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Bioconcentration And Translocation Of Heavy Metals In Therapeutic Flora: Ecological And Pharmacological Risks

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Abstract
This study investigates the accumulation patterns and health risk implications of four heavy metals such as Zinc (Zn), Iron (Fe), Cadmium (Cd), and Chromium (Cr) present in soil and eight medicinal plant species collected from two distinct locations across multiple sites. Soil and plant samples were analyzed to determine metal concentrations, bioconcentration factors (BCF), and daily intake of metals (DIM). Results showed that Zn levels in soil were within the critical range (1.12-1.70 mg/kg), while plant Zn content varied significantly across species (16.48-25.94 mg/kg), with Adhatoda vasica exhibiting the highest accumulation. Fe concentrations in soil were moderate (10.91-16.04 mg/kg), whereas plant Fe ranged from 12.96 to 23.25 mg/kg, exceeding FAO/WHO limits for edible plants but remaining within therapeutic safety margins. Cd levels in soil (3.45-4.63 mg/kg) exceeded some older permissible limits, yet its uptake by plants remained very low (0.025-0.086 mg/kg), reflecting limited bioavailability. Chromium concentrations in soil (0.028-0.041 mg/kg) were above the critical threshold, with high accumulation in plants (3.40-4.65 mg/kg), surpassing international permissible limits. However, DIM values for all metals were within safe human intake levels. BCFs varied widely among metals and species, with Cr showing the highest accumulation potential. Overall, findings suggest that while the studied soils pose no immediate risk, certain medicinal plants, particularly

for Cr and Fe, may require controlled use to avoid chronic exposure. These plants also show potential for use in phytoremediation and bio-monitoring applications.

Keywords: Heavy metals, Medicinal plants, Daily Intake of Metal (DIM), Phytoremediation, Soil-plant interaction

Introduction

Pakistan has an extremely diverse wild flora, most of which has traditionally been valued for its medicinal and fragrant characteristics. Local populations, particularly in rural and poor areas, have traditionally used these herbs to cure a variety of health issues. Despite widespread ethno-medical use, most of this botanical wealth has yet to be properly investigated in terms of pharmacological activity and chemical makeup (Shinwari, 2000).

According to estimates, more than 1,000 plant species in Pakistan have therapeutic potential and play an important role in indigenous healthcare systems, particularly among economically marginalized groups (Mushtaq et al., 2009). This great floral diversity offers a significant opportunity to find new bioactive chemicals and create plant-based medicines.

Ethnobotanical surveys and research projects across the country have highlighted the importance of preserving traditional knowledge while scientifically confirming the medical use of indigenous plants (Sheikh &Hussain, 2008). Pakistan currently has about 5,700 medicinal plant species, with 372 species known for their widespread use and distribution. Importantly, over 456 of these plants are widely traded and used as basic materials in nearly 350 herbal formulations used in traditional and commercial medicine.

With few exceptions, heavy metals are primarily found in the d and f blocks. Heavy metals have a density of more than 5 grams per cubic centimeter. Heavy metals include group transition elements, lanthanides, actinides, and several metalloids. (Hutchings et al., 2003; Jann, 2004). Small amounts of heavy metals play an important role in the bodies of all living organisms because they are involved in various biochemical reactions that are required for organism survival; however, the requirements of heavy metals such as copper, zinc, iron, manganese, and cobalt vary depending on the species Vijver et al. (2001).

Metals are important in medicinal plants because they are utilized to form a variety of colloids that are used not only in the plants to carry out various physiological processes, but also on the bodies of the organisms who eat these medicinal plants. The concentration of different metals in medicinal plants varies depending on the growing conditions, drying method, and geographical location of the species. Excessive quantities of these metals can harm living creatures. Other heavy metals, such as mercury and plutonium, can accumulate in the bodies of animals over time and cause serious illness (Broyer and Paull, 1997; Malani and Ichikawa, 1998).

Some heavy metals, such as vanadium, tungsten, and cadmium, are hazardous to organisms but useful under certain situations (Malani and Ichikawa, 1998). Some of these elements are essential for human health in trace amounts (Co, Cu, Cr, and Ni), while others are carcinogenic, harming the central nervous system (Hg, Pb, and As), kidneys and livers (Cd and Cu), and skin, bones, and teeth (Ni, Cd, Cu, and Cr) (Girish and Shridhar, 2007). Current environmental issues include potential harm to animals and human health (Radojevicand Bashkin, 1999).

The present study aims to investigate the accumulation of heavy metals such as zinc (Zn), iron (Fe), cadmium (Cd), and chromium (Cr), in selected medicinal plants growing naturally in the semi-arid region of KallarKahar, Punjab, Pakistan. By analyzing both soil and plant samples from two distinct locations, this research seeks to evaluate the extent to which these metals are absorbed by the plants and transferred into biologically significant parts. Specific objectives include: (1) determining the concentration levels of these metals in soil and plant tissues; (2) calculating the bioconcentration factor (BCF) to assess metal uptake efficiency; and (3) estimating the daily intake of metals (DIM) to evaluate potential human health risks associated with the consumption of these plants. The findings aim to provide insight into the safety and sustainability of using wild medicinal flora for therapeutic purposes in the face of increasing environmental pollution.

Material and methods

Study area and plant selection

This study was conducted in Kallar Kahar, a sub-division of Chakwal District in Punjab, Pakistan. Located approximately 25 km southwest of Chakwal and 125 km from Rawalpindi, Kallar Kahar is known for its scenic landscapes, peacocks, and a natural saline lake. Geographically, the area lies between 32°26′11″ to 32°41′18″N latitude and 71°50′33″ to 72°30′07″E longitude, encompassing about 200 acres rich in floristic diversity.

The region falls within the Pothohar Plateau, characterized by a semi-arid climate with hot, dry summers, moderate winters, and irregular rainfall, mainly during the monsoon season. The soils are generally calcareous and moderately fertile, supporting a wide range of indigenous plant species. The unique microclimatic conditions of this undisturbed ecosystem make it suitable for investigating plant-soil interactions and the accumulation of heavy metals in wild medicinal flora.

Eight commonly used medicinal plant species were selected for this study: *Dodonaea viscosa, Withania somnifera, Solanum nigrum, Calotropis procera, Mentha spicata, Peganum harmala, Cannabis sativa*, and *Adhatoda vasica*.

Sample Collection

A total of 48 soil samples were collected from 24 randomly selected sites across two distinct locations, with each site covering approximately 8.3 acres. Using a stainless-steel auger, soil was excavated to a depth of 12-15 cm to capture representative topsoil samples. The collected samples were air-dried, labeled, sealed in brown paper bags, and incubated at 60°C for 15 days for preservation.

Plant samples were collected from the same locations using sterilized tools to avoid cross-contamination. One representative plant specimen was collected per site, resulting in 24 plant samples per location. To remove adhered dust and loosely bound metals, samples were washed initially with distilled water followed by rinsing in 0.1 N hydrochloric acid.After cleaning, the plant materials were shade-dried to avoid photodegradation, then oven-dried at 50°C for 15 days. The dried material was ground into a fine powder using a stainless-steel grinder and stored in airtight plastic containers for chemical analysis.

Sample preparation and digestion

Soil Digestion:Following the protocol of Vukadinović and Bertić (1988), 1 g of air-dried soil was transferred to a digestion flask and treated with 4 mL of concentrated H_2SO_4 and 8 mL of H_2O_2. The mixture was heated until the emission of fumes ceased. An additional 2 mL of H_2O_2 was added, and heating continued until the solution turned clear, indicating complete oxidation of organic matter. The digest was filtered using Whatman No. 42 filter paper and diluted to 50 mL with double-distilled water. Samples were then stored in labeled plastic bottles for mineral analysis.

Plant Digestion: Similarly, 1 g of each dried plant sample was digested using 2 mL of concentrated H_2SO_4 and 4 mL of H_2O_2, following the same procedure until a clear solution was obtained. The final volume was brought up to 50 mL with double-distilled water, filtered, and stored for further examination.

Instrumentation and mineral analysis

The digested soil and plant extracts were analyzed for concentrations of Zinc (Zn), Iron (Fe), Cadmium (Cd), and Chromium (Cr) using an Atomic Absorption Spectrophotometer (Perkin-Elmer AAS-5000). Analytical procedures followed the guidelines outlined by Lindsay and Norvell (1978) to determine heavy metal concentrations in soil and medicinal plants commonly consumed in rural communities.

Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) quantifies the transfer efficiency of metals from soil to plants and was calculated using the formula by Cui et al. (2004):

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

Daily Intake of Metals (DIM)

To estimate potential human exposure to heavy metals through medicinal plant consumption, the Daily Intake of Metals (DIM) was calculated as:

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

Where M = the concentration of metal in the vegetables.
F=Conversion factor of 0.085.
V= Vegetable consumption per person per day for humans was 0.345 kg
W= 60 kg is the average human body weight for an adult

Statistical analysis

All data were statistically analyzed using SPSS software. One-way Analysis of Variance (ANOVA) and Pearson correlation coefficients were applied to determine the significance of metal variation among plant species and soil samples. Significance levels were tested at p values of 0.05, 0.01, and 0.001, following the procedures of Steel and Torrie (1980).

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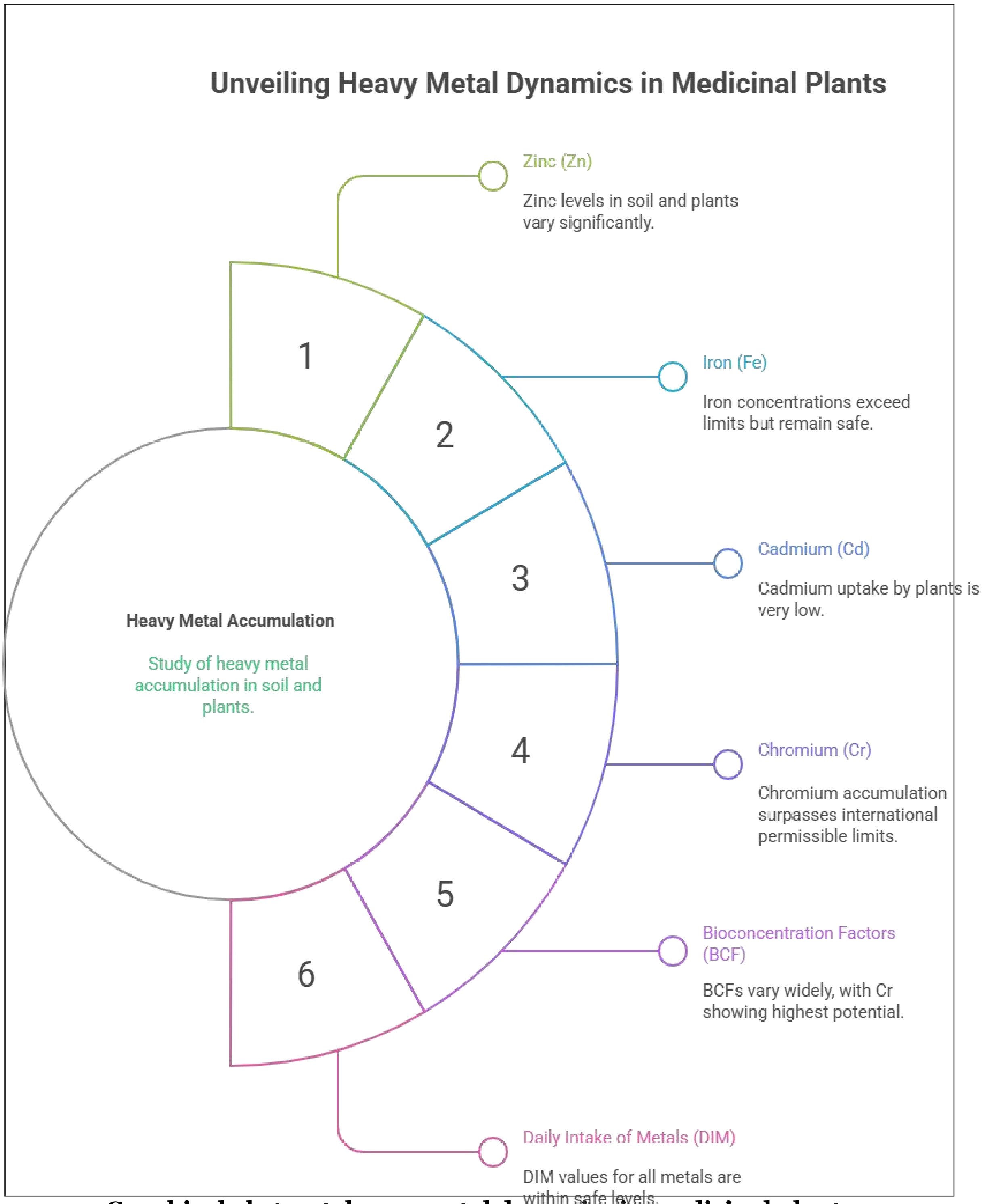

Graphical abstract, heavy metal dynamics in medicinal plants

Results and Discussion

Zinc (Zn) analysis in plants and soil

The statistical analysis revealed a non-significant variation ($p > 0.001$) in Zn concentrations across sampling sites at Location-I, indicating relatively uniform Zn distribution in this area. Conversely, a statistically significant difference ($p < 0.001$) was observed at Location-II, reflecting site-specific fluctuations in soil Zn levels (Table 1). Soil Zn concentrations ranged from 1.23 to 1.70 mg/kg at Location-I and 1.12 to 1.65 mg/kg at Location-II, with the maximum level recorded at S1 of Location-I, and minimum at S3 of Location-II (Fig. 1a). These concentrations fall near or just above the critical Zn level of 1.5 mg/kg (McDowell et al., 1985), suggesting an adequate baseline for plant uptake. Similar trends have been reported by Singh et al. (2025), reinforcing that the studied soils do not pose a risk of zinc deficiency.

Zn accumulation in plant tissues varied highly significantly ($p < 0.001$) across all medicinal species at both locations (Table 1). At Location-I, Zn levels in plants ranged from 16.605 to 25.94 mg/kg, and at Location-II from 16.48 to 25.75 mg/kg. In both locations, *Adhatoda vasica* exhibited the highest Zn accumulation, while *Calotropis gigantea* had the lowest (Fig. 1b).

Zinc is essential for both plant physiology (enzyme activity, gene expression, protein synthesis) and human health, where it regulates immune function, wound healing, and neurological processes. All measured concentrations were significantly below the dietary toxicity threshold of 100 ppm and also well within the FAO/WHO limit of 27.4 ppm for edible crops. Whereas there is no established limit for medicinal plants by WHO (2005), Zn values remained within the recommended agricultural range of 15-200 ppm (Ajah et al., 2022), indicating no risk for therapeutic use.

Bioconcentration factor (BCF) and daily intake of metal (DIM)

The BCF values for Zn varied from 0.362 to 0.231(Fig. 1c), suggesting a strong Zn uptake potential by certain plants. The highest BCF observed in P8 signifies a species with high bioaccumulation capacity, possibly suitable for phytoextraction applications, whereas the lowest (P4) could indicate metal exclusion or resistance.The calculated DIM for Zn across all plants was well below the permissible daily intake for humans, affirming the nutritional safety and therapeutic reliability of these species in traditional medicine systems.

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Table 1: Analysis of variance for Zn 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	0.072ns	0.098*	33.618***	34.118***
Error	16	0.043	0.036	1.676	1.601

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

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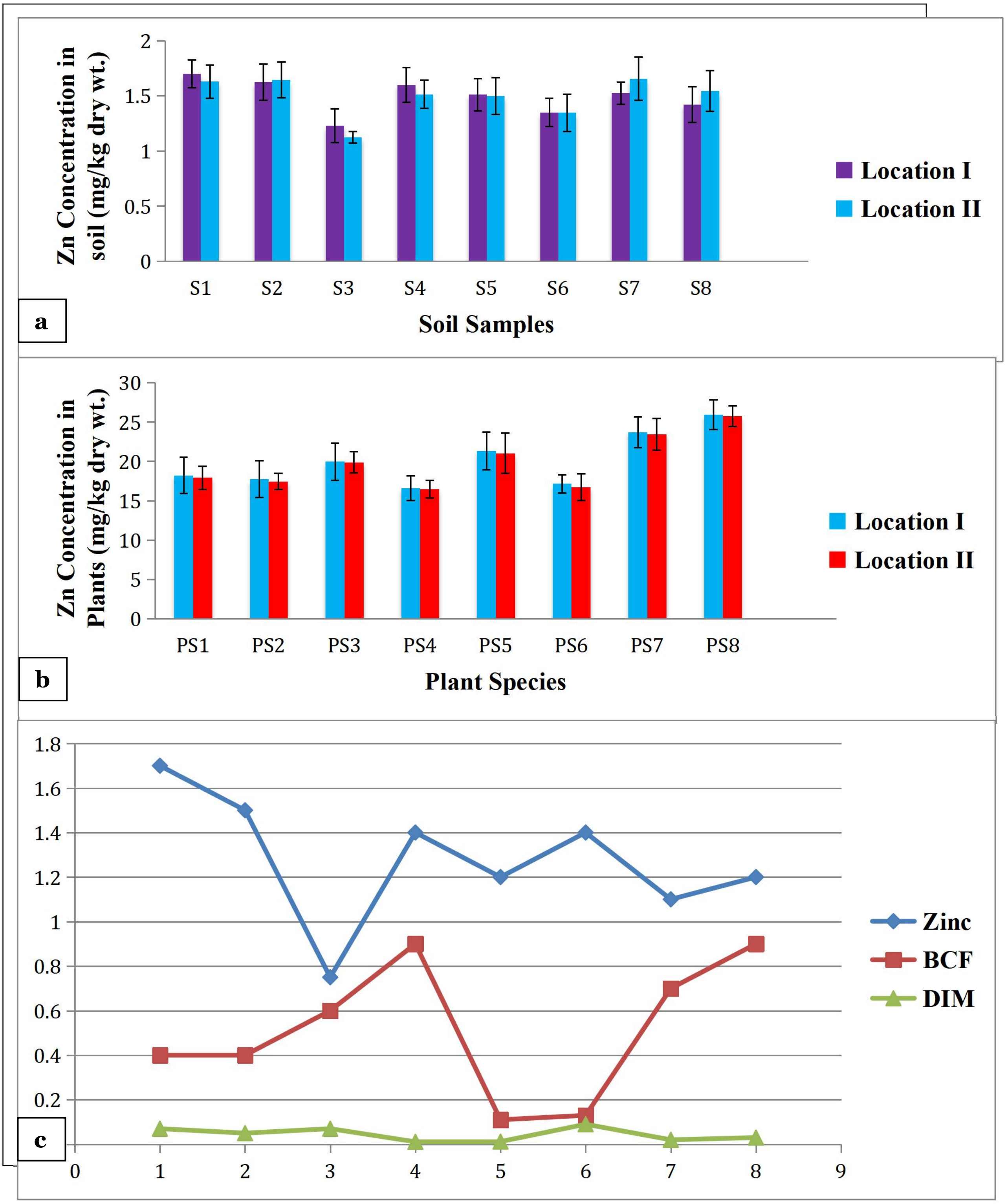

factor (BCF) and DIM scattered plot(c) at two different locations and eight different study sites (S-P = soil to plant)

Iron (Fe) analysis in plants and soil

Iron concentrations in soil did not vary significantly (p > 0.001) across both locations (Table 2), implying spatial homogeneity in Fe distribution. At Location-I, values ranged from 10.91 to 15.65 mg/kg, while at Location-II, they spanned 12.58 to 16.04 mg/kg (Fig. 2a). The highest Fe content was observed at S4, and the lowest at S2 of Location-II.All observed values were below the critical threshold of 20 mg/kg (McDowell et al., 1985). However, they were notably higher than values reported by Mogwasi et al. (2023), suggesting mild enrichment in the study area. It's important to note that iron solubility drops 1000-fold with each unit increase in soil pH, limiting its availability despite total content (Sharma et al., 2025).

Fe concentrations in plants showed highly significant variation at Location-I (p < 0.001) and significant differences (p < 0.05) at Location-II. In Location-I, Fe content ranged from 13.02 to 23.25 mg/kg, with *Adhatoda vasica* showing the highest value and *Calotropis gigantea* the lowest (Fig. 2b). In Location-II, values ranged from 12.96 to 21.48 mg/kg, with Cannabis sativa at the upper end.All plant values were below the critical threshold of 30 mg/kg (McDowell, 1985), but exceeded the FAO/WHO safety limit of 20 ppm for food plants. However, the toxicity threshold for iron in medicinal or edible plants is much higher, *i.e.*,around 1000 mg/kg (Gatasheh *et al*., 2025; Abbas *et al.*, 2023;Karahan, 2023). Given that iron deficiency causes a range of disorders, from fatigue to impaired cognitive function, the elevated Fe content may actually be beneficial in nutraceutical contexts, particularly in anemia-prone regions.

Bioconcentration factor (BCF) and daily intake of metal (DIM)

BCF values ranged from 0.934 to 1.743 (Fig. 2c), indicating moderate Fe uptake efficiency, with P2 exhibiting the highest value. The low to moderate BCF range may result from antagonistic interactions with other metals (e.g., Zn, Cd) in the rhizosphere.DIM calculations show that all plants provided iron levels well within the recommended dietary intake (10-60 mg/day), thereby making them safe for use in herbal formulations and even functionally valuable as bioavailable iron sources.

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Table 2: Analysis of variance for Fe 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	9.373 ns	3.915ns	45.883***	32.619***
Error	16	6.075	6.977	3.698	3.456

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

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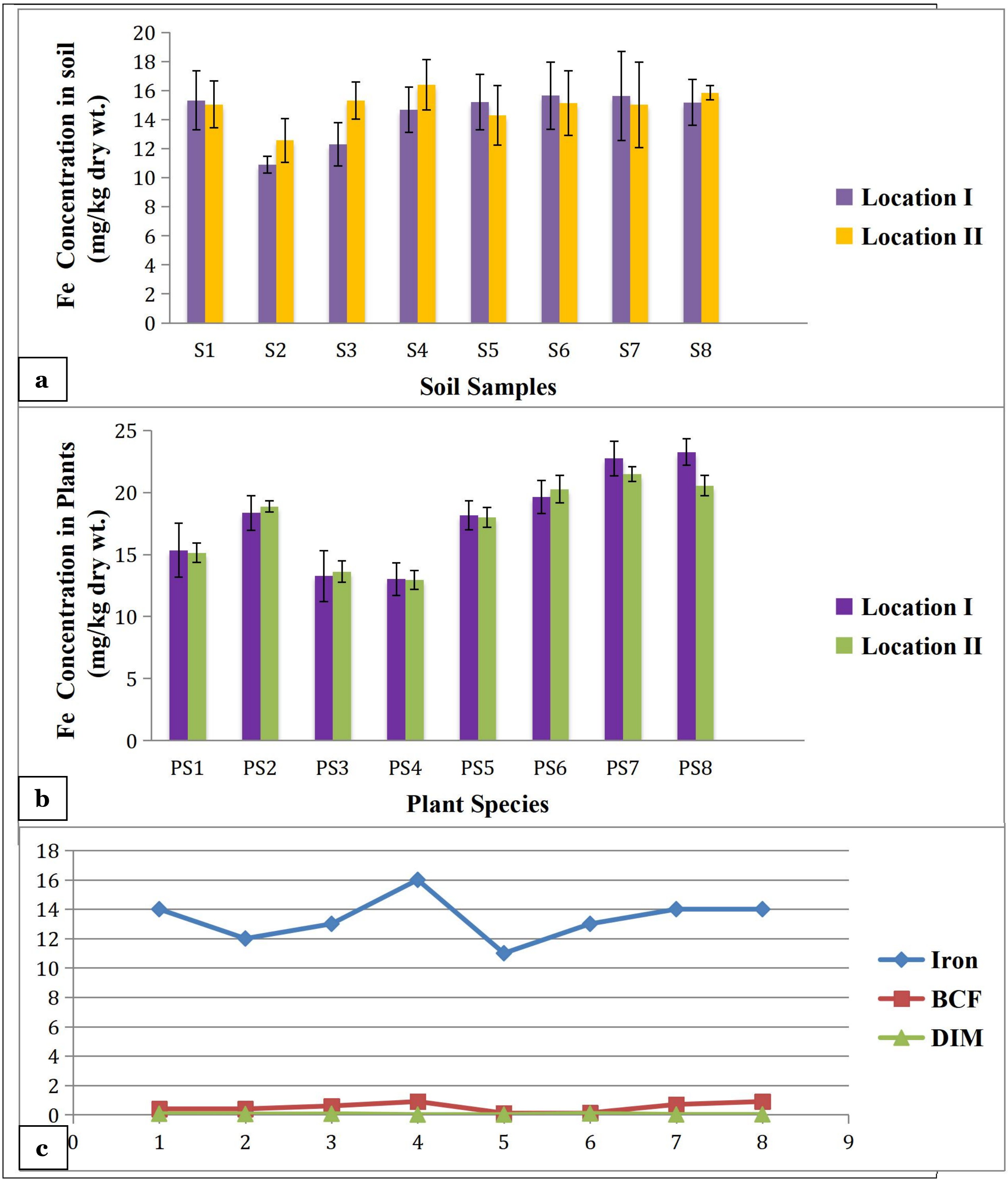

Fig. 2 (2a-2c): Sodium level in soil (a) and plants (b), Fe, Bioconcentration factor (BCF) and DIM (c) at two different locations and eight different study sites (S-P = soil to plant)

Cadmium (Cd) analysis in plants and soil

Soil Cd levels showed significant variation at Location-I ($p < 0.001$) but were non-

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significant at Location-II (Table 3). Values ranged between 3.45 and 4.63 mg/kg at Location-I and 3.68 and 4.46 mg/kg at Location-II (Fig. 3a). These concentrations fall within the Dwivedi (1997) range of 3-8 mg/kg, though they surpass older benchmarks like 0-1 mg/kg (Yasin et al., 2025).Despite this, soil Cd levels remained below toxic thresholds and are comparable to levels recorded by Mengdi et al. (2021). However, their elevated presence, even within limits, warrants long-term environmental monitoring due to Cd's cumulative toxicity.

Plant Cd levels were low and showed no significant variation across species at either location ($p > 0.05$). Values ranged from 0.025-0.040 mg/kg (Location-I) and 0.066-0.086 mg/kg (Location-II) (Fig. 3b). Maximum uptake was recorded in *Mentha spicata* and *Peganum harmala*.

These values are below the FAO/WHO limit of 0.21 ppm and WHO's PTWI of 60 µg/day for a 60 kg adult (Abdul Reda, 2022), indicating minimal health risk. Cd concentrations here are also significantly lower than levels reported in vegetables from Italy and Turkey (Ismal et al., 2023), reflecting relatively clean environmental conditions in the study sites.

Bioconcentration factor (BCF) and daily intake of metal (DIM)

BCF values ranged from 0.0159 to 0.021 (Fig. 3c), indicating very low Cd bioavailability and limited plant accumulation. P8 exhibited the highest BCF, while P3 showed the lowest. DIM values confirmed that daily intake from these plants remains far below toxic levels, ensuring safety in therapeutic use, especially for patients vulnerable to heavy metal exposure.

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

Source of Variation (S.O.V)	**Degreesof Freedom (df)**	**Mean Squares**			
		Soil		**Plant**	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.499*	0.218ns	0.003ns	0.004ns

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Error	16	0.108	0.085	0.001	0.002

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

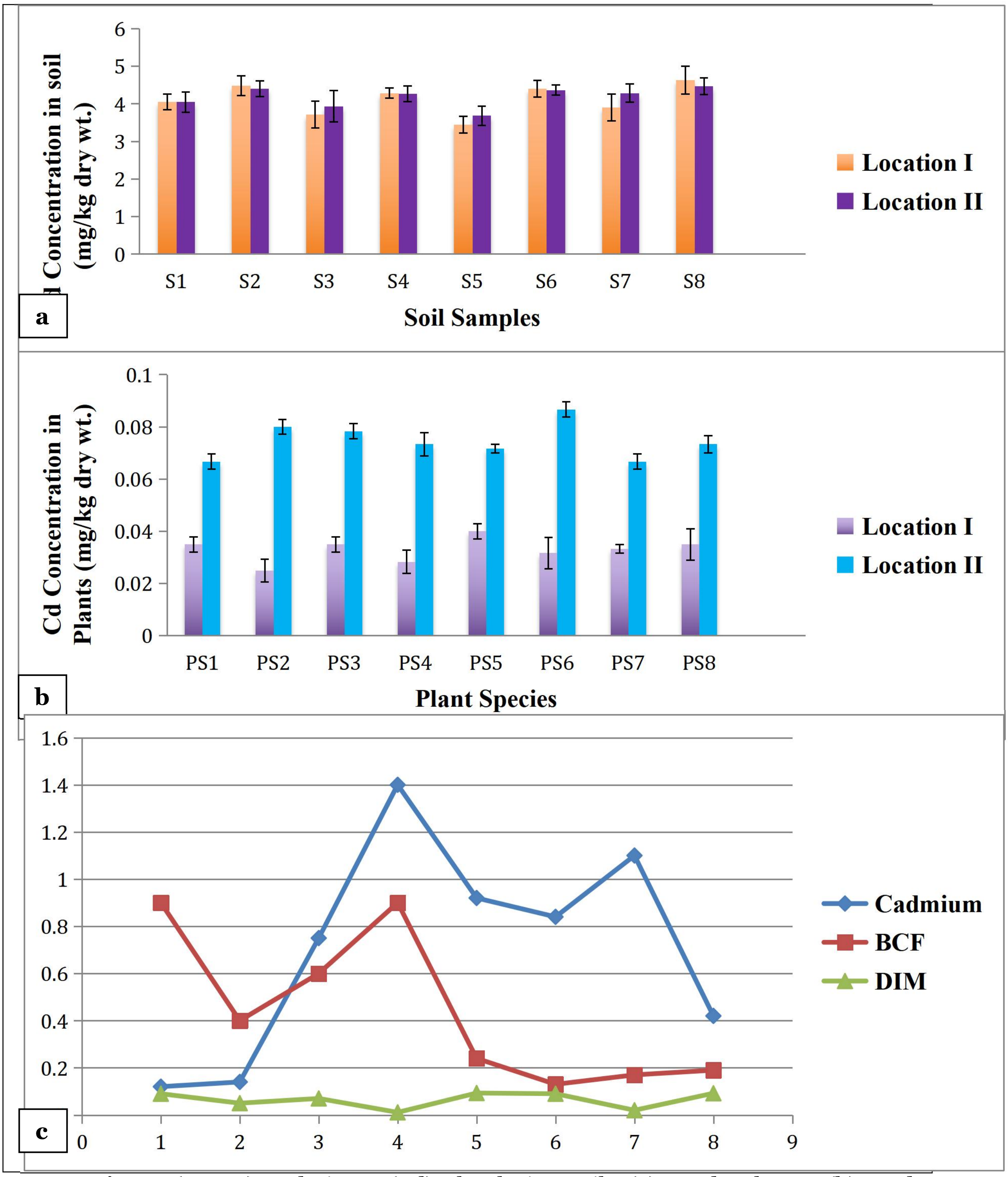

Fig.3 (3a-3c): Cadmium (Cd) level in soil (a) and plants (b), Cd, Bioconcentration factor (BCF) and DIM correlation (c) at two different locations and eight different study sites (S-P = soil to plant)

Chromium (Cr) analysis in plants and soil

Chromium concentrations in soil showed no statistically significant variation across all sites ($p > 0.001$) at both locations (Table 4). Soil Cr levels ranged from 0.028-0.041 mg/kg (Location-I) and 0.030-0.040 mg/kg (Location-II) (Fig. 4a). These values were slightly above the critical value of 0.02 mg/kg (Lopez et al., 2025), yet much lower than the toxic levels (2-50 mg/kg) reported by Ofomatah (2023), and lower than other studies in Pakistan (Ali et al., 2022).

Cr levels in medicinal plants showed no significant location-based variation ($p > 0.05$) at either site. At Location-I, plant Cr levels ranged from 3.40 to 4.65 mg/kg, and at Location-II from 3.87 to 4.27 mg/kg (Fig. 4b). *Cannabis sativa* and *Peganumharmala* showed maximum accumulation.

All observed values exceed the FAO/WHO threshold of 0.02 ppm, necessitating careful consumption guidance. However, they remain below toxic doses that might cause dermatological, respiratory, renal, and hepatic disorders (McGrath, 2000). The recommended dietary Cr intake is 50-200 µg/day (Genchi, 2021), well above any potential intake through these plants under typical consumption scenarios.

Bioconcentration factor (BCF) and daily intake of metal (DIM)

BCF values for Cr were exceptionally high, ranging from 102.79 to 149.57 (Fig. 4c), indicating strong bioaccumulation capacity. P1 demonstrated the highest accumulation, suggesting potential for biomonitoring or phytoremediation. DIM assessments showed that despite elevated uptake, actual human exposure remains within safe boundaries.

Table 4: Analysis of variance for Cr concentrations in soil and plants at different locations

Source of Variation (S.O.V)	**Degreesof Freedom (df)**	**Mean Squares**			
		Soil		**Plant**	
		Location-I	Location-II	Location-I	Location-II
Locations	7	0.011ns	0.021ns	0.736***	0.077ns

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Error	16	0.002	0.001	0.109	0.070

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

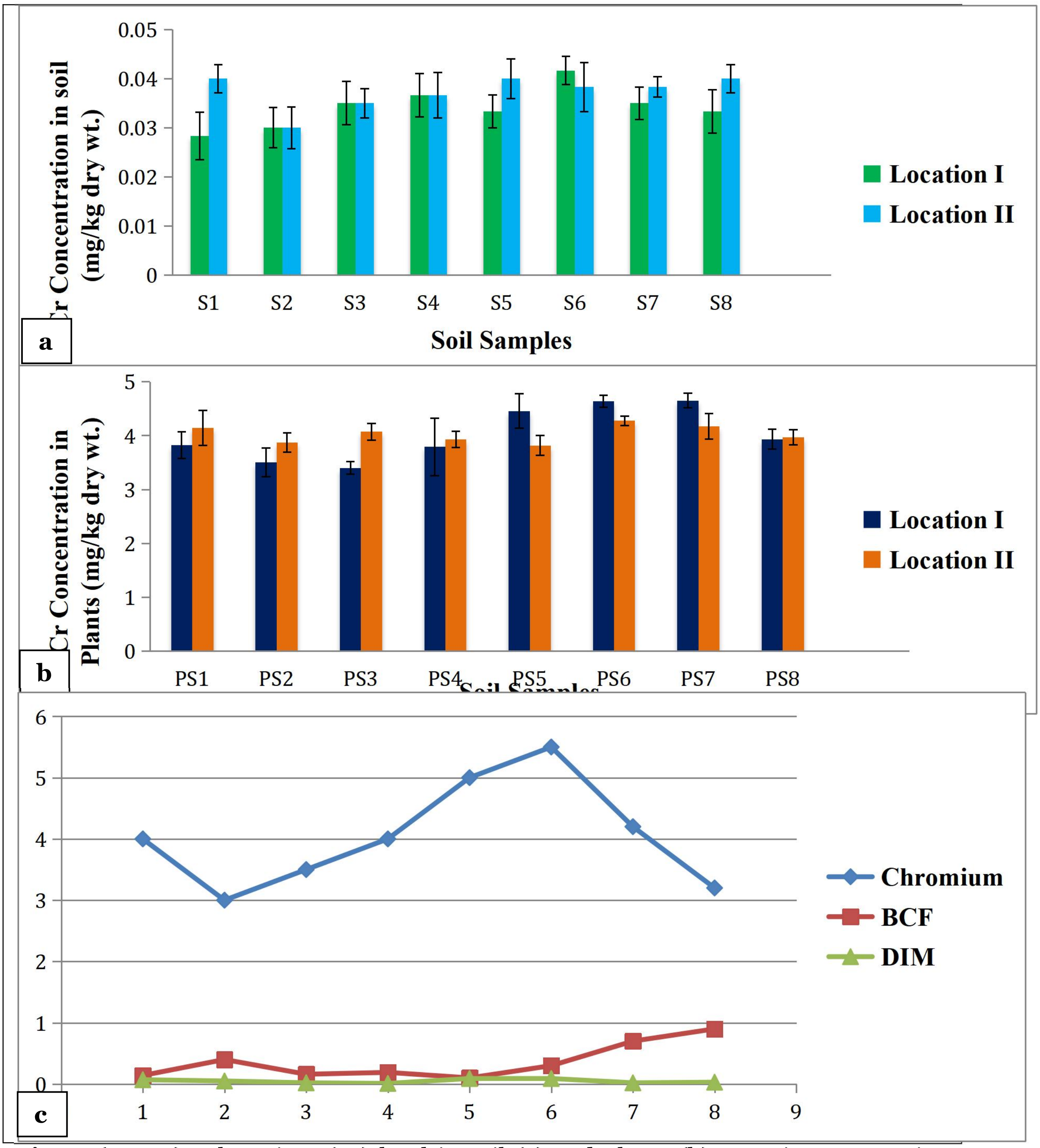

Fig. 4 (4a-4c): Chromium (Cr) level in soil (a) and plants (b), Cr, Bioconcentration factor (BCF) and DIM (c) at two different locations and eight different study sites (S-P = soil to plant).

Principal component analysis (PCA)

The biplot illustrates the principal component analysis (PCA) results, highlighting the relationships between heavy metal concentrations (Zinc, Iron, Cadmium, Chromium), bioconcentration factor (BCF), daily intake of metals (DIM), and eight medicinal plant species (PS1-PS8). The green vectors indicate the direction and magnitude of each variable's contribution to the principal components.

Notably, Iron and BCF exhibit strong positive loadings along the X-axis, correlating with species PS4, PS7, and PS8, suggesting these plants have higher metal accumulation and bioconcentration capacity. In contrast, Cadmium and Chromium are more aligned with PS5 and PS6, indicating potential toxic metal uptake in these species. Zinc and DIM display negative loadings, clustering closer to PS1 and PS2, which may reflect lower levels of metal accumulation and minimal dietary intake risk. The spatial separation among plant species in the plot reflects their varying metal uptake behavior, offering insights into both environmental safety and the suitability of these species for medicinal use or phytoremediation strategies (Fig. 5a).

Correlation Matrix analysis

The correlation matrix plot visually represents the strength and direction of relationships among heavy metals (Zinc, Iron, Cadmium, Chromium), Bioconcentration Factor (BCF), and Daily Intake of Metals (DIM). The color intensity and size of each circle indicate the strength of the correlation, with blue denoting positive correlations and red indicating negative ones. Strong positive correlations are evident between Zinc and Iron, as well as among Iron, Chromium, and Cadmium, suggesting these metals may co-occur in plants or share uptake pathways.

BCF shows strong positive correlation with Zinc and Iron, indicating that plants absorbing more of these metals also exhibit higher accumulation efficiency. DIM also positively correlates with Chromium and Cadmium, pointing to potential dietary risks when consuming plants contaminated with these elements. Conversely, negative correlations, such as between Chromium and BCF or between Zinc and Cadmium, suggest contrasting uptake behaviors or competitive inhibition among these elements. Overall, the matrix provides an integrative view of inter-element dynamics and their implications for plant uptake and human health exposure (Fig. 5b).

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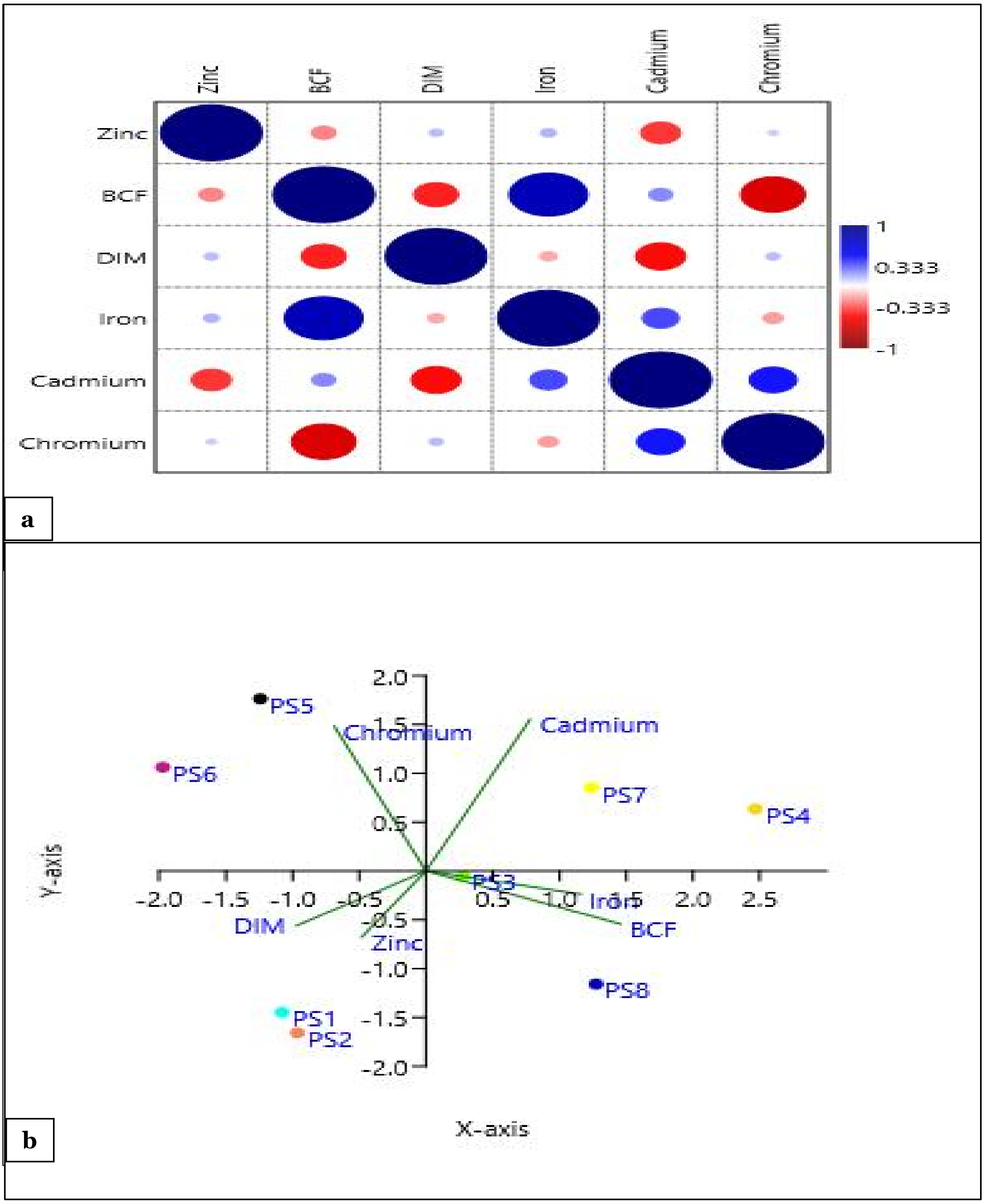

(b) for different studied medicinal plants at different sites

Conclusion

This study provides a comprehensive assessment of heavy metal accumulation in medicinal plants and their corresponding soils across two distinct locations. The

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analysis confirms that Zn and Fe are present in sufficient quantities in soil and are absorbed by plants in concentrations that support both nutritional and therapeutic roles without exceeding toxic thresholds. Cd, although present in moderate levels in soil, exhibited minimal transfer to plant tissues, indicating low environmental mobility and low risk of human exposure through these species.Cr, however, displayed high bioconcentration and uptake values, with plant tissue concentrations exceeding permissible limits for edibles. Despite this, the calculated daily intake values for Cr and all other metals remain within tolerable safety margins, suggesting that short-term use of these plants does not pose significant health risks. Nevertheless, long-term or high-frequency use particularly of plants like *Cannabis sativa* and *Peganum harmala* may lead to cumulative exposure, especially for Cr and Fe, and should be carefully monitored.

The variability in BCF values across species highlights the differential metal uptake capabilities of medicinal plants, offering useful insight for species selection in phytoremediation and bio-monitoring strategies. This study underscores the need for regulatory guidelines on heavy metal thresholds in medicinal plants, and recommends periodic monitoring of soils and plant materials, especially in areas where these species are harvested for medicinal or dietary purposes.

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