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Elemental Distribution in Soils, Irrigation Water, and Vegetable Seeds and Associated Health Risk AssessmentinKhairpur Mir's, Pakistan

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Abstract

This study investigated the concentrations of essential macronutrients (Na, K, Mg, Ca) and trace elements (Al, Zn, Mn, Se, Sn, Cr) in seed samples of okra (*Abelmoschusesculentus* (L.) Moench), brinjal (*Solanummelongena* L.), cucumber (*Cucumissativus* L.), Indian squash (Benincasafistulosa), and bitter gourd (*Momordicacharantia* L.), soil, and water samples were collected. All samples were analyzed using the inductively coupled plasma optical emission spectroscopy (ICP-OES) method. The highest concentrations in seeds were observed for Na (525.51 mg/kg in okra) and K (1028.53 mg/kg in cucumber). The study also found a wide range of concentrations for Mg, Ca, and other trace elements. To evaluate human health risks, the Estimated Daily Intake (EDI), Hazard Quotient (HQ), Total Hazard Quotient (THQ), and Total Cancer Risk (TCR) were calculated. The EDI values for all analyzed elements were found to be low, contributing insignificantly to daily nutritional needs. The Total Hazard Quotient (THQ) for Zn, Mn, Se, and Cr ranged from 3.16×10^{-3} to 8.47×10^{-2}, and the Cancer Risk for Cr was between 5.00×10^{-7} and 4.15×10^{-5}, both below safety thresholds.The consumption of these vegetable seeds poses negligible non-carcinogenic and carcinogenic health risks from Zn, Mn, Se, and Cr.

Keywords: Chandia Mor, Thari Mirwah, ICP-OES, Estimated Daily Intake (EDI), Total Cancer Risk (TCR).

Introduction

The issue of environmental contamination, particularly by potentially toxic elements (PTEs) or heavy metals, has emerged as a significant global concern, threatening both ecological balance and human health [1]. The distribution of elements, both essential micronutrients and toxic non-essential elements within the soil-water-plant continuum, is a critical pathway for human exposure [2,3]. Soils, the foundation of agriculture, act as major sinks for both naturally occurring elements and those introduced through anthropogenic activities, such as the use of untreated wastewater for irrigation, excessive application of fertilizers and pesticides, and industrial effluents. The inherent geological composition of the region can also contribute to elevated background levels of certain elements, such as arsenic, which has been identified as a significant contaminant in the groundwater of Khairpur [4,5].

Irrigation water, often drawn from groundwater sources like tube wells and hand pumps in this area, serves as a direct conduit for elemental transfer to the soil and, subsequently, to growing vegetable crops. If this water is contaminated, the risk of elemental uptake and accumulation in edible plant tissues is high. Vegetables, which form a crucial part of the human diet, are highly susceptible to bioaccumulation, meaning they can concentrate these elements in their tissues far beyond the levels found in the surrounding soil or water [6,7]. The final and most critical link in this chain is the consumption of contaminated vegetable seeds and produce. Elements like Cadmium (Cd), Lead (Pb), and Arsenic (As), even at low concentrations, are non-essential and highly toxic, posing serious non-carcinogenic and carcinogenic health risks to the local population, especially to vulnerable groups like children [8]. High concentrations of even essential micronutrients like Iron (Fe), Copper (Cu), and Zinc (Zn), while beneficial at low levels, can also become hazardous when ingested in excess [9]. Therefore, understanding the elemental distribution across these three compartments, soil, irrigation water, and vegetable seeds,is indispensable for quantifying the exposure risk and devising effective public health strategies [10]. The primary aim of this research is to comprehensively characterize the elemental contamination status in the agricultural environment of Khairpur Mir's and evaluate the associated health hazards to the local consumers.

The aims of the present study include: determining the concentration levels of selected trace elements in the vegetable seeds, agricultural soils, and irrigation water used for vegetable cultivation across the study area. To compare the measured trace element concentrations in soil and water against the WHO/ FAO permissible limits to identify the extent of contamination. To estimate the daily intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI) of trace elements by the local population through the consumption of the contaminated vegetables and to calculate the carcinogenic risk of known carcinogens.

Materials and Methods

Chemicals / Reagents

The chemicals and reagents used for sample preparation and analysis in this study primarily consisted of various concentrations of nitric acid (HNO_3) and hydrochloric acid (HCl. For the digestion of agricultural soil samples, 70% HNO_3 acid and 32% HCl acid were provided by Fisher Scientific Canada and combined in a 1:3 ratio to form aquaregia. Additional HNO_3 solutions were utilized for preparation steps, including 10% v/v HNO_3 acid for cleaning glassware and 3% HNO_3 acid for rinsing the filtered soil digests. The seed samples were digested using a combination of 6 mL of concentrated HNO_3 (Fisher Scientific) and 2 mL of concentrated hydrogen peroxide (H_2O_2) (Fisher Scientific), where the H_2O_2 was specifically included to aid in breaking down the organic matrix. Water samples, processed via microwave digestion, were diluted with a 0.2 M HNO_3 solution after cooling, and ultrapure water was universally used for cleaning and final dilution of both seed and water samples.

Instruments

The study utilized a primary analytical system and a sample preparation system. Elemental analysis was conducted using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The ICP-OES system consisted of the iCAP 7000 Series ICP Spectrometer (Model: iCAP 7000 Series ICP Spectrometer) from Thermo Fisher Scientific (United States), which was integrated with the Teledyne CETAC Technologies ASX-280 (Model: ASX-280) autosampler from Nebraska, USA. For water sample preparation, a closed vessel microwave digestion system was employed, specifically the Milestone Ethos D model from Milestone (Sorisole-Bg, Italy).

Study Area

District Khairpur exhibits a hot desert climate, characterized by extremely hot summers with temperatures frequently surpassing 50°C, accompanied by hazy conditions and persistent dry heat leading into the monsoon season. Winters are relatively warm, contrasting starkly with the intense summers. Rainfall is sparse, primarily concentrated during the monsoon, and exhibits significant annual variability [11]. The region experiences low wind speeds and abundant sunshine year-round, while the monsoon season, though not bringing substantial rainfall, significantly increases humidity and heat indices, culminating in a typical desert climate with scorching summers, mild winters, and limited precipitation (Fig. 1) [12].

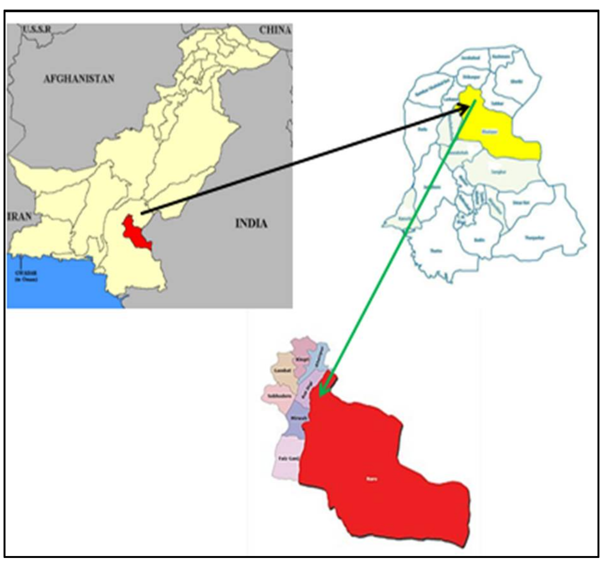

Figure 1. **Map of area under study**

Sampling
Soil sampling

Twenty agricultural soil samples were collected from locations used for the cultivation of Indian squash (*Benincasa fistulosa*), bitter gourd (*Momordica charantia* L.), brinjal (*Solanum melongena* L.), okra (*Abelmoschus esculentus* (L.) and cucumber (*Cucumis sativus* L.) were grown. The samples were taken using the procedure outlined in [13]. At each sampling site, four sub-samples, each containing two to three kilograms of soil, were removed using a shovel and pickaxe from the corners of a 1 m^2 area to a maximum depth of 25 cm. The four sub-samples were visually inspected and cleaned of gravel, debris, plant residues, and other odd objects before being combined to create a single homogenous soil mixture and put into a clean zip-lock polyethylene bag. After drying and grinding the samples with a clean mortar and pestle, they were filtered through a 200 µm nylon sieve to create a fine powder free of gravel and large particles. The samples were then taken to Shah Abdul Latif University Khairpur's research laboratory for digestion purposes.

Soil Sample Digestion and Analysis

The reagents, 70% nitric acid (HNO_3) and 32% hydrochloric acid (HCl), were provided by Fisher Scientific Canada. To make aqua-regia, concentrated HNO_3 and HCl acids were combined in ratios of 1:3. The 28 mL of aqua-regia and around one gram of each dry powdered soil were combined in a 250 mL conical glass flask. The flask was heated to 120°C on a hot plate for five hours while being gently spun to combine the reactants.Once the dissolved samples had cooled, they were filtered through filter paper (Whatman # 42), rinsed with 3% HNO_3 acid, and then placed in 100 mL HDPE bottles for analysis using ICP-OES. Before being used, every piece of glassware used to prepare the samples was cleaned by submerging it in 10% v/v HNO3 acid for a whole day and then washing it with de-ionized water [13].

Seed sampling

The study focused on analyzing heavy metal concentrations in Okra (*Abelmoschusesculentus* (L.), Brinjal (*Solanummelongena* L.), Cocumber (*Cucumissativus* L.), Indian Squash (Benincasafistulosa) and Bitter Guard (*Momordicacharantia* L.), which were collected from Kingri, Kotdeji, Khuhra, Pano-Akil, Wada Machhion, Pir –Jo – Goth, ChandiaMor, and ThariMirwah district Khairpur, utilizing ICP-OES to quantify metal levels. The seed samples were collected from the different agricultural lands under study crops cultivated when they were at full maturity stage.Thissignifies a multi-site study designed to capture variations in seed quality across diverse growing environments, ensuring samples were collected at the optimal harvest time to minimize developmental differences and accurately reflect mature seed characteristics, thus providing a representative snapshot for meaningful comparisons and analyses of the studied crops.This targeted selection of widely consumed vegetables within a defined geographic area suggests an investigation into potential local contamination sources, such as agricultural practices or industrial activities, with the ultimate goal of assessing food safety and potential health risks associated with heavy metal accumulation in these commonly consumed vegetables [14] (Table 1).

Table 1.**Collection and coding of different samples from various locations of District Khairpur.**

S. No.	Sample Name	Sample Code	*Taluka*	Scientific Name	Image
1	Okra-1	O-1	Kingri		
2	Okra-2	O-2	Kotdeji	*Abelmoschusesculentus* (L.) Moench	
3	Okra-3	O-3	Khuhra		
4	Brinjal-1	B-1	Pano-Akil		
5	Brinjal-2	B-2	Wada Machhion	*Solanummelongena* L.	
6	Brinjal-3	B-3	Kengri		
7	Cocumber-1	Cu-1	Pir –Jo – Goth		
8	Cocumber-2	Cu-2	Khuhra	*Cucumissativus* L.	
9	Cocumber-3	Cu-3	Kingri		
10	Indian Squash-1	I.S-1	Pir –Jo – Goth		
11	Indian Squash-2	I.S-2	Chandia Moor	Benincasa fistulosa	
12	Indian Squash-3	I.S-3	ThariMirwah		
13	Bitter Gourd -1	BG-1	ThariMirwah		
14	Bitter Gourd -2	BG-2	Khuhra	*Momordicacharantia* L.	
15	Bitter Gourd -3	BG-3	Kingri		

Seed Sample preparation and analysis

Each sample was weighed (1.0 g) and placed into a PTFE (polytetrafluoroethylene) high pressure jar before being digested. HNO_3 (6 mL, concentrated, Fisher Scientific) and H_2O_2 (2 mL, concentrated, Fisher Scientific) were then added. The acid was used to carefully wipe away any debris that had adhered to the vessel's walls. To help break down the organic matrix, hydrogen peroxide was used. The digestion was done using a microwave digestion system that had a temperature sensor and a segmented rotor. For 15 min, the samples were heated to 200°C. Following digestion, every sample was moved to a 50 mL volumetric flask, and the digestion vessel was cleaned with ultrapure water before the wash solution was moved to the flasks [15]. The flask was then filled to capacity with ultrapure water before being subjected to ICP-OES. In ICP systems, the gas used for the plasma, auxiliary, and nebulizer flows was almost exclusively Argon (Ar). The Plasma Gas Flow was the highest at 10 to 18 L/min to sustain the plasma and cool the torch, while the Auxiliary Gas Flow and Nebulizer Gas Flow were much lower, generally between 0.5 and 1.5 L/min. RF Power typically ranged from 1.0 to 1.5 kW, providing the energy necessary to sustain a plasma discharge at temperatures between 6,000 and 10,000 K. The Sample Uptake Rate, controlled by a peristaltic pump, typically delivered the liquid sample at 1.0 to 2.0 mL/min. Regarding the Viewing Mode, the choice between axial and radial configurations significantly impacts analytical performance. Axial viewing aligns the detector along the length of the plasma, providing a longer path length that increases sensitivity and lowers detection limits. In contrast, radial viewing captures light from the side of the plasma, which reduces background noise and chemical interferences, making it more robust for samples with high concentrations of dissolved solids(ICP-OES Parameters for Macro and Trace Elements are given in Table 2.

*Table 2.***ICP-OES Parameters for Macro and Trace Elements under study.**

Trace Element	Typical IDL (µg/L)	Wavelength (λmax, nm)
Sodium (Na)	0.5 – 5	589.592
Potassium (K)	2 – 10	766.491
Magnesium (Mg)	0.05 – 0.5	279.553
Calcium (Ca)	0.05 – 0.5	393.366
Aluminum (Al)	0.5 – 5	396.152
Zinc (Zn)	0.1 – 1.0	213.856
Manganese (Mn)	0.1 – 1.0	257.610
Selenium (Se)	5 – 20	196.026
Tin (Sn)	10 – 50	189.989
Chromium (Cr)	0.2 – 2.0	267.716

Water sampling

The irrigation water samples were collected from the same locations as the vegetable seeds, which were the agricultural lands where Okra, Brinjal, Cucumber, Indian Squash, and Bitter Gourd were cultivated across various sites in District Khairpur, including Kingri, Kotdeji, Khuhra, Pano-Akil, Wada Machhion, Pir–Jo–Goth, ChandiaMor, and ThariMirwah. A total of 45 irrigation water samples were collected for metal analysis from 15 locations. For the sample collection and preparation procedure, 500 mL of the water samples were placed into PTFE flasks. These sealed flasks were then subjected to analysis using a closed vessel microwave digestion system with a programmed heating sequence to prepare them for final elemental quantification by ICP-OES.

Microwave digestion method for water analysis

In a closed vessel microwave digestion system using the Milestone Ethos D model (Sorisole-Bg, Italy), 500 mL of water samples were placed in PTFE flasks, which were then sealed and exposed to microwave irradiation. The microwave oven’s digestion program was applied at 100 W (2 min), 250 W (6 min), 400 W (5 min), and 550 W (8 min), with ventilation for 8 min.Following cooling, the flask contents were diluted with (0.2 M) HNO_3 to 10 mL. Similarly, the same process was used to create reagent blanks. The microwave digestion method is superior to the conventional method because it extracts elements from samples more accurately and requires less time to digest water samples. It also has a lower possibility of element evaporation. Moreover, it requires less acid to digest [16].

Risk Assessment

A human health risk assessment for heavy metals from edible seeds involves several key formulas to quantify the potential health risks. These formulas are generally categorized into non-carcinogenic and carcinogenic risks.The core concepts are the estimated daily intake (EDI), the target hazard quotient (THQ), the total hazard quotient (TTHQ), and the target cancer risk (TCR) [17].

Estimated Daily Intake (EDI)

The formula is:

$$EDI = \frac{Cmetal \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

Where C_{metal}, IR, EF, ED, BW, and AT are the concentration of the metal (or element) in the vegetable, Ingestion Rate of the vegetable, Exposure Frequency, Exposure Duration, Body Weight, and Averaging Time, respectively.

Hazard Quotient (HQ)

The THQ is the ratio of the EDI to the reference dose (RfD) for a specific heavy metal.

The formula is:

$$HQ = \frac{EDI}{RfD} \quad (2)$$

Where EDI and RfD are Estimated Daily Intake and Oral Reference Dose, respectively.

Target Cancer Risk (TCR)

The TCR is calculated by multiplying the EDI by the cancer slope factor (CSF) of the heavy metal.

The formula is:

$$TCR = EDI \times SF^{0} \quad (3)$$

Table 3 comprehensively outlines the mathematical framework and variable inputs required for the human health risk assessment in the study. Section I lists the definitions and adopted values for the key variables used across these risk formulas, including the element concentration in the vegetable (C_{veg}) from the study data, the vegetable ingestion rate (IR_{veg}), the Exposure Frequency (EF) of 365 days/year, the Exposure Duration (ED) of 70 years, the standard Body Weight (BW) of 65 kg, and the Averaging Time (AT) of 25,550 days, along with references to later sections for the Oral Reference Dose (RfD) and the Oral Slope Factor (SF^{o}) values.

Section II details the daily consumption rates (kg/person/day) specifically adopted for the risk assessment of the five studied vegetables, Eggplant, Okra, Cucumber, Bitter Gourd, and Indian Squash, justifying the rates based on common regional intake practices [19]. Finally, Section III presents the critical toxicological data, listing the Oral Reference Dose (RfD) values (mg/kg/day) for the non-carcinogenic risk calculations for Aluminum, Zinc, Manganese, Selenium, Tin, and Chromium (specifically Cr(VI)), drawing these reference values from sources [20, 21].

Table 3. **Integrated Parameters for Human Health Risk Assessment.**

I. Definition of Variables [18]

Variable	Value	Unit
C_{veg}	Study Data	mg/kg
IR_{veg}	See Ingestion Rates Below	kg/day
EF	365	days/year
ED	70	years
BW	65	kg
AT	25550	days
RfD	See Section IV	mg/kg/day
SF^{o}	See Section IV	$mg/kg/day^{-1}$

II. Daily consumption rates adopted for risk assessment [19]

Vegetable Type	Common Regional Intake Rate (kg/person/day)	Source Type
Eggplant (Brinjal)	approx 0.25	Used widely in Pakistani/Indian risk studies.
Okra (Bhindi)	approx 0.20	Used widely in Pakistani/Indian risk studies.
Cucumber	approx 0.25	Used widely in Pakistani/Indian risk studies.
Bitter Gourd	approx 0.10	Consumption is typically lower due to taste.
Indian Squash (Tinda)	approx 0.15	Rate is comparable to other common gourds/squashes.

III. Oral Reference Dose (RfD) Values [20,21]

Element	Chemical Symbol	Oral Reference Dose (RfD) (mg/kg/day)
Aluminum	Al	1.0
Zinc	Zn	0.3
Manganese	Mn	0.14
Selenium	Se	0.005
Tin	Sn	0.6
Chromium	Cr(VI)	0.003

Statistical Analysis

Statistical Analysis Pearson's correlation coefficients (r) were computed using IBM SPSS Statistics (version 18.0; PASW, Inc., Chicago, IL, USA) to evaluate the relationships between heavy metal concentrations in vegetable/fruit seeds, agricultural soil, and irrigation water samples from Khairpur Mir's, Sindh. Statistical significance was evaluated at the $p < 0.05$ and $p < 0.01$ levels.

Results and Discussion

Sodium (Na), Potassium (K), Magnesium (Mg), and Calcium (Ca)

The data for the macronutrients Na, K, Mg, and Ca reveal a substantial range in concentrations across the soil-water-seed matrices, which is characteristic of diverse environmental settings and species-specific uptake. The wide variation in water Na (up to 386.93 mg/L in B-3) and soil Na (up to 127.88 mg/kg in O-3) is a key finding that signals a potential for soil salinity and osmotic stress in the Khairpur Mir's agricultural fields, which is a significant agronomic concern due to the risk of soil structure deterioration and reduced water infiltration [22,23]. The high seed Na detected in O-1 (525.51 mg/kg) is considerably lower than the outlier value of 1240 mg/kg reported for Okra in external literature [24], suggesting that while salinity is a localized problem in this study area, it is not as severe as documented elsewhere. Conversely, K consistently showed high seed concentrations (peaking at 1028.53mg/kg in Cu) compared to Na, underscoring its essential and dominant role as a macronutrient in seed development. The observed high soil Mg (up to 2234.67 mg/kg) is a finding consistent with literature on the lithogenic nature of Mg in agricultural soils[25]. Similarly, the high variability in Ca (max 848.3 mg/kg in seed) is largely attributed to natural soil composition. Overall, the calculated EDI values for all four macronutrients were consistently low, confirming that while these seeds contribute to the diet, their consumption is not expected to significantly fulfill the full recommended daily nutritional requirements, but neither do they pose a toxicity risk (Table 4 – 6).

Table 4. **Concentration of Various metals present in seeds of different vegetables collected from District Khairpur.**

Code	Na (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Al (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Se (mg/kg)	Sn (mg/kg)	Cr (mg/kg)
B-1	14.07±1.14	79.62±0.68	180.29±1.06	101.5±1.76	1.04±0.06	1.44±0.07	0.243±0.021	0.063±0.007	21.12±1.07	0.004±0
B-2	9.03±0.39	62.02±1.18	28.11±1.43	48.6±0.06	4.25±0.42	0.92±0.13	0.293±0.034	0.042±0.006	17.28±1.78	0.01±0.001
B-3	24.54±2.6	45.94±2.57	167.18±1.68	31.55±3.57	3.55±0.4	1.5±0.07	0.337±0.015	0.053±0.004	29.52±1.32	0.129±0.001
Cu-1	2.24±0.28	52.49±0.95	20.09±0.73	49.45±1.16	1.45±0.14	0.24±0.01	0.341±0.067	0.07±0.005	6.24±0.02	0.005±0.001
Cu-2	4.48±0.7	35.97±3.21	15.15±1.01	18.56±2.59	1.07±0.14	0.38±0.06	0.266±0.044	0.046±0.006	3.12±0.2	0.004±0
Cu-3	0.52±0.06	23.92±0.94	12.47±0.97	17.11±0.78	0.69±0.07	0.12±0.01	0.175±0.014	0.07±0.009	4.5±0.55	0.003±0.001
O-1	525.51±5.42	81.55±1.44	278.5±12.05	184.07±9.78	13.54±0.58	3.14±0.12	1.817±0.115	0.183±0.012	39.13±7.61	0.056±0.003
O-2	271.11±6.01	36.25±1.35	297.33±0.31	2.27±0.25	1.02±0.2	0.85±0.09	0.016±0.003	0.072±0.006	39.36±1.72	0.004±0
O-3	482.03±10.87	15.77±0.74	158.07±10.3	1.25±0.06	3.47±0.32	0.23±0.03	0.014±0	0.048±0.006	3.59±0.55	0.475±0.058
I-S-1	1.43±0.21	6.37±0.87	29.75±1.06	35.05±1.58	4.13±0.58	0.64±0.11	0.375±0.045	0.083±0.01	18.34±1.11	0.324±0.039
I-S-2	0.89±0.11	9.58±0.55	13.15±0.9	11.1±0.85	1.51±0.16	0.08±0.01	0.159±0.014	0.068±0.004	46.41±1.2	0.013±0.001
I-S-3	1.76±0.3	67.63±2.17	41.69±2.06	36.61±1.26	5.64±0.63	0.4±0.06	0.499±0.051	0.829±0.114	35.2±1.95	0.054±0.004
BG-1	53.98±1.13	16.57±2.18	381.07±2.86	263.33±12.77	6.7±1.05	1.79±0.07	1.564±0.09	0.055±0.007	61.89±3.48	0.134±0.001
BG-2	24.86±3.51	27.16±1.95	339.57±3.25	177.57±13.15	4.2±0.54	5.19±0.78	1.247±0.046	0.068±0.004	95.6±3.67	0.145±0.014
BG-3	42.22±0.09	26.2±0.43	441.73±0.32	848.3±0.4	3.89±0.12	6.13±0.01	1.664±0.005	0.126±0.02	100.54±0.14	0.206±0

Table 5. **Concentration of Various metals present in Soil of different vegetables sites collected from District Khairpur**

	Na (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Al (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Se (mg/kg)	Sn (mg/kg)	Cr (mg/kg)
WHO Limit	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*300*	*2,000*	*0.8 - 3*	*N/A*	*100 - 150*
B-1	7.14±0.75	463.2±8.81	1396.9±10.94	228.26±3.75	360±7.2	1.64±0.15	120.42±3.1	0.041±0.005	2.52±0.08	0.32±0.035
B-2	ND ±0.0	1029.87±15.28	1404±17.52	222.93±0.46	259.8±1.6	1±0	17.58±1.14	0.044±0.003	2.24±0.03	0.042±0.005
B-3	6.73±0.51	214±15.01	1054.33±14.01	153.47±3.17	173.1±1.8	0.3±0.01	25.52±0.07	0.028±0.003	2.68±0.12	0.04±0.01
Cu-1	8.13±0.85	273.3±11.63	980.7±4.25	241.52±8.17	265.6±3.7	1.4±0.03	50.52±7.19	0.09±0.012	3.59±0.54	0.147±0.018
Cu-2	6.35±0.87	882.05±10.44	996.43±12.87	260.6±14.3	405.2±2.7	1.19±0.01	25.16±0.61	0.061±0.003	4.44±0.31	0.365±0.03
Cu-3	ND ±0	1028.53±9.21	1805±11.14	123.87±12.08	186.1±6.7	0.41±0.04	53.8±3.99	0.236±0.008	1.02±0.03	0.095±0.013
O-1	ND ±0	600.07±4.01	1407.33±15.01	204.87±8.6	334.9±6.2	0.57±0.02	18.29±0.16	0.02±0.004	11.37±0.58	0.283±0.024
O-2	ND ±0	289.04±8.51	1597.33±19.66	260.33±1.19	520.8±2.6	0.61±0.04	12.27±0.09	0.05±0.007	2.78±0.42	0.448±0.004
O-3	127.88±1.16	236.59±5.84	2234.67±12.34	497.5±5.05	690.3±3.4	4.55±0.17	18.97±0.51	0.523±0.035	5.42±0.16	0.229±0.027
I-S-1	14.39±0.12	596.5±5.51	1385.93±0.93	629.3±7.07	175.11±0.77	0.48±0	12.46±0.03	0.057±0.006	1.77±0.08	0.0469±0.0085
I-S-2	24.54±1	418±9.76	1691.67±19.5	334.5±5.53	290±0.69	1.05±0.15	11.19±0.04	0.144±0.022	16.28±1	0.0608±0.0052
I-S-3	8.23±0.3	138.6±38.6	1022.81±0.76	485.47±9.11	613.2±16.81	2.46±0.21	18.58±1.6	0.655±0.055	12.15±1.79	0.168±0.013
BG-1	0.35±0.04	230.78±3.54	1552.94±16.12	638.03±12.03	258.08±35.28	0.91±0.01	11.05±0.04	0.318±0.007	6.86±0.64	17.9±2.136
BG-2	ND ±0	318.89±16.86	1239.85±7	860.13±10.76	128.8±22.75	2.69±0.11	31.91±1.21	0.219±0.012	5.02±0.91	1.426±0.138
BG-3	0.82±0.08	409.03±3.76	1629.43±3.27	611.83±17.09	128.86±4.06	0.75±0.05	21.61±0.67	0.436±0.015	4.47±0.56	17.665±1.145

Table 6. **Concentration of Various metals present in water of different vegetables sites collected from District**

WHO Limit	Na (mg/L)	K (mg/L)	Mg (mg/L)	Ca (mg/L)	Al (mg/L)	Zn (mg/L)	Mn (mg/L)	Se (mg/L)	Sn (mg/L)	Cr (mg/L)
	N/A	*N/A*	*N/A*	*N/A*	*5.0*	*2.0*	*0.20*	*0.02*	*N/A*	*0.10*
B-1	32.32±2.85	12.99±0.72	22.44±0.45	63.71±2.52	1.608±0.088	0.06±0.01	0.01±0	0.12±0.02	8.98±0.83	0.0017±0.0002
B-2	371.6±18.46	15.13±2.09	48.37±0.27	77.55±11.2	1.515±0.533	0.06±0.01	0.02±0.001	0.15±0	13.33±0.02	0.0004±0.0001
B-3	386.93±12.98	48.73±1.22	54.76±0.23	76.89±0.9	1.378±0.196	0.3±0.01	0.019±0.001	0.1±0.01	10.23±0.02	0.001±0.0001
Cu-1	0.81±0.05	7.86±0.75	9.46±0.42	3.14±0	0.807±0.052	0.1±0	0.102±0.003	0.06±0.01	2.33±0	0.0035±0.0002
Cu-2	0.51±0.1	14.85±1.01	8.78±0.73	3.76±0.14	0.441±0.06	0.05±0	0.032±0.005	0.05±0.01	4.98±0.01	0.0029±0.0003
Cu-3	0.64±0.02	22.01±1.17	5.18±0.23	2.19±0.01	0.623±0.003	0.05±0.01	0.06±0.002	0.05±0	1.93±0.05	0.0031±0.0004
O-1	493.4±15.78	7.86±0.75	90.32±6.33	98.93±14.71	3.06±0.004	0.23±0.04	0.05±0.008	ND ±0	5.58±0.62	0.0006±0.0001
O-2	57.8±1.57	5.73±0	114.2±8.34	27.86±2.99	1.672±0.156	3.92±0.55	0.024±0	ND ±0	4.06±0.67	0.0089±0.0007
O-3	14.6±2.8	9.84±0	26.6±1.17	128.97±6.67	0.617±0.005	4.19±0	0.027±0.004	0.12±0.02	12.1±0	0.0015±0.0002
I-S-1	0.31±0.03	7.86±0.75	1.2±0	0.38±0.01	1.137±0.589	0.08±0.01	0.006±0	0.05±0	0.5±0.01	0.0058±0.0004
I-S-2	2.53±0.08	5.63±0.01	9.46±0.42	0.37±0	0.441±0.06	0.1±0	0.102±0.003	0.05±0.01	0.31±0	0.0029±0.0003
I-S-3	4.51±0	9.91±0	8.4±0.5	0.28±0	1.913±0	0.09±0	0.005±0	0.03±0	4.09±0.02	0.0023±0.0004
BG-1	0.31±0.03	7.86±0.75	9.46±0.42	3.66±0.01	0.441±0.06	0.1±0	0.102±0.003	0.05±0	0.37±0	0.0029±0.0003
BG-2	2.53±0.08	13.06±1.61	10.26±0.84	0.38±0.01	1.137±0.589	0.08±0.01	0.006±0	0.05±0.01	0.44±0	0.0058±0.0004
BG-3	0.35±0.01	9.79±0.3	19.67±1.28	0.64±0.01	0.803±0.018	0.05±0.01	0.006±0	0.02±0	0.72±0	0.0027±0.0001

Aluminum (Al), Zinc (Zn), and Manganese (Mn)

The trace elements Al, Zn, and Mn presented a mix of nutritional status and toxicity mitigation. Aluminum showed a high concentration in soil (O-3 at 690.3 mg/kg), which is typical of its natural abundance in the Earth's crust, but its seed concentration was drastically lower (O-1 at 13.54 mg/kg. This discrepancy, also reflected in a very low EDI (max 2.371 mg/kg/day), indicates a highly effective physiological barrier in the vegetables, demonstrating a very low Transfer Factor (TF) from soil to seed, and conclusively dismissing any potential neurotoxicity risk as the EDI is over 100×below the WHO/FAO guideline of 286 mg/kg/day [26]. In contrast, the key finding for Zinc is the exceptionally low concentration in agricultural soil (max 4.55 mg/kg), which is far below the reported global average for Zn in soil of 70 mg/kg [27]. This suggests a state of widespread Zn deficiency in the Khairpur Mir's agricultural lands, posing a major risk to crop productivity and nutritional quality, despite the low THQ confirming no toxicity risk. For Mn, the soil concentration (max 120.42 mg/kg) is consistent with its lithospheric origin [28], but the calculated EDI (max 0.318 mg/kg/day) is over 440×lower than the WHO/FAO Provisional Tolerable Daily Intake (PTDI) of 140 mg/kg/day. This low EDI indicates that Mn consumption from these seeds is well within safe limits, posing no risk of Mn toxicity [29], with variability in seed concentrations being primarily controlled by plant-specific uptake rather than uniform environmental loading (Table 4 – 8).

Selenium (Se), Tin (Sn), and Chromium (Cr)

The findings for Se, Sn, and Cr highlight specific localized risks and a wide disparity with reported literature. For Selenium, the calculated EDI (max 0.145 mg/kg/day) is far below the WHO/FAO PTDI of 7 mg/kg/day, confirming no toxicity risk. However, given Se's role as an essential micronutrient [30, 31], these low EDI values suggest the possibility of Se nutritional deficiency in the local population, contrasting sharply with the exceptionally high Se levels (272.0 mg/kg) reported in other literature [32].

Table 7. **EDI (µg/kg/day) present in seeds of different vegetables collected from District Khairpur.**

Code	Na	K	Mg	Ca	Al	Zn	Mn	Se	Sn	Cr
B-1	2.459	13.945	31.597	17.781	0.182	0.252	0.043	0.011	3.696	0.001
B-2	1.580	10.852	4.920	8.508	0.744	0.161	0.051	0.007	3.023	0.002
B-3	4.296	8.046	29.255	5.522	0.622	0.262	0.059	0.009	5.171	0.023
Cu-1	0.392	9.192	3.516	8.657	0.254	0.042	0.060	0.012	1.091	0.001
Cu-2	0.784	6.294	2.651	3.250	0.187	0.066	0.047	0.008	0.547	0.001
Cu-3	0.091	4.184	2.183	2.996	0.121	0.021	0.031	0.012	0.787	0.001
O-1	92.001	14.269	48.729	32.208	2.371	0.550	0.318	0.032	6.849	0.010
O-2	47.464	6.347	52.012	0.397	0.178	0.149	0.003	0.013	6.892	0.001
O-3	84.341	2.761	27.640	0.219	0.607	0.040	0.002	0.008	0.628	0.083
I-S-1	0.250	1.115	5.204	6.134	0.723	0.112	0.066	0.015	3.211	0.057
I-S-2	0.156	1.675	2.302	1.944	0.264	0.014	0.028	0.012	8.127	0.002
I-S-3	0.308	11.838	7.299	6.408	0.988	0.070	0.087	0.145	6.155	0.009
BG-1	9.447	2.900	66.689	46.096	1.171	0.313	0.274	0.010	10.835	0.023
BG-2	4.351	4.752	59.431	31.066	0.735	0.908	0.218	0.012	16.732	0.025
BG-3	7.387	4.588	77.307	148.650	0.681	1.073	0.291	0.022	17.585	0.036

Table 8. **Comparison of Elemental Concentrations in Vegetable Seeds (mg/kg) Reported in the Literature.**

Code	Na	K	Mg	Ca	Al	Zn	Mn	Se	Sn	Cr
Brinjal reported	660 [38]	820 [38]	300.13 [38]	230 [38]	39.8 [39]	38 [38]	12.87 [38]	272.0 [40]	-	-
Brinjal Present Study	15.880	62.527	125.193	60.550	2.947	1.287	0.291	0.053	22.640	0.048
Cucumber Reported	525 [38]	465 [38]	392 [38]	180 [38]	4.997 [38]	28.418 [40]	16.311 [40]	206.9 [40]	9.33 [35]	1.282 [42]
Cucumber present study	2.413	37.460	15.903	28.373	1.070	0.247	0.261	0.062	4.620	0.004
Okra reported	1240 [38]	455 [38]	330 [38]	210 [38]	18.543 [39]	105 [38]	14.35 [38]	158.1 [40]	-	2092 [42]
Okra Present	426.217	44.523	244.633	62.530	6.010	1.407	0.616	0.101	27.360	0.178
Indian Squash reported	-	-	-	-	6.503 [38]	9.425 [38]	1.082 [38]	179.7 [40]	-	19.01 [38]
Indian Squash Present study	1.360	27.860	28.197	27.587	3.760	0.373	0.344	0.327	33.317	0.130
Bitter Gourd reported	690 [38]	0.84 [41]	0.57 [41]	2.35 [41]	-	40 [38]	13.98 [38]	0.24 [43]	7.48 [35]	3.7 [42]
Bitter Gourd Present Study	40.353	23.310	387.457	429.733	4.930	4.370	1.492	0.083	86.010	0.162

Tin presents the most critical finding for non-essential elements, with a dramatic accumulation in Bitter Guard seed (BG-3 at 100.54 mg/kg), which is significantly higher than the local soil and water concentrations, pointing to an extremely high bioaccumulation factor (BAF) for this particular species at that site. This is a crucial outlier, as external literature reports much lower Sn concentrations (e.g., 7.48 mg/kg for Bitter Gourd [33]), and Sn compounds are known neurotoxins [34]; therefore, the EDI of 17.585 mg/kg/day in BG-3 warrants urgent investigation into the source of this localized contamination. Finally, Chromium concentrations in both soil (max 17.9 mg/kg) and seeds (max 0.475 mg/kg) were low and within background ranges [35].The "Reported" values [36] for both Brinjal (272.0 mg/kg) and Cucumber (206.9 mg/kg) are extraordinarily high for Seed samples. In contrast, the "Present Study" result for Brinjal (0.053 mg/kg) aligns more closely with typical micronutrient levels found in vegetables. The very low EDI (max 0.083 mg/kg/day) is over 300×lower than the WHO/FAO guideline, conclusively demonstrating that Cr intake from these vegetable seeds poses no non-carcinogenic or carcinogenic health risk, a finding that stands in stark contrast to the highly exaggerated literature value of 2092 mg/kg for Okra [37] (Table 4 – 8).

Non-Carcinogenic and Carcinogenic Risk Assessment

The HQ is a ratio of the estimated exposure to a substance to its RfD. An HQ value less than 1 indicates that the daily exposure is unlikely to cause adverse non-carcinogenic health effects. The THQ is the sum of the HQs for all combined substances, assessing the cumulative non-carcinogenic risk. For all the samples and all the metals (Zn, Mn, Se, and Cr), the individual HQ values are significantly less than 1. The highest HQ value is 8.30×10^{-2} for Cr in sample O-3. Since all individual HQs are well below the threshold of 1, the intake of any single one of these metals from the samples is not expected to pose a non-carcinogenic health risk. The THQ values, which represent the cumulative risk from all four metals, are also all less than 1. The THQ values range from a low of 3.16×10^{-3} (Cu-2) to a high of 8.47×10^{-2} (O-3). Since all THQ values are below the critical value of 1, the combined exposure to these metals from the food samples is unlikely to result in adverse non-carcinogenic health effects (Table 9).

Table 9. **HQ (Zn, Mn, Se & Cr), THQ (Zn, Mn, Se & Cr) and TCR (only Cr) in various vegetable seeds.**

Code	HQ (Zn)	HQ (Mn)	HQ (Se)	HQ (Cr)	THQ	TCR (Cr)
B-1	8.40×10^{-4}	3.07×10^{-4}	2.20×10^{-3}	1.00×10^{-3}	4.35×10^{-3}	5.00×10^{-7}
B-2	5.37×10^{-4}	3.64×10^{-4}	1.40×10^{-3}	2.00×10^{-3}	4.30×10^{-3}	1.00×10^{-6}
B-3	8.73×10^{-4}	4.21×10^{-4}	1.80×10^{-3}	2.30×10^{-2}	2.61×10^{-2}	1.15×10^{-5}
Cu-1	1.40×10^{-4}	4.29×10^{-4}	2.40×10^{-3}	1.00×10^{-3}	3.97×10^{-3}	5.00×10^{-7}
Cu-2	2.20×10^{-4}	3.36×10^{-4}	1.60×10^{-3}	1.00×10^{-3}	3.16×10^{-3}	5.00×10^{-7}
Cu-3	7.00×10^{-5}	2.21×10^{-4}	2.40×10^{-3}	1.00×10^{-3}	3.69×10^{-3}	5.00×10^{-7}
O-1	1.83×10^{-3}	2.27×10^{-3}	6.40×10^{-3}	1.00×10^{-2}	2.05×10^{-2}	5.00×10^{-6}
O-2	4.97×10^{-4}	2.14×10^{-5}	2.60×10^{-3}	1.00×10^{-3}	4.12×10^{-3}	5.00×10^{-7}
O-3	1.33×10^{-4}	1.43×10^{-5}	1.60×10^{-3}	8.30×10^{-2}	8.47×10^{-2}	4.15×10^{-5}
I-S-1	3.73×10^{-4}	4.71×10^{-4}	3.00×10^{-3}	5.70×10^{-2}	6.08×10^{-2}	2.85×10^{-5}
I-S-2	4.67×10^{-5}	2.00×10^{-4}	2.40×10^{-3}	2.00×10^{-3}	4.65×10^{-3}	1.00×10^{-6}
I-S-3	2.33×10^{-4}	6.21×10^{-4}	2.90×10^{-2}	9.00×10^{-3}	3.89×10^{-2}	4.50×10^{-6}
BG-1	1.04×10^{-3}	1.96×10^{-3}	2.00×10^{-3}	2.30×10^{-2}	2.80×10^{-2}	1.15×10^{-5}
BG-2	3.03×10^{-3}	1.56×10^{-3}	2.40×10^{-3}	2.50×10^{-2}	3.20×10^{-2}	1.25×10^{-5}
BG-3	3.58×10^{-3}	2.08×10^{-3}	4.40×10^{-3}	3.60×10^{-2}	4.61×10^{-2}	1.80×10^{-5}

The TCR is a measure of the probability of developing cancer due to exposure to a carcinogenic substance over a lifetime. For this analysis, Chromium is the only metal for which a cancer risk was assessed, assuming it is present in its hexavalent form Cr(VI), which has a known Cancer Slope Factor. Regulatory agencies, such as the Environmental Protection Agency (EPA), typically consider a cancer risk to be negligible if it is below 10^{-6} (or one in a million). A risk between 10^{-6} and 10^{-4} is generally considered an acceptable range, while risks above 10^{-4} may warrant intervention. The TCR values calculated for Chromium range from 5.00×10^{-7} (B-1, Cu-1, Cu-2, Cu-3, O-2) to 4.15×10^{-5} (O-3). Several samples (B-1, Cu-1, Cu-2, Cu-3, O-2) have a TCR of 5.00×10^{-7}, which is below the negligible risk threshold of 10^{-6}. The majority of samples have TCR values within the acceptable range of 10^{-6} to 10^{-4}. The highest TCR value is 4.15×10^{-5} for sample O-3. This value is still within the acceptable risk range defined by regulatory bodies (Table 9).The THQ values for all samples are well below 1, indicating no significant non-carcinogenic health risk from the combined intake of Zn, Mn, Se, and Cr. The TCR values for Cr are all within or below the acceptable risk range of 10−6 to 10−4. These findings suggest that the intake of these metals from the analyzed samples is not likely to pose a significant health concern to consumers, according to the established guidelines (Table 9).

Statistical Analysis

Correlation coefficient

Correlation coefficients between pairs of elements are given, ranging from -1 to +1, showing the direction and strength of the linear relationship between two metals. The strong positive correlations, moderate positive correlations, and weak or no correlations (≥0.7, 0.5 – 0.7 and <0.5) are shown in the Tables: 8 to 10 at significance levels of 0.05 and 0.01, respectively.

Correlation coefficient in seed samples

Thestrong positive correlation was observed between pairs Ca – Mg (0.703**), Zn – Mg (0.803**), Zn – Ca (0.826**), Mn – Mg (0.728**), Mn – Ca (0.731**), Mn – Al (0.738**), Mn – Zn (0.797**), Sn – Mg (0.787**), Sn – Ca (0.739**), Sn – Zn (0.866**) and Sn – Mn (.723**) in seed samples which were statistically significant at the 0.01 level, indicating that sources or interactions in the seed samples are similar. While moderate positive correlation and Negative correlations were not observed in seed samples. The correlation coefficient data is extremely valuable to understand the interaction of metals in seed samples, which may be significant for nutritional, agricultural and environmental studies (Table 10).

Correlation coefficient in Soil samples

The strong positive correlation in soil samples was observed only in one pair as, Zn – Na (0.769**) at 0.01 significant levels, while some of the metals displayed moderate correlation coefficient which include; Mg – Na (0.619*), Al – Na (0.592*), Zn – Al (0.608*) and Se – Zn (0.602*) at of 0.05 significant level (Table 11).

Correlation coefficient in water samples

The strong positive correlation was found between pairs of metals, as given in Table 12, are Sn – Ca (0.854**) and Sn – Se (0.742**) at a 0.01 significant level. The moderate correlation coefficient was found in pairs of metals as, Mn – Na (0.619*), Ca – Na (0.645**), Ca – Mg (0.518*), Al – Na (0.682**), Al – Mg (0.657**) and Sn – Na (.557*) at 0.05 and 0.01 significant level, whereas moderate negative correlation was displayed among pairs Cr – Ca (-0.525*) and Cr – Sn (-0.553*) at 0.05 significant level. This is helpful data to understand the interactions between metals in water samples, which may be important for public health, hydrological and environmental studies.

Table 10. **Correlation coefficients between pairs of metals in seed samples collected from district Khairpur Mir's.**

Correlations										
	Na	***K***	***Mg***	***Ca***	***Al***	***Zn***	***Mn***	***Se***	***Sn***	***Cr***
Na	1									
K	.147	1								
Mg	.325	-.041	1							
Ca	-.041	-.079	.703**	1						
Al	.537*	.342	.379	.239	1					
Zn	.065	.033	.803**	.826**	.374	1				
Mn	.186	.101	.728**	.731**	.738**	.797**	1			
Se	-.059	.368	-.139	-.019	.285	-.072	.079	1		
Sn	-.084	-.177	.787**	.739**	.279	.866**	.723**	.068	1	
Cr	.375	-.505	.225	.178	.162	.159	.083	-.100	.083	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 11. **Correlation coefficients between pairs of metals in soil samples collected from district Khairpur Mir's.**

Correlations										
	Na	***K***	***Mg***	***Ca***	***Al***	***Zn***	***Mn***	***Se***	***Sn***	***Cr***
Na	1									
K	-.255	1								
Mg	.619*	.058	1							
Ca	.134	-.409	.104	1						
Al	.592*	-.287	.232	-.132	1					
Zn	.769**	-.377	.297	.401	.608*	1	.			
Mn	-.115	.107	-.107	-.308	-.078	.058	1			
Se	.425	-.409	.307	.492	.418	.602*	-.213	1		
Sn	.096	-.319	.026	.117	.281	.164	-.349	.309	1	
Cr	-.170	-.226	.186	.475	-.302	-.161	-.192	.368	.013	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 12. **Correlation coefficients between pairs of metals in water samples collected from district Khairpur Mir's.**

Correlations										
	Na	***K***	***Mg***	***Ca***	***Al***	***Zn***	***Mn***	***Se***	***Sn***	***Cr***
Na	1									
K	.436	1								
Mg	.638*	.072	1							
Ca	.645**	.248	.518*	1						
Al	.682**	.003	.657**	.430	1					
Zn	-.096	-.176	.503	.461	-.002	1				
Mn	-.115	-.221	-.160	-.214	-.368	-.135	1			
Se	.174	.373	-.205	.509	-.205	.016	-.138	1		
Sn	.557*	.402	.371	.854**	.290	.335	-.344	.742**	1	
Cr	-.503	-.338	.137	-.525*	-.143	.345	-.084	-.510	-.553*	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Conclusion

The study concludes that, despite wide variations in elemental concentrations across soil, irrigation water, and a vegetable seed in Khairpur Mir's, the consumption of the analyzed vegetable seeds does not pose a significant health risk to the local population. Health risk indicators were reassuring: the Estimated Daily Intake (EDI) for all measured elements was consistently low, and the Total Hazard Quotient (THQ) for non-carcinogenic risk (ranging from 3.16×10^{-3} to 8.47×10^{-2}) and the Total Cancer Risk (TCR) for Cr (between 5.00×10^{-6} and 3.00×10^{-7}) were all well below the established safety threshold of 1.0. However, the findings reveal two key environmental and nutritional concerns: salinity stress is suggested by high Na levels in some water samples (up to 386.93mg/L) and the seeds (up to 525.51 mg/kg), and potential nutritional deficiency is indicated by very low Zn levels in the agricultural soil (max 4.55 mg/kg). Furthermore, the outlier concentration of Sn in Bitter Gourd seeds (100.54 mg/kg) necessitates urgent follow-up investigation to identify the localized source of this contamination and reassess its site-specific health implications. It is recommended that urgently investigate the source of high Sn contamination in the Bitter Gourd seeds and simultaneously implement soil management strategies to address the localized salinity stress and widespread Zn deficiency in the agricultural lands.

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