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Heavy Metal (Cu, Co, Mn, Pb) Uptake and Health Risk Associated with Medicinal Plants from different Contrasting Locations

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Abstract
This study explored the accumulation of copper (Cu), cobalt (Co), manganese (Mn), and lead (Pb) in soils and commonly used medicinal plants from two distinct locations and eight different sites. Metal uptake was assessed through Bioconcentration Factor (BCF), whereas health risks were evaluated using Daily Intake of Metals (DIM) in eight different medicinal plants. Cu levels in plants were high, especially in *Adhatoda vasica*, with BCFs reaching up to 10.78. Co showed the least uptake with BCFs below 0.13. Mn concentrations, although below toxic limits, had considerable BCFs, indicating active absorption. Pb accumulation was moderate but still raised concern due to its toxicity and presence in several plant species. The DIM values suggest that continued, unregulated use of these plants could pose health risks, particularly from Cu and Mn exposure. Overall, the results point to significant variability in metal accumulation across plant species and locations, emphasizing the importance of site-specific monitoring to ensure the safe use of medicinal plants. Future studies should include a wider range of heavy metals (e.g., Cd, Zn, Cr) and assess seasonal variations in metal uptake to build a more comprehensive understanding of risks.

Keywords: Pothohar, medicinal plants, heavy metals, dietary risk assessment, toxicity

Introduction

Pakistan is home to a rich diversity of wild flora, many of which hold immense medicinal and aromatic value. While some of these species are widely recognized and utilized by local communities for traditional remedies, a substantial portion remains scientifically unexplored and under-documented. More than 1,000 plant species in Pakistan have been identified for their therapeutic applications, serving as primary healthcare resources for rural and marginalized populations (Ali., 2008; Mushtaq et al., 2009). Globally, the use of medicinal plants spans across continents, forming the foundation of traditional healing systems and serving as precursors for modern pharmaceuticals (Mohanta et al., 2003).

Among these botanicals, *Dodonaea viscosa*, locally known as "aliar," stands out due to its wide distribution in the Salt Range and Soon Valley. This evergreen shrub or small tree has been traditionally employed for a variety of pharmacological purposes, including analgesic, anti-inflammatory, spasmolytic, antiviral, laxative, antimicrobial, and antihypertensive effects (Ghisalberti, 1998). Indigenous knowledge, especially from regions like New South Wales and parts of Pakistan, attests to its safety and efficacy, although formal toxicological studies remain limited (Khalil et al., 2006).

Parallel to the exploration of medicinal flora is the growing concern over heavy metal contamination, particularly in soils and plants. Heavy metals, typically defined as elements with a density greater than 5 g/cm^3, include a range of transition metals, lanthanides, actinides, and certain metalloids (Jann, 2004; Hutchings et al., 2003). While essential trace elements such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) are vital micronutrients involved in enzymatic functions, bone development, metabolism, and immune response, their imbalance, either through deficiency or excess, can have detrimental effects on both human and animal health (Birla &Taneja, 2010;Okoye, 2011). To mitigate deficiencies, livestock diets are often supplemented with these minerals (Drinceanu et al., 2011), yet their bioavailability can be compromised by dietary antagonists or environmental contaminants.

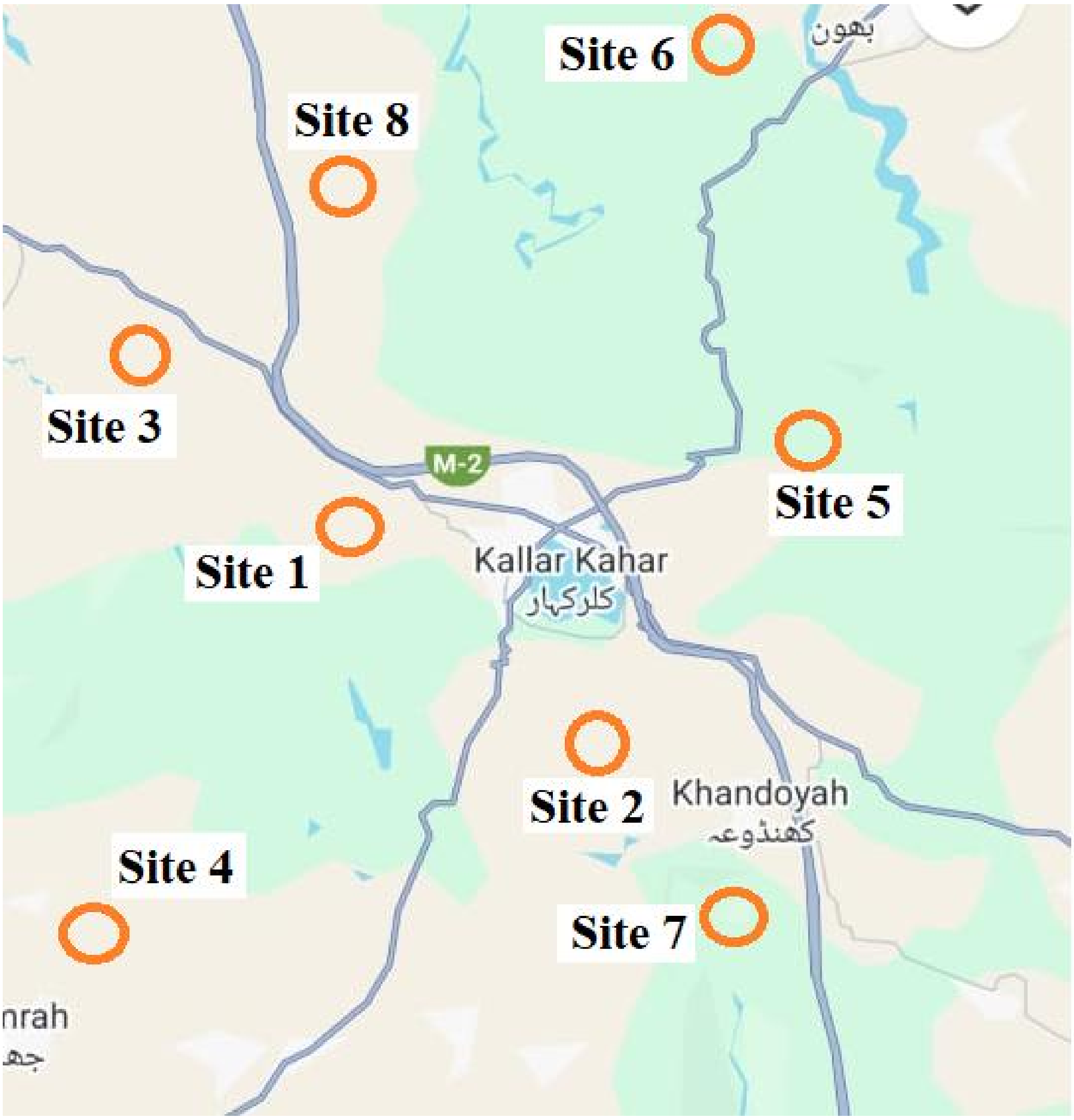
This ethnobotanical investigation aimed to document the indigenous knowledge associated with medicinal plant use in and around the KallarKahar region, particularly before such traditional practices and plant habitats are lost due to environmental degradation and modernization. The collected data contribute to the conservation of ethnomedicinal heritage and advocate for the sustainable cultivation and systematic farming of native medicinal species. This, in turn, can facilitate biodiversity conservation and ensure the rational, long-term utilization of valuable plant resources.

Material and Methods

Study Area

This research was carried out in KallarKahar, a sub-division of the Chakwal District in Punjab, Pakistan. Situated approximately 25 km southwest of Chakwal and about 125 km from Rawalpindi, the region is renowned for its scenic beauty, native peacocks, and a prominent natural saline lake. Geographically, the area lies between latitudes 32°26′11″ to 32°41′18″N and longitudes 71°50′33″ to 72°30′07″E, encompassing roughly 200 acres of diverse vegetation.
The region is part of the Pothohar Plateau, characterized by a semi-arid climate marked by hot, dry summers, moderate winters, and erratic rainfall

predominantly concentrated during the monsoon season. Soils in the region are typically calcareous and moderately fertile, supporting a wide array of native flora. These unique microclimatic and edaphic conditions make the region ideal for studying plant-soil interactions and the bioaccumulation of heavy metals in wild medicinal species.



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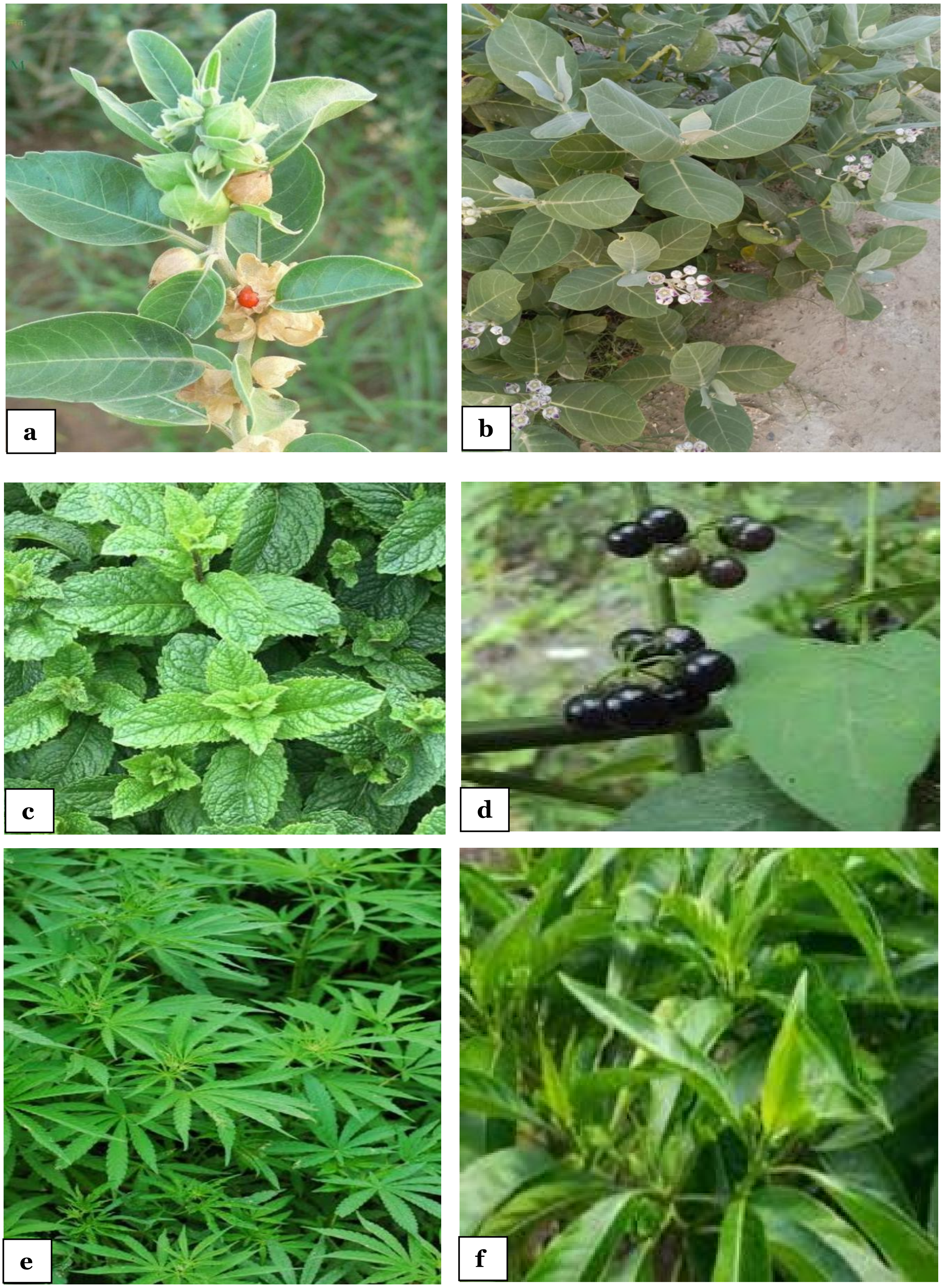


Fig.1: Fews medicinal plant species selected for this study:*Withania somnifera* (**a**) *Calotropis procera* (**b**) *Mentha spicata* © *Solanum nigrum* (**d**) *Cannabis sativa* (**e**) *Adhatoda vasica* (**f**).

Sample Collection

A total of 48 soil samples were collected from 24 randomly selected sites distributed across two ecologically distinct zones, with each site spanning approximately 8.3 acres. Topsoil (12-15 cm depth) was extracted using a sterilized stainless-steel auger to ensure representative sampling. Collected soil samples were air-dried, labeled, and stored in brown paper bags before being oven-incubated at 60°C for 15 days for moisture removal and stabilization.

Simultaneously, plant samples were harvested from the same sites using sterile tools to avoid cross-contamination. One representative medicinal plant specimen was collected per site, resulting in 24 plant samples per location. To remove adhered particulates and minimize contamination, plant samples were thoroughly rinsed with distilled water followed by a 0.1 N HCl solution. Samples were then shade-dried to prevent photodegradation, followed by oven-drying at 50°C for 15 days. The dried samples were ground into fine powder using a stainless-steel grinder and stored in airtight plastic containers for subsequent chemical analysis.

Sample Preparation and Digestion

Soil Digestion

Soil digestion was carried out following the procedure described by Vukadinović and Bertić (1988). One gram of air-dried soil was transferred to a digestion flask and treated with 4 mL of concentrated sulfuric acid (H_2SO_4) and 8 mL of hydrogen peroxide (H_2O_2). The mixture was heated until fume emission ceased. An additional 2 mL of H_2O_2 was added and heating continued until the digest turned transparent, indicating complete organic matter decomposition. The solution was filtered using Whatman No. 42 filter paper and diluted to 50 mL with double-distilled water, then stored in pre-labeled plastic bottles for analysis.

Plant Digestion

Similarly, 1 g of dried plant powder was digested using 2 mL of concentrated H_2SO_4 and 4 mL of H_2O_2. The mixture was heated until a clear solution was obtained, filtered, and diluted to 50 mL using double-distilled water. These digests were then preserved in labeled bottles for heavy metal quantification.

Instrumentation and Mineral Analysis

The concentrations of Zinc (Zn), Iron (Fe), Cadmium (Cd), and Chromium (Cr) in soil and plant digests were determined using an Atomic Absorption Spectrophotometer (Perkin-Elmer AAS-5000). Analytical protocols were followed according to the standardized methodology outlined by Lindsay and Norvell (1978), ensuring accurate quantification of heavy metals in both soil and plant tissues.

Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) was calculated to evaluate the ability of each plant species to uptake and accumulate metals from soil, using the following equation as described by Cui et al. (2004):

BCF = Metal value in the edible part/metal value in the soil

Daily Intake of Metals (DIM)

To assess potential human exposure through the consumption of medicinal plants, the Daily Intake of Metals (DIM) was estimated using the formula:

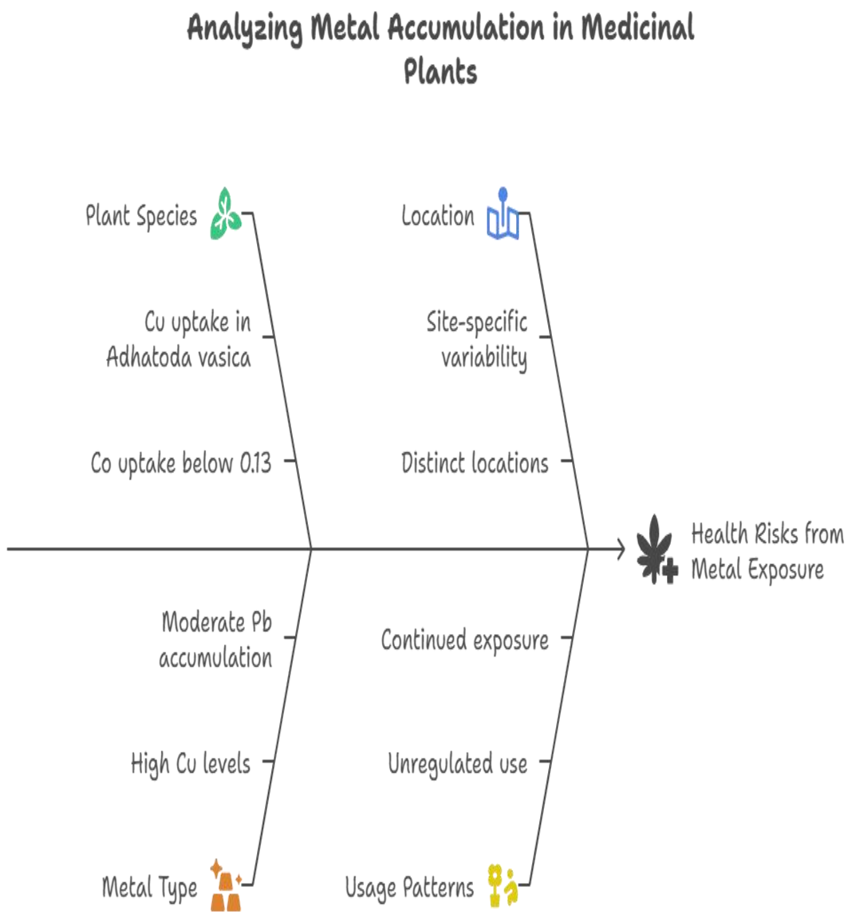
$$\text{DIM} = \frac{M \times F \times V}{W}$$

Where:

M = concentration of metal in plant material (mg/kg)
F = conversion factor (0.085)
V = daily vegetable intake per person (0.345 kg/day)
W = average adult body weight (60 kg)

Statistical Analysis

All experimental data were subjected to statistical evaluation using SPSS software. One-way Analysis of Variance (ANOVA) was employed to detect significant differences in metal concentrations among plant and soil samples. Additionally, Pearson correlation coefficients were calculated to examine the relationships between metal levels in soil and plant tissues. Significance was determined at p-values of 0.05, 0.01, and 0.001, following the procedures outlined by Steel and Torrie (1980).



Graphical Abstract for Heavy Metal Accumulation, Bioconcentration, and Dietary Risk Assessment in Medicinal Plants from Contrasting Locations

Results and Discussion

Copper (Cu)

Soil

The concentration of copper (Cu) in soil samples across both locations was not significantly affected by site variation ($p > 0.05$), as shown in Table 1. At Location I, mean soil Cu levels ranged from 1.18 to 1.37 mg/kg, while at Location II, values ranged between 1.19 and 1.39 mg/kg. The lowest Cu content was observed at Site S8 in Location I, whereas the highest was recorded at Site S5 in Location II. No clear trend of increase or decrease was observed, with an erratic distribution across all sampling sites (Fig. 2a).

All observed soil Cu concentrations slightly exceeded the critical threshold of 1 mg/kg reported by McDowell (1985). However, these levels were lower than those reported by Sinha and Gupta (1995), yet still above the critical range identified by Abbas et al. (2023).

Plants

At Location I, Cu concentration in plant samples varied significantly with site ($p < 0.05$), as shown in Table 4.9a. Mean plant Cu levels ranged from 10.80 to 12.99 mg/kg. *Adhatoda vasica* exhibited the highest Cu accumulation, whereas *Dodonaeaviscosa* recorded the lowest (Fig. 2b).

At Location II, Cu concentrations across plant species showed no significant variation ($p > 0.05$), with levels ranging between 11.24 and 12.71 mg/kg. As at Location I, *Adhatoda vasica* had the highest Cu content, while *Dodonaea viscosa* showed the lowest.

The Cu concentrations observed in all medicinal plants substantially exceeded the permissible limit of 3.00 ppm established by WHO (1992). These elevated levels may pose toxicological risks, potentially causing metal fume fever, dermatological and respiratory issues, a metallic taste sensation, and gastrointestinal disturbances (WHO, 2005). The World Health Organization recommends a lower acceptable daily intake threshold of 20 µg/kg body weight. Copper deficiency, although less common, can lead to anemia and Wilson's disease, a genetic disorder associated with impaired copper excretion (Litwin et al., 2023).

A possible synergistic effect between Cu and Zn could explain elevated Cu levels, as high Zn content in plants may enhance Cu uptake. The results further suggest that Cu accumulation in plants is influenced by the soil environment, soil type as a key determinant in trace mineral uptake. Additionally, high molybdenum (Mo) levels can antagonize Cu absorption, potentially disrupting animal physiology (Underwood &Suttle, 1999).

Bioconcentration Factor (BCF)

The BCF for Cu, indicating metal uptake efficiency from soil to plant, ranged from 8.72 to 10.78 (Fig. 2c). The highest Cu bioconcentration was recorded at plant sample P8, whereas the lowest was observed in P1. This variability reflects species-specific uptake capacities and sensitivity to Cu stress. Excessive accumulation may lead to phytotoxicity, which in extreme cases could result in plant mortality in affected sites.

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Soil-Plant Correlation

The correlation coefficient between soil and plant Cu concentrations was positive (r = 0.146), indicating a weak but direct relationship between Cu availability in soil and its accumulation in plants (Table 2).

Principal Component Analysis (PCA)

The PCA biplot (Figure 2c) illustrates the spatial distribution of Cu concentration, BCF, and Daily Intake of Metal (DIM) across eight plant-soil systems (PS1–PS8) from both locations. Vectors representing Cu, BCF, and DIM demonstrate their influence on different sampling sites. Sites PS4, PS7, and PS8 aligned strongly with Cu and BCF, indicating high metal accumulation and transfer efficiency. In contrast, PS2 and PS3 showed stronger association with DIM, suggesting a greater potential for human exposure upon medicinal plant consumption. Sites PS5 and PS6 were less correlated with any of the vectors, indicating relatively lower Cu uptake and associated health risks. This multivariate analysis highlights the spatial heterogeneity in metal accumulation and underscores the varying ecological and toxicological implications of Cu in wild medicinal plants.

Table 1:Analysis of variance for Cu concentrations in soil and plants at different locations

Source of Variation (S.O.V)	Degrees of Freedom (df)	Mean Squares			
		SOIL		PLANT	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.012^{ns}	0.013^{ns}	1.819*	0.686^{ns}
Error	16	0.017	0.015	0.580	0.511

**=significant at 0.01 level, ns= non-significant

Table 2: Copper correlation between soil and plants

		Soil	Plants
Soil	Pearson Correlation	1	-.287
	Sig. (2-tailed)		.174
	N	24	24
plants	Pearson Correlation	-.287	1
	Sig. (2-tailed)	.174	
	N	24	24

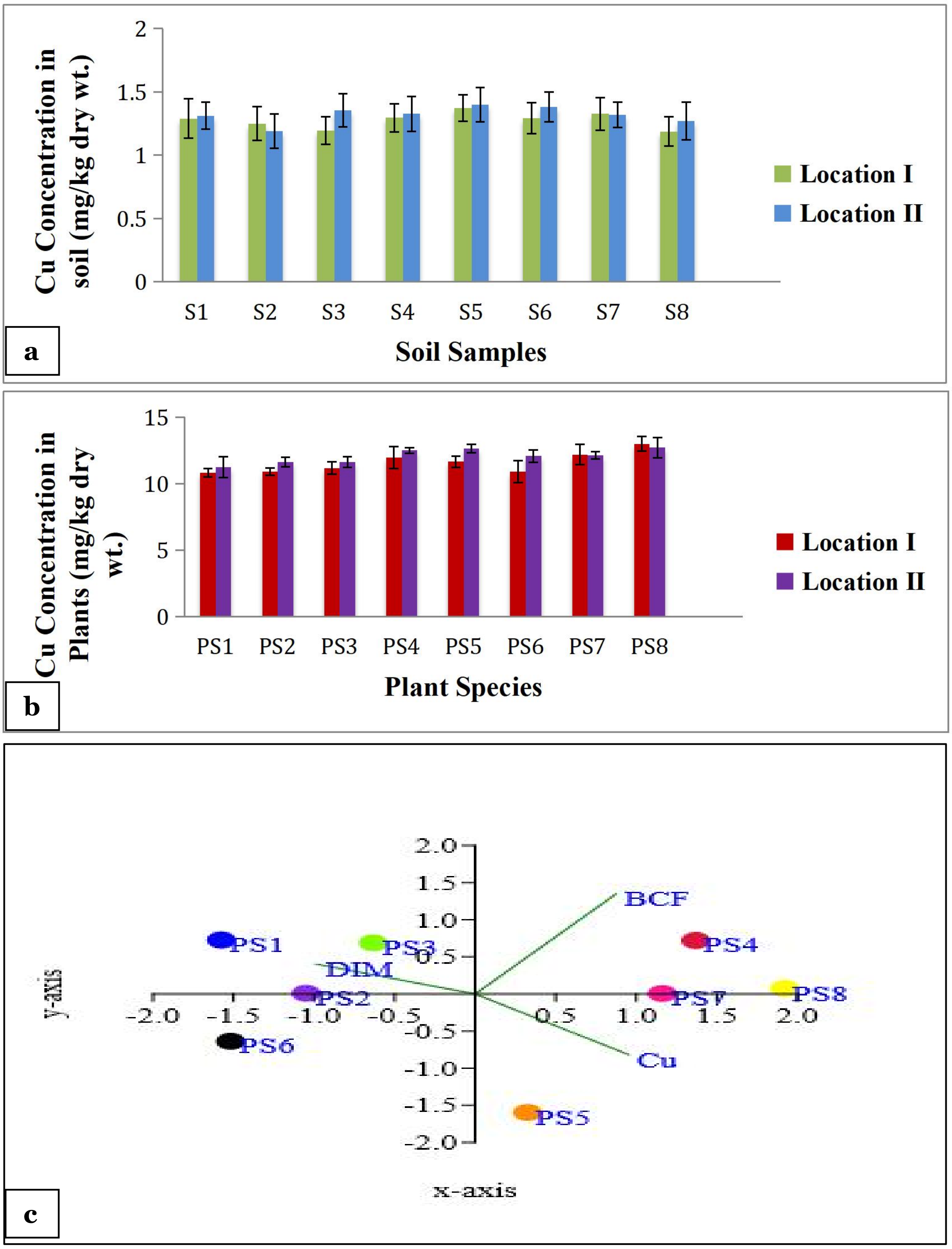

Fig. 2 (2a-2c): Copper (Cu) level in soil (a) and plants (b), Cu, Bioconcentration factor (BCF) and DIMbiplot (c) at two different locations and eight different study sites (S-P = soil to plant)

Cobalt (Co)
Soil
Cobalt concentrations in soil were significantly influenced by site variability at Location I ($p < 0.001$), while no significant differences were observed across sites at Location II (Table 3). At Location I, mean Co values ranged from 5.36 to 5.88 mg/kg, whereas at Location II, concentrations varied between 5.39 and 5.91 mg/kg. The minimum soil Co content was recorded at Site S2 (Location I), and the maximum at Site S4 (Location II) (Fig. 3a).

These findings reveal that Co concentrations in the soils of both locations exceed the critical threshold of 5 mg/kg as reported by Li et al. (2009). Nonetheless, the levels observed were lower than those previously documented by Mico et al. (2008) in the Bengal region but align closely with values reported by Vargas et al. (1992), who highlighted Co deficiency in similar pasturelands of Punjab, Pakistan. This suggests that while Co is present above the deficiency threshold, its marginal availability in some sites may necessitate Co fertilization to enhance soil fertility and support optimal plant development.

Plants
At Location I, plant Co concentrations were significantly influenced by site differences ($p < 0.05$) (Table 3). Mean Co values ranged from 0.093 to 0.235 mg/kg. Among the plant species, Cannabis sativa exhibited the highest Co accumulation, whereas *Menthaspicata* had the lowest (Fig. 3b).

In contrast, Co levels in plant samples from Location II varied non-significantly across sites ($p > 0.05$), with concentrations ranging from 0.48 to 0.70 mg/kg. *Calotropisgigantea*recorded the lowest Co uptake, while *Dodonaeaviscosa* exhibited the highest (Fig. 3b).

Although cobalt is required in trace amounts for biological functions, such as vitamin B12 synthesis and red blood cell production, excessive intake can pose cardiovascular risks. The concentrations observed in this study were above the critical level of 0.01 mg/kg established by the NRC (1984) and also exceeded the 0.05-0.06 mg/kg. However, these values fall within the general physiological range of 0.02-1.0 mg/kg reported by Abu-Abu-Darwish et al. (2009). It is important to note that there are currently no internationally defined permissible limits for cobalt in medicinal plants, which underscores the need for cautious interpretation and further toxicological assessment.

Bioconcentration Factor (BCF)
The BCF values for Co, representing the efficiency of metal transfer from soil to plant, ranged from 0.082 to 0.126 (Fig. 3c). The lowest BCF was observed in sample P4, suggesting limited bioavailability of Co, potentially due to competitive inhibition by other metals or antagonistic interactions. In contrast, the highest BCF was found in sample P1, indicating a species or site-specific affinity for cobalt uptake.

Soil-Plant Correlation
A significant negative correlation ($r = -0.426$) was observed between soil and plant Co concentrations (Table 4). This inverse relationship suggests that higher soil Co levels do not necessarily result in increased uptake by plants. Such trends may be attributed to physicochemical barriers in the rhizosphere, metal

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antagonism, or selective metal exclusion by certain plant species.

Principal Component Analysis (PCA)

The PCA biplot (Figure 3c) elucidates the spatial variation and interdependence of Co, BCF, and DIM across eight plant-soil systems (PS1-PS8) from both locations. PS6 aligned closely with the Co vector, indicating elevated cobalt accumulation. PS1 and PS3 correlated strongly with the DIM vector, highlighting a higher estimated daily intake of cobalt via these plant species. PS4, PS7, and PS8 were positioned near the BCF vector, reflecting enhanced metal uptake efficiency. Conversely, PS2 and PS5 showed minimal association with any of the vectors, suggesting low cobalt absorption and reduced health risk. These spatial patterns emphasize the localized nature of metal dynamics and underscore the need for site-specific risk assessments when evaluating the medicinal safety of wild plants.

Table 3: Analysis of variance for Co concentrations in soil and plants at different locations

Source of Variation (S.O.V)	Degrees of Freedom (df)	Mean Squares			
		SOIL		PLANT	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.080*	0.074ns	0.006***	0.014ns
Error	16	0.014	0.032	0.001	0.015

**=significant at 0.01 level, ns= non-significant

Table 4: Cobalt correlation between soil and plants

		Soil	Plants
Soil	Pearson Correlation	1	-.426*
	Sig. (2-tailed)		.038
	N	24	24
Plants	Pearson Correlation	-.426*	1
	Sig. (2-tailed)	.038	
	N	24	24

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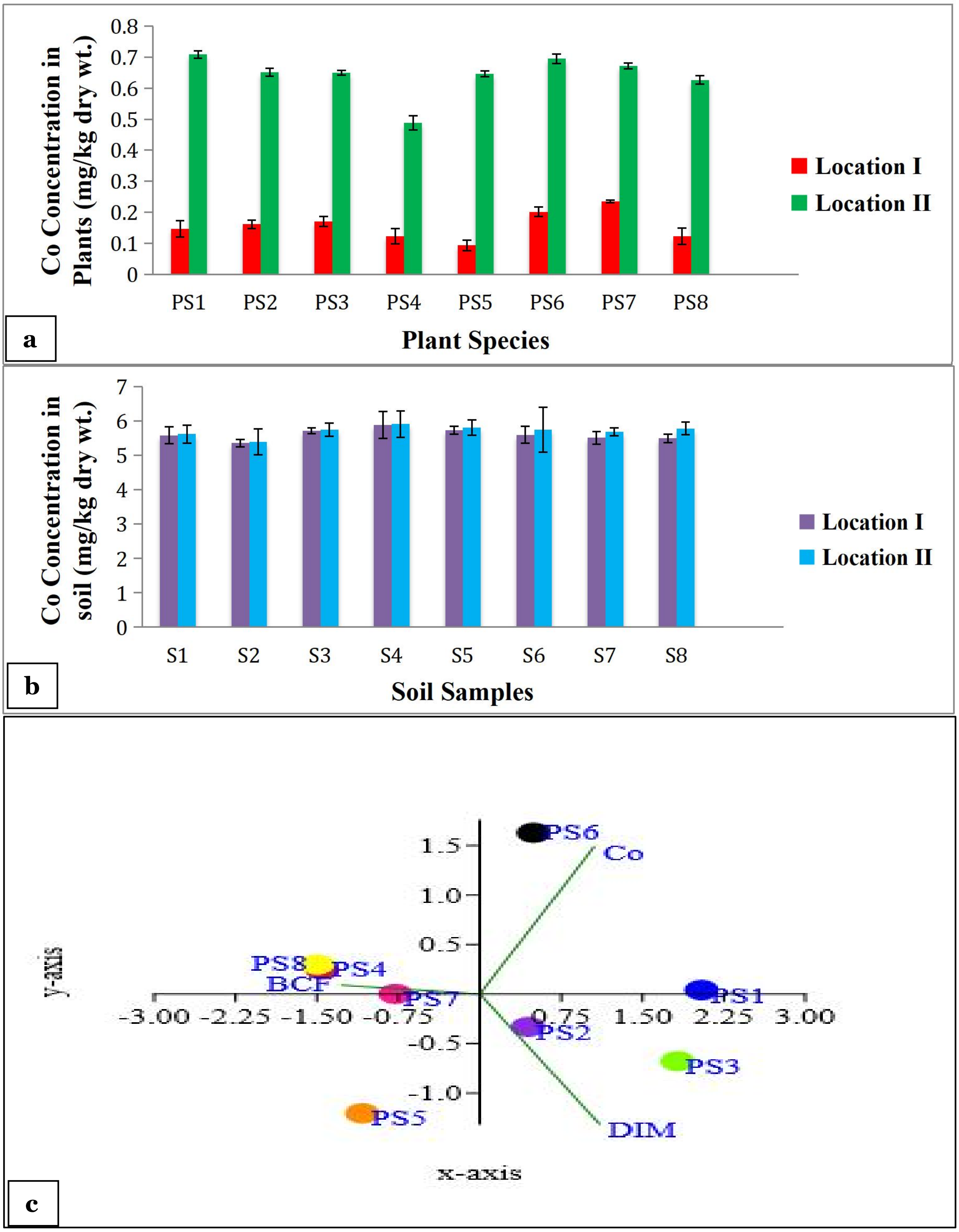

Fig. 3 (3a-3c): Cobalt (Co) level in soil (a) and plants (b), Co, Bioconcentration factor (BCF) and DIMbiplot (c) at two different locations and eight different study sites (S-P = soil to plant)

Manganese (Mn)

Soil

Analysis of variance revealed a statistically significant effect ($p < 0.001$) of site on manganese (Mn) concentrations in soil at both sampling locations (Table 5). Soil

Mn levels fluctuated irregularly across the sites, ranging from 3.20 to 4.14 mg/kg at Location I and 3.14 to 3.99 mg/kg at Location II. The lowest Mn concentration was recorded at Site S3 in Location II, while the highest was found at Site S7, also in Location II (Fig. 4a).

All measured Mn concentrations in soil were below the critical threshold of 5 mg/kg as reported by McDowell (1985). Comparatively, Supriatin and Salam (2024) reported considerably higher Mn values in Indonesian soils, whereasAnda (2012) also recorded greater concentrations elsewhere. The relatively low Mn content in this study may be attributed to its antagonistic interaction with soil organic matter. Additionally, manganese availability in soil is influenced by various environmental parameters including pH, aeration, nutrient levels, temperature, and moisture, which likely contributed to the observed variability across farms (Abba et al., 2023).

Plants

At Location I, manganese concentrations in plant samples showed no significant variation among sites ($p > 0.05$), with values ranging from 20.63 to 23.75 mg/kg (Table 5). The highest Mn accumulation was found in Calotropisgigantea, while the lowest was in Menthaspicata (Fig. 4b).

In contrast, plant Mn levels at Location II were significantly affected by site differences ($p < 0.05$), ranging between 21.68 and 23.62 mg/kg. Interestingly, *Menthaspicata* showed the highest Mn content at this location, whereas *Calotropisgigantea* recorded the lowest.

Despite being below the critical toxicity threshold of 40 mg/kg in plants (McDowell, 1985), the Mn concentrations in the studied species far exceeded the FAO/WHO (1984) permissible limit for edible plants, which is just 2 ppm. Nonetheless, the observed values remain within the typical background range of 300-500 ppm dry weight, considered safe for plant tissues. Manganese plays an essential role in plant metabolism, and its deficiency may result in chlorosis and other physiological disorders.

In humans, manganese is vital for bone formation, enzymatic function, and immune health. Its recommended dietary intake for adults is approximately 11 mg/day (Rondanelli et al., 2021). Chronic Mn deficiency has been associated with myocardial infarction, impaired skeletal development in infants, and conditions like immunodeficiency and rheumatoid arthritis in adults (Barceloux, 1999). The elevated Mn levels observed in this study exceed permissible limits for medicinal use and may pose health risks if not monitored, indicating the need for regulating Mn concentrations in medicinal plants from this region.

Bioconcentration Factor (BCF)

BCF values for manganese varied across plant samples, ranging from 5.650 to 7.287 (Fig. 4c). The highest Mnbioconcentration was found in plant sample P3, suggesting efficient uptake, whereas the lowest was observed in P5. These differences likely reflect species-specific metal uptake mechanisms and the metal's bioavailability in the soil matrix. Plants with higher BCFs may act as bioaccumulators and require monitoring for potential toxicity.

Soil-Plant Correlation

Correlation analysis revealed a weak and statistically non-significant negative association between soil and plant Mn concentrations (r = -0.133) (Table 6). This suggests that soil Mn availability alone does not directly govern uptake in plants, possibly due to complex plant-specific physiological controls or environmental antagonisms affecting Mn mobility.

Principal Component Analysis (PCA)

The PCA biplot (Figure 4c) provides a multivariate representation of manganese dynamics across the eight plant-soil systems (PS1-PS8). PS7 and PS5 aligned closely with the Mn and BCF vectors, indicating high accumulation and bioconcentrationpotential at these sites. Conversely, PS3, PS2, and PS8 were more closely associated with the DIM vector, implying elevated human exposure risk via plant consumption. PS1, PS4, and PS6 were positioned away from all vectors, reflecting relatively low Mn levels, minimal uptake, and reduced dietary risk. This analysis highlights the spatial variability in manganese behavior across sampling sites and underscores the importance of localized assessment for potential health impacts.

Table 5: Analysis of variance for Mn concentrations in soil and plants at different locations

Source of Variation (S.O.V)	Degrees of Freedom (df)	Mean Squares			
		SOIL		PLANT	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.299^{*}	0.227^{*}	2.926^{ns}	1.172^{ns}
Error	**16**	0.057	0.054	2.381	2.461

**=significant at 0.01 level, ns= non-significant

Table 6: Manganese Correlation between Soil and Plants

		Soil	Plants
Soil	Pearson Correlation	1	-.133
	Sig. (2-tailed)		.535
	N	24	24
Plants	Pearson Correlation	-.133	1
	Sig. (2-tailed)	.535	
	N	24	24

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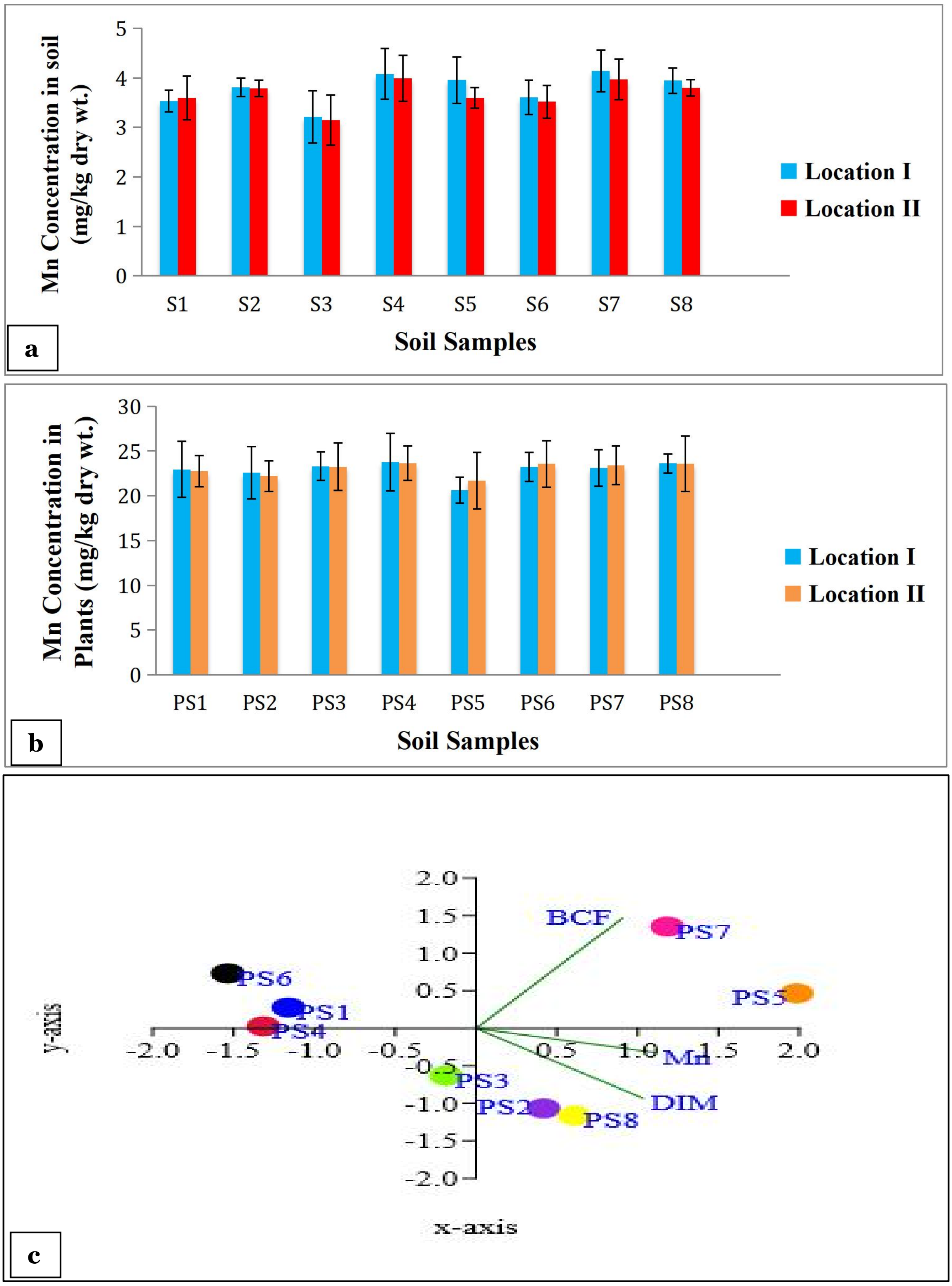

Fig. 4 (4a-4c): Manganese (Mn) level in soil (a) and plants (b), Mn, Bioconcentration factor (BCF) and DIMbiplot (c) at two different locations and eight different study sites (S-P = soil to plant)

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Lead (Pb)
Soil
Analysis of variance revealed a statistically significant ($p < 0.001$) effect of site on soil lead (Pb) concentrations across both study locations (Table 7). At location-I, mean Pb levels ranged from 6.80 to 7.83 mg/kg, while at location-II, values fluctuated between 6.25 and 7.86 mg/kg, with the highest concentrations observed at Site S8 and the lowest at Site S4 (Fig. 5a). These values are generally within the baseline range of 5-25 mg/kg reported by Hayashi (1985).

The Pb concentrations in this study were lower than those documented by Abbas et al. (2023) in riparian soils, yet exceeded those reported by Pichtel et al. (2000) during a bio-monitoring investigation of heavy metal pollution. Variations in soil lead content are likely influenced by both intrinsic soil characteristics and extrinsic anthropogenic activities, which may elevate Pb levels above natural background thresholds (Osweiler, 1996).

Plants
At location-I, Pb accumulation in plant species showed no statistically significant variation ($p > 0.05$) across different sites (Table 7). Concentrations ranged from 5.78 to 6.27 mg/kg, with *Peganumharmala* showing the highest Pb content and *Menthaspicata* the lowest (Fig. 5b).

Similarly, site-II also exhibited non-significant variation in Pb content among plant samples ($p > 0.05$), where concentrations ranged from 6.28 to 6.69 mg/kg. *Dodonaea viscose* demonstrated the highest mean value, while *Mentha spicata* again exhibited the lowest Pb levels (Fig. 5b).

These levels exceeded the critical phytotoxic threshold of 0.05 mg/kg and remained below the maximum tolerable limit of 10 ppm set by WHO (1998) for herbal medicinal plants. While the FAO/WHO (1984) limit for Pb in edible plants is 0.43 ppm, chronic exposure above 1 ppm in foodstuffs may pose health hazards, including kidney, liver, immune, and vascular toxicity (Sarlak et al., 2023). The observed values also fell within the yield reduction range of 5-30 ppm and were lower than the levels linked to adverse effects (80 mg/kg).

Although the Pb concentrations recorded here are within safe consumption limits, the presence of antagonistic elements such as nickel (Ni), calcium (Ca), and selenium (Se) may affect Pb bioavailability and uptake. Overall, the Pb concentrations observed in the current study were within a range that does not pose significant risk to human or animal health.

Bioconcentration Factor (BCF)
The BCF for Pb, reflecting the plant's efficiency in translocating Pb from soil, ranged between 0.847 and 0.932 (Fig. 5c). The highest BCF was recorded in P4, indicating increased Pb accumulation potential and possible plant sensitivity. Conversely, P6 showed the lowest BCF, suggesting a degree of resistance or exclusion capability, possibly due to the presence of competing metal ions in the rhizosphere, which may inhibit Pb uptake.

Soil-Plant Correlation
A non-significant positive correlation ($r = 0.081$) was noted between soil and plant Pb levels (Table 8), implying a weak relationship between Pb availability in soil and its subsequent assimilation into plant tissues. This weak correlation may

suggest metal imbalance or differential uptake mechanisms among species, influenced by soil chemistry and plant-specific traits.

Principal Component Analysis (PCA)
The PCA biplot (Figure 5c) illustrates the spatial variation in Pb concentration, BCF, and daily intake of metals (DIM) across the eight plant-soil systems (PS1-PS8). PS6 clustered strongly with Pb concentration, indicating a potential contamination hotspot. PS4 and PS7 were aligned with the BCF vector, revealing high bioconcentration potential in those areas. In contrast, PS1 and PS2 were more closely associated with DIM, indicating higher exposure risks through plant consumption. PS3 and PS5, distanced from all three vectors, exhibited lower Pb levels, reduced bioaccumulation, and minimal health risks. This multivariate approach underscores the site-specific behavior of Pb, informing targeted risk mitigation strategies for medicinal plant safety and public health.

Table 7: Analysis of variance for Pb concentrations in soil and plants at different locations

Source of Variation (S.O.V)	Degrees of Freedom (df)	Mean Squares			
		SOIL		PLANT	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.312*	0.843**	0.124ns	0.064ns
Error	16	0.106	0.224	0.076	0.049

**=significant at 0.01 level, ns= non-significant

Table 8: Lead Correlation between Soil and Plants

		Soil	Plants
Soil	Pearson Correlation	1	.081
	Sig. (2-tailed)		.707
	N	24	24
Plants	Pearson Correlation	.081	1
	Sig. (2-tailed)	.707	
	N	24	24

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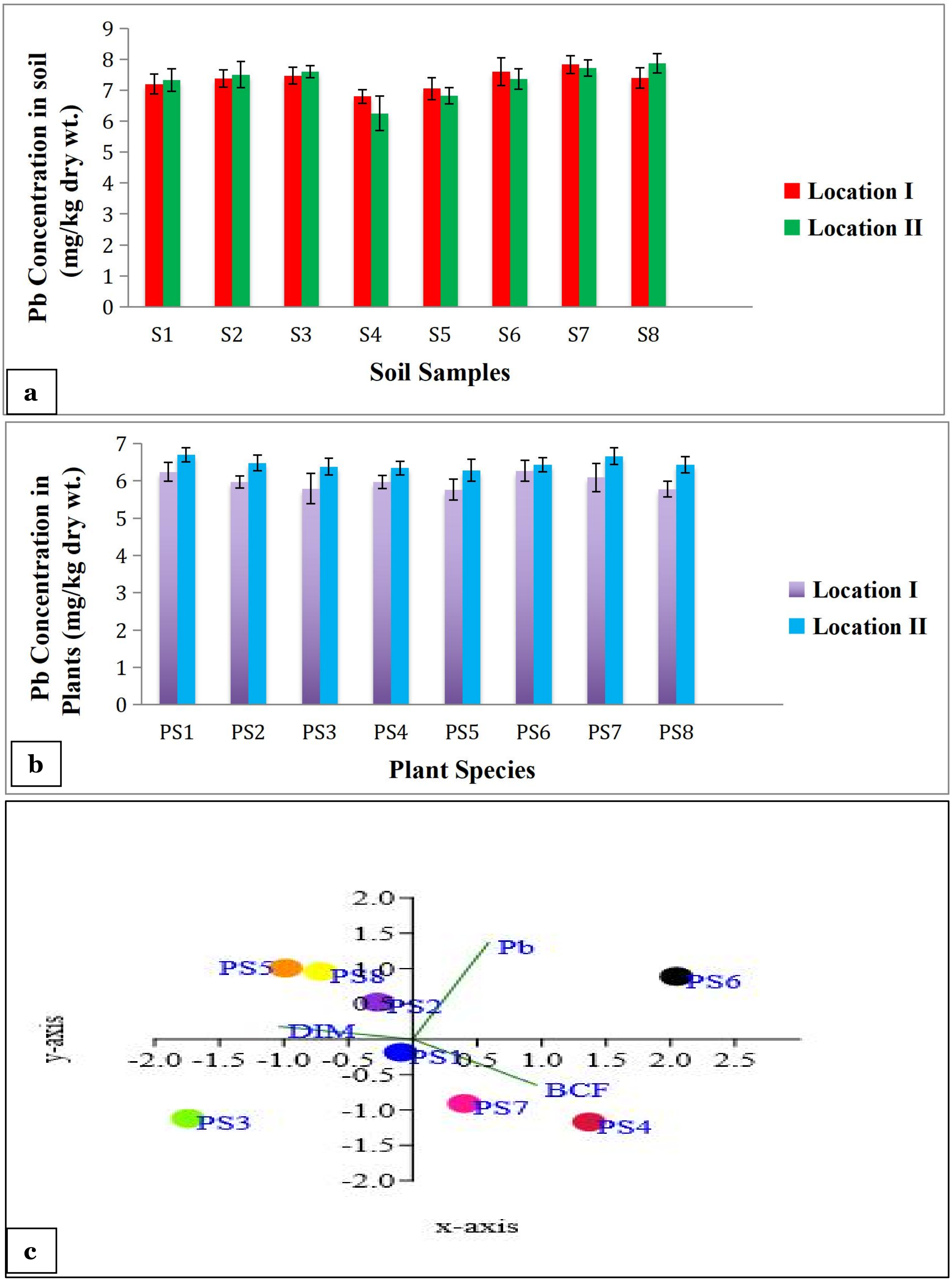

Fig. 5 (5a-5c): Lead (Pb) level in soil (a) and plants (b), Pb, Bioconcentration factor (BCF) and DIMbiplot (c) at two different locations and eight different study sites (S-P = soil to plant)

Conclusion

This investigation revealed that medicinal plants from both locations absorb heavy metals at varying rates, depending on species and soil composition. Cu and Mn were taken up in substantial amounts, often exceeding safety thresholds, while Co remained comparatively low. Although Pb levels were not alarmingly high, their persistent presence is a concern due to their cumulative toxicity. BCF data highlighted that plants like *Adhatodavasica* and Menthaspicata are particularly efficient in metal uptake, making them potential indicators of soil contamination, but also increasing the risk of transmitting metals into the human body. DIM estimates further confirmed that daily exposure to Cu and Mn through herbal use could exceed recommended intake levels, especially if consumption is frequent and unregulated. These findings underscore the need for strict quality control of medicinal plants, especially those harvested from metal-rich environments.

Future Perspective and Recommendations

To ensure the safe use of medicinal plants, future research should expand to include a broader range of heavy metals and seasonal variations to fully assess ecological and health risks. Certain species with high bioconcentration potential, such as *Adhatoda vasica* and *Mentha spicata*, could be explored for phytoremediation or biomonitoring purposes. Regular surveillance of heavy metal levels in medicinal plants, especially those sold in local markets is essential to prevent chronic exposure. Cultivation should be encouraged in low-contamination areas, and public awareness campaigns are needed to educate communities about the risks associated with consuming plants from polluted soils. Furthermore, national policies must establish clear safety thresholds and enforce quality standards for herbal medicines in alignment with international guidelines.

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