible limit of evaluated RE. In this case, the preventive levels of elements in soil, as

TABLE 1 | Environmental description of study sites.

Site	Description	Geographical location	Elevation (m)	Annual temp. (°C)	Annual precipitation (mm)	Soil	Parent bedrock
A	Location with heaps of mine waste	49.710 N; 13.987 E	501	6.5	700	Modal Cambisol	Schists, sandstones, graywackes, and quartz
B	Drátovna—residues of smelter waste spills	49.678 N; 13.975 E	501	6.5	712	Modal Cambisol	Schists, sandstones, graywackes, and quartz
C	Jince—control site (with no physical relics of metallurgy)	49.785 N; 13.987 E	502	6.5	600–800	Fluvisol	Sandstones, graywackes, and quartz

Statistical description of the contents of Cd, Cu, Ni, Pb, and Zn in the studied sites

Site		Cd	Cu	Ni	Pb	Zn
				$mg\,kg^{-1}$		
A $N=6$	Mean	44 a	127 a	30 a	3898 a	6310 a
	Minimum	13.2	77.6	16.3	3368	4078
	Maximum	84.6	162	41.7	4250	8542
	SD	26.2	35	10.4	329	1724
B $N=6$	Mean	27 b	59 b	23 b	2559 b	3822 b
	Minimum	21	44.8	16.3	2064	3094
	Maximum	47.7	108	35	3166	5546
	SD	11.6	27.6	7.3	485	997
C $N=6$	Mean	1.6 c	16.4 c	14.6 c	241 c	274 c
	Minimum	0.48	13.7	12	114	116
	Maximum	2.9	20	17.5	387	437
	SD	1.3	3.1	2.4	145	178
	p	0.014	0.04	0.024	0.003	0.005

Note: The p values were obtained from nonparametric Kruskal–Wallis text. Using the Tukey post hoc test, the mean contents of PTM in each treatment with different letters are significantly different.
Abbreviations: A: location with heaps of mine waste, B: Dratovna-residues of smelter waste spills, C: Jince-control without physical relics of metallurgy, N: number of samples, SD: standard deviation.

given by the Czech Public Notice No. 153/2016 (Cd—0.5, Cu—50, Pb—60, Ni—50, Zn—50 mg kg^{-1}) and WHO 1996, (Cd—0.8, Pb—85, Cu—60, Ni—50, Zn—120 mg kg^{-1}) are considered Si.

i. Ii ≤ 0.7, safe pollution level
ii. 0.7 < Ii ≤ 1.0, clean environment
iii. < Ii ≤ 2.0, slight pollution
iv. < Ii ≤ 3.0, moderate pollution
v. Ii ≥ 3.0, serious/very high pollution

2.7 | Accumulation Index

Bioaccumulation factor was estimated according to Zárubová et al. (2015).

$$BAF_{leaf} = \frac{\text{content of element in shoot (dry weight)}}{\text{content of element in soil}}$$

$BAF_{leaf} > 1$ indicates enrichment of the plant structures by PTEs (accumulators).

2.8 | Statistical Analysis

The experimental data were processed using STATISTICA version 10. Kruskal–Wallis tests were used with $p < 0.05$ as the criterion for significance. A nonparametric test was used as there was no normality in the soils and plants datasets.

3 | Results

3.1 | PTE Contents in Soils

There were marked significant differences in the Cd, Cu, Ni, Pb, and Zn content at all sites (Table 2). The mean Cd content was highest (44 mg kg $^{-1}$) at Site A, followed by B with a content of 28.4 mg kg^{-1} (Figure 1a).

The lowest (1.6 mg kg^{-1}) content of Cd was in C. However, the contents of soil Cd in this study were higher than the permissible limit according to WHO and the Czech Public Notice No. 153/2016. Furthermore, the Cu content was highest (127 mg kg^{-1}) at Site A with 62 mg kg in B. Except for the content of Cu in control (17.3 mg kg^{-1}), Sites A and B were higher than the permissible limits. Nickel (Ni) contents ranged from 30 mg kg^{-1} in Site A to the lowest of 15 mg kg^{-1} in C. The content of Ni in all the sites was below the allowable limit in uncontaminated soils (WHO 1996; Czech Public Notice No. 153/2016).

Furthermore, the highest content of Pb was 3776 mg kg^{-1}, recorded in soils of Site A followed by 2682 mg kg^{-1} in B, while C recorded the lowest of 241 mg kg^{-1}. In all three sites, the content of Pb was above the permissible limits in soils.

The Zn content was again the highest (6310 mg kg^{-1}) at Site A, with B recording 3866 mg kg^{-1}. The lowest content (274 mg kg^{-1}) of Zn was recorded in the control. In addition to Ni and Cu content in control, the remaining PTE content in soils threatens human health, flora, and fauna.

3.2 | Contamination Level of Soils

The contamination levels of soils in the three sites are given in Table 1. According to the Czech Public Notice No. 153/2016, Cd content in Sites A and B indicate serious contamination.

Cu content was moderately contaminated in Site A per the regulatory limits in this study with slight pollution in B following the Czech Public Notice No. 153/2016. Meanwhile, Ni content in all the sites indicates a clean environment. Sites A–C are seriously contaminated with Pb but still to a diverse extent. However, according to the WHO limit, Pb content in Site C indicates moderate contamination. Additionally, the content of Zn in all sites indicates serious contamination except moderate pollution in C, according to the WHO limit.

3.3 | PTE Accumulation and Bioaccumulation Factor (BAF) of Herbaceous Species

The accumulation of studied PTEs in herbaceous species is presented in Figures 2–5. After critical scrutiny, Sites A, B, and C recorded 34, 51, and 33 herbaceous plants, respectively (Table S1).

In Site A, 43% (15 species) accumulated above the Cd permissible limit in plants (0.02 mg kg^{-1}; WHO 1996; Kabata-Pendias 2011), with the highest (0.27 mg kg^{-1}) content recorded in *Geum*

TABLE 2 | The pollution index of soils from different sites.

PTE	Czech public notice no. 153/2016	PTE content in the study sites			Contamination level in the study sites			WHO limit	Contamination level in the study site		
		A	B	C	A	B	C		A	B	C
Cd	0.5	44	28.4	1.6	88	56.8	3.2	0.8	55	35.5	2
Cu	50	127	62	16.4	2.54	1.24	0.33	60	2.1	1.03	0.27
Ni	50	30	23	15	0.6	0.5	0.3	50	0.6	0.5	0.3
Pb	60	3898	2682	241	65	44.7	4	120	32.4	22.4	2
Zn	50	6310	3866	274	126	77.3	5.5	120	52.6	32.2	2.3

Abbreviations: A: heaps of mine waste, B: Drátovna, C: Jince (control).

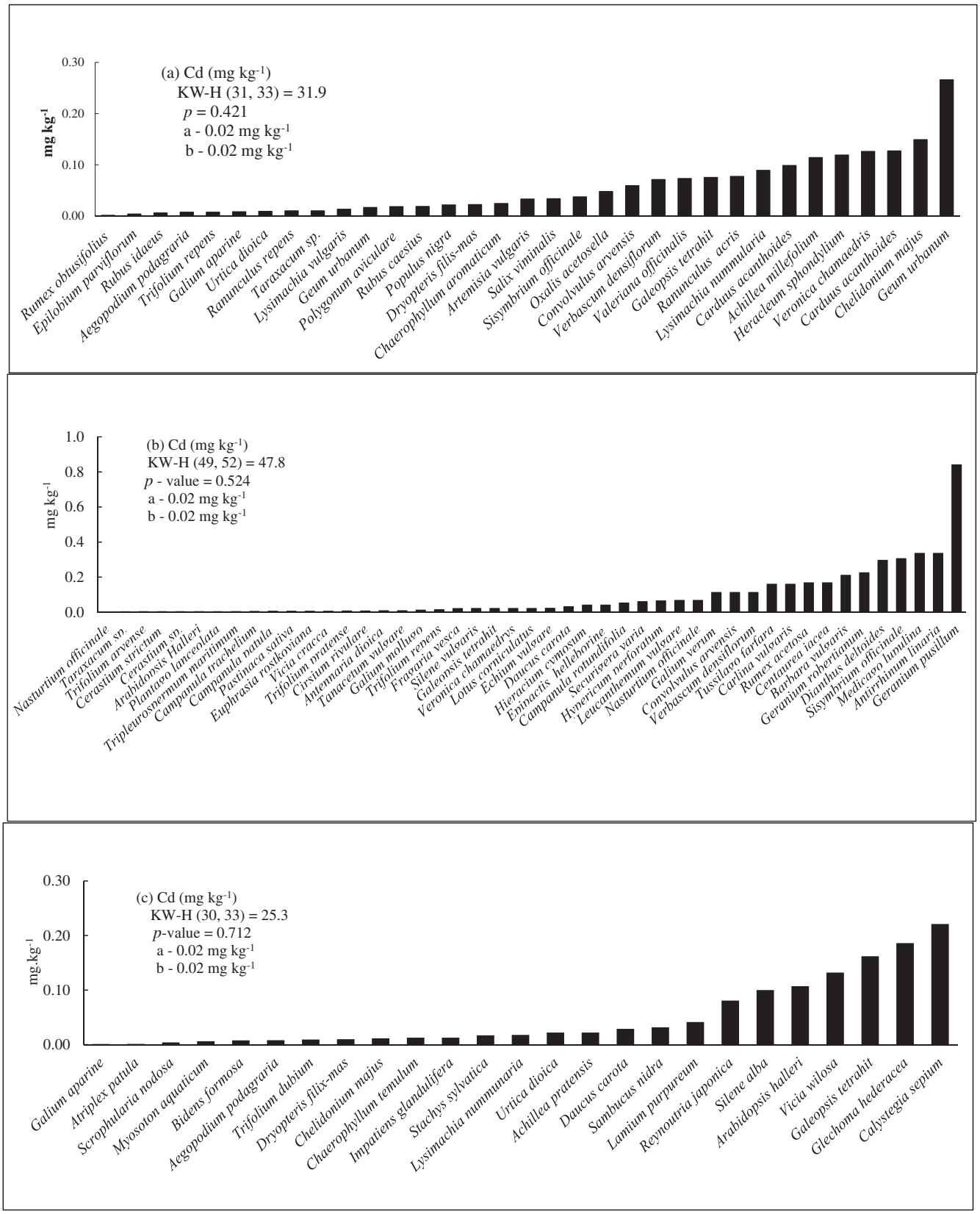
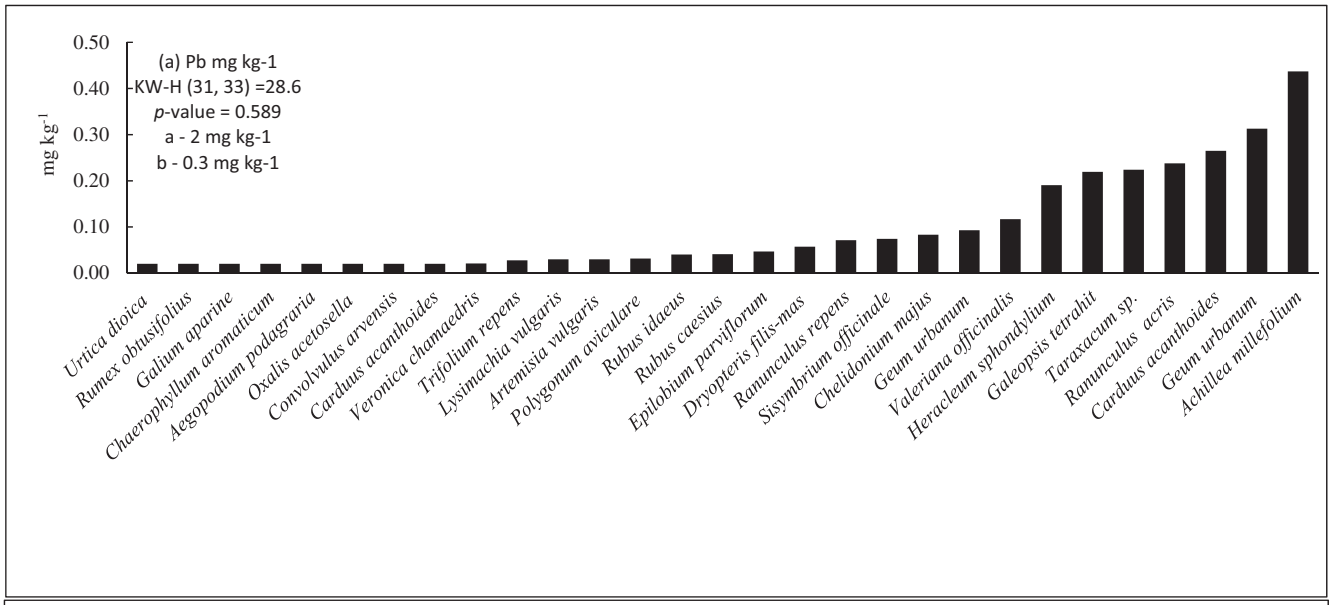
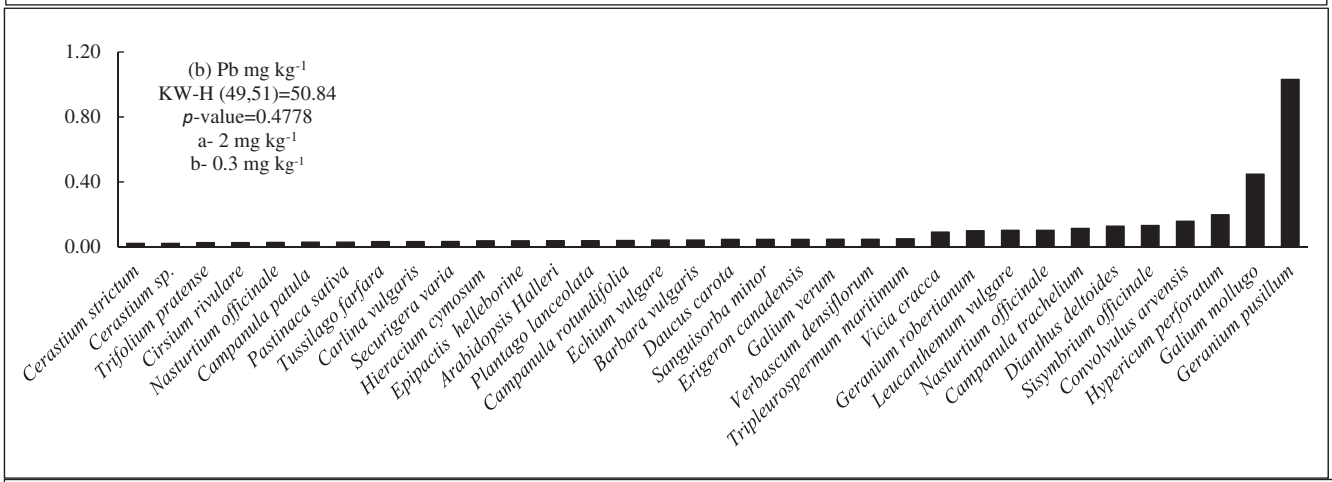


FIGURE 1 | The content of Cu in the leaves of herbaceous plants in sites (a) A—location with heaps of mine waste, (b) B—Dratovna—residues of smelter waste spills, and (c) C—Jince—control without physical relics of metallurgy. The *p* value was obtained from nonparametric Kruskal–Wallis text. (a) according to the permissible limit of the World Health Organisation (WHO) and (b) according to the upper limit value in plants by Kabata-Pendias (2011).





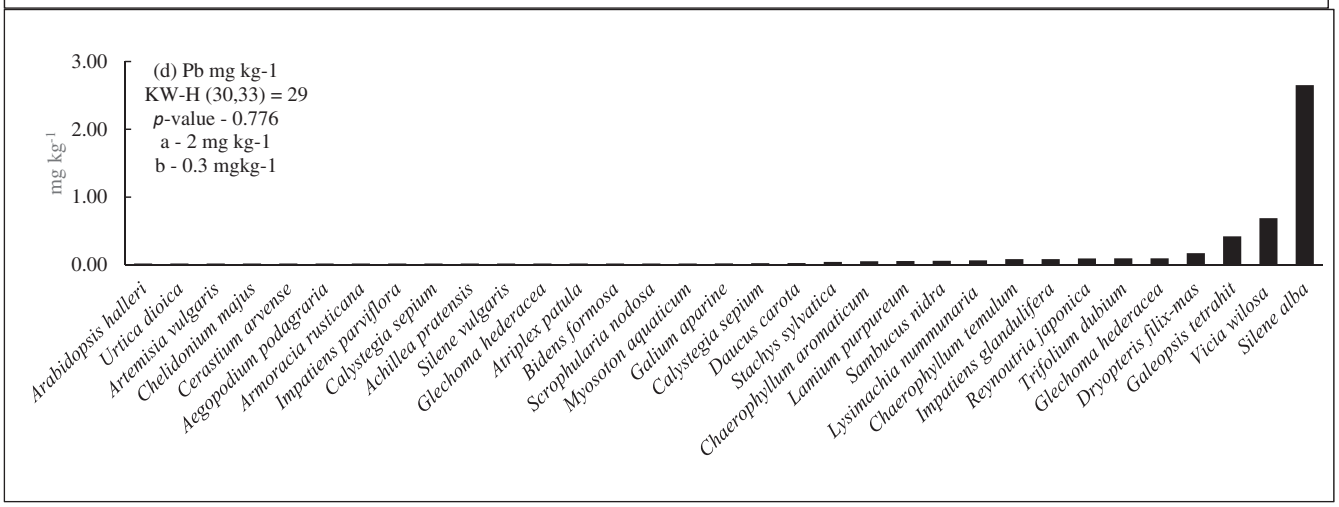


FIGURE 2 | The content of Cd in the leaves of herbaceous plants in sites. (a) A—location with heaps of mine waste, (b) B—Dratovna—residues of smelter waste spills, and (c) C—Jince—control without physical relics of metallurgy. The *p* value was obtained from nonparametric Kruskal–Wallis text. (a) according to the permissible limit of the World Health Organisation (WHO) and (b) according to the upper limit value in plants by Kabata-Pendias (2011).

urbanum (Figure 2a). In Site B, approximately 56% (27 species) had leaf accumulation above the permissible limit of Cd in plants following the WHO with *Geranium pusillum* Burm. f. having the highest content (0.84 mg Cd kg^{-1}; Figure 2b). Of the total herbaceous species in Site C, *Daucus carota, Sambucus nidra*, *Lamium purpureum*, *Reynoutria japonica*, *Silene alba*, *A. helleri*, *Vicia wilosa*, *Galeopsis tetrahit*, *Glechoma hederacea*, *and Calystegia sepium* representing approximately 29% (10 species) recorded Cd content higher than the acceptable limits in plants according to WHO (0.02 mg kg^{-1}; Figure 2c). The highest Cd content

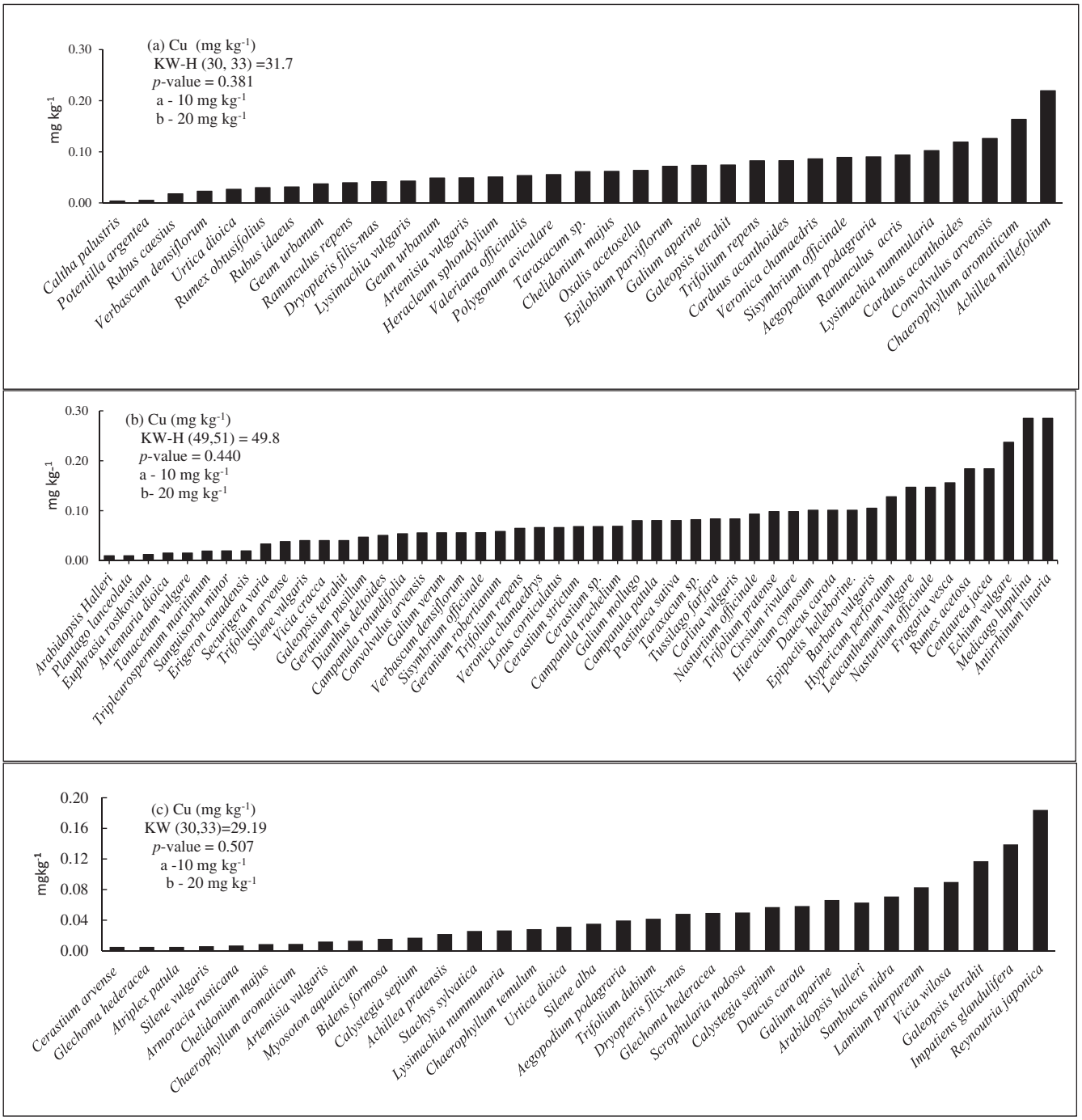


FIGURE 3 | The content of Ni in the leaves of herbaceous plants in sites (a) A—location with heaps of mine waste, (b) B—Dratovna—residues of smelter waste spills, and (c) C—Jince—control without physical relics of metallurgy. The *p* value was obtained from nonparametric Kruskal–Wallis text. (a) according to the permissible limit of the World Health Organisation (WHO) and (b) according to the upper limit value in plants by Kabata-Pendias (2011).

(0.22 mg kg^{-1}) in Site C was recorded in *C. sepium*. Furthermore, the contents of Cu and Ni in the leaves of all the studied herbaceous plants were below the permissible limits in plants, according to WHO (10 mg Cu kg^{-1} and 75 mg Ni kg^{-1}; Figures 1 and 3).

The content of Pb in the leaves in all sites was below the permissible limit in plants (2 mg kg^{-1}) and vegetables (0.3 mg kg^{-1}) following the WHO guidelines (Figure 4a–c). Except for *G. urbanum* L. (132 mg kg^{-1}) and *G. pusillum* (166 mg kg^{-1}) in Sites A and B, respectively, there were no species above the permissible limit (99.4 mg kg^{-1}) of Zn in plants, especially in vegetables (WHO 1996; Mensah et al. 2009; Figure 5a–c). The BAF in all the studied species was > 1 (Tables S2–S4).

4 | Discussion

The study provides a field situation of leave-PTE accumulation by herbaceous plants in contaminated soils, which resulted from metallurgical activities (mining and smelting). Natural

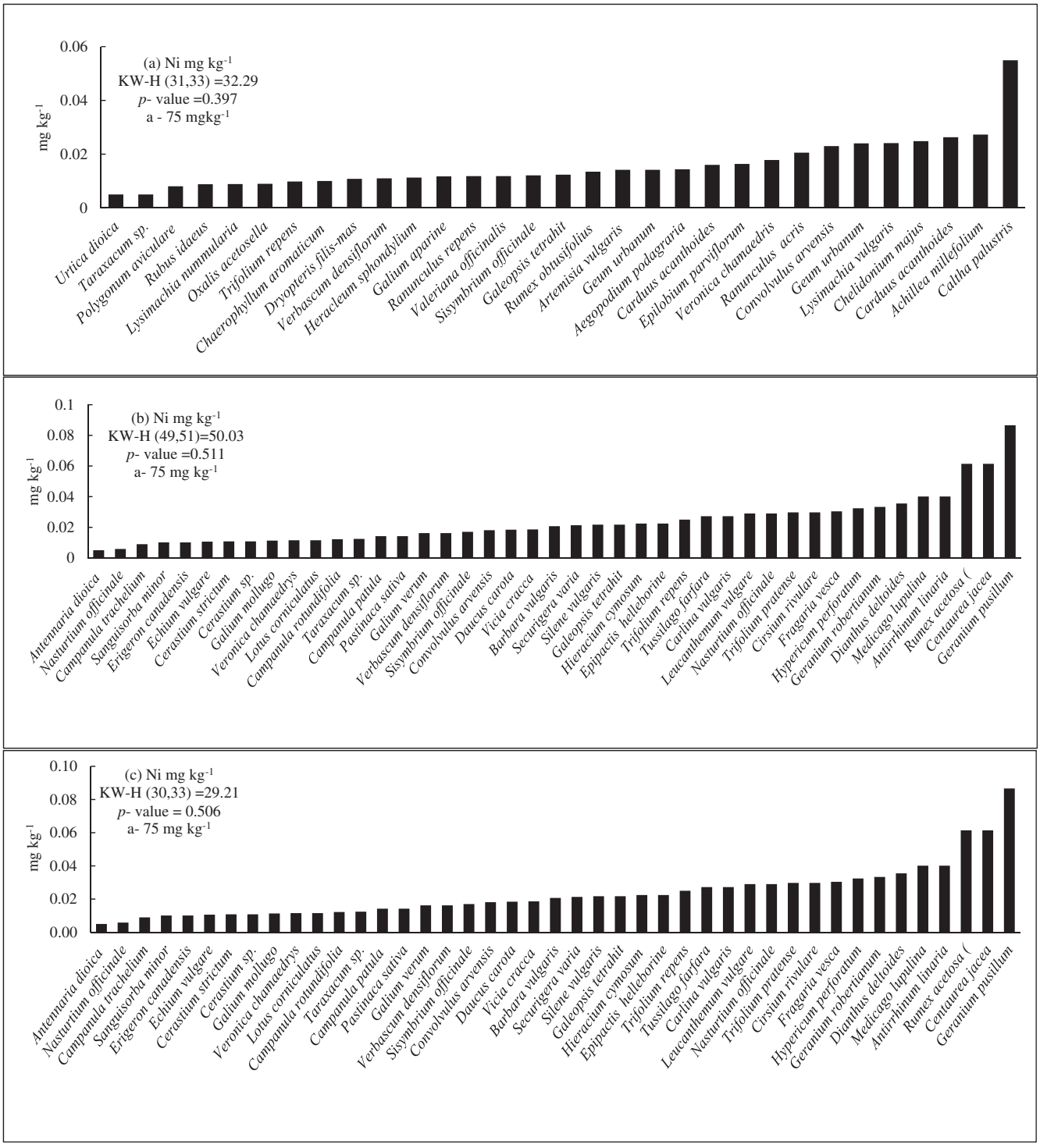


FIGURE 4 | The content of Cd in the leaves of herbaceous plants in sites (a) A—location with heaps of mine waste, (b) B—Dratovna—residues of smelter waste spills, and (c) C—Jince—control without physical relics of metallurgy. The *p* value was obtained from nonparametric Kruskal–Wallis text. (a) according to the permissible limit of the World Health Organisation (WHO) and (b) according to the upper limit value in vegetables (WHO).

soil conditions without additional amendments in this study have revealed the growth of herbaceous species suitable under exogenous factors/natural conditions, for example, environmental effects on excess PTEs accumulation, especially Cd (e.g., *G. urbanum*) and Zn (e.g., *G. pusillum*).

Firstly, the PTE content in soils of this study, especially for Sites A and B threatens human health, and the accumulation of these elements in plant tissue suggests that the sites are unsuitable for agricultural production. Even though, field surveys and recap of metallurgical relics preclude the control site. Cd, Pb, and Zn illustrate some level of soil contamination. This provides a forewarning of surrounding sites to metallurgy as deposits from the atmosphere can finally settle in the soils and leaves of plants (Fida et al. 2022). Similarly, the sloppiness of the control could contribute to the gradual wash-away of some of the PTEs, for example, Cd.

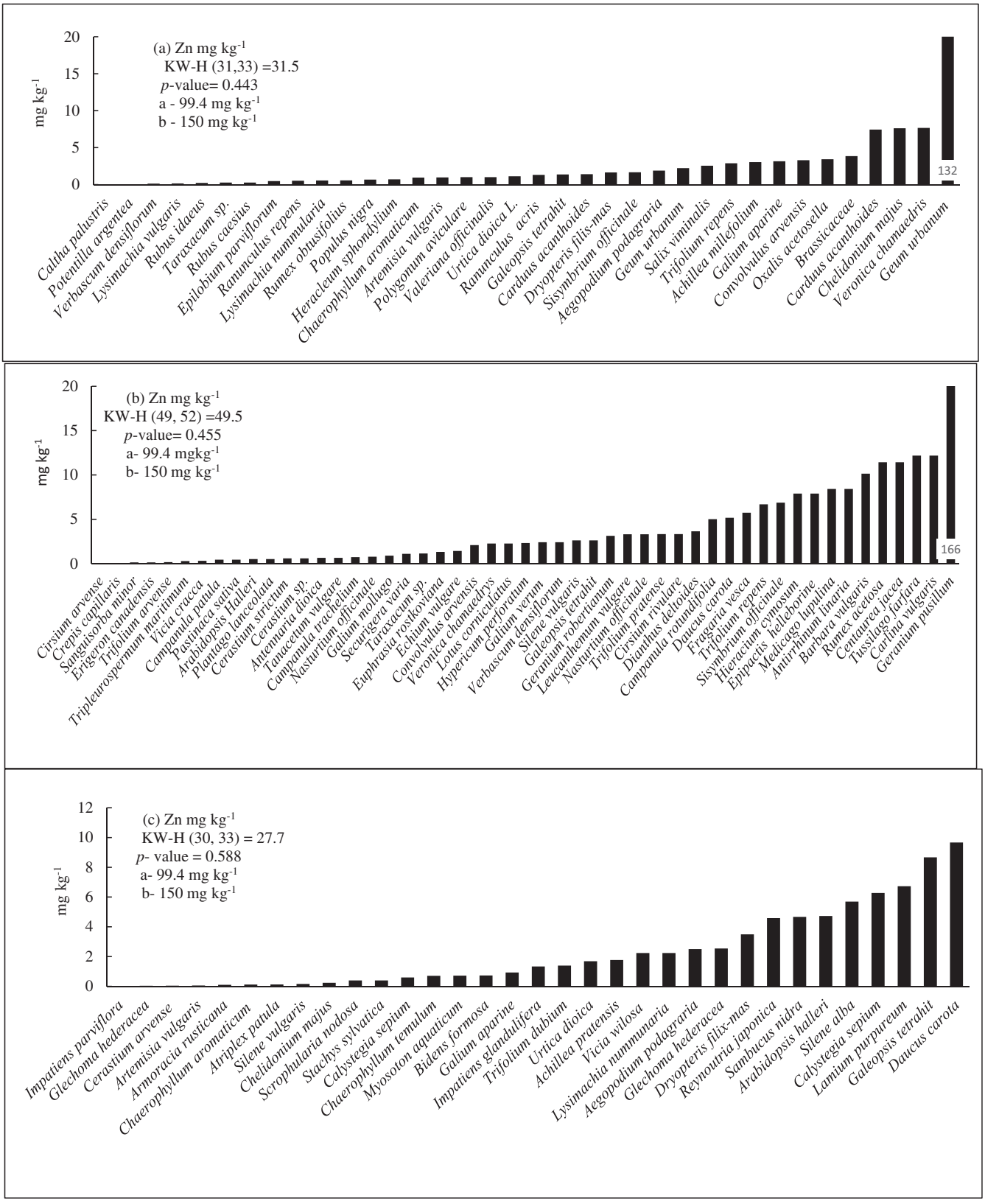


FIGURE 5 | The content of Zn in the leaves of herbaceous plants in sites (a) A—location with heaps of mine waste, (b) B—Dratovna—residues of smelter waste spills, and (c) C—Jince—control without physical relics of metallurgy. The *p* value was obtained from nonparametric Kruskal–Wallis text. (a) according to the permissible limit of the World Health Organisation (WHO) and (b) according to the upper limit value in plants by Kabata-Pendias (2011).

The study is vital considering that parts of the sites serve as arable land and alert farmers and agronomists about the possibility of PTE uptake and fodder consumption. Additionally, some of the studied plants are used to obtain essential oils, for example, *A. vulgaris* with therapeutic effects (Anwar et al. 2016), and secondary metabolites (Asare, Száková, and Tlustoš 2023a).

The comparatively high Pb and Zn contents in Sites A and B were evident in mining minerals including schists, sandstones, and graywackes (Czech Geological Survey 2012). Meanwhile, the extent of PTE accumulation in the soils can relate to the level of metallurgy and the closeness to metallurgical activities. Although, the study involved the leaves of several herbaceous plants, comparatively fewer plants could accumulate above the permissible limits according to prescribed guidelines such as WHO (1996) and Kabata-Pendias (2011)—0.2 mg Cd kg^{-1}, Cu, Pb, and 150 mg Zn kg^{-1}. This is also visible according to the BAF < 1 for all the herbaceous plants analyzed in this study.

4.1 | Cadmium

Under natural soil conditions, in this study, the accumulation of Cd, Pb, and Zn was comparatively lower than in recent research conducted within the same area by Asare et al. (2024). Although Cd content in this study (A—44 and B—27 mg kg^{-1}) was higher than in the previous research, within the same locality, the latter accumulated up to 30 mg kg^{-1} in the shoot of some herbaceous plants (e.g., *Hypericum maculatum* L.) at an acidic pH of 5.25. The solubility of Cd usually occurs at increased acidity of soils for plant uptake (Kubier, Wilkin, and Pichler 2019).

Cd forms chelate with ligands in the cell wall, cytoplasm, and vacuole resulting in its immobilization, thereby losing toxicity (Ali, Khan, and Sajad 2013). Root vacuole storage of Cd reduces its toxicity and long-distance transport to shoot. Cd is sequestered in shoots, and detoxification occurs in cell walls or plant vacuoles, which may result in low leaf accumulation. Meanwhile, according to Baldantoni et al. (2016), leafy vegetables have a relatively high potential for Cd uptake and translocation and are thus considered Cd accumulators, which implies leaves of herbaceous plants used as vegetables, for example, *Rumex* spp must be verified for PTE contents. Additionally, Cd chelates with organic ligands in the leaf and migrates within cells (Yamaguchi, Park, and Inouye 2011). Free Cd^{2+} also interacts with the cell walls of xylem vessels and partly adsorbs on them or can migrate through the cell walls of the xylem to stem and leaf cells, mainly under the influence of transpiration flow (Sterckeman and Thomine 2020).

4.2 | Copper

Although Cu mining and smelting directly preclude the area, it is a component of the minerals—galenite (PbS), sphalerite (ZnS), boulangerite ($Pb_5Sb_4S_{11}$), and antimonite (Sb_2S_3) (Borůvka and Vacha 2006). Again, no plant could accumulate Cu above the WHO and other regulatory limits. Meanwhile, similar studies in the same study region with Cambisol Cu accumulation in the shoot of *Equisetum arvense* L. at an uncontaminated was 37.1 mg kg^{-1}, which suggests it can be used in remediating Cu-contaminated soils (Asare et al. 2024). This can be supposed that different compartments of plants can support different accumulations of PTE (Dastogeer et al. 2020) as various parts of the shoot can support the accumulation of diverse contents.

4.3 | Other PTEs

Additionally, studies have confirmed that herbaceous species such as *A. halleri* are plants suitable for hyperaccumulating Zn (Huguet et al. 2012). This species is present in the metal-contaminated and non-contaminated soils of Europe. It is constitutively Zn-tolerant and hyperaccumulator, although displaying minor quantitative variations in Zn tolerance among and within populations. Again, *A. halleri* is moderately tolerant to Cd, and its ability to accumulate Cd is variable between populations and within a given population (Bert et al. 2002). Although *A. halleri* can accumulate up to 6000 mg kg^{-1} DW (dry weight) Cd when grown in hydroponic solution (Küpper et al. 2000), meanwhile, the current study recorded only 0.0024 mg kg^{-1} Cd and 0.51 mg kg^{-1} Zn in the leaf. Although the accumulation of PTEs such as Pb and Zn was comparatively lower in studies such as Asare et al. in the same study area, it is evident that bioindication resulting from herbaceous plants' accumulation of these metals indicates the use of plants in the sites for herbs and fodder will be detrimental. It must also be noted that in most contaminated sites, the sustenance of herbaceous plants may result from the accumulation of PTEs in other aboveground (e.g., stem) and belowground biomass (e.g., root). Notably, Cd exhibited excess leave accumulation in many species compared to Zn, Pb, and Cu. Pb accumulation by all studied plants was below the limit of WHO. Comparing the accumulation ability from the study of Kabata-Pendias (2011) in most cases the PTEs are higher than the limit for health reasons. Thus, it is feasible to associate Cd values with the phytoextraction of PTEs in moderately contaminated soils. Therefore, for health reasons, the permissible limits according to WHO are the most suitable option for using herbaceous plants.

G. pusillum recorded the highest leave accumulation for Zn above the most recorded amount in plants.

Meanwhile, soil dynamics on physicochemical properties, for example, high acidic pH, can cause dissolution and immobility of PTEs, thereby affecting the high uptake of these elements in accumulating in different compartments of herbaceous plants (Moreno-Lora and Delgado 2020). For example, the content of Zn in plants depends on the plant genotype. Plant Zn is required from 25 to 150 mg kg^{-1} to stimulate various physiological activities for optimum growth (Balafrej et al. 2020). However, different plant compartments vary in the translocation of Zn. Pb accumulation in all the sites was relatively minimal considering the contamination level. Restricted translocation of Pb results from blockage by the apoplastic barrier in the root (Asare, Száková, and Tlustoš 2023b).

5 | Conclusions

The current exploratory study demonstrated the growth of herbaceous plants even in Cd, Cu, Zn, and Pb-contaminated

soil from metallurgical activities (mining and smelting) after long-term abandonment. The contamination factor of the studied PTEs in the sites shows that Cd, Cu, Pb, and Zn contents threaten human health, flora, and fauna. Therefore, the studied site cannot be used for arable fields or cultivation of food crops due to the bioaccumulation of the PTEs.

Notably, approximately 43% of the total herbaceous species (107 species) accumulated above the Cd permissible limit according to WHO in plants (0.02 mg kg^{-1}) with the highest (0.22 mg kg^{-1}) in *C. sepium* (0.27 mg kg^{-1}) *G. urbanum* content, and *G. pusillum* Burm. f. (0.84 mg kg^{-1}) in all the three sites. The accumulation of Zn is more pronounced in *G. urbanum* L. (132 mg kg^{-1}), and *G. pusillum* (166 mg kg^{-1}) above the permissible limit of Zn in plants, especially in vegetables.

Meanwhile, the leaves of all the plants accumulated below the permissible limit of Ni, especially Cu and Pb even though they were indicated contaminated sites.

Under natural soil conditions, plants have innate modalities that support the level of (high or low) uptake, translocation, and accumulation of PTEs, especially, in the leaf. Therefore, hyperaccumulation connected to the leaves of plants can be misleading as different plants were characterized by diverse and relatively low leave accumulation even below permissible limits.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.