

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

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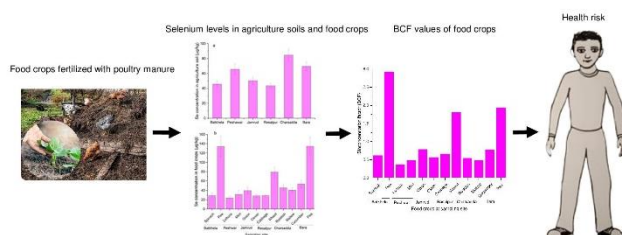
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Received: 25/01/2025, Accepted: 08/08/2025, Available online: 12/09/2025

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<https://doi.org/10.30955/gnj.07288>

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*, *Brassicajuncea*, *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 (Figure 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).

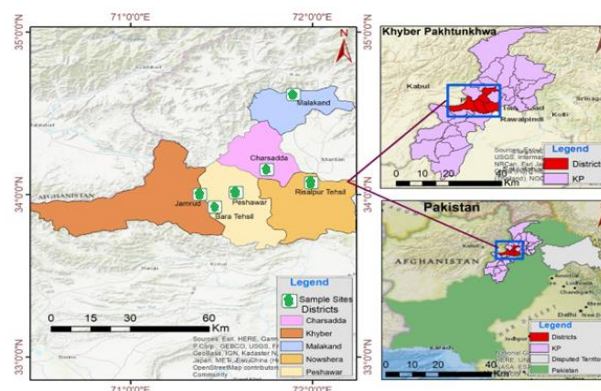


Figure 1. Location map shows the study areas from where agricultural soils and food crops were collected

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 HNO₃ 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 HClO₄ 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 HNO₃ 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 HClO₄ 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 µm, and then dilution with milliQ H₂O until the volume reached 25 mL. The solutions were further quantified for the presence of Se and then stored 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 $\mu\text{g kg}^{-1}$.

2.5. Bio-concentration factor (BCF)

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

$$\text{BCF} = \frac{C_{\text{Plant}}}{C_{\text{Soil}}} \quad (1)$$

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).

$$\text{DISE} = \frac{C_{\text{se}} \cdot C_{\text{factor}} \cdot D_{\text{food intake}}}{\text{BW}_{\text{average weight}}} \quad (2)$$

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, $D_{\text{food intake}}$: is the consumption rate for food plants ingested by children and adults, and $\text{BW}_{\text{average weight}}$: is the average body weight. For children and adults, the average daily intake of food plants was reported as 0.232 and 0.345 $\text{kg person}^{-1} \text{ day}^{-1}$, 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).

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

Where DISE is the daily intake of Se, and RfD is the Se reference dose of 55 $\mu\text{g kg}^{-1}$. 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 (**Figure 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 (**Figure 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 Batkhela 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 ($40\text{--}300\text{ }\mu\text{g kg}^{-1}$) 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).

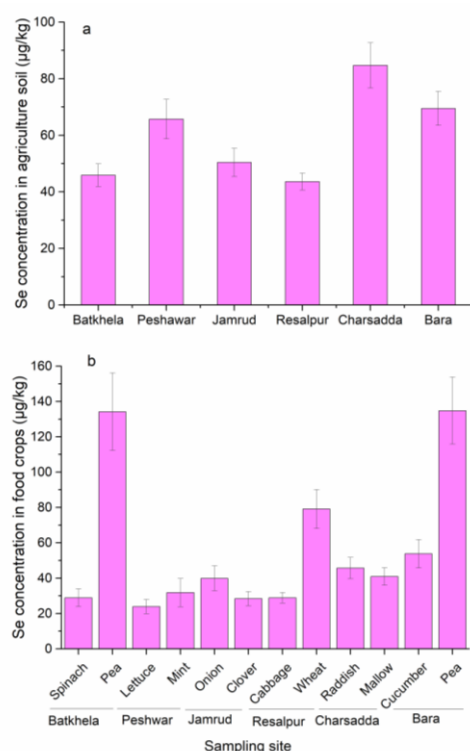


Figure 2. (a) Se concentration in agriculture soils and (b) in different food crops in the study sites.

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 (Figure 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.

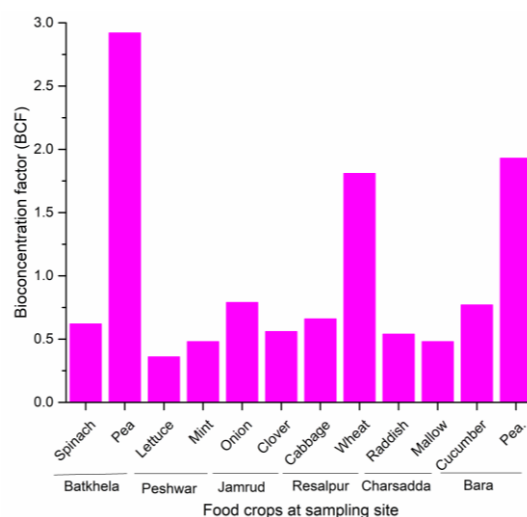


Figure 3. The BCF values of food crops grown in agriculture soils treated with poultry manure.

3.3. Daily intake of selenium

The DISe values for children (32.7 kg) and adults (73 kg) were assessed to determine the possible health hazards related to Se ingestion through food crops are shown in Table 1. In adults, DISe ranged from $8.38\text{E-}06\text{ kg/person/day}$ to $1.173\text{ E-}05\text{ kg person}^{-1}\text{ day}^{-1}$, with spinach and lettuce having the highest DISe and cabbage and clover having the lowest. In children, DISe ranged from $7.84\text{E-}05\text{ kg person}^{-1}\text{ day}^{-1}$ to $1.2\text{E-}05\text{ kg person}^{-1}\text{ day}^{-1}$, with peas having the highest DISe and lettuce and spinach the lowest. Overall, Bara showed the highest DISe values, and Peshawar had the lowest DISe values for adults and children (Table 2). Various organizations have established different acceptable thresholds for the DISe to keep optimum nutrition. As a result, there is significant variation in the suggested diurnal confines for a healthy population. For example, the expert panel of the National Academy of Sciences (2000), the Food and Agriculture Organization (FAO 2004), and the Canadian Council of Ministers of the Environment (CCME 2009) agree that a DISe from $50\text{ to }200\text{ }\mu\text{g}$ is adequate and harmless (Qin *et al.* 2013). Similarly, $70\text{ }\mu\text{g}$ of Se intake for men (≥ 14 years),

65 µg for pregnant women, 75 µg for breastfeeding women and 60 µg for women were recommended by the Superior Health Council of Belgium (2012) (Waegeneers et al. 2013). FAO/WHO (1999) suggested that women need at least 16 µg/day of Se, men need 21 µg/day, and other individuals need 40 µg/day (Fisinin *et al.* 2008). A maximum Se intake limit of 60 µg is recommended for children (1-3 years old), 250 µg for adolescents (15-17 years old), and 300 µg for adults by the European Food Safety Authority (EFSA, 2023) has established. In contrast, for adults, the Institute of Medicine (2000) recommended the maximum Se intake level of 400 µg day⁻¹. These outcomes collectively indicate that all food crops in the present study areas are safe for consumption, as they do not contain toxic levels of selenium.

The HRI linked to Se intake from food crops was <1 for both adults and children in all of the selected sites (Table 1), with values ranging from 0.002 kg/person/day to 0.011

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

Table 1. Daily intake of Se (DISe) and Health risk index (HRI) through ingestion of vegetables 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	Adult				Children			
				Wt Kg	DFI (Kg person ⁻¹ day ⁻¹)	DISe	HRI	Wt Kg	DFI (Kg person ⁻¹ day ⁻¹)	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.

Acknowledgments

This authors extend their appreciation for the support of the Higher Education Commission of Pakistan funded project entitled "Technology development for the prevention and mechanisms of mix-pollution caused by toxic elements and antibiotic/resistance genes in agricultural soils" (NRPU-17206), and the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU252912).

Funding

This work is financially supported by the Higher Education Commission of Pakistan funded project entitled “Technology development for the prevention and mechanisms of mix-pollution caused by toxic elements and antibiotic/resistance genes in agricultural soils” (NRPU-17206), and the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU252912).

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