HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS VIA CONSUMPTION OF MARKETED VEGETABLES IN ISLAMABAD AND RAWALPINDI

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Dedication

I dedicate this Thesis to my family. I love you deeply with all my heart. To my husband Abeer Khan Kundi, you have been a listener and a supporter of all my endeavors. Your partnership, steadfastness, and love sustain me. To my brother, Haisum Javed, who kept reminding me that all things are possible. Never be afraid to pursue your dreams and goals. You both are precious gifts from the ALLAH. I love you without measure. Thank you for your patience as I pursued and completed this degree. To my parents, thank you for raising your children to think for themselves. I am grateful for my father who always told me I could achieve anything I chose to do.

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Azka Irum

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Abstract

The relationship between human beings and soil is very complex. About 95% of food comes from the soil, while 99% of freshwater passes from the soil. Despite such a complex relationship, the soil has not gained much attention for ages. Soil act as a sink to many pollutants such as heavy metals (Pb, Cd, Zn, As, Cr), due to which it is much more prone to degradation. These heavy metals in soil are transferred into vegetables, through which they enter the food chain. Due to this fact, the objective of the study was set to detect the concentration of heavy metals such as Pb, Cd, Zn, As, and Cr in vegetables by using atomic absorption spectroscopy and to assess the potential health risk associated with the consumption of these contaminated vegetables using carcinogen and non-carcinogen equations. The mean concentration of Cd and Cr in all vegetables was below the permissible limit set by WHO except for tomato, while Pb and As were detected to be above the permissible limits, and Zn was below the permissible limit in all vegetables except spinach. The estimated daily intake (EDI) of all the vegetables for both adults and children was below the tolerable daily intake. The target hazard quotient (THQ) for Pb, and As, were greater than 1 showing severe health risks to both children and adults, while for Cr, Cd, and Zn, it was within safe limits (<1). Target carcinogenic risk for metals such as Pb, Cd, Cr, and As was higher than 10^{-6} showing potential cancer risk to both adults and children. The hazard quotient (HQ) for Pb and Cd was higher than 1 posing a health risk to both adults and children, while HQ for As and Cr was <1 for both adults and children, thus the exposed population has no risk of adverse effects. Incremental lifetime cancer risk (ILCR) for Cd, Cr, and As calculated for both children and adults were $>10^{-5}$, showing high potential cancer risk in the exposed population, while for Pb, ladyfinger, spinach, and zucchini showed high risk while others were calculated to be less than 10^{-5}. The study helped in the evaluation of cancer risk due to the intake of fresh vegetables marketed in Islamabad and Rawalpindi. The study concluded that the exposed population is at risk due to the consumption of vegetables contaminated with metals specifically As, Pb, Cd, and Cr. Furthermore, there is a need for regular monitoring of metal concentration in marketed fresh vegetables, and to develop advanced approaches to restrict the accumulation of metals in agricultural land.

Keywords: Heavy metals, Risk Assessment, Vegetables, Human Health

Chapter 1

Introduction

Vegetables are an essential component of our diet and contain high concentrations of trace minerals, ascorbic acid, calcium, iron, and other key biochemicals and nutrients like carotene (Jimoh & Oladiji, 2005). These are inexpensive source of energy in developing countries (Wadood et al., 2021). Pakistan's population is expanding rapidly, creating a huge need for food to meet its demands (Wadood et al., 2021). To meet both domestic and foreign demand, Pakistan is producing a wide range of vegetables. Being an agricultural economy, this sector affects around 70% of our population directly or indirectly. Vegetable farming accounts for around 2% of all crop production area, whereas their export proportion is only 0.22% GOP (2013). These are the primary energy sources, particularly in developing nations. According to the World Health Organization (WHO), in order to acquire all the nutrients needed for maximum health as reported by Lock et al. (2005), each person should consume at least 400 g of fruits and vegetables every day. The average daily vegetable intake in Pakistan is 134 g, which is 66.5% below the daily minimum recommended intake of 400 g GOP (2013). Particularly in developing nations, food safety is the main concern. Providing safe food is a difficult task and crucial to both public health and food quality assurance. In general, groundwater and other freshwater reservoirs are used to irrigate fruits and vegetables. The dependence on groundwater, which is typically expensive and of poor quality due to sodium adsorption, high electrical conductivity, sodium carbonate residues, and heavy metals, has increased as a result of water constraints in developing countries. Additionally, especially in the peri-urban areas, domestic wastewater and industrial effluents have emerged as substitute sources of irrigation. According to Ensink et al. (2004), it is estimated that 80% of the wastewater produced in underdeveloped nations is utilized to irrigate crops without first being treated. About 30% of Pakistan's wastewater is used for cultivation, while the other water is discharged into rivers untreated (FAO, 2002). Due to the benefits and potential health risks, the use of wastewater for irrigation is debatable. The application of wastewater has a number of drawbacks, such as contaminating groundwater and the food chain since edible plants may accumulate metal (Wadood et al., 2021). Additionally, the majority of farmers are uneducated and are not aware of the non-legal use of agrochemicals. One of the top nations where agrochemicals are extensively employed to boost crop productivity is Pakistan. The soil and water sources are also polluted by the regular application of agrochemicals. The edible sections of vegetables can bioaccumulate metals from contaminated soil (Alam et al., 2018). In comparison to other food crops like grains and fruits, vegetables might accumulate more HMs (Mapanda et al., 2005).

One key topic of environmental research is the trophic transmission of dangerous heavy metals and metalloids in food chains and webs. As a result of heavy metals being transported from the abiotic environment (water, sediments, and soils) to living things and building up in the biota, food chains become contaminated with these substances. The biomagnification of these substances in the food chains occurs when heavy metals are elevated at successive trophic levels of food chains. Hazardous heavy metal and metalloid trophic transfer, bioaccumulation, and biomagnification in food chains have significant effects on wildlife and human health. Toxic non-essential heavy metals and metalloids can enter the body by a variety of means, including ingestion, inhalation, and skin absorption. In addition to providing farmers with a means of livelihood, urban vegetable growing is essential for meeting the city's nutritional demands. In most nations and locations, consumers of vegetables are exposed to heavy metals through skin contact and ingestion, which can have harmful consequences on their health.

Through the consumption of these contaminated vegetables, dietary exposures to hazardous metals (lead, cadmium, and copper) and metalloids (arsenic) have been recognized as a risk to human health. Depending on the level of exposure, this condition causes varying degrees of health problems (Demirezen and Aksoy 2006). Sulfur atoms have a strong affinity for the majority of heavy metals. By forming bonds with the sulfur in enzymes, they interfere with their function (Waseem and Arshad 2016). Additionally, consuming contaminated food can substantially affect the immune systems of people who have malnutrition-related diseases, intrauterine growth retardation, and a high prevalence of upper gastrointestinal cancer (Iqbal et al., 2010). Additionally, trace elements are needed in small amounts for metals including iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). Among these, zinc and copper are critical to maintain health throughout one's life and play crucial physiological and biochemical roles. Rapid industrialization and urbanization in emerging nations are to blame for the high concentration of heavy metals in urban environments (Wong et al., 2003). These heavy metals once accumulated in the human body, cause adverse effects such as exposure to As across the globe, including arsenicosis, diabetes, peripheral vascular disease, neurologic and neurobehavioral disorders, hearing loss, portal fibrosis, and hematologic abnormalities (Tchounwou, et al., 2003; Tchounwou, et al., 2004). The skin, lungs, liver, and blood systems are the organs in which chronic effects are most prominent. There have been numerous health impacts linked to As use across the globe, including humans are genotoxic to arsenical compounds, and the methylated form of As inhibits DNA repair while also causing the spleen and liver to create reactive oxygen species (ROS) (Kim et al., 2015). Ingestion of inorganic As can lead to cancer in a variety of body parts (Ng et al., 2003). According to reports, elemental As can cause cancer in a variety of human organs, including the bladder, kidney, skin, lung, and liver (Xiong et al., 2013). Proliferative prostatic lesions, bone fractures, renal illness, hypertension, and lung cancer can all occur in people exposed to high levels of Cd (Satarug et al., 2003; Kolluru et al., 2019). Numerous studies examined additional potential risks to human health associated with the ingestion of vegetables contaminated with HMs in other parts of the world, including Ethiopia (Gebeyehu and Bayissa, 2020), China (Ji et al., 2018), Pakistan (Alam et al., 2018), India (Gupta et al., 2021), and Bangladesh (Shaheen et al., 2016; Alam et al., 2003).

According to Jarup (2003), exposure to Cd may harm the kidneys. Additionally, Cd is a known potential carcinogen and the cause of several disorders linked to blood, bone, nervous system, liver, kidney, and other organ disorders. It can cause endocrine system disruption, especially with regard to reproductive hormones like progesterone and testosterone, and it also raises the risk of ovarian and breast cancer (Itoh, et al., 2014; Jahan, et al., 2014; Yang, et al., 2015).

Even at blood lead levels (BPb) less than 5 mg/dL, Pb is nephrotoxic, especially in populations that are vulnerable, such as those with hypertension, diabetes, and renal disease (Ekong et al., 2006). Additionally, the harmful effects of Pb on human health have been well-documented. Its acute and chronic exposure can result in encephalopathy, ataxia, and a lower level of awareness, which may cause coma and death, as well as weakness in the fingers, wrists, or ankles and miscarriage in pregnant women. It can also cause learning and concentration problems in children, as well as long-term exposure to humans might affect memory (USEPA, 1999). Recent research on the assessment of the toxicity of low-dose exposures to As, Cd, and Pb has shown that hazardous metal interactions in combinations were synergistic and that heavy metal mixtures exhibited higher toxicities than individual heavy metals (Cobbina et al. 2015).

Zn toxicity causes development retardation, delayed sexual maturation, increased susceptibility to infection, diarrhea, and changes in enzymatic function in humans (Hambidge & Krebs, 2007). Pb and Cd are listed by the Environmental Protection Agency as potential human carcinogens (USEPA, 2009) (group B2). IARC classed inorganic Pb compounds as "probably carcinogenic to people" (group 2A), while Pb was classified as "potentially carcinogenic to humans," placing Cd and its compounds in group 1 (carcinogenic to humans) (group 2B). As is categorized as an established carcinogen (USEPA group A). Therefore, it is unquestionably crucial to evaluate the levels of metals in vegetables and related health hazards.

The amount of potentially harmful HMs a person consumes through the diet of vegetables impacts the level of toxicity in humans. Several researchers have adapted the probability risk assessment technique to fully use the available toxicity and exposure data (Solomon et al., 1996; Cardwell et al., 1999; Hall et al., 1999, 2000). However, these methods are only limited to quantifying the health risk of carcinogenic pollutants and not to quantifying the health risks of non-cancer risks. At present, methods of non-cancer risk assessment do not provide quantitative estimates on the probability of experiencing non-cancer effects from contaminated exposure (Chauhan, & Chauhan, 2014). Several indices and indicators are used to assess the possible risks to human health posed by HMs when consumed through vegetables (Gupta et al., 2019). The indices that can help identify the noncarcinogenic and carcinogenic risks of HMs to human health include estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), health risk index (HRI), hazard quotient (HQ), and cancer risk (CR). The chances that children or adults may develop cancer after being exposed to potentially carcinogenic elements throughout a lifetime is estimated by CR, while HI summarizes noncarcinogenic risks. This method was provided by the US EPA region III risk-based concentration table (US EPA, 2000). A further assumption was made that the consumed dose via ingestion is equal to the absorbed contaminant dose as stated in US EPA guidance (1989).

An important step in developing controls to better manage soil quality while simultaneously protecting the environment and human health is to identify the sources of any potential toxins in the soil. In soil, HMs concentrations come from both anthropogenic and natural sources. The anthropogenic sources include untreated industrial effluents, fertilizers, pesticides, aerosols in the atmosphere, vehicular emissions, and wastewater irrigation, while the major natural source is geological parent material (Lu et al., 2012; Sun et al., 2013; Huang et al., 2015). Principal component analysis (PCA), absolute principal component scoring/multiple linear regression (APCS/MLR) (Qu et al., 2013; Gholizadeh et al., 2016), stochastic models (Hu and Cheng, 2013), isotope labeling (Cheng and Hu, 2010), ensemble models (Wang et al., 2015), and geo-statistical models (Sun et al., 2013) were all used in various types of research to identify pollution sources.

The present study deals with the evaluation of heavy metal concentration in fresh vegetables collected from different markets in Islamabad and Rawalpindi. Sampling was done randomly from different local vegetable markets of Islamabad and Rawalpindi and health risk assessment was assessed through cancer and non-cancer risk assessment. The significant use of phosphorus-based fertilizers and continuous use of wastewater for irrigation purposes has led to an increase in heavy metal concentration in food crops. However, to the best of our knowledge, no or few major systematic investigations of metal concentration and assessment of human health risk have been conducted to date. To obtain a better understanding of HMs accumulation in vegetables as well as their possible health risk, it is necessary to make efforts to minimize the effects associated with heavy metal pollution. The significance of this study cannot be overstated given the nation's recent pledge to increase local production for food security. Our findings could contribute to the development of legislation that safeguards the environment and human health, as well as policies relating to agricultural output. Additionally, it has highlighted the necessity of long-term development and political commitment to establishing a hospitable climate. The study also provides a reference document for the policy makers on the anticipation and treatment of heavy metal pollution.

1.1. Objectives

Human health is closely related to the quality of soil and especially to its degree of pollution. Estimated daily intake, target hazard quotient, hazard index, cancer risk, and health risk index show that consumption of vegetables grown on soil or irrigated with water, contaminated with HMs can cause severe health hazards.

Therefore, the objectives of the study were:

1. To determine the concentration of selected HMs (As, Cd, Cr, Pb and Zn) in fresh vegetables marketed in Islamabad and Rawalpindi.
2. To evaluate the human health risk assessment of HMs via the consumption of fresh vegetables marketed in Islamabad and Rawalpindi.

Chapter 2

Literature Review

2.1. Heavy Metals

There are many definitions of heavy metals found in literature among which some of them defined metals as “metals with a density greater than $5g/cm^3$ (Csuros & Csuros 2016). Duffus (2002) define heavy metals as “a group of metals and metalloids which are associated with the contamination and ecotoxicity of the environment”. Recently Ali and Khan (2018) defined heavy metals as “naturally occurring metals having a density greater than $5g/cm^3$ and an atomic number greater than 20 (Ali & Khan 2018).

Heavy metals based on their role in the human body are classified into two groups: essential and non-essential elements. Essential elements are those elements that are required by the human body in a very low concentration such as; manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn), while non-essential heavy metals play no essential function in the human body and are toxic to the human body such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), mercury (Hg) (Ramírez 2013; Türkmen et al., 2009; Ali et al., 2019).

2.2. Environmentally Relevant Most Toxic Heavy Metals

Heavy metals are among the most investigated pollutants. The toxicity of heavy metals to biota depends on the dosage and duration of exposure to the metal. Many heavy metals are included in the list of toxic environmentally relevant heavy metals such as chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) (Barakat, 2011). Commonly occurring heavy metals in the environment are chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), and cadmium (Cd) (Khan et al., 2011). Cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and arsenic (As) are enlisted as the highest priority pollutants by China in their “12th 5-year plan for comprehensive prevention and control of heavy metal pollution “(Fu et al., 2017).

2.3. Sources of Heavy Metals

Sources of heavy metals in the environment can be natural as well as anthropogenic. The natural sources include weathering and volcanic eruptions. Industrialization and urbanization have led to an increase in heavy metal contamination of the environment (Nagajyoti et al., 2010). The anthropogenic sources include mining, industrial and agricultural activities. During mining, these heavy metals are released while extracting them from their respective ores. Once released into the environment from different natural and anthropogenic sources, these metals are deposited on land through dry and wet deposition. Discharge of wastewater from industries, excessive use of chemical fertilizers, and combustion of fossil fuel add heavy metals into the environment in different proportions (Ali et al., 2019).

Among the different sources of heavy metals in the environment, one of the major contributions to the environment is of commercial chemical phosphate fertilizers. Phosphate fertilizers are extracted from phosphate rock, and its extraction is made possible by acidification with sulfuric acid in the case of single superphosphate (SSP), while perchloric acid is used to acidify triple superphosphate (TSP). As a result, the final product contains all of the heavy metals and metalloids present in the rock (Mortvedt, 1996). These inorganic fertilizers, when used for agricultural purposes, add heavy metals to the ground (Carnelo et al., 1997). From land, these heavy metals leach into the groundwater and also to the plants including crops. The transfer pathway of potentially toxic metals from phosphate fertilizer to the human body is shown below (Dissanayake & Chandrajith, 2009).

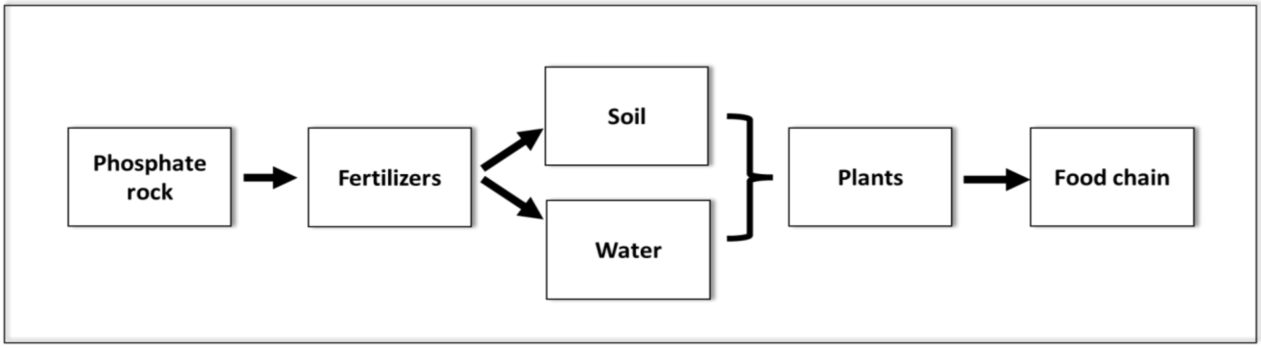

Fig 2.1. Pathways of toxic metals from phosphate fertilizers to the food chain (Dissanayake & Chandrajith, 2009)

Burning of fossil fuel in homes, industries and transportation is a man-made source of heavy metal contamination. Among the major anthropogenic sources of heavy metals such as chromium (Cr), cadmium (Cd) lead (Pb) and zinc (Zn) are vehicular emissions (Ferretti et al., 1995). Emissions from coal, combustion, and other burning processes are very important anthropogenic sources of heavy metals (Merian 1984). Cadmium, lead, and arsenic are partially volatile in the combustion process (Ali et al., 2019).

Chromium is released into the environment from electroplating industries, leather tanneries, and textile and steel industries (Palaniappan et al., 2009). Worldwide approximately 50,000t/year of chromium maybe released into the atmosphere from coal and wood burning and refuse incineration (Merian 1984). A significant concentration of chromium is also present in fertilizers (Krüger et al., 2017). Cadmium is released into the environment from natural such as weathering and volcanic eruptions, while anthropogenic sources including non-ferrous metal mining, specifically treating lead- zinc ores (Hutton 1983). Around 7,000t/year of cadmium may be released globally from coal burning and incineration of sewage sludge ((Merian 1984). Excessive use of chemical fertilizer is also a source of an increase in cadmium concentrations (Wang et al., 2015). Chemical fertilizers containing phosphorus as an ingredient also have cadmium in trace quantity ranging from 300 ppm on a dry weight basis and consequently maybe a major source of introducing this metal to agriculture (Grant & Sheppard, 2008). Anthropogenic sources of lead include acid batteries, old plumbing systems, and bullets of lead used in hunting, and burning of leaded gasoline, which is banned globally, but some of the developing countries still use tetraethyl lead as an antiknock agent in gasoline (Ale et al., (2019).

2.4. Contamination of Natural Waters and Soil by Heavy Metals

Heavy metals pose a significant threat to both the lives of aquatic as well as terrestrial ecosystems (Slaveykova & Cheloni, 2018). Once these heavy metals release into the environment they contaminate water, sediments, and soil. Heavy metals released into the atmosphere either from natural such as volcanic eruptions or anthropogenic sources such as industrial emissions of agricultural activities, contaminate the water bodies, air, and soil and ultimately, they are ended in the soil, as it acts as a sink to many contaminants (Jehan et al., 2020). Since heavy metals are non-biodegradable in nature, they remain within the earth system, stored in biota, or filtered into groundwater. The presence of toxic heavy metals such as lead, cadmium, chromium, and arsenic even in minute quantities in living organisms or groundwater, has a significant effect on human health. It is important to assess the degree of contamination in the riverine ecosystem by investigating the concentration of heavy metals and their distributions (Islam et al., 2018). Physiological and climatic conditions play an important role in the dynamics and biogeochemical cycling of heavy metals in the environment.

2.4.1. Water

Water is known as the “life-blood of the biosphere”. Due to the universal nature of water, different organic and inorganic chemicals, and environmental pollutants dissolve in water, and because of this reason aquatic ecosystems including both marine as well as freshwater, are vulnerable to contamination. Water contamination with heavy metals is a serious environmental issue, which directly or indirectly effects plants, animals, and human health even at a very low concentration (Rezania et al., 2016: Abrar et al., 2011). Once an aquatic ecosystem is contaminated with heavy metals, it can cause histopathological changes in the tissue of aquatic fish (Ahmed et al., 2014). Water contamination is caused by many anthropogenic sources such as mining operations, industrial and domestic effluents, and agricultural runoff (Zhuang et al., 2013). Among all, the release of untreated industrial wastewater is one of the major sources of contamination of groundwater and surface water (Afzal et al., 2018). Due to its persistence, bioaccumulation, and biomagnification in the food chain, and the toxicity nature of heavy metals, its pollution is a worldwide problem (Rajaei et al., 2012).

2.4.2. Soil

The source of contaminating soil with heavy metals comes from both natural as well as anthropogenic sources (Alloway, 2013). The composition of rock, degree of weathering, physical, chemical, and biological characteristics of soil, and climatic conditions are some of the factors that affect the presence and distribution of heavy metals in soil (Arunakumara et al., 2013). Many studies show that the soil of agricultural land, where more fertilizers and Cu-fungicides are used is more contaminated with heavy metals as compared to the soil of virgin soil and soil where fewer fertilizers are used (Semu & Singh, 1995). In urban areas, the contaminated soil with heavy metals maybe mostly contaminated by vehicular traffic on roads and reports show that the soil is heavily contaminated with lead, about 45%-85% of which is available for the plants to uptake bio-accessible (Mackay et al., 2013). The fate of heavy metals in the environment and their uptake by the plant is dependent on the bioavailability of the heavy metals. The bioavailability of heavy metals in the soil varies from each other and is dependent on the metal speciation and the different physicochemical properties of soils.

2.5. Heavy Metals as Toxic Materials

Non-essential heavy metals such as As, Pb, Cd, and Cr are harmful to plants, animals, and humans at very low concentrations, while essential heavy metals such as Zn can cause severe health effects at high concentrations (Mahboob et al., 2014). Three characteristic features which are: persistence, bioaccumulation, and toxicity, are considered while developing a procedure for hazard identification of chemical contaminants in the aquatic environment. Those chemical substances which are bio-accumulative and non-biodegradable, are more toxic in nature (DeForest, et al., 2007).

The toxicity of a substance is defined as the property of a substance to affect the survival, growth, and reproduction of an organism. Depending on dose or duration, some of the heavy metals are classified as carcinogenic, mutagenic, or teratogenic to different species depending on dose and duration. Heavy metals not only affect human health but wildlife is also affected. The mechanism affecting different tissues, organs, and systems of the human body is very complex, and many of them are still not fully investigated. A report shows that Bivalve (*Anodonta anatine*), when exposed to cadmium, affected an enzyme carbonic anhydrase (CA) in tissues, that plays a role in osmoregulation and Ca metabolism (Ngo et al., 2011). It is also reported that a decrease in freshwater mussels is also due to the high toxicity and bioaccumulation potential of cadmium in water (Ngo, 2008).

2.6. Trophic Transfer of Heavy Metals

Due to the non-biodegradable nature of heavy metals in the environment, they accumulate in the body of living organisms and are transferred from one trophic level to another in the food chain. Different heavy metals have different half-lives in different species, depending on the extent of the rate of accumulation and rate of elimination in the body of living organisms.

Heavy metals enter the body of an organism either directly from the abiotic environment such as water, sediments, and soil, or may enter the human body indirectly through food or prey. For instance, fish uptake heavy metals directly from water or sediments through their gills or skin or it may uptake it as food/prey through their alimentary canal. The nature of heavy metal to biomagnify make it more complex, as the concentration of heavy metals is lower at a lower trophic level and increases at a higher trophic level in a food chain. Speciation of the metal of interest, the physiological mechanism developed by the organism for regulation, homeostasis, and detoxification of heavy metals are the factors affecting the retention of heavy metals in an organism's body. Heavy metals such as mercury which is in methylated forms are accumulated to a greater extent and thus biomagnified in food chains due to their nature of lipophilicity.

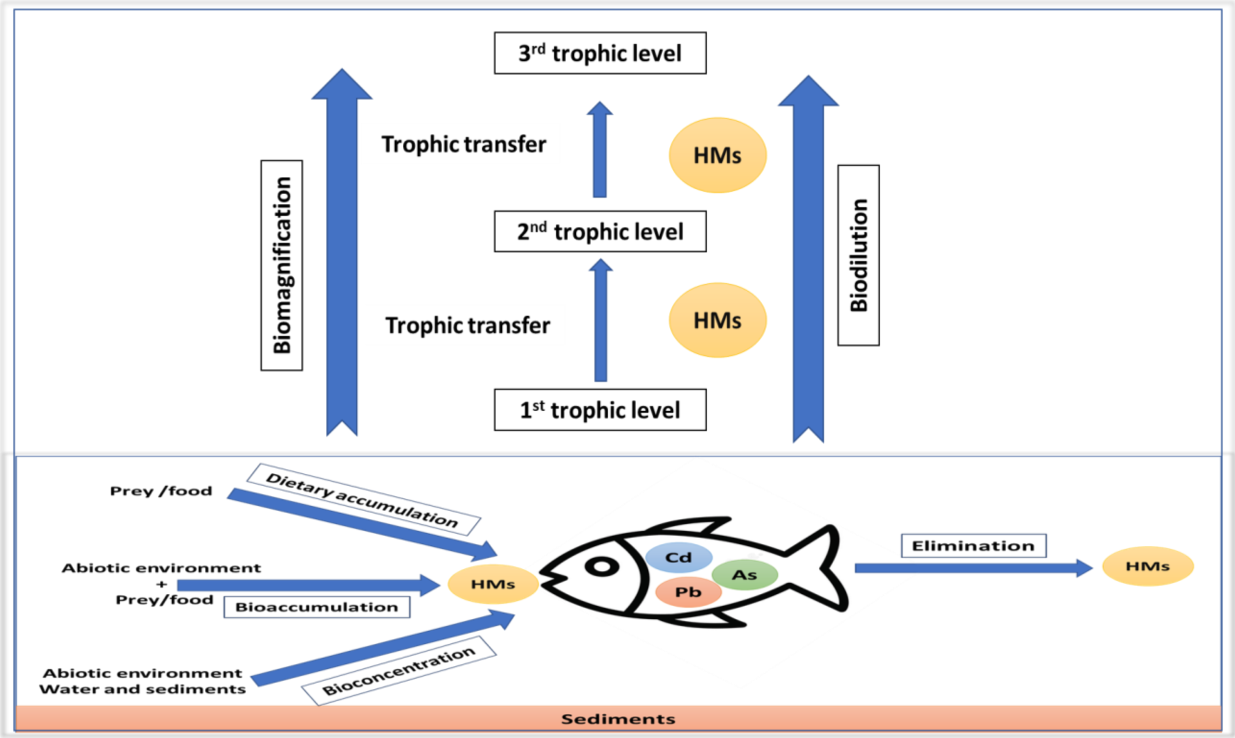

Fig. 2.2. Schematic diagram summarizing terms and concepts involved in trophodynamics of heavy metals (Ali & Khan, 2019).

2.7. Heavy Metals Transfer from Soil to Plants

Transfer of heavy metal from soil to plant is a very crucial step in the trophic transfer of metals in food chains. These metals come from different sources such as atmospheric deposition, livestock manure, irrigation with wastewater, chemical pesticides, phosphate-based fertilizers, and sewage sludge-based amendments (Elgallal et al., 2016; Woldetsadik et al., 2017). These heavy metals are taken up by the plants from contaminated soil and consequently taken up by the herbivorous animals along the food chain (Nica et al., 2012). Considering the contamination of the human food chain, contamination of food crops such as cereals and vegetables is a major concern. Consumption of these contaminated vegetables and cereals may cause a risk to human health (Orisakwe et al., 2012). Vegetables and cereal crops irrigated with wastewater have a high concentration of heavy metals as compared with vegetables irrigated with groundwater. Moreover, leafy vegetables have a higher concentration of heavy metals than bulbs and tubers (Mahmood & Malik, 2014). A study conducted by Khan et al. (2017) reported that consumption of vegetables such as ladyfinger, maize, and brinjal irrigated with wastewater in district Bannu, Khyber Pakhtunkhwa, Pakistan may pose a potential health risk to the local population (Khan et al., 2017). Significant evidence has been reported in previously done studies showing that irrigation with wastewater, and sewage sludge as a soil amendment for cereal crops, poses a risk to global food safety. There is a need to develop a consistent strategy and a stable treatment plant to solve the problem of increasing domestic and industrial wastewater, due to the increasing population. Due to the major issue of water scarcity globally, many developing countries do not have adequate water resources and hence are dependent on wastewater for irrigation purposes. Many farmers prefer wastewater over groundwater for irrigation sources, as wastewater has nutrients as well, however, along with nutrients several toxic metals are also present, which affect the quality and safety of food crops. Commonly grown crops such as vegetables, fodder, cotton, and to some extent rice receives wastewater irrigation almost twice a week, fodder once a week, and cotton once after 3 weeks (Murtaza & Zia, 2012). The consumption of these vegetables and food crops contaminated with metals may pose adverse health effects to the consumers (Khan et al., 2017). Wastewater-irrigated spinach has shown significantly higher accumulation of iron, manganese, and copper, compared to the freshwater-irrigated vegetables, indicating the highest metal absorption for the vegetables (Arora et al., 2008). The vegetables irrigated with wastewater are either consumed by farmers themselves or transported to urban markets for sale purposes (Alam et al., 2018).

2.8. Health Effects and Risks from Metals in Food Crop System

Due to the non-degradable nature of heavy metals and long half-lives, there is a potential to bioaccumulate in the human body as there is an inadequate elimination mechanism (Jarup 2003). Major sources of these heavy metals entering the vegetables are volcanic erosion of the earth crust, deposition of ores, and forest fires as natural sources, while fertilizers, herbicides, pesticides, insecticides, manure, industrial effluents, and domestic wastewater are major anthropogenic sources (Rayhan & Hosna, 2021). Moreover, rapid increase in industrialization and urbanization has led to an increase in traffic activities and consequently contribute to the accumulation of heavy metals in the roadside environment (Rai, 2012). Consumption of these vegetables contaminated with heavy metals may cause serious health problems and is a major exposure to heavy metals in the human body.

Prolonged intake of these heavy metals in the human diet may lead to a chronic accumulation of heavy metals as these metals may combine with proteins, nucleic acids, enzymes, and bio-membrane and, affect the normal functions of the human body, due to their persistent nature and toxicity (Lima et al., 2014; Khan et al., 2015; Miryousefi et al., 2020). Some of the heavy metals in trace quantities are essential for biological functions (co-factor) of the human body such as Ni, Cu, and Zn (Nafees & Amin, 2014; Harmanescu et al., 2011), while other metals such as Cd, Pb, As and Cr which are toxic to human body even at a very low concentration, are considered as carcinogenic (Mehrgan et al., 2019). Exposure to these heavy metals may affect the brain and nervous system by affecting functions of the brain, neurotransmitters, membrane transport, and binding of calcium ions, may cause Parkinson's disease, and Alzheimer's disease, headache, fatigue, drowsiness, confusion, memory loss, and IQ reduction. Continuous heavy metal intake may also affect the digestive system by resulting in vomiting, diarrhea, stomach irritation, mucus lining and degeneration, hyperplasia, inflammation, oxidation, and increases blood cholesterol levels in the liver. Kidney function is also affected by the consumption of heavy metals, causing nephropathies, tubular dysfunctions, and affecting homeostasis (Shamsi et al., 2020). Endometrial cancer, endometriosis, and spontaneous abortions, stillbirths, hypotrophy, affect endocrine regulatory mechanisms, induce atrophy of reproductive organs, alter spermatogenesis and sperm functions, musculoskeletal disorder, affect bone metabolism, enhance osteoporosis, in cell mitochondria, phosphorylation, and ATP synthesis are all affected. While in the whole cell, it affects lipids and protein synthesis, enzymatic and metabolic activities, cell functions and DNA via the production of free radicals (Kumar et al., 2019). Among these nutrients, zinc is a useful mineral due to its extraordinary biological and public health significance but increased ingestion may affect the concentration levels of high-density lipoproteins and disturb the immune system (Zhou et al., 2016). On the other hand, other metals like arsenic, cadmium, chromium, and lead are considered carcinogenic to the human body (Nafees et al., 2014). Toxic metal like lead has no biological function in the human body even at low concentrations. Long-term exposure to lead may cause anemia, hypertension, brain damage, abnormal hemoglobin synthesis, bone, renal, reproductive, and nervous system disorders, and decrease in children's learning ability, neuro-hematological damage, and cancers (Mehrgan et al., 2019; Harmanescu et al., 2011). The combined exposure to cadmium and zinc is harmful to bone and teeth growth as it inhibits the absorption of calcium ions (Shamsi et al., 2020). Exposure to cadmium causes liver and kidney damage, prostate cancers, arthritis and bone pains, anemia, and blood pressure in humans (Mehrgan et al., 2019). Acute and chronic exposure to arsenic could also cause numerous human health-related problems such as dermal, respiratory, cardiovascular,

cardiovascular, hematological, hepatic, renal, neurological, development, reproductive, immunological, genotoxic, mutagenic, and carcinogenic effects such as liver cancer (Kapaj et al., 2006; Lin et al., 2013).

One of the major pathways of metal entry into the food chain is through ingestion of contaminated food crops cultivated on contaminated soil or irrigated with wastewater and, drinking of water contaminated with heavy metals. In order to assess the potential health risk associated with contaminant exposure from soils and soil-like materials, human health risk assessment have also been developed (US EPA, 1989, 2000, 2001). The accumulation of metals in food crops, vegetables, fruits, milk, and water has a direct impact on the health of the local residents (Khan et al., 2013).

Keeping in view of the literature, there is a need to further explore the impacts of metals present in the food chain and highlight the associated risks. Multiple parameters considered for assessing the risks have been devised and are being practiced in different countries. This work also focused on the risks of metals taken up through the dietary route by humans.

Chapter 3

Materials and Methods

3.1. Study Area

The study was carried out in the selected markets of twin cities (Islamabad and Rawalpindi). The study included several vegetables that are most frequently used in Pakistan. In the majority of the markets, the supply of vegetables was from Punjab and Sindh, Pakistan. The selected vegetables to conduct the study were brinjal, bottle gourd, cabbage, cucumber, green chili, ladyfinger, onion, potato, spinach, tomato, and zucchini. The local, botanical, and family names are presented in table 1. The selected vegetables were collected from 4 different markets in twin cities. The markets are Khanna Pull, Tramari, Karachi Company, and Mandi Morh.

Table 3.1. Local, common, and botanical of selected vegetables

Local	**Common**	**Botanical**	**Family**
Baingan	Brinjal	*Solanum melongena* L.	Solanaceae
Qadu	Bottle gourd	*Lagenaria siceraria*	Cucurbits
Band gobhi	Cabbage	*Brassica oleracea*	Brassicaceae
Kheera	Cucumber	*Cucumis sativus*	Cucurbits
Hari mirch	Green chili	*Capsicum annuum*	Solanaceae
Bhindi	Ladyfinger	*Abelmoschus esculentus*	Malvaceae
Pyaz	Onion	*Allium cepa*	Amaryllidaceae
Aalu	Potato	*Solanum tuberosum*	Solanaceae
Palak	Spinach	*Spinacia oleracea*	Amaranthaceae
Tamatar	Tomato	*Solanum lycopersicum*	Solanaceae
Tori	Zucchini	*Cucurbita pepo*	Cucurbits

Fig. 3.1. Sampling points from different markets of Islamabad and Rawalpindi

3.1. Vegetables Sampling

A number of 44 samples of 11 different vegetables were collected from the selected markets of twin cities, Islamabad and Rawalpindi. The collected sample was stored in ziplocked polyethylene bags and brought to the IESE Biotechnology lab, where the vegetable samples were first washed with tap water and then with deionized water to remove dust particles.

3.2. Sample Preparation

3.2.1. Washing and Drying

All the vegetable samples collected from different markets were washed twice with tap water and distilled water to remove dust particles. The samples were then air-dried, cut into small pieces, and placed in an oven for 72 h at 70^{o}C (Nawab et al., 2017).

Fig. 3.2. Collected vegetable samples

Fig. 3.3. Vegetables kept in oven for drying

3.2.2. Grinding

The dried samples were then powdered using an electric grinder and then stored in ziplocked bags to prevent them from moisture (Nawab et al., 2017).

Fig. 3.4. Powdered vegetable samples

3.3. Moisture Content

The moisture content of all the selected vegetables was determined by simply finding the difference between the weight before and after oven drying. The wet weight of samples was determined using a weighing balance and then all the moisture was removed by placing them in an oven at 60-70^{o}C for 72 h, after which the samples were weighed again.

$$MC = wet\ weight - dry\ weight \qquad (3.1)$$

3.4. Sample Digestion

For acid digestion 10 mL of Nitric acid (HNO_3) was added to each 0.5g sample and kept overnight. The next day samples were heated on a hotplate to evaporate the acid. Once the sample volume was reduced to half, 4 mL of Perchloric acid ($HClO_4$) was added to it and again it was heated on a hot plate, till a clear solution was obtained. After the endpoint is reached, the sample is cooled and filtered using Whatman filter paper. Deionized water was added to the samples to increase the volume of samples up to 50 mL (Randhawa et al., 2014; Rizwan et al., 2016).

3.5. Analysis of Heavy Metals

Atomic absorption spectrophotometer was used for heavy metal analysis. This device works on the principle of Beer-Lamber law. Depending on the wavelength of electromagnetic radiation, a substance can either absorb or transmit the radiation when it is exposed to it.

Fig. 3.5. Atomic absorption spectrophotometer (AAS)

Following the guidelines outlined in AOAC (1990), elements in the prepared samples were identified using an atomic absorption spectrophotometer (Analytik Jena novAA 800D). Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Zinc (Zn) were the selected metals. Table 1 provides a summary of the said elements' instrumental operating conditions.

Table 3.2. Analytical conditions of atomic absorption spectrophotometer for analysis of selected heavy metals

Metals	Acetylene (L/min)	Air (L/min)	Wavelength (Nm)	Silt width (nm)	Lamp current (mA)	Detection limit (mg/L)
As	2.0	17.0	193.7	1.2	6.0	0.15
Cd	2.0	17.0	228.8	1.2	2.0	0.0012
Cr	2.0	17.0	357.9	0.8	4.0	0.0054
Pb	2.0	17.0	217	1.2	4.0	0.013
Zn	2.0	17.0	213.9	0.8	2.0	0.0014

3.6. Standard Solution Preparation

Calibrated standards were prepared from the commercially available stock solution (Merck) in the form of an aqueous solution (1000 mg/L). Highly purified de-ionized water was used for the preparation of working standards. All the glass apparatus used throughout the process of analytical work were immersed in HNO_3 overnight and washed with several changes of de-ionized water prior to use.

3.7. Quality Control

Quality assurance and quality control (QA/QC) procedures were conducted by using standard reference materials of plants. Duplicate samples were performed simultaneously. In addition, blank samples were also performed throughout all the experiments. High-grade purity of 99.9% chemicals were used (Merck). Certified standard solutions (Merck) of all five metals was diluted in 1000mg/L to check the quality assurance of multiple runs of sample in triplicates with blanks that were made under optimized conditions. Samples were analyzed at the wastewater lab IESE, NUST.

3.8. Human Health Risk Assessment

To evaluate human health risk, different equations were used which are as follows.

3.8.1. Estimated Daily Intake

The following equation was used to calculate the estimated intake (EDI) of metal via vegetable consumption (Ashfaq et al., 2021):

$$EDI = \frac{C_m \times IR \times C_f \times Ef \times ED}{B_w \times At} \qquad (1)$$

Conversion factor – C_f (0.085) which is the conversion of fresh vegetable into dry weight, IR_{veg} is the food ingestion rate, which was considered to be 0.345 kg/person/day for adults and 0.232 kg/ person/day for children (Orisakwe et al., 2012; Nawab et al., 2017), Ef is the exposure frequency which is 365 days/year and ED is the exposure duration which is 70 years (Ashfaq et al., 2021). While B_W represents the average body weight of an individual and is considered as 73 and 32.7 kg for adults and children, respectively (Nawab et al., 2017; Khan et al., 2013).

3.8.2. Total Estimated Daily Intake

The total estimated daily intake of all the vegetables consumed by adults and children was determined by the following equation:

$$\begin{aligned} Total\ EDI = {} & EDI_{brinjal} + EDI_{Bottle\ gourd} + EDI_{Cabbage} + EDI_{cucumber} + EDI_{greenchili} \\ & + EDI_{ladyfinger} + EDI_{onion} + EDI_{potato} + EDI_{spinach} + EDI_{Tomato} \\ & + EDI_{zucchini} \qquad (2) \end{aligned}$$

3.8.3. Target Hazard Quotient

The target hazard quotient is the ratio of exposure to the toxic element and the reference dose which is the highest level at which no adverse effects are observed. THQ was calculated using the following equations (Ashraf et al., 2021):

$$THQ = \frac{EDI \ \times Cm}{rfd} \qquad (3)$$

Where EDI is the estimated daily intake calculated in eq (1), Cm is the concentration of metal in a particular vegetable and rfd is the oral reference dose and its value for As & Cd, Cr, Pb, and Zn are 0.001, 1.5 ,0.0035, and 0.300 mg/kg/day, respectively (USEPA, & IRIS 2006). If the value of THQ >1, it poses a severe health risk to the population.

3.8.4. Hazard Index

The hazard index is used to estimate the potential human health risk when multiple heavy metals are involved. HI is calculated as the sum of hazard quotients (HQ) of the elements assessed for each food type.

$$HI = (THQ_1 + THQ_2 + THQ_3 \ldots \ldots, THQ_n) \qquad (4)$$

Here THQ_1, THQ_2, THQ_3, and THQ_n represent the total hazard quotient of metal 1, 2, 3, and so on. If the HI is >1, there is the potential for adverse non-carcinogenic health effects.

3.8.5. Health Risk Index

To calculate the health risk index of selected heavy metals in vegetables, the following equation was used (Adedokun, et al., 2016).

$$HRI = \frac{EDI}{rfd} \qquad (5)$$

Where EDI is the estimated daily intake which is calculated as per equation (1), and rfd is the oral reference dose, its values for As & Cd, Cr, Pb, and Zn are 0.001, 1.5, 0.0035, and 0.300 mg/kg/day, respectively (USEPA, & IRIS. 2006). HRI < 1 shows that the exposed population is at no risk.

3.8.6. Target Carcinogen risk

Target carcinogen risk is used to evaluate the carcinogenic risk. TCR methodology was provided by USEPA region III risk-based concentration (Sarker & Sultana, 2020). The model to estimate TCR is shown as follows:

$$TCR = EDI \times Csfo \qquad (6)$$

Here Csfo is the cancer slope factor and its value for As, Cd, Cr, and Pb while other parameters have been explained previously. A range of 10^{-6} to 10^{-4} target cancer risk is assumed as an acceptable risk for developing cancer.

Chapter 4

Results and Discussions

4.1. Moisture Content

The moisture content of all the selected vegetables was found greater than 80%. Following table 4.1. shows the average percentage of moisture content of different vegetables.

Table 4.1 Average percentage of moisture content in selected vegetables

Vegetables	Khaana pull	Tramari	Karachi company	Mandi morh
Brinjal	93.8	93.5	93.4	92.6
Bottle gourd	96.0	94.9	95.8	94.9
Cabbage	91.1	91.2	93.0	92.5
Cucumber	97.4	96.4	96.9	97.2
Green chili	89.1	89.5	90.9	89.7
Lady finger	90.7	87.1	90.5	90.3
Onion	88.3	90.3	87.6	89.6
Potato	81.2	80.4	81.1	81.2
Spinach	71.5	91.0	81.8	91.2
Tomato	94.7	96.6	95.6	95.1
Zucchini	--	95.9	95.9	95.9

4.2. Heavy Metal Concentration

4.2.1. Concentration of Arsenic in Fresh Vegetables

Arsenic concentration in vegetables ranged from 1.21 – 1.54 g/kg. The highest mean concentration was found in spinach (4.87 mg/kg) while the lowest was found in bottle gourd (1.23 mg/kg). The concentration of As in selected vegetables was in the following increasing order: bottle gourd > brinjal > cabbage > cucumber > potato > zucchini > tomato > ladyfinger > green chili > Onion > spinach. Concentrations of Arsenic (As) in all selected vegetables were found to be higher than permissible limits set by FAO/WHO (1999).

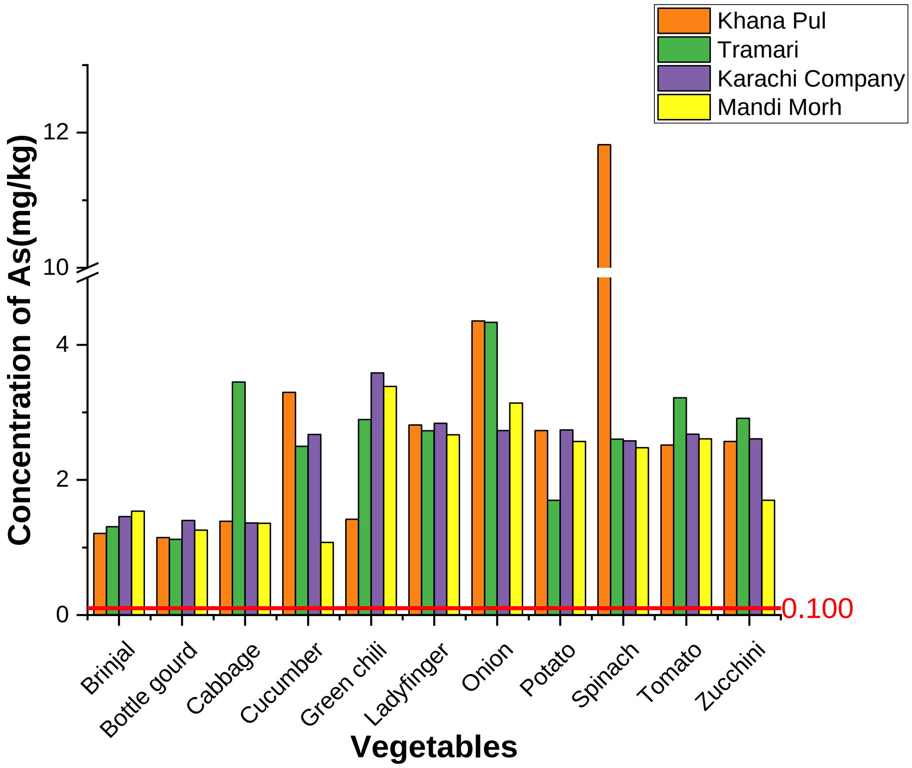

Fig. 4.1. Arsenic concentration in selected vegetables

Brinjal showed a lower concentration than observed from agricultural land irrigated with groundwater, in Kasur Pakistan (Ashraf et al., 2021), while it was found to be higher than observed in brinjal samples collected from industrial, non-industrial, and local markets in Bangladesh (Haque et al., 2021) and from agriculture land of Malatya province, Turkey (Varol et al., 2022). Bottle gourd showed arsenic concentration to be less than observed by Haque et al. (2021) in Bangladesh. Cabbage showed a higher concentration than observed in agricultural land in Northeast Nigeria (Akan et al., 2021), while it was lower than observed in agricultural land of Kasur, Pakistan (Ashraf et al., 2021) and in markets of Jamaica (Antoine et al., 2017). Varol et al. in 2022 reported a lower concentration of Arsenic in cucumbers from agricultural land in Malatya province, Turkey than observed in the current study (Varol et al., 2022). Ladyfinger showed a higher concentration than observed in agricultural land in Kasur, Pakistan by Ashraf et al. (2021) and in markets of Delhi, India by Kumar et al. (2022). Onion showed a higher concentration than observed in agricultural land in Northeast Nigeria by Akan et al. (2021). The concentration of As in Potato showed higher than observed in markets of Jamaica (Antoine et al., 2017) and in potato samples collected from industrial, non-industrial, and local markets in Bangladesh (Haque et al., 2021), in marketed potatoes in Delhi, India (Kumar et al.,

2022) and in spinach irrigated with groundwater in district Lahore (Hussain et al., 2022). While the observed concentration was lower than investigated in spinach irrigated with river Ravi, Hudiara drain, and domestic sewage water (Hussain et al., 2022). Spinach showed a higher concentration than observed in agricultural land in Northeast Nigeria (Akan et al., 2021) and in markets of Jamaica (Antoine et al., 2017), while it was lower than observed in spinach irrigated with groundwater, river Ravi, domestic and from Hudaira Drain in district Lahore, Pakistan (Hussain et al., 2022), in marketed spinach in Delhi, India (Kumar et al., 2022) and in markets of Kathmandu, Nepal (Shakya et al., 2013). Tomato showed a higher concentration than observed in agricultural land in Northeast Nigeria (Akan et al., 2021), in tomato samples collected from agricultural land irrigated with groundwater in district Kasur, Pakistan (Ashraf et al., 2021), in marketed tomatoes in Delhi, India (Kumar et al., 2022) and in markets of Jamaica (Antoine et al., 2017). Zucchini showed a lower concentration than observed by Varol et al., (2022) in agricultural land of Malatya province, Turkey**.**

4.2.2. Concentration of Cadmium in Fresh Vegetables

The concentration of Cadmium in all the vegetables was within the limits of FAO/WHO 2001, except for tomato samples collected from Tramari, Rawalpindi. The mean and range of Cd in all vegetables are represented in table 4.1. The mean concentration of all the vegetables was in increasing order; cucumber > lady finger > onion > potato > brinjal > green chili > cabbage > > zucchini >Bottle gourd> spinach > tomato.

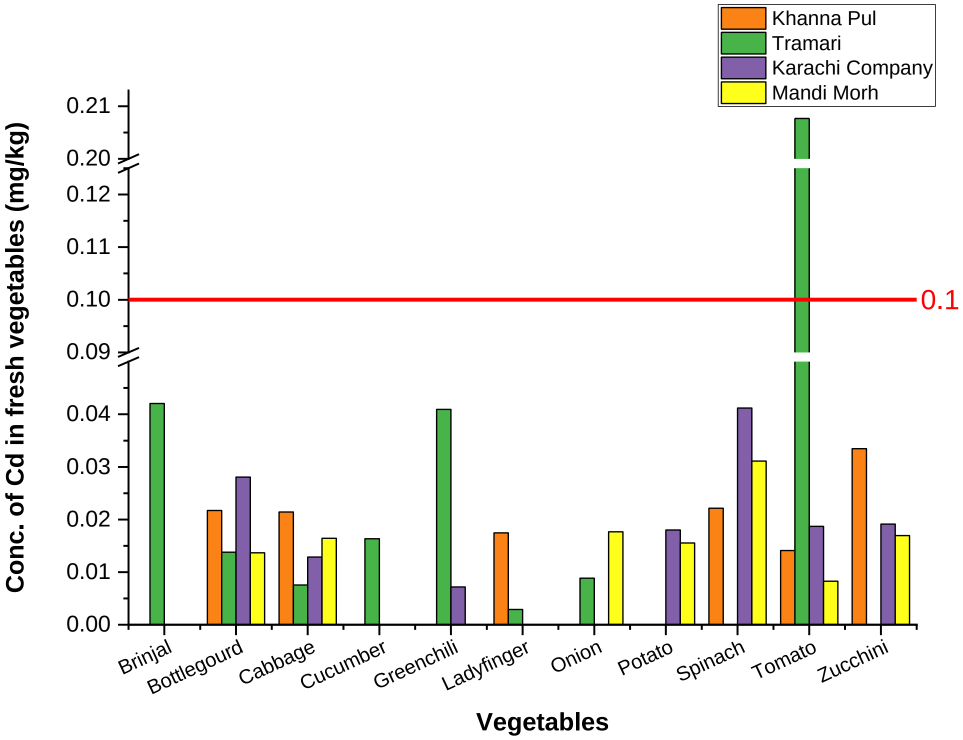

Fig. 4.2. Cadmium Concentration in Selected Vegetables

The results of brinjal showed a lower concentration than the values observed in Bangladesh (Haque et al., 2021), in samples collected from agricultural land in Malatya province, Turkey (Varol et al., 2022), and in brinjal collected from agricultural land in district Kasur, Pakistan (Ashraf et al., in 2021), in Bahawalpur, where irrigation is mainly done via tube well water (Iqbal et al., 2016), in wastewater irrigated agriculture land in Dera Ghazi Khan, Pakistan (Latif et al., 2013) and in district Vehari (Sarwar et al., 2020). A similar study conducted by Haque et al. (2021) in industrial and non-industrial areas of Bangladesh concluded that cadmium concentration in bottle gourd was found to be higher than the concentration observed in the current study. Cabbage showed a lower concentration observed by Akan et al. (2021) in agricultural land in Northeast Nigeria (Akan et al., 2021), in markets of Jamaica (Antoine et al., 2017), in district Kasur Pakistan (Ashraf et al., 2021), in agricultural land irrigated with wastewater as well as areas where cabbage farm is irrigated with groundwater in district Lahore, Pakistan (Khanum et al., 2017; Mahmood and Malik 2014), and in district Vehari, Pakistan where the main source of irrigation is wastewater (Sarwar et al., 2020). Cucumbers showed higher concentration than observed in Malatya province, Turkey (Varol et al., 2022), and in markets of Faisalabad, Pakistan (Ejaz et al., 2020), and in district Vehari, where irrigation is done using wastewater (Sarwar et al., 2020). Green chili showed a lower concentration than reported by Ejaz et al. (2020) in Faisalabad markets. Ladyfinger showed a higher concentration than observed in ladyfinger from agricultural land in the district Kasur, Pakistan (Ashraf et al., 2021) and a higher concentration than observed was reported in Peshawar, Pakistan (Ali et al., 2021). Onion showed lower concentration than observed in a study conducted by Akan et al. (2021) in Northeast Nigeria, onion grown near the highway in Jhansi, India (Gupta et al., 2022), and onions collected from a market in the Tamale metropolis, Ghana (Ametepey et al., 2018), while it was lower than reported in Bahawalpur, where turbine water and sewage water was used for irrigation. The concentration of cadmium in Potato was found lower than the concentration observed in potatoes irrigated with groundwater, domestic sewage water, Ravi river, and Hudiara Drain in district Lahore, Pakistan (Hussain et al., 2022) in agriculture land irrigated with wastewater as well as groundwater in district Lahore, Pakistan (Mahmood, & Malik, (2014), and in Swat, Pakistan (Khan et a., 2013). In Spinach, the concentration of cadmium was lower than observed in the district Vehari, Pakistan where irrigation was done using wastewater (Sarwar et al., 2020) and in agricultural land irrigated with groundwater as well as with wastewater in the district Lahore, Pakistan (Mahmood, & Malik, 2014) and from different sites in Gilgit (Khan et al., 2010), in groundwater, domestic sewage water, river Ravi and Hudiara drain in Lahore (Hussain et al., 2022), and in wastewater irrigated land in Dera Ghazi Khan, Pakistan (Latif et al., 2018) and in Bahawalpur, Pakistan where irrigation source was tube well and sewage water (Iqbal et al., 2016). Tomato showed a lower concentration of cadmium than observed in industrial, non-industrial, and local markets in Bangladesh (Haque et al., 2021), in Northeast Nigeria (Akan et al., 2021), in agricultural land in Malatya province, Turkey (Varol et al., 2022) in tomato grown near the highway in Jhansi, India (Gupta et al., 2022), in marketed tomatoes in Delhi, India (Kumar et al., 2022), in district Kasur, Pakistan (Ashraf et al., 2021), a higher concentration was reported in Peshawar (Ali et al., 2021) and in markets of Faisalabad, Pakistan (Ejaz et al., 2020). Zucchini showed a higher concentration than observed in agricultural land of Malatya province, Turkey (Varol et al., 2022), and in agricultural land irrigated with wastewater in Dera Ghazi Khan, Pakistan (Latif et al., 2018).

4.2.3. Concentration of Chromium in Fresh Vegetables

The concentration of Cr in all the vegetables was within the limits of FAO/WHO 2001, EU 2001, and Australian regulation, except for tomato samples collected from Tramari, Rawalpindi, exceeding Australian standards. The mean and range of Cr in all vegetables are represented in table 4.1. The mean concentration of all the vegetables was in increasing order; ladyfinger> potato > onion > cabbage >cucumber > green chili > brinjal > bottle gourd > zucchini > spinach > tomato.

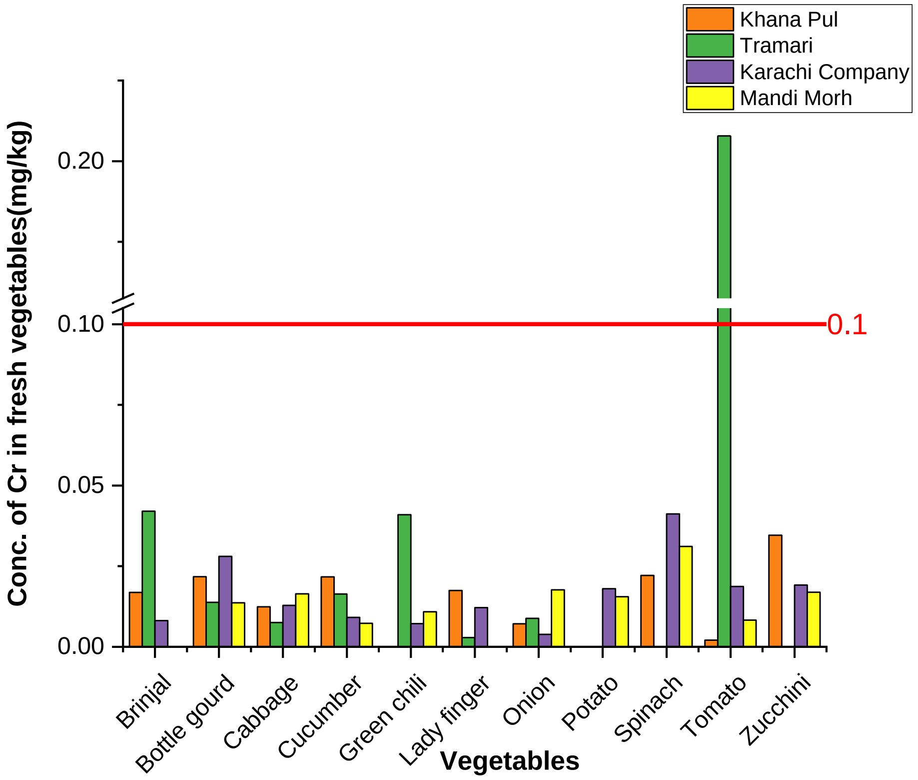

Fig. 4.3. Chromium concentration in selected vegetables

Brinjal showed a lower concentration than observed in agricultural land irrigated with groundwater in district Kasur, Pakistan (Ashraf et al., 2021) and in wastewater-irrigated agricultural land in Dera Ghazi Khan, Pakistan (Latif et al., 2018), in Bahawalpur where tube well and sewage water was the main source of irrigation (Iqbal et al., 2016) and in Vehari district (Sarwar et al., 2020). Bottle gourd showed a lower concentration than observed by Wadood et al. (2021) in the markets of Multan. Cabbage showed a lower value than observed in district Kasur, Pakistan (Ashraf et al., 2021), Northeast Nigeria (Akan et al., 2021), in markets of Tamale metropolis, Ghana (Ametepey et al., 2018) and in cabbage irrigated with wastewater as well as groundwater in Lahore, Pakistan (Mahmood & Malik, 2014), in Bahawalpur, where irrigation sources are tube well water as well as sewage water (Iqbal et al., 2016). Cucumber showed a greater Cr concentration than observed in Multan and Rawalpindi (Wadood et al., 2021), Vehari (Sarwar et al., 2020), and in markets of Faisalabad (Ejaz et al., 2020). Green chili showed a lower Cr concentration than observed in the markets of Faisalabad (Ejaz et al., 2020). Ladyfinger showed a lower concentration than observed in the district Kasur, Pakistan (Ashraf et al., 2021) and in Peshawar, Pakistan (Ali et al., 2021). Onion showed a higher concentration than observed in the Tamale metropolis, Ghana **(**Ametepey et al., 2018), while it was lower than observed in agricultural land irrigated with freshwater in Northeast Nigeria (Akan et al., 2021) and a quite lower concentration than observed in Vehari district, Pakistan where wastewater irrigation is common (Sarwar et al., 2020). While in Bahawalpur, it was reported to be lower than observed, where the irrigation source was tube well water as well as sewage water (Iqbal et al., 2016). Potato showed a lower concentration than observed in Lahore, Pakistan via wastewater irrigation (Khanum et al., 2017; Mahmood and Malik 2014), and in marketed potatoes in Delhi, India (Kumar et al., 2022). While it was reported to be in accordance with the reported in Bahawalpur, where tube well water was the irrigation source (Iqbal et al., 2016). Spinach showed a lower concentration than observed in Bahawalpur, where the irrigation source was tube well water and sewage water (Iqbal et al., 2016) and in spinach collected from agricultural land irrigated with wastewater as well as groundwater in Lahore, Pakistan (Mahmood & Malik, (2014). While the concentration was observed to be higher than observed in the markets of Kathmandu, Nepal (Shakya et al., 2013). Tomato showed lower concentration than reported in Peshawar, Pakistan (Ali, et al., 2021). While it was observed to be higher than tomato samples collected from agricultural land in the district Kasur, Pakistan (Ashraf et al., 2021) irrigated with groundwater, and in markets of Faisalabad, Pakistan (Ejaz et al., 2020). Zucchini showed lower concentration than observed in wastewater-irrigated land in Dera Ghazi Khan, Pakistan (Latif et al., 2018).

4.2.4. Concentration of Lead in Fresh Vegetables

Lead has many harmful effects on human health such as it affects kidney, liver, lungs, and spleen that affects several biochemical defects. Occupational or accidental exposure to excessive levels of lead in adults may cause neuropathology. Research study shows a relationship between Pb in the human body and the increase in blood pressure of adults (Ametepey et al., 2018).

The Pb concentrations in vegetables ranged from 0.53 to 2.41 mg/kg. The highest concentration was observed in spinach and ladyfinger (2.41 mg/kg), while the lowest was observed in onion (0.53 mg/kg). The concentration of Pb in vegetables was observed in the order of ladyfinger> spinach> zucchini > tomato> cabbage> potato>bottle gourd> cucumber > brinjal > green chilly >onion as shown in Table 4.1. The concentration of Pb in all vegetable samples were exceeding the permissible limit set by FAO/WHO (2001) and Brazilian regulations.

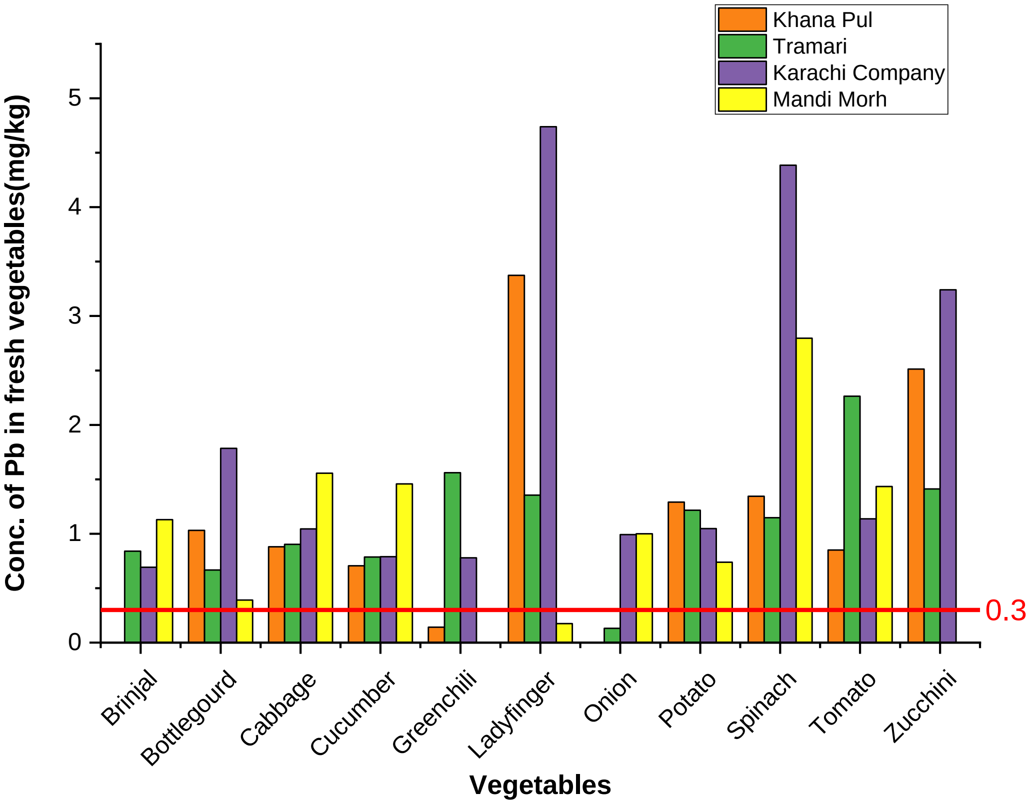

Fig. 4.4. Concentration (mg/kg) of Pb in selected vegetables

The mean concentration of Brinjal (0.69) in the current study was found to be higher than the study conducted in vegetables irrigated from fresh water, while was lower than the concentration observed in brinjal irrigated through wastewater in Bahawalpur (Iqbal et al., 2016), in different urban and peri-urban areas of Multan (Randhawa et al., 2014) and in samples from agricultural land irrigated with groundwater in district Kasur, Pakistan (Ashraf et al., 2021). A study conducted by Haque et al. (2021) in Bangladesh, Sarwar et al. (2020) in district Vehari, and Wadood et al. in markets of Multan, Pakistan showed a higher concentration of Pb in bottle gourd than the mean concentration in the present study. A similar study was conducted by Ur Rehman et al. (2019), on vegetables grown with groundwater and wastewater in Sahiwal, Pakistan. The results of Pb concentration in cabbage grown with groundwater was lower than the current study, while the cabbage grown on wastewater had a high concentration and was also lower than a study conducted in agricultural land of district Kasur, Pakistan (Ashraf et al., 2021). The concentration of cucumber was found to be lower than the concentration observed by Ejaz et al. (2020) in Faisalabad, Pakistan, and in the district Vehari, Pakistan, where irrigation was done using wastewater (Sarwar et al., 2020). While, Pb concentration in cucumber was higher than observed in agricultural land of Malatya province, Turkey (Varol et al., 2022). The concentration of green chili in the present study was found to be lower than observed in Faisalabad by Ejaz et al. (2020) and in the market along the highway in India (Basu et al., 2013). A study conducted by Nawab et al. (2017) in Khyber Pakhtunkhwa revealed the concentration of Pb in ladyfinger to be less than in the present study. They were also found to be lower than the study conducted by Randhawa et al. (2014) in urban and peri-urban regions of Multan and Wadood et al. (2021) on marketed ladyfinger in Multan, while higher than a study conducted in the agricultural land of district Kasur, Pakistan by Ashraf et al. in (2021). The mean Pb concentration of onion was found to be lower than values observed in Khyber Pakhtunkhwa and in district Vehari where irrigation was done using wastewater (Sarwar et al., 2020), and the vegetables grown with freshwater in Bahawalpur (Iqbal et al., 2016), while it was lower than vegetables irrigated with wastewater in Bahawalpur (Iqbal et al., 2016). In the present study, the Pb concentration in potatoes was found to be higher than in the study conducted in Khyber Pakhtunkhwa, Pakistan (Nawab et al., 2017), while lower than the values reported by Khanum et al. (2017) and Mahmood and Malik (2014) in research carried out on wastewater irrigated potato collected from Lahore District, Pakistan. Moreover, the concentration of Pb in spinach were lower than those observed by Haque et al. (2021), Iqbal et al. (2016) and Ur Rehman et al. (2019) in Bangladesh Bahawalpur and Sahiwal, Pakistan, while higher than those observed in Lahore, Pakistan irrigated with groundwater as well as with wastewater (Mahmood, & Malik, 2014). A study conducted by Nawab et al. (2018) in Khyber Pakhtunkhwa and Ejaz et al. (2020) in Faisalabad observed Pb concentration in tomatoes to be lower than the present study, while it was observed higher in a study conducted by Ashraf et al. (2021) in agricultural land of district Kasur, Pakistan. Zucchini showed a higher concentration than observed in Dera Ghazi Khan, where wastewater was used for irrigation.

4.2.5. Concentration of Zinc in Fresh Vegetables

Zinc is a useful mineral due to its extraordinary biological and public health significance, but increased ingestion may cause adverse effects on human health (Rahman et al. 2014). Zinc has the power to lower the immunity and also reduces high-density lipoproteins (Harmanescu et al., 2011). Reports show that its deficiency causes health-related diseases in about two billion people in the developing world (Parsad, 2003). On the other hand, its deficiency causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhea in children. A report by Hambidge and Krebs (2007) shows that about 800,000 children die every year due to zinc deficiency (Hambidge and Krebs, 2007; Ametepey et al., 2018).

The concentration of Zinc in all vegetable samples was within permissible limits set by SEPAC 1995; EU 2001, except for spinach collected from Khanna pull, Rawalpindi. The mean and range of Zn concentration in all vegetables are represented in table 4.1. The mean concentration of all the vegetable samples was in decreasing order of spinach > zucchini > tomato > onion > ladyfinger > potato > green chili > cucumber > cabbage > bottle gourd > brinjal.

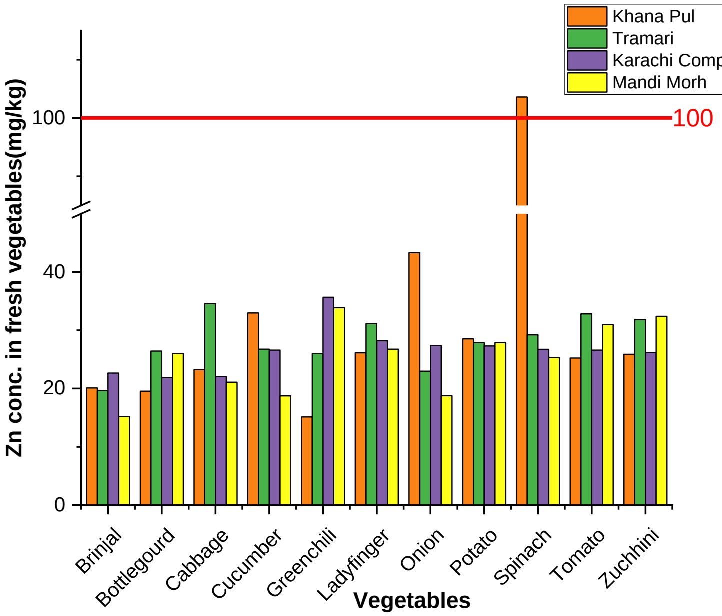

Fig. 4.5. Concentration (mg/kg) of Zn in selected vegetables

A similar study conducted in industrial, non-industrial and markets in Bangladesh (Haque et al 2021), and in Malatya province Turkey (Varol et al., 2022) showed that the concentration of zinc in brinjal was found to be lower than the values observed in the current study, while a high concentration was observed in a study conducted in agricultural land of district Kasur, Pakistan (Ashraf et al., 2021) and in wastewater irrigated agriculture land in Dera Ghazi Khan, Pakistan (Latif et al., 2018) and in Vehari district (Sarwar et al., 2020). In the case of bottle gourd, a similar study conducted by Haque et al. (2021) in Bangladesh showed the zinc concentration to be lower than the observed in the present study, while the concentration observed in a study conducted by Sarwar et al. (2020) in district Vehari, Pakistan was found to be higher than current observed values. Cabbage showed a higher concentration than observed in agricultural land irrigated with groundwater in the district Kasur (Ashraf et al., 2021), and district Lahore (Mahmood, & Malik, 2014), Pakistan, while lower than observed in cabbage cultivated with wastewater in district Lahore, Pakistan (Mahmood, & Malik, 2014) and in district Vehari, Pakistan (Sarwar et al., 2020). Cucumbers showed comparatively similar concentrations than observed in the markets of Rawalpindi and Multan (Wadood et al., 2021). A similar concentration of Zn in ladyfinger was observed in Peshawar, Pakistan (Ali et al., 2021), while a relatively lower concentration was observed in District Kasur, Pakistan (Ashraf et al., 2021) and Dar es Salaam, Tanzania (Kacholi and Sahu 2018). Onion showed lower Zn concentration than investigated in marketed onions collected from the Tamale metropolis, Ghana (Ametepey et al., 2018) and in the district Vehari Pakistan, where wastewater irrigation is done. The concentration of zinc in potato samples was observed higher than the observed concentration in Bangladesh investigated by Haque et al. (2021), and in agriculture land irrigated with groundwater and wastewater in district Lahore, Pakistan (Mahmood, & Malik, 2014) and in Rawalpindi and Multan markets (Wadood et al., 2021), and in Dar es Salaam, Tanzania (Kacholi and Sahu 2018). In Spinach, the zinc concentration was higher than observed by Haque et al. 2021 in Bangladesh, and in agricultural land irrigated with wastewater and groundwater in the district Lahore, Pakistan (Mahmood, & Malik, 2014). While it was lower than investigated in the district Vehari, Pakistan where wastewater is used for irrigation purposes (Sarwat et al., 2020). Tomato showed a higher concentration of zinc than the concentration observed by Haque et al. (2021) in Bangladesh and observed in markets of tamale metropolis, Ghana (Ametepey et al., 2018) and in Malatya province, Turkey (Varol et al., 2022), comparatively low concentration was observed in Peshawar, Pakistan (Ali et al., 2021). Zucchini showed a higher concentration than observed in wastewater-irrigated land in Dera Ghazi Khan, Pakistan (Latif et al., 2018), and in Malatya province, Turkey (Varol et al., 2022).

Table. 4.2. Mean and Range of selected HMs (mg/kg) via consumption of vegetables from open markets

Vegetables	Statistics	As	Cd	Cr	Pb	Zn
Brinjal	Range	1.21 – 1.54	0 – 0.042	0- 0.042	0.69 – 1.13	15.2 – 22.6
	Mean ±	1.38 ± 0.15	0.010 ± 0.02	0.017 ± 0.02	0.66 ± 0.48	19.4 ± 3.09
Bottle gourd	Range	1.12 – 1.40	0.014 – 0.028	0.01 – 0.03	0.3 – 1.78	19.5 – 26.4
	Mean ±	1.23 ± 0.13	0.019 ± 0.007	0.019 ± 0.01	0.97± 0.60	23.5 ± 3.33
Cabbage	Range	1.36 – 3.45	0.007 – 0.021	0.007 – 0.022	0.88- 1.55	21.1 – 34.6
	Mean ±	1.89 ± 1.04	0.014 ± 0.006	0.012 ± 0.003	1.09 ± 0.31	25.2 ± 6.28
Cucumber	Range	1.07 – 3.30	0 – 0.016	0.007 – 0.022	0.70- 1.45	18.7 – 33.0
	Mean ±	2.38 ± 0.94	0.004 ± 0.008	0.014 ± 0.01	0.93 ± 0.35	26.3 ± 5.82
Green chili	Range	1.42 – 3.58	0 – 0.041	0 – 0.04	0 – 1.56	15.1 – 35.6
	Mean ±	2.82 ± 0.98	0.012 ± 0.02	0.015 ± 0.02	0.62 ± 0.71	27.7 ± 9.35
Ladyfinger	Range	2.67 - 2.84	0 – 0.017	0 – 0.02	0.17 – 4.73	26.1 – 31.1
	Mean ±	2.76 ± 0.08	0.005 ± 0.008	0.008 ± 0.01	2.41 ± 2.03	28.0 ± 2.23
Onion	Range	2.73 – 4.35	0 – 0.018	0.003 – 0.02	0.14 – 0.99	18.7 – 43.3
	Mean ±	3.64 ± 0.83	0.007 ± 0.008	0.009 ± 0.01	0.53 ± 0.53	28.1 ± 10.7
Potato	Range	1.70 – 2.74	0 – 0.018	0 – 0.041	0.74 – 1.29	27.3 – 28.5
	Mean ±	2.43 ± 0.50	0.008 ± 0.01	0.008 ± 0.01	1.07 ± 0.24	27.9 ± 0.50
Spinach	Range	2.48 – 11.8	0 - 0.041	0.002 – 0.208	1.15 – 4.38	25.3 – 103.6
	Mean ±	4.87 ± 4.63	0.024 ± 0.017	0.023 ± 0.10	2.42 ± 1.50	46.2 ± 38.3
Tomato	Range	2.52 – 3.22	0.008 – 0.208	0 – 0.034	0.85- 2.26	25.2 – 32.8
	Mean ±	2.75 ± 0.31	0.062 ± 0.10	0.059 ± 0.014	1.42 ± 0.61	28.9 ± 3.57
Zucchini	Range	1.70 – 2.91	0 – 0.033	0 - 0.015	1.41 – 3.24	25.9 – 32.4

	Mean ±	2.45 ± 0.52	0.017 ± 0.01	0.004 ± 0.007	1.79 ± 1.41	29.1± 3.52

4.3. Statistical Analysis

4.3.1. Correlation between Heavy Metals

The interrelationship between all the selected metals (As, Cd, Cr, Pb, and Zn) and vegetables was determined by using Pearson's correlation matrix, as shown in table 4.2. A very significant correlation was found between Cd and Cr (0.978) and As and Zn (0.923) at $p<0.01$. A strong relationship was found between Cd and Cr and Zn and As, while Zn and Pb showed a negative relationship. The metals Cd and Cr, As, and Zn are grouped showing that the anthropogenic sources of these metals are closely related.

Table 4.3. Correlation coefficient matrix of heavy metals in the vegetables

Metals	As	Cd	Cr	Pb	Zn
As	1				
Cd	0.064	1			
Cr	0.060	**0.978****	1		
Pb	0.044	**0.277****	**0.280****	1	
Zn	**0.923****	0.058	0.074	-0.002	1

*Correlation is significant at $P< 0.05$

**Correlation is significant at $P< 0.01$.

4.3.2. ANOVA

One-way Analysis of Variance was used to determine whether there was a significant difference ($p \leq 0.05$) in the amounts of heavy metals in vegetables between the various sampling sites (p 0.05). (ANOVA). Post-hoc Tukey's test was used to separate the means.

4.4. Human Health Risk Assessment

4.4.1. Estimated Daily Intake

There are several exposure pathways of heavy metals in our bodies. These may enter our body through different routes such as ingestion of HMs-contaminated food, drinking water, and air, and affect human health even at a very low concentration. These heavy metals are toxic to human health as they are non-competitive inhibitors of several enzymes (Esposito et al., 2001). Table 4.3 summarizes the estimated daily intake (EDI) of five metals (As, Cd, Cr, Pb, and Zn).

4.4.1.1. Estimated Daily Intake of Arsenic

The estimated daily intake of Arsenic via consumption of fresh vegetables in adults and children was observed less than the maximum tolerable limit of 0.13 mg/kg/person/ day (Shaheen et al., 2016). The range and mean of the estimated daily intake of As are shown in table 4.3. The estimated daily intake of As in adults was observed in the following decreasing order spinach > onion > green chili > brinjal > cabbage > tomato > ladyfinger > zucchini > cucumber > potato > bottle gourd. While for children the pattern was different and is in decreasing order: spinach > onion > green chili > ladyfinger > tomato > zucchini > potato > cucumber > cabbage > brinjal > bottle gourd.

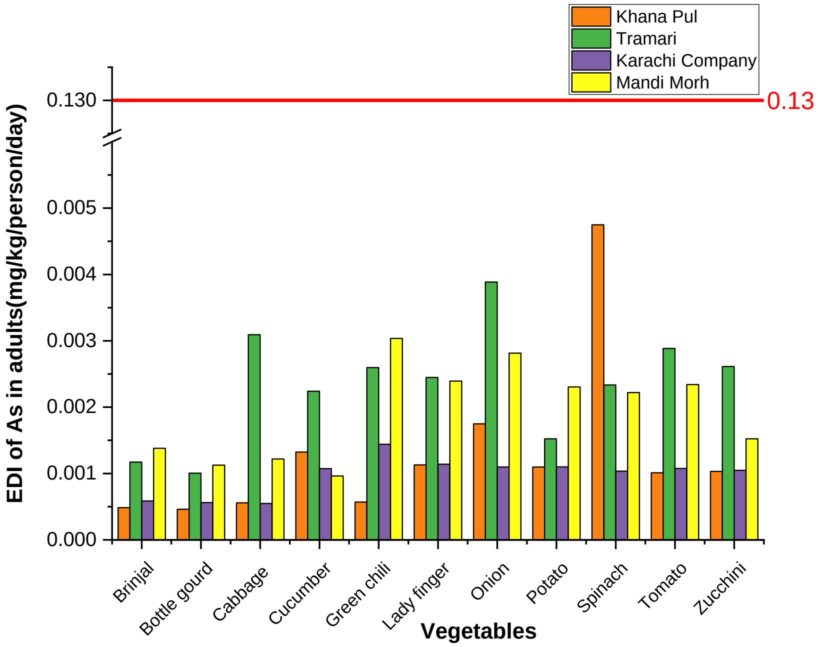

Fig. 4.4. Estimated daily intake of Arsenic via consumption of fresh vegetables in adults

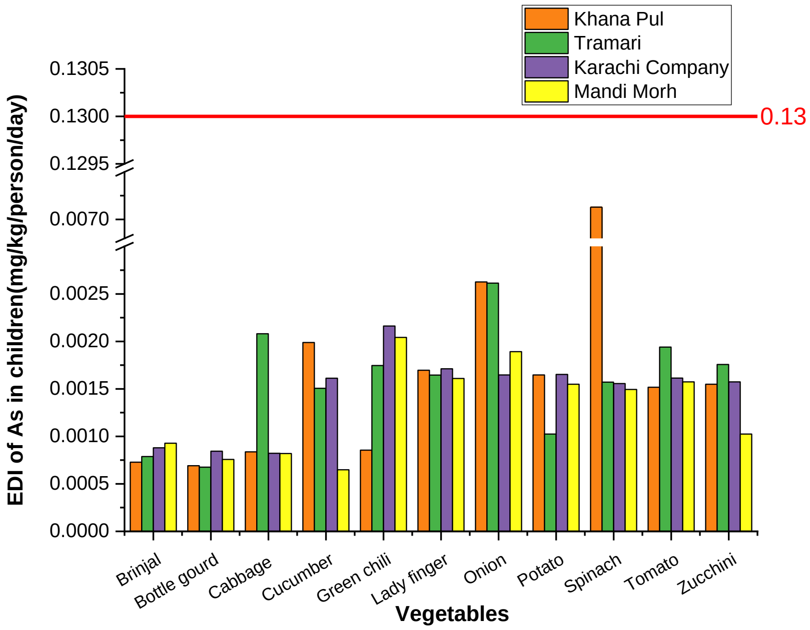

Fig. 4.5. Estimated daily intake of Arsenic via consumption of fresh vegetables in children

Estimated daily intake of Brinjal in adults as well as in children was observed to be lower than MTL, and similar results were reported in the population of district Kasur, Pakistan (Ashraf et al., 2021). Estimated daily intake of arsenic incase of bottle gourd in adults showed a lower value than MTL, Haque et al. (2021) reported similar results in an adult population of Bangladesh consuming bottle gourd from industrial, non-industrial, and local markets. Estimated daily intake of arsenic via consumption of cabbage incase of adults as well as children were observed to below MTL, Ashraf et al. (2021) and Rizwan et al. (2016) reported similar results in the population of district Kasur, and Gujranwala, Pakistan. Arsenic intake via consumption of cucumber was also observed to be below MTL, a similar result was also reported by Varol et al. (2022) in the population of Malatya province Turkey. Estimated daily intake of arsenic via consumption of ladyfinger in adults as well as children both showed EDI below MTL. Ashraf et al. (2021) reported a similar result in the population of the district Kasur, Pakistan. The estimated daily intake of As via consumption of Potato in adults as well as children were below MTL, Antoine et al. (2017) in the population of Jamaica via consumption of potato form the local market also observed EDI to be below MTL. Estimated daily intake of As via ingestion of spinach in adults as well as children was below maximum tolerable limits, Haque et al. (2021) also reported similar results showing that EDI was below maximum tolerable limits in the population of Bangladesh consuming spinach from industrial and non-industrial areas, while a lower value was observed in case of adult population consuming spinach from local markets (Haque et al., in 2021). Estimated daily intake of arsenic via consumption of tomatoes in adults as well as in children were observed to be below maximum tolerable limits, similar results were also reported in the population of district Kasur, Pakistan (Ashraf et al., 2021). Varol et al. (2022) in Turkey reported similar results of EDI of arsenic via the consumption of zucchini below the maximum tolerable limit.

4.4.1.2. Estimated Daily Intake of Cadmium

The estimated daily intake of cadmium via consumption of fresh vegetables in adults and children was observed less than the maximum tolerable limit of 0.02 mg/kg/person/ day (Shaheen et al., 2016). The range and mean of the estimated daily intake of cadmium are shown in table 4.3. The estimated daily intake of cadmium in children and adults was observed in the following decreasing order tomato > spinach > bottle gourd> zucchini > cabbage > green chili > brinjal > potato > onion > ladyfinger > cucumber. The present study revealed that daily uptake of cadmium via ingestion was free of danger, due to its low consumption on daily basis.

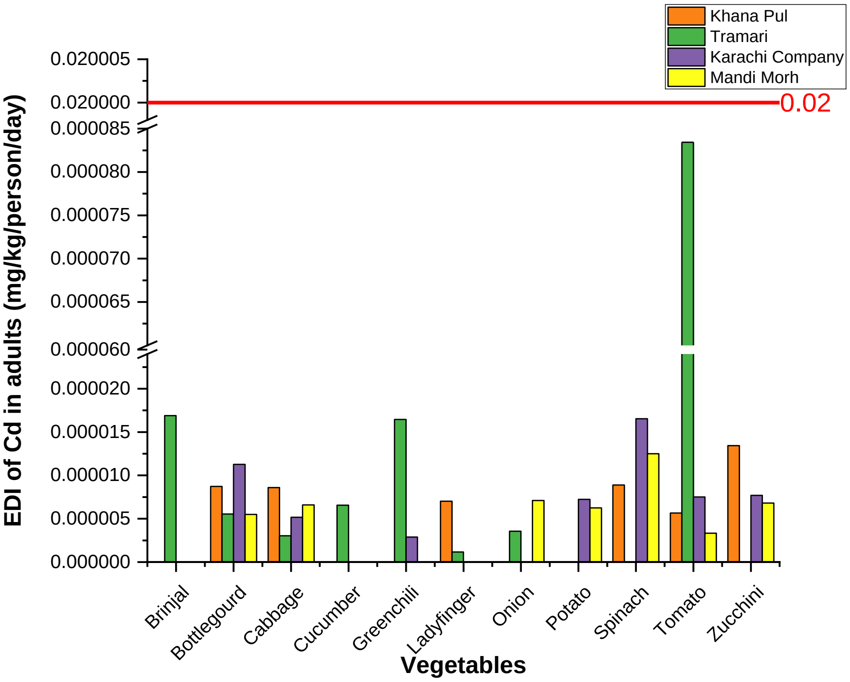

Fig 4.6. Estimated daily intake of Cadmium via consumption of fresh vegetables in adults

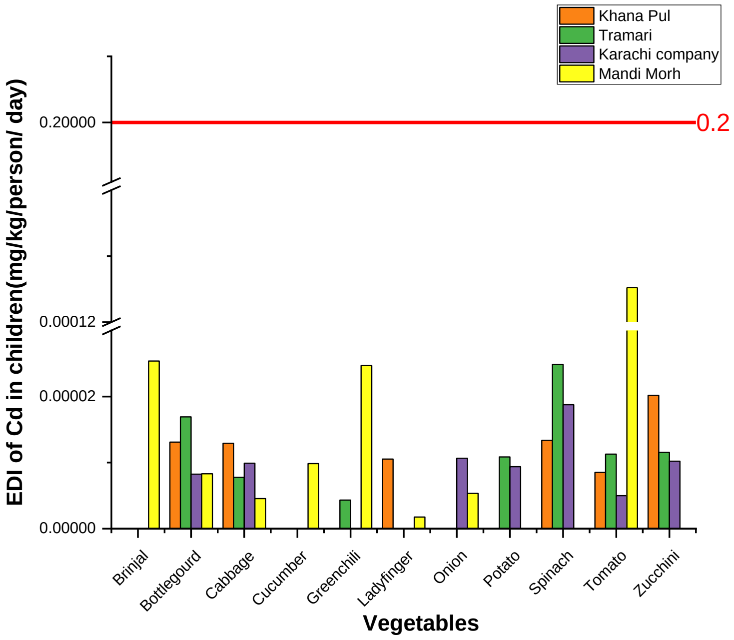

Fig 4.7. Estimated daily intake of Cadmium via consumption of fresh vegetables in Children

The estimated daily intake of cadmium via consumption of brinjal was observed to be below MTL. Similarly, Ashraf et al. (2021), and Randhawa et al. (2014) also reported EDI of Cd via the consumption of brinjal in the population of district Kasur and urban and peri-urban areas of Multan. The result of EDI of Cd via ingestion of bottle gourd was found to be similar to a study conducted by Haque et al. (2021) in industrial and non-industrial markets of Bangladesh and Wadood et al. (2021) in markets of Rawalpindi. The estimated daily intake of cadmium via ingestion of cucumber in markets of Multan and Rawalpindi was found similar to the present study, which was below MTL (Wadood et al., 2021). Results Intake of cadmium via Cabbage in adults as well as children were found to be in the same way as the EDI of Cd reported by Ashraf et al. (2021), Khanum et al. (2017), and Rizwan et al. (2016) in Kasur, Lahore, and Gujranwala. Ingestion of ladyfinger contaminated with Cd reported by Ashraf et al. (2021), and Ali et al. (2021) in the population of Kasur, and Peshawar showed an estimated daily intake in both adults and children to be similar to the current study. Similarly, incase of onion Wadood et al. (2021) reported similar results in the markets of Rawalpindi and Multan. The estimated daily intake of potatoes showed similar results as reported by Khan et al. (2013), and Khanum et al. (2017) in the population of Swat, and Lahore, Pakistan. Rizwan et al. (2017) and Khan et al. (2010) reported similar results of spinach in the population of Gujranwala and Gilgit, Pakistan. Haque et al. (2021) and Ashraf et al. (2021) also reported the estimated daily intake of cadmium via ingestion of potatoes to be below the maximum tolerable limit. Varol et al. (2022) also reported the EDI of zucchini to be below the maximum tolerable limit.

4.4.1.3. Estimated Daily Intake of Chromium

The estimated daily intake of chromium was observed below the maximum tolerable intake, 0.035 mg/kg/person/day (Shaheen et al., 2016). The mean estimated daily intake of Chromium is shown in table 4.3. The decreasing order of estimated daily intake of chromium in adults and children is as follows; tomato > spinach > bottle gourd > zucchini > brinjal > green chili > cucumber > cabbage > onion > potato > ladyfinger.

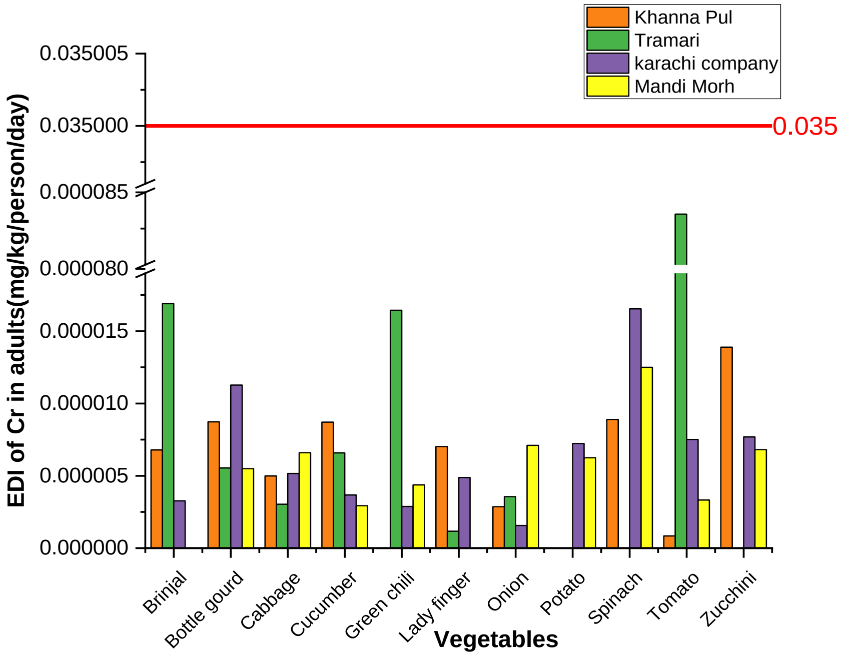

Fig 4.10 Estimated daily intake of Chromium via consumption of fresh vegetables in adults

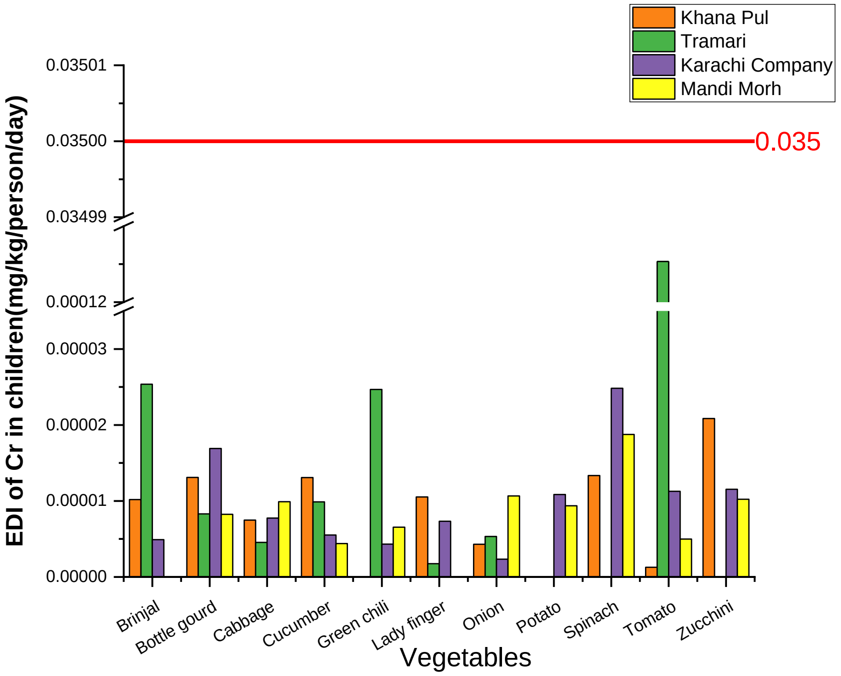

Fig 4.11 Estimated daily intake of Chromium via consumption of fresh vegetables in children

Estimated daily intake of brinjal in adults as well as in children both was observed to be similar as reported in the population of district Kasur, Pakistan (Ashraf et al., 2021), and in the adult population of Malatya, Turkey (Varol et al., 2022). Wadood et al. (2021) in Multan reported similar results as observed in the population of the present study. Estimated daily intake of cabbage in adults as well as in children was observed to be similar as observed in the population of district Kasur, Pakistan (Ashraf et al., 2021), in the population of Dera Ismail Khan, Pakistan (Ullah, et al., 2022) via consumption of cabbage irrigated with groundwater, in agricultural land irrigated with groundwater as well as wastewater in district Lahore, Pakistan (Mahmood, & Malik, 2014; Khanum et al., 2017) and children and adult population of Gujranwala, Pakistan (Rizwan et al., 2016). Cucumber showed EDI to be similar as reported by Wadood et al. (2021) in Rawalpindi and Multan markers, while EDI of Cr via consumption of cucumber reported by Varol et al. (2022) in the population of Malatya province, Turkey was beyond the maximum tolerable limit. Estimated daily intake of ladyfinger in adults as well as in children was observed to be similar as reported in the population of district Kasur, Pakistan (Ashraf et al., 2021), in district Peshawar, Pakistan (Ali et al., 2021), and in adult and children population of district Swat, Pakistan (Khan et al., 2013). The estimated daily intake of cadmium via consumption of onion in adult and children population of the current study shows a similar intake value reported in the district Swat, Pakistan by Khan et a., 2013). Estimated daily intake of chromium via consumption of potatoes showed a similar value as reported in the population of Swat (Khan et al., 2013), and in Gujranwala (Khanum et al., 2017). Estimated daily intake of Cr via consumption of spinach in adults and children was found to be similar as reported by Ullah and his coworkers in Dera Ismail Khan, Pakistan (Ullah, et al., 2022), and in adult and children population of Gujranwala, Pakistan (Rizwan et al., 2016). Ullah et al. (2022), Ali et al. (2021), and Khan et al. (2013) in the population of Dera Ismail Khan, Peshawar, and Swat reported similar results as observed in the exposed population of the current study. The estimated daily intake of zucchini observed in the current study was similar to the EDI reported by Varol et al. (2022) in the population of Malatya province Turkey.

4.4.1.4. Estimated Daily Intake of Lead

The estimated daily intake of Pb in adults and children was observed to be within the maximum tolerable intake, 0.21 mg/kg/person/day (Shaheen et al., 2016). The mean estimated daily intake of lead is shown in table 4.3. the decreasing order of estimated daily intake of lead in adults and children is as follows; spinach > ladyfinger > zucchini > tomato > cabbage > potato > bottle gourd > cucumber > brinjal > green chili > onion.

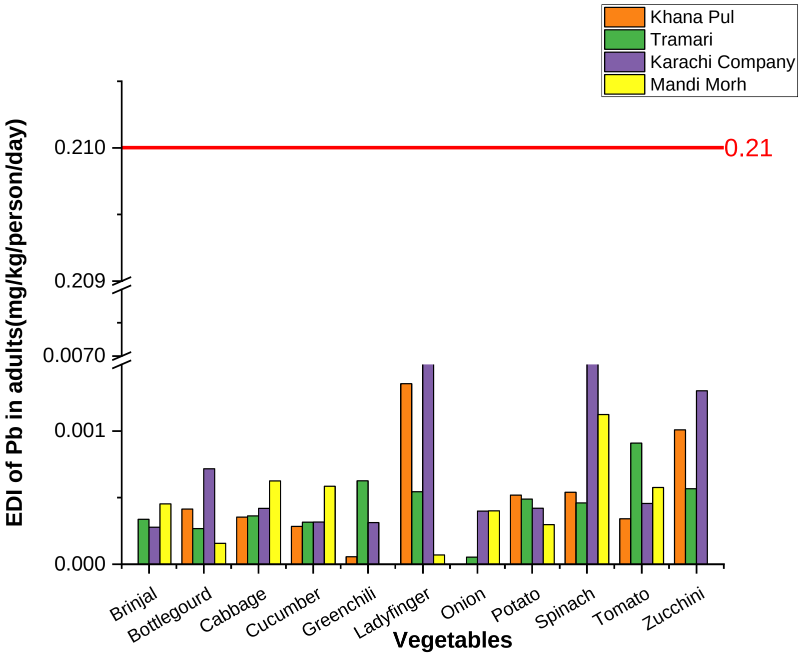

Fig 4.12 Estimated daily intake of Lead via consumption of fresh vegetables in adults

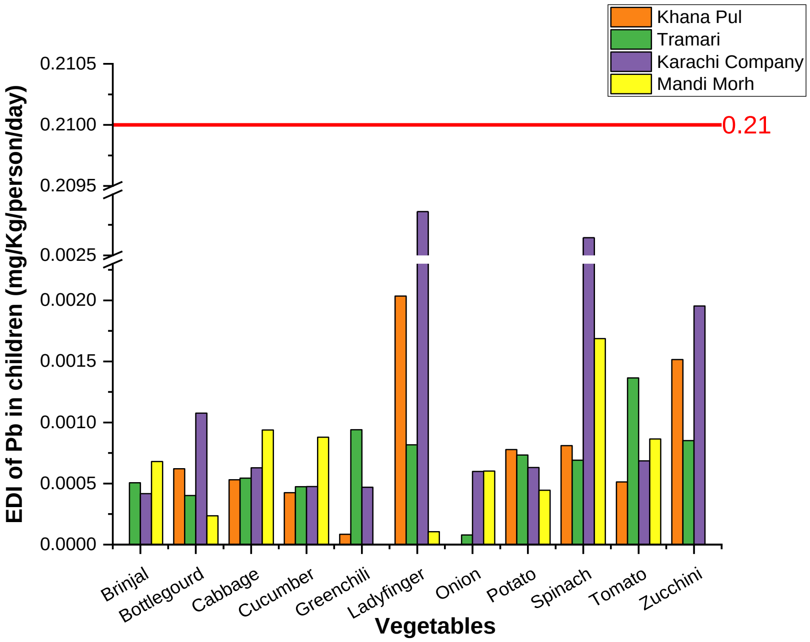

Fig 4.13 Estimated daily intake of Lead via consumption of fresh vegetables in children

Brinjal showed an EDI similar to that observed in the population of district Kasur (Ashraf et al., 2021), Multan, Pakistan (Wadood et al., 2021), Malatya province, Turkey (Varol et al., 2022), and in Bangladesh (Haque et al., 2021). Intake of Pb via bottle gourd in the reported population of Bangladesh (Haque et al., 2021), and in Multan (Wadood et al., 2021) was observed to be similar to the EDI of the present study. Cabbage showed an EDI to be similar as reported by Ullah et al. (2022), Ashraf et al. (2021), Khanum et al. (2017), and Rizwan et al. (2016), in Dera Ismail Khan, Kasur, Lahore, and Gujranwala Pakistan. Wadood et al. (2021) in Rawalpindi and Multan reported similar daily intake of Pb via ingestion of cucumber. The estimated daily intake of ladyfinger in adults as well as in children both was observed to be close to the EDI reported in the population of district Kasur, Pakistan (Ashraf et al., 2021), and observed population of Peshawar, Pakistan (Ali et al., 2021). A similar study conducted in the exposed population of Multan, Rawalpindi (Wadood et al., 2021) reported EDI to be lower than MTL as observed in the present study. Potato showed EDI close to the one reported in the population of Jamaica consuming vegetables from fresh markets (Antoine et al., 2017), in the adult population of Bangladesh via consumption of potatoes from industrial, non-industrial and markets (Haque et al., in 2021), in adult and children population of Gujranwala, Pakistan (Rizwan et al., 2016) and in the adult population of Lahore (Khanum et al.,2017). Estimated daily intakes of spinach in adults and children were found to be similar to those reported in the populations of Bangladesh (Haque et al., in 2021), Dera Ismail Khan, Pakistan (Ullah, et al., in 2022), Gilgit, Pakistan (Khan, et al., in 2010), and Gujranwala, Pakistan (Rizwan et al., 2016). Similar EDI results of Pb via tomato consumption were reported by Ashraf et al. (2021), Wadood et al. (2021), Haque et al. (2021), Varol et al. (2022) in district Lahore, Rawalpindi, and Multan, Bangladesh, and Malatya province, Turkey. Varol et al. (2021) found a similar EDI of zucchini in the population of the Malatya province of Turkey.

4.4.1.5. Estimated Daily Intake of Zinc

The estimated daily intake of Zn in adults and children was observed to be within the maximum tolerable intake, 60 mg/kg/person/day (Shaheen et al., 2016). The mean estimated daily intake of Zn is shown in table 4.3. The decreasing order of estimated daily intake of Zn in adults and children is as follows; spinach > zucchini > tomato > potato > onion > ladyfinger > green chili > cucumber > cabbage > bottle gourd > brinjal.

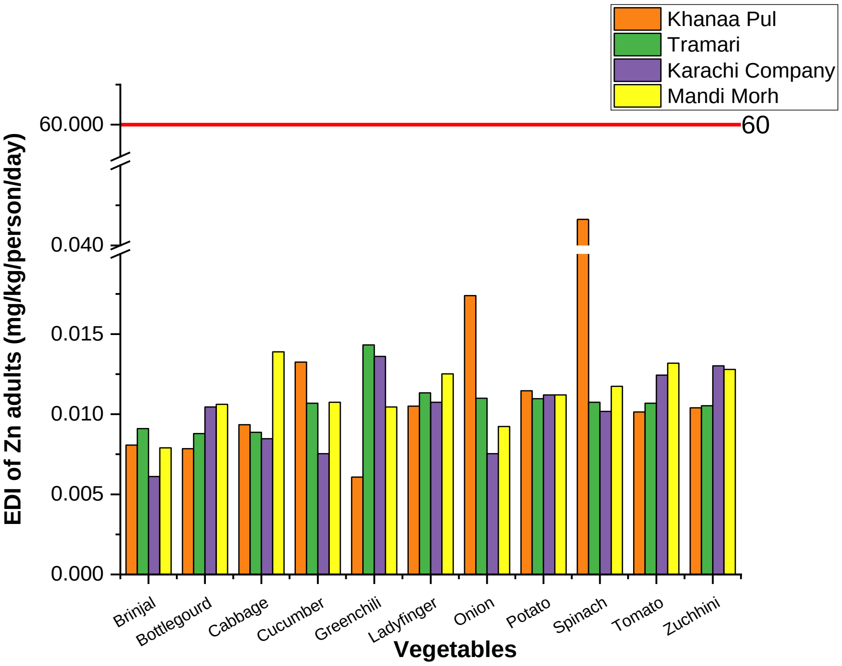

Fig 4.14. Estimated daily intake of Zn via consumption of fresh vegetables in adults

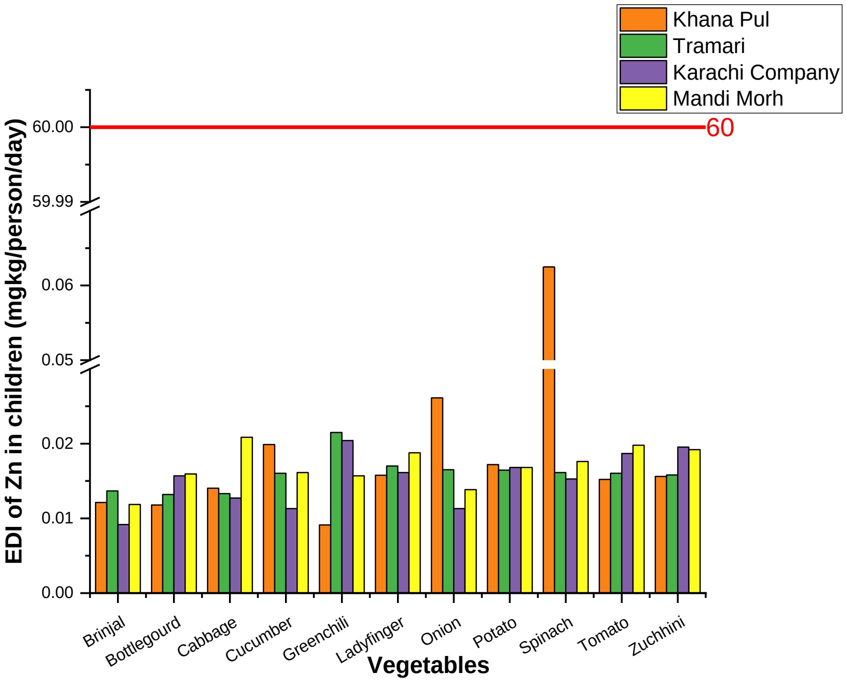

Fig. 4.15. Estimated daily intake of Zn via consumption of fresh marketed vegetables in children

Brinjal showed an EDI similar to that observed in the populations of Kasur (Ashraf et al., 2021), Multan (Wadood et al., 2021), Malatya province, Turkey (Varol et al., 2022), and

Bangladesh (Haque et al., 2021). Bottle gourd showed a similar EDI as observed in the adult population of Multan via consumption of marketed bottle gourd, and in Bangladesh via consumption of vegetables from different sources such as from markets, industrial and non-industrial areas (Haque et al., 2021). In the population of the districts of Kasur (Ashraf et al., 2021), Dera Ismail Khan (Ullah et al., 2022), Gujranwala (Rizwan et al., 2016), and Lahore (Mahmood and Malik, 2014), the EDI of Zn from cabbage intake was found to be comparable to the current study. EDI of Zn via consumption of cucumber was found to be similar as reported by Varol et al. (2022) and Wadood et al. (2021) in the population of Malatya province, Turkey, and Rawalpindi and Multan. Similarly, the EDI of zinc via consumption of ladyfinger was observed close to the EDI reported by Ali et al. (2021), Wadood et al. (2021), and Khan et al. (2013) in the district Peshawar, Multan, and Swat, Pakistan. Intake of onion showed similar results as observed in Rawalpindi and Multan (Wadood et al., 2021) and in Swat (Khan et al., 2013). Daily intake of zinc via consumption of potatoes reported in Multan and Rawalpindi (Wadood et al., 2021), Gujranwala (Rizwan et al., 2016), and Lahore (Mahmood & Malik, 2014) showed similar results. Spinach showed an EDI similar to the studies conducted in Dera Ismail Khan (Ullah et al., 2022), and in Gujranwala (Rizwan et al., 2016). Ashraf et al. (2021), Wadood et al. (2021), and Ali et al. (2021) in the population of districts Kasur, Multan and Rawalpindi, and Peshawar reported daily intake of Zn via consumption of tomatoes to be close to the EDI result calculated in the present study.

Table 4.4. Mean EDI of selected heavy metals via consumption of selected fresh vegetables in adults and children

Vegetables		As	Cd	Cr	Pb	Zn
Brinjal	Adults	9.06×10^{-4}	4.2×10^{-6}	6.73×10^{-6}	2.67×10^{-4}	7.80×10^{-3}
	Children	8.31×10^{-4}	6.3×10^{-6}	1.01×10^{-5}	4.01×10^{-4}	1.17×10^{-2}
Bottle Gourd	Adults	2.77×10^{-4}	7.7×10^{-6}	7.76×10^{-6}	3.89×10^{-4}	9.43×10^{-3}
	Children	7.43×10^{-4}	1.2×10^{-5}	1.16×10^{-5}	5.84×10^{-4}	1.41×10^{-2}
Cabbage	Adults	8.70×10^{-4}	5.8×10^{-6}	4.94×10^{-6}	4.4×10^{-4}	1.01×10^{-2}
	Children	1.14×10^{-3}	8.8×10^{-6}	7.42×10^{-6}	6.61×10^{-4}	1.52×10^{-2}
Cucumber	Adults	4.20×10^{-4}	1.6×10^{-6}	5.47×10^{-6}	3.76×10^{-4}	1.05×10^{-2}
	Children	1.44×10^{-3}	2.5×10^{-6}	8.22×10^{-6}	5.64×10^{-4}	1.58×10^{-2}
Green chili	Adults	9.06×10^{-4}	4.8×10^{-6}	5.92×10^{-6}	2.49×10^{-4}	1.11×10^{-2}
	Children	1.70×10^{-3}	7.2×10^{-6}	8.89×10^{-6}	3.74×10^{-4}	1.67×10^{-2}
Ladyfinger	Adults	6.43×10^{-4}	2.0×10^{-6}	3.27×10^{-6}	9.68×10^{-4}	1.13×10^{-2}
	Children	1.67×10^{-3}	3.1×10^{-6}	4.90×10^{-6}	1.45×10^{-3}	1.69×10^{-2}
Onion	Adults	9.63×10^{-4}	2.7×10^{-6}	3.77×10^{-6}	2.13×10^{-4}	1.13×10^{-2}
	Children	2.19×10^{-3}	4.0×10^{-6}	5.66×10^{-6}	3.2×10^{-4}	1.69×10^{-2}
Potato	Adults	4.08×10^{-4}	3.4×10^{-6}	3.37×10^{-6}	4.31×10^{-4}	1.12×10^{-2}
	Children	1.47×10^{-3}	5.0×10^{-6}	5.06×10^{-6}	6.47×10^{-4}	1.68×10^{-2}
Spinach	Adults	1.08×10^{-3}	9.5×10^{-6}	9.48×10^{-6}	9.71×10^{-4}	1.86×10^{-2}
	Children	2.94×10^{-3}	1.4×10^{-5}	1.42×10^{-5}	1.46×10^{-3}	2.79×10^{-2}
Tomato	Adults	7.85×10^{-4}	2.5×10^{-5}	2.38×10^{-5}	5.71×10^{-4}	1.16×10^{-2}
	Children	1.66×10^{-3}	3.7×10^{-5}	3.57×10^{-5}	8.57×10^{-4}	1.74×10^{-2}
Zucchini	Adults	5.29×10^{-4}	7.0×10^{-6}	7.10×10^{-6}	7.2×10^{-4}	1.17×10^{-2}
	Children	1.48×10^{-3}	1.05×10^{-5}	1.06×10^{-5}	1.08×10^{-3}	1.75×10^{-2}

4.1.2. Total Estimated Daily Intake of Heavy Metals

The total estimated intake of arsenic in adults was observed 8.00 $\times 10^{-3}$, while for children it was 1.7 $\times 10^{-1}$ mg/kg/person/day. For total EDI of cadmium was observed as 7.38 $\times 10^{-5}$ and 1.11×10^{-4} for adults and children respectively, while the total EDI of chromium was 8.16 $\times 10^{-5}$

and 1.22×10^{-4} for adults and children respectively. For lead, it was observed as 5.60×10^{-3} and 8.40×10^{-3} in adults and children respectively. The total estimated intake of zinc was found to be 1.24×10^{-1} mg/kg/person/day, while for children it was 1.87×10^{-1} mg/kg/person/day. The results of the total estimated daily intake (TEDI) were similar to those reported in Bangladesh (Haque et al., 2021) and in the district Kasur, Pakistan (Ashraf et al., 2021).

The 90% total estimated daily intake in adults was from zinc followed by arsenic (6%), lead (4%), chromium (0.06%), and cadmium (0.05%). While for children 88% of total EDI was zinc followed by arsenic lead, chromium, and cadmium 8, 4, 0.06, and 0.05% respectively.

The combined estimated daily intake of all the metals via consumption of vegetables was also observed to be within the maximum tolerable limits.

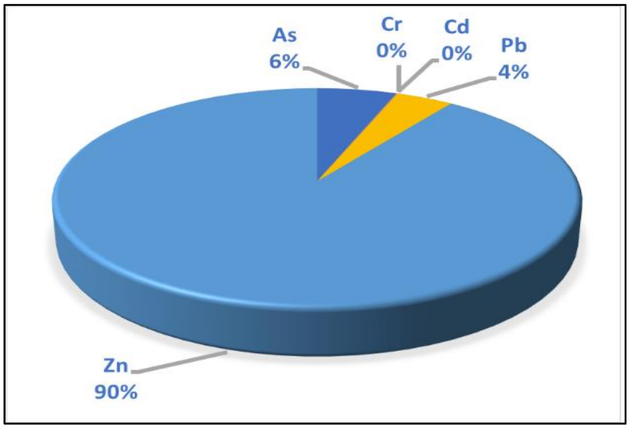

Fig 4.16. Total estimated intake of all the selected metals in adults via fresh vegetable consumption

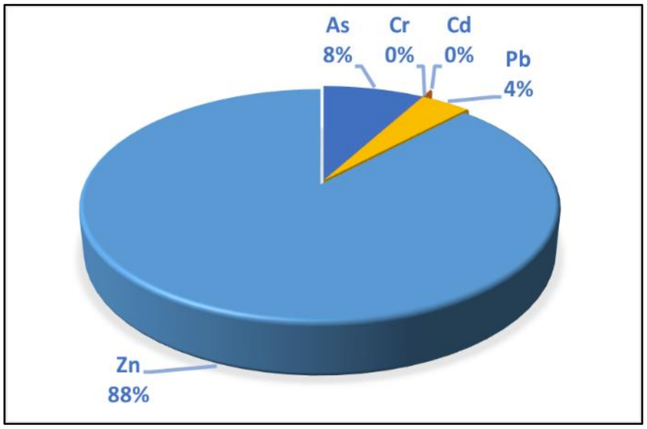

Fig. 4.17. Total estimated intake of all the selected metals in children via fresh vegetable consumption

4.4.2. Target Hazard Quotient

To determine the noncancer health effects on the exposed population via the consumption of selected vegetables THQ was calculated. The results are as follows

4.4.2.1. Target Hazard Quotient of Arsenic

The mean target hazard quotient of metals via the consumption of vegetables is given in table 4.3. The THQ of all the metals via consumption of vegetables are all greater than 1, which shows that all vegetable consumption poses a health risk to adults as well as children. The increasing trend of all the vegetables via consumption in adults is as follows; bottle gourd > brinjal > cabbage > cucumber > potato > zucchini > tomato > ladyfinger > green chili > onion > spinach.

While for children the increasing trend is bottle gourd > brinjal > cabbage > green chili > potato > zucchini > cucumber > tomato > ladyfinger > onion > spinach.

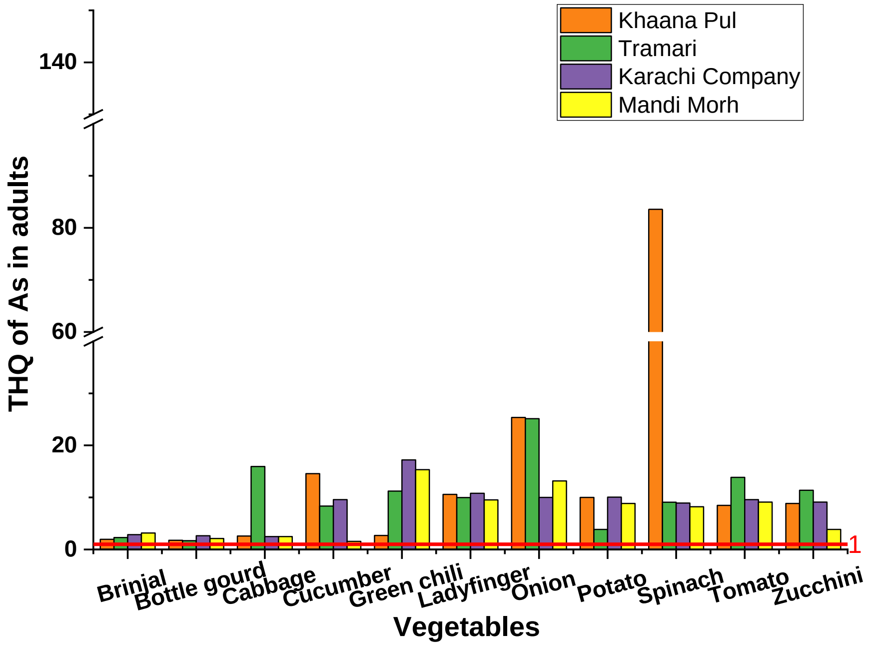

Fig 4.18 Target Hazard Quotient of Arsenic in adults via consumption of Fresh vegetables

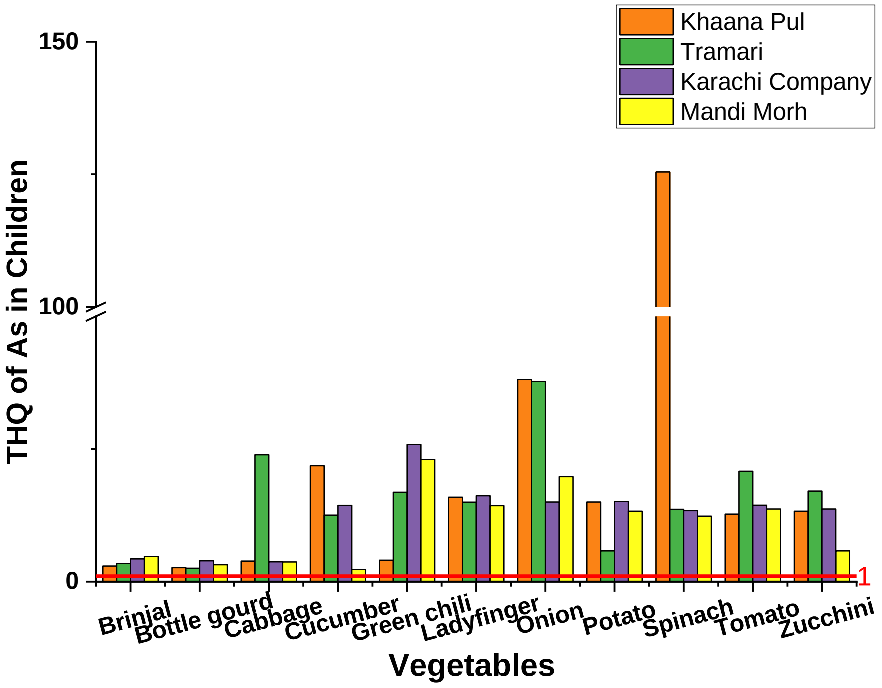

Fig 4.19 Target Hazard Quotient of Arsenic in children via consumption of Fresh vegetables

THQ of As via consumption of brinjal was observed to be similar as reported in the population of district Kasur (Ashraf et al., 2021), and in Bangladesh (Haque et al., 2021), while in the population of Malatya province Turkey, THQ value reported was less than 1 showing that exposed population has no risk of developing non-cancer related health effects (Varol et al., 2022). Haque et al. (2021) in Bangladesh reported similar results of developing non-cancer-related health effects via the consumption of bottlegourd and spinach. THQ of As via consumption of cabbage was observed to be similar as reported in the adults and children population of district Kasur, Pakistan, while in the population of Jamaica, THQ values were reported greater than 1 (Antoine et al., 2017). Varol et al. (2022) reported THQ values of As via the consumption of cucumber. Ashraf et al. (2021) reported THQ values of As via consumption of ladyfinger and tomato to be similar to the present study. Potato showed a THQ value greater than 1, showing that there will be non-cancer effects associated with consumption, similarly adult population in Bangladesh also showed THQ >1, showing a non-Cancer risk (Haque et al., 2021). While the adult population of Jamaica consuming vegetables from markets has a THQ < 1 showing no risk (Antoine et al., 2017). Ashraf et al. (2021), and Haque et al. (2021) in the population of district Kasur, Pakistan and Bangladesh, reported similar results, while Antoine et al. (2017) reported that the population of Jamaica, is at risk of non-cancer health effects. Varol et al. (2021) reported that the THQ of arsenic via consumption of zucchini was less than 1, showing no non-cancer-related health effects to the population.

4.4.2.2. Target Hazard Quotient of Cadmium

The target hazard quotient of cadmium in all vegetable samples collected from different markets of Islamabad and Rawalpindi was below 1 which means they have no health impact on children as well as on adults' health except for tomato collected from Tramari, Rawalpindi. The decreasing trend of chromium in vegetable consumption by adults and children are as follows; tomato < spinach< bottle gourd < zucchini <brinjal < green chili < cucumber < cabbage < green chili < brinjal < potato < ladyfinger < cucumber.

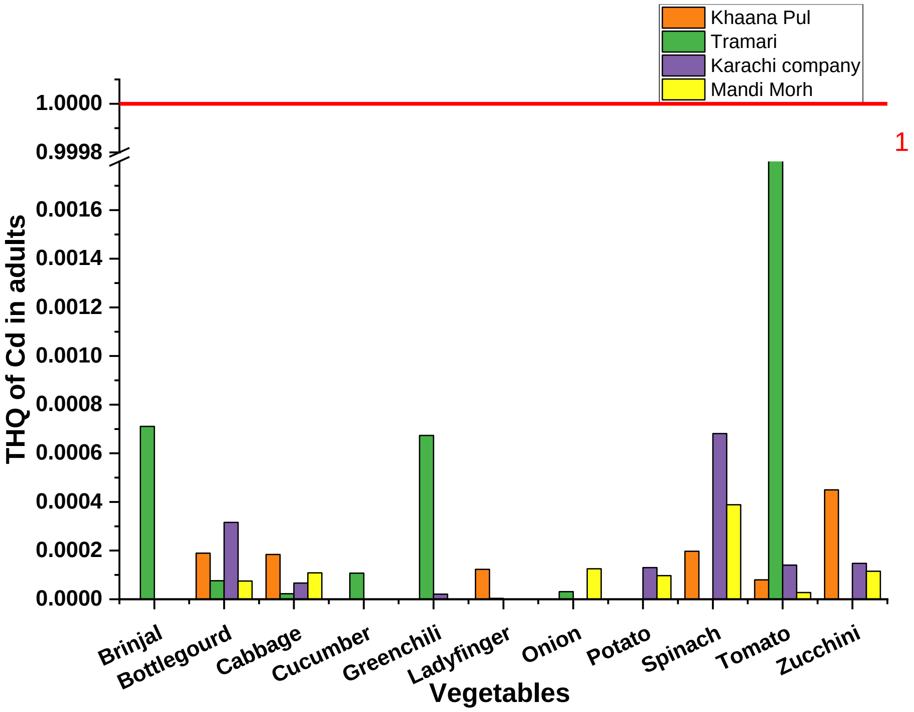

Fig 4.20 Target Hazard Quotient of cadmium in adults via consumption of Fresh vegetables

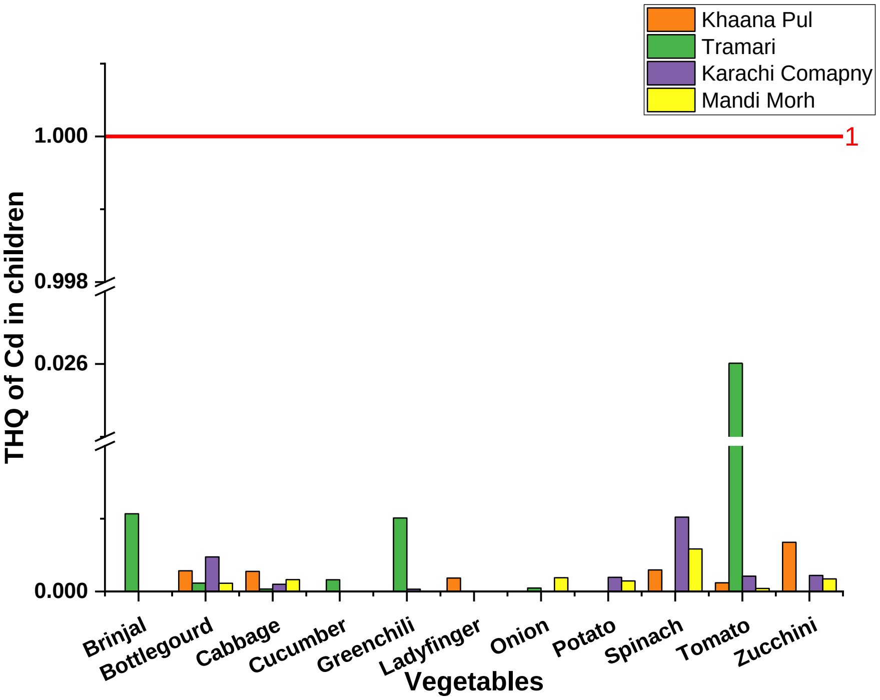

Fig 4.21 Target Hazard Quotient of cadmium in children via consumption of Fresh vegetables

Brinjal showed a THQ <1, posing no harmful non-cancer risks to adults as well as children similar results were reported adult population of Malatya, Turkey (Varol et al., 2022) and the population of district Kasur, Pakistan (Ashraf et al., 2021). Haque et al. (2021) in the population of Bangladesh reported similar results of THQ of Cd via consumption of bottlegourd. THQ of Cd via consumption of cabbage was observed to be similar as compared to the results of THQ exposed population of district Kasur, Pakistan (Ashraf et al., in 2021) and in the adult population of Jamaica consuming vegetables from markets (Antoine et al., 2017). THQ of Cd via consumption of ladyfinger was observed to be similar as observed in adults and children of district Kasur, Pakistan (Ashraf et al., in 2021). Gupta et al. (2022) reported similar THQ values in the population of Jhansi, India consuming onion cultivated near the highway. Potato showed THQ<1 which means that the exposed population is safe similar results were reported in the population of Jamaica consuming marketed potatoes (Antoine et al., 2017), while the adult population of Bangladesh is exposed to non-cancer risk via consumption of potatoes from industrial, non-industrial and markets (Haque et al., 2021). THQ values of Cd via consumption of spinach in population of Handan city, China (Meng et al., 2021) and in Bangladesh (Haque et al., 2021) were observed to be similar as observed in the current study. THQ of Cd via consumption of tomato was observed to be similar as compared with the study conducted in the population of district Kasur, Pakistan (Ashraf et al., 2021) and in the population of Jhansi, India (Gupta et al., 2022). Varol et al. (2022) in the population of Malatya province, Turkey reported similar results as obtained in the population of the present study via the consumption of cucumber and zucchini.

4.4.2.3. Target Hazard Quotient of Chromium

The target hazard quotient of chromium in all vegetable samples collected from different markets of Islamabad and Rawalpindi was below 1 which means they have no health impact on children as well as on adults' health except for tomato collected from Karachi Company, Islamabad. The decreasing trend of chromium in vegetable consumption by adults and children is as follows; tomato < spinach < bottle gourd < zucchini <brinjal < green chili < cucumber < cabbage < onion < potato < ladyfinger.

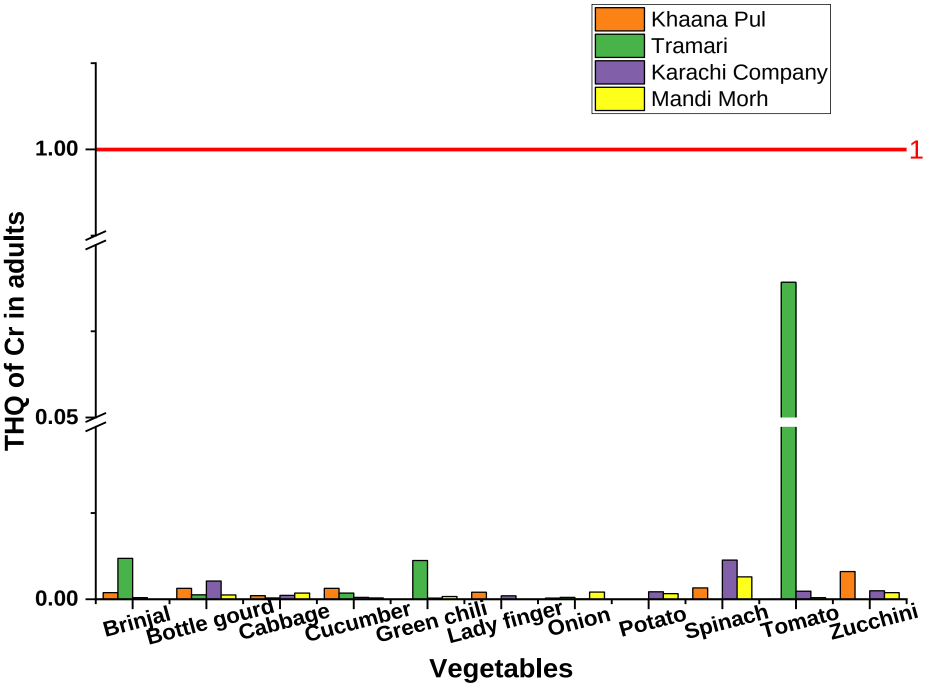

Fig 4.22 Target Hazard Quotient of chromium in adults via consumption of Fresh vegetables

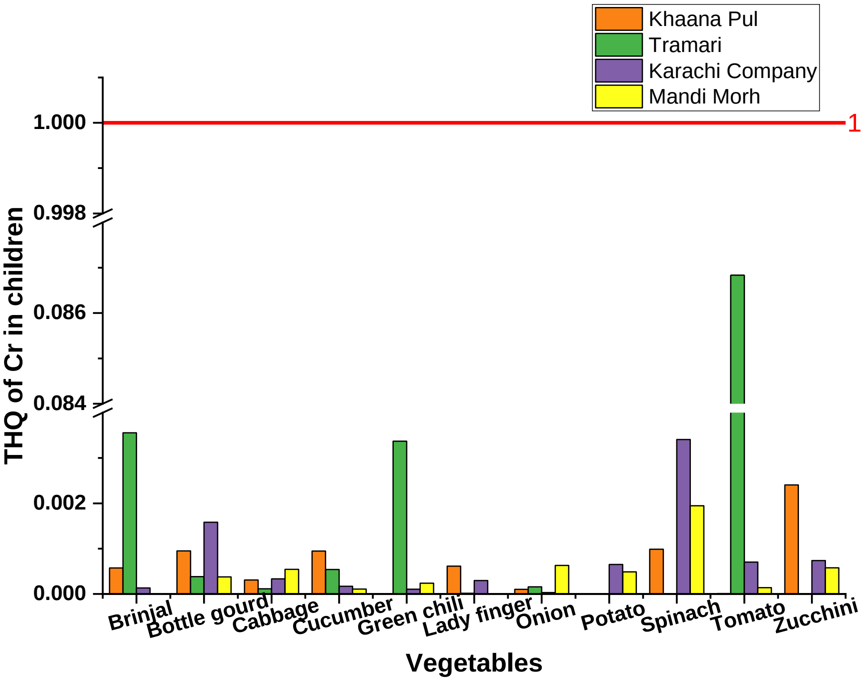

Fig 4.23 Target Hazard Quotient of chromium in children via consumption of Fresh vegetables

The Target Hazard Quotient (THQ) value of Cr via consumption of brinjal, cabbage, tomato, and ladyfinger reported in the population of district Kasur, Pakistan (Ashraf et al., 2021) was observed to be similar to the present study. In the case of THQ values of spinach, the values reported in Handan city, China (Meng et al., 2021) were similar compared to the present study.

4.4.2.4. Target Hazard Quotient of Lead

The mean Target Hazard Quotient of lead in selected vegetables is given in table 4.4. All the vegetable samples showed THQ <1, showing no risk to the exposed adult population except for ladyfinger from Karachi company, and tomato and zucchini from mandi morh were observed higher than 1, posing a risk to the adult population.

In the case of children, all the vegetable samples were posing no risk to the exposed population except ladyfinger from Khanna Pul and Karachi company, spinach from mandi morh and Karachi company, and zucchini from Karachi company. The decreasing trend observed for adults and children is as follows; spinach < ladyfinger < zucchini < tomato < cabbage < potato < bottlegourd < cucumber < brinjal < green chili < onion.

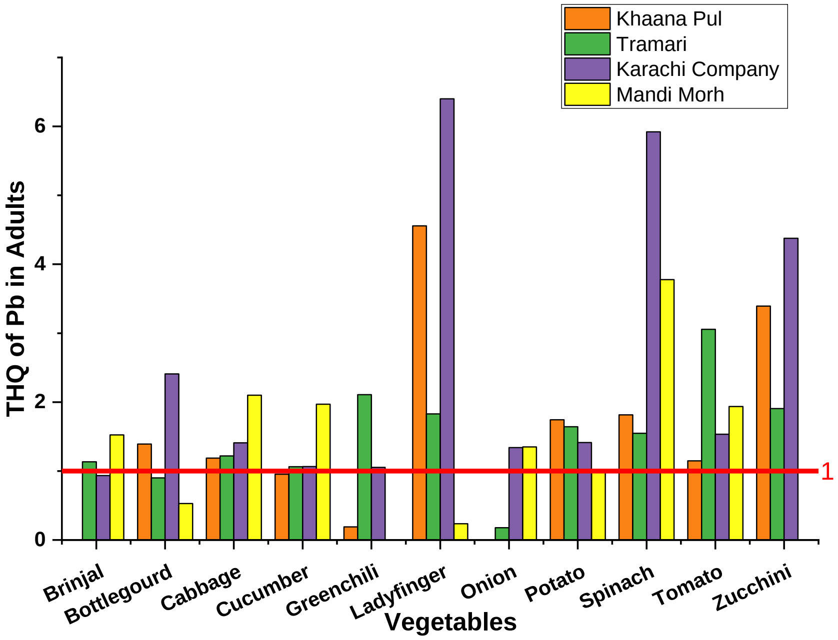

Fig 4.24 Target Hazard Quotient of lead in adults via consumption of Fresh vegetables

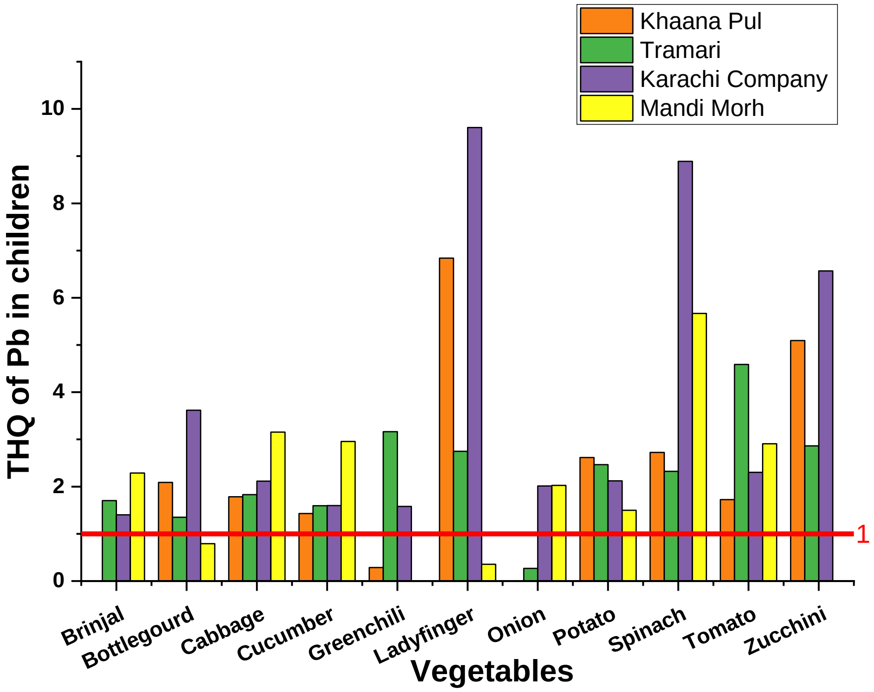

Fig 4.25 Target Hazard Quotient of lead in children via consumption of Fresh vegetables

Ashraf et al. (2021) reported similar results of THQ of lead via the consumption of brinjal, cabbage, and tomatoes in the population of district Kasur, Pakistan. THQ values of bottlegourd, tomatoes, and spinach in the population of Bangladesh were reported >1, showing a risk to the exposed population (Haque et al., 2021), while the current study shows THQ < 1 in the exposed population. THQ values of Pb via consumption of ladyfinger were observed to be less than 1, showing no non-cancer risk to the exposed population, while in the present study consumption of ladyfinger is showing a potential non-cancer risk to the exposed population of Islamabad and specifically Rawalpindi. Gupta et al. (2022) reported similar results of THQ via the consumption of onion and tomatoes in the exposed population of Jhansi, India. According to Antoine et al. (2017), THQ levels of less than 1 were reported by the exposed population of Jamaica, consuming potatoes, tomatoes, and spinach.

4.4.2.5. Target Hazard Quotient of Zinc

Table 4.4 shows the mean zinc Target Hazard Quotient for a variety of vegetables. With the exception of spinach from Khanna Pul, Rawalpindi, all vegetable samples had THQ values below 1, indicating no risk to the exposed adult population. In the case of children, all the vegetable samples were posing no risk to the exposed population except spinach and onion from

Khaana Pul. The decreasing trend observed for adults and children is as follows; spinach < potato < tomato < zucchini < onion < ladyfinger < green chili < cucumber < cabbage < bottle gourd < brinjal.

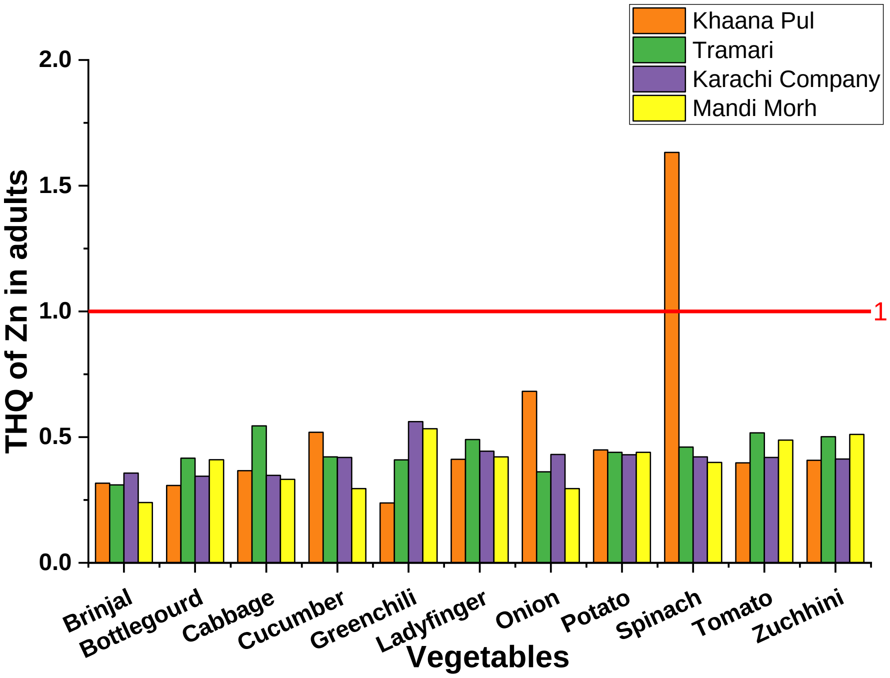

Fig.4.26 Target Hazard Quotient of zinc in children via consumption of Fresh vegetables

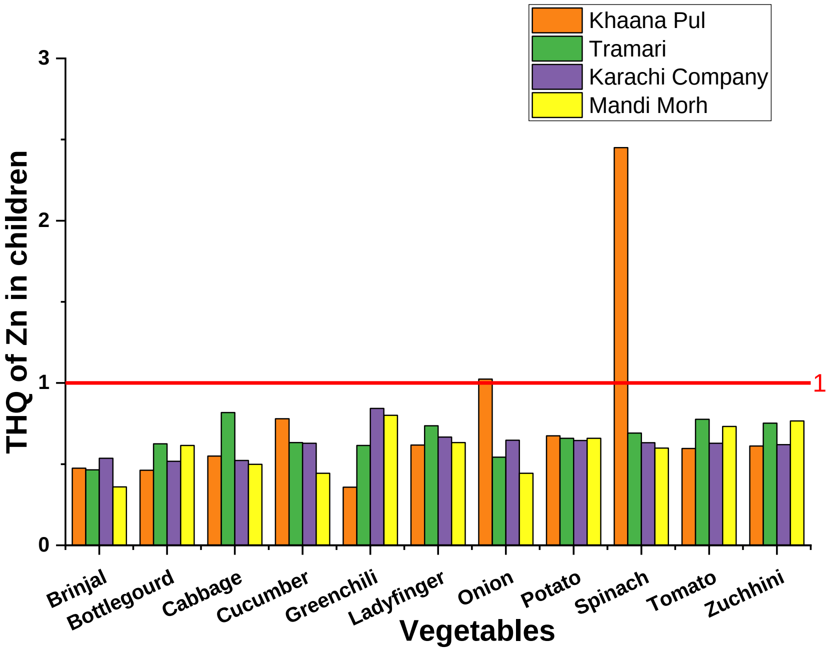

Fig.4.27 Target Hazard Quotient of zinc in children via consumption of Fresh vegetables

Similar findings of THQ of zinc by consumption of brinjal, cabbage, ladyfinger, and tomatoes in the people of district Kasur, Pakistan, were published by Ashraf et al. (2021). The exposed population of Jhansi, India, experienced similar THQ results from eating onions and tomatoes (Gupta et al., 2022). According to Meng et al. (2021), the THQ value of Zn acquired from spinach in the exposed population of Handan City, China, was less than 1, however, in the current investigation, the value is greater than 1, posing a risk to the exposed population.

4.4.3. Hazard Index

4.4.3.1. Hazard Index of Arsenic

The arsenic hazard index via consumption of selected vegetables was estimated to be higher than one, indicating that combined consumption of all vegetables poses a non-cancer risk to the exposed adult and child population. The contribution of vegetables in adult and children's diets to the hazard index (HI) was in the following increasing order bottle gourd > brinjal > cabbage > cucumber > potato > zucchini > ladyfinger > tomato > green chili > onion > spinach.

Similar results of the hazard index were found by Haque et al. (2021) and Ashraf et al. (2021) in populations of Bangladesh and district Kasur, Pakistan, respectively, whereas HI < 1 was reported in populations of Northeast Nigeria (Akan et al., 2021) and in Jamaica (Antoine et al., 2017.

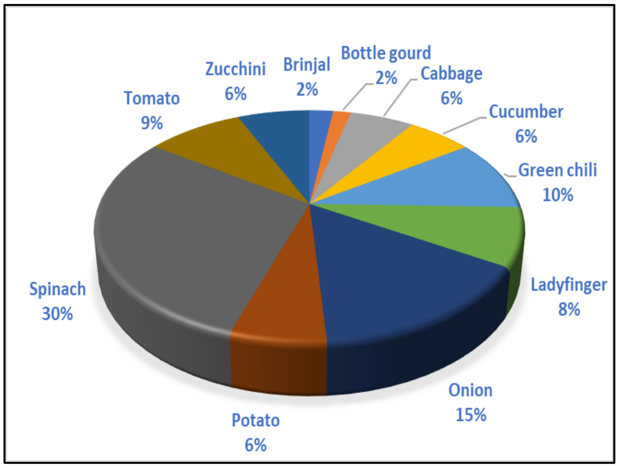

Fig. 4.28. Percentage contribution of each vegetable in adult diet to the hazard index of Arsenic

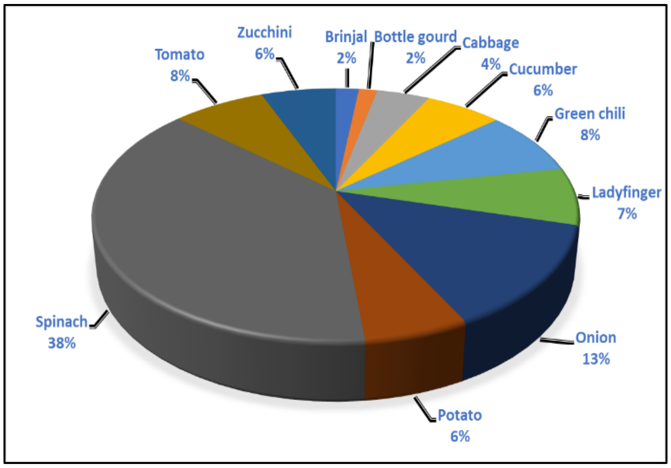

Fig. 4.29. Percentage contribution of each vegetable in children diet to the hazard index of Arsenic

4.4.3.2. Hazard Index of Cadmium

The hazard index of cadmium via consumption of selected vegetables was observed to be less than 1, hence combined consumption of all the vegetables poses a non-cancer risk to the exposed adult and children population. The contribution of selected vegetables in adult and children's diets to hazard index was in increasing order of cucumber > ladyfinger > onion > potato > cabbage > bottle gourd > green chili > brinjal > zucchini > spinach > tomato. Both Ashraf et al. (2021) and Akan et al. (2021) found comparable results of HI of cadmium through the consumption of several vegetables in the Kasur district of Pakistan and Northeast Nigeria, respectively. While Antoine et al. (2017) reported HI > 1 in the Jamaican population, affecting the exposed population's health.

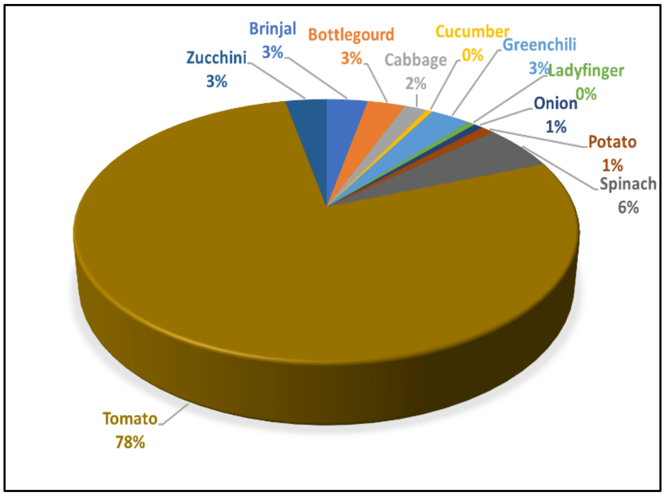

Fig. 4.30. Percentage contribution of each vegetable in adult diet to the hazard index of Cadmium

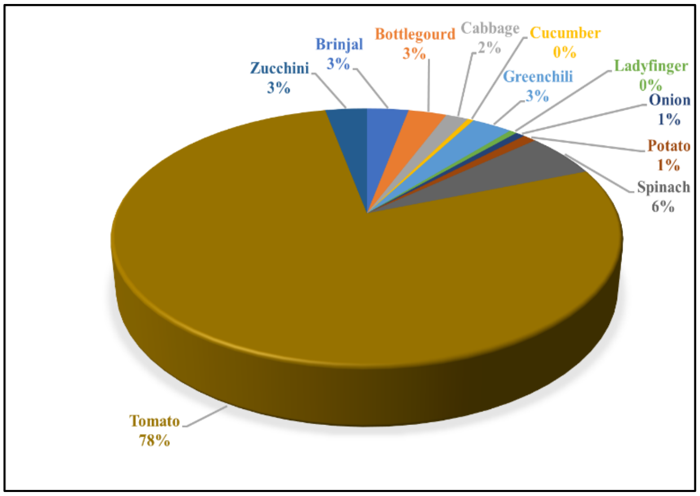

Fig. 4.31. Percentage contribution of each vegetable in children diet to the hazard index of Cadmium

4.4.3.3. Hazard Index of Chromium

The hazard index of lead via consumption of selected vegetables was observed to be less than 1, hence combined consumption of all the vegetables poses a non-cancer risk to the exposed adult and children population. In order of increasing contribution to the hazard index, several vegetables in the diets of adults and children were onion > ladyfinger > potato > cabbage > cucumber > bottle gourd > green chili > zucchini > brinjal > spinach > tomato. Ashraf et al. (2021) and Akan et al. (2021) reported in the population of Kasur, Pakistan, and Northeast Nigeria, similar results of HI of Cr via consumption of vegetables.

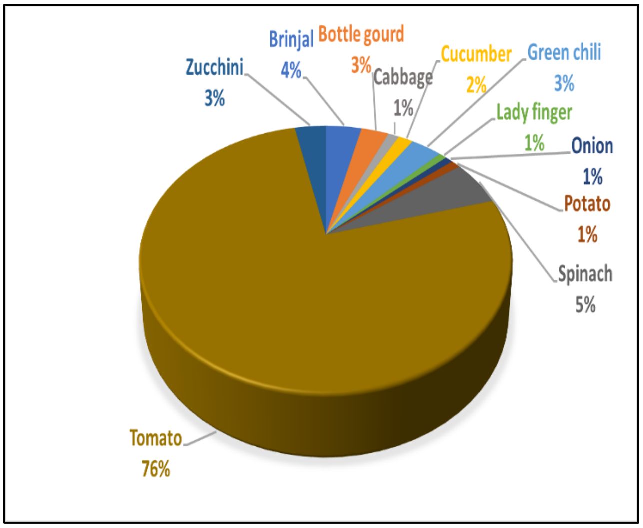

Fig. 4.32. Percentage contribution of each vegetable in adult diet to the hazard index of chromium

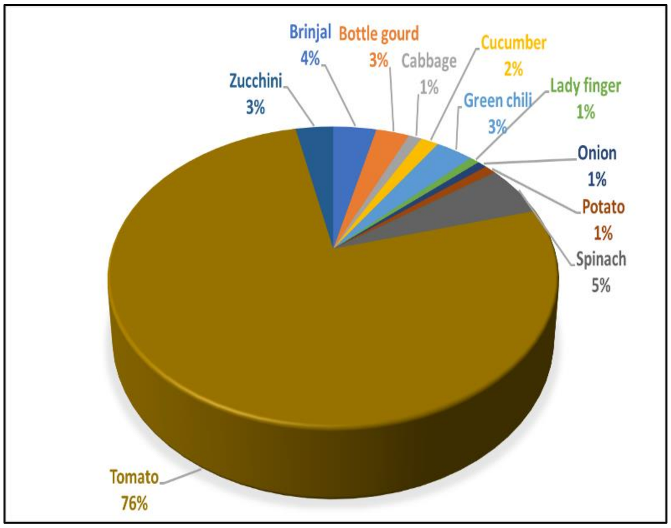

Fig. 4.33. Percentage contribution of each vegetable in adult diet to the hazard index of chromium

Hazard Index of Lead

The hazard index of lead via consumption of selected vegetables was observed to be greater than 1, hence combined consumption of all the vegetables poses a non-cancer risk to the exposed adult and children population. Ladyfinger showed the highest contribution to adult and children's diets followed by spinach > zucchini > tomato > potato > cabbage > bottle gourd > cucumber > brinjal > green chili > onion. Akan et al. (2021) and Haque et al. (2021) reported HI>1in the population of Northeast Nigeria and Bangladesh. Ashraf et al. (2021) reported similar results of HI > 1 in the children population of Kasur, Pakistan, while in the case of the adult population HI > 1 was observed.

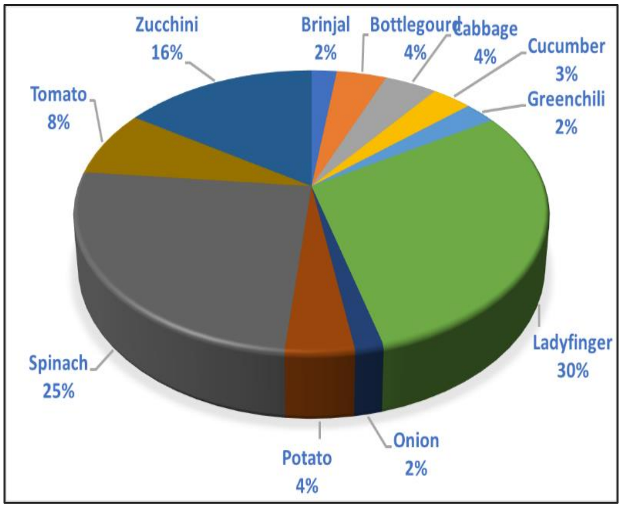

Fig. 4.34. Percentage contribution of each vegetable in adult diet to the hazard index of lead

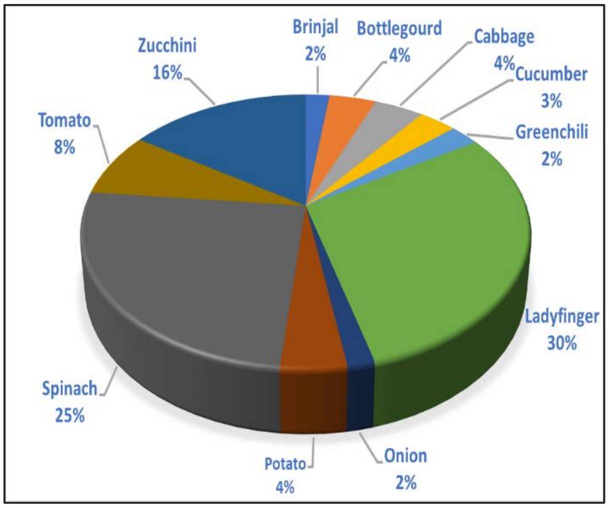

Fig. 4.35. Percentage contribution of each vegetable in children diet to the hazard index of lead

Hazard Index of Zinc

The hazard index of lead via consumption of selected vegetables was observed to be greater than 1, hence combined consumption of all the vegetables poses a non-cancer risk to the exposed adult and children population. Ladyfinger showed the highest contribution to adult and children's diets followed by Spinach > zucchini > tomato > onion > ladyfinger > potato > green chili > cucumber > cabbage > bottle gourd > brinjal. HI of the population consuming vegetables in Pakistan's district of Kasur was less than 1, suggesting no non-cancer risk to the exposed adult and child population (Ashraf et al., 2021), populations of Northeast Nigeria (Akan et al., 2021) and Bangladesh (Haque et al., 2021) showed similar results.

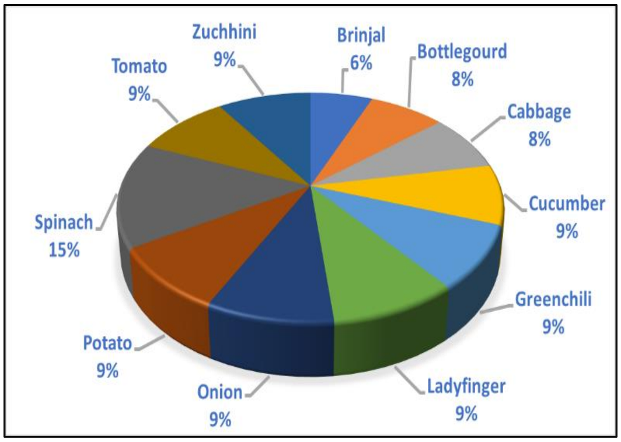

Fig. 4.36. Percentage contribution of each vegetable in adult diet to the hazard index of zinc

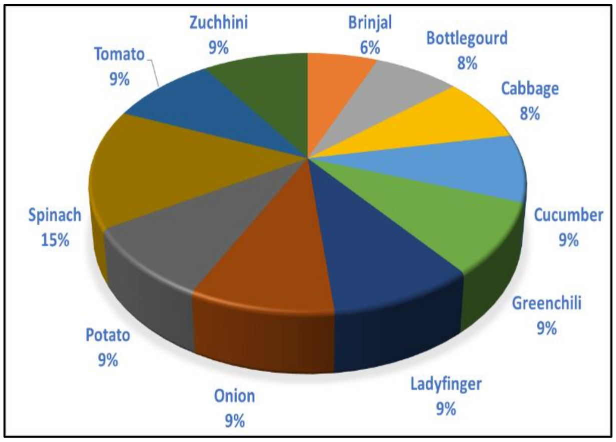

Fig. 4.37. Percentage contribution of each vegetable in children diet to the hazard index of zinc

Table 4.4. Mean THQ of selected HMs via consumption of selected fresh vegetables in adults and children

Vegetables		As	Cd	Cr	Pb	Zn
Brinjal	Adults	4.25	1.78×10^{-4}	7.1×10^{-4}	7.05×10^{-2}	3.05×10^{-1}
	Children	3.85	2.67×10^{-4}	1.07×10^{-3}	1.05×10^{-1}	4.59×10^{-1}
Bottle Gourd	Adults	3.22	1.64×10^{-4}	5.48×10^{-4}	1.39×10^{-1}	3.69×10^{-1}
	Children	3.07	2.47×10^{-4}	8.22×10^{-4}	2.08×10^{-1}	5.55×10^{-1}
Cabbage	Adults	11.5	9.55×10^{-5}	2.16×10^{-4}	1.46×10^{-1}	3.98×10^{-1}
	Children	8.81	1.43×10^{-4}	3.25×10^{-4}	2.19×10^{-1}	5.97×10^{-1}
Cucumber	Adults	11.5	2.68×10^{-5}	2.93×10^{-4}	1.11×10^{-1}	4.14×10^{-1}
	Children	8.81	4.02×10^{-5}	4.40×10^{-4}	1.66×10^{-1}	6.21×10^{-1}
Green chili	Adults	19.8	1.74×10^{-4}	6.18×10^{-4}	8.79×10^{-2}	4.36×10^{-1}
	Children	17.4	2.61×10^{-4}	9.28×10^{-4}	1.32×10^{-1}	6.54×10^{-1}
Ladyfinger	Adults	16.2	3.15×10^{-5}	1.55×10^{-4}	1.02	4.42×10^{-1}
	Children	15.3	4.73×10^{-5}	2.32×10^{-4}	1.54	6.63×10^{-1}
Onion	Adults	30.2	3.93×10^{-5}	1.53×10^{-4}	0.05×10^{-2}	4.42×10^{-1}
	Children	27.7	5.89×10^{-5}	2.3×10^{-4}	8.62×10^{-2}	6.65×10^{-1}
Potato	Adults	12.1	5.69×10^{-5}	1.9×10^{-4}	1.37×10^{-1}	4.39×10^{-1}
	Children	12.3	8.54×10^{-5}	2.85×10^{-4}	0.20×10^{-1}	6.60×10^{-1}
Spinach	Adults	58.7	3.17×10^{-4}	1.06×10^{-3}	8.65×10^{-1}	7.28×10^{-1}
	Children	80.1	4.76×10^{-4}	1.58×10^{-3}	1.30	1.09
Tomato	Adults	17.3	4.39×10^{-3}	1.46×10^{-2}	2.64×10^{-1}	4.55×10^{-1}
	Children	15.4	6.60×10^{-4}	2.19×10^{-2}	3.96×10^{-1}	6.83×10^{-1}
Zucchini	Adults	13.0	1.78×10^{-4}	6.19×10^{-4}	5.40×10^{-1}	4.58×10^{-1}
	Children	12.4	2.67×10^{-4}	9.3×10^{-4}	8.10×10^{-1}	6.88×10^{-1}
HI	Adults	1.97×10^{2}	5.64×10^{-3}	1.916×10^{-2}	3.44	4.88
	Children	2.09×10^{2}	8.49×10^{-3}	2.88×10^{-2}	5.17	7.34

4.4.4.Health Risk Index

The health risk index of heavy metals via consumption of vegetables is used to assess the cancer risk of the exposed population.

4.3.1.1. Health Risk Index of Arsenic

The mean health risk index of arsenic is given in table 4.4. the value of the health risk index of all the vegetable samples was greater than 1 showing a high risk to both children and adults. The increasing trend of the health risk index of adults via vegetable consumption is bottle gourd > brinjal > cabbage > cucumber > potato > zucchini > ladyfinger > tomato > green chili > onion > spinach. In the case of children, the trend is as follows; bottlegourd > brinjal > cabbage > cucumber > potato > zucchini > tomato > ladyfinger > green chili > onion > spinach.

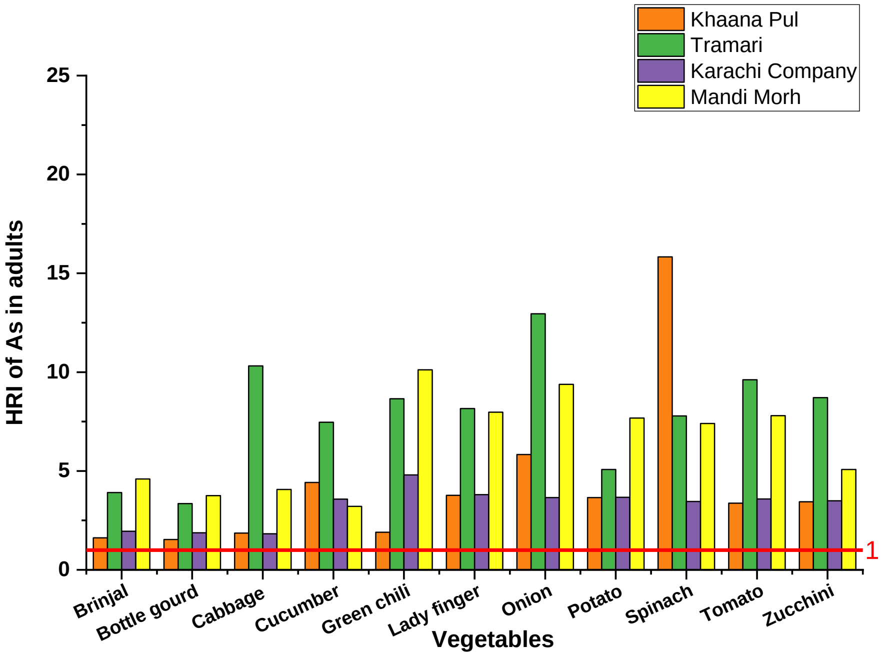

Fig. 4.38 HRI of Arsenic in adults via consumption of fresh vegetables

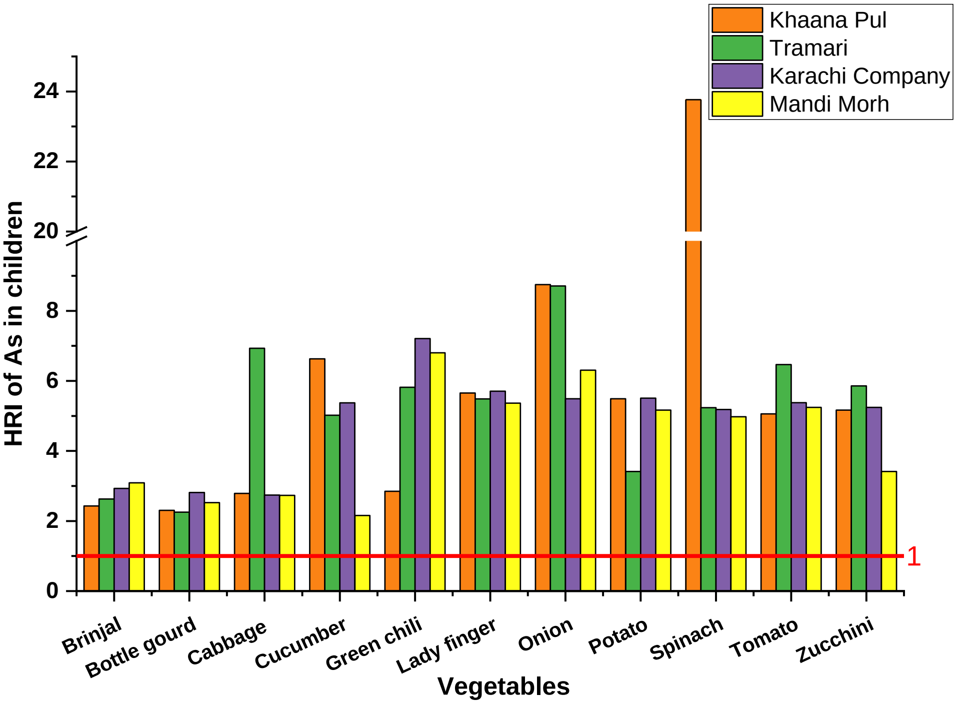

Fig. 4.39 HRI of Arsenic in adults via consumption of fresh vegetables

4.3.1.2. Health Risk Index of Cadmium

The mean health risk index of cadmium in adults and children is presented in table 4.4. The health risk index of cadmium is less than 1 showing no potential risk to the exposed population. The increasing trend of HRI in children and adults is as follows; cucumber > ladyfinger > onion > potato > brinjal > cabbage > green chili > zucchini > bottlegourd > spinach > tomato.

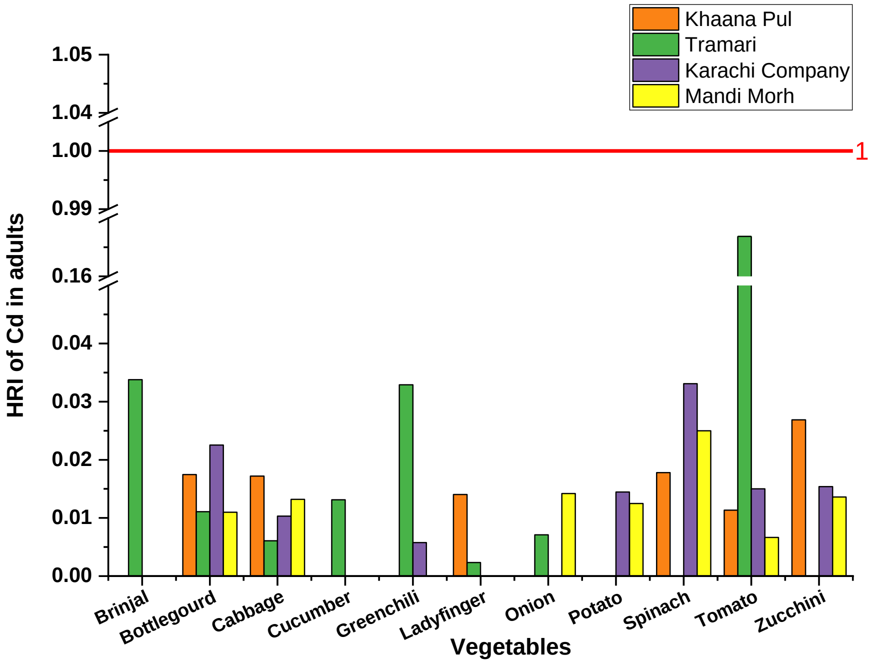

Fig. 4.40 HRI of Cadmium in adults via consumption of fresh vegetables

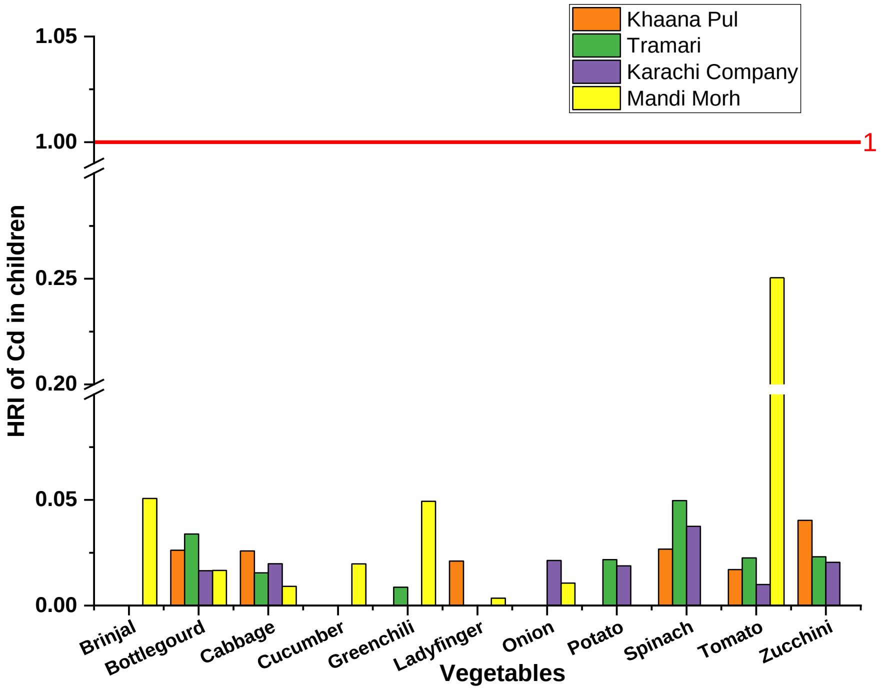

Fig. 4.41 HRI of cadmium in children via consumption of fresh vegetables

Chauhan et al. (2014) reported similar results in the population of Rewa MP, India consuming wastewater irrigated brinjal, bottle gourd, and ladyfinger. HRI <1 was observed via consumption of bottlegourd in the population of Gujranwala (Rizwan et al., (2017), Lahore (Khanum et al.,

2017) and Dera Ismail Khan (Ullah et al., 2022). Ali et al. (2021) in the population of Peshawar and Swat reported similar HRI of Cd, via consumption of ladyfinger, and tomato. Khan et al. (2013) reported similar HRI of Cd via the consumption of onion, potato, and ladyfinger in the population of Swat. HRI of Cd via consumption of potatoes was recorded to be less than 1 in the adult and children population of Gujranwala (Rizwan et al., 2016) and in the adult population of Lahore (Khanum et al., 2017). HRI of Spinach was found similar to the study conducted in the population of Dera Ismail Khan (Ullah et al., 2021), and in Gujranwala (Rizwan et al., 2016).

4.3.1.3. Health Risk Index of Chromium

The mean health risk index of chromium in adults and children is presented in table 4.4. The health risk index of chromium is less than 1 showing no potential risk to the exposed population. The increasing trend of HRI in children and adults is as follows; ladyfinger > potato > onion > cabbage > cucumber > green chili > brinjal > zucchini > bottle gourd > spinach > tomato.

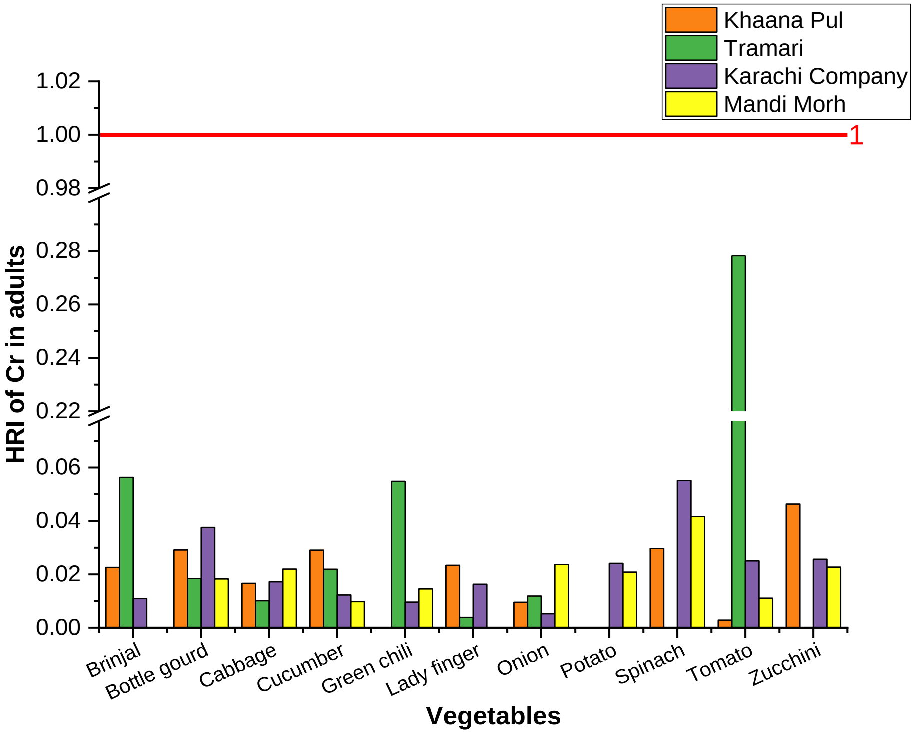

Fig. 4.42 HRI of chromium in adults via consumption of fresh vegetables

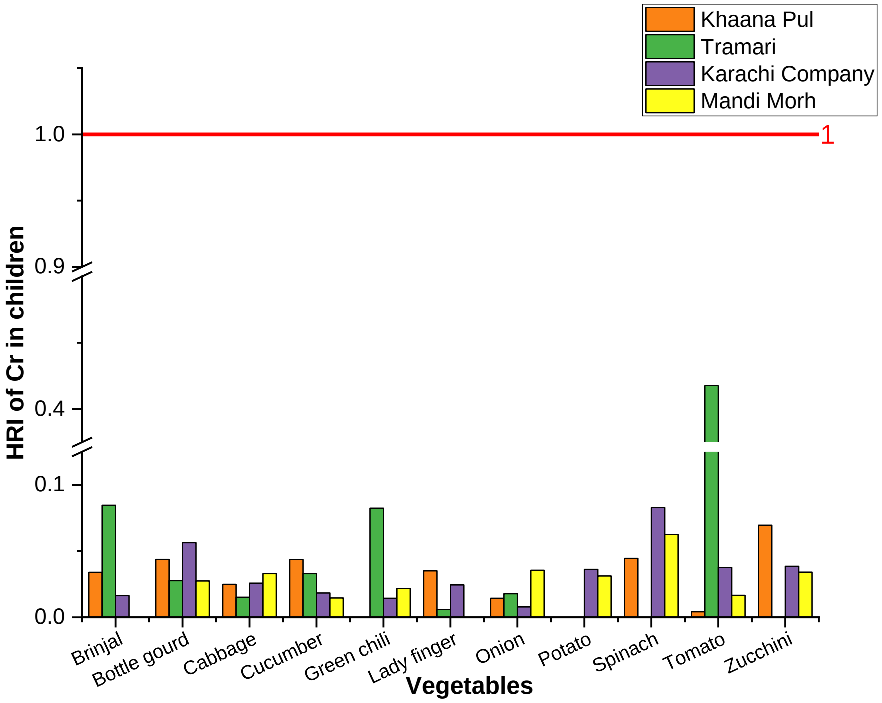

Fig. 4.43 HRI of chromium in children via consumption of fresh vegetables

Potato showed HRI of less than 1, showing no cancer risk. Similar results were reported by Khanum et al. (2017) in the adult population of Lahore. Ullah et al. (2022) in Dera Ismail Khan reported similar HRI via the consumption of spinach, and cabbage irrigated with freshwater. Similar HRI < 1 was also reported in the population of Gujranwala via the consumption of spinach, cabbage, and potato collected from an industrial site (Rizwan et al., 2016). Ali et al. (2021) reported HRI < 1 via the consumption of tomatoes and ladyfinger in the population of Peshawar. Khan et al. (2013) in the population of Swat also reported similar results via the consumption of ladyfinger, onion, tomato, and potato.

4.3.1.4. Health Risk Index of Lead

The mean health risk index of lead in adults and children via consumption of selected vegetables is shown in table 4.4. The health risk index of lead in both adults and children is less than 1, hence showing no risk to the exposed population. The increasing trend of HRI in adults and children is as follows; onion > green chili > brinjal > cucumber > bottle gourd > cabbage >potato > tomato > zucchini > ladyfinger > spinach. While for children it was onion > green chili > brinjal > cucumber > bottle gourd > cabbage > potato > tomato > zucchini > spinach ladyfinger.

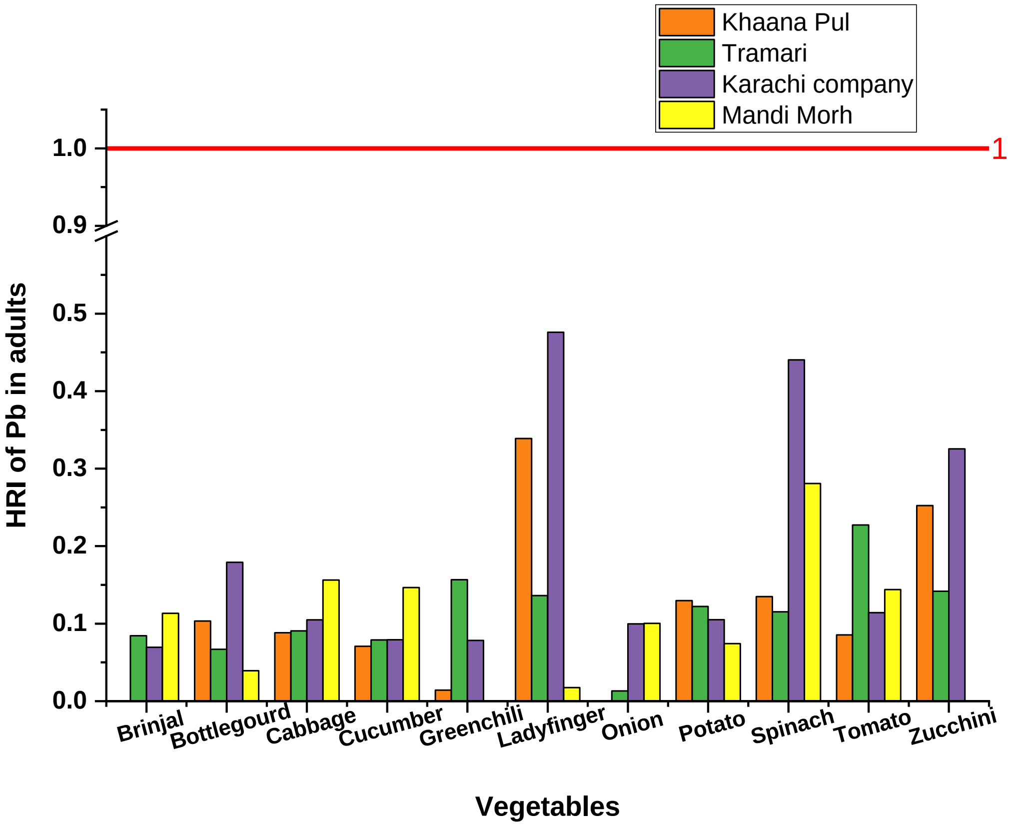

Fig. 4.44 HRI of lead in adults via consumption of fresh vegetables

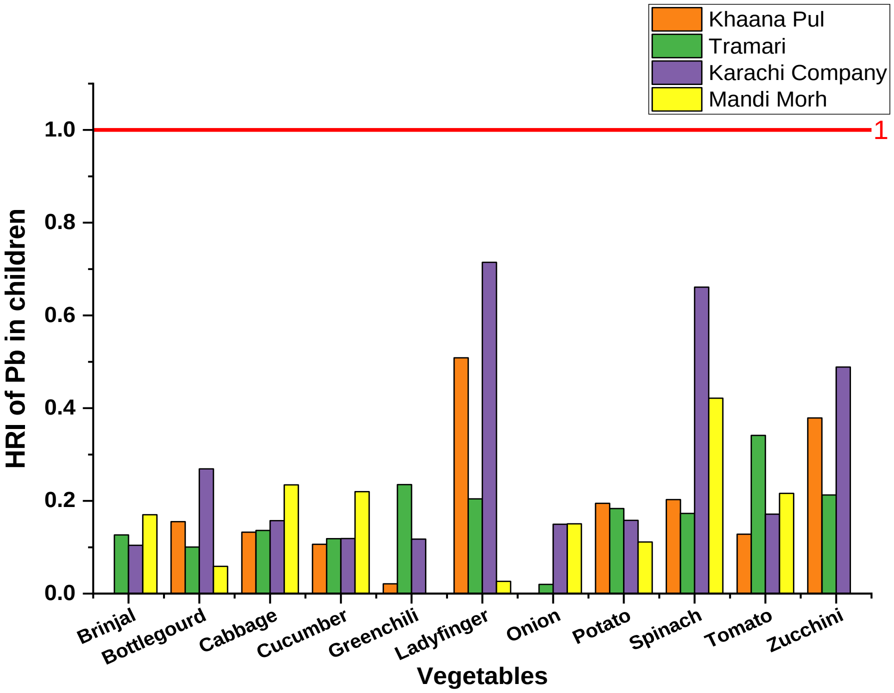

Fig. 4.45 HRI of Lead in children via consumption of fresh vegetables

Similar results were reported by Khanum et al. (2017) in the population of Lahore consuming wastewater-irrigated potatoes, and cabbage. In the population of Dera Ismail Khan, HRI was reported similar to the consumption of spinach and cabbage (Ullah et al., 2021). HRI was reported to be similar in a population of Gujranwala consuming spinach, cabbage, and potato collected from industrial sites (Rizwan et al., 2016). Similar results were reported in the population of Peshawar via consumption of tomatoes irrigated with wastewater, while ladyfinger showed HRI > 1(Ali et al., 2021).

Chauhan et al. (2014) observed similar HRI in the population of Rewa, MP, India, via consumption of wastewater-irrigated brinjal, tomatoes, and ladyfinger, while the population consuming spinach and cabbage at another site showed HRI greater than 1 posing cancer risk to the exposed adult and children population.

4.3.1.5. Health Risk Index of Zinc

The mean HRI of Zn is given in table 4.5. The HRI of adults and children via consumption of fresh vegetables is greater than 1 showing potential risk to human health. The increasing trend of HRI in adults and children are as follows; brinjal > bottle gourd > cabbage > cucumber > green chili > potato > ladyfinger > onion > tomato > zucchini > spinach.

Khan et al. (2018) in the population of Swat reported HRI < 1, via the consumption of onion, tomato, ladyfinger, and potato. HRI was reported to be within the safe range in the population of Gujranwala via the consumption of Potatoes, cabbage, and spinach (Rizwan et al., 2016). Ullah et al. (2022) reported HRI < 1 in the population of Dera Ismail Khan via the consumption of spinach and cabbage. Ali et al. (2021) reported HRI < 1 in the population of Peshawar via the consumption of tomatoes and ladyfinger.

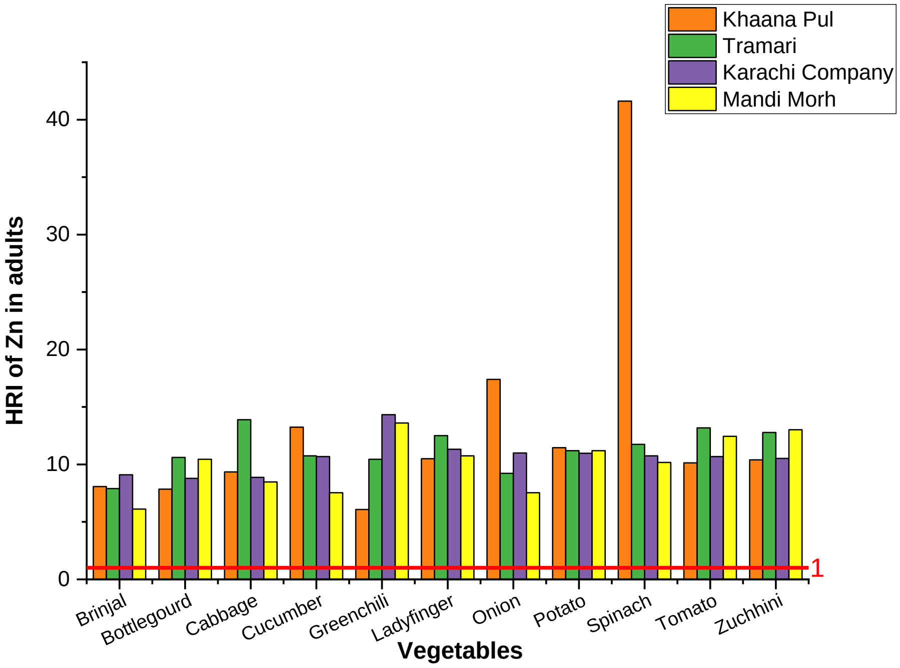

Fig. 4.46 HRI of Zinc in adults via consumption of fresh vegetables

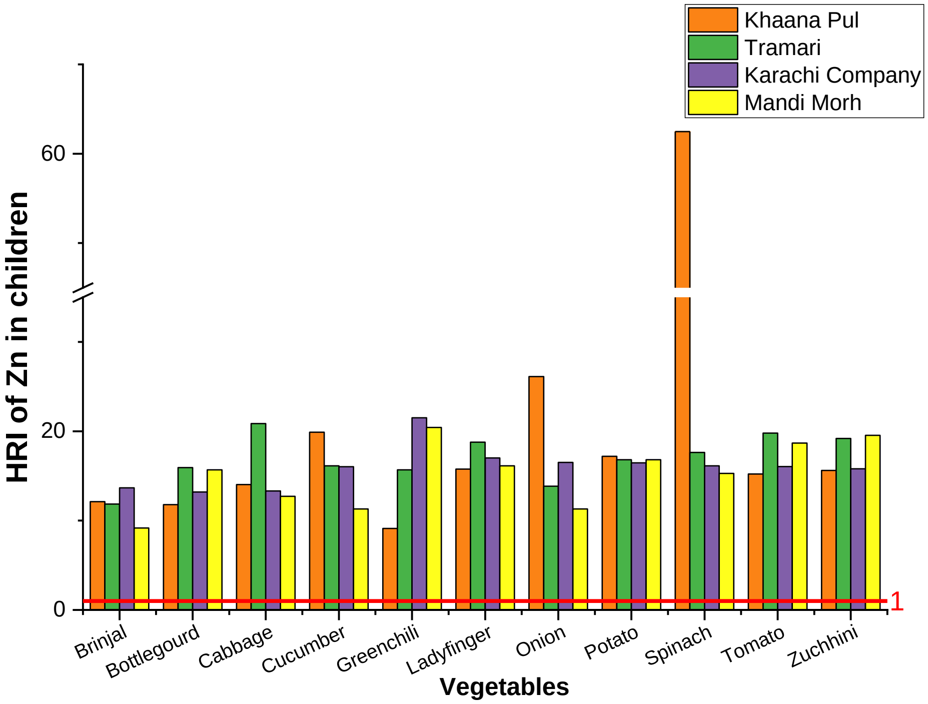

Fig. 4.47 HRI of Zinc in children via consumption of fresh vegetables

Table 4.5. Mean HRI of selected HMs via consumption of selected fresh vegetables in adults and children

Vegetables		As	Cd	Cr	Pb	Zn
Brinjal	Adults	3.02	8.45×10^{-3}	2.24×10^{-2}	6.68×10^{-2}	7.80
	Children	2.77	1.27×10^{-2}	3.37×10^{-2}	1.00×10^{-1}	11.7
Bottle Gourd	Adults	2.63	1.55×10^{-3}	2.58×10^{-2}	9.72×10^{-2}	9.43
	Children	2.47	2.33×10^{-2}	3.88×10^{-2}	1.46×10^{-1}	14.1
Cabbage	Adults	4.51	1.17×10^{-2}	1.65×10^{-2}	1.10×10^{-2}	10.1
	Children	3.79	1.76×10^{-2}	2.47×10^{-2}	1.65×10^{-1}	15.2
Cucumber	Adults	4.67	3.28×10^{-3}	1.82×10^{-2}	9.39×10^{-2}	10.5
	Children	4.79	4.93×10^{-3}	2.74×10^{-2}	1.41×10^{-1}	15.8
Green chili	Adults	6.37	9.67×10^{-3}	1.97×10^{-2}	6.23×10^{-2}	11.1
	Children	5.67	1.45×10^{-2}	2.96×10^{-2}	9.35×10^{-2}	16.7
Ladyfinger	Adults	5.93	4.09×10^{-3}	1.09×10^{-2}	2.42×10^{-2}	11.3
	Children	5.55	6.15×10^{-3}	1.63×10^{-2}	3.63×10^{-1}	16.9
Onion	Adults	7.95	5.33×10^{-3}	1.26×10^{-2}	5.33×10^{-2}	11.3
	Children	7.31	8.00×10^{-3}	1.89×10^{-2}	8.00×10^{-2}	16.9
Potato	Adults	5.02	6.74×10^{-3}	1.12×10^{-2}	1.08×10^{-1}	11.2
	Children	4.89	1.01×10^{-2}	1.69×10^{-2}	1.62×10^{-2}	16.8
Spinach	Adults	8.62	1.90×10^{-2}	3.16×10^{-2}	2.43×10^{-1}	18.6
	Children	9.79	2.85×10^{-2}	4.75×10^{-2}	3.64×10^{-1}	27.9
Tomato	Adults	6.09	4.99×10^{-2}	7.93×10^{-2}	1.43×10^{-1}	11.6
	Children	5.53	7.50×10^{-2}	1.19×10^{-2}	$2.14 \times 10^{`1}$	17.4
Zucchini	Adults	5.18	1.40×10^{-2}	2.36×10^{-2}	1.80×10^{-1}	11.7
	Children	4.92	2.10×10^{-2}	3.55×10^{-2}	2.70×10^{-1}	17.5

4.4.5.Target Carcinogen Risk

4.4.5.1. Target Carcinogen risk of As

The target carcinogenic risk of arsenic in both children and adults via consumption of fresh vegetables was beyond the range of 10^{-4} and 10^{-6}, which shows that there is a high risk of developing cancer. The mean TCR values are given in table 4.6. The increasing trend of Target carcinogen risk in adults and children via fresh vegetable consumption is as follows; bottle gourd> brinjal > cabbage > cucumber > potato > zucchini > tomato > ladyfinger >green chili > onion > spinach.

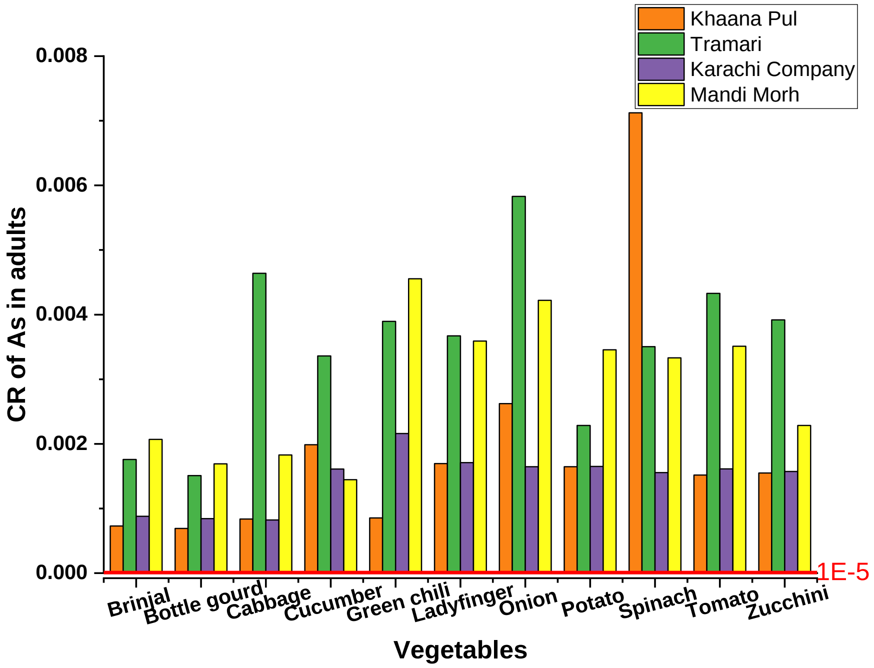

Fig 4.48 Target Carcinogen Risk of Arsenic in adults via consumption of fresh vegetables

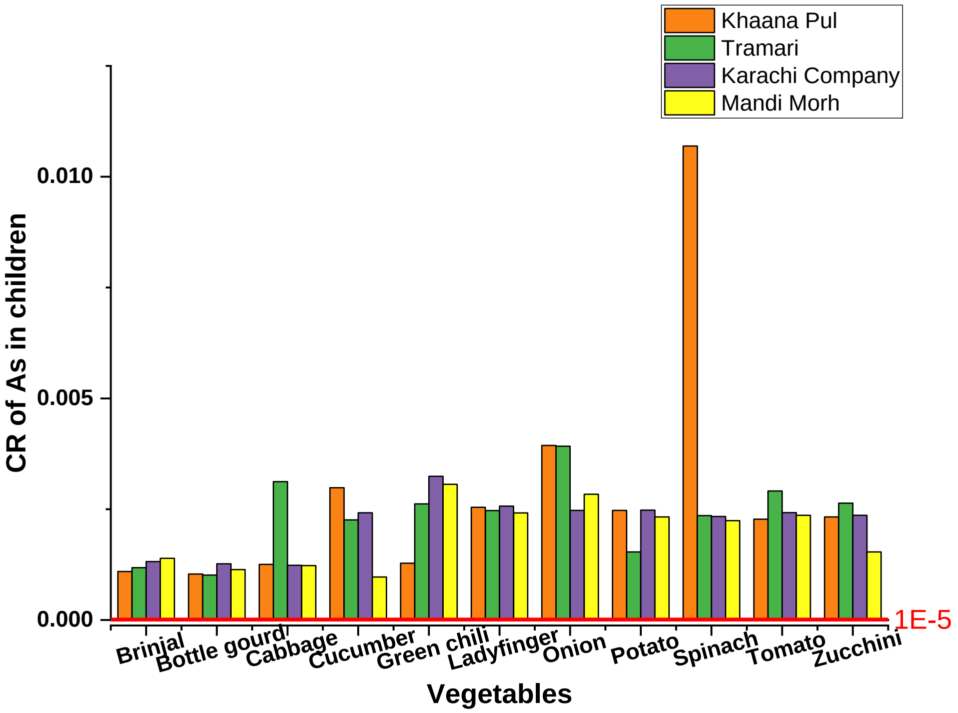

Fig 4.49 Target Carcinogen Risk of Arsenic in children via consumption of fresh vegetables

A similar TCR was seen in the Northeast Nigerian adult population, (Akan et al., 2021) consuming tomato, spinach, cabbage, and onion. Tomato, spinach, bottle gourd, brinjal, and potato intake from commercial, noncommercial, and market sources were used to detect comparable TCR in the population of Bangladesh (Haque et al., 2021). A similar TCR of As was reported in Pakistan's district Kasur by eating brinjal, cabbage, tomato, and ladyfinger (Ashraf el., 2021).

4.4.5.2. Target Carcinogen risk of Cd

The target carcinogenic risk of chromium in both children and adults via consumption of fresh vegetables was beyond the range of 10^{-6}, which shows that there is a high risk of developing cancer, except for ladyfinger from Tramari (Rawalpindi). The mean TCR values are given in table 4.6. The increasing trend of Target carcinogen risk in adults and children via fresh vegetable consumption is as follows; ladyfinger > onion > potato > brinjal > cucumber >green chili > cabbage > zucchini > bottle gourd > spinach > tomato.

Similar TCR results for Cd via consumption of brinjal, cabbage, tomato, and ladyfinger were reported in the population of district Kasur, Pakistan (Ashraf et al., 2021). In the northeast

Nigerian population, TCR was reported similar, via the consumption of cabbage, onion, tomato, and spinach (Akan et al., 2021).

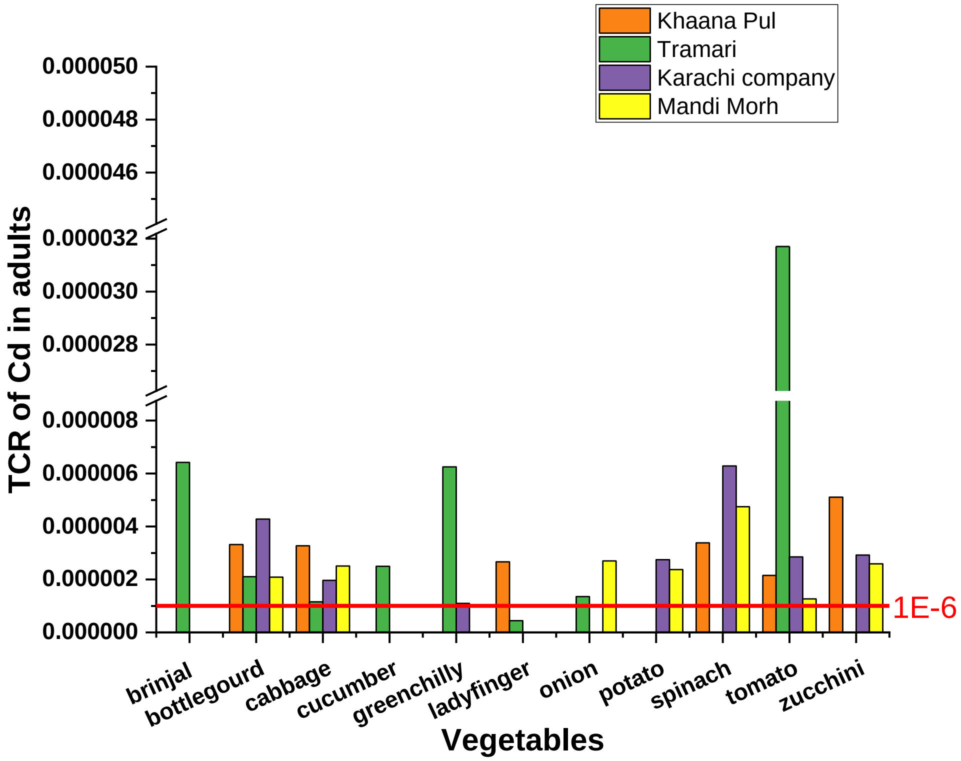

Fig. 4.50 Target Carcinogen Risk of Cadmium in adults via consumption of fresh vegetables

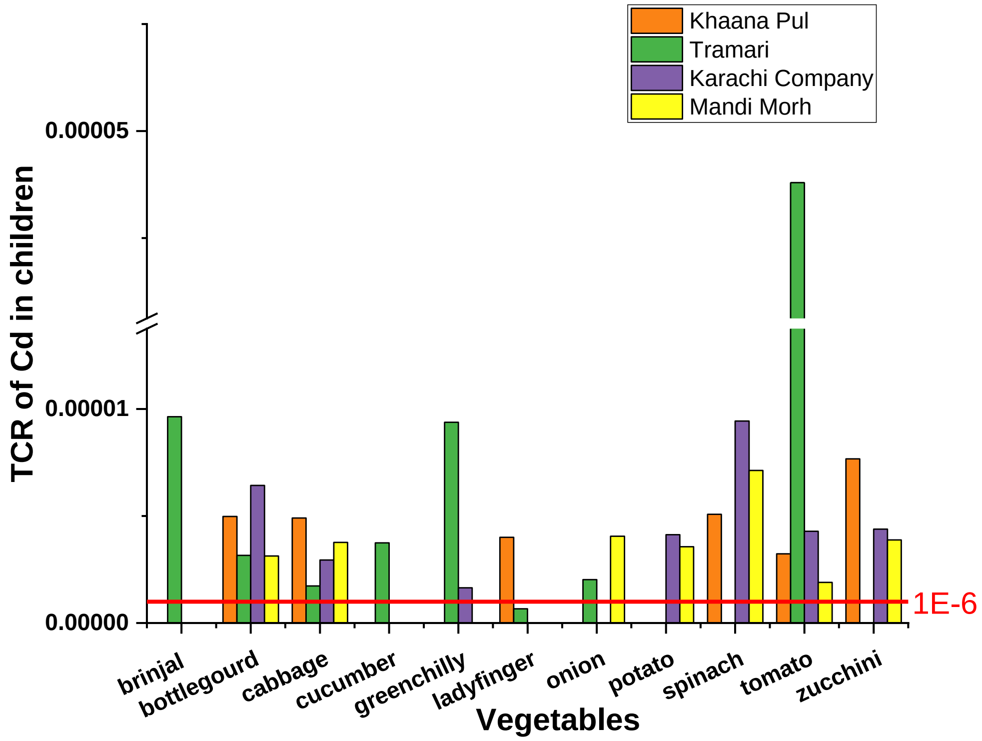

Fig. 4.51 Target Carcinogen Risk of Cadmium in children via consumption of fresh vegetables

4.4.5.3. Target Carcinogen risk of Cr

The target carcinogenic risk of chromium in both children and adults via consumption of fresh vegetables was beyond the range of 10^{-6}, which shows that there is a high risk of developing cancer. The mean TCR values are given in table 4.6. The increasing trend of target carcinogen risk in adults and children via fresh vegetable consumption is as follows; ladyfinger > potato > onion > cabbage >cucumber > green chili >brinjal > zucchini > bottle gourd > spinach > tomato. TCR of Cr via consumption of cabbage, onion, tomato, and spinach in the population of Northeast Nigeria was reported to be similar to the present study (Akan et al., 2021). District of Kasur, Pakistan showed similar TCR of Cr via consumption of brinjal, cabbage, tomato, and ladyfinger.

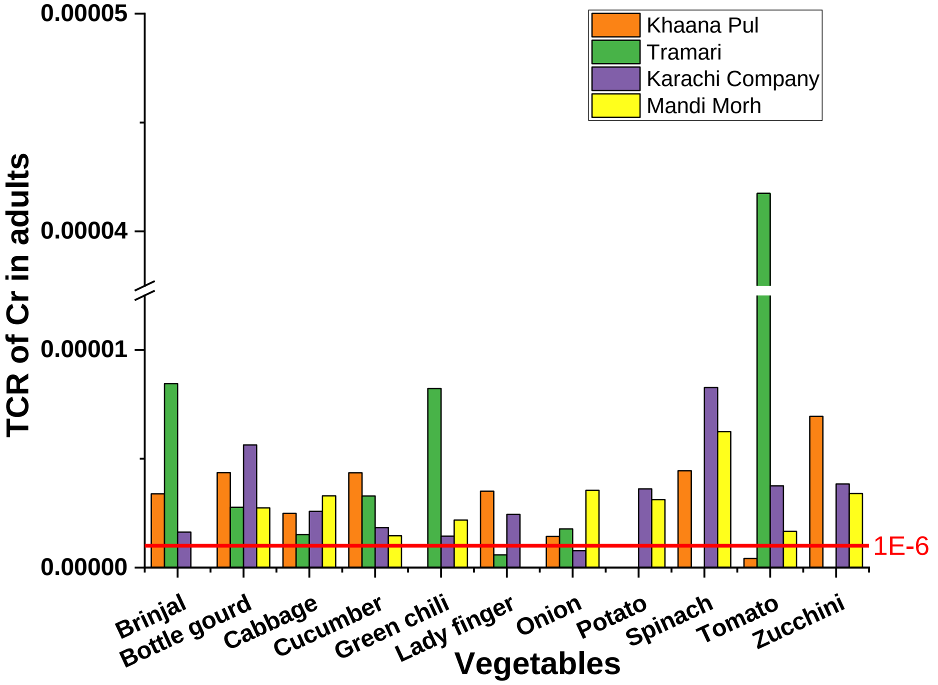

Fig. 4.52 Target Carcinogen Risk of Chromium in adults via consumption of fresh vegetables

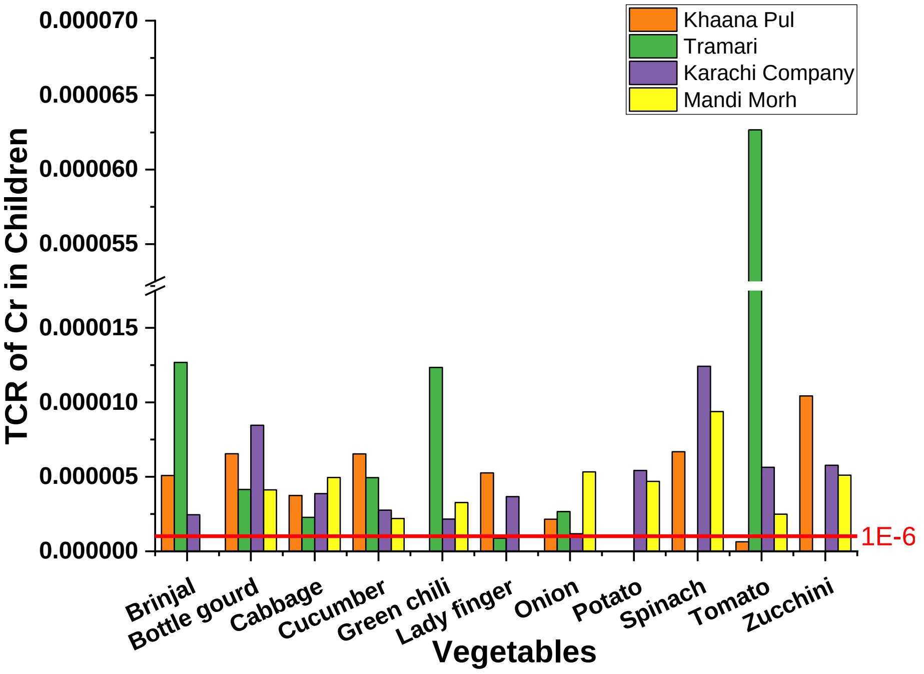

Fig. 4.53 Target Carcinogen Risk of Cadmium in children via consumption of fresh vegetables

4.4.5.4. Target Carcinogen risk of Pb

The target carcinogen risk of Pb via consumption of selected vegetables was beyond the safe limits of 10^{-6}, except for green chili, ladyfinger, and onion samples collected from Khaana pul, Mandi morh, and Tramari. Mean TCR values are presented in table 4.5. Increasing risk via consumption of selected vegetables was in the order of onion > green chili > brinjal >cucumber > bottlegourd >potato > cabbage > tomato > zucchini > ladyfinger > spinach. Ashraf et al. (2021) also reported similar results related to the consumption of brinjal, cabbage, tomatoes, and ladyfinger irrigated with wastewater in the district Kasur. This continuous consumption of these vegetables may cause the probability of developing cancer in the exposed population. TCR of Pb in the population of Bangladesh was reported similar via consumption of brinjal, bottlegourd, potatoes, tomatoes, and spinach from markets, industrial and non-industrial sites (Haque et al., 2021). Akan et al. (2021) in Northeast Nigeria and Haque et al. (2021) in Bangladesh reported that consumption of cabbage, onion, tomatoes, and spinach contaminated with lead, may increase the probability of developing cancer in the exposed population.

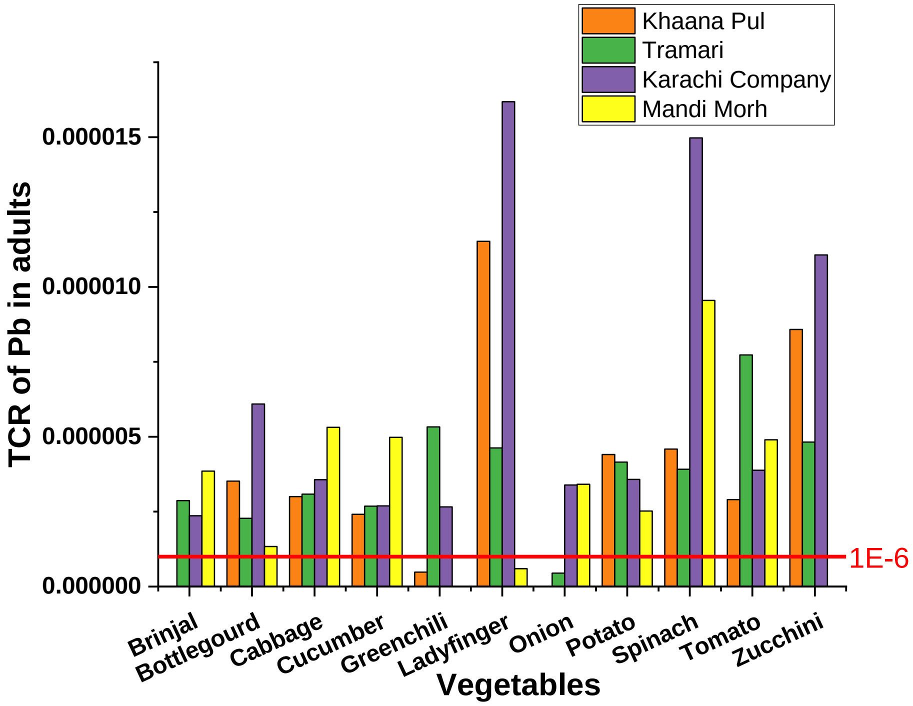

Fig. 4.54 Target Carcinogen Risk of Lead in adults via consumption of fresh vegetables

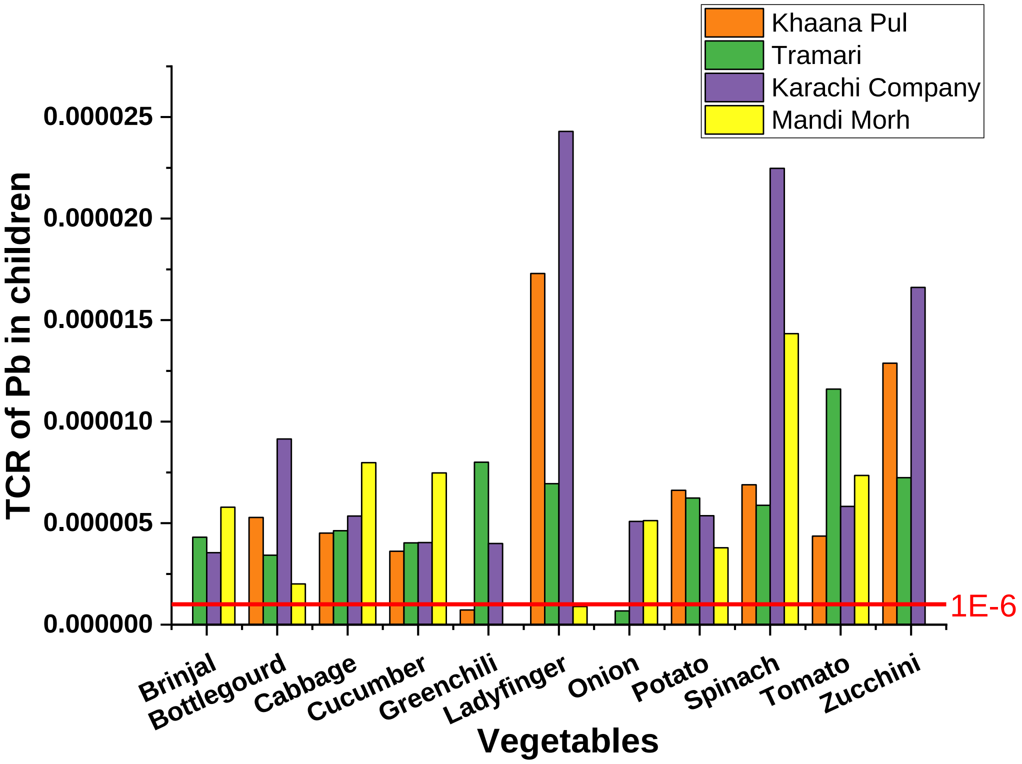

Fig. 4.55 Target Carcinogen Risk of Lead in Children via consumption of fresh vegetables

Table 4.6. Mean Target carcinogen risk of adults and children via consumption of fresh vegetables contaminated with heavy metals

Metals		As	Cd	Cr	Pb	Zn
Brinjal	Adults	1.36×10^{-3}	1.60×10^{-6}	3.37×10^{-6}	2.27×10^{-6}	--
	Children	1.25×10^{-3}	2.41×10^{-6}	5.06×10^{-6}	3.41×10^{-6}	
Bottle gourd	Adults	1.18×10^{-3}	2.95×10^{-6}	3.88×10^{-6}	3.31×10^{-6}	--
	Children	1.11×10^{-3}	4.42×10^{-6}	5.82×10^{-6}	4.96×10^{-6}	
Cabbage	Adults	2.03×10^{-3}	2.22×10^{-6}	2.47×10^{-6}	3.74×10^{-6}	--
	Children	1.71×10^{-3}	3.34×10^{-6}	3.71×10^{-6}	5.62×10^{-6}	
Cucumber	Adults	2.10×10^{-3}	6.23×10^{-7}	2.74×10^{-6}	3.19×10^{-6}	--
	Children	2.16×10^{-3}	9.36×10^{-7}	4.12×10^{-6}	4.79×10^{-6}	
Green chili	Adults	2.87×10^{-3}	1.84×10^{-6}	2.96×10^{-6}	2.12×10^{-6}	--
	Children	2.55×10^{-3}	2.76×10^{-6}	4.45×10^{-6}	3.18×10^{-6}	
Lady finger	Adults	2.67×10^{-3}	7.78×10^{-7}	1.63×10^{-6}	8.23×10^{-6}	--
	Children	2.50×10^{-3}	1.17×10^{-6}	2.45×10^{-6}	1.24×10^{-5}	
Onion	Adults	3.58×10^{-3}	1.01×10^{-6}	1.89×10^{-6}	1.81×10^{-6}	--
	Children	3.23×10^{-3}	1.52×10^{-6}	2.83×10^{-6}	2.72×10^{-6}	
Potato	Adults	2.26×10^{-3}	1.28×10^{-6}	1.68×10^{-6}	3.67×10^{-6}	--
	Children	2.20×10^{-3}	1.92×10^{-6}	2.53×10^{-6}	5.5×10^{-6}	
Spinach	Adults	3.88×10^{-3}	3.60×10^{-6}	4.74×10^{-6}	8.26×10^{-6}	--
	Children	4.40×10^{-3}	5.41×10^{-6}	7.12×10^{-6}	1.24×10^{-5}	
Tomato	Adults	2.74×10^{-3}	9.49×10^{-6}	1.19×10^{-5}	4.85×10^{-6}	--
	Children	2.49×10^{-3}	1.42×10^{-5}	1.78×10^{-5}	7.29×10^{-6}	
Zucchini	Adults	2.33×10^{-3}	2.65×10^{-6}	3.55×10^{-6}	6.12×10^{-6}	--
	Children	1.25×10^{-3}	3.98×10^{-6}	5.33×10^{-6}	9.18×10^{-6}	--

Chapter 5

Conclusions and Recommendations

5.1. Conclusions

Results of the study show the presence of heavy metals in vegetables sold in different markets of Islamabad and Rawalpindi. It can be related to the use of wastewater for irrigation purposes that could negatively affect human health. Heavy metals such as As and Pb concentration in all the selected vegetables were exceeding permissible limits. In the case of Cd and Cr, tomato samples collected from Tramari exceeded the permissible limit, while Zn concentration was also within safe limits except for spinach from Khaana Pul. The estimated daily intake of all the five metals via consumption of selected vegetables was within the safe ranges.

THQ values of arsenic were exceeding safe limits, while all other heavy metals such as Cd, Cr, Pb, and Zn were within the safe limits, posing no non-cancer risk to the exposed population. The health risk index (HRI) of arsenic and zinc via consumption of selected vegetables was found to be greater than 1, posing a cancer risk to the exposed adult and children population. The increasing risk of cancer in the exposed population via ingestion of As in Islamabad and Rawalpindi was: Karachi company > Khaana pul > Mandi morh > Tramari, while increasing order of Zn was Mandi morh > Karachi company > Tramari > Khaana pul. The cancer risk due to ingestion of Cd, Cr, and Pb in the exposed adult and children population was lower than safe limits. Cadmium and Cr were in increasing order of Mandi Morh > Khaana Pul > Karachi company > Tramari. An increasing trend of Pb was observed as follows: Morh mandi > Tramari > Khaana pul > Karachi company.

Target carcinogen risk (TCR) of As, Cd, Cr, and Pb via ingestion of selected vegetables was higher than the safe limits. The increasing order of TCR of Cd, Cr, and Pb at different sites was following Mandi morh > Khaana pul> Karachi company > Tramari.

Hence the result of the study revealed that there is a high risk of cancer effects in the adult and children population as compared to non-cancer health effects. Consumption of these selected vegetables for a long time may increase the chances of developing cancer in exposed adults and children population.

5.2. Recommendations

Considering the risks highlighted in the results of the present study, there is a need for remediative or mitigative actions. Some of the suggestions are as follows:

- Toxic heavy metals should be removed from the water using appropriate measures.
- If using wastewater is required, treating it is the most crucial stage. Every city should have a wastewater treatment plant so that wastewater can be cleaned and disinfected before being disposed of into the environment.
- Once heavy metals (toxic metals) have entered the soil system, it is exceedingly expensive and challenging to remove them using various ways. The remediation of contaminated soils should also be focused on avoiding health risks from heavy metal exposure through the food chain.

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