



## Human Health Risk Assessment of Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Lead (Pb) from Vegetables of District Ghotki, Sindh, Pakistan

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### Abstract

The current study investigated the accumulation of heavy metals (HMs) including Cadmium (Cd), Chromium (Cr), Nickel (Ni), and Lead (Pb) in eight common vegetables (tomato, bitter gourd, mustard, fennel, sunflower, brinjal, capsicum, and cluster beans). The vegetable samples were collected from three agricultural regions (Ghotki, Mirpur Mathelo, and Ubauro) of Sindh, Pakistan. The concentrations of HMs were determined using standard analytical methods. The contents of Cd, Cr, Ni, and Pb were observed up to 0.090, 0.880, 0.850, and 0.68 mg/kg, respectively. The resulting data indicated the high contamination of Cr in vegetable samples of Ubauro, especially in bitter gourd, mustard, and cluster beans with a significantly maximum content of Cr in capsicum compared to other studied agricultural regions. Estimated Daily Intake (EDI) values for all metals remained within safe limits. Target Hazard Quotient (THQ) values were less than 1.00 in all vegetable samples, indicating a low non-carcinogenic risk. However, bitter gourd showed the highest Health Index (HI = 1.53) and Total Target Hazard Quotient (TTHQ = 0.0154–0.753), suggesting it is the most significant contributor to overall metal exposure. Total Cancer Risk (TCR) values of HMs showed variation. The maximum estimated TCRs for Cd, Cr, and Pb from vegetables reached up to 0.430, 1.18, and 0.018, respectively, indicating a possible contribution of cancer risk. Tomato showed the highest TCR for Cd, while capsicum showed the highest TCR for Cr. Even though the individual health risks from heavy metals in most vegetables were low, the study concludes that certain vegetables and locations pose potential non-carcinogenic and carcinogenic risks, warranting ongoing monitoring and further research.

**Keywords:** Vegetables; heavy metals; estimated daily intake; total target hazard quotient, total cancer risk

### Introduction

Concern over heavy metal pollution in the environment has grown as a result of the quickening rates of industrialization, urbanization, and agricultural intensification [1]. Due to their toxicity, persistence, and ability to bioaccumulate in food chains, heavy metals pose significant risks to human health, even at trace levels [2]. Because of

their well-established detrimental effects on health, Cadmium (Cd), Chromium (Cr), and Nickel (Ni) are particularly concerning. This study focuses on these three metals in vegetables grown in District Ghotki, Sindh, Pakistan—an area that may be affected by a variety of anthropogenic activities [3].

Because they provide vital nutrients, vegetables are an essential part of the human diet. However, they can also be a major source of exposure to heavy metals. HMs from contaminated soil, water, and air can be absorbed and accumulated by plants [4]. The bioavailability of the metal, the pH and organic matter content of the soil, the type of plant, and the surrounding environment all affect how much of the metal is absorbed. After being absorbed, these metals may build up in various vegetable components, endangering consumers [5,6]. One of the most harmful metals, Cd, is known to induce kidney damage, bone demineralization, and a number of malignancies. It may also interfere with calcium metabolism and disrupt the endocrine system [7].

Cd, Cr, Ni, and Lead (Pb) are toxic heavy metals that can accumulate in vegetables through contaminated soil, water, or fertilizers and pose serious health hazards when consumed over time. Cd can damage the kidneys, liver, and bones, and long-term exposure may lead to skeletal deformities, anemia, and cancer due to its high toxicity and bioaccumulation. Cr, particularly in its hexavalent form ( $\text{Cr}^{6+}$ ), is a potent carcinogen that can cause liver and kidney damage, respiratory problems, and skin irritation; although trivalent ( $\text{Cr}^{3+}$ ) is an essential nutrient in trace amounts, excessive intake is harmful [8]. Ni exposure through vegetables may result in allergic reactions, skin dermatitis, respiratory issues, and in higher doses, may affect the liver, kidneys, and gastrointestinal tract. Pb is highly toxic even at low concentrations and can cause severe neurological disorders, particularly in children, leading to learning disabilities, behavioral problems, and reduced cognitive function; in adults, it can induce hypertension, kidney dysfunction, and reproductive toxicity. Continuous ingestion of vegetables contaminated with these metals can therefore

pose significant risks to human health and overall well-being [9].

Various types of vegetables and fruits are grown in the agriculturally important areas of Sindh, Pakistan. District Ghotki particularly, is significant for vegetable growth. Although, the district Ghotki is facing enhancing ecological threats, like industrial effluents, fertilizers and pesticides from agricultural runoff and potential toxic metal contaminants from automobile exhaust [10]. These experiences may lead to the amassing of HMs in soil and irrigation, potentially affecting the vegetables cultivated in the area. To save health and lives of people, it is important to assess the threat of HMs in fruits and vegetables [11]. This needs measuring the HMs concentrations found in vegetables used by the local people and measuring the possible exposure of humans via vegetable consumptions [12]. To assess the probable health hazards, carcinogenic and non-carcinogenic associated to HM exposure, risk assessment procedures for instance, Total Cancer Risk (TCR), Total Targeted Hazard Quotient (TTHQ) and Estimated Daily Intake (EDI) are usually used [13]. This study aims to assess the HM levels, mostly Cd, Cr, Ni and Pb in the district Ghotki, Sindh. The present work assessed the potential health risks of these metals including, EDI via consumption of locally cultivated vegetables. Moreover, this study analyzed the carcinogenic and non-carcinogenic health risks using the THQ, TTHQ, and TCR methods. The values of EDI for HMs were compared with the Maximum Tolerable Daily Intake (MTDI) and the WHO/FAO guidelines.

## **Materials and Methods**

### ***Study Area***

District Ghotki, situated in Sindh province, Pakistan is described by its, flat, dry landscape and a mostly rural population

(Fig. 1). District Ghotki is separated into three major areas: Desert, Ravine, and Cultivable. The environment is dry and hot, with notable temperature difference between winter and summer. The economy is sound, mainly determined by agriculture, with wide-ranging cultivation of crops, for instance cotton, sugarcane and rice. The study area is also identified by its fruit production and numerous industries including sugar mills, rice processing factories. The presence of toxic metal pollutants is an environmental concern that could harmfully impact locally cultivated vegetables and threaten the health of the people [14]. The focus of this work on HM contamination is noteworthy because it believes local environmental factors and dependence of community on agriculture. This approach is critical for guiding HM analysis and developing strategies to mitigate health hazards [10].



Figure 1. Map of the Study area of District Ghotki, Sindh

### Sample Collection

Triplicate samples of eight varieties of vegetables which include tomatoes, bitter gourd, mustard, fennel, sunflower, eggplant, bell peppers, and cluster beans were collected from district Ghotki. To remove surface contaminants, all vegetable samples were first

washed with tap water and then with de-ionized water. Thus, 72 samples were collected from study area. After proper washing, samples were stored in clean, airtight polyethylene bags and shifted to the laboratory for further analysis. The vegetable samples were then dried in an oven at a controlled temperature to remove water content. All samples were labeled appropriately with information including sample ID, sample location, sample collection date and vegetable type [15].

### Sample Preparation

#### Wet digestion

Vegetable samples were washed first with tap water and then with double-distilled water and stainless steel blade was used to cut them into pieces for preparation process. For moisture removal the resulting pieces were placed in an oven at 100 °C 24 hours. The dried vegetable samples were cooled, then ground using a clean non-metallic mortar and pestle to obtain a fine, uniform powder. The powder was sieved through 75 µm (200 mesh size), homogenized, and stored in clean, airtight containers inside a desiccator until further analysis. To avoid metal contamination a non-metallic mortar and pestle made of ceramic, rather than stainless steel or other metallic materials was used, as these could introduce trace metal impurities and affect the accuracy of results. The wet digestion process were applied using an acid mixture (1:4 of - HNO<sub>3</sub>: - H<sub>2</sub>SO<sub>4</sub>; v:v). 5 mL of the acid mixture was introduced to a digestion tube containing 2 g of each homogenized dried sample. After that, the mixture was heated for an hour between 130 and 170 °C until a transparent solution was visible. 2 mL of 30% concentrated H<sub>2</sub>O<sub>2</sub> were added after cooling, and the mixture was heated until a clear solution was obtained. Following filtration using Whatman No. 42 filter paper, the clear solution was diluted with 250 mL of de-ionized water. In order to prepare the samples

for analysis using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), they were then collected in falcon tubes [16, 17].

### ICP-OES Analysis

Standard solutions of known concentrations were prepared for Cd, Cr, Ni, and Pb to calibrate the ICP-OES instrument. The digested and diluted vegetable samples were then introduced into the *Thermo Scientific iCAP 7000 Series, ICP Spectrometer, Thermo Fisher Scientific, United States, Name: Teledyne CETAC Technologies ASX-280 Model: ASX-280, Nebraska, USA* ICP-OES. The instrument measured the intensity of light emitted by excited metal atoms in the plasma, which is directly proportional to the metal's concentration in the sample. The ICP-OES software then calculated the metal concentrations in the samples based on the established calibration curves [18, 19]. Operating parameters for HM Analysis are given in Table 1.

**Table 1.** Key ICP-OES Operating Parameters for Heavy Metal Analysis (Cd, Cr, Ni, and Pb).

Sr. No:	Parameter	Specification/Detail
1	Instrument Model	Thermo Scientific iCAP 7000 Series ICP Spectrometer
2	Autosampler Model	Teledyne CETAC Technologies ASX-280
3	Metals Analyzed	Cd, Cr, Ni, Pb
4	RF Power	1.2–1.5 kW
5	Plasma Gas Flow	10–15 L/min (Argon)
6	Auxiliary Gas Flow	0.5–1.5 L/min (Argon)
7	Nebulizer Gas Flow	0.5–1.0 L/min (Argon)
8	Sample Uptake Rate	0.5–1.5 mL/min
9	Replicate Readings	3 to 5 per sample
10	Integration Time	5–10 seconds
11	Recommended Wavelengths	Cd: 214.438 nm Cr: 267.716 nm Ni: 231.604 nm Pb: 220.353 nm
12	Internal Standard	Yttrium (Y) (1.0 mg/L at 371.029 nm) or Scandium (Sc)

### Quality Control

To ensure the reliability of the analysis, several quality control measures were implemented. Digestion acids and reagent blanks were analyzed to check for any contamination during the process. The precision of both the digestion and analysis methods was assessed by analyzing replicate samples. To determine the accuracy and recovery efficiency of the method, spiked samples were also analyzed. For ICP-OES analysis, quality control (QC) samples were analyzed at defined frequencies to ensure data accuracy and precision. Typically, a method blank, calibration verification standard, and spike recovery sample were analyzed after every 10 to 15 samples. A certified reference material (CRM) was also analyzed at the beginning and end of each analytical batch.

### Risk Assessment

Human health risk assessment from vegetable consumption is a scientific process used to evaluate the potential for adverse health effects caused by consuming vegetables contaminated with toxic substances, such as, EDI of HMs, THQ, TTHQ, HI, and TCR.

### Estimated Daily Intake (EDI) of Heavy Metals

EDI was measured in mg/kg-weight by following formulae [20].

$$EDI = \frac{MC \times IR}{BW}$$

MC = Metal Concentration

IR = Ingestion rate (g/day/person)

BW = Body Weight

### Non-Carcinogenic Risk

The HI, THQ, and TTHQ were used to quantify the non-carcinogenic hazards of HMs from vegetable consumption.

### Target Hazard Quotient (THQ)

The THQ was evaluated using the formula given below:

$$THQ = \frac{EF \times ED \times FIR \times CM}{BW \times AT \times RfD} \times 10^{-3}$$

In this study, EF = Exposure Frequency (365 days/year),

ED = Exposure Duration (70 Years), as indicated by [21].

CM = Heavy Metal Concentration (mg/kg),

BW = Average Body Weight (60 kg),

AT = Average Exposure Time (EF × ED), (AT = 25,550 days)

RfD = Reference dose of Metals, (Cd = 0.001mg/kg/day, Cr = 1.5mg/kg/day, Ni = 0.02 mg/kg/day, Pb = 0.0035 mg/kg/day),

FIR = Food Ingestion Rate (160 g/person/day) [22].

Although there is a possible health risk and related treatments and protective measures must be implemented, exposed customers are not to suffer any detrimental health risks if the THQ is less than 1. According to USEPA Region III Risk-based Concentration, formula used to estimate THQ was computed as below [23,24]

### Total Targeted Hazard Quotient (TTHQ)

The TTHQ for each individual from THQs as described by following equation: [23].

$$TTHQ = THQ(Cd) + THQ(Cr) + THQ(Ni) + THQ(Pb)$$

### Hazard Index (HI)

To calculate the total risk of non-carcinogenic health risks from consuming many metals, HI is evaluated as below [23].

$$HI = TTHQ(Food1) + TTHQ(Food2) + TTHQ(Food3) + TTHQ(Food4) \dots \dots n$$

### Carcinogenic Risk Assessment

Targeted Cancer Risk (TCR)

TCR can be calculated by using the following formula;

$$TCR = EDI \times CPSo$$

Where,

EDI = Estimated Daily Intake,

CPSo = Carcinogenic Potency Slope (Pb = 0.0085 (mg/kg – day)<sup>-1</sup> (Cd = 1.5 (mg/kg – day)<sup>-1</sup>, Cr = (0.42 (mg/kg – day)<sup>-1</sup>) and Ni = 1.7 (mg/kg–day)<sup>-1</sup> [25].

Generally speaking, a CR value below 1.0E-06 is regarded as insignificant, one beyond 1.0E-04 as unsatisfactory, and one falling between 1.0E-06 and 1.0E-04 as a normal range [26, 27].

## Results and Discussion

### Concentration of heavy metals in commonly consumed vegetables

The mean heavy metal concentrations across eight commonly consumed vegetables—tomato, bitter gourd, mustard, fennel, sunflower, brinjal, capsicum, and cluster beans—grown in Ghotki, Mirpur Mathelo, and Ubaro were found in ranges of 0–0.09 mg/kg for Cd, 0–0.88 mg/kg for Cr, 0–0.85 mg/kg for Ni, and 0.019–0.68 mg/kg for Pb. When compared to the maximum permissible limits (MPL) established by the WHO and FAO, Cadmium levels remained within safe boundaries, whereas Lead concentrations in certain samples exceeded the permissible limit of 0.3 mg/kg, indicating a potential health risk in specific crops or locations (Table 2). Despite this, research has shown that leafy vegetables accumulate a lot of heavy metals because of their wide surface and rapid soil absorption [28].

**Table 2.** Heavy metal contents in various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubauro.

Vegetables	Cd (mg/kg)			Cr (mg/kg)			Ni (mg/kg)			Pb (mg/kg)		
	Ghotki	Mirpur Mathelo		Ghotki	Mirpur Mathelo		Ghotki	Mirpur Mathelo		Ghotki	Mirpur Mathelo	
			Ubaro			Ubaro			Ubaro			Ubaro
Tomato	0.287	0.255	0.287	0.127	0.159	2.483	0.000	0.000	0.064	0.226	0.223	0.064
Bitter guard	0.096	0.127	0.255	ND	0.191	1.687	0.064	0.159	0.096	0.277	2.069	2.165
Mustard	ND	0.096	0.127	0.096	0.096	1.783	0.096	0.891	0.096	0.207	0.223	0.223
Fennel	ND	ND	ND	0.127	0.096	0.127	0.013	0.446	0.032	0.060	0.064	0.064
Sunflower	ND	ND	ND	0.096	0.127	0.159	0.096	1.114	0.127	0.140	0.127	0.159
Brinjal	ND	ND	ND	0.414	0.096	0.096	1.019	0.923	0.096	0.115	0.159	0.191
Capsicum	0.191	ND	ND	1.592	2.228	2.801	0.127	1.242	0.127	0.092	0.096	0.096
Cluster		ND	ND									
Beans	ND			0.159	0.191	2.165	2.451	2.451	2.706	0.207	1.369	1.146
Average	0.072	0.060	0.084	0.326	0.398	1.413	0.483	0.903	0.418	0.166	0.541	0.514
WHO/FAO												
Limit	0.01 mg/kg			0.10 mg/kg			0.05 mg/kg			0.20 mg/kg		

Vegetable accumulation capabilities and soil characteristics determine the amount of heavy metals present in various vegetable varieties [29]. Cd exceeded the WHO limit in tomato, bitter gourd and capsicum. Cr surpassed the WHO/FAO recommendations in tomato, bitter gourd, mustard, capsicum, and cluster beans, whereas Ni showed the alarming level in sunflower, brinjal, capsicum, and cluster beans. The Pb crossed the safe limit of WHO/FAO only in bitter gourd and cluster beans. Ubauro showed a tendency for elevated Cr levels, particularly in bitter gourd, mustard, and cluster beans. Capsicum exhibited relatively high Cr concentrations across all locations, with the highest levels observed in Ubaro. These findings suggest potential regional differences in heavy metal bioavailability and accumulation in vegetables, highlighting the need for ongoing monitoring to assess potential dietary exposure and associated health risks (Table 2). Results of Cd, Cr, Ni and Pb from different vegetables are compared with present work as given in literature of different countries like China, Pakistan, Bangladesh and Nigeria are given Table 7.

The present work's Cd content, ranging from ND – 0.287 mg/kg, are usually

lower as compared to reported in other regions. Such as, studies from Pakistan show Cd content of 0.021 mg/kg [30]. When compared to internationally reported results, the contents of Cd in this study are comparable to China (0.106 mg/kg) [31] and to some extent lower than another reported Chinese study (1.6 mg/kg) [32]. and Bangladesh (1.696 mg/kg) [12], however results are also considerably lower than those found in Nigeria (26.87–33.50 mg/kg) [34]. The Cr concentrations in the present study (0.0 - 2.801 mg/kg) are notably lower than those reported elsewhere. A study from Pakistan reported 0.163 mg/kg [30], while another showed a much wider range of 9.84-22.30 mg/kg [30]. Similarly, Cr levels from China (4.3 mg/kg) [33] and Nigeria (27.0–38.67 mg/kg) [34] are significantly higher. The concentration in this study is also higher than that reported in Bangladesh (0.529 mg/kg) [12].

The Ni concentrations (0.0 – 1.242 mg/kg) in the present study are lower than most comparable findings. Studies from Pakistan reported levels of 0.412 mg/kg [30]. International data also shows higher concentrations, such as 7.7 mg/kg in China [33] and 0.78 mg/kg in Bangladesh [12]. The

Pb concentrations in this study (0.092 – 2.069 mg/kg) are relatively low when compared to other studies.

A study from Pakistan reported a wide range of Pb as 0.631 mg/kg [30], while another study from Pakistan showed an extremely high range of 47.12-123.97 mg/kg [31]. In comparison, levels from China (0.54 mg/kg [32] and 105 mg/kg [33]), Nigeria (4.17–10.90 mg/kg) [34], and Bangladesh (4.4113 mg/kg) [12] indicate significant variability, with the current study's values falling on the lower end of the spectrum. This comparative analysis shows that while heavy metal concentrations are present in the vegetables of the Ghotki District, they are generally at the lower end of the range when compared to similar studies from other regions of Pakistan and internationally. However, some studies from Nigeria [34] report alarmingly high concentrations, highlighting significant regional differences in heavy metal contamination (Table 7).

### Estimated Daily Intake (EDI)

EDI is a calculated value representing the average daily exposure of a person to a contaminant. It's determined by multiplying the average concentration of a heavy metal in a food item by the average daily consumption rate of that food. The goal is for the EDI to be

significantly lower than the MTDI, creating a safety margin. When EDI approaches or exceeds the MTDI, it indicates a potential for adverse health effects over a lifetime. The EDI values for Pb from this studies (0.01 - 0.77  $\mu\text{g}/\text{kg}$  body weight/day) are well below the WHO/FAO's Provisional Tolerable Daily Intake (PTDI) of 3.5  $\mu\text{g}/\text{kg}$  body weight/day (Table 3(a) and Table 3(b)). This suggests that in the specific study areas, the intake of Pb from the vegetable sources is generally within safe limits. However, even low-level chronic exposure to Pb is a concern due to its neurotoxic effects, especially in children. Similarly, the EDI for Cd (0.003 - 0.016  $\mu\text{g}/\text{kg}$  body weight/day) is considerably lower than the MTDI of 0.83  $\mu\text{g}/\text{kg}$  body weight/day. This is a positive finding, as Cd is a known carcinogen that can accumulate in the kidneys and bones, causing long-term damage.

While there is no universally adopted MTDI for all forms of Cr, some studies found that the EDI for Cr from vegetables was low (0.005 - 0.02  $\mu\text{g}/\text{kg}$  body weight/day). Exceeding a safe intake of Cr<sup>VI</sup> can lead to severe health problems, including DNA damage. The EDI for Ni (0.01 - 0.03  $\mu\text{g}/\text{kg}$  body weight/day) is well below the Tolerable Daily Intake of 12  $\mu\text{g}/\text{kg}$  body weight/day. This is reassuring, as high exposure to Ni can cause allergic reactions, and some Ni compounds are considered carcinogenic (Table 3 (a) and Table 3 (b)).

**Table 3 (a).** Estimated Daily Intake (EDI) ( $\mu\text{g}/\text{kg}$  bw/day) of Cd and Cr of various vegetables (dry weight) collected from Ghotki, MirpurMathelo and Ubauro.

Vegetables	Cd			Cr		
	Ghotki	MirpurMathelo	Ubaro	Ghotki	Mirpur Mathelo	Ubaro
Tomato	$7.75 \times 10^{-4}$	$6.88 \times 10^{-4}$	$7.75 \times 10^{-4}$	$3.43 \times 10^{-4}$	$4.29 \times 10^{-4}$	$6.70 \times 10^{-3}$
Bitter guard	$2.59 \times 10^{-4}$	$3.43 \times 10^{-4}$	$6.88 \times 10^{-4}$	0.00	$5.16 \times 10^{-4}$	$4.55 \times 10^{-3}$
Mustard	0.00	$2.59 \times 10^{-4}$	$3.43 \times 10^{-4}$	$2.59 \times 10^{-4}$	$2.59 \times 10^{-4}$	$4.81 \times 10^{-3}$
Fennel	0.00	0.00	0.00	$3.43 \times 10^{-4}$	$2.59 \times 10^{-4}$	$3.43 \times 10^{-4}$
Sunflower	0.00	0.00	0.00	$2.59 \times 10^{-4}$	$3.43 \times 10^{-4}$	$4.29 \times 10^{-4}$
Brinjal	0.00	0.00	0.00	$1.12 \times 10^{-3}$	$2.59 \times 10^{-4}$	$2.59 \times 10^{-4}$
Capsicum	$5.16 \times 10^{-4}$	0.00	0.00	$4.30 \times 10^{-3}$	$6.01 \times 10^{-3}$	$7.56 \times 10^{-3}$
Cluster Beans	0.00	0.00	0.00	$4.29 \times 10^{-4}$	$5.16 \times 10^{-4}$	$5.84 \times 10^{-3}$
MTDI Limit		0.046 (mg/day)			0.2 (mg/day)	

**Table 3 (b).** Estimated Daily Intake (EDI) ( $\mu\text{g}/\text{kg}$  bw/day) of Ni and Pb of various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubaro.

Vegetables	Ni Ghotki	Ni Mirpur Mathelo	Ni Ubaro	Pb Ghotki	Pb Mirpur Mathelo	Pb Ubaro
Tomato	0.00	0.00	$1.73 \times 10^{-4}$	$6.10 \times 10^{-4}$	$6.02 \times 10^{-4}$	$1.73 \times 10^{-4}$
Bitter guard	$1.73 \times 10^{-4}$	$4.29 \times 10^{-4}$	$2.59 \times 10^{-4}$	$7.48 \times 10^{-4}$	$5.58 \times 10^{-3}$	$5.84 \times 10^{-3}$
Mustard	$2.59 \times 10^{-4}$	$2.40 \times 10^{-3}$	$2.59 \times 10^{-4}$	$5.59 \times 10^{-4}$	$6.02 \times 10^{-4}$	$6.02 \times 10^{-4}$
Fennel	$3.51 \times 10^{-5}$	$1.20 \times 10^{-3}$	$8.64 \times 10^{-5}$	$1.62 \times 10^{-4}$	$1.73 \times 10^{-4}$	$1.73 \times 10^{-4}$
Sunflower	$2.59 \times 10^{-4}$	$3.01 \times 10^{-3}$	$3.43 \times 10^{-4}$	$3.78 \times 10^{-4}$	$3.43 \times 10^{-4}$	$4.29 \times 10^{-4}$
Brinjal	$2.75 \times 10^{-3}$	$2.49 \times 10^{-3}$	$2.59 \times 10^{-4}$	$3.10 \times 10^{-4}$	$4.29 \times 10^{-4}$	$5.16 \times 10^{-4}$
Capsicum	$3.43 \times 10^{-4}$	$3.35 \times 10^{-3}$	$3.43 \times 10^{-4}$	$2.48 \times 10^{-4}$	$2.59 \times 10^{-4}$	$2.59 \times 10^{-4}$
Cluster Beans	$6.62 \times 10^{-3}$	$6.62 \times 10^{-3}$	$7.30 \times 10^{-3}$	$5.59 \times 10^{-4}$	$3.70 \times 10^{-3}$	$3.09 \times 10^{-3}$
MTDI Limit		0.3 (mg/day)			0.21 (mg/day)	

### *Non-Carcinogenic and Carcinogenic Health Risk*

#### *Non-carcinogenic health risk*

The determination of the THQ values reveal important insights into the potential non-carcinogenic health risks associated with consuming the studied vegetables. A THQ value is a ratio of a chemical's exposure to its reference dose; if the value is less than one, it suggests that the daily intake is below the level at which adverse health effects are expected to occur. Conversely, a THQ value greater than one signals that the daily intake exceeds the safe reference dose, indicating a potential health risk.

The data shows that the majority of the THQ values for Cd, Cr, and Ni are below the critical threshold of 1. THQ values for Cr are uniformly low across all vegetables and locations, suggesting that the risk of non-carcinogenic effects from this metal is negligible. While all Ni THQ values also remain below 1, a closer look reveals that the values for Cluster Beans are notably higher than for other vegetables, with THQ reaching 0.361 in Ubaro. Similarly, Cd THQ values, especially in Tomato, are

relatively elevated in Ghotki and Ubaro, at 0.765, approaching the safety limit, but still remaining below it. This indicates that while the current intake levels might not pose an immediate risk, they should be monitored to prevent future concerns.

A more significant finding emerges when examining the THQ values for Pb. The data for this metal presents several instances where the THQ exceeds 1. Specifically, the THQ for Pb in Bitter Guard is alarming, with values of 1.576 in Mirpur Mathelo and 1.650 in Ubaro. These figures are well above the safe threshold and imply a potential for adverse non-carcinogenic health effects from consuming this vegetable in these regions. Similarly, the THQ for Pb in Cluster Beans in Mirpur Mathelo is 1.043, also indicating a potential health risk. The elevated THQ values for Pb are the primary cause for concern. This suggests that while most of the heavy metal concentrations are at levels that do not pose an immediate non-carcinogenic risk, Pb contamination in certain vegetables and locations is a clear health issue. This type of exposure can affect various organs in

the human body, with a particular risk to the nervous and renal systems (Table 4).

The (Table 5) presents the TTHQ, also known as the Hazard Index (HI), for various vegetables across three different locations: Ghotki, Mirpur Mathelo, and Ubaro. The TTHQ is a cumulative risk assessment metric that sums the individual THQ values for multiple heavy metals. A TTHQ value of less than 1 suggests that the total exposure from all assessed heavy metals is below the level at which a non-carcinogenic health risk is expected. Conversely, a TTHQ greater than 1 indicates a potential for adverse health effects from the combined exposure.

The data shows that the consumption of most of these vegetables in all three locations is generally safe in terms of cumulative non-carcinogenic risk, as their TTHQ values are below the critical threshold of 1. For example, vegetables like Fennel, Sunflower, and Mustard consistently exhibit very low TTHQ values, ranging from a minimum of 0.048 for Fennel in Ghotki to a maximum of 0.545 for Mustard in Mirpur Mathelo. Even vegetables with relatively

higher individual THQ values for certain metals, such as Tomato in Ghotki (0.938), remain just below the safety threshold when the total risk is considered.

However, a significant health concern is highlighted for Bitter Guard and Cluster Beans in two of the locations. The TTHQ for Bitter Guard is notably elevated in Mirpur Mathelo at 1.937 and in Ubaro at a staggering 2.345. These values are well above the safety limit, indicating that the combined daily intake of heavy metals through Bitter Guard consumption in these regions poses a potential non-carcinogenic health risk. Similarly, the TTHQ for Cluster Beans in Mirpur Mathelo is 1.370, and in Ubaro it is 1.238, both surpassing the threshold. These elevated TTHQ values are primarily driven by the high concentrations of lead and nickel in these specific vegetables, as evidenced by the individual THQ calculations. This suggests that while a majority of the vegetables are safe for consumption, the cumulative exposure from certain crops in these particular areas warrants public health attention and potential regulatory action.









**Table 4 (a).** Targeted Hazard Quotient (THQ) of Cd and Cr from various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubaro.

Vegetables	Cd			Cr		
	Ghotki	Mirpur Mathelo	Ubaro	Ghotki	Mirpur Mathelo	Ubaro
Tomato	0.7650	0.6800	0.7650	0.0002	0.0003	0.0044
Bitter guard	0.2560	0.3390	0.6800	0.0000	0.0003	0.0030
Mustard	0.0000	0.2560	0.3390	0.0002	0.0002	0.0032
Fennel	0.0000	0.0000	0.0000	0.0002	0.0002	0.0002
Sunflower	0.0000	0.0000	0.0000	0.0002	0.0002	0.0003
Brinjal	0.0000	0.0000	0.0000	0.0007	0.0002	0.0002
Capsicum	0.5090	0.0000	0.0000	0.0028	0.0040	0.0050
Cluster Beans	0.0000	0.0000	0.0000	0.0003	0.0003	0.0038

**Table 4 (b).** Targeted Hazard Quotient (THQ) of Ni and Pb from various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubauro.

Vegetables	Ni			Pb		
	Ghotki	Mirpur Mathelo	Ubaro	Ghotki	Mirpur Mathelo	Ubaro
Tomato	0.0000	0.0000	0.0085	0.1720	0.1700	0.0490
Bitter guard	0.0085	0.0212	0.0128	0.2110	<b>1.5760</b>	<b>1.6500</b>
Mustard	0.0128	0.1188	0.0128	0.1580	0.1700	0.1700
Fennel	0.0017	0.0595	0.0043	0.0460	0.0490	0.0490
Sunflower	0.0128	0.1485	0.0169	0.1070	0.0970	0.1210
Brinjal	0.1359	0.1231	0.0128	0.0880	0.1210	0.1460
Capsicum	0.0169	0.1656	0.0169	0.0700	0.0730	0.0730
Cluster Beans	0.3268	0.3268	0.3608	0.1580	1.0430	0.8730

**Table 5.** Total targeted Hazard Quotient (TTHQ) of heavy metals from various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubauro.

Vegetables	Scientific Name	Image	Ghotki	Mirpur Mathelo	Ubaro
Tomato	<i>Solanumlycopersicum</i>		0.938	0.850	0.827
Bitter guard	<i>Solanumpubescens</i>		0.476	<b>1.937</b>	<b>2.345</b>
Mustard	<i>Brassica nigra</i>		0.171	0.545	0.525
Fennel	<i>Foeniculumvulgare</i>		0.048	0.108	0.053
Sunflower	<i>Helianthus annuus</i>		0.120	0.246	0.138
Brinjal	<i>Solanummelongena</i>		0.224	0.244	0.159
Sweet pepper	<i>Capsicum annum</i>		0.599	0.243	0.095
Cluster Beans	<i>Cyamopsistetragonoloba</i>		0.485	1.370	1.238

### Carcinogenic health risk

The TCR is a crucial metric in health risk assessments, as it quantifies the increased

probability of an individual developing cancer over a lifetime due to exposure to a carcinogen, in this case, heavy metals. The US Environmental Protection Agency (EPA)

generally considers a TCR below  $10^{-6}$  (one in a million) to be a negligible risk and a value up to  $10^{-4}$  (one in ten thousand) to be acceptable. Values exceeding this threshold warrant a closer look due to potential health concerns. The table shows varying levels of TCR across different heavy metals and locations. The highest risks are associated with Ni and Cr, while Cd also presents significant risk, and Pb poses the lowest risk. The distribution of risk is not uniform and depends on the specific vegetable and its cultivation location. In Ghotki and Mirpur Mathelo, the highest TCRs are consistently found in Cluster Beans for Ni, with values of  $1.13 \times 10^{-2}$  at both locations. These figures are significantly above the acceptable risk threshold of  $10^{-4}$  and indicate a high potential carcinogenic risk from consuming these vegetables. Similarly, Capsicum and Brinjal show high TCR values for Ni and Cr, particularly at Mirpur Mathelo, where the TCR for Ni in Capsicum is  $5.70 \times 10^{-3}$ , and in Brinjal, it is  $4.23 \times 10^{-3}$ . In contrast, Pb consistently shows very low TCR values, with a maximum of  $4.96 \times 10^{-5}$  in Bitter Guard from Ubaro, which is still well within the acceptable range.

The data suggests that the contamination levels and the associated health risks are site-specific. For instance, the TCR for Cr in Tomato is highest in Ubaro at  $2.81 \times 10^{-3}$ , whereas for Bitter Guard, the highest Cr TCR is also in Ubaro at  $1.91 \times 10^{-3}$ . These elevated values highlight potential environmental contamination issues in the Ubaro region that are impacting the food chain. The high TCRs for Nickel in Cluster Beans in all three locations, with the highest in Ubaro at  $1.24 \times 10^{-2}$ , are a major concern. Such risks could be a consequence of the vegetables absorbing heavy metals from contaminated soil or irrigation water. Briefly, while the carcinogenic risk from Pb seems negligible across all samples, the TCRs for Ni, Cr, and Cd in certain vegetables, especially Cluster Beans and Capsicum, exceed the acceptable thresholds, indicating a significant health risk for consumers. These findings could inform public health policy, such as implementing stricter monitoring of heavy metal concentrations in soil and water or advising the public on the consumption of specific vegetables from these contaminated areas (Table 6a and b), (Fig.2 and Fig. 3).

**Table 6 (a).** Target Cancer Risk (TCR) of Cd and Cr from various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubaro.

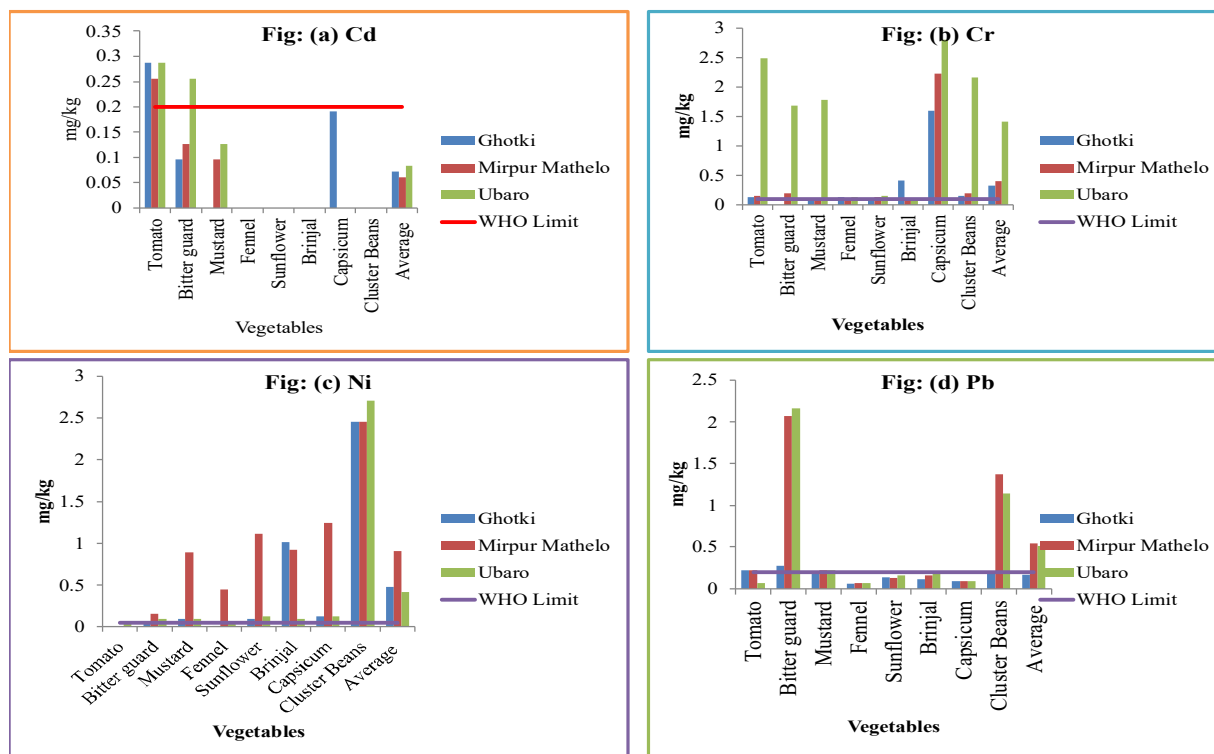
Vegetables	Cd			Cr		
	Ghotki	Mirpur Mathelo	Ubaro	Ghotki	Mirpur Mathelo	Ubaro
Tomato	$1.16 \times 10^{-3}$	$1.03 \times 10^{-3}$	$1.16 \times 10^{-3}$	$1.44 \times 10^{-4}$	$1.80 \times 10^{-4}$	$2.81 \times 10^{-3}$
Bitter guard	$3.89 \times 10^{-4}$	$5.14 \times 10^{-4}$	$1.03 \times 10^{-3}$	0	$2.17 \times 10^{-4}$	$1.91 \times 10^{-3}$
Mustard	0.00	$3.89 \times 10^{-4}$	$5.14 \times 10^{-4}$	$1.09 \times 10^{-4}$	$1.09 \times 10^{-4}$	$2.02 \times 10^{-3}$
Fennel	0.00	0.00	0.00	$1.44 \times 10^{-4}$	$1.09 \times 10^{-4}$	$1.44 \times 10^{-4}$
Sunflower	0.00	0.00	0.00	$1.09 \times 10^{-4}$	$1.44 \times 10^{-4}$	$1.80 \times 10^{-4}$
Brinjal	0.00	0.00	0.00	$4.70 \times 10^{-4}$	$1.09 \times 10^{-4}$	$1.09 \times 10^{-4}$
Capsicum	$7.74 \times 10^{-4}$	0.00	0.00	$1.81 \times 10^{-3}$	$2.52 \times 10^{-3}$	$3.18 \times 10^{-3}$
Cluster Beans	0.00	0.00	0.00	$1.80 \times 10^{-4}$	$2.17 \times 10^{-4}$	$2.45 \times 10^{-3}$

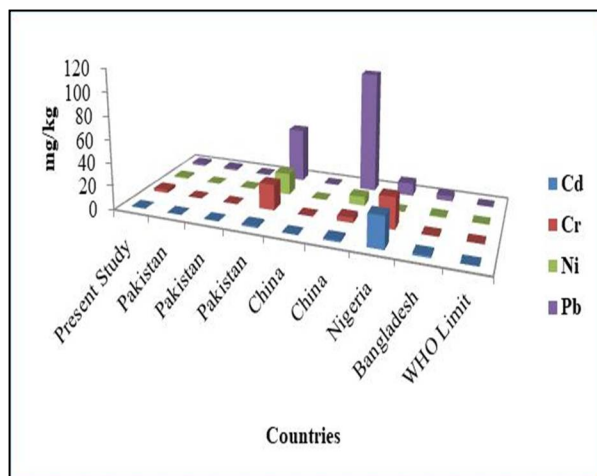
**Table: 6 (b).** Target Cancer Risk (TCR) of Ni and Cd from various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubaro.

Vegetables	Ni			Pb		
	Ghotki	Mirpur Mathelo	Ubaro	Ghotki	Mirpur Mathelo	Ubaro
Tomato	0.00	0.00	$2.94 \times 10^{-4}$	$5.19 \times 10^{-6}$	$5.12 \times 10^{-6}$	$1.47 \times 10^{-6}$
Bitter guard	$2.94 \times 10^{-4}$	$7.29 \times 10^{-4}$	$4.40 \times 10^{-4}$	$6.36 \times 10^{-6}$	$4.74 \times 10^{-5}$	$4.96 \times 10^{-5}$
Mustard	$4.40 \times 10^{-4}$	$4.08 \times 10^{-3}$	$4.40 \times 10^{-4}$	$5.59 \times 10^{-6}$	$5.12 \times 10^{-6}$	$5.12 \times 10^{-6}$
Fennel	$5.97 \times 10^{-5}$	$2.04 \times 10^{-3}$	$1.47 \times 10^{-4}$	$1.38 \times 10^{-6}$	$1.47 \times 10^{-6}$	$1.47 \times 10^{-6}$
Sunflower	$4.40 \times 10^{-4}$	$5.12 \times 10^{-3}$	$5.83 \times 10^{-4}$	$3.21 \times 10^{-6}$	$2.92 \times 10^{-6}$	$3.65 \times 10^{-6}$
Brinjal	$4.68 \times 10^{-3}$	$4.23 \times 10^{-3}$	$4.40 \times 10^{-4}$	$2.64 \times 10^{-6}$	$3.65 \times 10^{-6}$	$4.39 \times 10^{-6}$
Capsicum	$5.83 \times 10^{-4}$	$5.70 \times 10^{-3}$	$5.83 \times 10^{-4}$	$2.11 \times 10^{-6}$	$2.20 \times 10^{-6}$	$2.20 \times 10^{-6}$
Cluster Beans	$1.13 \times 10^{-2}$	$1.13 \times 10^{-2}$	$1.24 \times 10^{-2}$	$4.75 \times 10^{-6}$	$3.15 \times 10^{-5}$	$2.63 \times 10^{-5}$

**Table: 7** Comparison of average heavy metals (mg/kg) in vegetables with previous studies in various locations.

Place of Study	Cd mg/kg	Cr mg/kg	Ni mg/kg	Pb mg/kg
Present Study	0.0 – 0.287	0.0 – 2.801	0.0 – 1.242	0.092 – 2.069
Pakistan	0.021 [30]	0.163 [30]	0.412 [30]	0.631 [30]
Pakistan	0.70-1.61 [31]	9.84-22.30 [31]	4.03-18.60 [31]	47.12-123.97 [31]
China	0.106 [32]	-----	-----	0.041 [32]
China	1.6 [33]	4.3 [33]	7.7 [33]	105 [33]
Nigeria	26.87–33.50 [34]	27.00–38.67 [34]	-----	4.17–10.90 [34]
Bangladesh	1.696 [12]	0.529 [12]	0.78 [12]	4.4113 [12]

**Figure 2.** (a), (b), (c) and (d) Graphical representation of concentration of heavy metals from vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubaro



**Figure 3.** Comparison of concentration (mg/kg) of heavy metals from vegetables of study area and various countries

### Study of Principal Component Analysis

#### *Cadmium in ubauro, mirpur mathelo, and ghotki*

The first plot, with an impressive 98.72% of the variance explained by the first two principal components, shows the distribution of Cd. The plot suggests that samples from Ghotki have significantly different Cd levels compared to those from Ubauro and Mirpur Mathelo. "Cd in Ubauro" and "Cd in Mirpur Mathelo" are less influential and appear closer to the center. The vegetable samples are spread out, showing that different vegetables accumulate Cd to varying degrees (Fig. 2).

#### *Chromium (Cr) in ubauro, mirpur mathelo, and ghotki*

This plot, explaining 99.24% of the variance, highlights the distribution of Cr. The vectors for "Cr in Ubauro" and "Cr in Mirpur Mathelo" are pointing in different directions from "Cr in Ghotki," which has a shorter vector, suggesting that the Cr concentration profiles are quite distinct across these locations. This visual separation implies that the sources of Cr contamination or the soil

conditions affecting Cr uptake are different in these areas (Fig. 4).

#### *Nickel in ubauro, mirpur mathelo, and ghotki*

With 97.43% of the variance explained, this plot shows the Ni distribution. The "Ni in Mirpur Mathelo" vector is long, pointing to a distinct group of samples, suggesting that some vegetables from this location are particularly high in nickel. The "Ni in Ghotki" vector is also significant, indicating that Ni levels in Ghotki samples are different from those in Ubauro and Mirpur Mathelo. This reinforces the idea that metal contamination is location-specific (Fig.4).

#### *Lead in ubauro, mirpur mathelo and ghotki*

The plot explaining 99.7% of the variance, demonstrates the highest explanatory power among all four. The long vectors for "Pb in Ubauro" and "Pb in Ghotki" are nearly opposite each other, suggesting a strong contrast in lead accumulation between these two locations. The "Pb in Mirpur Mathelo" vector is shorter and positioned differently, indicating a unique Pb profile for that area. This plot vividly shows that lead contamination is highly variable depending on the specific location (Fig. 4).

These plots provide a powerful visual summary, revealing that the type and concentration of heavy metal contamination are highly dependent on both the specific location and the type of vegetable being studied. The PCA successfully distinguishes the metal profiles of each location, providing critical insights for further research and risk assessment (Fig.4).

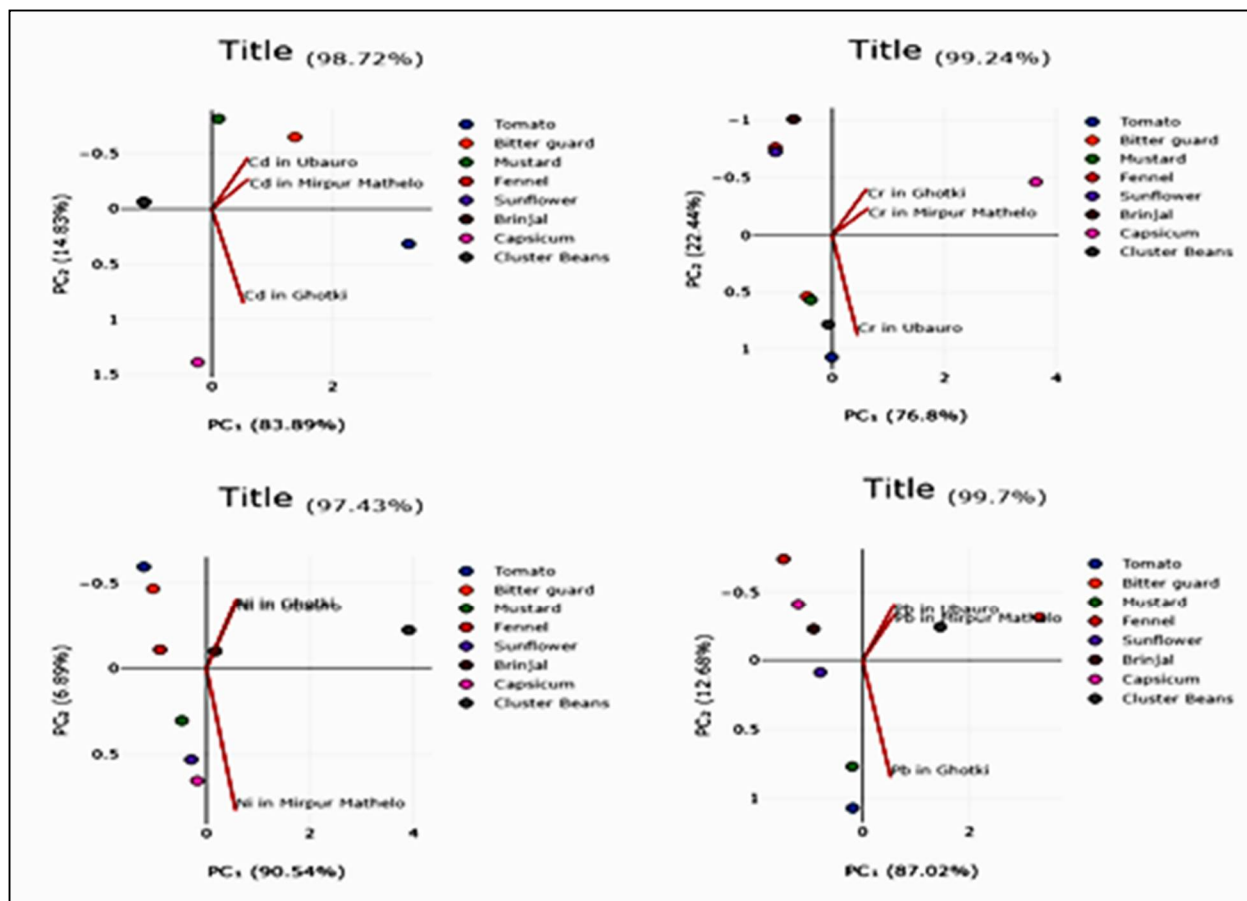


Figure 4. Principal Component Analysis of heavy metals in various vegetables (dry weight) collected from Ghotki, Mirpur Mathelo and Ubauro

## Conclusion

The findings of the study reveal that HMs contamination in vegetables from the Ghotki, Mirpur Mathelo, and Ubaro regions is generally lower than in other international studies, specific vegetables like bitter gourd, capsicum, and cluster beans still contain concerning levels of Pb, Cd, Cr, and Ni that exceed WHO/FAO safe limits. The research identified a significant non-carcinogenic health risk associated with consuming bitter gourd and, more importantly, a substantial carcinogenic risk from Cr and Cd in several vegetables, with the TCR values surpassing the safe threshold set by the USEPA. This indicates a potential for a lifetime risk of developing cancer for consumers. The PCA

confirmed that heavy metal contamination is highly site-specific. The concentration and type of heavy metal varied significantly between the three agricultural regions, suggesting that different sources of contamination and soil conditions exist in each area. The analysis also confirmed that HM pollution is highly specific to location, with distinct contamination profiles across the three agricultural areas, emphasizing the need for focused regional monitoring to safeguard public health.

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### Conflict of Interest

The authors declare no conflict of interest.

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