

Selenium in agriculture soils and food crops, and its potential human health risks

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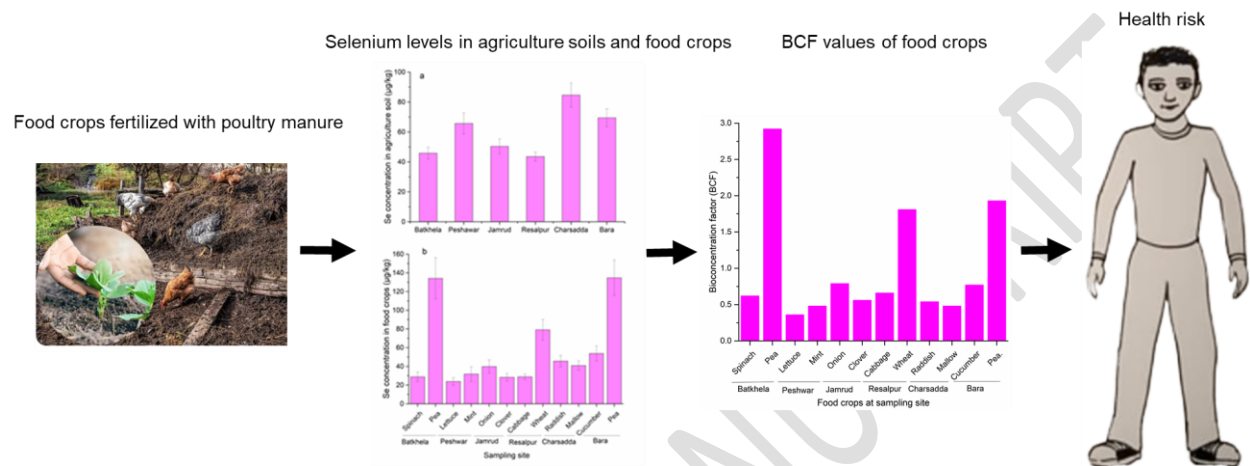
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Graphical abstract



Abstract

This study determined the levels of Se in agricultural soils (that are typically fertilized with poultry manure) and food crops such as peas, wheat, cucumbers, radishes, mallow, onions, mint, cabbage, spinach and clover were explored in Khyber Pakhtunkhwa Province (Pakistan), with inductively coupled plasma mass spectrometry. Selenium concentrations varied substantially ($P<0.05$) among all samples taken from the study area. In agricultural soils, high Se levels were observed in Charsadda and low in Resalpur, ranging from 45.9 to 84.7 $\mu\text{g kg}^{-1}$ with an order of; Charsadda > Bara > Peshawar > Jamrud > Batkhela > Resalpur. In food crops, Se concentration was highest in peas of Batkhela and Bara, ranging from 23.9 $\mu\text{g kg}^{-1}$ to 134.2 $\mu\text{g kg}^{-1}$ with an order of; pea > wheat > cucumber > radish > mallow > onion > mint > cabbage > spinach > clover > lettuce. However, daily food intake and health risk indices showed that Se levels in all the collected samples were within the permissible guidelines (40-300 $\mu\text{g kg}^{-1}$) established by the National Food Safety Standards US (2010). These findings indicate that food crops in the current study areas are free from the toxicity of Se and safe for human consumption.

Keywords

Soil, crops, selenium, health risk, vegetables

1. Introduction

Selenium (Se) is a naturally occurring mineral required for various biological roles such as antioxidant defense, reproduction, and production (Surai 2002; Fernández-Lázaro *et al.* 2020). Its nutritional value makes it indispensable for various health-related functions (Zia *et al.* 2016). Plant and animal-derived food are the key Se sources for humans (Surai & Fisinin, 2014). Deficiency of Se is associated with several human ailments that affect the muscular, immune, nervous and reproductive systems. It can also create problems in the thyroid gland and heart and can lead to cancer (Kieliszek & Bano, 2022). While, Se toxicity may cause hair and nail loss and produce disorders in humans and animals digestive and nervous systems (Tinggi 2005).

In agricultural soils, the concentration of Se is controlled by anthropogenic and natural processes, where soil parent material is the main natural source (Fordyce *et al.* 2007), and mining, agriculture, transportation, land use, metallurgy, etc. are the anthropogenic sources (Pan *et al.* 2023). Irrigation, chemical fertilizers and natural/farmyard manure are the prime agriculture activities (Bajaj *et al.* 2011). The prolonged use of these fertilizers and manures can enhance the concentration of Se soil (Wang *et al.* 2016). Selenium concentration in food crops depends on the plant's accumulating capacity and soil properties such as soil Se levels, pH, texture, mineral composition, organic matter, and competing ions (Dumont *et al.* 2006). Fruits mostly contain less Se compared to vegetables, while cereals contain Se ranging from 0.01 and 0.55 $\mu\text{g g}^{-1}$ (Qin *et al.* 2013). Brassica species, Garlic and Brazil nuts are excellent Se dietary sources (Bodnar *et al.* 2012). However, Se concentration in food crops still needs to be understood.

In plants, the aboveground parts accumulate more Se than the roots but less than the seeds. The availability of Se in soil and its subsequent accumulation in plant tissues depends on pH,

cation exchange capacity, organic matter, texture, micronutrients, climate, plant physiology and translocation mechanisms (Xu et al., 2024). However, in some cases, plant stems and leaves accumulate much more Se than grains (Kapital'chuk & Golubkina, 2008). Moreover, during seedling growth, younger leaves have greater Se concentration than older ones (Harris *et al.* 2014). Selenium is mostly stored in plant cell vacuoles (Chauhan *et al.* 2019). It can also be transported out through sulfate transporters present in the tonoplast (Somagattu *et al.* 2024). Plants can be categorized into hyperaccumulators, secondary accumulators, and non-accumulators based on the amount of Se stored in their cells (Galeas *et al.* 2007). Hyperaccumulator are those plants that store high quantities of Se in their cells (i.e., >1000 mg Se Kg⁻¹ DW). These plants prefer to grow in regions of the world that have high Se contents. They contain methylated forms of selenocysteine (SeCys) and selenomethionine (SeMet), provide the plants with Se tolerance, and they can be further vaporized as dimethyl diselenide (DMDSe). Hyperaccumulators consist of *Astragalus species*, *Neptunia*, *Stanleya*, *Conopsis*, *Xylorhiza*, etc. Secondary-accumulators are those plants that store Se and show no signs of toxicity upto 100-1000 mg Se Kg⁻¹ DW; e.g., *Brassicajuncea*, *Brassicanapus*, *Broccoli*, *Aster*, *Helianthus*, *Medicago sativa*, *Camelina*, etc. Non-accumulators are those plants that store < 100 mg Se Kg⁻¹ of their DW. These plants cannot survive or grow on Se-rich soils, exhibit retarded growth, and volatilize Se to dimethyl selenide (DMSe); for example, grasses and crops (Galeas *et al.* 2007).

Selenium is known as a two-edged sword worldwide due to its deficiency and toxicity to both humans and animals. Therefore, this research aimed to assess Se concentration in agricultural soils, food crops, and their potential health risks.

2. Materials and methods

2.1. Samples collection

Soil samples were randomly collected from a depth of 20 cm from each field. After transportation, samples were air dried and sieved through 2 mm mesh. These samples were sealed in Kraft-paper envelopes and stored until analyses (Khan et al., 2008). The food crops (n=36, edible parts) were also collected from the same agricultural fields where poultry manure was used as a fertilizer such as pea (*Pisum sativum*) and spinach (*Spinacia oleracea*) from Batkhela, lettuce (*Lactuca sativa* L) and mint (*Mentha arvensis* L) from Peshawar, onion (*Allium cepa*) and clover (*Trifolium pratense*) from Jamrud, wheat (*Triticum aestivum*) and cabbage (*Brassica oleracea* L) from Resalpur, radish (*Raphanus sativus*) and mallow (*Malva neglecta*) from Charsadda, cucumber (*Cucumis sativus*) and pea (*Pisum sativum*) from Bara in Khyber Pakhtunkhwa, Pakistan (Fig. 1). Plants were cleaned with ordinary tap water and then with deionized water. After air drying for a day, the samples were baked for 24 hours at 70-80°C. Dry samples were ground and sieved through a 2 mm mesh. Soil samples were also air-dried, sieved through a 2 mm mesh and then kept in polythene bags. All the samples were stored for further analysis (Rehman et al. 2017). The basic characteristics of soil, like EC and pH were determined in a solution of soil and deionized water by Accumet XL 60 m equipped with both electrodes (pH and EC). Mastersizer 2000 (Malvern Instruments Ltd, UK) was used to determine the soil particle size (sand, silt, and clay) according to the operational manual of the instrument (Waqas et al. 2014).

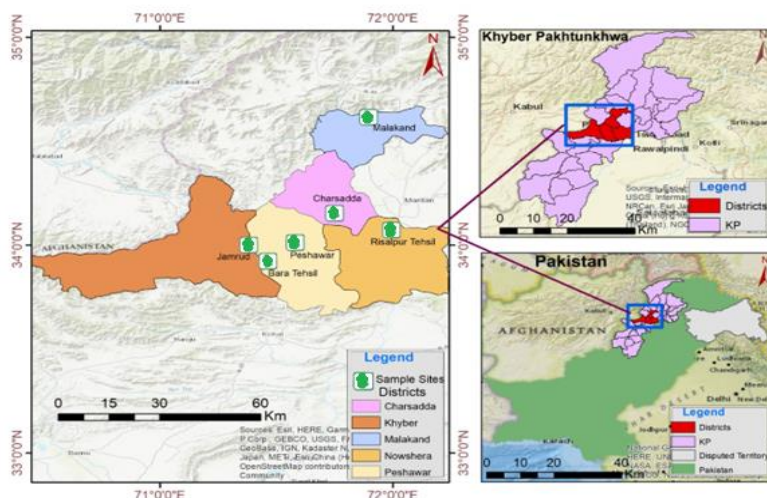


Fig1. Location map shows the study areas from where agricultural soils and food crops were collected

2.2. Sample extraction

The soil samples were extracted using strong acids extraction method. Specifically, 0.5 g of soil was placed in a flask and mixed with a 15 mL solution of HNO_3 and HCl in a 1:3 ratio (Ravindran *et al.* 2017). After 24 h, the samples were heated on a hot plate at 80°C to obtain clear extracts. The extract was then heated with 5 mL HClO_4 and again heated until close to dry. Extracts were diluted once again, cooled, filtered again, and added with milliQ water to make 50 ml the total volume of the sample.

About 2 g of dry powder extract from food plants were placed in Teflon tubes. Then, 10 ml of HNO_3 solution was added to it and left overnight. These samples were then heated at 110°C for 2 hours on a hot plate until they were almost dry. The transparent fluid was cooled and then about 05 ml of HClO_4 solution was added and again heated to pursue the process of digestion (Marwa *et al.* 2012). Lastly, this sample underwent filtration with a membrane exhibiting pore diameter of $0.22\ \mu\text{m}$, and then dilution with milliQ H_2O until the volume reached 25 mL. The solutions

were further quantified for the presence of Se and then stowed at normal room temperature (Khan *et al.* 2008; Rehman *et al.* 2017).

2.3. Analysis of selenium

The quantity of Se in the extracted samples was detected by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies, 7500 CX, USA). The ICP-MS was operated based on our previously published parameters (Saeed *et al.* 2024). All samples were examined at the College of Resource and Environment, Hainan Agriculture University, Changsha, China.

2.4. Quality control measures

Preparing all the solutions and standards in ultrapure water to ensure the experiment's accuracy and precision. The reagent blanks and standard reference materials were included in each batch. Plant and soil reference materials (GBW07603-GSV-2 and GBW07406-GSS-6, respectively) were purchased from the National Research Center for Standards in China. Recovery rate for Se was ranged from 95.3 to 103.1%. The minimum detectable level of Se was between 0.5-10 µg

2.5. Bio-concentration factor (BCF)

The bio-concentration factor (BCF) was quantified using equation (1) (Sawut *et al.* 2018).

$$BCF = \frac{C_{\text{plant}}}{C_{\text{soil}}} \quad (1)$$

Where C_{plant} : Se concentration in plant, and C_{soil} : Se concentration in soil.

2.6. Daily intake of selenium (DISe)

The DISe was computed with equation (2) (Khan *et al.* 2008).

$$DISe = \frac{C_{se} \times C_{factor} \times D_{food\ intake}}{BW_{average\ weight}} \quad (2)$$

Where C_{se} is Se concentration in eatable parts, C_{factor} is the conversion factor for fresh to dry plants, and it is 0.085, $D_{food\ intake}$: is the consumption rate for food plants ingested by children and adults, and $BW_{average\ weight}$: is the average body weight. For children and adults, the average daily intake of food plants was reported as 0.232 and 0.345 kg person⁻¹ day⁻¹, respectively. Based on these figures, for adults and children the average body weight was estimated to be 70 kg and 32.7 kg, respectively (Zhong *et al.* 2018).

2.7. Health Risk Index (HRI)

The HRI was calculated by consuming Se in the eatable plants using equation (3) (Khan *et al.* 2008).

$$HRI = \frac{DISe}{RfD} \quad (3)$$

Where $DISe$ is the daily intake of Se, and RfD is the Se reference dose of 55 ug kg⁻¹. According to the United States Environmental Protection Agency (USEPA 2002), the oral reference dose values for different chemical elements vary. If the Hazard Quotient (HRI) is less than 1, then the underexposure population will have no significant health risks. However, if the HRI is greater than 1, then the underexposure population will have substantial health risks. (Kamunda *et al.* 2016).

2.8. Statistical analysis

Origin Lab (Northampton, MA) was used for bar graph construction. SPSS 21 (SPSS Chicago IL., USA) was used to calculate one-way ANOVA. The study areas map was prepared using ArcGIS version 2.18.

3. Results and discussion

3.1. Concentration of Se in soil and food crops

Selenium concentration in agriculture soils ranged from 45.9 to 84.7 $\mu\text{g kg}^{-1}$, where Se concentration was maximum in Charsadda and minimum in Resalpur, following the order of: Charsadda > Bara > Peshawar > Jamrud > Batkhela > Resalpur (Fig. 2a). According to soil-related Se threshold recommendations, regional differences in Se concentrations exist due to differing geological and environmental factors (Pan *et al.* 2023). However, it is generally recognized that Se concentrations of >200 mg/kg in soil could expose cultivated plants to toxicity, which can ultimately pass into the human food chain (Lokeshappa *et al.* 2012). A large proportion of soils worldwide possess low contents of Se (usual range 0.01–2.0 mg Se kg^{-1} ; mean 0.4 mg Se kg^{-1}), whereas seleniferous soils can have concentrations ≤ 1200 mg Se/kg (Fordyce 2005; Spallholz *et al.* 2008). Geology mostly controls the amount of Se in most soils, and sandstones, specific shales, limestone, coal series and slate are linked to high Se soils (Broadley *et al.* 2006). The soils in the current study sites are sandy loam with a pH ranging from 7-8. In contrast, sandy loam soils have been previously observed to contain low Se contents (Antanaitis *et al.* 2008), because soils like peat soils or colloid-poor sandy sediments may be poor in Se due to leaching. Selenium also enters soils through rock weathering, sea spray, volatilization, the recycling of Se from biota, and atmospheric deposition of Se from volcanic activity (Antanaitis *et al.* 2008). The current study did not cover the sources mentioned above; therefore, further study is recommended to investigate the contribution of these resources to the

Se input in the study areas. Anthropogenically, Se enters soils through metal processing, fossil fuel combustion, lime and manure, applications of fertilizers, and disposal of sewage sludge (Fordyce 2005). In the current locations, poultry manure has been extensively used in the agriculture fields, which could be the potential cause of Se concentration in these soils. Earlier studies have shown high Se enrichment through manure fertilization applications (Kieliszek & Sandoval, 2023). The fluctuations of Se contents in agriculture soils in the current sites could be due to the improper (i.e. scale/rate) utilization of poultry manure.

Selenium concentration in food crops ranged from 23.9 $\mu\text{g kg}^{-1}$ to 134.2 $\mu\text{g kg}^{-1}$, following the order of pea > wheat > cucumber > radish > mallow > onion > mint > cabbage > spinach > clover > lettuce (Fig. 2b), which is consistent with the findings observed by Iqbal *et al.* (2008). However, the current findings are higher than previously reported results. Moatkhef (2020) reported that average mean concentrations of Se in vegetables ranged from 0.02- 2.30 $\mu\text{g g}^{-1}$. It may be because the agricultural lands in the current study sites are mostly fertilized with poultry manure. Pea crops from Bara and Batkehl indicated the maximum Se levels and lettuce the minimum. These results support previous findings of high Se concentration in pea plants (Ragályi *et al.* 2023) while contradicting previous findings in lettuce, where Se content was high and increased up to 302% mainly with fertilization (Puccinelli *et al.* 2022; Kieliszek & Sandoval, 2023). In plants, Se can accumulate above the permissible limits (40-300 $\mu\text{g kg}^{-1}$) set by national food safety standards US (2010) (Bugang & Woolsey, 2010). Selenium uptake in plants relies on the species and is influenced by several factors, including CaCO_3 content, pH, salinity, and competing ions such as sulfate and phosphate (Kabata Pendias 2001; Dhillon & Dhillon, 2003; Wu 2004; Kaur *et al.* 2014).

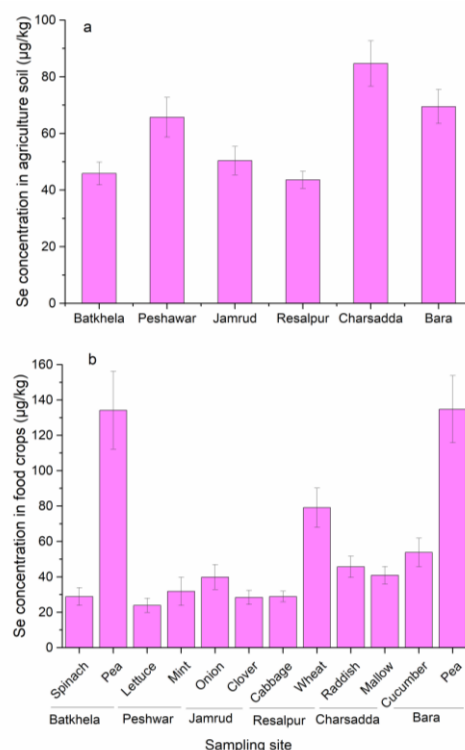


Fig. 2. (a) Se concentration in agriculture soils and (b) in different food crops in the study sites.

3.2. Bioconcentration factor (BCF)

The BCF values ranged from 0.36 to 2.92 across different food crops, with notable BCF values (>1) observed for peas both in Batkhela and Bara sites, followed by wheat in Resalpur (Fig. 3). This finding is consistent with prior research showing that leguminous plants collect more Se than cereals and leafy vegetables (Chilimba *et al.* 2014; Ngigi *et al.* 2019), because, legumes contain a higher protein levels ranging between 20-40% than cereals that have less than 15% protein levels (Poblaciones *et al.* 2014, 2015). These results contradicted to the findings reported by Kielisze *et al.* (2021), which summarized that Se concentration is greater in green grasses and leafy vegetables than in leguminous plants as more surface area is available. The use of poultry manure as an organic fertilizer in agricultural fields holds the dual potential of increasing the

availability of crucial minerals while posing harmful metal accumulation risks in food crops (Siddiqui *et al.* 2021; Muhammad *et al.* 2020). The occurrence of soil-related elements including nitrogen (N), sulfur (S), phosphorus (P), and Se have a vital role in modulating the bioavailability of trace elements (Gupta *et al.* 2018). Additionally, variations in BCF among food crops can be linked to differences in their accumulation rates, the variability of heavy metals, and nutrients present in poultry manure, applied in agricultural fields. Furthermore, soil characteristics such as pH, organic matter content, microbial community composition, water solubility, and sorption capability as well as physiological features intrinsic to different plant species contribute to the variability in trace elements uptake and accumulation levels by plants and their subsequent transfer factors (Nawab *et al.* 2018). These findings collectively indicated that various factors and mechanisms including, physicochemical and biological parameters and soil/plant types influence BCF values.

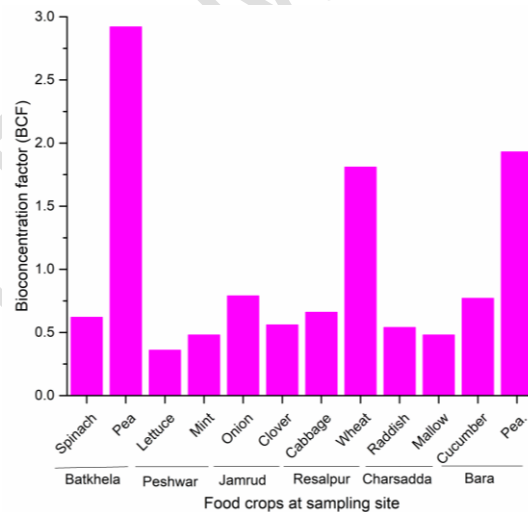


Fig. 3. The BCF values of food crops grown in agriculture soils treated with poultry manure.

3.3. Daily intake of selenium

208 The DISe values for children (32.7 kg) and adults (73 kg) were assessed to determine the
 209 possible health hazards related to Se ingestion through food crops are shown in Table 1. In
 210 adults, DISe ranged from 8.38E-06 kg/person/day to 1.173 E-05 kg person⁻¹ day⁻¹, with spinach
 211 and lettuce having the highest DISe and cabbage and clover having the lowest. In children, DISe
 212 ranged from 7.84E-05 kg person⁻¹ day⁻¹ to 1.2E-05 kg person⁻¹ day⁻¹, with peas having the
 213 highest DISe and lettuce and spinach the lowest. Overall, Bara showed the highest DISe values,
 214 and Peshawar had the lowest DISe values for adults and children (Table 2). Various
 215 organizations have established different acceptable thresholds for the DISe to keep optimum
 216 nutrition. As a result, there is significant variation in the suggested diurnal confines for a healthy
 217 population. For example, the expert panel of the National Academy of Sciences (2000), the Food
 218 and Agriculture Organization (FAO 2004), and the Canadian Council of Ministers of the
 219 Environment (CCME 2009) agree that a DISe from 50 to 200 µg is adequate and harmless (Qin
 220 *et al.* 2013). Similarly, 70 µg of Se intake for men (≥14 years), 65 µg for pregnant women, 75 µg
 221 for breastfeeding women and 60 µg for women were recommended by the Superior Health
 222 Council of Belgium (2012) (Waegeneers *et al.* 2013). FAO/WHO (1999) suggested that women
 223 need at least 16 µg/day of Se, men need 21 µg/day, and other individuals need 40 µg/day (Fisinin
 224 *et al.* 2008). A maximum Se intake limit of 60 µg is recommended for children (1-3 years old),
 225 250 µg for adolescents (15-17 years old), and 300 µg for adults by the European Food Safety
 226 Authority (EFSA, 2023) has established. In contrast, for adults, the Institute of Medicine (2000)
 227 recommended the maximum Se intake level of 400 µg day⁻¹. These outcomes collectively
 228 indicate that all food crops in the present study areas are safe for consumption, as they do not
 229 contain toxic levels of selenium.

230 The HRI linked to Se intake from food crops was <1 for both adults and children in all of
 231 the selected sites (Table 1), with values ranging from 0.002 kg/person/day to 0.011
 232 kg/person/day and from 0.002 kg person⁻¹ day⁻¹ to 0.016 kg person⁻¹ day⁻¹, respectively. These
 233 indices indicate that the amount of Se is within the permissible guidelines and pose no health
 234 hazards. The Hazard Quotient (H.Q.) values (<1) indicate a negligible potential risk from
 235 exposure through these food chains (Li *et al.* 2017). Previous research stated selenium's
 236 antitoxic, antiviral, anti-carcinogenic, antioxidant, radio-protective, and immune-stimulating
 237 properties (Saeed *et al.* 2024). The chronic and subchronic reference doses (RfDs) for Se and
 238 selenious acid (H₂SeO₃) compounds are set at 0.055 mg kg⁻¹ day⁻¹, and they are non-carcinogenic
 239 to humans (U.S. Environmental Protection Agency 1992). Conversely, SeS₂ is categorised as a
 240 likely human carcinogen and assigned to Group B2. Still, insufficient quantitative information
 241 is available to determine the SeS₂ slope factor (US Environmental Protection Agency
 242 Recommendations for Exposure Risk Assessment 1992).

243 **Table 1.** Daily intake of Se (DISE) and Health risk index (HRI) through ingestion of vegetables
 244 taken from the study sites

| Part | Cse (mg kg ⁻¹) | CF | Adult | | | | Children | | | |
|----------|-------------------------------|-------|----------------|---|----------|-------|----------------|---|----------|-------|
| | | | Weight (Kg) | DFI (Kg person ⁻¹ day ⁻¹) | DISE | HRI | Weight (Kg) | DFI (Kg person ⁻¹ day ⁻¹) | DISE | HRI |
| Spinach | 0.02 | 0.085 | 70 | 0.345 | 8.00E-06 | 0.002 | 32.7 | 0.232 | 1.20E-05 | 0.002 |
| Pea | 0.13 | 0.085 | 70 | 0.345 | 5.40E-05 | 0.011 | 32.7 | 0.232 | 7.80E-05 | 0.016 |
| Lettuce | 0.02 | 0.085 | 70 | 0.345 | 8.00E-06 | 0.002 | 32.7 | 0.232 | 1.20E-05 | 0.002 |
| Mint | 0.03 | 0.085 | 70 | 0.345 | 1.30E-05 | 0.003 | 32.7 | 0.232 | 1.80E-05 | 0.004 |
| Raddish | 0.045 | 0.085 | 70 | 0.345 | 1.90E-05 | 0.004 | 32.7 | 0.232 | 2.70E-05 | 0.005 |
| Mallow | 0.04 | 0.085 | 70 | 0.345 | 1.70E-05 | 0.003 | 32.7 | 0.232 | 2.40E-05 | 0.005 |
| Cabbage | 0.028 | 0.085 | 70 | 0.345 | 1.20E-05 | 0.002 | 32.7 | 0.232 | 1.70E-05 | 0.003 |
| Wheat | 0.079 | 0.085 | 70 | 0.345 | 3.30E-05 | 0.007 | 32.7 | 0.232 | 4.80E-05 | 0.01 |
| Onion | 0.039 | 0.085 | 70 | 0.345 | 1.60E-05 | 0.003 | 32.7 | 0.232 | 2.40E-05 | 0.005 |
| Clover | 0.028 | 0.085 | 70 | 0.345 | 1.20E-05 | 0.002 | 32.7 | 0.232 | 1.70E-05 | 0.003 |
| Cucumber | 0.053 | 0.085 | 70 | 0.345 | 2.20E-05 | 0.004 | 32.7 | 0.232 | 3.20E-05 | 0.006 |

Table 2. Area-wise daily intake of Se (DISE) and health risk index (HRI) through ingestion of vegetables

| Part | Region | Cse (mg kg ⁻¹) | CF | Wt t | DFI (Kg person ⁻¹ day ⁻¹) | Adult DISE | HRI | Wt Kg | DFI (Kg person ⁻¹ day ⁻¹) | Children DISE | HRI |
|-----------|-----------|----------------------------|-------|---------|---|---------------|-------------|----------|---|------------------|-------------|
| Vegetable | Batkhela | 0.08 | 0.085 | 70 | 0.345 | 3.35143E-05 | 0.006702857 | 32.7 | 0.232 | 4.82446E-05 | 0.00964893 |
| | Peshawar | 0.03 | 0.085 | 70 | 0.345 | 1.13111E-05 | 0.002262214 | 32.7 | 0.232 | 1.62826E-05 | 0.003256514 |
| | Jamrud | 0.03 | 0.085 | 70 | 0.345 | 1.42436E-05 | 0.002848714 | 32.7 | 0.232 | 2.0504E-05 | 0.004100795 |
| | Resalpur | 0.05 | 0.085 | 70 | 0.345 | 2.26221E-05 | 0.004524429 | 32.7 | 0.232 | 3.25651E-05 | 0.006513028 |
| | Charsadda | 0.04 | 0.085 | 70 | 0.345 | 1.67571E-05 | 0.003351429 | 32.7 | 0.232 | 2.41223E-05 | 0.004824465 |
| | Bara | 0.09 | 0.085 | 70 | 0.345 | 3.77036E-05 | 0.007540714 | 32.7 | 0.232 | 5.42752E-05 | 0.010855046 |

4. Conclusions

In this study, the levels of Se were analyzed in agricultural soils (usually fertilized with poultry manure) and food crops across various districts of Khyber Pakhtunkhwa, Pakistan. Selenium in the soil samples and food crops was within the permissible limits. Food crops in the study area are safe for consumption, as they do not contain any toxic levels of Se according to health risk assessments, however, pea crops accumulated more Se than other food crops. More research is needed in the remaining provinces of Pakistan to comprehend the levels of Se exclusively in agricultural soils and food crops. This research will provide a thorough evaluation of Se levels in food crops and the associated hazards to health.

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References

- Antanaitis A., Lubyte J., Antanaitis S., Staugaitis G. and Viskelis P. (2008), Selenium concentration dependence on soil properties, *Journal of Food, Agriculture and Environment*, 6, 163.
- Bajaj M., Eiche E., Neumann T., Winter J. and Gallert C. (2011), Hazardous concentrations of selenium in soil and groundwater in North-West India, *Journal of Hazard Material*, 189, 640-646. [10.1016/j.jhazmat.2011.01.086](https://doi.org/10.1016/j.jhazmat.2011.01.086)
- Bodnar M., Konieczka P. and Namiesnik J. (2012), The properties, functions, and use of selenium compounds in living organisms, *Journal of Environmental Science and Health, Part C: Toxicology and Carcinogenesis*, 30, 225-252. <https://doi.org/10.1080/10590501.2012.705164>
- Bolan N., Mahimairaja S., Kunhikrishnan A., Seshadri B. and Thangarajan, R. (2015), Bioavailability and ecotoxicity of arsenic species in solution culture and system: implications to remediation, *Environmental Science and Pollution Research*, 22, 8866-8875. <https://doi.org/10.1007/s11356-013-1827-2>
- Broadley M.R., White P.J., Bryson R.J., Meacham M.C., Bowen H.C., Johnson S.E, Hawkesford M.J., McGrath S.P., Zhao F.J., Breward N. and Harriman M. (2006), Biofortification of UK food crops with selenium, *Proceedings of the Nutrition Society*, 65, 69-181. <https://doi.org/10.1079/PNS2006490>
- Bugang W. and Woolsey M. (2010), National Food Safety Standard-Maximum Levels of Contaminants in Foods (GB 2762-2010), Global Agricultural Information Network, USDA Washington DC, 2-7.
- Chauhan R., Awasthi S., Srivastava S., Dwivedi S., Pilon-Smits EA., Dhankher O.P. and Tripathi R.D. (2019), Understanding selenium metabolism in plants and its role as a beneficial element, *Critical Reviews in Environmental Science and Technology*, 49, 1937-1958. <https://doi.org/10.1080/10643389.2019.1598240>
- Chilimba A.D., Young S.D. and Joy E.J. (2014), Agronomic biofortification of maize, soybean and groundnut with selenium in intercropping and sole cropping systems, *African Journal of Agricultural Research*, 9, 3620-3626. <https://doi.org/10.5897/AJAR2014.8978>
- da Silva G.P., Costa L.C., Carmona V.V., Silva S.L.O. and Traspadini E.I.F. (2017), Selenium and agricultural crops, *African Journal of Agricultural Research*, 12, 2545-2554. <https://doi.org/10.5897/AJAR2016.11884>
- Dhillon K.S. and Dhillon S.K. (2003), Quality of underground water and its contribution towards selenium enrichment of the soil-plant system for a seleniferous region of northwest India, *Journal of Hydrology*, 272, 120-130. [https://doi.org/10.1016/S0022-1694\(02\)00259-7](https://doi.org/10.1016/S0022-1694(02)00259-7)
- Dumont E., Vanhaecke F. and Cornelis R. (2006), Selenium speciation from food source to metabolites: a critical review, *Analytical and Bioanalytical Chemistry*, 385, 1304-1323. <https://doi.org/10.1007/s00216-006-0529-8>
- EPA U. (2002), A review of the reference dose and reference concentration processes. In Risk Assessment Forum, U. The Environmental Protection Agency.
- Fernández-Lázaro D., Fernandez-Lazaro C.I., Mielgo-Ayuso J., Navascués L.J., Córdova Martínez A. and Seco-Calvo J. (2020), The role of selenium mineral trace element in exercise: Antioxidant defense system, muscle performance, hormone response, and athletic performance, A systematic review. *Nutrients*, 12, 1790. <https://doi.org/10.3390/nu12061790>

- Fisinin V.I., Papazyan T.T. and Surai P.F. (2008), Producing specialist poultry products to meet human nutrition requirements: selenium-enriched eggs, *World's Poultry Science Journal*, 64, 85-98. <https://doi.org/10.1017/S0043933907001742>
- Fordyce F.M., Guangdi Z., Green K. and Xinping L. (2000), Soil, grain and water chemistry in relation to human selenium-responsive diseases in Enshi District, China, *Geochemistry*, 15, 117-132. [https://doi.org/10.1016/S0883-2927\(99\)00035-9](https://doi.org/10.1016/S0883-2927(99)00035-9)
- Galeas M.L., Zhang L.H., Freeman J.L., Wegner M. and Pilon-Smits, E.A.H (2007), Seasonal fluctuations of selenium and sulfur accumulation in selenium-hyperaccumulators and related non-accumulators, *New Phytologist*, 173:517-525. [https://doi.org/10.1016/S0883-2927\(99\)00035-9](https://doi.org/10.1016/S0883-2927(99)00035-9)
- Gupta S.K., Ansari F.A., Nasr M., Chabukdhara M. and Bux F. (2018), Multivariate analysis and health risk assessment of heavy metal contents in foodstuffs of Durban, South Africa, *Environmental Monitoring Assessment*, 190, 1-15. <https://doi.org/10.1007/s10661-018-6546-1>
- Harris J., Schneberg K.A. and Pilon-Smits E.A. (2014), Interactions distinguish hyperaccumulator *Stanleya pinnata* from non-hyperaccumulator *Brassicajuncea* (Brassicaceae), *Planta*, 239, 479-491. doi:10.1007/s00425-013-1996-8
- Iqbal S., Kazi T.G., Bhanger M.I., Akhtar M. and Sarfraz R.A. (2008), Determination of selenium content in selected Pakistani foods, *International Journal of Food Science and Technology*, 43, 339-345. <https://doi.org/10.1111/j.1365-2621.2006.01447.x>
- Kabata-Pendias A. (2001), Trace elements in soils and plants. 3rd ed. Boca Raton (FL): CRC Press; 241–252.
- Kamunda C., Mathuthu M. and Madhuku M. (2016), Health risk assessment of heavy metals in soils from Witwatersrand Gold Mining Basin, South Africa, *International Journal of Environmental Research and Public Health*, 13, 663. <https://doi.org/10.3390/ijerph13070663>
- Kapital'chuk M.V., Golubkina N.A. (2008), Selenium bioaccumulation by plants on different soil types in Moldova, *Agro HHI*, 4-6.
- Kaur N., Sharma S., Kaur S. and Nayyar H. (2014), Selenium in agriculture: a nutrient or contaminant for crops? *Archives of Agronomy and Soil Science*, 60, 1593-1624. <https://doi.org/10.1080/03650340.2014.918258>
- Khan S., Cao Q., Zheng Y.M., Huang Y.Z. and Zhu Y.G. (2008), Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China, *Environmental Pollution*, 152, 686-92. <https://doi.org/10.1016/j.envpol.2007.06.056>
- Kieliszek M. and Sandoval S.N.S. (2023), The importance of selenium in food enrichment processes. A comprehensive review, *Journal of Trace Elements in Medicine and Biology*, 127260. <https://doi.org/10.1016/j.jtemb.2023.127260>
- Kieliszek M., Bano I. and Zare H. (2022), A comprehensive review on selenium and its effects on human health and distribution in middle eastern countries, *Journal of Trace Elements in Medicine and Biology*, 200, 971-87. <https://doi.org/10.1007/s12011-021-02716-z>
- Kieliszek M. and Bano I. (2022), Selenium as an important factor in various disease states-a review, *Experimental and Clinical Sciences*, 21, 948. [10.17179/excli2022-5137](https://doi.org/10.17179/excli2022-5137)
- Li Z., Liang D., Peng Q., Cui Z., Huang J. and Lin Z. (2017), Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: A review, *Geoderma*, 295, 69-79. <https://doi.org/10.1016/j.geoderma.2017.02.019>
- Liu W., Yang X., Duan L., Naidu R., Yan K., Liu Y. and Chen Y. (2021), Variability in plant trace element uptake across different crops, soil contamination levels and soil properties in

the Xinjiang Uygur Autonomous Region of northwest China, *Scientific Reports*, 11, 1-13.
<https://doi.org/10.1038/s41598-021-81764-w>

Lokeshappa B., Shivpuri K., Tripathi V. and Dikshit A.K. (2012), Assessment of toxic metals in agricultural produce, *Public Health Nutrition*, 2, 24-29.

Moatkhef F., Ismail H., Agamy N. and Aborhyem S. (2020), Quantitative determination of selenium in the most common food items sold in Egypt, *The Journal of the Egyptian Public Health Association*, 95, 1-9. <https://doi.org/10.1186/s42506-020-00044-z>

Muhammad J., Khan S., Lei M., Khan M.A., Nawab J., Rashid A. and Khisro S.B. (2020), Application of poultry manure in agriculture fields leads to food plant contamination with potentially toxic elements and causes health risk, *Environmental Technology & Innovation*, 19, 100909. <https://doi.org/10.1016/j.eti.2020.100909>

Nawab J., Farooqi S., Xiaoping W., Khan S. and Khan A. (2018), Levels, dietary intake, and health risk of potentially toxic metals in vegetables, fruits, and cereal crops in Pakistan, *Environmental Science and Pollution Research*, 25, 5558-5571.
<https://doi.org/10.1007/s11356-017-0764-x>

Ngigi P.B., Lachat C., Masinde P.W. and Du Laing G. (2019), Agronomic biofortification of maize and beans in Kenya through selenium fertilization, *Environmental Geochemistry and Health*, 41, 2577-2591. <https://doi.org/10.1007/s10653-019-00309-3>

Pan Z., Feng Y., Wang M., Meng W. and Chen J. (2023), Geochemical characteristics of soil selenium and evaluation of selenium-rich land resources in Guiyang area, *Frontiers in Geochemistry*, 1, 1094023. <https://doi.org/10.3389/fgeoc.2023.1094023>

Poblaciones M.J., Rodrigo S. and Santamaria O. (2015), Biofortification of legumes with Selenium in Semiarid Conditions.

Poblaciones M.J., Rodrigo S., Santamaría O., Chen Y. and McGrath S.P. (2014), Agronomic selenium biofortification in *Triticum durum* under Mediterranean conditions: from grain to cooked pasta, *Food Chemistry*, 146, 378-384.
<https://doi.org/10.1016/j.foodchem.2013.09.070>

Puccinelli M., Malorgio F., Pintimalli L., Rosellini I. and Pezzarossa B. (2022), Biofortification of lettuce and basil seedlings to produce selenium enriched leafy vegetables, *Horticulture*, 8, 801. <https://doi.org/10.3390/horticulturae8090801>

Qin H.B., Zhu J.M., Liang L., Wang M.S. and Su H. (2013), The bioavailability of selenium and risk assessment for human selenium poisoning in high-Se areas, China, *Environment International*, 52, 66-74. <https://doi.org/10.3390/horticulturae8090801>

Qingyun W.A., Zhang J., Bingzi Z.H., Xiuli XI., Xihai DE. and Zhang H. (2016), Influence of long-term fertilization on selenium accumulation in soil and uptake by crops, *Pedosphere*, 26, 120-9. [https://doi.org/10.1016/S1002-0160\(15\)60028-5](https://doi.org/10.1016/S1002-0160(15)60028-5)

Ragályi P., Takács T., Soós Á., Kovács B., Dernovics M., Lončarić Z., Dobosy P., Záray G. and Rékási M. (2023), Quantitative analysis of selenium species in the edible parts of cabbage, carrot, tomato and green pea treated with selenate-enriched irrigation water, *Plant and Soil*, 1-20. <https://doi.org/10.1007/s11104-023-06365-0>

Ravindran B., Mupambwa H.A., Silwana S. and Mnkeni P.N. (2017), Assessment of nutrient quality, heavy metals and phytotoxic properties of chicken manure on selected commercial vegetable crops, *Heliyon*, 3. [10.1016/j.heliyon.2017.e00493](https://doi.org/10.1016/j.heliyon.2017.e00493)

Saeed S., Khan A., Haider I., Waqas M., Nawab J., Kamran M. and Khan S. (2024), Assessment of selenium in poultry food, chicken, eggs and their potential health risks in

- Khyber Pakhtunkhwa, Pakistan, *Journal of Food Composition and Analysis*, 125, 105767.
<https://doi.org/10.1016/j.jfca.2023.105767>
- Sawut R., Kasim N., Maihemuti B., Hu L., Abliz A., Abdujappar A. and Kurban M. (2018), Pollution characteristics and health risk assessment of heavy metals in the vegetable bases of northwest China, *Science of the Total Environment*, 642, 864-878.
<https://doi.org/10.1016/j.scitotenv.2018.06.034>
- Siddiqui H.J., Gul S., Shaheen U., Rehman G.B. and Khan N. (2021), Poultry manure as an organic fertilizer with or without biochar amendment: Influence on growth and heavy metal accumulation in lettuce and spinach and soil nutrients, *PHYTON INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY*, 90, 651. [10.32604/phyton.2021.011413](https://doi.org/10.32604/phyton.2021.011413)
- Somagattu P., Chinnannan K., Yammanuru H., Reddy U.K. and Nimmakayala P. (2024), Selenium dynamics in plants: Uptake, transport, toxicity, and sustainable management strategies, *Science of the Total Environment*, 175033.
<https://doi.org/10.1016/j.scitotenv.2024.175033>
- Spallholz J.E., Mallory B.L., David R.J., Smith L., Rahman M. and Hook J Rigdon R. (2008), Selenium and arsenic content of agricultural soils from Bangladesh and Nepal. *Toxicol Environmental Chemistry*, 90, 203-210. <https://doi.org/10.1080/02772240701419347>
- Surai P.F. (2002), Selenium in poultry nutrition: a new look at an old element. 1. Antioxidant properties, deficiency, and toxicity, *World's Poultry Science Journal*, 58, 333-347.
<https://doi.org/10.1079/WPS20020032>
- Surai P.F. and Fisinin VI. (2014), Selenium in poultry breeder nutrition: An update, *Animal Feed Science and Technology*, 191, 1-15. <https://doi.org/10.1016/j.anifeedsci.2014.02.005>
- Tinggi U. (2005), Selenium toxicity and its adverse health effects. In *Reviews in Food and Nutrition Toxicity*, Volume 4 (pp. 29-56). CRC Press.
- U.S. Environmental Protection Agency (U.S. Environmental Protection Agency Guidelines for exposure assessment. U.S.EPA: Washington, DC, USA, (1992).
<http://www.epa.gov/ncea/pdfs/guidline.pdf>.
- ur Rehman M.Z., Rizwan M., Ali S., Sabir M. and Sohail MI. (2017), Contrasting effects of organic and inorganic amendments on reducing lead toxicity in wheat, *Bulletin of Environmental Contamination and Toxicology*, 99, 642-647.
<https://doi.org/10.1007/s00128-017-2177-4>
- Waegeneers N., Thiry C., De Temmerman L., Ruttens A. (2013), Predicted dietary intake of selenium by the general adult population in Belgium, *Food additives and contaminants: Part A*, 30, 278-285. <https://doi.org/10.1080/19440049.2012.746474>
- Waqas M., Khan S., Qing H., Reid B.J. and Chao C. (2014), The effects of sewage sludge and sewage sludge biochar on PAHs and potentially toxic element bioaccumulation in *Cucumis sativa L*, *Chemosphere*, 105, 53-61. <https://doi.org/10.1016/j.chemosphere.2013.11.064>
- World Health Organization. (1996), *Trace elements in human nutrition and health*. World Health Organization.
- Wu L. (2004), Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium, *Ecotoxicology and Environmental Safety*, 57:257-269. [https://doi.org/10.1016/S0147-6513\(03\)00064-2](https://doi.org/10.1016/S0147-6513(03)00064-2)
- Zhang K., Guo X., Zhao Q., Han Y., Zhan T., Li Y. and Zhang J. (2020), Development and application of a HPLC-ICP-MS method to determine selenium speciation in the muscle of pigs treated with different selenium supplements. *Food Chemistry*, 302, 125371.
<https://doi.org/10.1016/j.foodchem.2019.125371>

- 451 Zhao C., Ren J., Xue C. and Lin E. (2005), Study on the relationship between soil selenium and
452 plant selenium uptake, *Plant and Soil*, 277,197-206. [https://doi.org/10.1007/s11104-005-](https://doi.org/10.1007/s11104-005-7011-9)
453 [7011-9](https://doi.org/10.1007/s11104-005-7011-9)
- 454 Zhong W., Zhang Y., Wu Z., Yang R., Chen X., Yang J. and Zhu L. (2018), Health risk
455 assessment of heavy metals in freshwater fish in the central and eastern North
456 China, *Ecotoxicology and Environmental Safety*, 157, 343-349.
457 <https://doi.org/10.1016/j.ecoenv.2018.03.048>
- 458 Xu Z, Zhou W, Zhou Y, Cui H, Liu R, Shang
459 G (2024) Factors controlling accumulation and bioavailability of selenium in paddy soils: A
460 case study in Luxi County, China *Environmental Pollution* vol 348
<https://doi.org/10.1016/j.envpol.2023.123196>
- 461 Khan, S., Q. Cao, Y.M. Zheng, Y.Z. Huang, Y.G. Zhu. Health risks of heavy metals in
462 contaminated soils and food crops irrigated with wastewater in Beijing, China.
463 *Environmental Pollution*. 152 (2008) 686-692