**Mineral and Heavy metal analysis of *Raphanus sativus* L. microgreen grown in soil of eight different districts of West Bengal**

Shubhadeep Hazra1, Amitesh Chakraborty1, Santanu Giri1, Aniruddha Sarkar1, Tushar Adhikari1\*

1 Department of Pharmaceutical Chemistry, Guru Nanak Institute of Pharmaceutical Science and Technology; 157/F Nilgunj Road, Sodepur, Kolkata 700114.

\*Email ID: [tushar.adhikari2022@gnipst.ac.in](mailto:tushar.adhikari2022@gnipst.ac.in)

**ABSTRACT**

Microgreens have emerged as potent ‘functional food’ or ‘superfood’ in 21st century. Bioactive compounds and biological activities of microgreens have been explored, but little is known about the mineral profiling. This research aims in comparison of element profile of Radish microgreen grown in soils of different districts of West Bengal. The radish microgreens were cultivated and the mineral content (Ca, Mn, Zn, Se), heavy metal (Cr, Cd, Pb) were analysed using Flame Atomic Absorption Spectrometer. Significant variations (p<0.05) in mineral profile were found in radish microgreens grown in soils of different districts. Major elements determined were Calcium (109.14±2.18 to 148.11±1.28 mg/100g); zinc (4.42±0.04 to 15.89±0.01mg/100g); manganese (6.62±0.02 to 8.37±0.00mg/100g) and Selenium (0.030.01 to 0.0980.02 to mg/100g). South 24 Parganas recorded exceptionally high zinc content in grown microgreens. Chromium concentration was below the toxic limits. Lead and Cadmium was found in negligible amount. Hazard index of microgreen grown in all districts were less than 1. Mostly all investigated microgreens were good sources of mineral with best profile in Purba Medinipur and Hoogly indicating alkaline and saline soil are best for growth. Based on the obtained result, these microgreens can act as dietary supplements for daily mineral intake in future.

**Keywords:** Radish, Microgreen, agroclimatic, Atomic Absorption Spectroscopy, Mineral, Heavy metal.

1. **INTRODUCTION**

Nature is the greatest source of remedies to manage and prevent progression of different diseases with 80% of the world rely on natural product for healing as per WHO [1–3]. In the 21st century, microgreens have become novel emerging class of ‘functional food’ characterized with tender, edible seedlings which are harvested at the cotyledonary or early true-leaf stage (2-4 leaf stage), within 7–14 days post-germination [4,5]. These are exceptionally rich in nutrient profile and bioactive components, usually much higher than their mature counterparts. The increased demand of microgreen is due to compact growth period, minimal space requirement, low input cost, year-round cultivation, ready to consume, and dense nutritional profile [6–8].

The microgreens germinated from *Raphanus sativus* L., (radish), belonging to the Brassicaceae family is promising for its nutritional content and therapeutic applications. These have peppery, mild flavor and crisp texture and dense phytochemical content including glucosinolates, ascorbic acid, anthocyanins, chlorophylls. The glucosinolate content in radish microgreens has cancer-preventive effects and boosts immune function [9,10]. Radish was selected for microgreen studies is due to several factors rapid germination, high adaptability of growth, consistent growth patterns, high germination rates exceeding 85%, and exceptional nutritional density. The mineral profile of these radish microgreens has not yet been explored which gives the novelty to the work.

Minerals are required for maintenance of normal functioning and homeostasis of human body [11]. Essential minerals are of two types, macrominerals (required >100mg/day) like Ca, Mg, Na, K, P, S and microminerals (required <100mg/ day) like Zn, Mn, Fe, Cu, Se. These minerals serve as cofactor of over 300 enzymes and are vital for human metabolism, immune modulation, enzymatic function, and antioxidant defense [12,13]. Deficiency in intake of mineral causes several health issues and suppressed immunity [14–17]. Thus, Food Drug Administration offers RDA or recommended daily intake of these minerals. Calcium being macromineral should be in-taken at least 1.3g per day, followed by microminerals like Zinc (11mg/day); Manganese (2.3mg/day); Selenium (5.5g/day). Excess of mineral intake also causes several health issues [18–21]. Conversely, consumption of heavy metals (density>4.0) like Cr(VI), Cd, Pb, As, Hg should be also monitored and if consumed above safe limits, causes neurological damage, kidney dysfunction, carcinogenic effects and most importantly bioaccumulation. Thus, regulation of the mineral intake should be strictly monitored [22,23].

Plants and natural products are sources of minerals. These minerals are up taken from the soil by roots during the growth [24]. Minerals play important role in structural development and physiological processes of plants too. For example, Manganese is important for chlorophyll functioning . The mineral content in soil depend on several factors: Edaphic (soil-related factors) like pH, texture, organic matter content, nitrogen content, moisture and aeration; Geographical factor like altitude, soil type, temperature, rainfall; Microecological factors (microbes in soil and their interactions); and anthropogenic factors (human activities) like fertilizer use, pesticides, industrial discharge, pollution and contaminants [25,26].

West Bengal, an eastern state of India is washed by Bay of Bengal at South and drained by river Ganges too. Himalayas provide completely different ecosystem in North of Bengal as compared to South Bengal. Thus, broad classification of the agroclimatic region formed seven zones including hill zone (Northern districts); Terai and Teesta Alluvial Zone, Gangetic Alluvial Zone (Old and New), Vindhyan Alluvial Zone, Undulating Red Laterite Zone (Western Districts), and Coastal Saline Zone (Southern Districts) [27,28]. These variations in soil characteristics directly influences the plant mineral content, affecting both essential nutrients and potentially harmful heavy metals. Thus, examining the mineral content of microgreen grown in soil of different district representing different agro-climatic zones helps in determining the region-specific dietary recommendations and understanding soil pattern suitable for growth of microgreen for best nutritional profile.

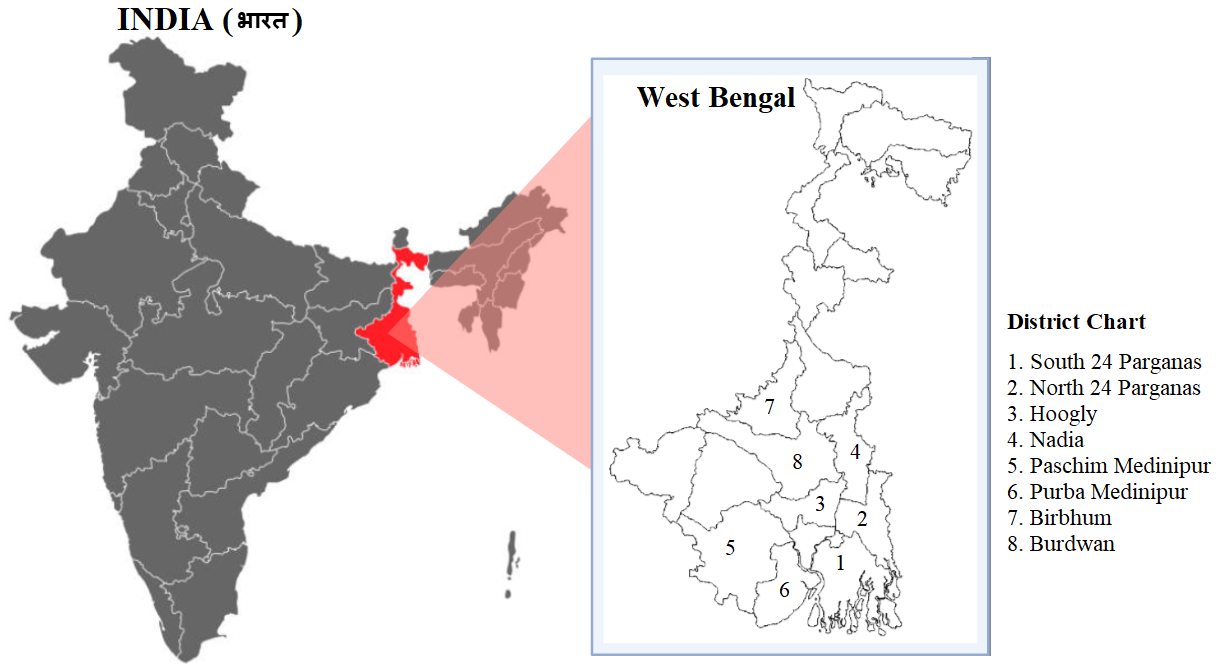
The current study aims in determining the difference in mineral content of *Raphanus sativus* L. microgreens when grown in soil of different district of West Bengal and establish correlations between soil characteristics and plant mineral content across different agroclimatic zones, thereby identify optimal growing regions for nutritionally superior and safe microgreen production.

1. **MATERIALS AND METHODS**
   1. **Soil collection of different districts**

Soil from eight different districts of West Bengal, India, representing four different agroeconomic zones were collected (Fig. 1). The agroclimatic zones represents significant difference in the soil composition [28–30]. The details of the soil collected from different district are shown in Table 1.

**Table 1.** Geographical location and agroeconomic zone of soil collection from different districts

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Different regions of West Bengal** | **Agroeconomic Zone** | **Latitude and Longitude of soil collection** |
| 01 | South 24 Parganas | Coastal and Saline Zone | 22.4043° N, 88.4937° E |
| 02 | North 24 Parganas | New Alluvial Zone | 22.8895° N, 88.4220° E |
| 03 | Hooghly | Old Alluvial Zone | 23.0781° N, 88.2789° E |
| 04 | Nadia | New Alluvial Zone | 23.4013° N, 88.5021° E |
| 05 | Paschim Medinipur | Coastal and Saline Zone | 22.6601° N, 87.7370° E |
| 06 | Purba Medinipur | Coastal and Saline Zone | 22.1887° N, 87.9190° E |
| 07 | Birbhum | Red and Laterite Zone | 23.6776° N, 87.6852° E |
| 08 | Burdwan | Old Alluvial Zone | 23.2325° N, 87.8634° E |

****

**Fig. 1.** Different districts representing different agroeconomic zone used for microgreen cultivation

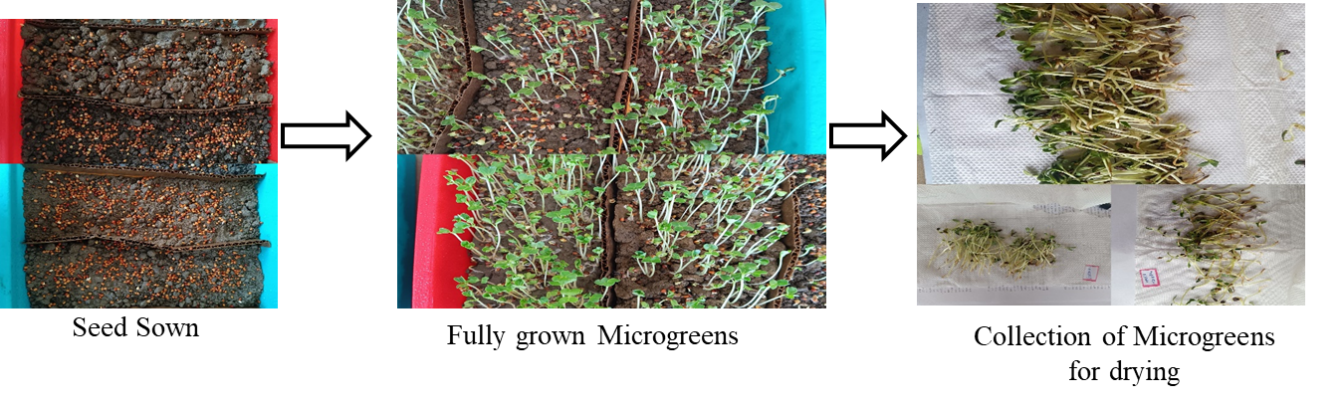
* 1. **Physio-chemical properties of soil analysis**

Different physical and chemical properties of soil collected from different locations as mentioned in Table 1 were examined [31]. The bulk density of the soil was determined followed by pH of the soil using pH meter (DBP ATC Model 10PHM11). The electrical conductivity was determined using conductivity bridge with Potassium Chloride (EMPARTA®) as standard [32]. The total nitrogen was determined using the Kjeldal methods [33]. The moisture content and organic carbon content of the soil was also determined by titrimetric analysis using diphenylamine indicator [34].

* 1. **Microgreen growth and collection**

The soil was placed in germination tray evenly with depth 8inches and length and breadth of 12 inches each. Radish or *Raphanus sativus* L. seeds were procured from registered vendors. These seeds were sown in the soil of different districts evenly and were watered for 3.00.4mL/cm2. The temperature of the growth was maintained around 352oC with at least 6 hours of direct-diffused sunlight exposure. Humidity of the growing condition was maintained around 70-74%.

Daily the growth of saplings was observed. The small saplings were harvested at 2-4 leaf stage (Fig. 2). They were cleaned using tissue paper, the roots were cut off and the aerial portion was dried in hot air oven for 24 hours at 60oC and the moisture content and dry weight were noted.

****

**Fig. 2.** Microgreen growth and collection

* 1. **Sample Digestion for mineral content analysis**

0.5g of the accurately weighed dried microgreens were taken using digital weighing balance (Wensar PGB220). They were ground in mortar and pestle and sieved through mesh size 100. These samples were heated with 10mL 69% nitric acid (Emplura (1.93406.2521)) at 130oC for 30 minutes on a hot plate (TEMPSTAR KH-701 C), followed addition of 5mL 60% perchloric acid (Emparta (1.93004.2500)) and heated for 10 minutes. The resultant solution was cooled and diluted with blank solution (2%v/v nitric acid) upto 50mL and filtered with Whatman filter paper Grade 1. This prepared the sample for analysis using Flame Atomic Absorption Spectroscopy (PerkinElmer – PinAAcle 500) to determine the mineral content [26,35].

* 1. **Calibration of Atomic Absorption Spectroscopy**

For determination of mineral content, Flame Atomic Absorption Spectrometer (PerkinElmer – PinAAcle 500) was used. Minerals that were analysed includes micromineral like Calcium; trace elements like Zn, Mn, Se and heavy metals like Cr, Cd and Pb. The working conditions of the instrument is summarized in Table 2.

**Table 2.** Working parameters of Flame AAS

|  |  |  |  |
| --- | --- | --- | --- |
| **Analytical parameter** | **Value/mode** | **Trace element** | **Wavelength (nm)** |
| Slit Width (nm) | 0.2 nm | Calcium (Ca) | 422.67 |
| Fuel | Nitrous oxide-acetylene | Zinc (Zn) | 213.86 |
| Oxidant flow (N2O) | 6 L/min | Manganese (Mn) | 279.48 |
| Acetylene flow | 7.5 L/min | Selenium (Se) | 196.00 |
| Flame temperature | 2100-2400 °C | Chromium (Cr) | 357.87 |
| Sample flow rate | 1.3 ml/min | Cadmium (Cd) | 228.82 |
| Replicate | 3 | Lead (Pb) | 217.08 |

The instrument was calibrated by developing calibration curve using different concentration (0.2 - 2mg/mL) standard stock solution of Calcium, Zinc, Manganese, Selenium, Chromium, Cadmium and Lead (LOBA Chemie). Reagent blank solution was used to correct the instrument reading. The Limit of Detection (LOD), Limit of Quantification (LOQ), Relative Standard Deviation (RSD) and regression coefficient was determined by the given formula [36].

LOD = 3.3 \* (SD / S) (1)

LOQ = 10 \* (SD / S) (2)

Here, SD is the standard deviation of ten blank reading for each mineral and S is the slope of calibration curve. 2.2 and 10 are the proportionality constants.

Further the instrument was validated recording the mineral content of known standard solutions.

* 1. **Dietary Value Calculation of microelement and microelements**

According to the reports of FAO-WHO, the limits of intake of Ca, Zn, Mn and Se are 1300, 11, 2.3mg/day and 5.5g/day. Based on these values, the percentage of Recommended Dietary Intake fulfilled by consumption of 100g of the microgreen was calculated as %RDA [37].

RDA (%) = (MV/RDAstd) 100% (3)

Where, RDA (%) is the percentage of recommended dietary allowance fulfilled by consuming 100g of microgreen; MV is the mineral value of the microgreen in mg/100g and RDAstd are the standard value of Dietary Intake mentioned by WHO in mg/day.

The Estimated Daily Intake is the amount of mineral consumed by consuming the RACC (Recommended Amount Customarily Consumed) amount of microgreen, which is 85g/day. So, EDI is given as:

EDI (mg/day) = (MVRACC)/100 (4)

The Nutrition Contribution is defined as the percentage of recommended daily intake consumed on consuming the RACC amount of plant material. It is given as

NC% = EDI/ RDAstd (5)

* 1. **Toxicity Value Calculation of Heavy Metals**

Toxicity induced by consumption of these microgreens can be expressed in terms of Estimated Daily Intake (EDI), THQ or the Target Hazzard Quotient and the Hazard Index (HI), which are given as [38]:

EDI (mg/kg/day) = [MV × RACC × TR]/ (BW × 1000) (6)

Here, MV is the Mineral content determined in mg/kg and RACC value is in g/day. BW is the body weight of adult human being with is 70kg in average [39]. TR is the transference rate of toxic metal which is normally given as 19.8% for lead, 6.6% for Cadmium and 42% for chromium [38,40,41].

The Target Hazzard Quotient is given by

THQ = EDI/RfD (7)

The RfD value is the reference allowed intake of heavy metals in mg/kg/day value for an adult human being and EDI is the Estimated Daily Intake calculated for heavy metal. The EDI and THQ for all different heavy metals were measured.

Hazzard Index is the summation of Target Hazzard Quotient of all the heavy metals. HI<1 indicates that the product is safe to be consumed with no chances of carcinogenicity and mutagenicity. If HI>1, it indicates that the toxic effects of the heavy metals are quite likely to occur when consumed [26].

HI = THQCr + THQCd + THQPb (8)

* 1. **Statistical Analysis**

All readings were taken in triplicates to maintain validity and were expressed in Mean SEM values. Correlation matrix was performed using the mean values. ANOVA studies were done using Graphpad Prism 10.4.2.

1. **RESULTS AND DISCUSSION**
   1. **Soil Analysis**

The different physiochemical properties including pH, bulk density, nitrogen and carbon content and other properties of soil collected from different districts were estimated. The details are shown in Table 3.

**Table 3.** Physiochemical analysis of soils of different districts

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl No.** | **Soil sample** | **pH in water** | **Electronic Conductivity (ds/m)** | **Organic**  **Carbon (%)** | **Available**  **N (kg/ha)** | **Moisture Content (%)** | **Bulk Density**  **(g/cm3)** |
| **1.** | South 24 Parganas | 7.3 | 0.17 | 0.363 | 120.18 | 6.04 | 1.23 |
| **2.** | North 24 Parganas | 7.8 | 0.28 | 0.909 | 210.52 | 9.68 | 1.10 |
| **3.** | Hooghly | 6.64 | 0.32 | 0.924 | 250.95 | 10.46 | 1.24 |
| **4.** | Nadia | 8.1 | 0.23 | 0.287 | 184.56 | 12.5 | 1.27 |
| **5.** | Paschim Medinipur | 8.13 | 0.24 | 0.921 | 238.33 | 13.51 | 1.04 |
| **6.** | Purba Medinipur | 8.23 | 0.19 | 0.454 | 112.90 | 10.01 | 1.07 |
| **7.** | Burdwan | 5.23 | 0.44 | 0.681 | 247.31 | 11.50 | 1.11 |
| 8. | Birbhum | 6.58 | 0.12 | 0.833 | 213.25 | 12.00 | 1.20 |

The detailed analysis revealed the differences in the characteristics of soil with soil of Burdwan to be most acidic (pH = 5.23) with quite high Nitrogen content (247.31 kg/ha) in it. The soil of South 24 parganas was the most neutral (pH = 7.3) with least organic carbon in it (0.363%). In terms of alkalinity, Purba Medinipur soil is highly alkaline with pH 8.23, indicating its salinity owing to its proximity to Bay of Bengal [42]. It is due to repeated leaching and sea water intrusion, that the soils of Purba Medinipur and South 24 Parganas have lower content of Nitrogen and Carbon. This also indicates its appropriateness of cultivating crops which suits best in saline region [43]. The soil of Birbhum and Burdwan district have comparable properties, except their pH. Agroclimatic zone of New Alluvial soil, like North 24 Parganas showed very high carbon content of about 0.909% indicating its rich and fertile alluvial layer. Moreover favourable pH of 7.8 boosts growth of most of crops.

* 1. **Collection of Microgreen**

The full-grown microgreens are harvested within the stipulated time of 7-14 days. Equal number of seeds were evenly distributed to assure uniformity in growing conditions of the microgreens. The germination pattern of the seeds in soils of different districts of West Bengal are shown in Table 4.

**Table 4.** Germination Details of Radish Microgreen in different districts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Different region of WB** | **Seed sowing density (Number /cm2 soil)** | **Total Number of seeds sown** | **Duration of growth of >50% total seeds (in days)** | **% of Germination (%)** | **Moisture content** | **Dry weight (g)** |
| **South 24 parganas** | 2 | 180 | 8 | 86.23 | 69.42±1.21% | 2.29±0.48 |
| **North 24 Parganas** | 2 | 180 | 9 | 89.46 | 67.03±2.16% | 2.31±0.31 |
| **Nadia** | 2 | 180 | 7 | 90.11 | 57.13±1.94% | 2.75±0.43 |
| **Hooghly** | 2 | 180 | 9 | 73.31 | 60.43±0.98% | 2.63±0.82 |
| **Paschim Medinipur** | 2 | 180 | 9 | 83.77 | 70.21±1.98% | 2.29±0.18 |
| **Purba Medinipur** | 2 | 180 | 9 | 79.03 | 61.29±1.87% | 2.61±0.25 |
| **Birbhum** | 2 | 180 | 8 | 86.97 | 62.76±1.77% | 2.58±0.71 |
| **Burdwan** | 2 | 180 | 10 | 80.21 | 51.98±2.03% | 2.19±0.20 |

It was found that the fastest growth of the microgreen was in Nadia district with highest germination percentage of 90% followed by North 24 Parganas. This indicates Alluvial soil to be the fastest grower of the microgreen. On the other hand, in soil of Burdwan district, the microgreens showed bit delayed growth and 50% of the microgreens were full grown within 10 days. Moisture content of microgreen grown in Paschim Medinipur was highest over 90%. The dry weight yield of the samples grown in Nadia district were the highest.

* 1. **Quality Assurance**

The instrument was calibrated to determine its linearity, precision, range and accuracy. The Table 5 shown indicates the LOD, LOQ and regression coefficient of the instrument.

**Table 5.** Calibration of the instrument

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Trace element** | **Relative SD (%RSD)** | **LOD (mg/100g)** | **LOQ (mg/100g)** | **Linearity (R2)** |
| 1 | Calcium (Ca) | 0.062 | 0.01058 | 0.03209 | 0.993728 |
| 2 | Zinc (Zn) | 0.042 | 0.00215 | 0.00654 | 1.000000 |
| 3 | Manganese (Mn) | 0.028 | 0.00318 | 0.00966 | 1.000000 |
| 4 | Selenium (Se) | 0.031 | 0.00104 | 0.00315 | 1.000000 |
| 5 | Chromium (Cr) | 0.024 | 0.00481 | 0.01459 | 0.999725 |
| 6 | Cadmium (Cd) | 0.012 | 0.00018 | 0.00054 | 0.999218 |
| 7 | Lead (Pb) | 0.003 | 0.00007 | 0.00021 | 0.999107 |

The low relative standard deviation (<0.06%) indicated indicate high repeatability and method precision. Similarly, the low LOD (0.00007 – 0.01058mg/100g) and LOQ (0.00021 – 0.032mg/100g) indicated good detectability and suitability for trace element analysis in sample over wide range. Regression coefficient in each case of mineral analysis (r2>0.999) showed excellent linearity of the instrument and this indicates high accuracy and reliability of the AAS method.

The further internal validation was assured by measuring the mineral content using the instrument for five different solutions of known mineral concentration. The recorded mineral contents were in permissible limits of recovery (90-110%).

* 1. **Mineral Content Analysis in Microgreen**

Table 6 represents the content of Ca, Mn, Zn, Se, Cr, Cd and Pb in microgreen grown in eight districts of West Bengal.

**Table 6.** Mineral content in radish microgreen grown in soil of different district of West Bengal in mg/100g [Mean SEM]

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Different Region of WB** | **Calcium (Ca)** | **Zinc (Zn)** | **Manganese (Mn)** | **Selenium (Se)** | **Chromium (Cr)** | **Cadmium (Cd)** | **Lead (Pb)** |
| 01 | South 24 Parganas | 121.33±2.12a | 15.89±0.01a | 6.62±0.02c | 0.064±0.01bc | 1.27±0.02c | 0.0011±0.00cd | 0.0006±0.00d |
| 02 | North 24 Parganas | 132.12±3.81e | 5.34±0.21ab | 7.74±0.10ab | 0.098±0.02e | 1.21±0.03bc | 0.0016±0.00c | 0.0004±0.00c |
| 03 | Hooghly | 138.29±1.85a | 5.23±0.00ab | 8.37±0.00ab | 0.048±0.01d | 1.59±0.01b | 0.0009±0.00b | 0.0002±0.00de |
| 04 | Nadia | 119.05±1.39ab | 6.66±0.03ac | 6.87±0.04ab | 0.074±0.00c | 1.78±0.00c | 0.0010±0.001ab | 0.0003±0.00c |
| 05 | Paschim Medinipur | 135.95±0.98a | 6.22±0.00ae | 8.06 ± 0.02cd | 0.069±0.02c | 1.37±0.01c | 0.0020±0.001c | 0.0008±0.00ab |
| 06 | Purba Medinipur | 148.11±1.28d | 4.42±0.04ab | 7.71 ± 0.00ad | 0.057±0.01bc | 1.33±0.02c | 0.0021±0.001c | 0.0002±0.00c |
| 07 | Birbhum | 109.14±2.18e | 8.76±0.09c | 6.73 ± 0.01b | 0.031±0.01abc | 0.62±0.00de | 0.0015±0.00c | 0.0007±0.00c |
| 08 | Burdwan | 120.13±2.95a | 6.52±0.11bc | 7.92 ± 0.00d | 0.094±0.01c | 2.51±0.01c | 0.0028±0.00c | 0.0008±0.00ab |

Values followed by different alphabets represents, they are statistically significant with p<0.05.

Microgreen of radish, grown in different soil of eight districts of West Bengal shows significance differences in them. This highlight the fact that change in agroclimatic zone is associated with change in mineral content in the plants grown on them.

* + 1. **Macroelement analysis**

Calcium was the only macronutrient that was analysed. Calcium, a crucial macronutrient involved in bone health, neuromuscular function, and intracellular signalling. It is important for bone health, skeletal muscle contraction, blood clotting and cardiac muscle contraction [44–46]. Normally, for human body, 1.3g Calcium should be daily consumed. It was found that growing the same plant in different soil caused variation in Calcium content from 109.14±2.18mg/100g (Birbhum) to 148.11±1.28 mg/100g (Purba Medinipur). The Calcium content of radish microgreen varies as Purba Medinipur> Pashchim Medinipur> North 24 Parganas> Hoogly> South 24 Parganas> Burdwan> Nadia> Birbhum. This emphasized on the fact that saline or alkaline soil have better calcium content than acidic soil. Birbhum due to lateritic and acidic soil composition have lesser Calcium content in them, leading to prone to calcium deficiency.

* + 1. **Trace element analysis**

Trace elements and microminerals have several significant roles in our body and are required less than 100mg daily. Zinc (Zn) is the most abundant trace element, with daily dietary intake of atleast 11mg. It plays important role as cofactor of several enzymes including antioxidants like superoxide dismutase and digestive enzymes like carboxypeptidase [47–49]. The content of zinc ranged significantly from 4.42±0.04mg/100g (Purba Medinipur) to 15.89±0.01mg/100g (South 24 Parganas). South 24 Parganas is a district drained by Gangetic alluvial soil at North and shore of Bay of Bengal at south. Thus, the higher zinc content might be due to favorable chelation with humous which increases zinc uptake of plant from soil. Constant leaching of soil nutrient and sea water entry to soil make Purba Medinipur soil lesser Zinc rich.

Manganese is also associated with antioxidant enzymes, blood clotting and several other biological processes [50–52]. It was found that highest Manganese content was in Hoogly district 8.37±0.00mg/100g which might be due to the rich fertile alluvial deposits of river Ganges. On the other hands South 24 Parganas showed lowest Manganese content (6.62±0.02 mg/100g). This might be because of organic matter that binds Mn that limit its uptake. Manganese is essential for photosynthesis. Thus, manganese rich microgreen may offer superior nutraceutical benefits. Overall manganese content is quite less in radish microgreens.

Therapeutic role of Selenium as trace element is known for its antioxidant, role in iodine metabolism and immune-modulating roles [53–55]. In the grown radish microgreens, North 24 Parganas showed highest Selenium content (0.0980.02mg/100g) which might be because of selenium-enriched soils or proximity to anthropogenic activities like industrial emissions. All the plants showed Selenium content in safe range of daily consumption.

Plants uptake minerals during their growth from soil through their root. So, apparently the mineral content in aerial part of plant is comparatively less concentrated than their roots. The variations in the mineral content might be because of several factors. The difference is attributed to differences in soil composition, fertility, pH, organic matter content, microecological and anthropogenic activities [26].

* + 1. **Heavy Metal analysis**

Limitation and regulation of heavy metal intake is absolutely necessary to maintain healthy well-being. Chromium apart from being a heavy metal, is extremely essential in glucose metabolism in trivalent form forming chromodulin complex. Chromium (III) is beneficial, while Chromium (VI) is generally toxic [56,57]. Birbhum (0.62 0.00mg/100g) and South 24 Parganas (1.27 0.02mg/100g) recorded lower Cr levels, while Burdwan showed highest concentration of chromium (2.510.01mg/100g) which might be due to industrial emissions or weathering of Cr-rich minerals.

Cadmium is non-essential toxic mineral [58,59]. In the grown radish microgreens, it ranged from 0.00090.00 mg/100g (Hooghly) to 0.00280.00 mg/100g (Burdwan), which might be due to industrial effluents and phosphate fertilizer overuse. Overall concentration of cadmium is in safe limits which is offered as 0.003mg/kg/day.

Lead content was significantly low in microgreens grown in all the districts and ranged from 0.0002–0.0008 mg/100g. Though Burdwan recorded the highest lead burden, it is within the safe limits indicating the safety in consumption and lesser chances of lead accumulation in human body [60,61].

* 1. **Pearson Correlation Matrix**

Based on the mineral content determined in microgreens grown in soil of different districts, Table 7 shows the Pearson correlation matrix.

**Table 7.** Correlation matrix of minerals found in radish microgreen

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Ca** | **Zn** | **Mn** | **Se** | **Cr** | **Cd** | **Pb** |
| **Ca** | 1.00000 |  |  |  |  |  |  |
| **Zn** | -0.51617 | 1.00000 |  |  |  |  |  |
| **Mn** | 0.696225 | -0.70503 | 1.00000 |  |  |  |  |
| **Se** | 0.14329\* | -0.21775 | 0.358625 | 1.00000 |  |  |  |
| **Cr** | 0.061414 | -0.22915 | 0.40717 | 0.659675\*\* | 1.00000 |  |  |
| **Cd** | 0.124036 | -0.32511 | 0.368795 | 0.360664 | 0.42854 | 1.00000 |  |
| **Pb** | -0.53259 | 0.364366 | -0.14207 | 0.062371 | 0.063831 | 0.506051 | 1.00000 |

\* Significant at 0.05 probability level and \*\* Significant at 0.01 probability level

The correlation matrix developed relationships among the minerals and heavy metals in radish microgreens. Strong positive correlation was found between calcium (Ca) and manganese (Mn) (r = 0.696); Selenium (Se) and chromium (Cr) (r = 0.659) indicating their uptake in synergistic manner and are influenced by similar physiochemical characteristics. Moderate negative correlation was found between Calcium and Zinc (r = -0.516); and Cadmium and zinc (r = -0.325) indicating potential competitive antagonism during absorption by roots. Notably Pb showed a negative correlation with Ca (r = –0.533), indicating Calcium uptake inhibited lead accumulation in roots. Positive correlation between all heavy metals exhibited similar contamination source or uptake nature. Negative correlation shows independence of the sources of mineral uptake [62].

* 1. **Dietary value calculation**

Table 8 elaborates the %RDI (Recommended Dietary Intake), EDI (Estimated Daily Intake), and Nutrient Contribution (NC) calculation of essential minerals found in microgreens.

The %RDI of Calcium ranged from 8.39% (Birbhum) to 11.39% (Purba Medinipur). Thus regular consumption of microgreens of radish can significantly supplement diets. The highest zinc contribution was from South 24 Parganas (144.46% RDI or 122.7% NC). Thus, it might excellent dietary source for immune support and growth. Across all regions, manganese NC exceeded 240% and thus implies strong antioxidant property of microgreens [63]. Radish microgreens offer overall Nutrient Contribution for trace elements like Zn, Mn, and Se, with regional variation (Fig. 3).

**Table 8.** Dietary values of macromineral and micromineral of microgreens grown in different districts

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **District** | **Calcium (Ca)** | | | **Zinc (Zn)** | | | **Manganese (Mn)** | | | **Selenium (Se)** | | |
| **%RDI (%)** | **EDI (mg/day)** | **NC (%)** | **%RDI (%)** | **EDI (mg/day)** | **NC (%)** | **%RDI (%)** | **EDI (mg/day)** | **NC (%)** | **%RDI (%)** | **EDI (mg/day)** | **NC (%)** |
| South 24 Parganas | 9.333 | 103.131 | 7.933 | 144.455 | 13.507 | 122.786 | 287.826 | 5.627 | 244.652 | 116.364 | 0.054 | 98.909 |
| North 24 Parganas | 10.163 | 112.302 | 8.639 | 48.545 | 4.539 | 41.264 | 336.522 | 6.579 | 286.043 | 178.182 | 0.083 | 151.455 |
| Hooghly | 10.638 | 117.547 | 9.042 | 47.545 | 4.446 | 40.414 | 363.913 | 7.115 | 309.326 | 105.455 | 0.049 | 89.636 |
| Nadia | 9.158 | 101.193 | 7.784 | 60.545 | 5.661 | 51.464 | 298.696 | 5.840 | 253.891 | 134.545 | 0.063 | 114.364 |
| Paschim Medinipur | 10.458 | 115.558 | 8.889 | 56.545 | 5.287 | 48.064 | 350.435 | 6.851 | 297.870 | 125.455 | 0.059 | 106.636 |
| Purba Medinipur | 11.393 | 125.894 | 9.684 | 40.182 | 3.757 | 34.155 | 335.217 | 6.554 | 284.935 | 103.636 | 0.048 | 88.091 |
| Birbhum | 8.395 | 92.769 | 7.136 | 79.636 | 7.446 | 67.691 | 292.609 | 5.721 | 248.717 | 56.364 | 0.026 | 47.909 |
| Burdwan | 9.241 | 102.111 | 7.855 | 59.273 | 5.542 | 50.382 | 344.348 | 6.732 | 292.696 | 170.909 | 0.080 | 145.273 |

**Fig. 3.** Nutrient Contribution from Radish microgreens

* 1. **Calculation of toxicities from heavy metals**

To calculate the health risks imposed by consumption of microgreen due to the heavy metal consumption, several parameters including Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) of Cd, Cr and Pb were estimated as shown in Table 9.

For chromium, Cr (VI) is generally toxic [64]. Based on the observed values, the THQ values of chromium varied from 0.1054 to 0.4267, with the highest in Burdwan and Nadia. The THQ values for cadmium were also very low (maximum 0.002244 in Burdwan), indicating a negligible health risk when consumed. Lead is a potent neurotoxin; the EDI value of lead was also minimalistic with highest of 1.92×10-6 mg/kg/day (Paschim Medinipur and Burdwan) indicating very less chances of lead accumulation [60].

The HI, ranged from 0.1070 (Birbhum) to 0.4294 (Burdwan) which indicated the risk of carcinogenicity. None of the values were close to or crossed 1, indicating that the overall exposure to Cr, Cd, and Pb due to consumption of radish microgreen does not impart carcinogenic health threat.

**Table 9:** Hazard Index Calculation of Heavy Metals

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **District** | **Chromium (Cr)** | | **Cadmium (Cd)** | | **Lead (Pb)** | | **HI** |
| **EDI (mg/kg/day)** | **THQ** | **EDI (mg/kg/day)** | **THQ** | **EDI (mg/kg/day)** | **THQ** |
| South 24 Parganas | 0.000648 | 0.2159 | 8.8157E-07 | 0.00088157 | 1.44257E-06 | 0.000361 | **0.217142** |
| North 24 Parganas | 0.000617 | 0.2057 | 1.2823E-06 | 0.00128229 | 9.61714E-07 | 0.00024 | **0.207223** |
| Hooghly | 0.000811 | 0.2703 | 7.2129E-07 | 0.00072129 | 4.80857E-07 | 0.00012 | **0.271142** |
| Nadia | 0.000908 | 0.3026 | 8.0143E-07 | 0.00080143 | 7.21286E-07 | 0.00018 | **0.303582** |
| Paschim Medinipur | 0.000699 | 0.2329 | 1.6029E-06 | 0.00160286 | 1.92343E-06 | 0.000481 | **0.234984** |
| Purba Medinipur | 0.000678 | 0.2261 | 1.683E-06 | 0.001683 | 4.80857E-07 | 0.00012 | **0.227903** |
| Birbhum | 0.000316 | 0.1054 | 1.2021E-06 | 0.00120214 | 0.000001683 | 0.000421 | **0.107023** |
| Burdwan | 0.00128 | 0.4267 | 2.244E-06 | 0.002244 | 1.92343E-06 | 0.000481 | **0.429425** |

**CONCLUSION**

Microgreens of *Raphanus sativus* L. (radish) of Brassicaceae family, shows wide variation in nutrient content when grown in soils of different districts representing agroclimatic regions of West Bengal. This indicate strong influence of edaphic (soil-related), agro-climatic, and anthropogenic factors on mineral content in plants. Microgreens grown South 24 Parganas showed highest Zinc content. Radish microgreens grown in soil of Purba Medinipur and Hooghly had highest concentration of minerals, indicating acidic or laterite soil limits mineral uptake in plant which might be due to chelation. So, alkaline and neutral soil of Alluvial zone and coastal saline zone the best soil for growth of radish microgreen. The low levels of Cd and Pb across all districts and the hazard index less than 1 in microgreens grown in all districts confirm the safety of these microgreens for regular consumption. In future, these microgreens can be suggested for dietary intake and nutraceutical derivatives can be prepared for it for human use.

**Acknowledgment:** The authors thank Guru Nanak Institute of Pharmaceutical Science and Technology for giving the opportunity to conduct the research and for permitting to avail the facilities of Acharya Prafulla Chandra Roy (APC) laboratory to conduct the research.

**Author Contribution:** S. Hazra: Conceptualization, Data curation, Methodology, Visualization, Validation; A. Chakraborty: Writing – Original draft, Data curation, Formal analysis; S. Giri and A. Sarkar – Methodology and Software; T. Adhikari – Project administration, supervision, Writing– review and editing.

**Data availability:** The author can confirm that all data generated or analysed are included in the article.

**DECLARATIONS**

**Ethical approval:** Not applicable.

**Conflict of Interest:** The authors declare no competing interests.

**REFERENCE**

[1] Chaachouay N, Zidane L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs and Drug Candidates 2024;3:184–207. https://doi.org/10.3390/ddc3010011.

[2] De Smet PAGM. The Role of Plant-Derived Drugs and Herbal Medicines in Healthcare. Drugs 1997;54:801–40. https://doi.org/10.2165/00003495-199754060-00003.

[3] Rates SMK. Plants as source of drugs. Toxicon 2001;39:603–13. https://doi.org/10.1016/S0041-0101(00)00154-9.

[4] Lone JK, Pandey R, Gayacharan. Microgreens on the rise: Expanding our horizons from farm to fork. Heliyon 2024;10:e25870. https://doi.org/10.1016/j.heliyon.2024.e25870.

[5] Bhaswant M, Shanmugam DK, Miyazawa T, Abe C, Miyazawa T. Microgreens—A Comprehensive Review of Bioactive Molecules and Health Benefits. Molecules 2023;28:867. https://doi.org/10.3390/molecules28020867.

[6] Choe U, Yu LL, Wang TTY. The Science behind Microgreens as an Exciting New Food for the 21st Century. J Agric Food Chem 2018;66:11519–30. https://doi.org/10.1021/acs.jafc.8b03096.

[7] Gunjal M, Singh J, Kaur S, Nanda V, Ullah R, Iqbal Z, et al. Assessment of bioactive compounds, antioxidant properties and morphological parameters in selected microgreens cultivated in soilless media. Sci Rep 2024;14:23605. https://doi.org/10.1038/s41598-024-73973-w.

[8] Gupta A, Sharma T, Singh SP, Bhardwaj A, Srivastava D, Kumar R. Prospects of microgreens as budding living functional food: Breeding and biofortification through OMICS and other approaches for nutritional security. Front Genet 2023;14. https://doi.org/10.3389/fgene.2023.1053810.

[9] Zhu Q, Zhang P, Liu D, Tang L, Yu J, Zhang C, et al. Glucosinolate extract from radish (Raphanus sativus L.) seed attenuates high-fat diet-induced obesity: insights into gut microbiota and fecal metabolites. Front Nutr 2024;11. https://doi.org/10.3389/fnut.2024.1442535.

[10] Yan C, Huang Y, Zhang S, Cui L, Jiao Z, Peng Z, et al. Dynamic profiling of intact glucosinolates in radish by combining UHPLC-HRMS/MS and UHPLC-QqQ-MS/MS. Front Plant Sci 2023;14. https://doi.org/10.3389/fpls.2023.1216682.

[11] Tsuji PA, Canter JA, Rosso LE. Trace Minerals and Trace Elements. Encyclopedia of Food and Health, Elsevier; 2016, p. 331–8. https://doi.org/10.1016/B978-0-12-384947-2.00699-1.

[12] Weyh C, Krüger K, Peeling P, Castell L. The Role of Minerals in the Optimal Functioning of the Immune System. Nutrients 2022;14:644. https://doi.org/10.3390/nu14030644.

[13] Gharibzahedi SMT, Jafari SM. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. Trends Food Sci Technol 2017;62:119–32. https://doi.org/10.1016/j.tifs.2017.02.017.

[14] Kiani AK, Dhuli K, Donato K, Aquilanti B, Velluti V, Matera G, et al. Main nutritional deficiencies. J Prev Med Hyg 2022;63:E93–101. https://doi.org/10.15167/2421-4248/jpmh2022.63.2S3.2752.

[15] Vural Z, Avery A, Kalogiros DI, Coneyworth LJ, Welham SJM. Trace Mineral Intake and Deficiencies in Older Adults Living in the Community and Institutions: A Systematic Review. Nutrients 2020;12:1072. https://doi.org/10.3390/nu12041072.

[16] Leung AKC, Lam JM, Wong AHC, Hon KL, Li X. Iron Deficiency Anemia: An Updated Review. Curr Pediatr Rev 2024;20:339–56. https://doi.org/10.2174/1573396320666230727102042.

[17] Vetlényi E, Rácz G. The physiological function of copper, the etiological role of copper excess and deficiency. Orv Hetil 2020;161:1488–96. https://doi.org/10.1556/650.2020.31854.

[18] Chen P. Manganese metabolism in humans. Frontiers in Bioscience 2018;23:4665–79. https://doi.org/10.2741/4665.

[19] Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. Mol Aspects Med 2005;26:353–62. https://doi.org/10.1016/j.mam.2005.07.003.

[20] Haase H, Rink L. Zinc signals and immune function. BioFactors 2014;40:27–40. https://doi.org/10.1002/biof.1114.

[21] Rayman MP. Selenium and human health. The Lancet 2012;379:1256–68. https://doi.org/10.1016/S0140-6736(11)61452-9.

[22] Tefera M, Teklewold A. Health risk assessment of heavy metals in selected Ethiopian spices. Heliyon 2021;7. https://doi.org/10.1016/j.heliyon.2021.e07048.

[23] Nkansah M, Amoako C. Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. American Journal of Scientific and Industrial Research 2010;1:158–63. https://doi.org/10.5251/ajsir.2010.1.2.158.163.

[24] Özkutlu F, Kara SM, Şekeroğlu N. DETERMINATION OF MINERAL AND TRACE ELEMENTS IN SOME SPICES CULTIVATED IN TURKEY. Acta Hortic 2007;756:321–8. https://doi.org/10.17660/ActaHortic.2007.756.34.

[25] Erdoğan A, Şeker ME, Kahraman SD. Evaluation of Environmental and Nutritional Aspects of Bee Pollen Samples Collected from East Black Sea Region, Turkey, via Elemental Analysis by ICP-MS. Biol Trace Elem Res 2023;201:1488–502. https://doi.org/10.1007/s12011-022-03217-3.

[26] Karahan F. Evaluation of Trace Element and Heavy Metal Levels of Some Ethnobotanically Important Medicinal Plants Used as Remedies in Southern Turkey in Terms of Human Health Risk. Biol Trace Elem Res 2023;201:493–513. https://doi.org/10.1007/s12011-022-03299-z.

[27] Adak S, Datta S, Bhattacharya S, Ghose TK, Lahiri Majumder A. Diversity analysis of selected rice landraces from West Bengal and their linked molecular markers for salinity tolerance. Physiology and Molecular Biology of Plants 2020;26:669–82. https://doi.org/10.1007/s12298-020-00772-8.

[28] Mandal KG, Thakur AK, Mohanty RK, Mishra AK, Sinha S, Biswas B. Policy perspectives on agricultural water management and associated technologies suitable for different agro-climatic zones of West Bengal, India. Curr Sci 2022;122:386. https://doi.org/10.18520/cs/v122/i4/386-395.

[29] Mebrate A, Kippie T, Zeray N, Haile G. Selected physical and chemical properties of soil under different agroecological zone in Gedeo Zone, Southern Ethiopia. Heliyon 2022;8:e12011. https://doi.org/10.1016/j.heliyon.2022.e12011.

[30] Sugathas S, Neththasinghe NASA, Sirisena DN, Thilakasiri R, Ariyarathna M, Kadupitiya HK, et al. Effects of agro-climatic zones, soil orders, and irrigation types on the exchangeable cadmium in paddy soils. Soil & Environmental Health 2024;2:100078. https://doi.org/10.1016/j.seh.2024.100078.

[31] Shirsath WB. Quantitatively Physico-Chemical Analysis of Some Soil Samples of Satana (Baglan)Tahsil, District Nashik, Maharashtra (India). JOURNAL OF SCIENTIFIC RESEARCH 2021;65:143–8. https://doi.org/10.37398/JSR.2021.650731.

[32] Li X, Wang X, Zhao Q, Zhang Y, Zhou Q. In Situ Representation of Soil/Sediment Conductivity Using Electrochemical Impedance Spectroscopy. Sensors 2016;16:625. https://doi.org/10.3390/s16050625.

[33] Pontes FVM, Carneiro MC, Vaitsman DS, da Rocha GP, da Silva LID, Neto AA, et al. A simplified version of the total kjeldahl nitrogen method using an ammonia extraction ultrasound-assisted purge-and-trap system and ion chromatography for analyses of geological samples. Anal Chim Acta 2009;632:284–8. https://doi.org/10.1016/j.aca.2008.11.011.

[34] Surendran U, Raja P, Liu K, Bilotto F, Sridevi G. Comparative analysis of soil organic carbon and soil properties in landscapes of Kerala: insights from the Western Ghats of India. Environ Monit Assess 2024;196:838. https://doi.org/10.1007/s10661-024-12984-6.

[35] Palma MNN, Rocha GC, Filho SCV, Detmann E. Evaluation of Acid Digestion Procedures to Estimate Mineral Contents in Materials from Animal Trials. Asian-Australas J Anim Sci 2015;28:1624–8. https://doi.org/10.5713/ajas.15.0068.

[36] Benavides LF, Marín JD, Rosales C, García J. Development and Validation of a Method for the Analysis of Zinc Oxide in Cosmetic Matrices by Flame Atomic Absorption Spectroscopy. J Anal Methods Chem 2021;2021:1–9. https://doi.org/10.1155/2021/8840723.

[37] Rani S, Singh N, Kaur C, Varghese E. Measurement of phytochemical content and nutritional characteristics of microgreens grown in high altitude region of India. Journal of Food Measurement and Characterization 2024;18:3113–27. https://doi.org/10.1007/s11694-024-02390-4.

[38] Zhang J, Yang R, Chen R, Peng Y, Wen X, Gao L. Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China. Int J Environ Res Public Health 2018;15:133. https://doi.org/10.3390/ijerph15010133.

[39] Peng C, Zhu X, Hou R, Ge G, Hua R, Wan X, et al. Aluminum and Heavy Metal Accumulation in Tea Leaves: An Interplay of Environmental and Plant Factors and an Assessment of Exposure Risks to Consumers. J Food Sci 2018;83:1165–72. https://doi.org/10.1111/1750-3841.14093.

[40] Li L, Fu Q-L, Achal V, Liu Y. A comparison of the potential health risk of aluminum and heavy metals in tea leaves and tea infusion of commercially available green tea in Jiangxi, China. Environ Monit Assess 2015;187:228. https://doi.org/10.1007/s10661-015-4445-2.

[41] Gruszecka-Kosowska A, Mazur-Kajta K. Potential health risk of selected metals for Polish consumers of oolong tea from the Fujian Province, China. Human and Ecological Risk Assessment: An International Journal 2016;22:1147–65. https://doi.org/10.1080/10807039.2016.1146572.

[42] Naskar M, Neogy S, Datta D. Spatially explicit environmental impact assessment of commercial brackishwater aquaculture along the northwestern Coast of Bay of bengal using a multi-parameter index approach. Integr Environ Assess Manag 2025. https://doi.org/10.1093/inteam/vjaf064.

[43] Mukherjee P, Das S, Mazumdar A. Introducing winter rice cropping by using non-saline tidal water influx in western basins of South 24 Parganas, India. Sci Rep 2021;11:553. https://doi.org/10.1038/s41598-020-80797-x.

[44] Baylor SM, Hollingworth S. Calcium indicators and calcium signalling in skeletal muscle fibres during excitation–contraction coupling. Prog Biophys Mol Biol 2011;105:162–79. https://doi.org/10.1016/j.pbiomolbio.2010.06.001.

[45] Tai V, Leung W, Grey A, Reid IR, Bolland MJ. Calcium intake and bone mineral density: systematic review and meta-analysis. BMJ 2015:h4183. https://doi.org/10.1136/bmj.h4183.

[46] WAKABAYASHI T. Mechanism of the calcium-regulation of muscle contraction — In pursuit of its structural basis —. Proceedings of the Japan Academy, Series B 2015;91:321–50. https://doi.org/10.2183/pjab.91.321.

[47] WILLIAMS RJP. Binding of Zinc in Carboxypeptidase. Nature 1960;188:322–322. https://doi.org/10.1038/188322a0.

[48] Plum LM, Rink L, Haase H. The Essential Toxin: Impact of Zinc on Human Health. Int J Environ Res Public Health 2010;7:1342–65. https://doi.org/10.3390/ijerph7041342.

[49] Sun Z, Shao Y, Yan K, Yao T, Liu L, Sun F, et al. The Link between Trace Metal Elements and Glucose Metabolism: Evidence from Zinc, Copper, Iron, and Manganese-Mediated Metabolic Regulation. Metabolites 2023;13:1048. https://doi.org/10.3390/metabo13101048.

[50] Holley AK, Bakthavatchalu V, Velez-Roman JM, St. Clair DK. Manganese Superoxide Dismutase: Guardian of the Powerhouse. Int J Mol Sci 2011;12:7114–62. https://doi.org/10.3390/ijms12107114.

[51] Oliveira-Paula GH, Martins AC, Ferrer B, Tinkov AA, Skalny A V., Aschner M. The impact of manganese on vascular endothelium. Toxicol Res 2024;40:501–17. https://doi.org/10.1007/s43188-024-00260-1.

[52] O’Neal SL, Zheng W. Manganese Toxicity Upon Overexposure: a Decade in Review. Curr Environ Health Rep 2015;2:315–28. https://doi.org/10.1007/s40572-015-0056-x.

[53] Steinbrenner H, Duntas LH, Rayman MP. The role of selenium in type-2 diabetes mellitus and its metabolic comorbidities. Redox Biol 2022;50:102236. https://doi.org/10.1016/j.redox.2022.102236.

[54] Schomburg L, Köhrle J. On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health. Mol Nutr Food Res 2008;52:1235–46. https://doi.org/10.1002/mnfr.200700465.

[55] Zhang X, Liu C, Guo J, Song Y. Selenium status and cardiovascular diseases: meta-analysis of prospective observational studies and randomized controlled trials. Eur J Clin Nutr 2016;70:162–9. https://doi.org/10.1038/ejcn.2015.78.

[56] Vincent JB. The Biochemistry of Chromium. J Nutr 2000;130:715–8. https://doi.org/10.1093/jn/130.4.715.

[57] Hua Y, Clark S, Ren J, Sreejayan N. Molecular mechanisms of chromium in alleviating insulin resistance. J Nutr Biochem 2012;23:313–9. https://doi.org/10.1016/j.jnutbio.2011.11.001.

[58] Charkiewicz AE, Omeljaniuk WJ, Nowak K, Garley M, Nikliński J. Cadmium Toxicity and Health Effects—A Brief Summary. Molecules 2023;28:6620. https://doi.org/10.3390/molecules28186620.

[59] Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia A-A. Cadmium toxicity and treatment: An update. Caspian J Intern Med 2017;8:135–45. https://doi.org/10.22088/cjim.8.3.135.

[60] Wani AL, Ara A, Usmani JA. Lead toxicity: a review. Interdiscip Toxicol 2015;8:55–64. https://doi.org/10.1515/intox-2015-0009.

[61] Surenbaatar U, Lee S, Kwon J-Y, Lim H, Kim J-J, Kim Y-H, et al. Bioaccumulation of Lead, Cadmium, and Arsenic in a Mining Area and Its Associated Health Effects. Toxics 2023;11:519. https://doi.org/10.3390/toxics11060519.

[62] Haro A, Trescastro A, Lara L, Fernández-Fígares I, Nieto R, Seiquer I. Mineral elements content of wild growing edible mushrooms from the southeast of Spain. Journal of Food Composition and Analysis 2020;91:103504. https://doi.org/10.1016/j.jfca.2020.103504.

[63] Kosuth T, Leskova A, Castaings L, Curie C. Golgi in and out: multifaceted role and journey of manganese. New Phytologist 2023;238:1795–800. https://doi.org/10.1111/nph.18846.

[64] Vincent JB. Mechanisms of Chromium Action: Low-Molecular-Weight Chromium-Binding Substance. J Am Coll Nutr 1999;18:6–12. https://doi.org/10.1080/07315724.1999.10718821.