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**Research Article**

# ANALYSIS OF METAL CONTENT IN BITTER GOURD (*MOMORDICA CHARANTIA* LINN.) CULTIVATED IN KALOI, THARPARKAR, SINDH, PAKISTAN

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**ABSTRACT**

In the present study, the metal content of different parts of *Momordica charantia* L. (bitter gourd), a widely used medicinal plant, was analyzed to assess its potential as both a source of essential minerals and a carrier of toxic metals. ICP-OES was used to determine the concentration of 22 metals in the root, stem, leaf, fruit, and seed of the plant. The results indicated high concentrations of Ca, K, and Fe, which are beneficial to human health. The root contained the highest concentration of Ca (2896.67 mg/kg), while the leaf had the highest concentration of K (575.75 mg/kg). However, toxic metals such as As, Cd, and Pb were found at levels exceeding FAO/WHO safety limits (0.5, 0.2, and 0.3 mg/kg, respectively). Specifically, As was detected at 1.371 mg/kg in the leaf and 0.784 mg/kg in the seed, Cd at 0.222 mg/kg in the root, and Pb at 7.52 mg/kg in the seed, far exceeding permissible concentrations. Moreover, Ni concentration was highest in the root (48.57 mg/kg), while Hg levels were below the regulatory limit but still pose a potential risk of neurological impairment. These findings underscore the dual role of *M. charantia* as both a valuable source of essential nutrients and a potential health hazard due to heavy metal contamination. Given its widespread use for nutritional and medicinal purposes, there is an urgent need for environmental monitoring and agricultural safety measures, particularly in regions like Tharparkar, to prevent heavy metal accumulation in medicinal plants and safeguard public health.

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## INTRODUCTION

In arid regions such as Tharparkar, Pakistan, medicinal plants play a vital role in local healthcare due to limited access to formal medical services (Bibi et al., 2014). *Momordica charantia* (commonly known as bitter gourd or bitter melon; locally called karela), a member of the Cucurbitaceae family, is widely used in traditional medicine for managing diabetes, cardiovascular diseases, and immune disorders (Villarreal-La Torre et al., 2020). However, the accumulation of heavy metals in medicinal plants has become an increasing concern in Tharparkar, where environmental factors such as soil contamination and the use of polluted water for irrigation influence agricultural practices.

The contamination of *M. charantia* with toxic metals such as arsenic (As), cadmium (Cd), and lead (Pb) is particularly concerning in semi-arid and arid regions like Tharparkar, where groundwater and soil quality are severely impacted by industrial activities and mining. High As levels have been reported in groundwater used for irrigation, often exceeding WHO limits (Brahman et al., 2016). These contaminants are absorbed by plants and accumulate in their edible parts, posing a significant health risk to consumers who rely on them for both nutritional and medicinal purposes.

In addition to toxic metals, *M. charantia* contains essential elements such as calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), zinc (Zn), and iron (Fe), which are vital for various physiological functions (Kumar et al., 2021). While these elements are beneficial in appropriate amounts, excessive accumulation of toxic metals in edible plants can lead to severe health issues, including organ damage, neurotoxicity, cardiovascular diseases, and an increased risk of cancer (Smereczański and Brzóska, 2023; Ganie et al., 2024).

Tharparkar faces unique challenges due to its mineral-rich soil and reliance on contaminated groundwater for irrigation. Anthropogenic activities such as coal mining and deforestation further exacerbate heavy metal accumulation in both soil and water, leading to the bioaccumulation of these metals in crops, including *M. charantia* (Mawari et al., 2022). Therefore, monitoring and regulating metal contamination in medicinal plants is crucial. The present study aimed to analyze the metal composition of *M. charantia* grown in Kaloi, Tharparkar, Sindh, with a particular focus on the bioaccumulation of toxic metals in different plant parts (root, stem, leaf, fruit, and seed) and to assess their potential health risks.

## MATERIALS AND METHODS

### Collection of plant material

Whole plant material of *M. charantia* was collected from agricultural fields in Kaloi, an administrative region of district Tharparkar, Sindh, Pakistan, bordering the Mirpurkhas and Badin districts. The taxonomic identification of the plant was verified by a botanist at the Arid Zone Research Institute (AZRI), Umerkot, Sindh, Pakistan (Voucher specimen: QK01/2023). The scientific name was cross-checked with the International Plant Name Index (IPNI) (www.ipni.org).

The collected plant material was transported to the Biochemical Laboratory at the Institute of Biochemistry, University of Sindh, Jamshoro. The plant parts were separated, washed with deionized water, and shade-dried at 37-40°C until a constant weight was achieved. Seeds were obtained by dissecting the fruit. Finally, all plant materials were ground into a fine powder using an electric mechanical grinder, sieved, and stored in amber-colored jars for further analysis.

### Chemical reagents

All chemicals and reagents used in the experiments were of analytical grade. Nitric acid ($HNO_3$) (65%) and hydrogen peroxide ($H_2O_2$) (37%) were purchased from Merck (Germany). Throughout the study, ultra-pure Milli-Q deionized water (>18.2 MΩ·cm resistance, Elga, USA) was used. Glassware and other materials were soaked in 20% nitric acid ($HNO_3$) for 24 h, thoroughly rinsed with Milli-Q ultrapure water, dried at 60°C, and stored in a dust-free environment.

### Digestion of plant material, sample, and standards preparation

A 0.5 g sample of powdered plant material was digested with 10 ml of $HNO_3$ and $H_2O_2$ mixture (2:1) in a conical flask. The mixture was heated on a hot plate under a fume hood until complete dissolution of organic matter. After digestion, the solution was cooled, filtered through Whatman No. 1 filter paper, and diluted to a final volume of 25 ml with Milli-Q deionized water. Working standards for metal analysis were prepared by diluting a 100-ppm stock solution to final concentrations of 0.01, 0.1, 1.0, 10.0, 50.0, and 100 ppm.

### Metals analysis

A total of 22 metals were analyzed in different plant parts using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Thermo Scientific, ICAP 7000 Plus Series, Cambridge, UK) at the National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro. The ICP-OES system was equipped with an Echelle polychromator (high resolution) and a large format program array detector (L-PAD). A ceramic nebulizer was used for sample introduction.

The following parameters were optimized for ICP-OES analysis:

1. Analysis pump rate: 50
2. Flush pump rate: 100
3. Pump stabilization time: 5 min
4. RF power: 1150 W
5. Nebulizer gas flow rate: 0.7 L/min
6. Auxiliary gas flow rate: 0.5 L/min
7. Sample uptake rate: 0.8 L/min

The wavelength lines used for analysis were selected based on previous interference studies (Otho et al., 2022), with the most intense lines utilized and secondary lines employed as backups in case of potential interferences. Results were obtained in ppm and subsequently converted to mg. The final results were expressed as mg/kg of the original samples.

**Calibration and validation**

For accurate measurement, the instrument was optimized following standard protocols to maximize sensitivity and minimize interference. Method validation (Table 1) was performed according to the user manual and standard procedures. Linearity was confirmed using calibration curves with six standard concentrations. Limits of detection (LOD) and quantification (LOQ) were determined according to IUPAC guidelines (Vessman et al., 2001). The method demonstrated satisfactory accuracy, with a standard deviation <10% for triplicate measurements and recovery rates exceeding 85% for spiked samples.

**Statistical analysis**

Data were processed using SPSS (IBM, 2022) and OriginPro 2014b software. Metal concentrations in different plant parts were analyzed in triplicate, and results were expressed as mean ± standard deviation (SD). Metal content in each plant part was calculated as mg/kg. The statistical significance of metal concentration differences among plant parts was assessed using one-way ANOVA (Single Factor Analysis). The level of significance was set at $p \leq 0.01$.

Table 1. Standard calibration parameters of analyzed metals.

| Metals | Wavelength (nm) | Linear Range (mg/L) | Regression Equation | $R^2$ Value | LOD (mg/L) | LOQ (mg/L) |
|---|---|---|---|---|---|---|
| Al | 308.215 | 0-100 | y = mx + b | 0.9997 | 0.01 | 0.03 |
| As | 193.759 | 0-100 | y = mx + b | 0.9998 | 0.01 | 0.03 |
| Ca | 422.673 | 0-100 | y = mx + b | 0.9987 | 0.02 | 0.06 |
| Cd | 226.502 | 0-100 | y = mx + b | 0.9985 | 0.02 | 0.06 |
| Co | 232.995 | 0-100 | y = mx + b | 0.9994 | 0.03 | 0.09 |
| Cr | 283.563 | 0-100 | y = mx + b | 0.9982 | 0.02 | 0.07 |
| Cu | 324.754 | 0-100 | y = mx + b | 0.999 | 0.01 | 0.03 |
| Fe | 259.994 | 0-100 | y = mx + b | 0.9992 | 0.01 | 0.04 |
| Hg | 254.438 | 0-100 | y = mx + b | 0.9991 | 0.02 | 0.05 |
| K | 766.49 | 0-100 | y = mx + b | 0.9988 | 0.03 | 0.09 |
| Li | 670.783 | 0-100 | y = mx + b | 0.9997 | 0.01 | 0.03 |
| Mg | 279.553 | 0-100 | y = mx + b | 0.9995 | 0.02 | 0.06 |
| Mn | 589.592 | 0-100 | y = mx + b | 0.9984 | 0.02 | 0.06 |
| Na | 589.592 | 0-100 | y = mx + b | 0.9992 | 0.01 | 0.03 |
| Ni | 231.604 | 0-100 | y = mx + b | 0.9996 | 0.01 | 0.03 |
| Pb | 283.209 | 0-100 | y = mx + b | 0.9993 | 0.02 | 0.07 |
| Pd | 340.458 | 0-100 | y = mx + b | 0.9991 | 0.02 | 0.06 |
| Se | 196.09 | 0-100 | y = mx + b | 0.9989 | 0.03 | 0.09 |
| Si | 288.158 | 0-100 | y = mx + b | 0.9995 | 0.02 | 0.06 |
| Ti | 334.941 | 0-100 | y = mx + b | 0.9991 | 0.02 | 0.06 |
| V | 292.402 | 0-100 | y = mx + b | 0.999 | 0.01 | 0.04 |
| Zn | 213.856 | 0-100 | y = mx + b | 0.9988 | 0.02 | 0.07 |

## RESULTS AND DISCUSSION

Table 2 presents the concentration of essential metals in different parts of *M. charantia*, revealing significant variation across the root, stem, leaf, fruit, and seed ($p < 0.01$).

Sodium (Na) was most concentrated in the fruit (688.5 mg/kg) and least in the seed (259.67 mg/kg), aligning with findings from India but differing from lower levels reported by Kosanovic et al. (2009). This suggests the role of Na in osmoregulation and cellular function within the fruit. A similar trend was observed in arid and semi-arid regions of Sindh, Pakistan, where saline irrigation contributed to elevated Na levels in fruit (Kazi et al., 2014).

Potassium (K) was highest in the leaf (575.75 mg/kg) and lowest in the stem (295.17 mg/kg), emphasizing the leaf's role in enzyme activation and photosynthesis (Erel et al., 2014). This pattern was consistent with studies from Kenya and Thailand and similar observations in *Cucumis sativus* in Sindh, likely due to high temperatures and water stress (Bibi et al., 2014; Gayathry and John, 2022).

Calcium (Ca) was most concentrated in the root (2896.67 mg/kg) and least in the leaf (2563.33 mg/kg), supporting findings from China and Indonesia, which reported high Ca in fruit and seed (Nagarani et al., 2014). Ca is vital for structural integrity and nutrient uptake (Raina et al., 2016). In contrast, some crops in arid Sindh, such as *Aloe vera*, accumulate excess Ca in roots as a response to soil salinity (Saeed et al., 2018).

Aluminum (Al) accumulated most in the leaf (908.25 mg/kg) and least in the seed (139.29 mg/kg), potentially influencing stress responses and nutrient interactions (Ofoe et al., 2023). Similar trends were noted in Sindh crops grown in acidic soils, particularly in Thar Desert regions (Mussarat et al., 2014).

Silicon (Si) was highest in the leaf (15,807.5 mg/kg), consistent with studies from Japan and the Philippines that highlighted its role in structural reinforcement and pathogen resistance (Kaushik and Saini, 2019). This trend was also observed in arid areas like Tharparkar, where plants accumulated Si to counteract drought and heat stress (Brahman et al., 2016).

Cobalt (Co) was most concentrated in the leaf (0.54 mg/kg) and least in the stem (0.043 mg/kg), likely due to its involvement in metabolic functions like nitrogen fixation (Hu et al., 2021). In Sindh, Co levels tend to be higher in leaves of crops grown with compost amendments, which enhance nutrient uptake (Shagufta et al., 2024).

Vanadium (V) was highest in the stem (6.08 mg/kg) and lowest in the fruit (0.669 mg/kg), suggesting a role in stress responses and stem integrity (Wu et al., 2021). This pattern aligned with Brazilian studies and findings in Sindh, where irrigation with trace metal-containing water lead to V accumulation in *C. melo* and *C. sativus* stems (Kazi et al., 2014; Silva et al., 2016).

Palladium (Pd) was highest in the leaf (264.08 mg/kg) and lowest in the seed (43.67 mg/kg), suggesting its involvement in specific metabolic processes or stress responses (Kińska et al., 2018; Masinire et al., 2022). While Pd accumulation in plants is understudied in Pakistan, similar trends were observed in Thar Desert species adapted to environmental stress.

Lithium (Li) is predominantly found in the leaf (6.33 mg/kg) and minimally in the seed (0.34 mg/kg), indicating its role in stress adaptation (Shahzad et al., 2016). In Sindh, Li accumulation in desert-adapted plants is linked to enhanced resistance to high temperatures and water stress.

These findings highlight differential metal accumulation in *M. charantia*, underscoring their physiological roles and therapeutic potential. Statistically significant variations in Na, K, Ca, and Co across plant parts ($p$ = 0.01) emphasize their importance in osmoregulation, enzyme activation, and structural integrity (Fan et al., 2021). High K in leaves and Ca in roots suggest potential dietary benefits for metabolic health and mineral deficiencies (Saeed et al., 2018). The presence of trace metals like Co and Li further suggests therapeutic applications, offering insights into the development of functional foods and nutraceuticals to address mineral imbalances (Li et al., 2024). Harnessing these properties could lead to innovative dietary and medicinal solutions promoting public health.

Table 2. Essential metal content in different parts of *M. charantia*.

| Metal | Concentration of metals (mg/kg) in different parts | | | | |
|---|---|---|---|---|---|
| | Root | Stem | Leaf | Fruit | Seed |
| Na | 499.25±17.8 | 462.91±14.34 | 540.5±4.131 | 688.5±6.57 | 259.67±7.43* |
| K | 331.75±0.75 | 295.17±12.51 | 575.75±38.24 | 559.42±18.4 | 444.58±35.73* |
| Ca | 2896.67±36 | 3456.67±12.33 | 2563.33±6.3 | 2634.17±108.6 | 3625.83±37.61* |
| Al | 543.92±8.7 | 130.042±0.81 | 908.25±8.05 | 159.88±1.63 | 139.292±1.65* |
| Si | 9275±23.8 | 8428.33±10.41 | 15807.5±16.9 | 9533.33±17.15 | 8217.5±12.02* |
| Co | 0.289±0.008 | 0.043±0.004 | 0.54±0.003 | 0.121±0.015 | 0.108±0.006* |
| V | 0.692±0.020 | 6.080±0.039 | 1.343±0.04 | 0.669±0.03 | 2.226±0.018* |
| Pd | 99.092±4.05 | 110.875±6.02 | 264.08±5.53 | 76.592±6.85 | 43.67±5.164* |
| Li | 1.259±0.008 | 1.545±0.003 | 6.331±0.045 | 0.793±0.007 | 0.340±0.09* |

*Shows the $p$-value <0.01 when all parts were statistically compared trough one way-ANOVA (single factor) tool.

Table 3 presents the concentrations of essential metals (Cr, Cu, Mg, Mn, Se, and Zn) with potential hypoglycemic effects across different parts of *M. charantia* (root, stem, leaf, fruit, and seed). Statistical analysis ($p$ = 0.01) reveals significant variations, indicating distinct accumulation patterns and potential metabolic roles.

Chromium (Cr), essential for insulin sensitivity and glucose metabolism (Sinha et al., 2018), was highest in the root (16.879 mg/kg) and lowest in the fruit (5.833 mg/kg), which supports the findings of Cr role in insulin regulation/action (Anderson et al., 1983). Similar Cr concentrations have been reported in medicinal plants from semi-arid Sindh (Kazi et al., 2014).

Copper (Cu) was most abundant in the seed (17.501 mg/kg) and least in the stem (12.423 mg/kg), suggesting a role in oxidative phosphorylation and glucose regulation (Farid et al., 2021). Studies highlight the role of Cu in preventing glucose deregulation (Linder and Hazegh-Azam, 1996). Elevated Cu in seeds of other medicinal plants from Sindh has also been linked to improved glucose regulation (Mawari et al., 2022).

Magnesium (Mg), critical for insulin signaling, was highest in the root (1465.75 mg/kg), aligning with research linking Mg intake to improved insulin sensitivity (Rodríguez-Morán and Guerrero-Romero, 2003). Other studies in Sindh also report high Mg levels in diabetes-managing plants (Bibi et al., 2014).

Manganese (Mn), essential for carbohydrate metabolism (Schmidt and Husted, 2019), peaked in the leaf (89.72 mg/kg) and was lowest in the stem (29.892 mg/kg), consistent with findings suggesting Mn supplementation reduces blood glucose (Du et al., 2018). Higher Mn concentrations in *M. charantia* leaves have been reported in Sindh (Brahman et al., 2016).

Selenium (Se), known for its antioxidant properties and role in reducing insulin resistance (Titov et al., 2022), was most concentrated in the seed (0.707 mg/kg) and lowest in the root (0.195 mg/kg), supporting research linking Se levels to reduced diabetes risk (Rayman, 2012). Previous studies also reported Se-rich *M. charantia* (Mawari et al., 2022).

Zinc (Zn), essential for insulin synthesis and secretion (Nakamura et al., 2024), was highest in the seed (74.967 mg/kg) and lowest in the fruit (44.5 mg/kg). Zn supports pancreatic function and insulin activity (Chausmer, 1998). Similar Zn accumulation in seeds of indigenous Sindh plants reinforces their role in diabetes management (Kazi et al., 2014).

These findings highlight the differential accumulation of essential metals in *M. charantia*, contributing to its hypoglycemic properties. The similarity in metal distribution with other medicinal plants from arid and semi-arid regions of Sindh underscores their shared metabolic functions, validating therapeutic potential of *M. charantia* in diabetes management (Zhan et al., 2023).

Table 3. Analysis of hypoglycemic elements in different parts of *M. charantia*.

| Metal | Concentration of metals (mg/kg) in different parts | | | | |
|---|---|---|---|---|---|
| | Root | Stem | Leaf | Fruit | Seed |
| Cr | 16.88±0.125 | 8.11±0.013 | 10.84±0.021 | 5.833±0.021 | 3.663±0.013* |
| Cu | 23.69±0.16 | 12.423±0.05 | 14.41±0.041 | 13.216±0.13 | 17.501±0.5* |
| Mg | 1465.75±20 | 1836.92±14.03 | 1284±6.79 | 1252.67±17.29 | 1786.17±24.65* |
| Mn | 40.35±0.37 | 29.892±0.058 | 89.72±0.388 | 28.125±0.13 | 40.76±0.65* |
| Se | 0.195±0.051 | 0.486±0.037 | 0.204±0.051 | 0.468±0.12 | 0.707±0.055* |
| Zn | 47.4±0.35 | 47.817±0.038 | 49.9±0.066 | 44.5±0.03 | 74.967±0.13* |

*Shows the $p$-value <0.01 when all parts were statistically compared trough one way-ANOVA (single factor) tool.

The data in Table 4 provides a comprehensive analysis of heavy metal concentrations in *M. charantia*, highlighting critical environmental and human health concerns. This underscores the need for stringent monitoring and management to address heavy metal contamination in edible plants. The contamination is likely influenced by environmental conditions of Tharparkar, particularly groundwater dependence for irrigation and the widespread use of contaminated water. Studies from the Thar desert report high As and fluoride ($F^-$) levels, both toxic at elevated concentrations. In Tharparkar, As levels in drinking water have been recorded at up to 235 times the WHO limit (Kazi et al., 2014), while fluoride often exceeds the 1.5 mg/L threshold (Brahman et al., 2016). Moreover, local soil, affected by natural mineral deposits and pollution from coal mining, contributes to Cd and Pb bioaccumulation in plants. Contaminated irrigation water further exacerbates toxic metal uptake in edible crops.

Arsenic (As) concentrations in *M. charantia* leaves (1.371 mg/kg) and seeds (0.784 mg/kg) exceed the FAO/WHO limit of 0.5 mg/kg (Mawari et al., 2022), consistent with similar studies in India and Pakistan (Shandana et al., 2024). As is a well-documented carcinogen linked to skin lesions, bladder and lung cancers, cardiovascular disease, and neurotoxicity (Ganie et al., 2024). Elevated As in *M. charantia* aligns with regional findings showing high As levels in Umerkot and other Sindh areas, where vegetable contamination exceeds limits by threefold (Brahman et al., 2016). High As levels in groundwater of Tharparkar (Kazi et al., 2014) directly contribute to plant accumulation.

Cadmium (Cd) concentration in roots (0.222 mg/kg) surpasses the FAO/WHO limit of 0.2 mg/kg (Mawari et al., 2022), aligning with concerns over soil contamination in China (Wang et al., 2019). Cd exposure primarily affects kidneys and bones, increasing risks of renal dysfunction, osteoporosis, lung damage, and cancer (Wang et al., 2019). Higher Cd levels in Sindh contrast with lower concentrations reported in Punjab, suggesting contamination is region-specific, linked to agricultural practices and irrigation with contaminated water (Mawari et al., 2022).

Iron (Fe) concentration in leaves (1591.83 mg/kg) exceeds the FAO/WHO limit of 425.5 mg/kg (Mawari et al., 2022). Although Fe is essential for health, excessive intake can cause toxicity, leading to organ damage, oxidative stress, and conditions like hemochromatosis (Omena et al., 2021). In Sindh, where Fe-rich medicinal plants are widely used, supplementation should be carefully monitored, especially for individuals with preexisting liver or cardiovascular conditions.

Mercury (Hg) concentrations in this study remain below the FAO/WHO limit of 0.05 mg/kg (Mawari et al., 2022). However, even low Hg exposure poses neurological and developmental risks, particularly in pregnant women and children (Gworek et al., 2020). Even though *M. charantia* contains minimal Hg, proximity of Tharparkar to mining and coal combustion sites necessitates stricter pollution controls to minimize exposure.

Nickel (Ni) levels remain below the FAO/WHO limit of 67.9 mg/kg, with the highest concentration in roots (48.57 mg/kg), consistent with previous findings (Rahim et al., 2024). Ni exposure can cause allergic reactions, dermatitis, respiratory issues, and increased cancer risks (Das et al., 2019). Since Ni is prevalent in polluted soils, controlling industrial waste and fertilizer application is crucial to minimizing contamination.

Lead (Pb) concentrations in seeds (7.520 mg/kg) significantly exceed the FAO/WHO limit of 0.3 mg/kg, posing severe health risks (Rehman et al., 2017). Pb toxicity affects multiple organ systems, causing developmental delays in children, hypertension, renal damage, and reproductive issues in adults (Kumar et al., 2020). Elevated Pb levels in Tharparkar likely result from coal mining activities and irrigation with contaminated groundwater. Compared to industrial regions, lower Pb levels in Kenyan vegetables suggest that industrialization is a key driver of Pb contamination (Gayathry and John, 2022). Addressing Pb pollution requires stricter industrial regulations, safer agricultural practices, and regular health screenings.

Although titanium (Ti) concentrations in leaves (16.6 mg/kg) are high, no specific FAO/WHO limits exist. Comparative studies indicate varying Ti levels in plants, and while generally less toxic than other heavy metals, prolonged exposure may cause skin irritation and respiratory issues (Bacilieri et al., 2017). Further research is needed to establish safety standards for Ti, particularly in mining-affected regions.

The heavy metal analysis of *M. charantia* underscores the urgency of monitoring and regulating environmental pollution to protect public health. Effective strategies, including industrial emissions control, safer agricultural practices, and routine environmental assessments, are critical in mitigating heavy metal risks. In Sindh, where agriculture and medicinal plants play a vital role, comprehensive monitoring systems are essential to prevent heavy metal exposure, particularly in vulnerable areas like Tharparkar.

The correlation and clustering analysis of metal concentrations in *M. charantia* reveal significant interactions between essential and toxic metals, providing insights into the plant's metal homeostasis and environmental influences. Figure 1 shows a strong positive correlation between Ca and Mg ($p$ = 0.98), suggesting a shared uptake mechanism consistent with their roles in enzyme activation and cell wall stability (Wagan et al., 2023). Similarly, the positive correlation between Fe and Al ($p$ = 0.98) may reflect environmental factors, such as soil pH, which affect metal solubility and bioavailability (Adamczyk-Szabela and Wolf, 2022). The negative correlation between Na and Zn ($p$ = −0.88) is particularly notable, indicating a potential competitive uptake mechanism. Na, as an antagonistic ion, may interfere with Zn absorption, possibly helping plants mitigate Na toxicity while optimizing Zn uptake for enzymatic functions (Kumar et al., 2021). However, further controlled studies are needed to confirm these interactions and clarify the underlying physiological mechanisms.

Table 4. Analysis of heavy metals in different parts of *M. charantia*.

| Metal | Concentration of heavy metals (mg/kg) in different parts | | | | | Acceptable limits (mg/Kg)** |
|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Fruit | Seed | |
| As | 1.137±0.040 | 1.36±0.023 | 1.371±0.123 | 0.758±0.027 | 0.784±0.04* | 0.5 |
| Cd | 0.222±0.002 | 0.136±0.003 | 0.223±0.004 | 0.090±0.003 | 0.158±0.00 | 0.2 |
| Fe | 886.25±6.7 | 397.25±2.29 | 1591.83±9.3 | 504.5±2.22 | 257.83±3.8* | 425.5 |
| Hg | 0.005±0.001 | 0.023±0.017 | 0.005±0.001 | 0.022±0.0004 | 0.017±0.00 | 0.05 |
| Ni | 48.57±0.38 | 17.78±0.040 | 19.51±0.029 | 15.382±0.023 | 8.778±0.02* | 67.9 |
| Pb | 3.247±0.016 | 3.05±0.020 | 4.622±0.061 | 4.649±0.018 | 7.520±0.06* | 0.3 |
| Ti | 2.796±0.385 | 0.798±0.003 | 16.6±0.120 | 2.063±0.035 | 0.713±0.01* | - |

*Shows the $p$-values <0.01 when all parts were statistically compared trough one way ANOVA (single factor) tool.
**FAO/WHO acceptable limits (mg/Kg) (Mawari et al., 2022).

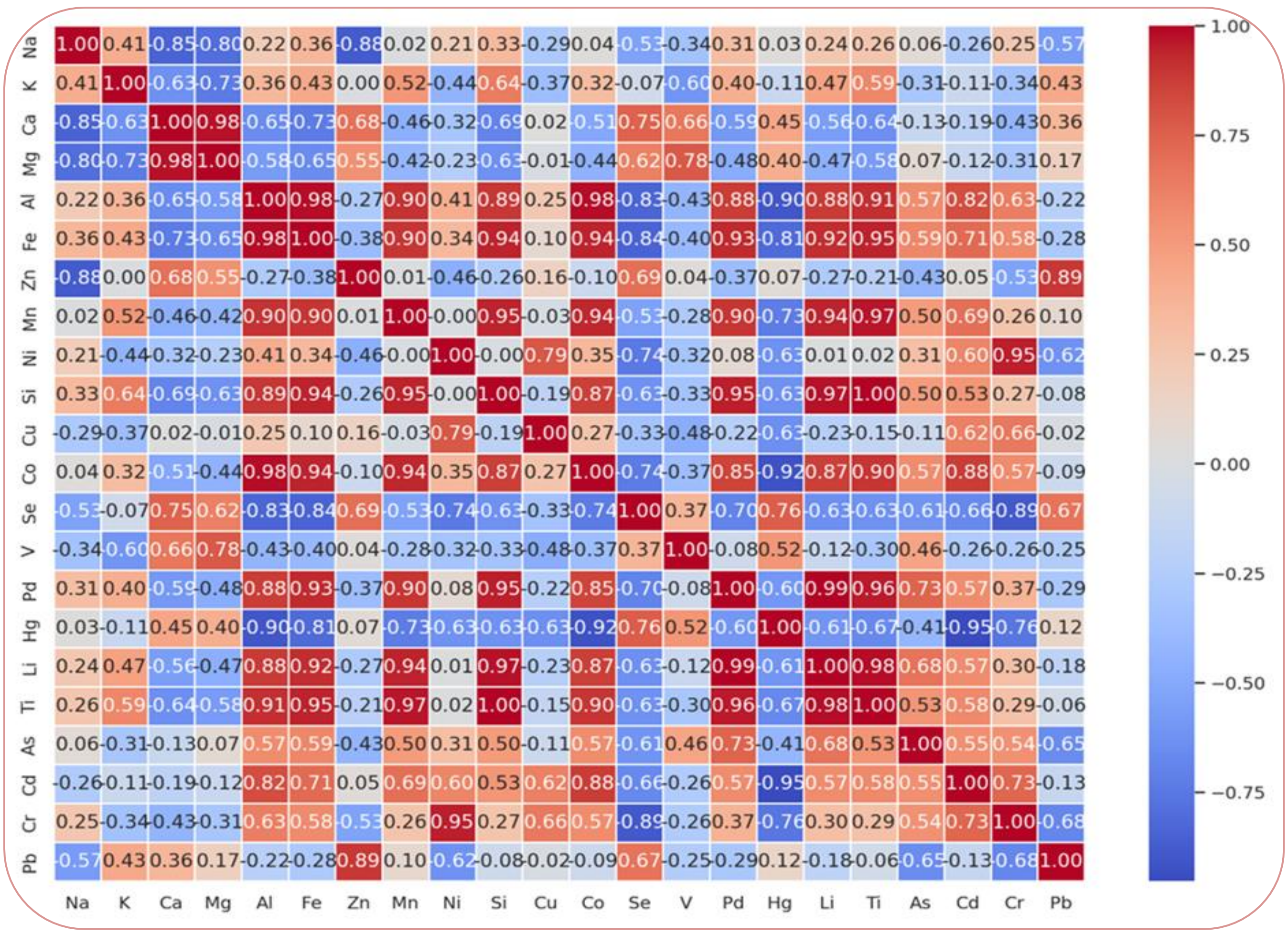


Figure 1. Correlation matrix of metal concentration in *M. charantia*.

Clustering analysis (Figure 2) further supports the idea of metal interactions in *M. charantia*. The close clustering of Na and K reflects their shared physiological roles in maintaining osmotic balance and regulating cellular turgor pressure, which are crucial for metabolic processes such as stomatal regulation and enzyme activation (White, 2013). The clustering of Al with Na and K may indicate potential competitive inhibition or modifications of ion channels in the roots of plant, as Al is known to influence nutrient uptake under stress conditions (Hajiboland et al., 2023).

The clustering of Zn and Mn is notable given their roles as cofactors in enzymatic processes and antioxidant defense. Zn contributes to the structural integrity of proteins and transcription factors, while Mn is essential for photosystem II in photosynthesis and acts as a cofactor for superoxide dismutase (Ahmed et al., 2024). The co-occurrence of these metals in the dendrogram suggests coordinated uptake and utilization, reflecting potential micronutrient synergy (Johnson and Mirza, 2020).

Furthermore, the clustering of Cr, Co, and Cd indicates similar uptake mechanisms or environmental interactions. Cd, for instance, mimics essential nutrients like Zn and Ca, leading to inadvertent uptake and accumulation in plants (Clemens and Ma, 2016). This clustering may reflect shared pathways of accumulation or detoxification strategies, as *M. charantia* likely employs similar mechanisms to manage these potentially harmful metals (Ali and Gill, 2022).

The distinct separation of Ca and Si in the dendrogram highlights their unique roles in plant physiology. Ca is crucial for cell wall stability, while Si enhances plant resistance to stress and improves structural integrity (Hosseini, 2022). Furthermore, the ability of Si to mitigate heavy metal stress further underscores its importance in plant health, particularly in metal-contaminated environments like Tharparkar.

In conclusion, correlation and clustering analyses reveal the complex metal interactions in *M. charantia*, highlighting its adaptive strategies for metal uptake and detoxification. These findings are essential for understanding plant nutrition, metal toxicity, and the potential use of *M. charantia* in phytoremediation, particularly in contaminated environments.

The Principal Component Analysis (PCA) of metal concentrations across different plant parts of *M. charantia* revealed distinct patterns of metal distribution. Figure 3 presents the PCA scores plot of plant samples (root, stem, leaf, fruit, and seed), projected onto the first two principal components (PC1 and PC2), which together explain 71.49% of the total variance (PC1: 52.84%; PC2: 18.65%). The root and stem samples cluster closely, indicating similar metal profiles, whereas the leaf and fruit samples show greater variation, reflecting differences in metal accumulation. This segregation suggests tissue-specific differences in metal concentrations, possibly regulated by physiological processes such as nutrient transport, sequestration, or storage. PC1 accounts for the majority of the variance among plant parts, with Ca, Mg, and Na identified as the primary contributors to this differentiation.

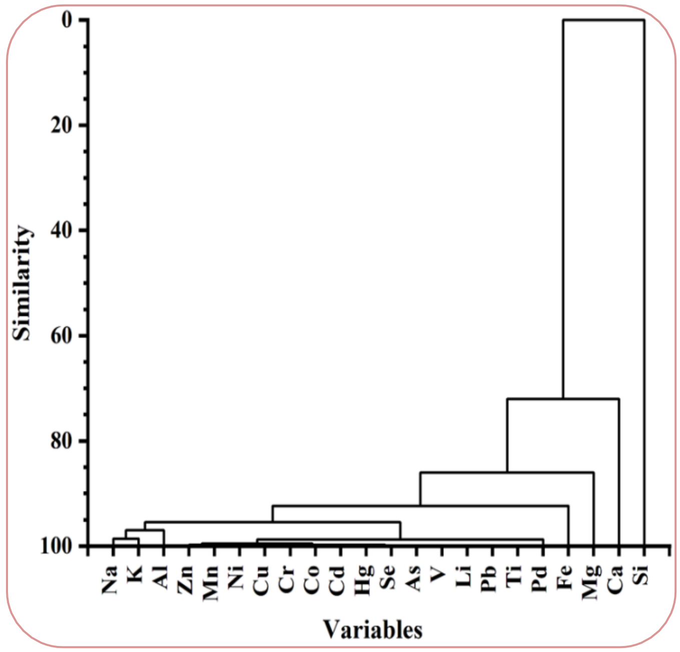


Figure 2. Hierarchical clustering dendrogram of metals analyzed in *M. charantia.*

Figure 4, the PCA loadings plot, illustrates the contribution of each metal to the separation observed in the scores plot. In this plot, metals are represented as vectors, with their direction and magnitude indicating their influence on PC1 and PC2. Ca, Mg, and Na exhibit high positive loadings on PC1, suggesting their significant role in clustering the root and stem samples. This implies that these metals play crucial roles in fundamental physiological processes such as cell wall formation and ion balance, which may be relatively uniform across these tissues. In contrast, Pb, Fe, and Si show stronger associations with PC2, indicating their greater influence on the differentiation between leaf and fruit samples. The presence of Pb, a potentially toxic metal, in leaves and fruits may suggest environmental contamination or metal stress, contributing to the variance captured by PC2.

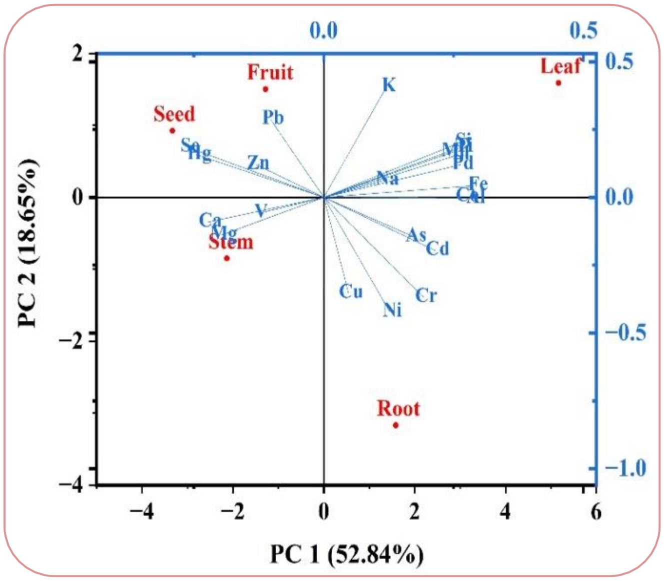


Figure 3. Score plot of PCA for metal concentrations in *M. charantia*.

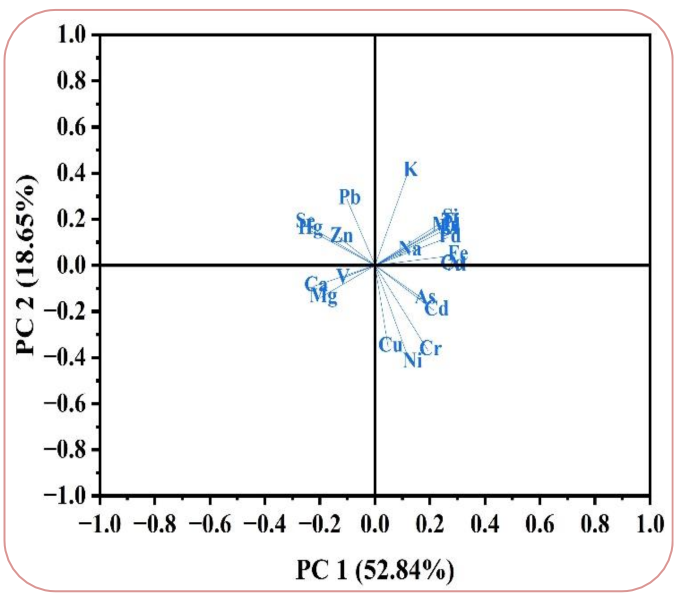


Figure 4. Loadings plot of PCA for metal concentrations in *M. charantia*.

The scree plot in Figure 5 displays the eigenvalues for each principal component, representing the proportion of total variance explained. The steep decline in eigenvalues after the first two components underscores the effectiveness of dimensionality reduction to PC1 and PC2 alone. PC1 has the largest eigenvalue, accounting for 52.84% of the variance, followed by PC2 with 18.65%. This confirms that these two components sufficiently capture the primary trends in the data, while higher-order components contribute minimally. The sharp decline in eigenvalues beyond PC2 justifies the selection of these two components for further exploration.

Collectively, these plots provide valuable insights into the metal concentration patterns across different plant tissues. The scree plot confirms that two principal components account for most of the variance, while the scores plot reveals distinct groupings of plant organs based on metal content. The loadings plot highlights that Ca, Mg, and Na contribute most to the variance along PC1, whereas Pb and Fe are key differentiators along PC2. These findings underscore the complex, tissue-dependent regulation of metal homeostasis in *M. charantia* and shed light on the roles of essential metals such as Ca, Mg, and Na in plant development, while also suggesting that Pb accumulation may be linked to environmental stress or pollution.

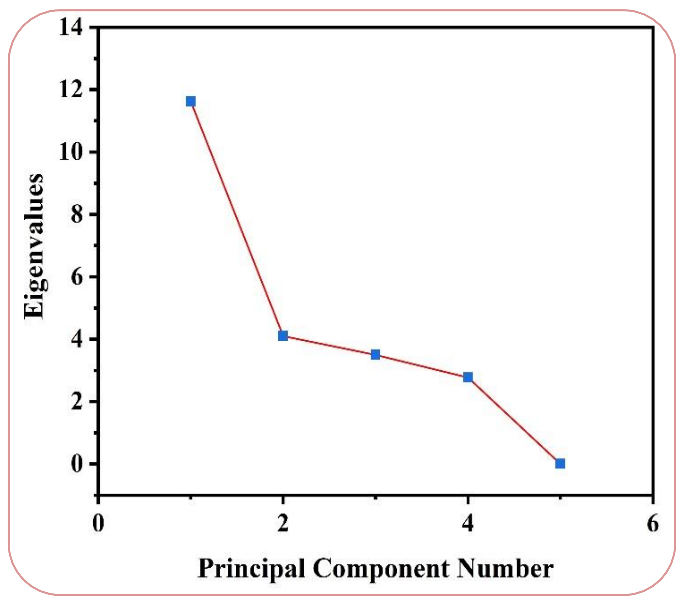


Figure 5. Scree plot of PCA for metal concentrations in *M. charantia*.

## CONCLUSION

The present research thoroughly examined the metal composition in different parts of *M. charantia* and identified it as a potential source of essential metallic elements, including Si, Ca, K, Na, and Fe. These findings support its role as a health-promoting plant due to its medicinal and nutritional value. However, the study also detected alarmingly high levels of toxic metals such as

As, Cd, and Pb, exceeding international safety standards (0.5, 0.2, and 0.3 mg/kg, respectively), in some parts of the plant, posing potential health risks to consumers, particularly in regions like Tharparkar, where environmental contamination is widespread.

The variation in metal concentration across different parts of the plant reflects both the metabolic susceptibility of the plant and the external environmental influences affecting metal accumulation. These results highlight the urgent need for stricter environmental monitoring and regulatory measures to prevent undesirable metal contamination in medicinal plants. Future research should focus on strategies to minimize metal contamination, particularly in irrigation water, through approaches such as phytoremediation. Moreover, studies on the impact of local farming and mining practices on soil and water contamination will be crucial for ensuring the safe cultivation and consumption of *M. charantia*. Addressing these challenges will help safeguard public health while preserving the medicinal properties of the plant.

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**AUTHORS' CONTRIBUTIONS**

QK, NAC and SAA designed the study; QK and SAA formulated the experiments, and MS and MP executed them; QK and SAA collected and organized the data, analyzed the results, and wrote the manuscript; NAC, MS, MP, and LN assisted in writing the manuscript and proofreading the paper.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**

Adamczyk-Szabela, D., Wolf, W.M., 2022. The Impact of soil ph on heavy metals uptake and photosynthesis efficiency in *Melissa officinalis*, Taraxacum officinalis, *Ocimum basilicum*. Molecules 27(15), 4671.

Ahmed, N., Zhang, B., Chachar, Z., Li, J., Xiao, G., Wang, Q., Hayat, F., Deng, L., Narejo, M.U.N., Bozdar, B., Tu, P., 2024. Micronutrients and their effects on Horticultural crop quality, productivity and sustainability. Scientia Horticulturae 323, 112512.

Ali, B., Gill, R.A., 2022. Editorial: Heavy metal toxicity in plants: Recent insights on physiological and molecular aspects, volume II. Frontiers in Plant Science 13, 1016257.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Roginski, E.E., Mertz, W., Glinsmann, W., 1983. Chromium supplementation of human subjects: Effects on glucose, insulin, and lipid variables. Metabolism 32(9), 894-899.

Bacilieri, F.S., de Vasconcelos, A.C.P., Lana, R.M.Q., Mageste, J.G., Torres, J.L.R., 2017. Titanium (Ti) in plant nutrition - a review. Australian Journal of Crop Science 11(4), 382-386.

Bibi, Z., Khan, Z.I., Ahmad, K., Ashraf, M., Hussain, A., Akram, N.A., 2014. Vegetables as a potential source of metals and metalloids for human nutrition: A case study of *Momordica charantia* grown in soil irrigated with domestic sewage water in Sargodha, Pakistan. Pakistan Journal of Zoology 46(3), 633-641.

Brahman, K.D., Kazi, T.G., Afridi, H.I., Baig, J.A., Arain, S.S., Talpur, F.N., Kazi, A.G., Ali, J., Panhwar, A.H., Arain, M.B., 2016. Exposure of children to arsenic in drinking water in the Tharparkar region of Sindh, Pakistan. Science of the Total Environment 544, 653-660.

Chausmer, A.B., 1998. Zinc, insulin and diabetes. Journal of the American College of Nutrition 17(2). 109-115.

Clemens, S., Ma, J.F., 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. Annual Review of Plant Biology 67, 489-512.

Das, K.K., Reddy, R.C., Bagoji, I.B., Das, S., Bagali, S., Mullur, L., Khodnapur, J.P., Biradar, M.S., 2019. Primary concept of nickel toxicity - an overview. Journal of Basic and Clinical Physiology and Pharmacology 30(2), 141-152.

Du, S., Wu, X., Han, T., Duan, W., Liu, L., Qi, J., Niu, Y., Na, L., Sun, C., 2018. Dietary manganese and type 2 diabetes mellitus: two prospective cohort studies in China. Diabetologia 61(9), 1985-1995.

Erel, R., Ben-Gal, A., Dag, A., Schwartz, A., Yermiyahu, U., 2014. Sodium replacement of potassium in physiological processes of olive trees (var. Barnea) as affected by drought. Tree Physiology 34(10), 1102-1117.

Fan, M., Lee, J.I., Ryu, Y.B., Choi, Y.J., Tang, Y., Oh, M., Moon, S.H., Lee, B., Kim, E.K., 2021. Comparative analysis of metabolite profiling of *Momordica charantia* leaf and the anti-obesity effect through regulating lipid metabolism. International Journal of Environmental Research and Public Health 18(11), 5584.

Farid, M., Farooq, M.A., Fatima, A., Abubakar, M., Ali, S., Raza, N., Alhaithloul, H.A.S., Soliman, M.H., 2021. Copper-INDUCED RESPONSES IN DIFFERENT PLANT SPECIES. In: Approaches to the Remediation of Inorganic Pollutants. https://doi.org/10.1007/978-981-15-6221-1_13

Ganie, S.Y., Javaid, D., Hajam, Y.A., Reshi, M.S., 2024. Arsenic toxicity: sources, pathophysiology and mechanism. Toxicology Research 13(1), 1-20.

Gayathry, K.S., John, J.A., 2022. A comprehensive review on bitter gourd (*Momordica charantia* L.) as a gold mine of functional bioactive components for therapeutic foods. Food Production, Processing and Nutrition 4(10), 2-14.

Gworek, B., Dmuchowski, W., Baczewska-Dąbrowska, A.H., 2020. Mercury in the terrestrial environment: a review. Environmental Sciences Europe 32, 128.

Hajiboland, R., Panda, C.K., Lastochkina, O., Gavassi, M.A., Habermann, G., Pereira, J.F., 2023. Aluminum toxicity in plants: present and future. Journal of Plant Growth Regulation 42, 3967-3999.

Hosseini, S.A., 2022. Silicon uptake, acquisition, and accumulation in plants. In: Silicon and Nano-silicon in Environmental Stress Management and Crop Quality Improvement: Progress and Prospects 37-42.

Hu, X., Wei, X., Ling, J., Chen, J., 2021. Cobalt: an essential micronutrient for plant growth? Frontiers in Plant Science 12, 768523.

Johnson, V.J., Mirza, A., 2020. Role of macro and micronutrients in the growth and development of plants. International Journal of Current Microbiology and Applied Sciences 9(11), 576-587.

Kaushik, P., Saini, D.K., 2019. Silicon as a vegetable crops modulator-a review. Plants 8(6), 148.

Kazi, T.G., Brahman, K.D., Afridi, H.I., Baig, J.A., Arain, S.S., Arain, N., 2014. Arsenic contamination of groundwater in Tharparkar, Pakistan: Implications for public health. Journal of Hazardous Materials 278, 75-80.

Kińska, K., Jiménez-Lamana, J., Kowalska, J., Krasnodębska-Ostręga, B., Szpunar, J., 2018. Study of the uptake and bioaccumulation of palladium nanoparticles by Sinapis alba using single particle ICP-MS. Science of the Total Environment 615, 1078-1085.

Kosanovic, M., Hasan, M.Y., Petroianu, G., Marzouqi, A., Abdularhman, O., Adem, A., 2009. Assessment of essential and toxic mineral elements in bitter gourd (*Momordica Charantia*) fruit. International Journal of Food Properties 12(4), 766-773.

Kumar, A., Kumar, A., Cabral-Pinto, M., Chaturvedi, A.K., Shabnam, A.A., Subrahmanyam, G., Mondal, R., Gupta, D.K., Malyan, S.K., Kumar, S.S., Khan, S.A., Yadav, K.K., 2020. Lead toxicity: Health hazards, influence on food Chain, and sustainable remediation approaches. International Journal of Environmental Research and Public Health 17(7), 2179.

Kumar, S., Kumar, S., Mohapatra, T., 2021. Interaction between macro- and micro-nutrients in plants. Frontiers in Plant Science 12, 665583.

Li, Y., Liu, Q., Zhang, D.X., Zhang, Z.Y., Xu, A., Jiang, Y.L., Chen, Z.C., 2024. Metal nutrition and transport in the process of symbiotic nitrogen fixation. Plant Communications 5, 100829.

Linder, M.C., Hazegh-Azam, M., 1996. Copper biochemistry and molecular biology. American Journal of Clinical Nutrition 63(5), 797S-811S.

Masinire, F., Adenuga, D.O., Tichapondwa, S.M., Chirwa, E.M.N., 2022. Palladium phytoremediation and phytomining potential of vetiver grass (*Chrysopogon Zizanioides*). Chemical Engineering Transactions 94, 1429-1434.

Mawari, G., Kumar, N., Sarkar, S., Daga, M.K., Singh, M.M., Joshi, T.K., Khan, N.A., 2022. Heavy metal accumulation in fruits and vegetables and human health risk assessment: Findings from Maharashtra, India. Environmental Health Insights 16, 1-10.

Mussarat, S., Abdel-Salam, N.M., Tariq, A., Wazir, S.M., Ullah, R., Adnan, M., 2014. Use of ethnomedicinal plants by the people living around Indus river. Evidence-Based Complementary and Alternative Medicine 2014, 212634.

Nagarani, G., Abirami, A., Siddhuraju, P., 2014. Food prospects and nutraceutical attributes of *Momordica* species: A potential tropical bioresources - a review. Food Science and Human Wellness 3, 117-126.

Nakamura, A., Kido, T., Seki, Y., Suka, M., 2024. Zinc deficiency affects insulin secretion and alters insulin-regulated metabolic signaling in rats. Journal of Trace Elements in Medicine and Biology 83, 127375.

Ofoe, R., Thomas, R.H., Asiedu, S.K., Wang-Pruski, G., Fofana, B., Abbey, L., 2023. Aluminum in plant: Benefits, toxicity and tolerance mechanisms. Frontiers in plant science, 13, 1085998.

Omena, J., Curioni, C., Cople-Rodrigues, C. dos S., Citelli, M., 2021. The effect of food and nutrients on iron overload: what do we know so far? European Journal of Clinical Nutrition 75(12), 1771-1780.

Otho, A.A., Memon, R.A., Abro, S.A., Memon, A.A., 2022. Elemental analysis of medicinal herb *Fagonia indica* Burm. f. and Its rhizospheric soil from six geographical locations of South-eastern Sindh Province, Pakistan, During Spring and Summer Seasons. Biological Trace Element Research 200(5), 2439-2454.

Rahim, N., Noor, A., Kanwal, A., Tahir, M.M., Yaqub, A., 2024. Assessment of heavy metal contamination in leafy vegetables: implications for public health and regulatory measures. Environmental Monitoring and Assessment 196(8), 684.

Raina, K., Kumar, D., Agarwal, R., 2016. Promise of bitter melon (*Momordica charantia*) bioactives in cancer prevention and therapy. Seminars in Cancer Biology 40-41.

Rayman, M.P., 2012. Selenium and human health. The Lancet 379(9822), 1256-1268.

Rehman, Z.U., Khan, S., Brusseau, M.L., Shah, M.T., 2017. Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. Chemosphere 168, 1589-1596.

Rodríguez-Morán, M., Guerrero-Romero, F., 2003. Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects: A randomized double-blind controlled trial. Diabetes Care 26(4), 1147-1152.

Saeed, F., Afzaal, M., Niaz, B., Arshad, M.U., Tufail, T., Hussain, M.B., Javed, A., 2018. Bitter melon (*Momordica charantia*): A natural healthy vegetable. International Journal of Food Properties 21(1), 1270-1290.

Schmidt, S.B., Husted, S., 2019. The biochemical properties of manganese in plants. Plants 8(10), 381.

Shagufta, Q., Shamim, G., Tariq, Z., Agha, W.Y., Raza U.K., Ali, A., Abdul, H.B., 2024. Influence of composted manures and co-composted biochar on growth performance of saffron and soil nutrients under varying electrical conductivity soil conditions: A two-year field study. Journal of Agriculture and Food Research 18, 101467.

Shahzad, B., Tanveer, M., Hassan, W., Shah, A.N., Anjum, S.A., Cheema, S.A., Ali, I., 2016. Lithium toxicity in plants: Reasons, mechanisms and remediation possibilities- a review. Plant Physiology and Biochemistry 107, 104-115.

Shandana, Khan, A., Waqas, M., Nawab, J., Idress, M., Kamran, M., Khan, S., 2024. Total arsenic contamination in soil, vegetables, and fruits and its potential health risks in the Chitral Valley, Pakistan. International Journal of Sediment Research 39(2), 257-265.

Silva, P.S.C., Francisconi, L.S., Gonçalves, R.D.M.R., 2016. Evaluation of major and trace elements in medicinal plants. Journal of the Brazilian Chemical Society 27(12), 2273-2289.

Sinha, V., Pakshirajan, K., Chaturvedi, R., 2018. Chromium tolerance, bioaccumulation and localization in plants: An overview. Journal of Environmental Management 206, 715-730.

Smereczański, N.M., Brzóska, M.M., 2023. Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: a comprehensive review of available data. International Journal of Molecular Sciences 24(9), 8413.

Titov, A.F., Kaznina, N.M., Karapetyan, T.A., Dorshakova, N.V., Tarasova, V.N., 2022. Role of selenium in plants, animals, and humans. Biology Bulletin Reviews 12(2), 189-200.

Vessman, J., Stefan, R.I., Van Staden, J.F., Danzer, K., Lindner, W., Burns, D.T., Fajgelj, A., Müller, H., 2001. Selectivity in analytical chemistry: (IUPAC Recommendations 2001). Pure and Applied Chemistry 73(8), 1391-1386.

Villarreal-La Torre, V.E., Guarniz, W.S., Silva-Correa, C., Cruzado-Razco, L., Siche, R., 2020. Antimicrobial activity and chemical composition of *Momordica Charantia*: A review. Pharmacognosy Journal 12(1), 213-222.

Wagan, F.A., Miano, T.F., Wagan, M.A., Channa, M.U.R., Channa, M.U.R., 2023. Comparison of bitter gourd

(*Momordica Charantia* L.) varieties under the influence of salt stress. Tropical Agroecosystems 4(1), 11-14.

Wang, P., Chen, H., Kopittke, P.M., Zhao, F.J., 2019. Cadmium contamination in agricultural soils of China and the impact on food safety. Environmental Pollution 249, 1038-1048.

White, P.J., 2013. Improving potassium acquisition and utilisation by crop plants. Journal of Plant Nutrition and Soil Science 176(3), 305-316.

Wu, Z.Z., Yang, J.Y., Zhang, Y.X., Wang, C.Q., Guo, S.S. and Yu, Y.Q., 2021. Growth responses, accumulation, translocation and distribution of vanadium in tobacco and its potential in phytoremediation. Ecotoxicology and Environmental Safety, 207, 111297.

Zhan, K., Ji, X., Luo, L., 2023. Recent progress in research on *Momordica charantia* polysaccharides: extraction, purification, structural characteristics and bioactivities. Chemical and Biological Technologies in Agriculture 10(58), 1-17.