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Research article

HEAVY METAL CONCENTRATION IN VEGETABLES AND THEIR POTENTIAL RISK FOR HUMAN HEALTH

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This study assesses heavy metal levels in the water, soil, and vegetables (swiss chard, lettuce, cabbage, collard green, tomato, green pepper, and carrot) irrigated with wastewater in Gamo, Ethiopia. The samples of soils, waters, and vegetables were randomly collected, processed, and analyzed for heavy metals using atomic absorption spectroscopy. The obtained results show that the mean concentrations of Cd, Cr, and Ni were the highest, and Pb, Zn, and Cu had the lowest concentration in irrigation waters. The levels of Cd in the Kulfo river area and Chamo Lake area and Cu in most of the farm soils were also found to be higher than the reference values. The study also revealed that the mean levels of Cd in most vegetables and Cr and Pb in some vegetables were higher than the maximum recommended limits set by the World Health Organization / Food and Agriculture Organization 2001. Among the vegetables, cabbage had the highest heavy metal content followed by Swiss-chard, carrot, tomato, collard green, green pepper, and lettuce. The hazard quotient obtained for Cu, and Ni in all vegetable samples and Cd in some vegetable samples exceeded 1. It indicates that there are potential health risks to consumers. This study recommends regular monitoring over heavy metals contents in soils, waters, and foodstuffs to prevent their excessive accumulation in food chains.

Key words: *heavy metals; pollution; vegetables; hazard quotient, risk assessment, safety, human health, FAAS.*

Vegetables as reported by [1] are essential sources that provide people with a wide range of vital micronutrients. Several researchers observed that vegetable consumption could prevent several chronic non-communicable diseases such as cardiovascular diseases, kidney, nervous as well as bone diseases. Vegetables also contribute substantially in providing a body with proteins, minerals, vitamins, fiber, and other nutrients which are usually in short supply in daily diets [2, 3]. The recent trend indicates that there is an increasing awareness regarding nutritive value of vegetables to the extent that many people now prefer eating vegetables to meat [4]. Vegetables have been recognized to have some medical properties due to antioxidant and antimicrobial effects. Many of them were even documented to possess anti-diabetic, anti-inflammatory, and anti-hypertensive potential [5, 6].

But at the same time safety and quality of agricultural products to a great extent depends on a place where they are grown, especially in case there is a threat that toxicants, heavy metals included, can occur in the environment. Heavy metals being introduced into the environment through anthropogenic sources are a serious growing problem throughout the world [7–9]. Anthropogenic sources of heavy metal contaminants include domestic, industrial, agricultural, bush burning, fossil fuels burning, etc.

Water gets contaminated with heavy metals on certain territories practically unavoidably since there are natural reasons for it (rock

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erosion) and anthropogenic activities (industries, agriculture, and households). Wastewaters from mining, electric industry, dye works, and chemical laboratories often contain heavy metals in high concentrations including cadmium (Cd), copper (Cu), and lead (Pb). Agricultural soils get contaminated with heavy metals due to irrigation with wastewaters and it is rather alerting since it can produce negative effects on people's health. A study was performed in Ghana that focused on water in irrigation systems used for growing cabbage, carrots, and lettuce that contained Cd and Pb; the study revealed that Cd and Pb concentrations grew considerably together with a growth in irrigation water concentration. In most developing countries it is a usual practice to grow vegetables along rivers that flow through urban territories. Water in such rivers is often reported to be contaminated with heavy metals. An extent to which metals are absorbed by plants from water depends, among other things, on a plant itself and chemical structure of a contaminant, element concentration in soil. pH, and interaction with other metals. Water gets contaminated with heavy metals mostly due to waste discharge from mining industry as well as from a wide range of other industries.

Heavy metals are found in soils in different chemical forms and it is closely connected to their solubility that directly depends on their mobility and biological availability. Soluble heavy metals easily penetrate plants. The highest concentrations in soils were detected for zinc (113 mg/kg), chromium (47.8 mg/kg), lead (17.7 mg/kg) and cadmium (0.250 mg/kg); they were detected on agricultural territories in Zivey, Burau, and Addis Ababa provinces. Heavy metals concentrations detected near Addis Ababa were the highest in comparison with Zivey province. It indicates that agricultural products are grown on territories where metals concentrations in soils occur due to natural reasons.

Some heavy metals such as Fe, Zn, Cu, and Se are essential for people especially when they are in small quantities. However, heavy metals are non-biodegradable and therefore readily accumulate to toxic levels in biological media and produce negative effects on animals, plants, and humans when they exceed a certain threshold [9]. Other heavy metals such as Pb, As, Cd, and Hg are toxic even in low concentrations and exposure to them results in certain health problems.

The human body can be easily contaminated by heavy metals such as Ni, Cd, Cr, Pb, and Cu through dietary exposure or exposure to the contaminated environment. Since fruits and vegetables can absorb heavy metal contents from the soil, even the same crops, fruits or vegetables, can contain minerals in different quantities depending on soils and a region where these plants are cultivated. The increased concentration of heavy metals is associated with the etiology of several diseases, especially cardiovascular, renal, and neurological disorders.

Cadmium is a non-essential element that occurs in foods and natural waters and it accumulates principally in the kidneys and liver. Cadmium causes acute and chronic poisoning, adverse effects on the kidneys, and liver, vascular and immune systems. Recently great attention has been paid to this element contents in water, soils, milk, food products and medicinal herbs and plants. Cadmium occurs on soils and plants most frequently from phosphate fertilizers, melting furnaces at nonferrous metallurgic enterprises, lead and zinc mines, discharges from industrial enterprises, and organic fuels burning.

Lead is a serious cumulative body poison that enters a body with air, water, and food and cannot be removed by washing fruits and vegetables.

Copper is an essential trace element required for proper health in a relevant quantity. Its high concentrations in fruits and vegetables can be harmful to human health and in the same way; low consumption can cause many symptoms like growth retardation, skin ailments, and gastrointestinal disorders.

Zinc is an essential element and an integral component in many coenzymes, essential for the synthesis of DNA, RNA, proteins, and insulin but toxic in high concentrations.

People may get exposed to chromium via inhalation, drinking water consumption, or eating food containing chromium or even through skin contact. Exposure to elevated levels of chromium leads to skin irritation, ulceration, damage to circulatory and nerve tissues and it causes health problems. However, daily intake of this metal within a certain range of concentrations (up to 200 μg/day) by human beings and animals is considered to be essential for carbohydrate & lipid metabolism.

Analyzing and predicting consequences caused by pollution requires identifying and determining a number of potential risk sources, estimating a number of risk factors that come into contact with the human-environment boundaries; estimating levels of exposure via detecting routes of exposure to a target organism and quantifying health risks caused by this exposure. However, the fact that the contaminant levels exceed the permissible limits set by such regulatory agencies as the *World Health Organization (*WHO) does not always indicates there is a risk for human health. For this reason, the target hazard quotient (THQ) method designed by the *United States Environmental Protection Agency (*USEPA) for assessing potential health risks associated with long-term exposure to heavy metals was used to assess health risks in this study [9]. THQ that was equal to or greater than one (1≥) indicated a health risk to exposed population.

Currently in Arba Minch Gamo-zone, Ethiopia there is no regulatory criterion of heavy metals in irrigation waters, soils & vegetables. However, due to businesses' economic, industrial, and other development activities, heavy metals concentrations are increasing in irrigation waters, agricultural soils, vegetables, and crops that are grown in and outside the city. The farmlands and land spots along the river bank are intensely used in crops and vegetable production, but still, very little information is available regarding heavy metals contents in irrigation waters, soils, and vegetables. Therefore, the present investigation aims to quantify the concentration of various metals in soils and commonly grown vegetables irrigated with wastewater to calculate the daily intake rate of metals (DIR) basing on intake of particular vegetables by local population and to estimate hazards for human health related to heavy metals through the consumption of vegetables grown on four irrigational sites in Gamo, Ethiopia. The results will provide invaluable baseline data for further investigation of heavy metals accumulation in foodstuffs, thereby improving food safety and more efficient health protection for people who live on the examined territory.

Materials and methods. Arba Minch located at 6°2′N 37°33′E is one of a town in Gamo Gofa Zone, Southern Nations Nationalities and Peoples Region (SNNPR) regional state in Ethiopia found around 500 km away from Addis Ababa, Ethiopia's capital city. The town got its present name Arba Minch, meaning «40 springs» since it has 40 natural springs which are major tourist attractions (Figure). Kulfo River, Abaya Lake, and Chamo Lake are the water source for many inhabitants in the Arba Minch area for farmlands, for domestic activities, etc. The river and lakes have their source from the highlands in Gamo region which have experienced high levels of agricultural development. The farmlands where these lakes and river water were used for irrigation were selected for this study.

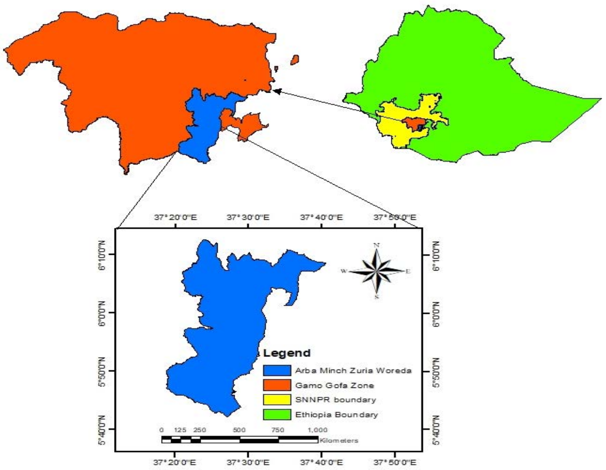

Figure 1. Location map of Arba Minch Zuriya district

Samples of soil, irrigating water, and seven commonly consumed vegetables namely, Swiss chard, lettuce, cabbage, collard green, tomato, green pepper, and carrot were collected from irrigational sites in Gamo, Ethiopia. All samples were randomly collected from the farmlands. Soil samples were collected at 15 cm depth. The collected soil and vegetable samples were put into clean polythene bags and labeled, while water samples were put in previously rinsed and dried bottles; then, all the samples were brought to the Chemistry Laboratory at the Arbaminch University (AMU) for preparation and subsequent atomic sbsorption spectroscopy analysis.

15 ml of a water sample was taken in a Teflon tube and then 5 ml HNO_3 and 1 ml. H_2O_2 were added to it. The vial was closed tightly and then inserted into a single safety shield carousel installed in a microwave chamber. The mixture was heated at 120 °C for over 3 hr in a microwave digester. After the process was completed, the clear and colorless solution was filtered using Whatman filter paper No. 42 and diluted with deionized water to raise the volume of the solution to 50 mL [10]. Finally, the samples were stored in plastic bottles until analysis.

500 g of a soil sample was placed in a polythene bag, dried in a microwave oven, and ground into a fine powder which was passed through a 2-mm mesh sieve. 1 g of a soil sample was diluted in a tri-acid mixture of (65 % of HNO_3: 70 % of $HClO_4$: 30 % of H_2O_2) with a 6:4:1 ratio respectively. The solution was heated to 200°C over 3 hr until brown fumes ceased to emit from the solution. The solution was filtered through Whatman filter paper No. 42 and then diluted with deionized distilled water to raise the volume of the solution to 50 mL [10]. The prepared samples were analyzed with flame atomic absorption spectroscopy (model AA 400P, Germany) in order to determine heavy metals concentrations in them.

The vegetable samples were cut into small pieces and then dried using the oven-dry procedure at 105 °C for 24 hr (Memmert UF 260 plus 230V Sunon model) to remove moisture. Dry samples were ground to powder and then bolted through a 1 mm sift. 0.5 g of a sample were taken in reference vessels, added with 6 ml of 65 % HNO_3, 3ml of 70 % $HClO_4$ and 1ml of 30 % H_2O_2 and then put onto a carousel installed in a microwave unit. The mixture was heated at 80 °C for over 3 hr in a microwave digester. After the process was completed, the clear and colorless solution was filtered using Whatman filter paper No. 42 and diluted with deionized water to raise the volume of the solution up to 50 mL [10]. Finally, the samples were stored in plastic bottles till analysis. The samples of vegetables, soils & waters collected from four sites in Ethiopia are illustrated in Table 1.

Table 1

Samples of vegetables, soils & waters were taken from four sites in Ethiopia

Samples		Waste water irrigated site			
		KRA	ATSHCA	ALA	CHLA
Vegetables	Swiss chard	✓	✓	✓	✓
	Lettuce	✓	✓	✓	✓
	Cabbage	✓	✓	✓	✓
	Collard Greens	✓	✓	✓	✓
	Tomato	✓	✓	✓	✓
	Green pepper	✓	✓	✓	✓
	Carrot	✓	✓	✓	✓
Soil		✓	✓	✓	✓
Irrigation water		✓	✓	✓	✓

Note: KRA – Kulfo river area, ATSHCA – Arbaminch textile share company area, ALA – Abaya lake area, CHLA – chamo lake area, Right mark (✓) indicates samples collected.

The prepared samples have analyzed for determining heavy metals concentrations in them using atomic absorption spectrometry with flame atomization, microwave oven, and dry oven (FAAS (*flame atomic absorption spectroscopy*) model AA 400P, Germany). Conventional auxiliary devices were applied. All the solutions were prepared using analytical chemical calibration reagents and deionized water. Graphical calibration standard solutions for all determinations were prepared by sequential dilution of conventional standards per 1,000 ppm.

All the procedures used in the present study were selected due to available data on them being successfully applied to determine heavy metals in different matrixes [11]. The instrumental operating conditions for the determination of heavy metals using FAAS are illustrated in Table 2. To provide high quality of experimental data, each sample was analyzed three times.

Table 2

Instrumental operating condition for determining heavy metals in wastewater, soil, and vegetable samples via FAAS [12, 13]

Element	Wavelength (nm)	Lamp Current (mA)	Slit Width (nm)	Flame type
Cd	228.8	2.0	1.2	Air – acetylene
Cr	357.9	6.0	0.7	Air – acetylene
Pb	283.3	5.0	0.7	Air – acetylene
Zn	213.9	5.0	0.7	Air – acetylene
Cu	324.7	4.0	0.5	Air – acetylene
Ni	232.0	7.0	0.6	Air – acetylene

The procedure was validated according to the protocol guidelines on the International Conference on Harmonization [14]. Analytical grade (Merck, Germany) reagents were used in all the experimental procedures of the research study.

Metals transfer from the contaminated soil to the edible parts of the vegetables was estimated via the transfer factor (TF) formula (1) [15–17].

$$\mathrm{TF} = \frac{C_{vegetables}}{C_{soil}} \quad (1)$$

where $C_{vegetables}$ is metals concentration in vegetables (mg/kg) and C_{soil} is metals concentration in soils (mg/kg).The higher is the value of TF, the more available heavy metals are. Hence, high TF values may indicate that heavy metals are poorly retained by soils or they are too efficiently absorbed by vegetables. Low TF values mean that there is a strong bond between heavy metals and colloids in soils.

Metals mobility when they are transferred from soils into plants is a function from physical and chemical properties of soils and vegetable types. This mobility changes when influenced by multiple anthropogenic and environmental factors. Differences in the transfer factor calculated for different vegetables may be due to different heavy metals concentrations in soils and differences related to elements consumption by different vegetables.

High accumulation factor (AF) values may indicate a potential health risk for consumers.

Assessment of health risk caused by consuming vegetables irrigated with wastewaters on the examined territories was performed taking into account daily intake rate (DIR). DIR were calculated basing on a questionnaire ($n = 400$ for each vegetable) (Table 3).

Table 3

Respondents interviewed for health risk assessment of wastewater irrigated vegetable consumption on the examined territories

Respondents	Age group (years old)		Total interviewed persons
	<16	>16	
Male	95	135	230
Female	76	94	170
Total interviews			400

Average body weight for an adult and a child was taken as 60 kg and 25 kg accordingly.

The daily intake rate of metals through consumption of selected vegetables (*Swiss chard, lettuce, cabbage, collard green, tomato, green pepper, and carrot)* was calculated with the equation (2) [15–19].

$$\mathrm{DIR} = \frac{C_{metals} \cdot D_{average\ vegetable\ intake}}{B_{average\ weight}} \quad (2)$$

where C_{metals} represents average element concentration in vegetable (mg/kg) and $D_{average\ vegetable\ intake}$ is average daily vegetable consumption or intake (kg/day-person) and $B_{average\ weight}$ is average body weight (kg). From our survey, the average daily each vegetable intake for adults and children was considered to be within 0.108–0.293 and 0.078–0.169 kg/(person-day) range, respectively, while the average adults and children body weights were considered to be 60 and 25 kg, respectively. All the data are summarized in Table 4.

Table 4

Average daily intake of each vegetable (kg/day-person)

Vegetables	Swiss Chard	Lettuce	Cabbage	Collard Green	Tomato	Green Pepper	Carrot	Total D_{ave}
Adults	0.227	0.163	0.246	0.293	0.231	0.108	0.187	1.455
Children	0.113	0.098	0.169	0.157	0.128	0.078	0.103	0.846

Hazard level for non-carcinogenic contaminants was determined by calculating a Hazard Quotient (*HQ*) [20]. Hazard quotient or chronic threat assessment in this study was calculated with the equation (3) of [15, 21, 22]. If a hazard quotient value is lower than one, then exposed consumers are assumed to be safe, but if it is equal to or higher than one, it is considered not safe for human health [22, 23].

$$HQ = \frac{DIR}{RfD} \quad (3)$$

where *DIR* represents the daily intake rate and *RfD* represents the reference dose of metals. The *RfD* values for Cd, Pb, Cr, Ni, Zn and Cu are 0.001, 0.004, 1.5, 0.02, 0.3, and 0.04 mg/kg-day, respectively; these values were taken from integrated risk information system [22, 24, 25].

Hazard index (*HI*) was developed to evaluate potential health risks for consumers that occurred due to exposure to more than one potential toxic element [25]. It was calculated by summating hazard quotients for each element in each vegetable as per the following formula (4) [25, 20].

$$HI = \sum HQ. \quad (4)$$

We took into account one-directional effects produced on critical organs and systems by the examined heavy metals according to the matrix presented in Table 5.

The data obtained in the research work were statistically processed via calculating average values. Average values for metal concentrations in water, soil, and vegetable samples were compared by using an independent sample *t*-test. Statistics applied software, version 8.1, was used for basic descriptive statistical analysis of the data.

Table 5

Data obtained from ATSDR on effects produced by heavy metals on critical organs and systems

Critical organs/systems	Zn	Cd	Cr	Cu	Ni	Pb
Central nervous system						+
Cardiovascular system					+	
Digestive system		+				+
Kidneys			+	+	+	
Blood	+	+	+			
Development					+	+
Reproductive system						+
Hormonal system						+

Results and discussion. Validation results indicated that calibration curves for the various concentration ranges showed good correlation coefficients ranged between 0.9987 and 0.9999, which was higher than the required limit (0.995) for trace element analysis [26, 27]. This showed that there was a strong correlation between concentration and absorbance indicating that the devices were properly calibrated (Table 6).

The instrumental detection limits (IDL) ranged between 0.0005 and 0.01 mg/kg and it was below the limit of detection (LOD) indicating good sensitivity of the measuring instrument used for analysis. The limit of detection (LOD) ranged from 0.075 to 0.372 mg/kg. The limit of quantification (LOQ) lied within 0.227–1.127 mg/kg range and the result shows both the LOD and LOQ values were greater than the IDL; hence, the results obtained via our analysis could be reliable [28].

Recovery results were within 80–120 % range and it was acceptable for metal analysis [29]. The precision of the method was expressed as

Table 6

Validation Parameters

Metals	IDL (mg/kg)[a]	LOD (mg/kg)	LOQ (mg/kg)	Regression Equation	Correlation Coefficient (R^2)	Recovery (%) [b]	RSD (%)
Cd	0.0005	0.153	0.465	$y = 0.1247x - 0.003$	0.9998	101.294 ± 3.398	3.355
Cr	0.002	0.184	0.558	$y = 0.0112x + 0.002$	0.9997	98.905 ± 3.578	3.618
Pd	0.01	0.372	1.127	$y = 0.0044x + 0.0009$	0.9987	99.436 ± 8.495	8.543
Zn	0.0008	0.075	0.227	$y = 0.1429x + 0.0277$	0.9999	99.265 ± 2.095	2.110
Cu	0.001	0.200	0.606	$y = 0.063x - 0.0013$	0.9996	98.273 ± 5.608	5.706
Ni	0.002	0.211	0.638	$y = 0.0243x + 0.0019$	0.9996	98.974 ± 4.515	4.562

Note: [a]Source for FAAS; [b]Mean ± SD, $n = 7$, SD = standard deviation, mg/kg: milligram per kilogram, RSD: relative standard deviation.

relative standard deviation (RSD) of the three replicate readings. The obtained RSD values ranged from 2.110 to 8.543 %, which was under the required control limits ≤ 15 % [30, 31]. These results indicate that the proposed method was precise and accurate.

Average concentrations of heavy metals detected in soil, water, and vegetables samples taken on four examined territories are given in Tables 7–9.

As we can see from the Table 7, average Cr, Pb, Zn, and Ni concentrations in soil samples from all irrigated sites were found to be lower than the recommended upper limits for soils by FAO/WHO & USEPA [32–35]. The situation is the same with Cd concentration in soil samples obtained from the ATSHCA and ALA and also Cu concentration in soil samples obtained from the KRA and ATSHCA.

Concentration of Cd from KRA and CHLA farms and Cu concentration from ALA and CHLA irrigated sites were higher than the recommended maximum limit for soil by FAO/WHO & USEPA [36–40].

The concentrations of Zn, Pb, and Cu in the wastewater samples that were detected on all irrigational sites were lower than the recommended maximum level for irrigation water set by USEPA & FAO/WHO. But at the same time, we detected relatively high concentrations of Cd, Cr, and Ni in wastewaters used for irrigation on all the examined territories and these concentrations were higher than maximum recommended levels for these metals. The results obtained show that the wastewater samples are substantially contaminated with these heavy metals. Therefore, attention should be focused on regular monitoring and control of wastewater used for irrigation.

Results obtained via quantifying metals in vegetables are shown in Table 8. Cd concentration in vegetables grown on the examined territories varied from 0.18 mg/kg (lettuce, ALA) to 0.45 mg/kg (Swiss chard, KRA) [41, 42].

Table 7

Average concentration of heavy metals found in soil farmlands and irrigation water

Heavy metals	*KRA*		*ATSHCA*		*ALA*		*CHLA*		Guidelines for max. levels in	
	water (mg/L)	Soil (mg/kg)	water (mg/L)	Soil (mg/kg)	water (mg/L)	Soil (mg/kg)	water (mg/L)	Soil (mg/kg)	water (mg/L) [a]	Soil (mg/kg) [b]
Cd	0.2605	3.9096	0.185	1.8261	0.1634	1.7512	0.245	3.2113	0.01	3
Cr	0.8402	19.897	0.2567	13.0664	0.4738	16.2893	0.6701	8.2187	0.1	50
Pb	0.0094	13.8313	0.0038	10.4406	0.0024	12.5333	0.0056	11.7341	0.015 [c]	100
Zn	0.3488	52.2735	1.3954	42.5626	0.1461	53.0685	0.0770	68.5274	2	300
Cu	0.4643	48.8125	0.6743	40.8878	1.2759	57.1213	0.366	51.2222	2 [d]	50 [c]
Ni	0.8767	42.1764	0.5609	31.3822	0.3953	19.5129	0.6811	31.3866	0.07 [d]	80 [c]

Note: *[a]WHO/FAO (2007), [b]WHO/FAO (2001), [c]USEPA (2010), [d]WHO (2008),* mg/kg: milligram per kilogram, mg/L: milligram per litter.

Table 8

Concentration of heavy metals (mg/kg) in vegetable samples in four sites of Ethiopia

Vegetables	Heavy metals						Heavy metals					
	Cd	Cr	Pb	Zn	Cu	Ni	Cd	Cr	Pb	Zn	Cu	Ni
Study area	KRA						ATSHCA					
Swiss Chard	0,4524	1,4062	0,4564	27,2354	11,2354	13,8213	0,3313	1,1245	0,1987	23,4859	9,5894	9,4152
Lettuce	0,2537	1,8213	0,3125	22,4562	16,4235	17,7014	0,1897	1,4526	0,1595	18,5642	13,8976	15,8631
Cabbage	0,4185	2,7801	0,4562	14,2563	16,5426	19,8916	0,3281	2,8456	0,2895	10,4586	14,5642	18,6542
Collard Green	0,2304	2,9945	0,2901	10,4568	13,9648	24,9745	0,1887	2,7589	0,1693	8,5642	9,5689	23,3456
Tomato	0,4324	1,8452	0,2812	13,6548	24,2345	26,8945	0,2791	1,5243	0,1623	10,2365	19,5642	24,4538
Green Pepper	0,2394	1,9452	0,2945	9,9865	21,4563	21,8916	0,1714	1,5624	0,1915	5,4568	16,5894	20,6542
Carrot	0,3515	2,0145	0,3912	12,4568	25,2345	25,7865	0,2988	1,7451	0,3056	9,4758	20,5642	21,5917
FAO/WHO (2001)	0,200	2,3	0,3	99,4	73,3	67	0,2	2,3	0,3	99,4	73,3	67
Study area	ALA						CHLA					
Swiss Chard	0,3041	1,2345	0,2956	28,3793	11,5564	5,3914	0,4194	1,0292	0,3477	31,4526	9,9524	11,3121
Lettuce	0,1797	1,5462	0,1895	24,3427	18,5462	9,8756	0,2165	1,6271	0,39	27,4589	17,4371	16,8123
Cabbage	0,2973	2,0461	0,3125	16,424	20,6542	11,9517	0,3685	2,1686	0,4218	21,5643	18,4682	17,9453
Collard Green	0,191	2,2451	0,1885	12,2014	16,2457	17,8501	0,2155	2,6527	0,2189	16,8956	14,3566	22,7254
Tomato	0,3152	1,4452	0,1812	15,0882	32,4568	15,3968	0,457	1,6977	0,2101	19,5641	26,3741	25,8745
Green Pepper	0,1885	1,7854	0,2282	10,6628	29,5463	16,7969	0,251	1,6362	0,2586	14,5623	23,1016	19,9453
Carrot	0,292	1,6123	0,3156	14,5447	31,4568	15,8259	0,3421	1,9557	0,3524	17,5659	26,0377	23,6895
FAO/WHO (2001)	0,2	2,3	0,3	99,4	73,3	67	0,2	2,3	0,3	99,4	73,3	67

Table 9

Average concentration of heavy metals (mg/kg) found in vegetables from all farms

Heavy Metals	Swiss Chard	Lettuce	Cabbage	Collard Green	Tomato	Green Pepper	Carrot	Guideline Value (mg/kg) [a]
Cd	0.38	0.21	0.35	0.21	0.36	0.20	0.32	0.2
Cr	1.20	1.61	2.46	2.66	1.63	1.73	1.83	2.3
Pb	0.32	0.24	0.37	0.22	0.21	0.24	0.34	0.3
Zn	27.64	23.21	15.67	12.03	14.64	10.17	13.51	99.4
Cu	10.58	16.58	17.56	13.53	25.66	22.67	25.82	73.3
Ni	9.99	15.06	17.11	22.22	23.15	19.82	21.72	67

Note: [a] *FAO/WHO-codex alimentarius commission (2001).*

Cd concentrations were not significantly high in Lettuce, Collard Green, and Green Pepper and didn't differ greatly on the examined territories [43–45]. However, Cd concentrations detected in Swiss chard, Cabbage, tomato, and carrot mostly from KRA and CHLA sites were higher than the permissibility level set by FAO/WHO. Among the sampling sites, the samples from the KRA site contained the highest Cd concentration which might be attributed to the sewage sludge discharge from the cement processing, metal manufacturing, stone crushing machine, and industrial waste from textile which dispose its wastes directly into the river [46, 47].

The concentration of Cr was observed to be the highest for collard green samples (2.99 mg/kg) from KRA. The concentrations of Cr detected in cabbage and collard green of the vegetables analyzed from KRA and ATSHCA sites and collard green from CHLA site were higher than the limit levels in food by FAO/WHO guidelines. Among the investigated vegetables, cabbage and collard green were found to be more Cr-loaded than other vegetables. High concentrations of this heavy metal were detected in vegetables from KRA, ATSHCA, and CHLA sites which might be due to sewage sludge discharged to the environment from metal production, cement production, textile production, and stone crashing sites or due to pollution from agronomic practices [48].

The Pb content was the highest in Swiss chard (0.46 mg/kg) and cabbage (0.46 mg/kg) collected on KRA farms and the lowest in lettuce (0.16 mg/kg) collected on ATSHCA farms. The Pb content in all of the vegetables from all farms, except Swiss chard and cabbage from KRA farms were found to be within the safe limit stated by FAO/WHO [49].

The highest concentrations of Cu were in tomato (32.46 mg/kg) follow by carrot (31.46 mg/kg) and green pepper (29.55 mg/kg) from ALA while the lowest Cu concentration was detected for collard green (9.57 mg/kg) from ATSHCA farms. Similarly, the highest levels of Ni were in tomato (26.89 mg/kg) from KRA while the lowest one was detected for Swiss chard (5.39 mg/kg) from ALA farms. The Cu and Ni content in all of the vegetable samples from all farms were found to be within the safe limit stated by FAO/WHO which is 73.3 mg/kg and 67 mg/kg respectively [50].

The results shown in Table 7 contain average concentration of zinc in all vegetable samples. CHLA had the highest concentration of 31.45 mg/kg in Swiss chard followed by 28.38 mg/kg in Swiss chard from the ALA site while the lowest concentration was obtained from the ATSHCA site with 5.46 mg/kg in green pepper [51]. This indicates that zinc was contained in all samples in concentrations that were within the stipulated limit stated by FAO/WHO which is 99.40 mg/kg.

All the obtained data allowed estimating transfer factor (TF) for various heavy metals transfer from soils into vegetables. The results are given in Table 10.

Swiss chard has the highest transfer factor values for Zn (0.52) followed by Ni (0.32). Lettuce has the highest transfer factor values for Ni (0.49) followed by Zn (0.43) [52]. As for the other vegetables, the highest TF values were detected for Ni (from 0.56 to 0.76) and Cu (from 0.27 to 0.52). Overall, TF values for heavy metals in the examined vegetable samples were in the following order:

$$Ni > Cu > Zn > Cr > Cd > Pb.$$

The highest TF values were found to be 0.76 and 0.52 for Ni and Zn respectively. These might be due to the higher mobility of these heavy metals together with natural occurrence in soil and their lower retention in soil than other toxic elements [53, 54].

Table 10

Transfer Factor (TF) of heavy metals from soil to vegetables

Heavy metals	Swiss Chard	Lettuce	Cabbage	Collard Green	Tomato	Green Pepper	Carrot	Average TF
Cd	0.12	0.08	0.12	0.08	0.12	0.08	0.13	0.104
Cr	0.09	0.12	0.19	0.21	0.13	0.13	0.14	0.14
Pb	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.024
Zn	0.52	0.43	0.29	0.22	0.27	0.18	0.25	0.31
Cu	0.22	0.34	0.35	0.27	0.51	0.45	0.52	0.38
Ni	0.32	0.49	0.56	0.74	0.76	0.67	0.72	0.61

We combined data on metals concentrations in vegetables with data on daily intake rate for each product; it allowed us to calculate daily intake of heavy metals into a body [55] (Table 11).

Daily intakes gave grounds for determining hazard quotients (Table 12). As we can see form these data, daily intakes of Cd, Cu, and Ni were higher than reference doses (*RfD*) recommended by USEPA and FAO/WHO both children and adults [24, 56]. The values of *HQ* were less than 1.0 for Cr, Pb, and Zn in all tested vegetables and Cd in lettuce and green pepper for both adults and children, thus indicating a risk was insignificant.

The highest hazard (*HQ*) quotients were revealed for Swiss chard, tomato, and cabbage for both adults and children. Therefore, consumption of Swiss chard, tomato, and cabbage grown on the examined territories can cause high health risks.

Hazard quotients were used as a basis for estimating hazard indexes that showed probability of functional disorders in specific organs and systems due to toxic elements prodding similar effects on them [57]. In the present study it was revealed (Table 13) that the highest health risks for people who daily consumed vegetables grown on the examined irrigated sites were related to functional disorders in the kidneys, cardiovascular system and overall development [58].

Table 11

Average daily intake of *Heavy metals* through consumption of vegetables (mg/kg-day)

Heavy metals	Individual	Swiss Chard	Lettuce	Cabbage	Collard Green	Tomato	Green Pepper	Carrot	Average DIR	Sum DIR	RfD
Cd	Adults	0.0022	0.0012	0.0020	0.0012	0.0021	0.0012	0.0019	0.0017	0.0072	0.001
	Children	0.0035	0.0020	0.0033	0.0020	0.0034	0.0019	0.003	0.0027	0.0101	
Cr	Adults	0.0070	0.0093	0.0143	0.0154	0.0095	0.0100	0.0106	0.0109	0.0471	1.5
	Children	0.0112	0.015	0.0229	0.0248	0.0152	0.0161	0.0171	0.0175	0.0662	
Pb	Adults	0.0019	0.0014	0.0022	0.0013	0.0012	0.0014	0.002	0.0016	0.0068	0.004
	Children	0.003	0.0022	0.0035	0.0021	0.002	0.0022	0.0032	0.0026	0.0096	
Zn	Adults	0.1603	0.1346	0.0909	0.0698	0.0849	0.0590	0.0784	0.0968	0.4072	0.3
	Children	0.2576	0.2163	0.1460	0.1121	0.1364	0.0948	0.1259	0.1556	0.5596	
Cu	Adults	0.0614	0.0962	0.1019	0.0785	0.1488	0.1315	0.1498	0.1097	0.4432	0.04
	Children	0.0986	0.1545	0.1637	0.1261	0.2391	0.2113	0.2406	0.1763	0.625	
Ni	Adults	0.0579	0.0874	0.0992	0.1289	0.1343	0.1150	0.1260	0.1069	0.4499	0.02
	Children	0.0931	0.1404	0.1595	0.2071	0.2158	0.1847	0.2024	0.1719	0.6293	

Table 12

Hazard Quotient of *Heavy metals* via intake of vegetables from wastewater irrigated farms

Heavy metals	Individual	Swiss Chard	Lettuce	Cabbage	Collard Green	Tomato	Green Pepper	Carrot
Cd	Adults	2.204	1.218	2.03	1.218	2.088	1.16	1.856
	Children	3.542	1.957	3.262	1.957	3.355	1.864	2.982
Cr	Adults	0.005	0.006	0.010	0.010	0.006	0.007	0.007
	Children	0.008	0.010	0.015	0.017	0.010	0.011	0.011
Pb	Adults	0.464	0.348	0.537	0.319	0.305	0.348	0.493
	Children	0.746	0.559	0.862	0.513	0.489	0.559	0.792
Zn	Adults	0.534	0.449	0.303	0.233	0.283	0.197	0.261
	Children	0.859	0.721	0.487	0.374	0.455	0.316	0.420
Cu	Adults	1.534	2.404	2.546	1.962	3.721	3.287	3.744
	Children	2.465	3.863	4.092	3.153	5.979	5.282	6.016
Ni	Adults	2.897	4.367	4.962	6.444	6.714	5.748	6.299
	Children	4.655	7.018	7.973	10.355	10.788	9.236	10.122

Table 13

Hazard Index of heavy metals that affect the same target organs and systems

Critical organs/systems	Group	$HI_{\text{макс}}$	$HI_{\text{средн}}$	Contribution made by specific metals into risks, %					
				Zn	Cd	Cr	Cu	Ni	Pb
Central nervous system	adults	1.7	0.24						100
	children	2.4	0.34						100
Cardiovascular system	adults	22.5	3.21					100	
	children	31.5	4.50					100	
Digestive system	adults	8.9	1.27		80.90				9.10
	children	12.5	1.78		80.90				9.10
Kidneys	adults	33.61	4.79			0.09	32.97	66.94	
	children	47.14	6.74			0.08	33.16	66.76	
Blood:	adults	14.11	1.23	16.01	83.63	0.36			
	children	22.81	1.71	15.91	83.73	0.36			
Development	adults	40.29	3.46					92.93	7.07
	children	64.70	4.84					92.97	7.03
Reproductive system	adults	1.7	0.24						100
	children	2.4	0.34						100

Conclusion. Results obtained in the present study allow concluding that consumption of vegetables irrigated with wastewaters results in unacceptable health risks, both for adults and children. The authors suggest regular monitoring over heavy metals contents in soils, irrigation water, and food products as it will allow avoiding substantial heavy metals accumulation in food chains and therefore health risks will be reduced.

Results obtained in the present study can become a stimulus for ecologists, managers, and public healthcare experts in their activities aimed at informing people about hazards related to consuming vegetables that are grown on contaminated territories; it can lead to reduction in health risks.

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