Toxicological assessment of Pb, Cd and Cr in lettuce and onion grown around Ellala River in Mekelle, Tigray, Ethiopia

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ABSTRACT

The purpose of this study was to investigate the toxicological effect of Pb, Cd and Cr in vegetables. Water, soil and vegetables samples were collected to determine heavy metal content using FAAS and its impact on human health via consumption of vegetables. The heavy metals for water samples were recorded in the order of Pb>Cd>Cr, and all, except Cd, were found under WHO/FAO limits. Similarly, the heavy metals of soil samples were also recorded in the order of Cr>Cd>Pb, which was under Ewers and EU permissible limits. On lettuce, mean levels of heavy metals were Pb 0.34 ± 0.04 mg/kg, Cd 1.07 ± 0.12 mg/kg and Cr 3.50 ± 0.23 mg/kg were recorded above the WHO/FAO limits. On onion, levels were 0.75 ± 0.05 mg/kg for Pb, 0.41 ± 0.04 mg/kg for Cd and 1.98 ± 0.27 mg/kg for Cr, which were above the WHO/FAO limits. The high metal content could be attributed to high anthropogenic activities. The TF (transfer factor) for the heavy metal in vegetables also showed a trend of Cd>Pb>Cr. The TF of Cd on lettuce ranged between 0.827 and 0.914, because of the high Cd mobility and its high bio-accumulation factor in lettuce. Thus, TF values of vegetables above 0.5 are considered to be contaminated and thus needs for continuous follow-up. However, the health risk index and daily intake rate of heavy metals of the study indicates the vegetables are safe to eat. But, due to the bioaccumulation nature of the heavy metals and the continual use of these vegetables with increase anthropogenic activities and demand of people for vegetables may pose health problem.

Keywords: Heavy metals, Vegetables, Transfer factor, Soil, Toxicological effect

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INTRODUCTION

As the most vital resource for all kinds of life on earth, water is essential for ensuring the integrity and sustainability of the earth's ecosystems. Rivers are important water bodies that play an important role in the sustenance of life in general and in the development of a given society in particular. In many countries, it is a common practice to grow vegetables along banks of rivers passing through urban centers. However, rivers are being polluted due to rapid industrialization, urbanization and other developmental activities. Disposal of sewage water and industrial wastes drained to agricultural lands poses a number of potential risks to human health via consumption of or exposure to the pathogenic microorganisms (WWAP, 2003).

Heavy metals, which are not only extremely persistent in the environment but also non-biodegradable in nature, are elements that are toxic and poisonous in relatively higher concentrations (Okweye, 2013). Heavy metal contamination, which is caused by weathering of soils, rocks and other anthropogenic activities, is a major problem of our environment and our food supply (Jarup, 2003). This problem is receiving increasingly more attention all over the world in general and in developing countries in particular. The biological half-lives of these heavy metals are long and have potential to accumulate in different body organs resulting in unwanted side effects (Sathawara *et al*., 2004; Shakeri *et al*., 2009; Tiwari *et al*., 2011).

Significant contamination of plants and plant products with toxic heavy metals emanating from contaminated soil and water has been observed through their release of these toxicants into the sea, rivers, lakes and irrigation channels. The consumption of contaminated vegetables constitutes an important route of animal and human exposure. Apart from their natural presence, vehicular emissions, pesticides, fertilizers, industrial effluents and other anthropogenic activities contaminate soils used for the cultivation of the vegetables within and by the sides of cities (Moraghan, 1993). Heavy metals in soils reduce the yield of vegetables by disturbing their metabolic processes (Gangwar, 2013). Soil, irrigation water and some vegetables from pre-urban sites are significantly contaminated by the heavy metals such as chromium (Cr), cadmium (Cd) and lead (Pb) which affect the nutritive values of vegetables as well as human health (Singh, 2006). To the best of our knowledge, the level of heavy metal contamination of lettuce *(Lactuca sativa* L.) and onion (*Allium cepa* L.) irrigated from Ellala River has not been reported. Therefore, the aim of this research work was to determine the concentration of heavy metals in water, soil, lettuce and onion vegetables irrigated with Ellala River. In addition, the risk of heavy metals through consumption of lettuce and onion cultivated in agricultural land along the river side was assessed.

MATERIALS AND METHODS

Description of the Study Area

Mekelle, the capital of Tigray National Regional State, is situated in northern Ethiopia. It is located at 13°29'45" N latitude and 39°28'26" E longitude. According to the Central Statistical Agency, its total population was 286,600 (Weldegebreal, 2015). The city is generally characterized as semi-arid with low rainfall for only two months per year. Mean daily temperature fluctuates between 11.5 and 31.7°C and mean annual rainfall received ranges between 24.0 and 486.0 mm/month (Mebrahtu and Zerabruk, 2011). The study was conducted in Ellala River which is found north of the city. The four sampling sites of the study area were randomly selected using the GPS (global positioning systems) along the river (Figure 1). Site 1 (S1), Feleg Daero, was a point where the river enters the city; Site 2 (S2), Qualay Kacha is found at the Mekelle-Adigrat highway on which sanitation activities of different vehicles and body hygiene takes place. Site 3 (S3), Wehnibet, is near to the metal work industries and Site 4 (S4), Mariam Dihan, is the point where the river leaves the city.

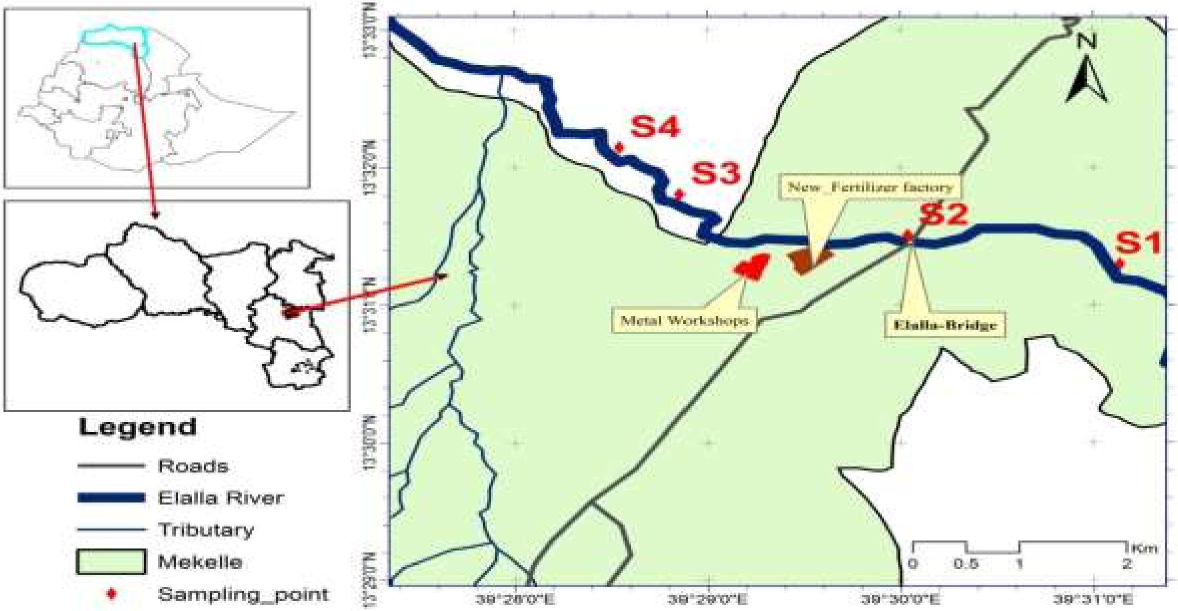

Figure 1. Map of the study area.

Instrument and chemicals

A drying oven with forced air and timer (FED 53, USA), hot plate, measuring cylinder, analytical balance (LARK LA114), volumetric flasks, filter paper Whatman No 42, burette and sample vials were used for sample preparation and analysis.

A Varian AA240 FS Fast Sequential Atomic Absorption Photometer (Varian, Australia) fully automated and PC controlled using a spectra AA base and PRO software versions equipped with fast sequential operation for multi-elemental flame determinations, using four lamp positions and automatic lamp selection, were used for the determination of heavy metals (Cd, Cr and Pb). The chemicals used were: 70% HNO_3 (BDH, England), 70% $HClO_4$ (Aldrich, Germany), 37% HCl (Riedel-de Hean, Germany), deionized water while Sigma flock was used in the analysis. All used chemicals were of analytical grade for analysis of elements in trace levels.

Collection and preparation of water samples

All water samples were collected using polyethylene bottles, which were pre-washed with 10% nitric acid and double distilled water. Before sampling, the bottles were rinsed three times with water from the sampling site and immersed in about 20 cm below the water surface. All water samples were immediately brought to the laboratory and filtered through Whatman No 42 filter paper. The samples were acidified with 5 mL HNO_3 and stored in a refrigerator at 4°C for analysis (APHA, 2005).

Collection and preparation of soil samples

About 1 kg soil samples were collected from each vegetable farm sampling site using stainless steel auger at 0-15 cm depth and stored in plastic bags. All the well mixed soil samples were dried in an oven at 105ºC for 12 hours to a constant weight, ground using mortar and pestle, passed through a 200 mesh (75 µm) sieve and stored in labeled containers till further analysis (Inoti, 2012).

Collection and preparation of vegetable samples

Root and leaf parts of the lettuce were separated with knife, washed with tap water and rinsed with deionized water to remove dust particles and air dried. The dried samples were pound into fine powder with porcelain mortar and pestle and packed till analysis (Gupta, 2008).

Onion sampling and pretreatment. Onion samples were collected from each of the three farm sites where the irrigation waters and soil were collected. Composite samples of onion, which is grown along the river, were collected depending on their availability. The samples were put in clean plastic bags and brought to the laboratory for further pretreatment. The edible parts of the onion samples were separated with Teflon knives, washed with a running tap water and rinsed with deionized water and air dried. After peeling the outer parts of the onion samples, the bulbs were cut into nearly equal smaller sizes and dried in oven at 80°C for 48 hours till it became brittle and crisp. Cooled to ambient temperature, the dried samples were ground into fine powder with porcelain mortar and pestle. The powdered sample was then placed in pre-cleaned polyethylene container and stored till digestion (Kitata and Chandravanshi, 2012).

Analytical Procedures

Heavy metals analysis. Selected heavy metals, i.e., Pb, Cd and Cr, were determined in triplicate using FAAS at Ezana Mining Laboratory PLC in Mekelle, Tigray, Ethiopia. The instrument working conditions (Table 1) and various instrumental parameters were optimized accordingly (Table 2).

Heavy metal analysis from water. Triplicate of 50 ml water sample was transferred into three beakers (400 mL) and 10 mL of conc. HNO3 was added to each beaker containing water sample which allowed evaporating at temperature of 80ºC in a fume hood until the solution became transparent. The mixture was cooled and then filtered into 100 mL volumetric flask using Whatman No 42 filter paper, and the flask was filled up to the mark with deionized water. Reagent blank was also prepared to check the contamination while heavy metals Cd, Pb and Cr were determined by FAAS (APHA, 2005).

Table 1. Instrument working conditions and limit of detection for the analysis of selected heavy metals in water, soil and vegetable samples of Ellala,

Element[a]	**Pb**	**Cd**	**Cr**
Lamp current (mA)	5	4	7
Wave length (nm)	217.0	228.8	357.9
Silt width (nm)	1.0	0.5	0.2
LOD[b], mg/L for water	0.01	0.01	0.02
LOD (mg/kg) for soil	1.0	1.0	1.0
LOD (mg/kg) for vegetables	0.02	0.01	0.02

[a]In all cases, “Air” was used as “support”, and C_2H_2 as fuel; [b]LOD stands for Limit of detection.

Heavy metal analysis from soil. About 50 g of oven dried ground soil sample was transferred into 400 mL beaker, and 80 mL of HCl (37%) and 30 mL of HNO_3 (70%) were added, respectively. The mixture was heated at160ºC for 1 hr in fuming hood and then cooled to room temperature. After cooling, 2 mL of sigma flock was added for settling purpose. Then, the mixture was transferred to a 250-mL volumetric flask filled with double distilled water to the mark, let to settle for at least 15 hours, filtered and analyzed for total Cr, Pb and Cd by FAAS (Aderinola and Kusemiju, 2012).

Heavy metal analysis from lettuce and onion samples**:** About 1.0 g of each ground lettuce and onion powder were taken into 100 mL beaker and digested using 3 mL HNO_3 (70%), 1 mL of HCl (37%) and 1 mL of $HClO_4$ (70%) at 85°C for 3 hours in fuming hood. After cooling, the solutions were filtered using Whatman No 42 filter papers, diluted to 50 mL and analyzed for total Cr, Cd and Pb by FAAS (Aderinola and Kusemiju, 2012). Blanks were prepared in the same way.

Method validation

The accuracy of digestion procedure and efficiency of the FAAS instrument for soil and water were checked by spiking samples with known concentration of the analyte. Spiked samples were prepared by adding a small known quantity of metal standard solutions to three sub-samples of water sample and soil sample by applying similar digestion procedure and analyzing for the levels of metals and calculating the recovery percent (Kitata and Chandravanshi, 2012). The procedure followed in the spiking of both irrigation water and soil is described below. Recovery study of the onion was carried out by using standard reference material (CRM IPE628) obtained from Ezana Mining Development PLC for all metals in plant and Pb in soil sample. The mean of each of the elements analyzed for the certified reference material is given in Table 2.

Table 2. Validation of analytical method using plant (CRM IPE682) and soil reference materials from Ezana Mining Plc (Mean ± SD in mg/L), n= 6.

Metals	**Sample**	**Measured**		**References**		**Percent recovery**
		Mean	**STDEV**	**Mean**	**STDEV**	
Pb	Plant	7.04	1.42	6.77	0.874	104.0
	Soil	8.14	1.02	9.00	0.89	90.4
Cd	Plant	0.345	0.026	0.319	0.028	108.2
Cr	Plant	0.574	0.116	0.615	0.153	93.3

Spiking of irrigation water samples. Three sub-samples of irrigation water from Ellala River, 50 mL each, were taken in 250 mL beakers and to each a known volume and a concentration of 0.01 mg/L of Pb, Cd and Cr, separately were added. Then after, the spiked samples were filled to the mark by the deionized water and digested using the optimized procedures. The spiked samples were run on FAAS for metal content determination to calculate the percent recoveries (Aderinola and Kusemiju, 2012).

Spiking of soil sample. Three sub-samples of soil, each weighing 50 g, were transferred to beakers (400 mL) and known concentrations of metal standard solutions (0.01 mg/L for Pb, Cd and Cr, respectively) were added to each of the three flasks. The spiked samples were digested using the optimized procedure for the soil sample and finally analyzed using FAAS to calculate the percent recoveries (Bigdeli and Seilsepour, 2008).

Health Risk Assessment

Transfer factor (TF). TF is the metal concentration in plant tissue above the ground divided by the total metal concentration in the soil. It signifies the amount of heavy metals in the soil that have ended up in the plant. TF was calculated for each metal according to the following equation (Arora et al., 2008):

$$TF = \frac{C(Vegetable)}{C(Soil)} \quad (1)$$

Where C (vegetable) stands for heavy metal concentration (mg/kg) in the extract of edible parts of vegetables; C (soil) represents the heavy metal concentration (mg/kg) in soils from where the vegetable was grown.

Daily intake rate (DIR). For the daily intake rate (DIR), the average metal content in vegetable was calculated and multiplied by the respective consumption rate. It was determined using Equation 2 (Kitata and Chandravanshi, 2012).

$$DIR = C\ (Metal\ conc.) \times C\ (Factor) \times D\ (Vegetable\ intake) \quad (2)$$

Where C (metal conc.) stands for heavy metal concentration in vegetables (mg/kg), C (factor) for conversion factor (0.085), D (vegetable intake) for daily intake of vegetable (kg $person^{-1}day^{-1}$). The conversion factor of 0.085 was set to convert fresh vegetable weight to dry weight based on Equation 3.

$$IRdw = IRww \left[\frac{100-W}{100}\right] \quad (3)$$

Where *IRdw* stands for dry-weight intake rate, *IRww* for wet-weight intake rate and *W* for percent water content (Arora *et al*., 2008; Bigdeli and Seilsepour, 2008; Khan *et al*., 2009).

Health risk index (HRI). The HRI was determined as per equation 4:

$$HRI = DIR/R_fDo \qquad (4)$$

Where DIR stands for daily intake rate, RfDo for reference oral dose for each trace elements (Rattan *et al*., 2005). Humans were considered to be safe if HRI < 1.

Statistical Analysis

All data were statistically analyzed by evaluating the mean and standard deviation. Mean values obtained for Pb, Cd and Cr from the sampling areas were compared by One-Way ANOVA using SAS version 9.1.3. Level of significance was set at α=0.05.

RESULTS AND DISCUSSION

Recovery test

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples in water and soil (Table 3). Each test was repeated at least three times to ensure precision. The recoveries of metals in the spiked samples were 100 to 106%. Thus, percent recoveries were under acceptable ranges of all studied methods, and each standard deviation was less than 10% (Kitata and Chandravanshi, 2012).

$$\textit{Percent-Recovery} = \frac{amount\ after\ spike - amount\ before\ spike}{amount\ added} \times 100\% \qquad (5)$$

Where AAS stands for amount after spike, ABS for amount before spike, and AA for amount added.

Heavy metals concentration of water from Ellala River

The concentration of heavy metals found in soils and water used for irrigation is summarized in Table 4. The mean concentration (mg/L) of heavy metals in river Ellala water was highest for Pb, followed by Cd and Cr. Cr was not detected from S1, S2 and S4 samples. The elevated concentration of Pb at S2 might be due to automobile exhaust, car wash practice and domestic waste disposal taking place at the river in this site. Generally, trends of Pb values in water from all sites were recorded in the order of S2 > S3 > S4 > S1. The mean calculated value of lead was found to be under the permissible limits set for irrigation water by the WHO/FAO (5 mg/L) (WHO/FAO, 2007) and Indian (0.01 mg/L) (Awashthi, 2000). However, the results were above USEPA (0.015 mg/L) irrigation water standards (USEPA, 2010). According to the results of one-way ANOVA, S2 was significantly different from the other studied sites. This might be due to the improper disposal of municipal as well as domestic sewage from garages and automobile exhaust of the high traffic area on Mekelle to Adigrat road.

The average values of Cd in water of this study were 0.015 ± 0.005 mg/L for S1, 0.025 ± 0.004 mg/L for S2, 0.033 ± 0.006 mg/L for S3 and 0.017±0.005 mg/L for S4. Even though Cd was found in highest concentrations among the three metals in S3, all the values were safe to use considering WHO/FAO (0.01 mg/L), Indian (0.01 mg/L) and USEPA (0.005 mg/L) standards for irrigation water (Awashthi, 2000; WHO/FAO, 2007; USEPA, 2010). The statistical analysis at 95% confidence level also showed S3 to be significantly different from the other study sites. This might be due to the disposal of wastes from the metal work industries in that area. Cr values in water from Ellala River were not detected for S1, S2 and S4. Nevertheless, S3 recorded Cr value of 0.025±0.005 which might be due to the result of effluents discharged from small scale metal works found around that area. The concentration of Cr in S3 was below the permissible level of WHO/FAO, UESPA and Indian standards set for irrigation water.

Concentration of heavy metals in soil from Ellala farms

Table 5 shows concentrations of the studied heavy metals (Pb, Cd and Cr) in soil samples. The mean concentration of Pb in soil sample of this study ranged from 4.56 ± 0.136 to 5.89 ± 0.080 mg/kg, Cd from 1.08 ± 0.024 to 1.28 ± 0.063 mg/kg and Cr from 16.70 ± 0.737 to 19.50 ± 0.453 mg/kg. Soil samples from Ellala farms gave high Cr mean values than other heavy metals. These concentrations were below permissible limits set by Ewers and EU (Ewers, 1991; European Union, 2002).

Pb concentration was 4.56 ± 0.136 mg/kg at S1, 5.89 ± 0.080 mg/kg at S2, 5.63 ± 0.125 mg/kg at S3 and 4.85 ± 0.281 mg/kg at S4. S2 and S3 had significantly higher concentration than S1 and S4. Reasons could be motor vehicle exhaust and car wash at S2 and waste disposal from the metal work industries at S3. At S1 and S4, anthropogenic activities were low. Pb values recorded in this study were lower than previous reports. Reports of Pb levels on vegetable farms of Addis Ababa area include 46.7 mg/kg at Peacock, 110 mg/kg at Kera, 32.7 mg/kg at Kolfe and 48.74 mg/kg at Akaki (Itanna, 2002). Other reports include 11.1 to 41.2 mg/kg from Koka village, near to Addis Ababa (Tamene and Seyoum, 2015). Therefore, Addis Ababa area had more Pb concentration than Ellala River suggesting more anthropogenic practice. Lower concentration of lead (3 to 5 mg/kg) was reported from Mayham, Adigrat area (Brhane *et al*., 2004).

Cd concentration varied from 1.28 ± 0.063 to 1.08 ± 0.024 mg/kg (Table 5). A mean of 1.28 ± 0.063 mg/kg at S2 and 1.27 ± 0.064 mg/kg at S3 were significantly the highest records (according to the results of the ANOVA). Reports from other areas of Ethiopia showed higher value of Cd than values from Ellala. For example, reports from Koka farms show more Cd value, i.e., 2.1 to 21.1 mg/kg (Tamene and Seyoum, 2015) than Ellala farms (1.1 to 1.3 mg/kg). On the other hand, Ellala farms had more Cd values than Addis Ababa (0.4-0.95 mg/kg) (Itanna, 2002), Ejersa area of East Shoa (0.03-0.35) (Asfaw, 2014) and Adigrat Mayham (1.00 mg/kg) (Brhane *et al*., 2014). This suggests that carcinogenic Cd occurs on soils of Ellala and needs early intervention.

Cr values on Ellala soils ranged from 16.7 ± 0.737 to 19.5 ± 0.453 mg/kg. Again, S2 (19.30 ± 0.237 mg/kg) and S3 (19.50 ± 0.453 mg/kg) gave high Cr values because of wastes discharged from small scale metal workshops in that area. More Cr concentration than Ellala has been reported from other areas of Ethiopia. In Addis Ababa area, for example, Cr concentration was very high that ranged between 81 and 283 mg/kg (Itanna, 2002). This shows that Addis Ababa area was more polluted than Ellala. At Ejersa of East Shoa values ranged between 10.3 and 125 mg/kg (Asfaw, 2014). The one at Ellala was lower than 20 mg/kg. Koka village had low Cr value, i.e., 1.7 to 11.4 mg/kg.

Heavy metal concentration in selected vegetables

Because of their broad leaf nature, leafy vegetables are more susceptible to pollutant accumulation, i.e., through dust from soil and rainwater (European Union, 2002). Table 6 shows the details. Elevated Pb is generally toxic to plants and human beings. Humans get the toxicants via vegetable consumption. Pb bioaccumulates over time and stays in the body for a long period of time. Mean values of Pb at S2 and S3 were statistically different on onion but not on lettuce. In this study, Pb content ranged from 0.157 ± 0.020 to 0.465 ± 0.042 mg/kg for lettuce and 0.300 ± 0.049 to 1.390 ± 0.089 for onion. Pb levels were higher on onion at S2 because of emissions from motor vehicles at the Adigrat to Mekelle main road and wastes from garages effluents from industries. Pb values were higher in onion than in lettuce in all sites of the study. Mean values of Pb on onion were 1.39 ± 0.089 mg/kg at S2, 0.547 ± 0.166 mg/kg at S3, 0.300 ± 0.049 mg/kg at S4. On lettuce, values were 0.0157 ± 0.02 mg/kg at S1, 0.465 ± 0.042 mg/kg at S2, 0.438 ± 0.059 mg/kg at S3 and 0.287 ± 0.053 mg/kg at S4. Pb values on vegetables from S2 and S3 exceeded maximum permissible levels of WHO/FAO standards for vegetables (FAO/WHO, 2001) suggesting the potential health risk from their consumption.

Table 3. Recovery test results for heavy metals determination in water and soil samples using spiking method (Mean ± SD), n=9.

Metals	**Sample types**	**Concentration (mg/L) in irrigation water**	**Concentration (mg/kg) in soil**
Pb	Unspiked sample	0.01	ND
	Spiking amount	0.01	
	Amount after spiked	0.02	
	% Recovery	100±0.0	
Cd	Unspiked sample	0.02	0. 82
	Spiking amount	0.01	1.30
	Amount after spiked	0.03	2.20
	% Recovery	100±0.0	106±0.0
Cr	Unspiked sample	0.04	18.42
	Spiking amount	0.01	19.00
	Amount after spiked	0.05	38.38
	% Recovery	100±0.0	105±0.0

ND = not determined

Table 4. Mean concentration of heavy metals (Mean ± SD, mg/L) in the water of Ellala River, n=9.

Sites	**Heavy metals**			**References**
	Pb	**Cd**	**Cr**	
S1	0.01± 0.001[b]	0.015 ± 0.005[ab]	BDL	
S2	0.04 ± 0.002[a]	0.025 ± 0.004 [b]	BDL	This study (S1 to S4)
S3	0.03 ± 0.011[ab]	0.033 ± 0.006[a]	0.025 ± 0.005	
S4	0.02 ± 0.001[b]	0.017 ± 0.005[ab]	BDL	
WHO/FAO	5.000	0.010	0.10	(WHO/FAO, 2007)
Indian standards	0.100	0.010	0.05	(Awashthi, 2000)
USEPA	0.015	0.005	0.10	(USEPA, 2010)

NB: Means with the same letter are not significantly different; BDL = below detection limit

Table 5. Mean concentration of heavy metals (Mean ± SD, mg/kg) in Soil farms of Ellala River; n=9

Sites	**Pb**	**Cd**	**Cr**	**Reference**
S1	4.56 ± 0.136[b]	1.08 ± 0.024[b]	16.7 ± 0.737[c]	
S2	5.89 ± 0.08[a]	1.28 ± 0.063[a]	19.3 ± 0.237[a]	
S3	5.63 ± 0.125[a]	1.27 ± 0.064[ab]	19.5 ± 0.453[a]	
S4	4.85 ± 0.281[b]	1.21 ± 0.055[b]	18.3 ± 0.620[b]	
Ewers	100	3.00	100	(1991)
EU	300	3.00	150	(2002)

Cd is easily absorbed and translocated to shoots of food crops and may lead to chronic Cd toxicity in human beings (He *et al*., 2005). It can also cause skeletal and kidney damage and induce cancer (Bubb and Lester, 1994). In the current study, Cd levels ranged from 0.893 ± 0.090 to 1.170 ± 0.027 mg/kg on lettuce and from 0.335 ± 0.026 to 0.445 ± 0.028 mg/kg on onion (Table 6), values that exceed FAO/ WHO (2001) standards. Cd levels were high at S2 and S3 than at other sites on both vegetables (Table 6). Reasons for the elevated levels must be the same; emissions and effluents. Levels at S2 and S3 did not vary significantly. Levels at S1 were significantly lower than other sites. Exposure to Cr includes breathing air, drinking water or consuming food containing Cr or even through skin contact. Exposure to elevated levels of Cr causes skin irritation, ulceration, damage to circulatory and nerve tissues, which in turn lead to health problems (Zhuang *et al*., 2009).

In this study, Cr content of the analyzed samples recorded in the range of 3.19 ± 0.172 to 3.76 ± 0.042 mg/kg for lettuce and 1.78 ± 0.069 to 2.35 ± 0.029 mg/kg for onion. Here too, Cr concentration was high at S2 and S3 because of emissions and effluents (Table 6). Lettuce accumulated greater Cr value than onion did. Bioaccumulation of leafy vegetables is normally higher than root vegetables.

While the Cr concentration in lettuce samples from the sampling sites S1 to S4 are in the order of 3.19 ± 0.172, 3.56 ± 0.035, 3.76 ± 0.042, 3.48 ± 0.123; its level in the onion samples showed the same trend 1.810 ± 0.076, 2.35 ± 0.029, 1.78 ± 0.069. S3 had significantly more Cr values than other sites. Concentrations were generally higher in lettuce than in onion. The concentration of carcinogenic heavy metals on lettuce from Ellala farms was in the order of Cr > Cd > Pb and on onion Cr > Pb > Cd (Table 6). All concentrations of heavy metals recorded from vegetables in this study were above FAO/WHO (2001) vegetable standards. Therefore, the use of vegetables from Ellala River is unsafe for human health. Benti (2014) reported the concentration of Pb, Cd and Cr in lettuce from farms around Adama Town. Results from the current study, however, showed lower value for Pb and Cd but higher value for Cr than the results from around Adama.

Table 6. Mean concentration of heavy metals (dry weight, Mean±SD, mg/kg) in lettuce and onion farms of Ellala River; n=9.

Sites		Pb	Cd	Cr
			Lettuce	
	S1	0.157 ± 0.020^{c}	0.893 ± 0.090^{c}	3.190 ± 0.172^{c}
	S2	0.465 ± 0.042^{a}	1.170 ± 0.027^{a}	3.560 ± 0.035^{bb}
	S3	0.438 ± 0.059^{a}	1.150 ± 0.032^{a}	3.760 ± 0.042^{a}
	S4	0.287 ± 0.053^{b}	1.070 ± 0.023^{b}	3.480 ± 0.123^{b}
WHO/FAO (2001)		0.3	0.2	2.3
			Onion	
	S1	ND	ND	ND
	S2	1.39 ± 0.089^{a}	0.427 ± 0.019^{a}	1.81 ± 0.076^{bb}
	S3	0.547 ± 0.166^{b}	0.445 ± 0.028^{a}	2.35 ± 0.029^{a}
	S4	0.300 ± 0.049^{c}	0.355 ± 0.026^{b}	1.78 ± 0.069^{b}
WHO/FAO (2001)		0.3	0.2	2.3

Higher Pb and Cr and lower values of Cd were also reported (Itanna, 2002) in lettuce from Addis Ababa vegetable farms than the results from the present study (Table 7). Besides, Tamene and Seyoum (2015) recorded the concentration of heavy metals in vegetables from Koka village. The finding of these researchers indicated lower concentration of Pb and Cr in onion than the results from the current study. Some previous studies reported higher value of Cd than the current study, e.g., Kitata and Chandravanshi (2012) reported higher value of Cr and no result for Pb from Meki Town. Generally, values of heavy metals in lettuce and onion obtained from Ellala farms showed comparable value to the results obtained from other parts of Ethiopia (Table 7).

Health risk assessment of Ellala River

Soil – plant transfer factor (TF). As vegetables are the source of human diet, the soil-to-plant transfer quotient is the main source of human exposure. A convenient way of quantifying the relative differences of bioavailability of metals to plants is the transfer factor (Zhuang *et al.*, 2009).

Cadmium had greater soil plant transfer rate to lettuce and onion than Cr and Pb (Table 8). Higher transfer quotient of heavy metal indicates stronger accumulation of the respective metal in that vegetable. Transfer quotient of 0.1 indicates that a plant is excluding the element from its tissues. The greater the transfer coefficient value (i.e. more than 0.50), the higher the chances for vegetables to be metal contaminated by anthropogenic activities requiring environmental monitoring of the area (Kitata and Chandravanshi, 2012). The accumulation of metals in vegetables in this study was in the order of: lettuce > onion which was in line with the fact that "leafy vegetables accumulate much higher contents of heavy metals as compared to other vegetables" (Zhuang *et al.*, 2009).

Table 7. Comparison of heavy metal concentrations (dry weight, Mean±SD, mg/kg) analyzed from lettuce and onion vegetable samples in Ellala River with other study reports in Ethiopia

Metals	**Lettuce**			**Onion**	
	Present study	**Benti (2014)**	**Itanna (2002)**	**Present study**	**Tamene & Seyoum (2015)**
Pb	0.37 ± 0.04	0.55 ± 0.20	0.99 ± 0.20	0.74 ± 0.45	0.37 ± 0.14
Cd	1.07 ± 0.22	0.36 ± 0.20	0.22 ± 0.10	0.40 ± 0.23	0.41 ± 0.30
Cr	3.50 ± 0.12	1.86 ± 0.30	5.34 ± 0.40	1.98 ± 0.27	1.30 ± 0.89

This is because leafy vegetables have higher translocation and transpiration rate as compared to other vegetables (Itanna, 2002).

Among the three metals, Cd recorded maximum values for TF, which ranged from 0.827 to 0.914 (for lettuce) whereas Cr was minimum ranging from 0.094 to 0.121 (for onion). Variations in TF among different vegetables may be attributed to differences in the concentration of metals in the soil and differences in element uptake by different vegetables (Lokeshwari and Chandrappa, 2006). In the vegetables, TF of Cd was highest except for onion at S3, which showed that Cd was more mobile than other metals. Lokeshwari and Chandrappa (2006) also reported that Cd is retained less strongly by the soil such that it is more mobile than other metals. Other reason to increased Cd contamination could be the input of added Cd in phosphate fertilizers to increase agricultural yields (Zhuang *et al*., 2009).

Daily intake rate of heavy metals (DIR). The degree of toxicity of heavy metals to human beings depends upon their daily intake. The intake values (Table 8) were calculated by taking the average value of metals in the two vegetables (lettuce and onion) and by considering the body weight of each person (65 kg) consuming approximately 116.7 g/day, which is called provisional tolerable daily intake (PTDI) (WHO, 1992a; WHO, 1992b). Thus, Cr recorded high value of DIM for S2 and S3 in both vegetables, but the lowest value was obtained in Pb and Cd for lettuce and onion, respectively. The general trend of DIM recorded is in the order of Cr > Pb > Cd for lettuce and Cr > Cd > Pb for onion. The highest DIM value for Cr in this study might be due to high value of the metal recorded in these vegetables especially for S3 and S2. The PTDI of heavy metals were below the WHO/FAO PTDI standards for heavy metal intake based on body weight (65 kg) for Pb, Cd and Cr.

Oral reference dose (RfDo). RfDo is an estimate of a daily oral exposure for the human population, which does not cause harmful effects during a lifetime.

Table 8. Transfer factor (TF), daily intake metals (DIM) (mg/kg), health risk index (HRI), of heavy metals via intake of lettuce and onion vegetables grown around Ellala River

Sites		Lettuce			Onion		
		Pb	Cd	Cr	Pb	Cd	Cr
S1	TF	0.034	0.827	0.191	ND	ND	ND
	DIM	2.5E-05	0.00015	0.00052	ND	ND	ND
	HRI	0.006	0.15	0.0003	ND	ND	ND
S2	TF	0.079	0.914	0.814	0.236	0.334	0.094
	DIM	7.6E-05	0.00019	0.00058	0.00023	0.00007	0.0003
	HRI	0.019	0.19	0.0005	0.058	0.018	0.0002
S3	TF	0.078	0.906	0.193	0.097	0.035	0.121
	DIM	7.1E-05	0.00019	0.00061	0.00009	0.00007	0.0004
	HRI	0.018	0.19	0.0004	0.023	0.017	0.100
S4	TF	0.059	0.884	0.19	0.062	0.293	0.097
	DIM	4.7E-05	0.00017	0.00057	0.00005	0.00006	0.0003
	HRI	0.012	0.17	0.0003	0.013	0.015	0.075

ND: Not determined

The values of oral reference doses (RfDo) for heavy metals (Pb 0.004, Cd 0.001 and Cr 1.5) used in the study were determined by FAO/WHO (2013) standards.

Calculated health risk index (HRI). Using the relationship between DIM and RfDo, the health risk index (HRI) was formulated using eqn (6).

$$HRI = \frac{DIM}{RfDo} \qquad (6)$$

Where HRI stands for health Risk Index, DIM for Daily Intake Metal, $RfDo$ for Reference oral Dose for non-carcinogenic toxicity value (mg/kg/day)

If the value of HRI is less than 1, the exposed population is said to be safe (USDA, 2007). Therefore, in the present study, the three heavy metals in all sites (on lettuce and onion) had HRI values lower than 1, suggesting low risk from them on the population. Based on HRI values, the local population did not face challenge by consuming vegetables grown around Ellala River. Nevertheless, because these heavy metals bioaccumulate in the body if consumed for a long time, care should be taken and consumption frequency of the vegetables there should be monitored.

CONCLUSION

Heavy metals such as Pb, Cd and Cr are the known pollutants that are found everywhere, particularly, in areas with high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms while their bio-accumulation in the food chain could be dangerous to human health. Water from Ellala River was found containing high level of Cd which was above the safe concentration limits for irrigation considering WHO/FAO, Indian and USEPA standards. However, heavy values for Pb and Cr were under the safe limits of irrigation water standards. In soil from the farms, concentration of Pb, Cd and Cr were below the EU and Ewers permissible limits, but high anthropogenic activities practiced in the river side areas contributed to the elevation of heavy metals at and around S2 and S3. In the assessment of heavy metal concentrations in onion and lettuce vegetables, lettuce exhibited higher Cd, whereas onion recorded higher Pb. On the other hand, Cr values from S2 and S4 were in border limit with vegetable standards. Nonetheless all values of Pb and Cd metals in the vegetables were above the WHO/FAO joint safe limits. The high recorded heavy metal content in the sample vegetables could be partly attributed to the intensive anthropogenic activities near Ellala River. Based on the findings, it is concluded that lettuce recorded relatively higher bioaccumulation of heavy metals than did onion. Although the value of DIM and HRI showed safe vegetable consumption, the high recorded value of heavy metals (Cd, Cr and Pb) posed health problems for the community. The irrigation water source of Ellala River was polluted by Cd and Pb as a result of automobile exhausts, car wash wastes, chemical fertilizers and waste disposal from metal work industries which could also contribute to concentration of these metals to the soil and vegetables. This has important implications for policy makers to aim at regular monitoring and controlling of heavy metal concentrations in irrigation water sources so that concentrations would not exceed the permissible limits.

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