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Authors' Affiliation:
Department of Botany, Faculty of Life Sciences, University of Okara - Pakistan

***Corresponding Author:**
Saba Younas
Email:
sabayounas34@gmail.com

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Assessment of heavy metal contamination in vegetables collected from selected localities of Okara, Pakistan

Saba Younas, Syeda Anjum Tahira, Muhammad Umer Farooq*

Abstract

Background: Human health is prone to heavy metals especially which become part of food chain by any means. Previously, no extensive nutritional studies are conducted on local food products and grains. The research work was carried out to observe level of heavy metals in vegetables sold or consumed in different localities of Okara city, Pakistan.

Methods: The concentration of heavy metals Nickel, Cadmium, Cobalt, Copper and Chromium in ten different types of vegetables collected from selected sites survey were analyzed using tri-acid method for atomic absorption spectrometer.

Results: The results showed that concentrations of all analyzed heavy metals were significantly ($P \leqslant 0.05$) different, except values of copper in collected vegetable samples. The average concentration ranged from 5.4 – 44.06 ppm of Cobalt, 5.7 – 9.63 ppm of Copper, 4.49 – 11.13 ppm of Cadmium, 4.59 – 33.77 ppm of Chromium and 8.58 – 13.68 ppm of Nickel. Mean concentrations of metals were found in following sequence Copper < Cadmium < Nickel < Chromium < Cobalt.

Conclusion: It was concluded that vegetables can accumulate high concentrations of Cobalt and Chromium while Nickel, Cadmium and Copper concentrates in low amount, from which level of chromium and cadmium surpasses the permissible limits by FAO/WHO especially in underground vegetables (Onion and Radish), which could be the cause of serious health issues. Hence, monitoring and assessment of contaminants in vegetables are periodically needed and public safety measures should be imposed.

Introduction

Heavy metals are the elements with relative density > 4g/cm3 [1] including; Chromium (Cr), Cobalt (Co), Copper (Cu), Cadmium (Cd), Lead (Pb), Nickel (Ni), Zinc (Zn), Arsenic (As), Iron (Fe) and elements of group of Platinum [2]. Heavy metal contamination of vegetables can't be disesteem as they are important part of our diet. Vegetables are part of the basic diet across the world, as they produce and are rich amount of minerals, carbohydrates, vitamins, calcium, iron, fibers and some antioxidants which help humans to protect them from diseases. Vegetable consumption by humans is one of the ways to get essential nutrients. However, vegetables may also accumulate heavy metals through foliar or root uptake which can be affected by environment. Whereas heavy metal contaminated food intake shows hazardous effects on human health. As we know that for food quality assurance, heavy metal contamination is an important aspect [3,4].

In last few years, understanding about food safety has stirred research related to the risk of consuming metals contaminated foods [5,6]. As urbanization and industrialization increases level of heavy metal contamination in our environment. [7]. Heavy metals are non-biodegradable pollutants which are deposited on surface of vegetable during production, transport and marketing and then absorbed by them into their tissues. They may be found in our food chain with less risk to metal toxicity on human and animal health in comparison with plants, but they have capability to concentrate heavy metals in them. Soil contamination caused by environmental pollution and crop irrigation with waste water plays important role in mixing of heavy metals in agricultural areas [8]. Soil irrigated with wastewater enriched with pollutants when they leached out of soil by plants in waterways and redistribution of heavy metals occur after plants death and decay.

Long term irrigation with wastewater may lead to biomagnification of heavy metals in plants. Metals uptake by plants from soil depends on factors like solubility, fertilizers, plant growth stages and also soil [9]. As we know, different plants have various pathways to uptake heavy metals. Here are some reports on heavy metals in vegetables examined in different parts of Asia including Bangladesh [10], China [11] and India [12].

For example, Gupta and his co-researcher [12] analyzed highest concentration of cadmium 17.8 mg/kg and of lead 57.6 mg/kg in waste water irrigated radish collected from Titagarh, India. Similarly, food survey report by Chen and colleagues[13] in Wuzhou, China found the content of Pb were 7% in leafy vegetables and 18% in root vegetables more than the Chinese national standard that are 0.3 mg/kg of Pb.

According to report given by Wenzel and Jackwer in 1999 [14] some plant species accumulating heavy metals, causing risks to health of humans after being consumed as food. Fumes exhausted from cars may also be a source of food contamination [15]. Mostly contaminated soils are found at landfills (especially that have industrial waste), gardens that used different insecticides having some metals as their active ingredient. Heavy metal causes serious health problems because of ability to store in various parts of our body, and is becoming worse day by day in countries like Pakistan and India [16].

High concentration of Co, Cu Cd and Pb in vegetables can be the cause of cancer, renal failure and hypertension. Whereas excess of Cd and Pb in food also causes some kidney and bone diseases [17]. Iron deficiency and destruction of membranes induced by toxicity of copper metal [18]. Heavy metals also increase blood pressure in humans. As vegetables are essential part of our daily food, FAO and WHO gives provisional tolerable weekly intake of metals in vegetables. However, there is very little data on metal concentration in vegetables. Thus, periodic monitoring of metal level in our food like vegetables is needed and it's important to ensure that our food is pollutant free.

In this research work, vegetables mostly consumed by locals of Okara city were selected, including carrot (*Daucus carota*), potato (*Solanum tuberosum*), Radish (*Raphanus sativus*), Onion (*Allium cepa*), Spinach (*Spinacia oleracea*), Turnip (*Brassica napus*), Mustard (*Brassica campestris*), Chenopodium (*Chenopodium album*), Coriander (*Coriandrum sativum*), Moongre (*Raphanus sativus*), and Spring onion (*Allium ampeloprasum*). In addition, coriander, spring onion, onion, carrot and radish were chosen due to large consumption in diet for by locals of Okara, potato as a source of Cd, and Spinach as wastewater irrigation is major practice for its growth. The aim of the study was comparison of heavy metals concentrations in vegetables to access the risk on health of consumers.

Methods

Study Area

Okara is 500m above the sea level and climate is hottest in May and June with maximum temperature of 44°C while coldest in January with minimum temperature of 2°C. 200mm is average annual rainfall of Okara. The city is located between latitudes 30.81°48' of North and longitude 73.45°27' of East and is 127 km away from Lahore and 100 km from Faisalabad. Okara city was selected as case study area. Project was aimed to evaluate metal contamination, if any, in vegetables consumed by local population of the area. Study samples were collected from farmer`s market (Suzi Mandi), local vegetable/fruit shops and from some fields or farms situated in vicinity of Okara during September 2016 to April 2017 i.e., the winter season.

Collection of Samples

Samples of ten available and mostly consumed fresh vegetables by locals of the area were collected from different sites. The concentration of heavy metals nickel, cadmium, cobalt, copper and chromium in vegetables i.e., Radish, Carrot, Potato, Onion, Spinach, Turnip, Mustard, Chenopodium, Coriander and Spring onion were analyzed using AAS. Five samples of each vegetable from each site were collected and packed in clean labeled polyethylene. The list of vegetables collected is given in Table 1 below six of which were underground vegetables and four are leafy vegetables.

Vernacular name	English name	Scientific name	Family
Underground vegetables			
Muli	Radish	*Raphanus sativus*-L.	Brassicaceae
Gajr	Carrot	*Daucus carota*- L.	Apiaceae
Saljm	Turnip	*Brassica napus* L.	Brassicaceae
Alu	Potato	*Solanum tuberosum* L.	Solanaceae
Payaz	Onion	*Allium cepa*	Amaryllidaceae
Sbz Payaz	Spring Onion	*Allium ampeloprasum* L.	Alliaceae
Leafy vegetables			
Palak	Spinach	*Spinacia oleracea* L.	Amaranthaceae
Sag	Mustard	*Brassica campestris* L.	Brassicaceae
Bathoo	Chenopodium	*Chenopodium album*	Amaranthaceae
Dhaniya	Coriander	*Coriandrum sativum*	Umbellifers

Table 1: Description of Vegetables collected from different localities of Okara.

Pretreatment of samples

All the collected samples were cleansed with water thoroughly many times to remove dust or any other contamination and again rinsed with distilled water about 2 to 3 times. After washing bruised, non-edible and rotten parts of samples were removed and were stored in labeled polyethylene packs till further processing.

Preparation of samples for metal analysis

All pretreated vegetable samples were sliced into small, uniform pieces with stainless steel knife and dried first in air to remove moisture then in Oven at 60 to 75°C until constant weight was attained. After drying they were grounded into powder using Pestle-mortar and homogenized by sieving through 2 mm mesh [2].

Acid digestion and heavy metal analysis

All the required apparatus, Glassware and containers were washed with distilled water followed by soaking in 10% HNO_3 for 24 hours to remove all contaminants. Homogenized preprocessed samples were digested using a method described by Allen and his colleagues in 1986. According to the procedure 15ml of triple-acid mixture (70% HNO_3, 70% H_2SO_4, & 65% $HClO_4$) was added in 250 ml titration flask having 1g sample of vegetable. After addition mixture was digested at 80°C to obtain transparent solution. Which was filtrated and filtrate was taken in 50ml volumetric flask and diluted with distilled water to maintain the volume up-to 50ml of solution [19]. The levels of heavy metals (Cu, Cr, Co, Ni and Cd) in all digested solutions were analyzed with Atomic Absorption Spectrometer (Perkin Elmer, AAS-analyst 400).

Data & Statistical analysis

Contents of heavy metals in vegetables were compared with corresponding permissible levels given by Food and Agriculture Organization (FAO) and World Health Organization (WHO). All the calculations of data were processed by using Microsoft Excel and Statistix 8.1® (Analytical Software, Tallahassee, USA). Results were expressed as mean of replicates with different analysis and samples means.

Results

A perusal of ANOVA in Table 2 showed heavy metal concentration interaction with sites of analyzed samples. Thus the probability showed that interaction between analyzed heavy metals in all samples was highly significant ($P \leqslant 0.05$) except the interaction between Copper and samples that was non-significant ($P \geqslant 0.05$). Similar to that F statistics showed linear relation between all independent variable, metal concentrations and dependent variables except with copper that showed a non-linear relation.

A contrast of heavy metals levels in collected samples of vegetables are presented in Figure 1. Results showed that heavy metal concentrations in all examined vegetables ranged from 5.4 – 44.06 ppm of Cobalt, 5.7 – 9.63 ppm of copper, 4.49 – 11.13 ppm of Cadmium, 4.59 – 33.77 ppm of Chromium and 8.58 – 13.68 ppm of Nickel.

A comparison of all selected vegetables along with collection sites (not published in this study), showed that mean of nickel and chromium concentration in all the samples collected from sampling Site4 was lowest and highest value of nickel analyzed in samples collected from Site1. Similarly, analysis showed that mean of cobalt and cadmium concentration in all the samples collected from Site4 was lowest but highest in samples collected from Site2. While in difference, copper level in all samples collected from Site1 was lowest and highest in samples collected from sampling Site3.

Results showed that different samples collected from selected sites contain different levels of metals. Thus according to Table 3, Carrot, Potato, Onion, Spinach and Chenopodium collected from Site3 contained highest concentration of nickel. Nickel level in Turnip and Coriander collected from Site3 was high. While highest concentration of copper in Radish, Potato, Onion, Spinach, Chenopodium and Spring onion has

-		Co		Cr		Cu		Cd		Ni	
SOV	Df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Sites	3	2.15^{NS}	0.09	7.37**	0.00	0.37^{NS}	0.78	2.05^{NS}	0.10	3.90**	0.01
Samples	9	2.67**	0.01	24.81**	0.00	1.35^{NS}	0.22	6.02**	0.00	4.09**	0.00
Sites* Samples	27	2.82**	0.00	13.93**	0.00	0.60^{NS}	0.94	5.78**	0.00	2.77**	0.00

Prob. < 0.05then **= Highly Significant Prob. > 0.05, NS= Non-significant

Table 2: ANOVA comparison for concentration of heavy metals (Cd, Cr, Co, Cu and Ni) (mg/kg) in vegetable collected from selected sites of Okara city.

Vegetable	Co	Cr	Cu	Cd	Ni
Underground vegetables					
Radish	44.06 a	17.25 de	6.61 h-j	11.13 d-j	16.37 d-g
Carrot	10.79 e-j	06.89 h-j	5.99 ij	04.75 j	10.87 e-j
Potato	27.06 c	04.59 j	7.08 h-j	05.14 j	09.54 f-j
Spring Onion	05.79 ij	07.72 h-j	5.69 ij	05.70 ij	08.58 h-j
Turnip	18.58 d	05.93 ij	5.74 ij	04.75 j	09.56 f-j
Onion	05.48 ij	33.77 b	6.85 h-j	05.87 ij	07.94 h-j
Leafy vegetables					
Spinach	08.79 g-j	09.48 f-j	7.06 h-j	05.59 ij	09.52 f-j
Coriander	16.68 d-f	07.25 h-j	8.09 h-j	04.80 j	13.68 d-h
Mustard	11.22 d-j	07.55 h-j	9.63 e-j	07.16 h-j	13.18 d-i
Chenopodium	16.59 d-f	11.59 d-j	7.38 h-j	04.49 j	10.29 e-j

In each column, means with same superscript (s) are not significantly different while those with different superscript(s) are significant.

Table 3: Comparison of five heavy metals (mg/kg) concentrations in collected vegetable samples.

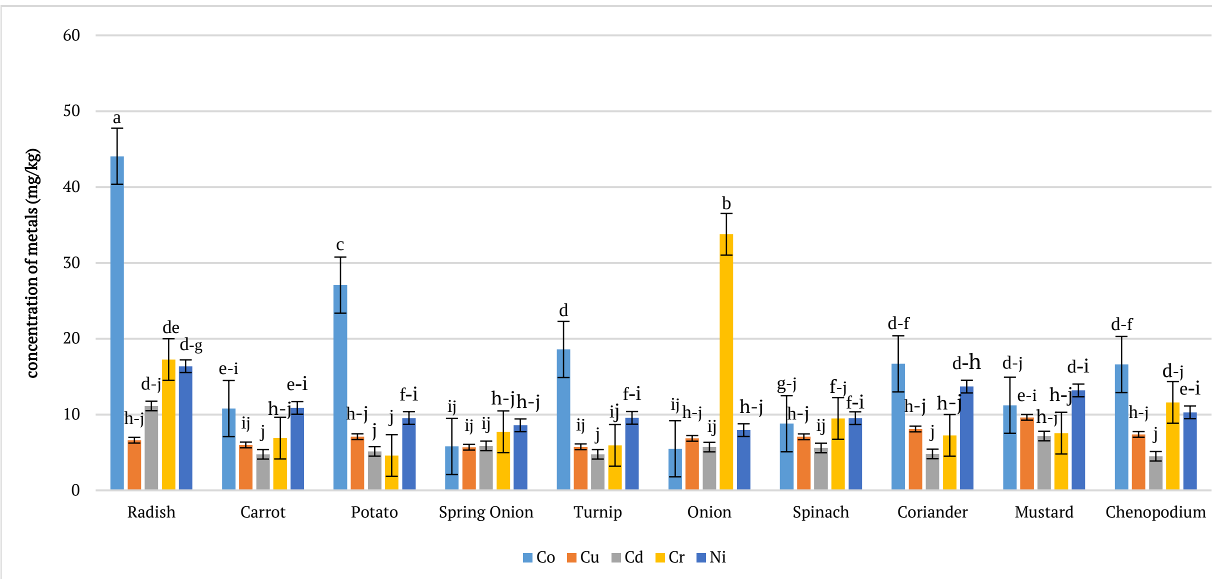

Figure 1: Comparison for five heavy metals concentrations (mg/kg) in vegetable samples collected during the study.

been detected in samples taken from Farmer`s market, whereas vegetables, collected from Local shops in Okara contained highest copper were Radish, Mustard and Coriander. In difference with samples collected from other sites copper level in Carrot, Turnip and Chenopodium collected from site3 was significantly high. The highest concentration of chromium in Onion was found in samples taken from Farmer`s market, whereas Mustard and Turnip collected from Site2 contained high level cobalt. In difference with vegetables from Site2, chromium level in Carrot, Potato and Spinach collected from Site3 was high.

In contrast with other metals, highest concentration of cobalt in Mustard and Chenopodium was detected in samples from Site1, whereas highest Cobalt level from Site2 was analyzed in Radish, Onion, Spring onion and Turnip. Cobalt concentration in Carrot, Potato and Coriander collected from Site3 was higher. Whereas cadmium detected in samples of Radish, Carrot, Potato, Spinach and Turnip brought from Site1 was with highest concentration.

Thus, result showed the sequence of heavy metal concentrations according to the collection sites for all analyzed vegetables in given order: S4<S3<S2<S1 for nickel and chromium concentrations, S1<S4<S2<S3 for copper concentrations and S4<S3<S1<S2 for cobalt and cadmium contents.

The results of the analyzed samples showed the mean concentration of metals in following sequence from high level of metal to low level in vegetable samples analyzed: Co>Cr>Ni>Cd>Cu. From which highest level of accumulated metals were analyzed in Radish and lowest in Onion and highest level of cadmium and nickel were also detected in Radish, whereas chromium and copper detected with highest concentration in Onion and Mustard respectively. According to comparison of metals with types of selected vegetables, underground and leafy vegetables, more level of accumulated metals were detected in underground vegetables especially in Radish and onion except the concentration of copper that was analyzed more in leafy vegetables.

Discussion

Now a day's use of wastewater irrigation and different types of fertilizers along with some other chemical treatments like pesticides are increased for crop production. Thus, heavy metals found in them bind to the soil which further up took by plants and accumulated in plants or maybe in edible parts of plants [20]. The determination of research work was to assess heavy metal level of vegetables and fruits that is most consumable edible by us, and to check whether the edibles as vegetables provided us are fit for us to eat or not. And if not then to what extents it's harmful or hazardous for our health.

Heavy metals contents of edibles are of concern because of their vital or lethal nature e.g., chromium, copper and cobalt are essential at lower limits but when they surpassed certain limits, they can be lethal [21], while some metals like arsenic, cadmium and nickel are hazardous at certain levels [22], change in effect of metals depends upon properties of metals. Mostly, formation of heavy metal complexes with some organic compounds causes toxicity [23].

As essential element cobalt is a constituent of Vitamin B and is useful in metabolism of human. Yes, it is true that there are some reports about cardiac effects and hemorrhage in lungs because of its short-term inhalation of high levels [24]. But there isn't any report found showing a relation between cobalt in food, water and cancer. Thus, cobalt is not classified carcinogenic by EPA.

As it is known, cobalt is being used for manufacturing of super alloys and some pigments [25] thus it can be used in fields and accumulated by plants. Cobalt level in all the tested samples was below the FAO/WHO limits and as it describes above about being non-hazardous. Similar to results of the study of Elbagermi and companions [26] perceived vegetables collected from market and production points in Misurata of Libya, cobalt concentration was within the safe limits given by FAO/WHO [27]. Chromium is also an essential metal, usually in food trivalent forms of chromium is found that is required for activity of insulin needed to stabilize the blood glucose levels, even though it can also be found in its hexavalent form, which is noxious [28]. The maximum level of chromium detected was 33.77 mg/kg in Onion and was noted above the recommended parameters established by FAO/WHO.

Chromium level of vegetable samples analyzed was much lesser than chromium concentrations ranged between 34.8 and 96 mg/kg reported in samples collected from Titagarh of India [12]. But chromium content of samples analyzed was more than the chromium reported 3.69 mg/kg in vegetables gathered from wastewater irrigated fields of India [16].

Copper, an important biocatalyst is essential for maintaining pigmentation of body and preventing anemia. Mostly plants had insufficient amount of copper in order to have a normal growth and are provided regularly by addition of different organic fertilizers [13]. Moreover, toxicity of copper can induce deficiency of iron and destruction of membrane in body [17]. The highest amount of copper analyzed in vegetables was 9.63 mg/kg in Mustard, Concentration of copper (2.06-33.22 µg/mg) in different vegetables collected from some cities of Saudi Arabia measured by Ali and Al-Qahtani in 2012 [29] were a lot higher than the copper level analyzed in the recent study.

It was observed that copper concentrations in all analyzed samples of vegetables were below the recommended limits by FAO/WHO just as report given by Al-Jassir and companions [30]. Whereas in difference with results, concentration of copper was high in some vegetable samples like spinach and turnip collected from different production sites and markets described by Ronaq and co-researchers [31] and were considered unsafe to intake.

Cadmium is one of the non-essential metals in food, as it accumulates in kidneys and liver. The concentrations of cadmium in vegetables analyzed ranged from 4.49 mg/kg to 15.6 mg/kg that was way more than the cadmium in samples of vegetables analyzed by Saha and his team [28] which were ranged from 0.15 mg/kg to 1.74 mg/kg.

The cadmium concentration in all analyzed samples of vegetables exceeds permissible limits by WHO/FAO several values for cadmium had been reported previously for vegetables included 10.4 mg/kg to 14.6 mg/kg in west Bengal, reported by Gupta and co-researchers [12], that were much higher than cadmium levels in vegetables analyzed in samples from Okara.

The level of nickel i.e., also a non-essential element for food measured was indicated in all collected vegetables were within the standard limits by FAO/WHO. The concentrations of nickel found in the vegetable samples analyzed by Akan and colleagues

[33] was ranged from 0.25 to 4.56mg/kg that were lesser than in samples studied.

The sequences Cu<Cd<Ni<Cr<Co was found in vegetables analyzed during recent study. The orders of the heavy metals contents in different kinds of vegetables, Pb>Mn>Cr>Cd>As, Mn>Cd>Cr>Pb>As tested by Saha and team [28] were in contrast with the results of the research. Comparison with results given by Kananke and his co-fellows [33] also showed similarity in order of metal abundance in vegetables as Cu>Ni>Cr>Pb>Cd only with the difference of copper level that was lesser in all vegetables analyzed.

Results also showed that cobalt, copper and nickel concentration in all the samples were within the permissible values given by FAO/WHO while cadmium and chromium level in samples exceeds given permissible limits. Thus, by comparing levels of metals reported by researchers in edibles like vegetables concluded that similar to the results chromium and cadmium contents in some of the collected samples exceed the allowable limit [34]. While in difference with results copper level analyzed by Kachenko and Singh in vegetable exceeded the standard limits given by different human health organizations [34]. Thus the study recommends periodic monitoring and assessment of heavy metals in vegetables along with prevention measures.

This study provides information on the concentration of heavy metals in vegetables sold or consumed in different localities of Okara city, Pakistan. The results indicated that some of the collected vegetable samples contained concentrations of cadmium and chromium that exceed the allowable limit given by FAO/WHO. However, cobalt, copper, and nickel concentration in all the samples were within the permissible values given by FAO/WHO. The consumption of vegetables contaminated with heavy metals can lead to various health problems such as cancer, kidney damage, and neurological disorders. Therefore, it is essential to take measures to mitigate these risks. Some of the steps that can be taken include monitoring and assessing heavy metal levels in vegetables periodically, avoiding the use of contaminated water for irrigation purposes, using organic fertilizers instead of chemical fertilizers, and promoting public awareness about the potential health risks associated with consuming contaminated vegetables. Additionally, it is recommended that people should wash their vegetables thoroughly before cooking or consuming them.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Saba Younas: Conducted research and compiled data

Syeda Anjum Tahira: Supervision, reviewing and evaluation of experiment

Muhammad Umer Farooq: Data analysis, proofediting and reviewing

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