

Mineral and Heavy Metal Profiling of Different Parts of the Insulin Plant (*Costus igneus*) Using ICP-MS

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ABSTRACT

Background: Medicinal plants and herb treatment have been emerging as a highly recommended method of disease management due to their therapeutic and nutritional properties. *Costus igneus*, the chosen plant, is commonly known as the Insulin plant in India, exhibiting antidiabetic, antioxidant, antimicrobial, and hepatoprotective properties. So far, most research has focused on identifying bioactive compounds and their biological effects of this plant, with little attention given to elemental analysis, which is critical for both nutritional value and safety. **Objective:** This study aimed to analyze the mineral and heavy metal composition of *C. igneus* using ICP-MS. We not only examine the presence of essential and nonessential elements but also find their distribution among different parts. **Materials and Methods:** Different parts of the insulin plant, including leaves, stems, and rhizomes, were collected, shade-dried, ground, and microwave-digested before undergoing ICP-MS analysis. **Results:** The maximum amounts of detected macroelements were K (1799.4 mg/100 g), Na (427.78 mg/100 g), Ca (1148.9 mg/100 g), and Mg (2131.4 mg/100 g). In contrast, maximum amounts of detected microelements were included Fe (263.00 mg/100 g), Mn (14.05 mg/100 g), Cu (2.86 mg/100 g), Zn (17.01 mg/100 g), Ni (0.67 mg/100 g), Se (0.23 mg/100 g), and Ag (0.006 mg/100 g). In addition, trace amounts of heavy metals As, Cd, Pb, Cr, Hg, and Sn were also detected. **Conclusions:** Results indicate that *C. igneus* could be a rich dietary source of essential minerals, complementing its known antidiabetic properties. Some of the heavy metals have been detected but remained below quantification limits, suggesting safety for human consumption. These findings would encourage researchers to find the correlation between mineral content and antidiabetic activity.

Keywords: Insulin Plant, *Costus igneus*, Macro- and Micro-Elements, Toxic Heavy Metals, ICP-MS.

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INTRODUCTION

Medicinal Plants (MPs) and herbs have long been recognized as a rich source of complex bioactive compounds. Historically, they have demonstrated a wide range of biological activities and significant therapeutic properties. The use of MP extract for treating and curing various diseases is among the oldest practices adopted by humans, as it contains phytochemicals such as alkaloids, flavonoids, coumarins, polysaccharides, phenols, tannins, and terpenes, as well as essential minerals (Rani *et al.*, 2023; Parvathy *et al.*, 2020; Spencer, 2008; Nostro *et al.*, 2000). The chosen medicinal plant, *Costus igneus* or *Costus pictus* or *Chamaecostus cuspidatus* (Figure 1) is commonly known as the insulin plant in India and belongs to the family of Costaceae. The plant leaves were mainly used for diabetes management (Shetty *et al.*, 2010). The recent review has highlighted its other properties,

such as antioxidant, antimicrobial, hepatoprotective, kidney stone-inhibitor, etc (Yadav *et al.* 2010; Devi *et al.*, 2010; Jothivel *et al.*, 2007). The phytochemical screening of *C. igneus* revealed that it is rich in protein, iron, ascorbic acid, -tocopherol, -carotene, terpenoids, steroids, saponins, flavonoids, etc (Hegde *et al.*, 2014; Vijaya *et al.*, 2022; George *et al.*, 2007). Apart from phytochemical content, medicinal plants and herbs can contain minerals, which are often essential for plant and human health, but also contain toxic heavy metals (Alawadhi *et al.*, 2024; Subramanian *et al.* 2012).

Today, consumers are increasingly conscious of their dietary choices. They often seek plant-based sources rich in nutrients with sufficient amounts of vital minerals. The growth of the mineral supplement industry reflects the rising consumer demand for mineral-rich products. Consequently, there is growing interest in identifying plant-based sources that not only possess medicinal properties but are also abundant in essential minerals.

The plant-based products play a vital role in traditional medicine systems. Many consumers mistakenly assume that they are safe to consume. However, plants can accumulate significant amounts of heavy metals when grown in contaminated soil (Hlihor *et al.*, 2022). This makes it essential to investigate the presence



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Figure 1: Photograph of the insulin plant (*Costus igneus*).

and concentration of undesirable metals in Medicinal Plants (MPs) used in traditional medicines and conventional foods. Previous studies have examined the mineral and heavy metal content in herbs, MPs, and vegetables (Aberoumand *et al.*, 2009; Miller-Cebert *et al.*, 2009; Tuzen *et al.*, 2007; Maiga *et al.*, 2005; Lavilla *et al.*, 1999). While certain heavy metals can provide specific health benefits at low concentrations, they can be toxic at elevated levels (Schuhmacher *et al.*, 1991). Therefore, quantifying these elements in commonly used medicinal plants is of critical importance.

Various analytical techniques have been employed for mineral profiling and heavy metal detection, including Flame Atomic Absorption Spectroscopy (FAAS) (Abou-Arab *et al.*, 2000), Energy Dispersive X-Ray Fluorescence (EDXRF) (Queralt *et al.*, 2005), Electrothermal Atomic Absorption Spectrometry (ETAAS) (Kalny *et al.*, 2007), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) (Borges *et al.*, 2017), Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICPOES) (Musa Özcan *et al.*, 2008; Bhat *et al.*, 2010; Ebrahim *et al.*, 2012), ICP-sector field-mass spectrometry (ICP-sf-MS), and hydride generation (HG)-ICP-OES (Ebrahim *et al.*, 2012), inductively coupled plasma mass spectrometry (ICP-MS) (Van Acker *et al.*, 2023; Arpadjan *et al.*, 2008), and Instrumental Neutron Activation Analysis (INAA) (Lokhande *et al.*, 2009).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) offers distinct advantages over other methods such as ICP-AES, flame atomic emission, FAAS, and GFAAS. The most significant advantage of ICP-MS is its ability to measure multiple elements simultaneously in a single analysis. In contrast, FAAS and GFAAS

require element-specific lamps, allowing only one or a few elements to be analyzed at a time. Additionally, ICP-MS provides very high sample throughput due to its short analysis time and minimal sample preparation requirements. In summary, ICP-MS is a highly efficient technique for multi-element determination at trace levels, delivering greater sensitivity than any other available method. The ICP-MS technique and its applications have been reviewed in references (Van Acker *et al.*, 2023; Nageswara *et al.*, 2007).

The composition and amounts of trace elements in MPs and herbs are crucial for human consumption since they affect both nutritional value and safety. Therefore, using highly sensitive analytical techniques is essential for determining multiple elements in MP samples. Chen *et al.*, (2022) reviewed the progress in ICP-MS analysis of minerals and heavy metals in traditional medicine. Potorti *et al.* (2020) used ICP-MS to investigate the concentrations of macro, micro, and heavy elements in spices and aromatic herbs. S. Tokalioglu (2012) employed ICP-MS in combination with multivariate analysis to quantify elements such as Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, and Pd in thirty medicinal herbs. Arpadjan *et al.* (2008) conducted quality control analysis for arsenic (As), cadmium (Cd), and lead (Pb) in medicinal herbs using ICP-MS and ETAAS techniques.

The elemental contents analysis in the studied plant (*Costus igneus*) was unreported. Additionally, no comparative analysis has been conducted on which parts of this plant contain what quantity of particular elements. In the present study, we aimed to determine the elemental contents, such as minerals and heavy metals, of the naturally grown medicinal plant insulin using ICP-MS. For comparative analysis, three parts of the studied plant were selected, including leaves, stem, and rhizomes. Therefore, this study not only examines the presence of essential and nonessential elements but also their distribution among different parts.

MATERIALS AND METHODS

Sample Collection

The insulin plant was collected from the central region of Gujarat, India. Various parts of the insulin plant, including leaves, stems, and rhizomes, were selected for the study. The samples were thoroughly washed with water, shadow-dried, and ground into a fine powder. The powdered plant material was stored at room temperature in airtight containers.

Reagents and Standards

Ultra-pure water from a Milli-Q purification system (Millipore Direct 8, Merck) was used to prepare all solutions. Standard analyte solutions were prepared from 1000 mg L⁻¹ stock solutions (Inorganic Ventures). All glassware was soaked in nitric acid for 24 hr after an initial rinse with ultra-pure water, and then thoroughly rinsed and rewashed before use. For calibration

curve preparation, concentrations in the range of 100-2000 ppb were used for the elements K, Na, Ca, Mg, and Mn, while concentrations of 50-1000 ppb were used for elements, including Fe, Mn, Cu, Zn, Ni, Se, Ag, As, Cd, Sn, Cr, Hg, and Pb.

Instrumentation

The mineral analysis was performed using an ICP-MS (Model - iCAPRQ, Thermo Fisher Scientific GmbH). A schematic diagram of the instrument is shown in Figure 2. The instrument comprises five basic components: (i) sample introduction system, (ii) inductively coupled plasma ion source, (iii) ion focusing optics, (iv) mass analyzer, and (v) ion detector. A brief overview of its working principle is given here.

Before analysis, the sample was prepared using the digestion method as described in the sample preparation section. The resulting solution was introduced into the nebulizer via a peristaltic pump, where it was converted into an aerosol with the aid of argon gas. This primary aerosol was then passed through a spray chamber, which removed larger droplets. Only the smaller droplets were allowed to enter the plasma source. Inside the plasma, these droplets underwent a series of sequential processes: desolvation, vaporization, atomization through molecular dissociation, and subsequent ionization of the atoms at high temperatures (approximately 10,000 K). Such elevated temperatures enable high ionization efficiency for most metals and metalloids. Ionization typically occurs within a residence time of 1 - 2 milliseconds.

Inductively Coupled Plasma (ICP) is generated at atmospheric pressure, after which plasma gas is introduced. This gas is drawn through the sampling cone orifice into an expansion chamber, where the pressure is maintained at approximately 1 mbar. At the vacuum interface, the gas undergoes supersonic expansion,

significantly increasing the average distance between particles and thereby reducing the probability of undesirable processes such as ion-electron recombination.

From the expansion chamber, a beam containing both charged and neutral species passed through the skimmer orifice and proceeded to the subsequent part of the instrument. Positive ions are then selectively attracted by an extraction lens, which separates them from negatively charged and neutral species via electrostatic forces. Additional ion optics guided the ion beam into the mass spectrometer, where it is bent by 90° and directed into a collision cell, followed by a quadrupole mass analyzer.

The collision cell, also known as the reaction cell, is a multipole-based ion guide filled with an inert gas such as helium (He). Molecular ions (e.g., ArCl^+) have a larger collision cross-section than their elemental isobars (e.g., As^+). As a result, the molecular ions collide more frequently with the He atom than their isobaric counterparts. By the time they exit the cell,

Table 1: Operating conditions for the ICP-MS instrument (Thermo iCAPRQ).

Parameter	Value
Forward power (RF)	1550 W
Nebulizer gas (Argon)	1.06 L/min
Auxiliary gas (Argon)	0.8 L/min
Cool gas flow (Argon)	14.0 L/min
CCT gas flow (He gas)	5.1 mL/min
CCT bias	21V
Sample uptake/wash time	60 sec
Dwell time	0.05 sec
Number of readings per sample	3 Main runs with 10 sweeps each

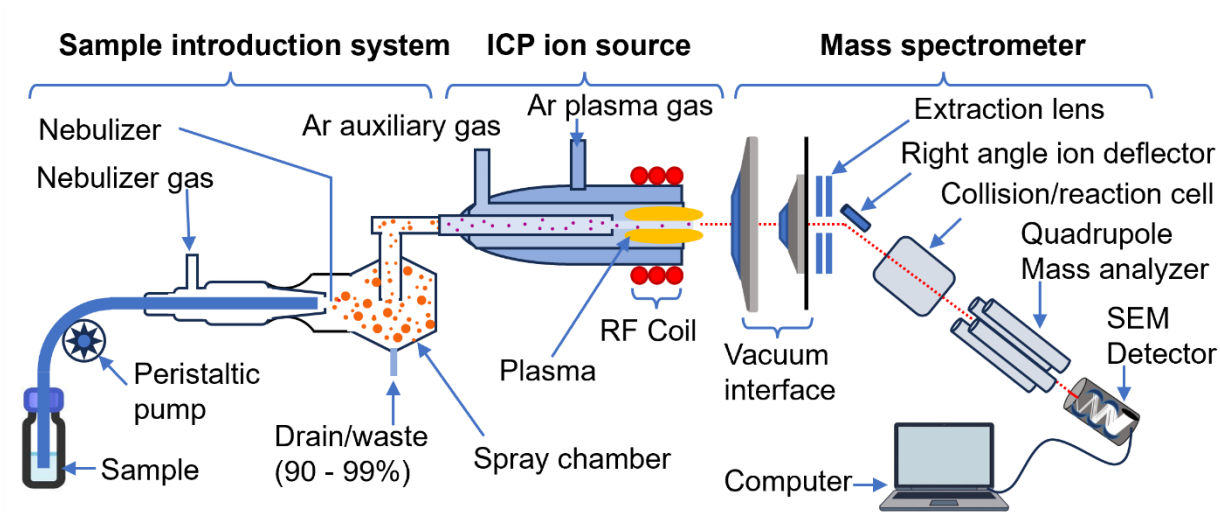


Figure 2: The schematic diagram of an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) containing five primary components: sample loading system, inductively coupled plasma ion source, the interface between the ion source and mass spectrometer, mass spectrometer, and detection system.

molecular ions have lower energy than the isobars. An energy barrier placed between the cell and the quadrupole prevents these low-energy molecular ions from entering the quadrupole. Only ions within the selected mass-to-charge (m/z) ratio window maintain a stable flight path through the quadrupole filter and ultimately reach the mass detector, while ions outside the selected m/z range are deflected out of the beam. The ions transferred by the mass spectrometer are detected using a dynode-based electron multiplier, which generates an electrical pulse for each ion striking its surface. These pulses are typically counted to quantify the analyte. The ICP-MS operating parameters are listed in Table 1. Elemental analysis was performed in triplicate under optimum conditions.

Sample Preparation

The microwave digestion method was employed to prepare the sample for ICP-MS analysis of minerals and heavy metals (Lavilla *et al.*, 1999). The sample preparation workflow is illustrated in Figure 3. Approximately 0.2 ± 0.005 g samples were weighed into a pre-cleaned, dry 50 mL microwave digestion vessel. For each recovery experiment, the sample was spiked with analytes before the addition of the extraction solvent. To stabilize mercury, 200 $\mu\text{g/L}$ of a gold solution was added. Subsequently, 2 mL of deionized water, 2 mL of HNO_3 , 1 mL of H_2O_2 , and 0.2 mL of

HCl were added. The mixture was allowed to stand for 10 minutes for pre-digestion in a fume hood.

After adding all components, the digestion vessels were sealed and placed in a microwave digester (Anton Paar GmbH, Austria) for digestion. Then, the digestion process was started with the temperature program mentioned in Table S1. Upon completion of digestion, the vessels were allowed to cool at room temperature for 15 minutes. Special care was taken when opening them, as pressurized acid fumes could be released. The vessels were gradually opened inside a fume hood. The digested solutions were then quantitatively transferred to a thoroughly cleaned 50 mL volumetric flask. Internal standards (10 mg/L of each element) were added, and the total volume was adjusted to 50 mL with deionized water, yielding a final concentration of 20 $\mu\text{g/L}$. The solutions were mixed thoroughly using a vortex mixer. A procedural blank solution was prepared in the same manner, omitting the sample matrix.

RESULTS

Minerals in the human diet are essential for the body's proper functioning. They participate in numerous metabolic processes and are required only in specific, well-defined quantities (Ceccanti *et al.*, 2021; Moodley *et al.* 2007). Therefore, their quantitative determination is essential. In this study, we analyzed

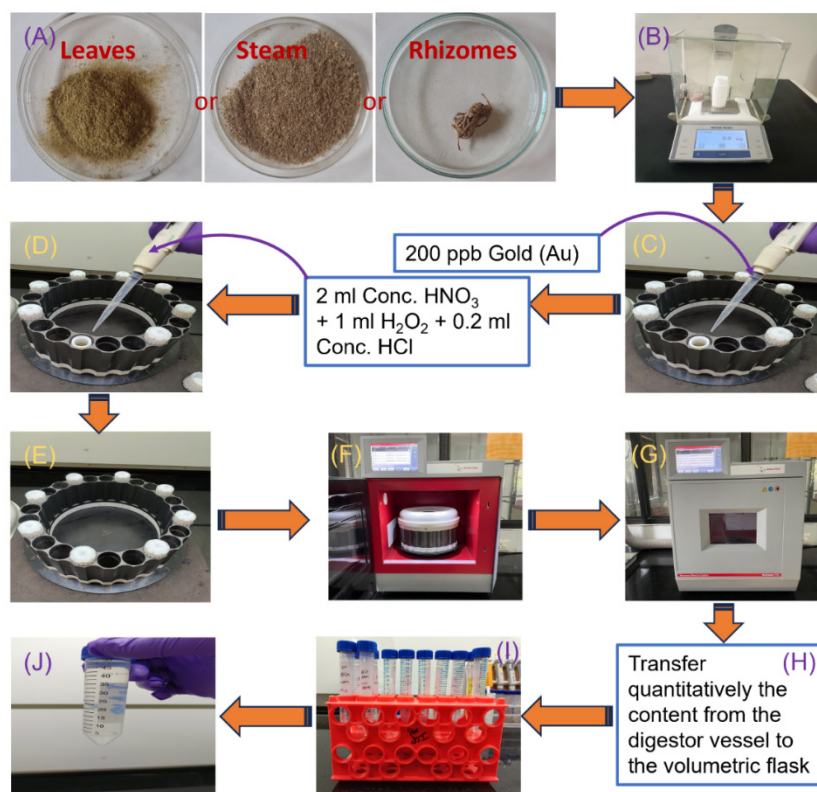


Figure 3: Flowchart of sample preparation for the ICP-MS study of insulin plant parts. (A) Powdered leaves or steam or un-crashed rhizomes, (B) weigh 0.2 g of any one component of (A) at a time, (C) treated plants part with 200 ppb gold solution, (D) then treated with HNO_3 , H_2O_2 , and HCl , (E) pre-digestion, (F) put vessels in the microwave, (G) start digestion process according to Table 2, (H) transfer from digester vessels to volumetric flasks, (I) added internal standard, and (J) finally store liquid sample after digestion in a volumetric flask.

macroelements, microelements, and heavy metals, and the results are presented in the following sections.

Potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), and phosphorus (P) are classified as macroelements (or macronutrients) due to their high concentrations in living organisms. In this study, we quantified the concentrations of four macroelements K, Na, Ca, and Mg, in the leaves, stem, and rhizomes of the insulin plant using ICP-MS. The results are presented in Table 2 and Figure 3, and the calibration curves for these elements are provided in the supplementary materials (Figure S1).

Essential trace metals are required for health in small amounts, but excessive intake can cause harm. The elements Fe, Mn, Cu, Zn, Ni, Se, and Ag are classified as microelements (or micronutrients). Except for silver, all are essential for human health and biological processes, though they can become toxic if their concentrations exceed recommended limits. In this study, the levels of these microelements in different parts of the insulin plant were analyzed, with results presented in Table 3. Calibration

curves of these microelements are provided in the supplementary materials (Figure S2). Overall, the microelements were detected in moderate amounts.

Toxic heavy metals have no beneficial role and can cause severe illness or death even at low levels. Tracing these metals is crucial for ensuring product quality, maintaining food safety, monitoring environmental contamination, and assessing human health risks. In this study, heavy metals such as As, Cd, Pb, Cr, Hg, and Sn were analyzed in insulin plant parts, with results presented in Table 4. The calibration curves for these metals are shown in the supplementary materials (Figure S3).

DISCUSSION

Macroelements

Macroelements are the elements that the body needs in larger quantities; examples include K, Na, Ca, Mg, P, etc. Among these elements, potassium (K) is the most prevalent ion in intracellular fluid and is essential for maintaining cell function. A potassium deficiency is associated with conditions such as hypertension and

Table 2: Quantification of macroelements present in insulin plant parts using ICP-MS. Each value is the Mean \pm Standard Deviation of triplicate.

Macroelement	Leaves	Stem	Rhizomes
K (in mg/100 g)	1246.6 \pm 35.1	741.65 \pm 17.05	1799.4 \pm 38.6
Na (in mg/100 g)	29.09 \pm 0.93	427.78 \pm 7.85	23.49 \pm 0.40
Ca (in mg/100 g)	1148.9 \pm 29.3	392.19 \pm 17.05	450.37 \pm 38.59
Mg (in mg/100 g)	905.12 \pm 28.62	2131.4 \pm 51.7	751.27 \pm 14.79

*A bold mark indicates the highest concentration of the element among the plant parts.

Table 3: Quantification of microelements present in the insulin plant using ICP-MS. Each value is the Mean \pm Standard Deviation of triplicate.

Microelement	Leaves	Stem	Rhizomes
Fe (in mg/100 g)	55.80 \pm 1.48	263.00 \pm 7.70	41.96 \pm 0.74
Mn (in mg/100 g)	7.91 \pm 0.15	14.05 \pm 0.33	3.22 \pm 0.07
Cu (in mg/100 g)	1.11 \pm 0.02	0.70 \pm 0.02	2.86 \pm 0.05
Zn (in mg/100 g)	2.02 \pm 0.04	17.01 \pm 0.25	4.77 \pm 0.18
Ni (in mg/100 g)	0.21 \pm 0.002	0.67 \pm 0.02	0.14 \pm 0.0002
Se (in mg/100 g)	0.03 \pm 0.004	0.23 \pm 0.02	0.018 \pm 0.002
Ag (in mg/100 g)	0.006 \pm 0.0002	0.002 \pm 0.0002	0.001 \pm 0.0001

*A bold mark indicates the highest concentration of the element among the plant parts.

Table 4: Quantification of heavy metals present in insulin plant parts using ICP-MS. Each value is the Mean \pm Standard Deviation of triplicate.

Heavy metal	Leaves	Stem	Rhizomes
As (in mg/100 g)	0.01 \pm 0.0001	0.04 \pm 0.001	0.004 \pm 0.001
Cd (in mg/100 g)	0.003 \pm 0.0004	0.008 \pm 0.0002	0.001 \pm 0.0001
Pb (in mg/100 g)	0.22 \pm 0.002	0.075 \pm 0.002	0.03 \pm 0.0003
Cr (in mg/100 g)	0.75 \pm 0.01	0.62 \pm 0.02	0.38 \pm 0.01
Sn (in mg/100 g)	0.079 \pm 0.002	0.009 \pm 0.0004	0.17 \pm 0.002
Hg (in mg/100 g)	ND	ND	ND

*A bold mark indicates the highest concentration of the element among the plant parts.

cardiovascular disease (Stone *et al.*, 2016). The K content in Idaho potatoes, bananas, and avocados exceeds 700 mg/100 g, while seeds and nuts contain significantly higher levels (2240 mg/100 g). Nutraceutical-rich plants, such as Piper longum, include as much as 1352.3 mg/100g (Bhat *et al.*, 2010). The recommended daily intake of potassium is 3500 mg. In the insulin plant, potassium exhibited the highest concentration in the leaves and rhizomes (Table 2). The lowest level (741.65 mg/100 g) was recorded in the stem, while the highest level (1799.4 mg/100 g) was found in the rhizomes.

Sodium (Na) is the dominant ion in extracellular fluid. Both Na and K play crucial roles in maintaining cellular homeostasis (Pohl *et al.*, 2013). Sodium levels in vegetables and fruit juices are generally low, ranging from 2.28 to 94.0 mg/100 g and 0.04 to 227 mg/100 g, respectively (Szefer *et al.*, 2026). In the nutraceutical-valued plant *Anethum Sowa*, sodium content was reported as 170.69 mg/100 g (Bhat *et al.*, 2010). The recommended daily intake of Na is 2400 mg. Deficiency is rare but can occur due to diarrhea, vomiting, or excessive sweating, and may lead to symptoms such as nausea, dizziness, muscle weakness, and poor concentration (Smith *et al.*, 2000). In the insulin plant, sodium content varied among parts, with the lowest concentration (23.49 mg/100 g) in rhizomes and the highest (427.78 mg/100 g) in the stem.

Calcium (Ca) is vital for numerous physiological processes, including maintaining a healthy skeletal system and the structure of cell organelles. It also plays a role in fluid homeostasis (Wawrzyniak *et al.*, 2021). Calcium deficiency can lead to osteoporosis, a condition that is more prevalent in underdeveloped countries (Rojas-Molina *et al.*, 2015). In plant-based foods, calcium levels vary widely, from approximately 8.7 mg/100 g in apples, green peppers, and potatoes to 100 mg/100 g in broccoli and 600 mg/100 g in spinach (Martínez-Ballesta *et al.*, 2010). In the nutraceutical-valued plant *Artemisia vulgaris*, calcium content reaches 3441.8 mg/100 g (Bhat *et al.*, 2010). The recommended daily intake is 800 - 1300 mg. In the insulin plant, calcium was highest in leaves, moderate in rhizomes, and lowest in stem (Table 2), ranging from 392.19 mg/100 g in the stem to 1148.9 mg/100 g in leaves. Calcium intake is commonly associated with dairy products such as milk, cheese, yogurt, etc (Cormick *et al.*, 2019). Notably, calcium levels in the insulin plant are comparable to those found in calcium-rich vegetables, such as broccoli, watercress, and kale, making it a valuable calcium source in regions where dairy products are not readily available.

Magnesium (Mg) is one of the abundant intracellular ions and plays a role in muscle relaxation and enhanced muscle function (de Baaij *et al.*, 2015). Vegetable-based foods generally have higher magnesium content. Magnesium levels in vegetables and fruits range from 5.5 to 191 mg/100 g, while the nutraceutical-valued plant *Momordica charantia* contains 236.72 mg/100 g (Bhat *et al.*, 2010). The recommended daily intake range is 200 - 400 mg.

In the insulin plant, magnesium concentration is considerably higher than in common edible plants (Ceccanti *et al.*, 2021; Disciglio *et al.*, 2017), ranging from 751.27 mg/100 g in rhizomes to 2131.4 mg/100 g in the stem.

Overall, macroelement content varied among the different parts of the insulin plant. As expected, potassium, calcium, sodium, and magnesium were the dominant elements.

Microelements

Microelements are those that are required in trace amounts, including Fe, Mn, Cu, Zn, Ni, and Se, among others. Iron (Fe) plays a vital role in various biological functions, including oxygen and electron transport, red blood cell production, and DNA synthesis (Abbaspour *et al.*, 2014). It is a key component of hemoglobin, myoglobin, cytochrome, heme proteins, and various enzymes (Bhat *et al.*, 2010; Hemalatha *et al.*, 2007). In vegetables and fruits, Fe content ranges from 0.13 - 3.01 mg/100 g, while nuts (16.1 mg/100 g) and cocoa powder (25.8 mg/100 g) are rich sources. Exceptionally high values have been reported in *Artemisia vulgaris* (357.1 mg/100 g) (Bhat *et al.*, 2010) and certain medicinal herbs (345.6 mg/100 g) (Tokaloğlu 2012). The Recommended Daily Intake (RDI) for Fe is 8 - 18 mg. In the insulin plant parts, Fe ranged from 41.96 mg/100 g (rhizomes) to 263.00 mg/100 g (stem).

Manganese (Mn) acts as a cofactor for many enzymes involved in carbohydrate metabolism and gluconeogenesis (Huskisson *et al.*, 2007). Fruits and vegetables typically contain low amounts of Mn, 0.01 - 0.078 mg/100 g and 0.01 - 0.66 mg/100 g, respectively (Szefer *et al.*, 2006). Higher amounts are found in the medicinal and edible plants *Hibiscus sabdariffa* flowers (24.3 mg/100 g) (Maiga *et al.*, 2005), nutraceutical-valued plant *Rauwolfia serpentina* (856.4 mg/100 g) (Bhat *et al.*, 2010), and in medicinal herb cinnamon (26.4 mg/100 g) (Tokaloğlu 2012). The RDI for Mn is about 2 mg, and deficiencies are rare. However, a shortage of it may lead to a reduction in cholesterol, red blood cells, and mucopolysaccharide irregularities. In the parts of the insulin plant, Mn content ranged from 3.22 mg/100 g (rhizomes) to 14.05 mg/100 g (Stem).

Copper (Cu) is essential for enzyme activity, connective tissue formation, and nerve health (Shenkin 2008). In vegetables, Cu content ranges from 0.004 to 0.24 mg/100 g, with legumes containing up to 0.5 mg/100 g and fruits 0.01 to 0.24 mg/100 g (Szefer *et al.*, 2006). High levels occur in nutraceutical-valued plant *Berberis aristata* (13.96 mg/100 g) (Bhat *et al.*, 2010) and medicinal herb *basil* (3.02 mg/100 g) (Tokaloğlu 2012). The RDI for Cu is 1.0 - 1.6 mg. In the insulin plants, Cu ranged from 0.70 mg/100 g (stem) to 2.86 mg/100 g (rhizomes).

Zinc (Zn) is a component of hundreds of enzymes, many of which are involved in energy metabolism. It plays a role in DNA-binding zinc finger proteins, hemoglobin, myoglobin, and cytochromes

Table S1: The temperature program used in AntonPaar microwave digester for the digestion of insulin plant parts.

Ramp time (min)	Hold time (min)	Temperature (°C)	Power (W)
40	30	195	

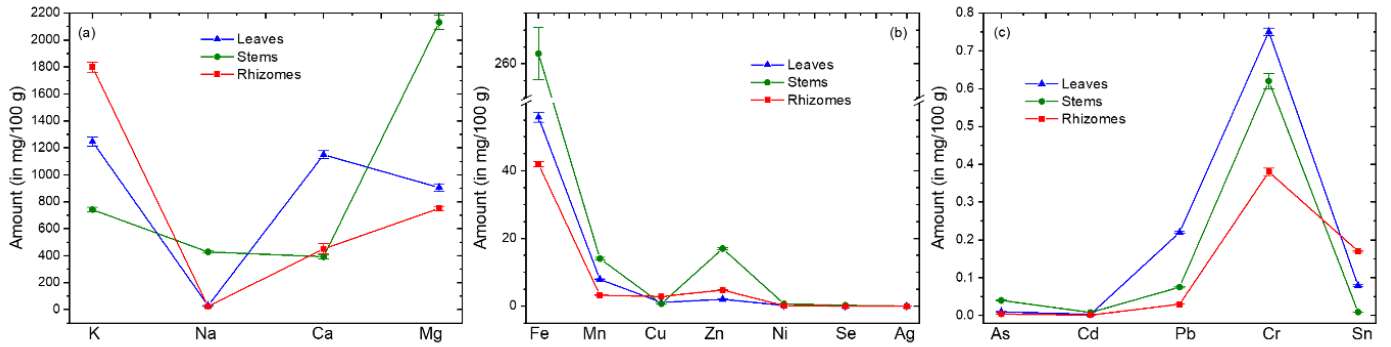


Figure 4: Concentration profile of macroelements (a), microelements (b), and heavy metals (c) among the insulin plant part leaves (blue), stem (green), and rhizomes (red). The error bars indicating measurement uncertainties based on triple replicates.

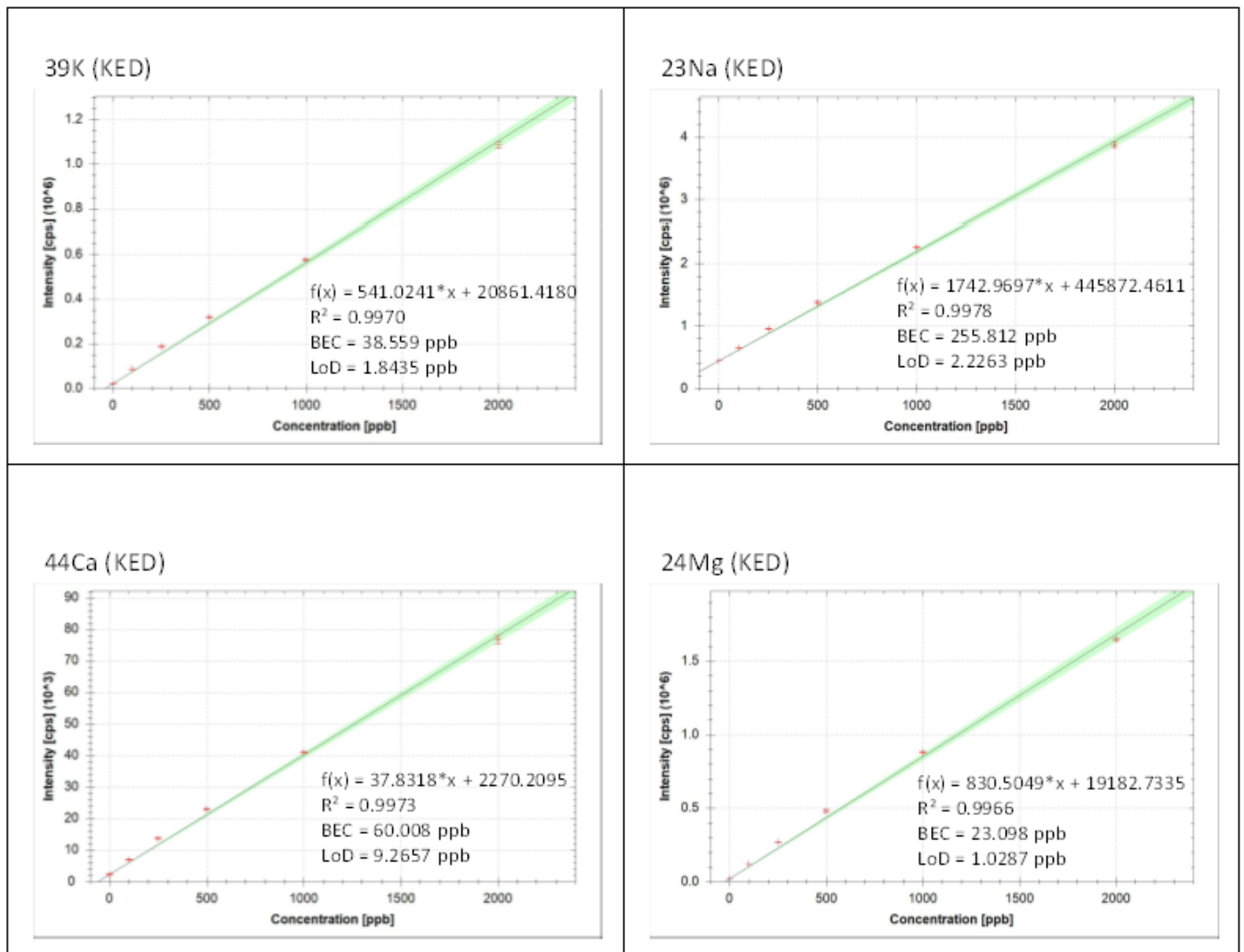


Figure S1: Calibration curve for the microelements K, Na, Ca, and Mg.

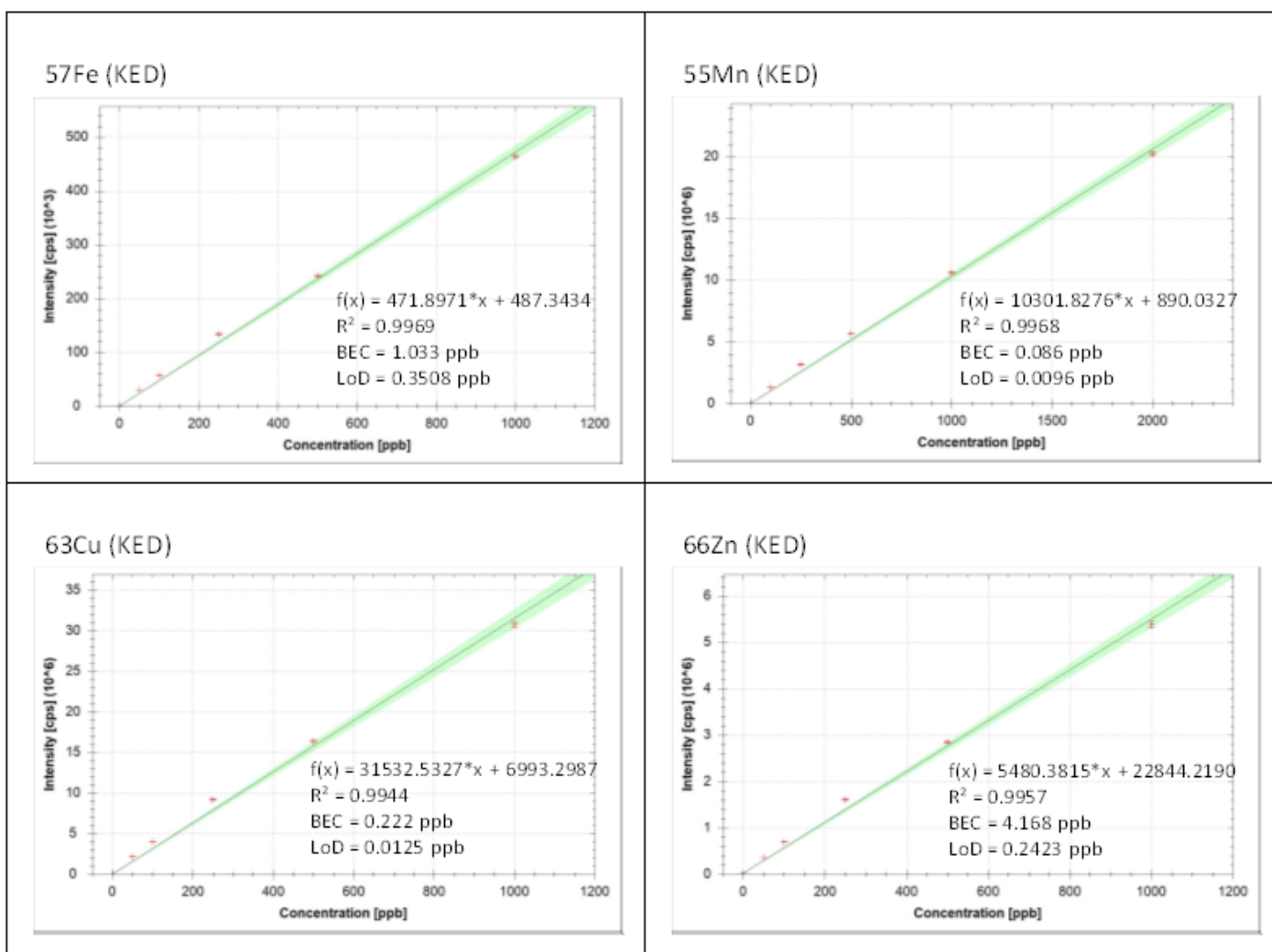
(Shenkin 2008). Zn levels in plant-based foods range from 0.05 - 11.8 mg/100 g, while fruit juices and beverages typically contain 0.01 - 0.27 mg/100 g (Szefer *et al.*, 2006). Moderate to high levels are reported in nutraceutical-valued plant *Hemidesmus indicus* (13.96 mg/100 g) (Bhat *et al.*, 2010), medicinal herb mistletoe (3.02 mg/100 g) (Tokaloğlu 2012), and medicinal and edible plant *B. costatum* flowers (6.7 mg/100 g) (Maiga 2005). The RDI for Zn intake is 8 - 11 mg. In the insulin plant, a lower amount (2.02 mg/100 g) of Zn was measured in leaves; whereas a higher amount (17.01 mg/100 g) was measured in the stem.

Nickel (Ni) participates in biochemical processes, though its precise biological role is not fully understood. It is often included in supplements to prevent blood deficiency (*NICKEL*). Ni levels in vegetables ranged from 5×10^{-4} - 0.28 mg/100 g, and in fruits from <0.004 to 0.05 mg/100 g (Szefer *et al.*, 2006). High amounts occur in *Momordica charantia*, 3.86 mg/100 g (Bhat *et al.*, 2010) and Nettle (1.31 mg/100 g) (Tokaloğlu 2012). Moderate levels of Ni were found in reddish orange (0.776 mg/100 g), dill (0.759 mg/100 g), and basil (0.664 mg/100 g) samples (Tokaloğlu 2012). The recommended intake is 0.302 - 0.735mg/day (Roychowdhury *et al.*, 2003), and excessive consumption can cause allergic

reactions or eczema. The WHO-recommended limit for Ni in foods is 0.1 mg/100 g (Haider *et al.*, 2004). In the insulin plants, Ni ranged from 0.14 mg/100 g (rhizomes) to 0.67 mg/100 g (stem), which is close to the recommended intake.

Selenium (Se) is a key component of selenoproteins (Guerrero-Romero *et al.*, 2005) and is essential for thyroid and immune function (Szefer *et al.*, 2006). It also plays a protective role against certain types of cancer (Combs *et al.*, 2004). Se deficiency can cause heart disease and hypothyroidism (Guerrero-Romero *et al.*, 2005), while excess intake may lead to gastrointestinal distress, hair loss, fatigue, and nerve damage. Plant-based foods typically contain 10^{-4} - 0.06 mg/100 g (Szefer *et al.*, 2006). The recommended daily intake is ~ 17 g. In the insulin plants, the measured Se ranged from 0.018 mg/100 g (rhizomes) to 0.23 mg/100 g (stem).

Silver (Ag) is not essential for human health but has antimicrobial properties (Sim *et al.*, 2018). It is used in wound dressings and medical devices to reduce infections. No RDI exists for Ag, although the estimated daily intake via food is 20-80 g (De Matteis 2017). The highest Ag content is reported in *Artemisia dracunculus* (0.063 mg/100 g) and the lowest in *Pimpinella*



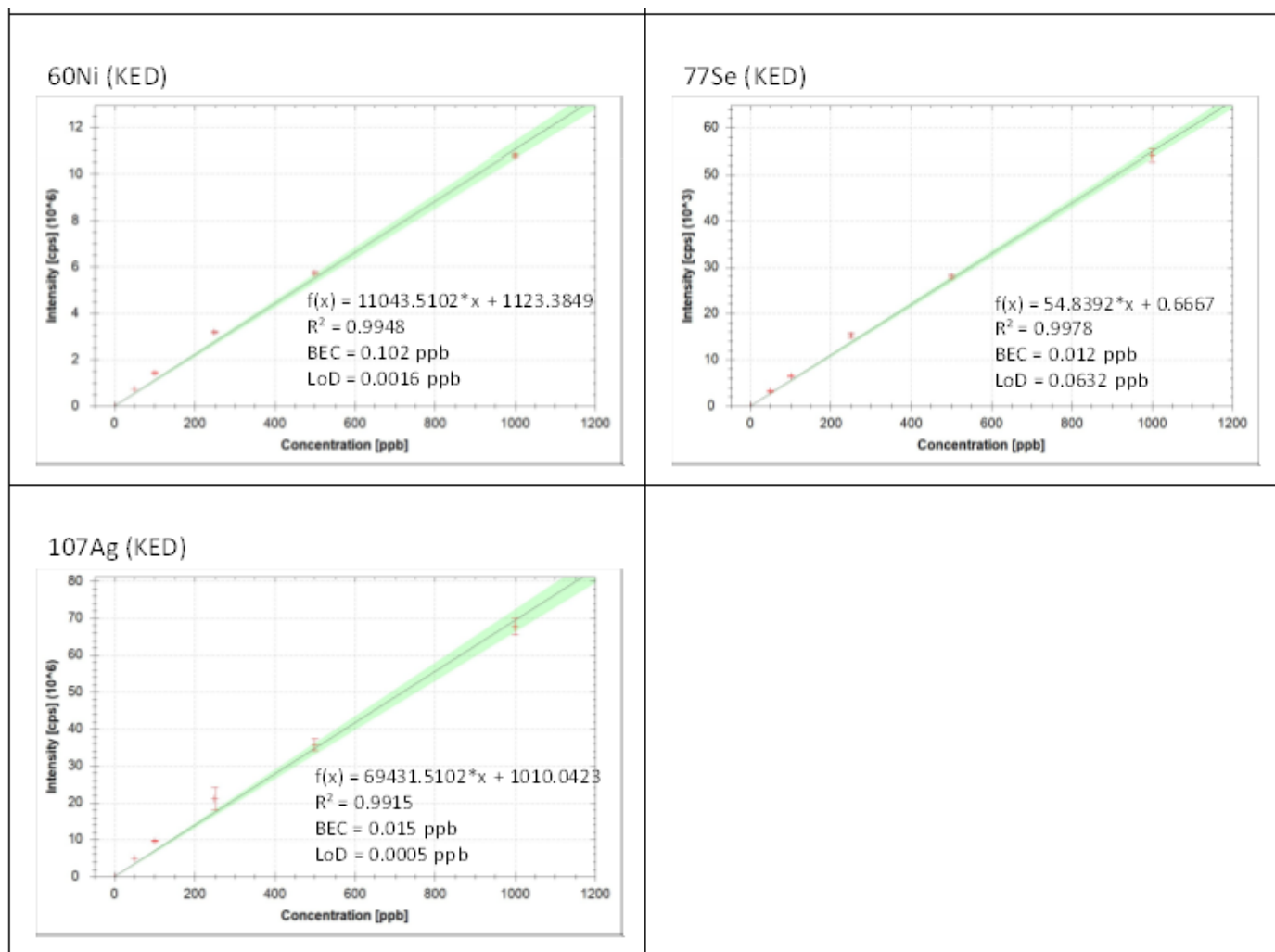


Figure S2: Calibration curve for the microelements Fe, Mn, Cu, Zn, Ni, Se, and Ag.

anisum and *Ocimum basilicum* (0.001 mg/100 g) (Musa Özcan *et al.*, 2008). In the insulin plants, Ag ranged from 0.001 mg/100 g (rhizomes) to 0.006 mg/100 g (leaves).

Overall, Table 3 shows that Fe, Zn, and Mn occur at comparatively higher levels in insulin plants, whereas Cu, Ni, Se, and Ag are present in lower amounts.

Heavy metals

The toxicity and health effects of several heavy metals, including As, Pb, Hg, and Cr, have been reported in the literature (Jaishankar *et al.*, 2014). However, only a few studies have addressed the speciation of toxic heavy metals in herbs (Sun *et al.*, 2003; Mattusch *et al.*, 2000) and medicinal plants (Arpadjan *et al.*, 2008). The concentration of heavy metals in medicinal plants used for health products is crucial, as it can serve as an indicator of environmental pollution (Jaishankar *et al.*, 2014). Additionally, direct consumption of these metals can have harmful effects (Briffa *et al.*, 2020). Several factors influence the existence of heavy metals in the environment: (i) emissions from industrial and vehicular activities, (ii) sludge purification processes, (iii)

agricultural practices such as the use of Cd-containing manure, (iv) application of organic fungicides containing mercury, and (v) insecticides containing Pd and As.

Arsenic (As) is recognized as a toxic and carcinogenic element. Human exposure to as occurs through air, food, and water. In medicinal plants, As content has been reported in the range of 1.2×10^{-3} to 0.0225 mg/100 g (Arpadjan *et al.*, 2008). The FAO/WHO has set the safe weekly intake limit for as at 0.015 mg/kg body weight and the WHO guidelines for drinking water specify that as should not exceed 10 g/L. In nutraceutical plants, As levels range from 1.01 to 53.1 mg/100 g (Bhat *et al.*, 2010). The present study found significantly lower as concentration (0.004 - 0.04 mg/100 g) in the insulin plant parts.

Cadmium (Cd) is a highly toxic heavy metal known for its detrimental effect on cellular enzymatic systems. The FAO/WHO has set a safe weekly intake for Cd at 0.007 mg/kg body weight. Due to its high soil-to-plant transfer rate, Cd is commonly found in fruits and vegetables. In medicinal plants, Cd concentrations range from 1.5×10^{-3} to 0.0268 mg/100 g (Arpadjan *et al.*, 2008). In the insulin plants, Cd levels ranged from 0.001 to 0.008 mg/100

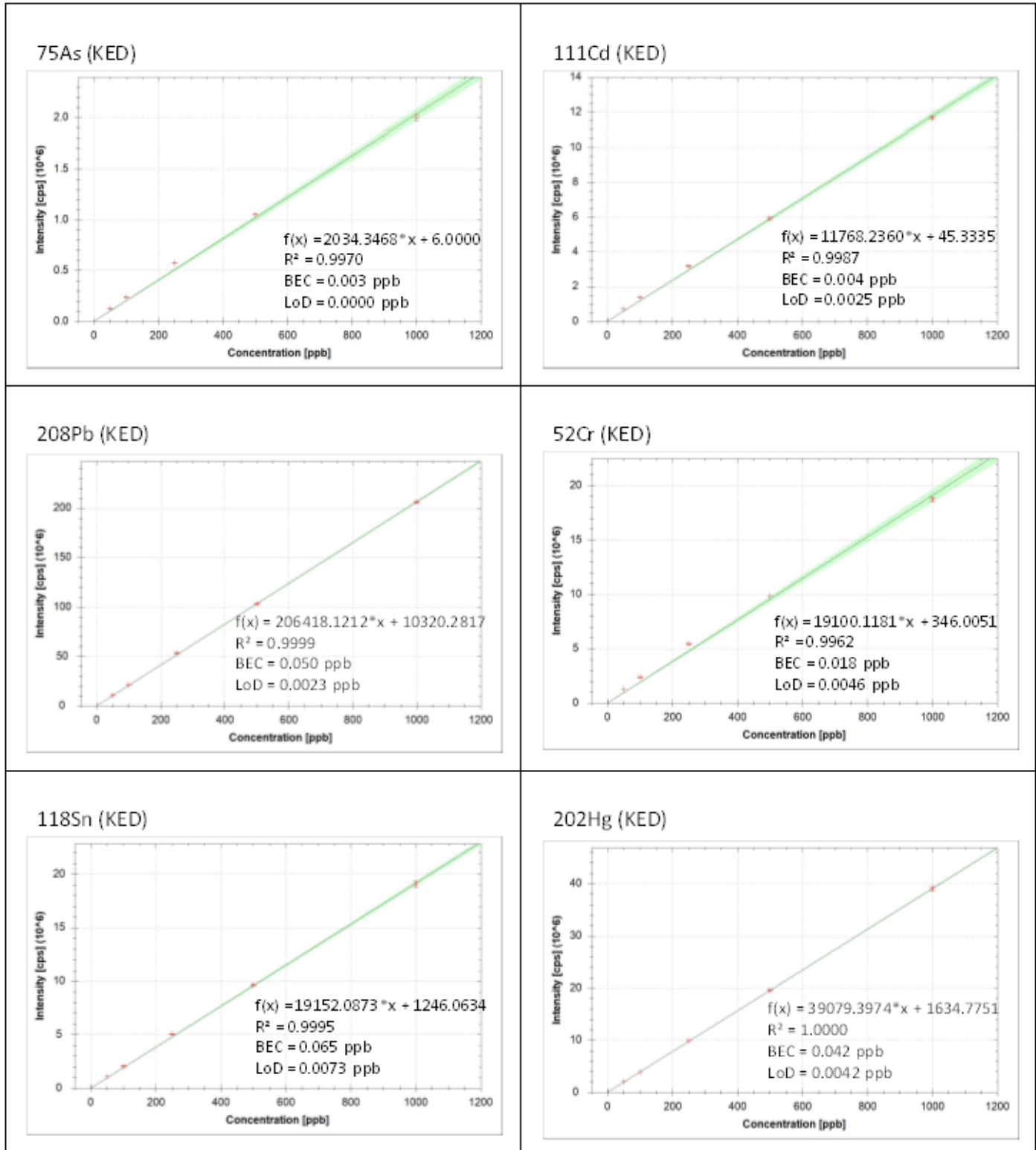


Figure S3: Calibration curve for heavy metals As, Cd, Pb, Cr, Sn, and Hg.

g, while in some medicinal and edible plants, Cd was below the detection limit (Maiga *et al.*, 2005).

Lead (Pb) is another highly toxic element. Significant sources of Pb exposure include industrial activities, food, smoking, drinking water, gasoline, and house paints (Jaishankar *et al.*, 2014). The FAO/WHO has set the safe weekly intake for Pb at 0.025 mg/kg body weight. Pb concentration in some medicinal plants ranges from 0.2×10^{-4} to 8.6×10^{-4} mg/100 g (Arpadjan *et al.*, 2008), while ginger has been reported to contain as much as 0.301 mg/100 g (Tokaloğlu 2012). In insulin plants, Pb level ranged from 0.03 to 0.22 mg/100 g.

Chromium (Cr) exhibits a contrasting health impact depending on its oxidation state. Hexavalent chromium (Cr⁶⁺) is highly toxic and carcinogenic. In contrast, trivalent chromium (Cr³⁺) is an essential nutrient involved in glucose metabolism. In medicinal and edible plants, Cr levels range from 0.07 to 1.72 mg/100 g (Maiga *et al.*, 2005); in nutraceutical plants, from 0.008 to 0.674 mg/100 g (Bhat *et al.*, 2010); and in medicinal herbs, from 0.044 to 0.874 mg/100 g (Tokaloğlu 2012). In the insulin plants, Cr levels ranged 0.38 - 0.75 mg/100 g, which is lower than in *B. costatum* flowers (1.72 mg/100 g) (Maiga *et al.*, 2005) and Nettle (0.871 mg/100 g) (Tokaloğlu 2012).

Tin (Sn) is a nonessential element classified as potentially toxic. While it plays a significant role in dental health and is found in some foods and supplements, excessive exposure to inorganic Sn compounds can cause gastrointestinal and other health issues (Dopp *et al.*, 2013). In medicinal plants, Sn levels range from 0.2×10^4 to 8.45×10^2 mg/100 g (Ebrahim *et al.*, 2012), while in vegetables, they range from 0.471 to 1.086 mg/100 g (Mellawati *et al.*, 2023). In insulin plants, Sn concentrations are relatively low, ranging from 0.009 to 0.17 mg/100 g.

Mercury (Hg) is toxic and highly bioaccumulative, posing significant threats to the environment, particularly marine ecosystems. Primary sources of Hg pollution include agriculture, municipal wastewater, mining, incineration, and industrial effluents. In most foods and beverages, Hg levels range from $<1 \times 10^4$ to 50×10^4 mg/100 g. Among nutraceutical plants, only *V. zizinalis* has been reported to contain Hg at 3.6 mg/100 g (Bhat *et al.*, 2010). In the present study, Hg was below the quantification limit in all insulin plant parts (Table 4).

Overall, the results in Table 4 indicate that the concentrations of heavy metals in insulin plants were below the quantification limits, suggesting that the insulin plant is safe for consumption as part of a health-beneficial diet.

Comparison and future scope of study

The amounts of macroelements, microelements, and heavy metals in the various parts of the insulin plant are compared in Figures 4(a-b). This figure revealed that the macroelements, microelements, and heavy metals present in the insulin plants

were within the maximum limit of 2200 mg/100 g, 260 mg/100 g, and 1 mg/100 g, respectively. This assessment helps choose or avoid a particular plant part according to its requirements and in considering it as a source of essential elements.

The finding of essential minerals in insulin plant parts opens up several important research directions and applications in both basic science and the pharmaceutical field. One can consider correlating mineral content with antidiabetic activity, as some minerals, such as zinc (Zn) and chromium (Cr), are already known to play roles in insulin synthesis and glucose metabolism. It can help in developing a nutraceutical profile for safe dietary use. From a mechanistic study point of view, one can study synergistic effects of minerals and phytoconstituents on glucose uptake, insulin secretion, or oxidative stress reduction. From the pharmacological and clinical research direction, there are multiple scopes. First, one can consider designing *in vivo* and clinical studies to investigate how mineral-rich extracts affect blood sugar regulation, lipid metabolism, or antioxidant status. Secondly, one can compare whole plant extracts vs mineral-removed extracts to pinpoint their contribution to therapeutic effects. From an agricultural and biotechnological perspective, one can explore soil-plant interactions to determine whether mineral uptake can be optimized through soil enrichment or hydroponics, thereby enhancing the medicinal value of plants. In terms of product development, formulating MP/herbal supplements, functional foods, or teas enriched with specific minerals derived from plants.

CONCLUSION

We successfully conducted mineral profiling of various parts of the insulin plant, including leaves, stem, and rhizomes, using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the first time. The results revealed notably high concentrations of essential macronutrient elements, such as K, Ca, and Mg, across the plant parts. In contrast, microelements (Fe, Mn, Cu, Zn, Ni, etc.) were present at relatively lower levels. This study highlights that the insulin plant is a rich and cost-effective source of essential minerals, which can contribute to improving dietary intake of both macro- and micro-elements. Furthermore, the plant shows potential in addressing mineral deficiencies and combating nutrient shortages caused by modern nutritional patterns, either through dietary supplements or food biofortification. Although some heavy metals, such as As, Cd, Cr, Pd, Sn, and Hg, have been found in the insulin plant, their levels are below quantification limits, which provides confidence in consuming these plant parts without concern.

While the insulin plant is widely recognized for its role in diabetes management, our findings underscore its broader nutritional value. This study can help consumers identify the specific parts that best meet their needs. Additionally, it will aid the nutritional and Ayurvedic production industries in creating plant-based supplements for essential elements. Furthermore, ICP-MS is an

effective tool for precise elemental analysis, supporting accurate evaluation of the nutritional and safety profiles of medicinal plants.

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CONFLICTS OF INTEREST

There are no conflicts to declare.

AUTHOR CONTRIBUTIONS

All authors contributed to the project design and conceptualization. Maharshi Bhatt prepared materials, collected and analyzed data, and wrote the first draft of the manuscript. Dr. Prasanta Das supervised the project and reviewed and edited the manuscript.

DATA AVAILABILITY

The data for this article are presented in the main text in a table and figure format. The data supporting this article have been included as part of the Supplementary Information.

ABBREVIATIONS

MPs: Medicinal Plants; **FAAS:** Flame Atomic Absorption Spectroscopy; **EDXRF:** Energy Dispersive X-Ray Fluorescence; **ETAAS:** Electrothermal Atomic Absorption Spectrometry; **GFAAS:** Graphite Furnace Atomic Absorption Spectroscopy; **ICP-AES:** Inductively Coupled Plasma-Atomic Emission Spectroscopy; **ICP-OES:** Inductively Coupled Plasma-Optical Emission Spectroscopy; **ICP-sf-MS:** ICP-Sector Field-Mass Spectrometry; **(HG)-ICP-OES:** Hydride Generation Inductively Coupled Plasma-Optical Emission Spectroscopy; **ICP-MS:** Inductively Coupled Plasma Mass Spectroscopy; **INAA:** Instrumental Neutron Activation Analysis; **ND:** Not Detected.

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SUMMARY

In this study, the mineral and heavy metal composition of different parts of the insulin plant (*C. igneus*), including leaves, stems, and rhizomes, was analyzed using ICP-MS. The investigation

not only identified essential and nonessential elements but also assessed their distribution across plant parts. The findings suggest that *C. igneus* is a rich dietary source of essential minerals, complementing its well-documented antidiabetic properties. Although some heavy metals were detected, their levels remained below quantification limits, indicating safety for human consumption. These results provide valuable insights for pharmaceutical companies in formulating herbal supplements and developing a nutraceutical profile for safe dietary use. Furthermore, they may support the development of novel herbal drugs that combine essential minerals with antidiabetic benefits.

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