







Research Article

Effect of Water and Soil
Contamination by Heavy Metals in
Lettuce (Lactuca sativa), Cabbage
(Brassica oleracea var. capitate),
and Turnip (Brassica napus L.) at
Different Stage

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Received: 23 March, 2022 Accepted: 04 April, 2022 Published: 05 April, 2022

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Keywords: Heavy metals; Contaminated soil; Contaminated water; Vegetables (cabbage, lettuce and turnin)

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Abstract

Environmental pollution with heavy metals is very harmful to the human body and *other life forms*, even in low concentrations, as there is no effective removal mechanism. Urban agriculture utilizes this contaminated land for the cultivation of vegetable crops to facilitate their food security and entry of toxic heavy metals into the food chain. The objective of this study was to evaluate the impact of contaminated soil and water with heavy metals on the quality of vegetable crops at different growth ages. Microwave Plasma Atomic Emission Spectrometry (MP-AES) was used to determine the concentration of these heavy metals. The concentration of heavy metals (Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr) in contaminated soil was found to be 39434.9±30.0, 3183.7±43.5, 222.0±10.2, 22.28±2.5, 0.057±0.00, 5.8±0.03, 72.72±0.01, 148.6±15 mg/kg, respectively. In addition, the mean concentration of Fe, Mn, Zn, Cu, Co, Ni, and Cr in wastewater was found to be 5.2±0.5, 0.8±0.08, 0.72±0.01, 1.05±0.03, 0.01±0.00, 0.2±00, 0.72±0.01 and 0.14±0.02, respectively. The relative abundance of heavy metal in soil, water and vegetable samples were in the following order: Fe >Mn >Zn > Cr > Pb> Cu> Ni> Co, Fe > Cu > Mn >Zn = Pb> Ni > Cr > Co and Fe > Mn > Zn > Pb > Cu> Cr > Ni> Co, respectively, and the accumulation of heavy metals in three month growth. All lettuce, cabbage, and turnip vegetables grew faster than two months. Both two and three months of age, the accumulation of heavy metals in turnips > lettuce > cabbage. Most of the soil, water, and vegetable samples exceeded the permissible limit of heavy metals prescribed by the World Health Organization (WHO) standards.

Abbreviations

PW: Pure Water; PWCS: Pure Water Contaminated Soil; PS: Pure Soil; CWPS: Contaminated Water Pure Soil and CWCS: Contaminated Water Contaminated Soil

Introduction

Environmental pollution with heavy metals has become a major concern in the world due to urbanization, industrialization, high population density, *improved living conditions*, and economic development [1]. A large amount of organic and inorganic pollutants, mostly toxic heavy metals, are released into the environment either directly or indirectly as a result of natural or anthropogenic activity [2,3]. Heavy metals, such as mercury, chromium, cadmium, arsenic, nickel,

lead, zinc, copper, cobalt, and manganese, are among the most dangerous industrial pollutants in water and soil [4]. Inorganic pollutants (toxic heavy metals) are not biodegradable, and their persistence and accumulation in living organisms pose a number of health and environmental risks [5,6]. Toxic heavy metals are very harmful to the human body and other life forms, even in low concentrations, as there is no effective removal mechanism [1,2]. Wastewater discharged from industries, particularly in developing countries like Ethiopia, is the major challenge of the present day. Much of the literature in Ethiopia indicate that wastewater discharged from different sources is above the permissible limits given by WHO and EPA [7,8]. Urban agriculture utilizes this waste and contaminated soil for the cultivation of vegetable crops to facilitate food security. Like in other cities in Addis Ababa, Ethiopian urban farmers irrigate vegetables such as lettuce (Lactuca sativa), cucumbers (Cucumis

sativus), potatoes (Solanum tuberosum), cabbage (Brassica oleracea var. capitate), green onion (Allium porrum L.), turnips (Brassica napus L.), etc. for urban markets and their needs. Most toxic heavy metals accumulate in vegetables contaminated by contaminated water and soil. Apart from the health risk, heavy metal accumulation also affects the growth and yield of vegetable crops. The objective of this study is to determine the accumulation of heavy metals in lettuce (Lactuca sativa L.), turnip (Brassica napus L.), and cabbage (Brassica oleracea var. capitate) at different ages growing in the pot.

Materials and methods

Area of the study

This study (pot experiment) was conducted at the Ethiopian Institute of Agricultural Research (EIAR) in Addis Ababa, Ethiopia under environmental conditions. The pot experiment had four treatments such as pure water, pure soil, pure water contaminated soil, contaminated water pure soil, and contaminated water contaminated soil (PWS, PWCS, CWPS, and CWS), respectively.

Apparatus and instruments

A Multiwve 3000 microwave system (Anton Paar, Graz, Austria) programmable for time and power between 600 and 1400 W and equipped with 16 high-pressure polytetrafluoroethylene vessels (MF 100) and a 4100 MP-AES (Agilent, California, US) were used for the analysis of toxic heavy metals. The operating conditions of the instrument were forward plasma power of 1.0kW, nitrogen gas (spectral purity, 99.95%), and a flow rate of 16.0L/min (plasma), 1.2L/min (auxiliary), and 1.0L/min (nebulizer). The instrument was tuned for daily performance using the Elan 6100 DRC sensitivity detection limit solution (PerkinElmer Pure, USA).

Chemicals and reagents

The following chemicals were used: concentrated Hydrochloric Acid (HCl) 37% (Fluka, Germany), nitric acid (HNO₃) 69% (Fluka), hydrogen peroxide (H₂O₂, 30%), hydrofluoric acid (HF, 70%) (Dong Woo Fine-Chem Iksan, Korea), and ultrapure deionized water (18.2 M.cm) (Millipore, Bedford, MA, USA). A 10mg/L multi-elemental standard solution from Ana Pure Kriat, Daejeon, Korea was used for preparing standards for calibration curves.

Sample collection for vegetable cultivation

Sample collection: The wastewater and contaminated soil were collected from the Akaki kality sub-city in Addis Ababa, and non-contaminated soil was collected from Debre Birhan in the Amhara region. A total of 4 vegetable seed samples, namely lettuce (*Lactuca sativa L.*), turnip (*Brassica napus L.*), and cabbage (*Brassica oleracea var. capitate*), were purchased from the paisa market, in Addis Ababa.

Sample cultivation: The vegetables (lettuce, turnip, and cabbage) were grown in pots using (PWS, PWCS, CWPS, and CWS) as follows Figure 1.



Figure 1: Depicts the cultivation of vegetables.

The vegetable samples were collected two or three months ago, washed with deionized water to remove unwanted material, sliced with a stainless steel knife, and dried. The dried samples were then homogenized and powdered in a grinder with titanium blades (MR 350 CA, Braun, Spain). The powdered samples were properly labeled and stored in plastic bags at 20°C (Micom CFD-0622, Samsung, Korea) for microwave digestion. The soil and water samples were prepared for digestion before and after the cultivation of the vegetable crops.

Procedures: The dried vegetable and soil samples were mechanically ground and weighted at approximately 0.5 g and added into a Teflon closed vessel, then 7.0 mL of concentrated HNO $_3$ and 2.0 mL of H $_2$ O $_2$ were added for vegetable samples [9] and 9.0 mL of concentrated HNO $_3$ and 3.0 mL of HF were added for soil samples [10, 11], and digested by microwave–assisted digestion. The microwave–assisted digestion was set as follows to digest the samples: 1000W at 80°C for 5 min, 1000W at 50°C for 5 min, 1000W at 190°C for 20min, and 0W for 30min for cooling. After cooling, the contents of the tubes were diluted to 50 mL with deionized water.

Analysis of the sample and quality assurance: Water, soil, and vegetable samples were analyzed using Agilent microwave plasma atomic emission spectrometers (MP-AES). Each metal or element has been measured using its wavelength. Analysis of the samples was performed by standard calibrations. Standard solutions were prepared with the same concentration of acids present in digested samples for all vegetables, water, and soil, by diluting a multi-elemental standard containing the analytes. Each sample was measured in triplicate. The accuracy, precision, and calibration curves of each metal concentration were performed by the standard reference materials. Calibration conditions: calibration correlation coefficient limit of 0.995, bank subtraction: on, stabilization time: 15 min, sample uptake time: 15 min, pump speed: 15rpm.

The Hazard Quotient (HQ)

The hazard quotient (HQ) is the ratio between the daily intake of vegetables (Div) and the reference oral dose (R,D).

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If the ratio is lower than 1, there will be no obvious risk [12]. An estimate of the potential hazard of metals to human health (HQ) through the consumption of vegetables is calculated as follows:

$$HQ = (Div) \times (Cmetal)/RfD \times Bo$$
 (1)

Where (Div) is the daily intake of vegetables (kg per day), (C_{metal}) is the concentration of metal in the vegetable (mg/kg), R_fD is the oral reference dose for the metal (mg/kg of body weight per day), and Bo is the human body mass (kg). The hazard quotient (HQ) indicates the health risk level due to exposure to pollutants [13].

Plant-to-soil transfer factor

The metal concentration in the extracts of soils and plants was calculated on the basis of dry weight. The plant Transfer Factor (TF) was calculated as follows:

$$TF = C_{plant}/C_{soil}$$
 (2)

 C_{plant} and C_{soil} represent the toxic metal concentration in extracts of plants and soils on a dry weight basis, respectively.

Data examination

The obtained data were analyzed using one-way ANOVA and Minitab17 software. The results were reported within the 95% level of confidence or (p<0.05) significant difference level and mean \pm standard deviation of each triplicated data point.

Results and discussion

The average concentration ±SD of each heavy metal in pure water, wastewater, pure soil, and contaminated soil samples collected from the two sites is given in Table 1. In addition, Tables 2,3 also show the mean ±SD concentration of heavy metals, mainly Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr in vegetable crops, namely lettuce, cabbage, and turnip, two and three months ago, respectively. The accumulation of these heavy metals in the mentioned vegetable plants or crops was different in the different treatments (PWS, PWCS, CWPS, and CWS), respectively Figures 2-4.

The concentration of heavy metals (Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr) in contaminated soil was found to be 39434.9 ± 30.0 , 3183.7 ± 43.5 , 222.0 ± 10.2 , 22.28 ± 2.5 , 0.057 ± 0.00 , 5.8 ± 0.03 ,

Table 1: Concentration (mg/kg) of trace metals (mean ± SD) in soil and (mg/L) in water as pre analysis.

Sample	Area	Fe	Mn	Zn	Cu	Со	Ni	Pb	Cr
PS	Debre Birhan	15963.3±95.1	872.1±22.4	67.3±2.6	2.25±0.01	ND	ND	1.17±0.09	2.7±0.05
CS	Akaki kality	39434.9 ±30.0	3183.7±43.5	222.0±10.2	22.28±2.5	1.20±0.00	5.8±0.03	72.72±0.01	148.6±15
PW	Domestic water	2.2± 0.3	ND	0.08±0.00	ND	ND	ND	0.08±00	ND
CW	Akaki kality	5.2±0.5	0.8±0.08	0.72±0.01	1.05±0.03	0.01±0.00	0.2±00	0.72±0.01	0.14±0.02

Table 2: Concentration (mg/kg) of trace metals (mean ± SD) in vegetable crops in the pot experiment after two months of growing.

Vegetables	Treatments	Fe	Mn	Zn	Cu	Со	Ni	Pb	Cr
	PWS	465.4±77.8	80±11.8	7.1±0.25	0.96±0.05	ND	ND	0.65±0.08	0.08±0.01
Cabbage (Brassica oleracea var.	PWCS	1648.8±68.4	145.8±25.8	16.0±0.5	2.5±0.02	ND	0.65±0.00	10.5±1.8	1.18±0.03
capitate)	CWPS	624.5±125.3	87.0±38.2	8.3±0.04	1.1±0.00	ND	ND	0.93±0.03	0.27±0.04
	CWS	1657.8±45.0	155.8±45.9	16.1±0.6	3.8±0.01	ND	1.3±0.02	11.5±2.0	1.35±0.08
	PWS	460.6±69.5	120.3±15.7	10.2±0.4	1.03±0.08	ND	ND	0.36±0.06	0.22±0.00
Lettuce (Lactuca sativa L.)	PWCS	1651.0±39.8	141.8±19.8	26.3±10.2	2.08±0.06	ND	0.84±0.03	7.01±1.5	1.16±0.03
Lettuce (Lactuca Sativa L.)	CWPS	638.5±89.7	217.2±68.6	9.2±0.7	1.02±0.01	ND	ND	1.03±0.01	0.24±0.00
	CWS	1698.9±62.5	260.3±75.2	32.8±9.3	4.13±0.05	ND	1.3±0.08	12.3±0.8	1.75±0.04
	PWS	472.6±59.6	262±22.0	16.2±1.2	1.4±0.03	ND	ND	0.92±0.04	0.54±0.03
Turnin (Brassias nanus I.)	PWCS	1575.2±95.1	455±85.3	53.8±11.7	2.33±1.2	ND	1.02±0.05	23.0±5.3	1.25±0.07
Turnip (Brassica napus L.)	CWPS	1080.0±67.5	270.3±96.4	21.7±5.2	1.32±0.04	ND	ND	1.05±0.05	0.91±0.06
	CWS	1708.2±81.6	555.0±94.3	55.3±10.7	5.26±0.04	ND	1.42±0.07	24.5±10.2	6.25±0.08

Table 3: Concentration (mg/kg) of trace metals (mean ± SD) in vegetable crops in the pot experiment after three months of growing.

Table 3: Concentration (mg/kg) or trace metals (mean ± Sb) in vegetable crops in the pot experiment after three months of growing.									
Vegetables	Treatments	Fe	Mn	Zn	Cu	Co	Ni	Pb	Cr
	PWS	755.7±63.4	118.3±36.4	12.0±8.5	1.02±0.08	ND	ND	0.87±0.00	0.17±0.02
Cabbage (Brassica oleracea var.	PWCS	1838.8±48.3	171.6±43.5	34.0±11.0	2.7±0.05	ND	0.55±0.01	13.4±1.6	2.5±0.08
capitate)	CWPS	1464.5±55.6	134±11.8	27.0±13.4	1.1±0.02	ND	ND	1.08±0.05	0.28±0.03
	CWS	2440.5±74.1	183.0±39.5	42.0±10.2	4.5±0.04	ND	1.5±0.01	17.0±7.4	2.44±0.07
	PWS	830.6±87.5	237.7±18.7	15.0±12.8	2.4±0.00	ND	ND	0.89±0.01	0.18±0.01
Lettuce (Lactuca sativa L.)	PWCS	2831.3±75.8	374.0±63.4	66.0±17.0	2.8±0.07	ND	0.95±0.02	17.0±2.4	2.01±0.04
Lettuce (Lactuca sativa L.)	CWPS	2353.0±44.8	282.6±44.3	63.7±14.7	2.4±0.00	ND	ND	1.02±0.06	0.54±0.02
	CWS	3906.2±86.7	522.3±92.6	82.0±16.3	5.7±0.08	ND	1.5±0.03	22.0±8.5	2.77±0.08
	PWS	972.6±59.6	266.3±34.0	20.5±2.3	2.0±0.01	ND	ND	0.93±0.04	0.64±0.5
Turnin (Dananina namus I.)	PWCS	3904.7±64.4	363.4±54.6	52.0±25.0	3.5±0.12	ND	1.2±0.00	22.7±6.9	2.95±0.57
Turnip (Brassica napus L.)	CWPS	3262.65±82.5	293.6±87.1	48.0±12.6	2.1±0.06	ND	ND	1.06±0.03	1.01±0.04
	CWS	3977.8±78.2	485±75.7	63.0±14.5	5.84±0.08	ND	1.52±0.04	25.1±12.0	6.71±0.18

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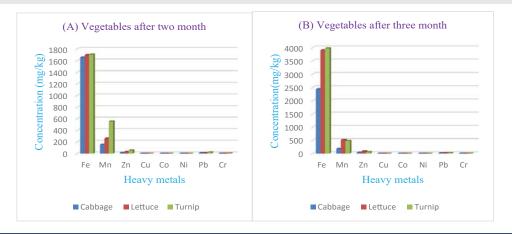


Figure 2: (A and B). The mean concentrations of heavy metals in cabbage, lettuce, and turnip growing in CWS collected the sample after two and three months' age, respectively.

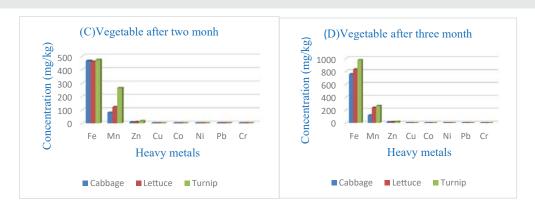


Figure 3: (C and D). The mean concentrations of heavy metals in cabbage, lettuce, and turnip growing in PWS collected the sample after two and three months' age, respectively.

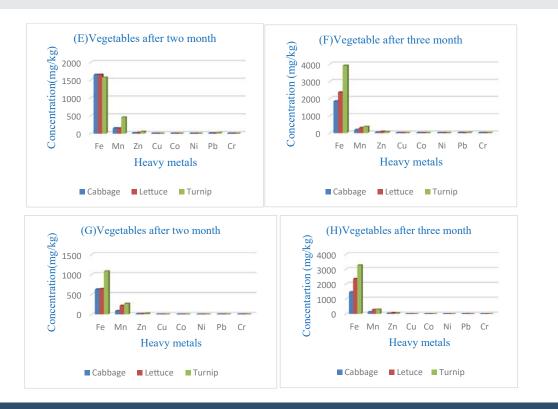


Figure 4: (E, F, and G, H). The mean concentrations of heavy metals in cabbage, lettuce, and turnip growing in PWCS and CWPS after two and three months' age, respectively.

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72.72±0.01, 148.6±15mg/kg, respectively. The obtained values of these heavy metals are compared with different organizations and literature as shown in Table 4. The mean concentration of Fe, Cu, and Ni were found to be which are less than the results of Fe, Cu, and Ni (43317.8, 83.70, and 294.2), respectively found in Cuba [16]. Most of the concentrations of trace metals in waste or contaminated water in the study were found to be higher than the different organization permissible limits [17-20]. In addition, the mean concentration of Fe, Mn, Zn, Cu, Co, Ni, and Cr in pure water was found to be less than the WHO permissible limits [21] but the concentration of Pb was found to be higher than the WHO standards. The concentration of (Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr) metals in cabbage growing in CWP were found to be 2440.5±74.1, 183.0±39.5, 42.0±10.2, 4.5±0.04, ND, 1.5±0.01, 17.0±7.4 and 2.44±0.07, respectively. The concentration of (Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr) metals in lettuce growing in CWP were found to be, 3906.2±86.7, 522.3±92.6, 82.0±16.3, 5.7±0.08, ND, 1.5±0.03, 22.0±8.5 and 2.77±0.08, respectively. The concentration of (Fe, Mn, Zn, Cu, Co, Ni, Pb, and Cr) metals in turnip grow in CWP were also found to be, 3977.8±78.2, 485±75.7, 63.0±14.5, 5.84±0.08, ND, 1.52±0.04, 25.1±12.0 and 6.71±0.18, respectively. The results of these metals in cabbage, lettuce, and turnip were not in good agreement with the literature, which means the metal

concentrations in cabbage lettuce, and turnips were found to be higher than the literature values [22,23].

Conclusion and recommendation

The accumulation of metal concentration in soil, water and vegetables in decreasing order is Fe >Mn >Zn > Cr > Pb> Cu> Ni> Co, Fe > Cu > Mn > Zn = Pb> Ni > Cr > Co and Fe > Mn > Zn > Pb > Cu> Cr > Ni> Co, respectively, and the accumulation of heavy metals in three month growth vegetables were found to be higher than two month growth in all lettuce, cabbage and turnip vegetables. At both two and three months of age, the accumulation of heavy metals in turnip > lettuce> cabbage. Soil-plant transfer factor of these metals in cabbage, lettuce, and turnip ranged from 0.01 to 0.34, which indicates most of the pollutants transferred from soil to plant than water to plants. This result indicates heavy metals had significant health risk effects to the consumer associated with the consumption of these vegetables, especially at the high maturity stage grown within contaminated areas. Therefore, we recommend that toxic heavy metals must be removed or reduced using different technologies before being discharged into the environment or there must be adulated treatments before and after releasing the waste from the sources.

Table 4: The comparison of concentration (mg/kg) of trace metals in soil, vegetables, and (mg/L) in water (grow within CWS after three months ago) results with the literature and standards.

Matrices		Organization /	- ·	Elements								
Matri	ces	Country	Reference	Fe	Mn	Zn	Cu	Со	Ni	Pb	Cr	
Soil			This study	39434.9 ±30.0	3183.7±43.5	222.0±10.2	22.28±2.5	0.057±0.00	5.8±0.03	72.72±0.01	148.6±15	
		US EPA	[14]	NG	NG	110.00	270.00	-	72.00	200.00	100.00	
		Ghana	[15]	NG	NG	37.33	202.99	-	72.00	183.66	-	
		Cuba	[16]	43317.80	1446.8	90.70	83.70	-	294.20	34.6	-	
			This study	5.2±0.5	0.8±0.08	0.72±0.01	1.05±0.03	0.01±0.00	0.2±00	0.72±0.01	0.14±0.02	
	Irrigation	WHO	[17,18]	5.00	0.400	5.00	2.00	-	0.02	0.01	0.10	
Water		FAOUN	[19]	5.00	0.20	2.00	0.20	0.05	0.20	5.00	0.10	
		US EPA	[20]	5.00	0.20	2.00	0.20	0.05	0.20	5.00	0.10	
	Drinking		This study	2.2± 0.3	ND	0.08±0.00	ND	ND	ND	0.08±00	ND	
		WHO	[21]	3.00	0.400	5.00	0.05	0.05	0.006	0.01	0.05	
Vegetables	Cabbage		This study	2440.5±74.1	183.0±39.5	42.0±10.2	4.5±0.04	ND	1.5±0.01	17.0±7.4	2.44±0.07	
		Ethiopia	[22]	43.81	72.45	1.08	2.66	0.21	0.38	2.80	0.11	
		Egypt	[23]	76.90	21.71	14.90	0.43	1.05	0.29	0.00	-	
		Brazil	[24]	-	-	-	-	0.51	0.54	1.66	0.39	
		China	[25]	-	-	2.42	0.25	-	0.048	0.038	0.013	
	Lettuce		This study	3906.2±86.7	522.3±92.6	82.0±16.3	5.7±0.08	ND	1.5±0.03	22.0±8.5	2.77±0.08	
		Ethiopia	[22]	112.95	118.65	4.82	5.30	0.21	0.70	1.55	0.32	
		Egypt	[23]	323.90	20.37	42.00	0.90	0.67	0.70	3.70	-	
	Turnip		This study	3977.8±78.2	485±75.7	63.0±14.5	5.84±0.08	ND	1.52±0.04	25.1±12.0	6.71±0.18	
		India	[26]	-	-	-	2.8	-	-	6.7	-	
		Brazil	[24]	-	-	-	-	0.33	0.26	0.58	0.21	

Table 4 Showing limits of selected heavy metals in soil, wastewater, and vegetables as recommended by US EPA, Ghana, Cuba, Egypt, India, China, Brazil, FAOUN, and WHO. Where NG stands for not given. *US EPA*- The United States Environmental Protection Agency, FAOUN- Food and Agriculture Organization of the United Nations, and WHO world Health Organization.

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Acknowledgement

The Ethiopian Institute of Agricultural Research's directorate of food science and nutrition research has provided financial assistance for this project. The author wishes to convey their heartfelt gratitude for their assistance and contribution to the completion of this article.

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