Phytoaccumulation of Heavy Metals and Nutritional Assessments of Terrestrial Plants in Contaminated Agro-ecosystem

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Abstract

The phytotoxicity of heavy metals is intense issue and heavy metals contribute significantly to driving the establishment of tolerant plant populations. The study investigated the nutritive and phytoremediation potential of some terrestrial plants, Parthenium hysterophorus, Chenopodium album and Solanum nigrum in contaminated soils of Central Punjab, Pakistan, using standard laboratory techniques including proximate analysis and spectrophotometric methods. Proximate analysis and the Kjeldahl method were used for nutritional analysis; wet digestion and atomic absorption spectrophotometry were used to determine phytoaccumulation of Cd, Pb, and Ni. Among the studied plants, the highest crude fiber content (39.633 %) was recorded for P. hysterophorus while it had the lowest concentration of protein (0.075 %) and the lowest amount of carbohydrate (10.812 %), and C. album had the highest protein (5.266%), while P. hysterophorus had the highest carbohydrate (27.397%). Results showed that P. hysterophorus had a high potential for removal of Cadmium and Lead heavy metals at different soil metal concentrations across the sampling sites. C. album showed evident phytoremediation potential for Cd and Pb. However, S. nigrum proved to be a good phytoremediator which was mainly beneficial for removing Cadmium (Cd) from the contaminated soil sites. P. hysterophorus showed high metal accumulation, with Pb removal at 93.6% and Ni at 43.5%. In the case of C. album, the sequence of metal accumulation followed the order of Pb > Ni > Cd. Furthermore, S. nigrum exhibited characteristics of a Cd hyperaccumulator, as evidenced by its substantial accumulation of total Cd. Soil Cd concentration decreased by 29.2%, with a Cd removal rate of 27.9% for S. nigrum. Variations in plant elemental profiles and soil composition may be attributed to spatial and agro-climatic differences among sites.

Keywords: Biomagnification, Phytoaccumulation Potential, Phytotoxicity, Elemental Profile, Nutritional Attributes.

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Introduction

Human health and wildlife can be adversely affected by the pollutants resulting from anthropogenic activities (Chua et al., 2019). Phytoremediation is widely regarded as a cost-effective environmental renewal technique driven by sunlight as the only energy source. It serves as an alternative to engineering methods, which are often more disruptive to soil (Sharma et al., 2023). Phytoremediation should ideally achieve acceptable contaminant levels within a decade. of contaminants in the environment. However, it is limited to the root zone of plants (Ma et al*.,* 2016). The concentrations of pollutants are toxic to plants also; therefore, this technology has restricted usage. For various environments and pollutants, phytoremediation technologies are available. (Greippson, 2011). Plants with high aboveground biomass can enhance phytoextraction efficiency; however, hyperaccumulators are specifically defined by their ability to accumulate exceptionally high concentrations of metals in their shoots (e.g., >1,000 mg kg^{-1} for Cd), irrespective of biomass. (DalCorso et al., 2019). These include weeds, trees, grasslands and vegetable crops. Weed species appear to be the greatest choice for metal accumulation, as these resilient tolerant plants may provide a significant amount of biomass as a secondary product and can survive in the severe conditions over large areas (Rajkumar et al., 2012). It's important to select native plants to analyze metal accumulation because they're often better in terms of growth, persistence, and reproduction in the wild.

Recent research has focused on identifying native plants that are resistant to and capable of accumulating heavy metals for sustainable remediation purposes. In the ideal condition; the shoots supplemented with the target pollutants can easily be reaped and burned for energy production and recycling the metal from leftover ash if economically reasonable (Tangahu et al.,2011). Heavy metals are toxic elements with a density at least five times greater than that of water. They are toxic to all living organisms (Sardar et al., 2013). Heavy metals have a higher potential for phytotoxicity and can act as an effective driver for the development of populations of tolerant plants (Milner et al., 2013). As a result, at diverse sites that have been contaminated with a number of heavy metals, it is possible to distinguish between metal-tolerant plant species and natural flora. Native plants should be selected in order to analyze metal accumulation because they usually show superior wild growth, persistence, and reproduction (Fahr et al., 2013).

There has been ongoing research into identifying native plants that are resistant to heavy metals. *P. hysterophorus* is a mature plant producing fertile seeds. In terms of remediation, *P. hysterophorus* is a competent herb. Cadmium is a major contaminant due to its bioaccumulation potential, as recognized by the EPA. (Agwu et al., 2018). *P. hysterophorus* is also regarded as an actual phytoremediator for Cadmium (Manoj et al., 2020). For the remediation of other heavy metals like Chromium (Cr) and Nickel (Ni) it is also considered effective (Sanghamitra et al., 2012). *C. album* is a weedy annual plant that grows extensively. It is positioned in the family Amaranthaceae. *C. album* could accumulate copper in large amounts. *C. album* leaves accumulated higher levels of Cr, Pb, and Cd compared to stems and roots (Bhargava et al., 2008).

Solanum nigrum is a member of the Solanaceae family. It is commonly known as Black Nightshade. *S. nigrum* is a 30-100 cm tall annual herbaceous plant. The best way to increase phytoremediation effectiveness is to increase plant biomass and Cd content (Yang et al., 2021). *S. nigrum* is a potential candidate for remediating Cd-polluted soils. It can store Cd in stems and leaves without reduced growth or phytotoxic symptoms. The variety of endophytic bacteria related with Cd hyper accumulator *S. nigrum* (Chen et al., 2012). Through phytoremediation, plants work in collaboration with various soil microorganisms to transform heavy metals into forms that are available to plants. In the end, it is lowering the levels of pollutants in the affected regions. Phytoremediation initiatives can increase the aesthetic value of contaminated sites. By replacing contaminated sites into green spaces that can be used for recreational or educational purposes, (Wang et al., 2011). Additionally, this development can have social benefits for the surrounding. The study focuses on finding potential phytoremediator terrestrial plants from different contaminated soils. It investigated the ability of plants to withstand and accumulate heavy metals such as Cadmium, Lead and Nickel.

Phytoremediation and plant nutrition are interrelated, as heavy metals affect nutrient uptake and plant composition. Studying both together helps understand how plants tolerate metal stress while maintaining nutritional value, identifying species useful for both soil cleanup and sustainable ecosystem recovery (Al-Obaidi et al., 2024). The research aimed to determine the capacity of these plants to accumulate cadmium (Cd), lead (Pb) and nickel (Ni), and to analyze their proximate nutritional attributes, thereby identifying effective native species for sustainable soil remediation and potential nutritional utilization.

Material and methods

Area of Study

This study was commenced in Sargodha Division which is situated in the Punjab province of Pakistan and is a Sargodha Division, Punjab, Pakistan, is an agricultural region known for citrus production. because of its significant contribution to the citrus fruit industry in the country, owing to its fertile agricultural lands.

Sample Collection

Three plants, *Parthenium hysterophorus* L. (P1; Asteraceae), commonly known as carrot grass, *C. album* L. (P2) it's referred to as Goosefoot belongs to family Chenopodaceae and *S. nigrum* L. (P3) typically famous as Black nightshade belongs to the family Solanaceae were collected from three selected sites, Khushab (S1), Bhalwal (S2), Bhera (S3). There were three replicates for each sample. Every sample was hand-picked randomly, wrapped and labeled in specific envelopes of brown color. For analysis samples were brought to the lab.

Soil samples were collected from three selected sites (Khushab, Bhalwal, Bhera) respectively and analysis was done in a soil testing laboratory. At each site, soil was collected from a depth of 0–20 cm, with five subsamples taken randomly and composited to form one representative sample per site. The following features of the soil samples pH, EC (Electrical conductivity), Organic matter, available phosphorus (P) and available potassium (K) collected were analyzed.

Determination of Proximate Attributes

In proximate analysis, moisture content, dry matter, protein and crude fiber contents, crude fat, and carbohydrates were calculated according to the standard method devised by (AOAC. 2006). The samples were placed in an oven for six to eight hours at 105°C to determine moisture content. The method for calculating the percentage of moisture content is given in formula (1). The percentage of moisture content calculated by a formula which is given in equation (1).

$$\text{Moisture (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight of sample}} \times 100 \qquad (1)$$

For determination of fat content, dried samples were treated with petroleum ether at 40^0C- 60^0C in the soxhlet apparatus to remove ether soluble components. Samples were dried in an oven at 70^0C until the constant weight was achieved. The method for calculating the percentage of fat content is given in formula (2).

$$\text{Crude fat (\%)} = \frac{\text{Weight of oven dried sample}}{\text{Weight of fresh sample}} \times 100 \qquad (2)$$

Nitrogen (%) was estimated by Kjeldhal method. Firstly, preparation of reagents such as 0.01N standard sulfuric acid, 4% Boric acid solution, Methyl red indicator and bromocersol green was done. In a digestion flask, 1g of plant material, 20 ml H2SO4, and 3g of digestion mixture (HgSO4 + K2SO4, 1:9) were taken, and boiled for 1-2 hours till material in it became clear. The material was diluted to 20ml for digestion. In Kjeldhal flask apparatus, 10 ml solution was poured to keep on Kjeldhal ammonia distillation unit. In a solution, 10 ml of 40% NaOH was added. This flask was instantly attached to distillation flask. In a conical flask, 10 ml of 4% boric acid solution was mixed with 100 ml alcohol mixed indicator. The conical flask was removed and chilled for a few minutes when the distillate had reached about 40-50 ml. A method (Kjeldahl, 1883) to estimate the amount of nitrogen content is given in formula (3).

$$\text{Nitrogen (\%)} = \frac{\text{Volume of } \frac{N}{10}}{\text{Weight of sample} \times 10} H_2SO_4 \times 0.0014 \times 250 \times 100 \quad (3)$$

All nitrogen components in plant samples were examined by the Kjeldhal method before protein determination. The protein contents were calculated by multiplication of a factor 6.25 with nitrogen. The method for calculating the percentage of protein content is given in formula (4).

$$Protein\ (\%) = Percentage\ of\ Nitrogen \times 6.25 \quad (4)$$

The determination of crude fiber content was carried out using the acid-base digestion method. Initially, fat was extracted from 3 g of oven-dried samples using the Soxhlet apparatus. Subsequently, the samples were separately digested with 1.25% H2SO4 and NaOH. After filtration, the residue was washed thrice with distilled water. The filtered residue was then placed in a China dish and dried in an oven at 105°C for 24 hours. The content of crude fibers was calculated as the difference in weight before and after the digestion process. Crude fibers were calculated by the given formula. The method for calculating the percentage of protein content is given in formula (5).

$$\text{Crude fiber (\%)} = \frac{\text{Weight of oven dried sample} - \text{Ash weight}}{\text{Weight of fresh sample}} \times 100 \quad (5)$$

Carbohydrates contents were calculated by deducting the moisture, ash, protein, fiber, and fats content from 100. The method for calculating the carbohydrates content is given in formula (6).

$$\text{Carbohydrates (\%)} = 100 - (\text{Protein (\%)} + \text{Fiber(\%)} + \text{Moisture (\%)} + \text{Fat(\%)} \quad (6)$$

Analyzing Elemental Profile (mg/kg)

Plant samples were ground to make fine powder and by using wet digestion method plant samples were digested. Plant material (0.5 g) was soaked into 10 ml HNO_3 in a digestion flask overnight. Next, perchloric acid (5 ml) was added to a digestion flask on a hot plate during the digestion process. The process was continued until the solution became transparent. Distilled water was added into a sample solution to make it a 50 ml final solution. After filtration of the sample solution, it was loaded into the atomic absorption Spectrophotometer for elemental analysis. Detection limits were set at 0.001 mg L^{-1} for Cd, 0.005 mg L^{-1} for Pb, and 0.002 mg L^{-1} for Ni, with instrument calibration performed before each run using certified standard solutions. Samples of known concentration were employed to obtain the standard curve for each metal. This standard curve was used to estimate the elemental content of samples (AOAC, 1998) (Table. 1). The method for preparing the 1000 ppm concentration stock solution for each metal (Cd - Cadmium, Ni - Nickel, and Pb - Lead) is as provided in formula (7).

$$X = \frac{\text{Molecular weight of salt}}{\text{Molecular weight of mineral}} \times 0.1 \quad (7)$$

Table 1: Summary of analytical methods and measured parameters.

Method/Technique	Parameter Measured	Reference/Source
Proximate analysis	Moisture, fat, fiber, carbohydrate	AOAC (2006)
Kjeldahl method	Nitrogen and protein content	Kjeldahl (1883)
Soxhlet extraction	Crude fat content	AOAC (2006)
Atomic absorption spectrophotometry	Cd, Pb, Ni concentrations	AOAC (1998)

Statistical Analysis

Data were analyzed using ANOVA in SPSS (v25.0) and Microsoft Excel (2016). Data obtained from the experiments were statistically analyzed using SPSS (Version 25.0) and Microsoft Excel (2016). Descriptive statistics such as mean and standard deviation were computed for all measured parameters. To determine significant differences among plant species, sites, and their interactions, Analysis of Variance (ANOVA) was performed, followed by post-hoc comparisons using Tukey's HSD test at a significance level of $p \leq 0.05$. Graphical representations of the results were generated using Microsoft Excel.

Results

Edaphic Physico-Chemical Attributes of Contaminated Sites

The composition of soil also varies among different sites which are given in Table 2. Analysis of soil of different sites showed that the PH value varies from 6.22-7.86. Results showed that electrical conductivity value ranges between 6.16 to 44.35 (mS/m), phosphorus value lies between 7.6-8.1 mg/kg and the potassium content of different terrestrial sites was between 26 to 255 mg/kg. There are highly significant differences ($p < 0.001$) in pH and E.C values among different sites while organic matter content, phosphorus levels, and potassium levels show significant differences ($p < 0.05$) across various sites.

Table 2: Physico-chemical properties of soil from various contaminated soils.

S.No.	Sites	PH	E.C (mS/m)	O. matter (%)	P (mg/kg)	K (mg/kg)
1	S1	7.7667	19.527	0.96	8.1	255
2	S2	6.2233	6.1633	0.82	7.6	227
3	S3	7.8667	44.353	0.76	7.8	26

Determination Nutritional Composition

Nutritional parameters such as moisture, crude fat, fiber, protein, and carbohydrate contents varied considerably among plant species and sites, as detailed in Table 3, showing that *C. album* generally exhibited higher nutritional quality than *P. hysterophorus* and *S. nigrum* (Figure 1). The analysis of variance in some terrestrial plants from different contaminated soils revealed significant differences. Specifically, for crude fiber and carbohydrates, there was a significant difference among sites, plants, and the interaction between plants and sites. While analyzing moisture and crude protein, significant differences were observed among sites and in the interaction between plants and sites; however, there was no significant variation among plants. Additionally, a significant difference was found for crude fat among sites, plants, and there was highly significant interaction between plants and sites.

Table 3: Comparison of means of nutritional attributes of some terrestrial plants from different contaminated soils.

Plants	Sites	M.O (%)	D.M (%)	Ash (%)	C.L (%)	C.F (%)	C.P (%)	Carb (%)
P1 *P. hysterophorus*	S1	11.467***	28.34	10.69	2.51	24.737	2.6533	15.577
	S2	60.533	32.66	7.083	3.79*	39.633***	2.7267**	13.487
	S3	66.493	38.69*	12.503**	2.297	10.283	3.0033	27.397***
P2 *C. album*	S1	72.697	30.98	10.77	17.157***	17.513**	3.7333	19.93**
	S2	55.32**	30.56	7.183	5.55	4.5	3.6	5.317
	S3	69.05	38.2**	12.03*	7.697	11.16	5.2667***	8.2*
P3 *S. nigrum*	S1	53.47	50.69	18.487	7.533	15.257*	2.21*	1.8
	S2	43.003*	65.62	20.363***	8.63**	11.593	2.2433	1.737
	S3	57.083	74.09***	18.487	5.78	11.467	2.31	1.423

*Keys: M. O= Moisture, D.M= Dry Matter, C. L= Crude Fats, C. F= Crude Fiber, C. P= Crude Proteins, Carb= Carbohydrates. *, **, *** within a column indicate significant differences at $p < 0.05$ (ANOVA ± Tukey test).*

Determination of Metal Absorption

Elemental content in different plants varies significantly (Table 4). Cd concentration varies among different plants collected from various soil-contaminated sites. In the findings, it was observed that the highest Cd concentration was found in P3 obtained from site S1. On the contrary, the lowest Cd content (0.0387 mg/kg) was found in P2 collected from site S2. Pb content varies among different plants collected from various soil-contaminated sites. The maximum quantity of Pb (1.5800 mg/kg) was detected in the P1 collected from S2 while the minimum concentration of Pb (0.5833 mg/kg) was observed in the P2 collected from site S3. The concentration of Ni varies among different plants collected from various soil-contaminated sites. The highest content of Ni (0.391 mg/kg) was found in the P2 collected from S2 and the minimum amount of Ni (0.164 mg/kg) was noted in the P3 collected from S3 (Figure 2). The analysis of variance revealed that for Cd concentration, there was no significant variation among sites, but there were significant differences among plants and the interaction between plants and sites was also significant. Similarly, for Ni concentration, there was no significant variation among sites, while plant difference and the interaction between plants and sites showed significant variation. However, for Pb concentration, significant variations were observed among sites, plants, and the interaction between sites and plants.

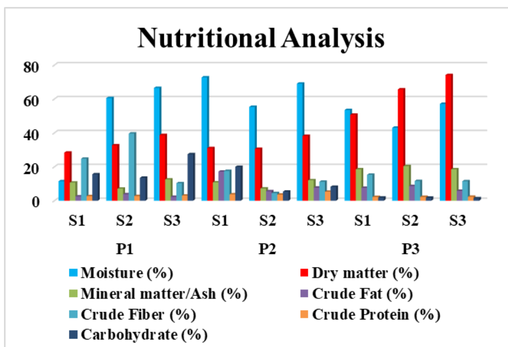

Figure 1: Nutritional composition (mean ± SD) of three plant species across sites."

Table 4: Comparison of means of elemental analysis of Cd, Ni, and Pb in terrestrial plants from different contaminated soils.

Plants	Sites	Cd (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
	S1	0.333	0.216	1.5767**
P. hysterophorus	S2	0.31*	0.215	1.58***
	S3	0.37	0.22	1.5433*
	S1	0.042**	0.217	0.602
C. album	S2	0.038	0.391***	0.7127
	S3	0.31*	0.363**	0.5833
	S1	0.503***	0.237*	0.8167
S. nigrum	S2	0.403	0.227	0.6317
	S3	0.443	0.164	0.6603

Keys: **, **, *** within a column indicate significant differences at $p < 0.05$ (ANOVA ± Tukey test).*

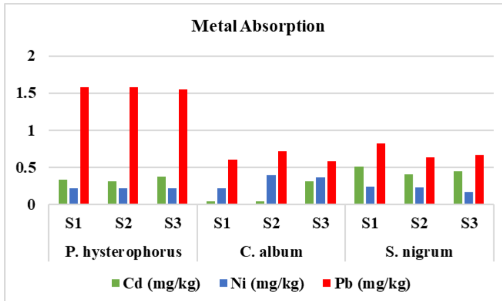

Figure 2: Metal accumulation (mg/kg) in three plant species across sites.

Discussion

To our knowledge, this is the first study to assess the nutritional and phytoremediation potential of some terrestrial plants from different contaminated soils of Central Punjab. Physico-chemical properties of soil from different contaminated soils indicate that the composition of soils in these studied contaminated areas is different from site to site. Soil analysis of different sites revealed that PH value is 6.22– 7.86. Several factors that can affect soil pH existed, including the type of contamination. To give an example, samples which can decrease soil pH, like acidic pollutants, are known and samples with a slighter impact are also known (Petelka et al., 2019). Moreover, locally vegetation and microbial activity can also affect pH. The conclusion of higher pH values could delineate that this soil is more alkaline or less acidic contamination (Nassary et al., 2020). Electrical conductivity value was shown between 6.16 to 44.35 (mS/m). Contamination can cause a soil sample having a high EC to be contaminated with soluble salts and ions in the soil. Significant differences ($p < 0.05$) were observed in the pH and E.C values among different sites whereas values observed for organic matter content (0.041), phosphorus (0.031) and potassium levels (0.021) were also significant, but lower than the 0.05 level at different sites. The considerable variability of EC values between sites shows different pollutants, and different types and amounts. Variation in phosphorus value between 7.6- 8.1 mg/kg and potassium content between 26 to 255 mg/kg had been found at different terrestrial sites. Variation of nutrient concentration might be a result from different agricultural practices, nutrient deposition, and type of contamination (Buta et al., 2019). Opoku et al (2020) explain that local climate, vegetation and hydrology can in turn influence soil characteristics and nutrient cycling patterns. Variations in organic matter content at each site could be related to different plant cover, land use history, and organic material decomposition rates (Liu et al., 2014). These results correspond to the (Xu et al., 2007) results.

Crude fat ranges from 2.29- 3.79% and crude fiber ranges from 10.2 – 39.6 % for results on nutritional composition of *P. hysterophorus*. There was however a variation of 13.4-27.3% in carbo hydrates, crude protein varied between 2.6-3.0% and 11-66% in moisture content. These differences may be due to environmental factors including temperature and humidity (Bhusal et al, 2020). These Results are consistent with Ahmad et al. (2011) and Ibrahim et al. (2017).

Also, the nutritional attributes of *C. album* under our study Cartop the plant with high content of crude fat (5.5 – 17.1 %) and crude protein (3.6 – 5.2 %). Results in percentage carbohydrate of *C. album* leaves are 5.3–19.9%. However, our results for crude fiber contents 17.5% were different. Variations in fatty acid composition of these oils come from plant species and maturity of the plant, and might be influenced by environmental factors including temperature (Adedapo et al., 2011). Nonetheless; our observations of moisture levels between 55.3 and 72.6% differ from theirs. Environmental factors such as rainfall, humidity, soil drainage and temperature have a very big influence on moisture content. This is consistent with the reports of (Liang et al. 2016) and (Adeolu et al., 2011). Results of this study indicated that moisture content of *S. nigrum* ranged between 43-57% and crude fiber content 11-15%. Results vary due to soil composition and humidity (Gqaza et al., 2013).

Results of the present study show *P. hysterophorus* has potential of removing heavy metals Cadmium (Cd) 65.7%, Nickel (Ni) 43.5% and Lead (Pb) 93.6% from the soil having different concentrations at different sites. Results have shown that *P. hysterophorus* was having a robust phytoremediation potential for Cadmium (Cd) and Lead (Pb), and has the potential to outperform the other two plants. This is attributed to root system architecture, transport proteins, metal transporters, and ligands. The results are consistent with those of (Sanghamitra et al., 2012) indicating that it has good phytoextraction capability for Zn and can remove other contaminants from contaminated soil.

We found that *C. album* has high potential phytoremediation capability for Cd 0.31 mg/kg and Pb 0.7 mg/kg, though varying concentrations were found to occur in different areas most likely ensuing from different environmental conditions, soil chemical and most likely the presence of these metals in the soil. This potential was attributed to a unique blend of genetic, physiological and biochemical adaptations including a suite of transport proteins. In addition, the increased root surface area and root hairs help out more their contact with soil and metal ions, increasing its ability for phytoremediation (Abdal et al., 2023). Zulfiqar et al. (2012) discussed results that collaborates with these findings.

Maximum Pb uptake was for *S. nigrum* with roots accumulation of Pb higher than that of shoots at maximum accumulation of 32.6 and 6.1 mg/kg, respectively. In addition, our study highlights the large capacity of *S. nigrum* to Phyto-remediate Cadmium (Cd) in multiple areas of contaminated soil. As some ion channels or metal transporters in the root system can allow the uptake of metals, there is this. Variations observed in metal accumulation could be caused by different environmental conditions or interactions with metals, the plant's ability to tolerate heavy metals, and the plant's growth of a root system with metal transporters present (Yang et al., 2022). The findings of this chapter are consistent with those of (Varun et al., 2016).

Conclusion

In the current study, *P. hysterophorus*, *C. album*, and *S. nigrum* have been assessed regarding the phytoremediation and nutritional potential in heavy metal-contaminated soils of Central Punjab, Pakistan. The highest protein (5.266%) was in *C. album*, while the highest carbohydrate (27.39%) was in *P. hysterophorus*. which also showed an exceptional Pb and Ni removal efficiency, whereas *P. hysterophorus* removed Pb (93.6%) and Ni (43.5%) most effectively. Overall, P. hysterophorus was the most effective accumulator of Pb, *C. album* showed strong affinity for Ni, while *S. nigrum* proved best at accumulating Cd. One hyperaccumulator *S. nigrum* was shown to be effective as a cadmium (Cd) hyperaccumulator, dissolving in the soil 29.2% of Cd with the removal rate 27.9%. Spatial and agro-climatic differences among sampling sites are probably responsible for the observed variability in metal uptake and the nutritional profiles of plants. However, these findings highlight the potential of these plants in sustainable phytoremediation strategies, specifically *P. hysterophorus* and *S. nigrum*, as heavy metal detoxifying agents for phytoremediation, and also as the avenue for further investigations to enhance their application. All selected plants have high phytoremediation potential for heavy metal in various soil contaminated sites. Additionally, heavy metal phytoaccumulation in these selected plants varies even within the same plant species from the same site or when collected from different sites. Different types of heavy metals (contaminants), different environmental conditions, varied soil composition, different sources of the contaminants and variations in metal uptake capacity of the plants could be attributed to this variability. The soil-contaminated regions that were selected vary possibly due to different types of contamination sources and different soil compositions. Future studies should validate these findings through large-scale field experiments, explore molecular mechanisms of metal uptake and assess plant–microbe interactions to enhance phytoremediation efficiency under varied environmental conditions.

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