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Assessment of concentrations of heavy metals in three leafy vegetables irrigated with wastewater in Hadnet district, Mekelle, Ethiopia

Hailekiros Tadesse *, Desta Berhe Sbhatu and Gebreselema Gebreyohannes

Department of Biological and Chemical Engineering, Mekelle Institute of Technology, Mekelle University, P.O. Box 1632; 231, Mekelle, Ethiopia
*Corresponding author. E-mail: hailekiros.tadesse@mu.edu.et; h.trick14@gmail.com

HT, 0000-0003-2007-0113

ABSTRACT

Mekelle is one of the Ethiopian cities suitable for urban and peri-urban agriculture for cultivating leafy vegetables using wastewater. The consumption of unprocessed and processed leafy vegetables is also very high in the city. Wastewater samples collected from four experimental sites (ESs) in Hadnet district of the city had higher concentrations in 4 (i.e., Cd, Cr, Cu, and Mn) of the 10 heavy metals tested than the permissible limit established by pertinent standards. Spring water samples collected from another site called Kallamino, designated as a comparison site, also had higher concentrations in 4 (i.e., Al, As, Cd, and Cu) of the 10 heavy metals tested. However, the leafy vegetables grown in the least contaminated ES had higher concentrations in 6-7 of the 10 heavy metals tested. The lettuce and spinach samples had elevated concentrations of As and Al, respectively. The wastewater used to irrigate vegetable farms in Hadnet district is not safe enough. More importantly, the soils of the farms might have accumulated far more heavy metals. The cultivated lettuce, spinach, and cauliflower are highly contaminated. Thus, the use of wastewater for irrigating urban and peri-urban farms needs to be regulated.

Key words: heavy metals, leafy vegetables, Mekelle city, wastewater

HIGHLIGHTS

- Tests of wastewaters used in urban agriculture for heavy metal contents.
- Tests of three leafy vegetables irrigated with wastewater for heavy metal contents.
- Contamination of the three leafy vegetables with high level of 6 to 7 heavy metals tested.

ABBREVIATIONS

Al	Aluminum
Alt.	Altitude
As	Arsenic
Cd	Cadmium
Cr	Chromium
CS	Control site
Cu	Copper
E	East
ES	Experimental site
FAO	Food and Agriculture Organization
Fe	Iron
g	Gram
H_2O_2	Hydrogen peroxide
Hg	Mercury
HNO_3	Nitric acid
Lat.	Latitude
Long.	Longitude
masl	Meters above sea level
mg/L	Milligram per liter
Min	Minutes
mL	Milliliter
Mn	Manganese

N	North
ND	Not detected
NIST	National Institute of Standards and Technology
Pb	Lead
SD	Standard deviation
SPSS	Statistical Package for Social Science
Zn	Zinc

INTRODUCTION

Heavy metals often enter into the environment due to anthropogenic activities. The toxicity, persistence, non-biodegradability, and bioaccumulation of the metals are regarded as the most severe environmental problems and human health risks (Souri *et al.* 2019). Toxic heavy metals enter the human body through the food chain and cause serious health problems. Human exposure to toxic heavy metals causes several health complications that can lead to death such as increased blood acidity, retardation of development, increased incidence of various cancers, interfering with the absorption of essential elements, and kidney damage.

Domestic and industrial wastewater is a critical medium of contaminant for the environment, which increases heavy metals and is disposed of into the environment (Souri *et al.* 2018, 2019). Wastewaters are discharged from wastewater treatment plants, industries, mining sites, power stations, and many other sites and facilities (Perrodin *et al.* 2013). Environmental contamination with heavy metals corresponds with the intensification of urbanization, agricultural activities, and industrialization that have the potential to accumulate heavy metals and contaminate the environment (Souri *et al.* 2018; Nkosi & Msimango 2022). Leafy and non-leafy vegetables irrigated with wastewater often accumulate heavy metals on their surfaces and tissues (Perrodin *et al.* 2013; Manzoor *et al.* 2018; Bahiru & Teju 2019; Rahman & Islam 2019; Gebeyehu & Bayissa 2020; Nkosi & Msimango 2022). The consumption of such produces contaminated with elevated levels of heavy metals has become a great concern for human health (Gebeyehu & Bayissa 2020).

Urban and peri-urban agriculture is being promoted as an important sector for livelihood and economic activities in many parts of the world including Ethiopia. Mekelle city, Tigrai, Ethiopia – endowed with many rivers and streams – is one of the fast-growing cities suitable for urban and peri-urban agriculture. In fact, urban and peri-urban agriculture for growing leafy and non-leafy vegetables is quite common in the city. Moreover, the consumption of unprocessed and processed leafy vegetables is very high. However, the quality of the produces has been of great concern. Some studies have reported that vegetables grown using the waters of some of the rivers and streams of the city have an excess accumulation of heavy metals (e.g., Gebeyehu & Bayissa 2020). Therefore, this study aimed to investigate the quality of water from unexplored rivers and streams used for irrigating leafy vegetables and the safety of three leafy vegetables grown there in terms of heavy metal accumulation. The findings of the study will help consumers to make good choices and take proper precautions when consuming leafy vegetables as well as relevant public entities to implement proper regulatory measures on urban and peri-urban agriculture.

MATERIALS AND METHODS

Description of the study area

The study was conducted in two districts of Mekelle city called Hadnet and Kallamino. Mekelle, the capital city of Tigrai, Ethiopia, is located about 200 km southeast of the historic city Aksum (alt.: 1,900 to 2,200 masl; lat.: 13° 30′ 0″ N; long.: 39° 28′ 11″ E). The climate of the city is semi-arid, with a mean annual temperature of 16–20 °C and an annual rainfall of 600 mm (Gebreyohannes 2019). Hadnet and Kallamino watersheds are about 6 km apart, with a plateau separating them.

Design of the study

The study was conducted in five farmlands. The first four farmlands are located in Hadnet district (experimental site (ES)), while the fifth one was located in Kallamino (comparison site (CS)). The farmlands in the ES are irrigated with wastewater, while that in the CS is irrigated with spring water. The farmlands in the ES are located around or at Adi Haqi (ES1), Enda Mikael (ES2), Tsa'eda Agam (ES3), and Fitsum Birhan Eye Hospital (ES4). Thus, the study compared the concentrations of 10 heavy metals in the (a) wastewater and spring water samples and (b) leafy vegetable samples collected from the least polluted ES and the CS.

Sample collection

Five samples of wastewater were collected from each of the four farms of the ES. Likewise, 10 samples of spring water were collected from the farm in the CS. Also, samples of mature cauliflower (*Brassica oleracea* var. *botrytis*) (10), lettuce (*Lactuca sativa)* (10), and spinach (*Spinacea oleracea*) (10) were collected from ES1 and the CS. While the wastewater samples were collected in clean polythene bottles, the vegetable samples were collected in separate sterile plastic bags. The vegetable and wastewater samples were taken to the geology lab in the College of Natural and Computational Sciences, Mekelle University for analyses. The vegetable samples were thoroughly washed with sterile distilled water, air-dried for 1 h, and put in an electric oven at 70 °C for 24 h. Dried samples were ground with a mortar and pestle into a fine powder, sieved, and kept in polythene bags for further analyses.

Analyses of heavy metals in vegetable samples

Vegetable samples such as cultivated lettuce, spinach, and cauliflower were prepared for extraction and were digested using the wet digestion method of protocol developed by scholars (Verlicchi *et al.* 2012; Perrodin *et al.* 2013; Aydin *et al.* 2019). A 1.25 g digested vegetable sample was taken in a glass beaker and dried at 103 °C for 24 h. About 25 mL of HNO_3 and three boiling chips were put into a destruction tube and heated at 100 °C for 1 h. The sample was subsequently heated at 125, 150, 175, and 200 °C for 15 min at each temperature. The solution was concentrated and cooled to about 5 mL. One milliliter of 30% H_2O_2 was added to the destruction tube and kept for 10 min. Then, 3 mL of 30% H_2O_2 and 25 mL of distilled water were added and boiled for 10 min to dissolve the sample. Finally, the sample was cooled and transferred to a 250 mL volumetric flask, settled for 15 h, and the absorbance of the clear supernatant was measured by atomic absorption spectroscopy. The precision and accuracy of the analyses were checked through repeated analysis against the NIST standard reference material 1570A.

Analyses of heavy metals in water samples

Each of the wastewater and spring water samples was subjected to the nitric acid digestion method (Verlicchi *et al.* 2012; Perrodin *et al.* 2013; Aydin *et al.* 2019). About 50 mL of the sample was transferred into a clean glass beaker, and then 10 mL of concentrated HNO_3 was added. The beaker was placed on a hot plate at 80 °C and the solution evaporated until it reduced to 20 mL. Then, the content was cooled, and 5 mL of concentrated HNO_3 was added and heated again on a hot plate. A small portion of concentrated HNO_3 was cautiously added to the beaker until the solution appeared transparent. After that, distilled water was added to the beaker until the volume of the solution reached the 50 mL mark and was filtered through Whatman No. 42 filter paper. Finally, the concentrations of heavy metals were determined using a flame atomic absorption spectrophotometer (Varian FAAS-240). For quality control analyses, glassware was washed with 10% HNO_3 and calibrated using standard solutions of each heavy metal. The precision and accuracy of analyses were checked through repeated analyses against the NIST standard reference material RM 1643E.

Data analyses

Acquired data were analyzed using inferential statistical methods with SPSS Version 23.0 software. Mean ($\pm$SD) values of heavy metal concentrations of the samples of wastewater, spring water, and leafy vegetables were computed using the analysis of variance and were compared against the values of the permissible limits established by the Food and Agriculture Organization (FAO 2004).

RESULTS

Concentrations of heavy metals in the waste and spring waters

Among the four sites of Hadnet district, copper (Cu) was recorded at the highest concentration above the permissible limit of the FAO (2004) at ES2 (1.1140 ± 0.0635 mg/L) followed by ES3 (1.0860 ± 0.0841 mg/L), ES4 (0.9420 ± 0.0390 mg/L), and ES1 (0.6120 ± 0.0249 mg/L) (Table 1). According to the FAO standards, the concentrations of Hg, Pb, Zn, Mn, Fe, and Al in the wastewater were within the safe limit. However, the concentrations of Cu ($0.5460 \pm 0.0241 – 0.7149 \pm 0.0058$ mg/L), Cr ($0.6120 \pm 0.0249 – 1.1140 \pm 0.0635$ mg/L), Cd ($0.0620 \pm 0.0045 – 0.1182 \pm 0.0176$ mg/L), and Mn ($0.1246 \pm 0.0112 – 0.1602 \pm 0.0035$ mg/L) were found to be above the permissible limit. On the other hand, the spring water of the CS had Al (0.1330 ± 0.0116 mg/L), As (0.0350 ± 0.0053 mg/L), Cr (0.2617 ± 0.0186 mg/L), and Cu (0.3752 ± 0.0191 mg/L) above the safe limit.

Table 1 | Concentrations of heavy metals in the waste and spring water samples

	Concentration (mg/L) (mean ± SD)					
Heavy metals	ES1 ($n=5$)	ES2 ($n=5$)	ES3 ($n=5$)	ES4 ($n=5$)	CS ($n=10$)	FAO
Al	0.0013 ± 0.0005	0.0011 ± 0.0002	0.0017 ± 0.0008	0.0010 ± 0.0001	0.1330 ± 0.0116	0.008
As	0.0019 ± 0.0002	0.0018 ± 0.0002	0.0022 ± 0.0012	0.0039 ± 0.0002	0.0350 ± 0.0053	0.004
Cd	0.0800 ± 0.0128	0.1182 ± 0.0176	0.0924 ± 0.0056	0.0620 ± 0.0045	0.2617 ± 0.0186	0.010
Cr	0.5460 ± 0.0478	0.7194 ± 0.0058	0.6300 ± 0.0316	0.5460 ± 0.0241	0.0250 ± 0.0053	0.050
Cu	0.6120 ± 0.0249	1.1140 ± 0.0635	1.0860 ± 0.0841	0.9420 ± 0.0390	0.3752 ± 0.0191	0.050
Fe	0.5520 ± 0.0541	0.6040 ± 0.0336	0.7760 ± 0.0385	0.6240 ± 0.0208	1.5120 ± 0.0148	260.000
Hg	0.0027 ± 0.0005	0.0044 ± 0.0006	0.0009 ± 0.0001	0.0020 ± 0.0001	0.0001 ± 0.0003	0.006
Mn	0.1310 ± 0.0288	0.1246 ± 0.0112	0.1602 ± 0.0035	0.1456 ± 0.0112	0.0128 ± 0.0013	0.100
Pb	0.0012 ± 0.0004	0.0021 ± 0.0002	0.0011 ± 0.0002	0.0021 ± 0.0002	0.0259 ± 0.0336	0.100
Zn	0.6560 ± 0.0513	0.6800 ± 0.0381	0.5760 ± 0.0643	0.7360 ± 0.0297	0.0534 ± 0.0059	2.000

ES, experimental site; CS, comparison site; SD, standard deviation; FAO, Food and Agriculture Organization.

Concentrations of heavy metals in vegetables irrigated with wastewater

Wastewater acquired from ES1 was relatively less polluted as compared to those acquired from the other three ESs. Thus, the investigation into the concentration of heavy metals in the three leafy vegetables was carried out by taking samples from this least polluted site. The three leafy vegetables grown in ES1 were found to have accumulated Al (0.0100 ± 0.0000 mg/L), As (0.0100 ± 0.0000 – 0.0300 ± 0.0000 mg/L), Cd (0.1500 ± 0.0300 – 0.5800 ± 0.0400 mg/L), Cr (1.8500 ± 0.2900 – 3.4900 ± 0.3800), and Fe (434.0000 ± 6.6700 – 503.4000 ± 12.3600) above the safe limit established by the FAO. Moreover, whereas Pb was found above the safe limit in lettuce and spinach, Zn was above the safe limit in lettuce and cauliflower (Table 2). The concentrations of some of the heavy metals are very high (e.g., As, Cd, Cr, and Pb). These imply that the heavy metal accumulation in such leafy vegetables will be much higher in the more contaminated sites of ES2, ES3, and ES4.

Concentrations of heavy metals in vegetables grown in spring water

With the exception of Al in spinach and As in lettuce, the concentrations of the heavy metals in the vegetables irrigated with spring water at the CS are below the permissible limits (Table 3). Even the concentrations of Al in spinach (0.0080 ± 0.0008 mg/L) and As in lettuce (0.0020 ± 0.0006 mg/L) are only slightly above the safe limits. The concentrations of Al, As, Cd, and Cu were above the permissible limits in the spring water of this site (Table 1).

Table 2 | Concentrations of heavy metals in leafy vegetables collected from ES1

	Concentration (mean ± SD) (mg/L)				
Heavy metals	Lettuce ($n=10$)	Spinach ($n=10$)	Cauliflower ($n=10$)	FAO	Levels of Contamination
Al	0.0100 ± 0.0000	0.0100 ± 0.0000	0.0100 ± 0.0000	0.006	1.67 times
As	0.0300 ± 0.0000	0. 0100 ± 0.0000	0.0100 ± 0.0000	0.001	10.0 – 30.0 times
Cd	0.5800 ± 0.0400	0.1500 ± 0.0300	0.3400 ± 0.0300	0.100	1.50 – 5.80 times
Cr	3.4900 ± 0.3800	2.3100 ± 0.3300	1.8500 ± 0.2900	1.000	1.85 – 3.49 times
Cu	0.8000 ± 0.0800	0.9200 ± 0.2200	1.3200 ± 0.2800	73.000	–
Fe	450.0000 ± 7.9100	503.4000 ± 12.3600	434.0000 ± 6.6700	425.00	1.02 – 1.18 times
Hg	ND	ND	ND	0.010	–
Mn	18.8000 ± 4.9700	17.2000 ± 1.9200	8.0000 ± 2.2400	500.00	–
Pb	0.6600 ± 0.1300	0.6100 ± 0.1100	ND	0.300	2.03 – 2.20 times
Zn	119.0000 ± 3.8100	ND	114.0000 ± 76.2800	100.00	1.14 – 1.19 times

SD, standard deviation; FAO, Food and Agriculture Organization; ND, not detected.

Table 3 | Concentrations of heavy metals in the vegetables collected from the CS

Heavy metals	Concentration (mean ± SD) (mg/L)			FAO
	Lettuce ($n = 10$)	Spinach ($n = 10$)	Cauliflower ($n = 10$)	
Al	0.0020 ± 0.0004	0.0080 ± 0.0008	0.0020 ± 0.0004	0.006
As	0.0020 ± 0.0006	0.0010 ± 0.0004	ND	0.001
Cd	0.0790 ± 0.0134	0.0580 ± 0.0148	0.0862 ± 0.0062	0.100
Cr	0.9000 ± 0.0381	0.8500 ± 0.0762	0.8400 ± 0.0822	1.000
Cu	ND	ND	ND	73.000
Fe	251.0000 ± 9. 6177	179.8000 ± 10.0349	194.4000 ± 8.8487	425.000
Hg	ND	ND	ND	0.010
Mn	0.9020 ± 0.2056	0.7700 ± 0.1723	0.8780 ± 0.0626	500.000
Pb	0.2670 ± 0.0120	0.0432 ± 0.0160	0.0720 ± 0.0192	0.300
Zn	83.6000 ± 5.5946	ND	ND	100.00

SD, standard deviation; FAO, Food and Agriculture Organization; ND, not detected.

DISCUSSIONS

This study has led to three important observations. First, the vegetables grown in farms irrigated with wastewater have a higher accumulation of heavy metals than those grown in farms irrigated with spring water. This is quite natural and many studies have come up with similar observations. For example, higher concentrations of heavy metals were observed in radish, spinach, turnip, cauliflower, and carrots grown in farms irrigated with wastewater as compared to those irrigated with spring water (Arora *et al.* 2008; Ahmad *et al.* 2016).

The second important observation is that the leafy vegetables grown in ES1 have higher levels of accumulation in 6 or 7 of the 10 heavy metals than the wastewater used to irrigate it. This implies that the wastewater is neither the direct nor the only source of the heavy metals for the vegetables. The soil, as continuously exposed to contaminated wastewater, can accumulate heavy metals over the course of time much more than the flowing wastewater. The physical and chemical properties and compositions of the soils such as pH, organic matter content, cation exchange capacity, nutrient balance, and metal permissibility can also lead to the accumulation of heavy metals in soils (Qureshi *et al.* 2016; Yu *et al.* 2018; Weber *et al.* 2019; Yang *et al.* 2020). Moreover, despite the concentrations of many of the heavy metals in the wastewater being below the safe limit, what has reached the vegetables can accumulate in their tissues to far greater levels. The bioaccumulation of heavy metals in vegetables is affected by many factors such as climate, atmospheric depositions, heavy metal concentrations in soil, the nature of the soils of the vegetable farms, and the degree of maturity of the plants at the time of harvest (Bhargava *et al.* 2012; Ali *et al.* 2013). Therefore, enhanced levels of heavy metals can be accumulated in the leafy vegetables through the soil and by way of bioaccumulation. In line with this, the higher accumulation of the heavy metals despite their relatively lower concentration in the wastewater can also be due to their higher transfer factor. The heavy metals of Cd, Cr, Fe, Zn, and Pb, detected in the vegetables above the permissible level, have a high transfer factor from the soil and water to the vegetables (Luo *et al.* 2011; Verlicchi *et al.* 2012; Perrodin *et al.* 2013; Aydin *et al.* 2019).

The third important observation is the difference in the accumulation of heavy metals in different vegetable types. The bioaccumulation of heavy metals of lettuce is higher than spinach and cauliflower and the bioaccumulation of cauliflower is lower than both the lettuce and spinach. These variations may account for differences in morphologies and anatomies, absorption and assimilation capacities, and sensitivities to the heavy metals of the vegetables (Pandey *et al.* 2012; Xiong *et al.* 2014; Khan *et al.* 2015; Qureshi *et al.* 2016). The findings of the present study are similar to previous ones that demonstrated that leafy vegetables that grow quickly with high transpiration and absorption rates bio-accumulate higher concentrations of heavy metals quickly (Luo *et al.* 2012; Durowoju *et al.* 2016; El-nour 2020).

CONCLUSION

The three vegetables grown in the ESs in the Hadnet district of Mekelle city provided us with important indications that the soils of the farms are accumulating heavy metals. The observation that the fast-growing lettuce has higher bioaccumulation capacity implies that other fast-growing produce cultivated in farms irrigated with wastewater can have similar contamination levels. Thus, urban and peri-urban agriculture in Mekelle city for growing leafy vegetables has to be monitored not to use wastewater for irrigation. Likewise, the soils of the farms growing such vegetables need to be examined if they are suitable. Furthermore, consumers need to be cautious in their consumption of vegetables growing in urban and peri-urban farms.

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AUTHORS' CONTRIBUTIONS

H.T.T., D.B.S., and G.G. were involved in conceiving, designing, and planning the study. H.T.T. carried out the collection and preparation of water and vegetable samples, the determination of heavy metal contents and data collection, the organization and analyses of the data, and the drafting of the manuscript. G.G. reviewed the draft manuscript and developed the final draft, and D.B.S. reviewed the manuscript for content, language, and style and readied it for publication.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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