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Assessment of Selected Heavy Metals in Onion Bulb and Onion Leaf (*Allium cepa* L.), in Selected Areas of Central Rift Valley of Oromia Region Ethiopia

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Abstract

This study was conducted to determine the levels of heavy metals (Pb, Cr, Cd, Fe, Cu, Zn, and Mn) in onion bulb and onion leaf around Mojo, Meki and Ziway areas to assess the concentrations of the heavy metals. The levels of the elements were determined using flame atomic absorption spectrometer. The concentrations of Cr in onion bulb and Fe in onion leaf were above the permissible level (2.3 mg/kg, 425.5 mg/kg) set by FAO/WHO at Mojo (4.87 mg/kg, 1090.40 mg/kg), Meki (4.13 mg/kg, 1836.47 mg/kg) and Ziway (3.33 mg/kg, 764.33 mg/kg) respectively. The results generally indicate that the consumption of these onion bulbs could be the health risk respective to Cr.

Keywords: Heavy metals; Onion bulb; Onion leaf

Introduction

Heavy metals are trace metals which are detrimental to human health and having a density at least five times that of water and are toxic or poisonous at low concentrations [1]. Heavy metals include cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), iron (Fe), and platinum group elements. If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of, then a gradual build-up of these toxins occurs.

Heavy metals are among the major contaminants of food supply and may be considered to be the major problem to our environment. Such problem is getting more serious all over the world especially in developing countries such as North and South Africa, Turkey, Yemen, Zimbabwe, Nigeria, Tanzania and Egypt. These metals are given special attention throughout the globe due to their toxic and mutagenic effects even at very low concentration. The implication associated with heavy metal contamination is of great concern, particularly in agricultural production system. They are not biodegradable, have long biological half-lives, toxic in nature and potential for accumulation in the different body organs leading to unwanted side effects [2]. Dietary exposure to heavy metals, namely chromium (Cr), cadmium (Cd), lead (Pb), arsenic(As), mercury(Hg), zinc (Zn), copper (Cu) and other has been identified as a risk to human health through the consumption of vegetable crops [3].

Even though, previous research was done [4] on the concentrations of heavy metals in the study area, information on the contamination of vegetables with heavy metals from the irrigation farms in Rift Valley region (Mojo, Meki and Ziway areas) of Ethiopia has not yet been fully established and no updated information exist. Therefore, the present study is carried out with the aim to assesses, compare and investigate the levels of heavy metals (Pb, Cd, Zn, Cu, Mn, Fe, and Cr) in onion bulb and onion leaf samples of study areas.

Materials and Methods

Description of the study area

The study was carried at Mojo (Lome woreda, Dunguge village), Ziway (Adami Tulu Jida combolcha wereda, Halaku-golba-boqe and Golbala-aluto villages) and Meki (Dugda Woreda, Shumi Gamo village).

Mojo is located 80 Kms South of Addis Ababa, in Oromia Regional state, Ethiopia. It has a latitude and longitude of 8°39′N 39°5′E with an elevation between 1788 and 1825 meters above sea level. A medium-sized leather factory and it is situated near the Mojo River and channels directly to the river course. It has been reported that Mojo river, is highly polluted by discharging effluent from Mojo tannery industry; and waste disposed from the town [4].

Meki is located in 140 km south from the capital city, Addis Ababa, Ethiopia. It has a latitude and longitude of 8°9′N 38°49′E/8.150°N. Meki River is crosses Meki Town which is most used for irrigation in the country.

Ziway is located on the road connecting Addis Ababa to Nairobi in the East Shewa Zone of the Oromia Region of Ethiopia. It has a latitude and longitude of 7°56′N38°43′E with an elevation of 1643 meters above sea level. Adjacent to Lake Ziway (Lake Dambal), the economy of the town is based on fishing and horticulture. Ziway is also home to caustic soda, castle, floriculture and pesticide factories.

Apparatus and instrument

Chopping board and Teflon knife were used to cut onion bulb and onion leaf samples in to pieces while air-circulating oven (WTC binder) were used for drying. Analytical balances (Sartorius analytic) were used to weigh the samples. Muffle furnace (Nabertherm) was used for dry-ashing process of vegetables samples. Round bottom flasks with ground glass joint fitted with reflux condenser was used for digesting the samples on block digester heating apparatus. Borosilicate volumetric flasks (50 ml and 100 ml) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders, pipettes, and micropipettes (100 μL-1000 μL) was used during measuring different quantities of volumes of sample solution, acid reagents and

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metal standard solutions. Metals concentration determination was done by flame atomic absorption spectrophotometer (FAAS) (Agilent Technologies, 200 Series AA) that used air-acetylene as fuel-oxidant mixture and that was equipped with deuterium background corrector and hollow cathode lamps.

Chemicals and reagents

All the reagents and chemicals used in this study were analytical grade. Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals Cu, Zn, Cr, Pb, Fe, Mn and Cd standard solutions were used to prepare 100 mg/L intermediate standard solutions. From 100 mg/L intermediate standard five different range working standard solution were prepared for each metals. Distilled water (chemically pure with specific resistance of 8.0 ms/cm at the working environment) was used for dilution of sample and intermediate metal standard solutions as well as for rinsing glassware and sample bottles.

Sample collection and preparations

Before sampling, there was survey assessment for identification of exact pollution source, distance cover, discharge sites and affected areas. Onion plant with emphasis on irrigation water was concern of the study because roots of onion are so shallow that; frequent and deep irrigation with water is required so as to keep the soil moisture level high. The large and frequent application of water might increase salt and mineral build-up in soil after evaporation. The area was highly productive of onion than the other vegetable.

Vegetable sample collection and preparation

Vegetable samples of Onion samples from one plot was collected from Mojo, Meki and Ziway and brought to one for composite. Therefore, from three plot three composite onion samples of 1 kg was purposely collected from the agricultural areas of Mojo, Meki and Ziway. These samples were purchased from farmer's field of agricultural sites that are freshly harvested, handpicked and packed in labeled paper bags and transported to the Holeta research laboratory for analysis. The samples were regularly collected in one month interval from December 2016-January 2017. The vegetables were washed thoroughly with tap water for the removal of soil particles and further were washed with distilled water. The onion bulb and onion leaf was separated and labeled. The vegetable samples were oven dried at 70°C for 24 h, crushed and grounded to powder.

Ashing of vegetable samples

Vegetable samples were digested using dry ashing method [5]. 0.5 g of grounded powder samples were weighted and transferred to a clean crucible, which was labeled according to the sample number and dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550°C and then left to ash at this temperature for 6 h [6]. The samples were removed from the furnace and allowed to cool in the hood carefully. The ash was wetted with 1 ml distilled water and 2.5 ml conc. HCI was added. After cooling filtered using Whatman filter No.41. The filtered samples were then diluted up the mark of 100 ml.

Instrumental calibration

Calibration curves were prepared to determine the concentration of the heavy metals in the sample solutions. Intermediate standard solutions (100 mg/L) of each metals were prepared from stock standard solutions containing 1000 mg/L of Cd, Cr, Pb, Cu, Zn, Fe and Mn. According to the instrument operation manual to attain its better sensitivity and working standards were then aspirated one after the other into the flame atomic absorption spectrometry and their absorbance was recorded. Calibration curves were plotted with different points for each of these metals standard using absorbance against concentrations (mg/L).

Analysis of samples

Concentrations of chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and lead (Pb) in the filtrate of digested vegetables samples were estimated by using an Atomic Absorption Spectrophotometer. The instrument was fitted with specific lamp of particular metal. Air-acetylene as fuel-oxidant mixture was used as the fuel and air as the support.

Data analysis

All the results of analysis were reported as mean ± standard deviation of triplicate measurements. The data was computed using Statistical Package for Social Science (SPSS) statistic 10.0 Microsoft window) for heavy metal analysis and in all statistical analyses descriptive statistics was used. As the level of heavy metal contamination might vary with sample collection site and vegetable type.

Bioconcentration factor (BCF)

The transfer of trace elements from soil to plant edible parts is best described by considering the bioconcentration factor. BCF is calculated as the ratio of the concentration of heavy metals in the vegetables to that in the corresponding soil where vegetables were obtained, all based on (dry weight) for each vegetable separately and calculated using equation bellow.

The bioconcentration factor=[C] plant/[C] soil

Where; [C] plant and [C] soil represent the heavy metal concentration in extracts of plants and soils on a dry weight basis respectively.

The BCF of above 1.0 indicates higher uptake of heavy metals in vegetables than in the soil and BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in vegetables and therefore low uptake of heavy metals to vegetables. Several reports have dealt with BCF determinations for vegetables [7].

Results and Discussion

In this study, the method validation was made by the spiking experiment in which known quantities of the metals standard solution were added to three samples which collected from sampling area for onion bulb sample and applied the whole procedure to the mixture (spiked samples) and calculated the percent recoveries (Table 1). The obtained percentage recovery varied from 80% to 120% in onion bulb samples which were in the acceptable range [8].

The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis of un-spiked sample and calculating the relative standard deviations for each metal. The % RSD results did not differ by more than 15% of the mean which indicated that the analytical method used was precise and reliable [9].

The correlation coefficients of all the calibration curves were>0.99 and these correlation coefficients showed that there was very good correlation (relationship) between concentration and absorbance.

Concentration of metals in onion bulb and onion leaf

The concentration of metals in vegetable samples of onion bulb and onion leaf irrigated with water were analyzed for trace essential (Cu, Fe, Mn, Zn and Cr), and trace non-essential metals (Cd and Pb) with

Concentration onion bulb sample (mg/kg)					
Metal	Un-spiked sample	Spiked amount	Recovered amount	% Recovery spiked amount	% RSD
Cr	4.87 ± 0.50	6.00	10.55 ± 0.05	94.67	7.90
Cu	3.93 ± 0.61	4.00	7.84 ± 0.004	97.75	2.32
Zn	12.42 ± 0.29	0.9	13.34 ± 0.4	102.22	8.64
Pb	0.33 ± 0.12	4.0	4.28 ± 0.2	98.75	4.64
Cd	0.05 ± 0.02	1.5	1.35 ± 0.07	86.67	3.32
Mn	8.20 ± 0.20	4.0	12.03 ± 0.5	95.75	2.44
Fe	20.87 ± 0.64	12.0	32.58 ± 0.93	97.58	3.08

Table 1: Recovery test results for the metals determination in onion bulb sample (mean ± SD).

FAAS. The levels of metals along with standard deviation of triplicate analysis for onion bulb and Onion leaf were given in Table 2.

ND not detected, * Means followed by different letters within the same row are significantly different at 5% probability level.

The pattern of concentrations of metals in onion bulb at Mojo was in the order of (Fe>Zn>Mn>Cr>Cu>Pb>Cd), Meki (Fe>Mn>Zn>Cr,>Cu,>Cd) and Ziway (Zn>Mn>Cu>Fe>Cd). Cr and Pb were also below the detection limit in all onion leaf at the three sites. The concentration of Cr in onion bulb at Mojo, Meki and Ziway areas were 4.87 mg/kg, 4.13 mg/kg and 3.33 mg/kg respectively and this result was above the permissible level of (2.3 mg/kg) FAO/WHO. But the other metals were within the ranges of FAO/WHO. Different vegetable species accumulate different metals depending on environmental conditions, metal species and plant available forms of heavy metals [10].

The trend in concentrations of metals in onion leaf at Mojo was (Fe>Mn>Zn>Cu), Meki (Fe>Mn>Zn>Cu) and Ziway (Fe>Zn>Mn>Cu). The average metal concentrations of onion leaf at Mojo were 1090.40 (Fe), 63.33 (Mn), 45.20 (Zn), 8.73 (Cu), for Meki 1836.47 (Fe), 63.73 (Mn), 31.20 (Zn), 1.93 (Cu), for Ziway (764.33) Fe, (92.27) Zn, (60.07) Mn and (1.20) Cu mg/kg (Table 2). From the above results only Fe were above the permissible level of FAO/WHO and was also comparable with literature value of [11]. Generally onion leaf had high concentration of Fe, Zn, Mn, and Cu than onion bulb (Table 2). Reta and Bhagwan reported 18 mg/kg for Zn, 8.8 mg/kg for Mn, 4.9 mg/kg for Cr and 3.2 mg/kg for Cu concentration in onion bulb, which is nearly similar to the values obtained in this study.

Chromium: Cr (III) is an essential element required for normal sugar and fat metabolism. It is effective to the management of diabetes and it is a cofactor with insulin. Cr (III) and its compounds are not considered a health hazard, while the toxicity and carcinogenic properties of Cr (VI) have been known for a long time [6]. The concentration level of Cr in onion at Mojo, Meki and Ziway were 4.87 mg/kg, 4.13 mg/kg and 3.33 mg/kg respectively. These values are above the maximum permissible limit of 2.3 mg/kg by FAO/WHO [12]. Therefore, the study showed that in the onion bulb, Cr contents were exceeded the permissible limit. So the levels of Cr obtained in the present study could be health risk to consumers. But in onion leaf the concentration of Cr was not detected. The reason is that Cr is retained in the roots and only a minor portion reaches the shoot [13].

Copper is an essential micronutrient involved in a number of biological processes needed to sustain life. However, it can be toxic when present in excess [14]. As can be seen from Table 2, the concentrations of copper in onion bulb and onion leaf at Mojo (3.93, 8.73), Meki (1.33, 1.93) and Ziway (0.87, 1.20) mg/kg respectively. The result was below the permissible limits of 73.3 mg/kg by FAO/WHO [12]. The highest concentration was observed in onion leaf. The reason is that several types of transporter proteins are involved in the root-to-shoot transport of metals. Metal ions are also trans located from source to sink tissue *via* phloem. Therefore, phloem sap contains metals arising from source tissue, like Fe, Cu, Zn, and Mn [15].

Zinc is essential to all organisms and has an important role in metabolism, growth, development and general well-being. It is an essential co-factor for a large number of enzymes in the body. Zinc deficiency leads to coronary heart diseases and various metabolic disorders [16]. As can be seen from Table 2, the average concentration levels of zinc in onion bulb and onion leaf at Mojo were 12.42, 45.20 at Meki 7.71, 31.20 and at Ziway 13.47, 92.27 mg/kg respectively. Onion leaf contains high concentration of Zn than onion bulb. This was similar with Cu translocation. The content of Zn reported in this study is generally lower than the permissible levels set by FAO/WHO [12].

Lead: Results obtained in the present study showed that high concentration of Pb were obtained in onion bulb at Mojo (0.33) mg/kg, but not detected at other sites including in onion leaf. These results were above the permissible limit 0.3 mg/kg by FAO/WHO [12]. So, the levels of Pb obtained in the present study indicate health risk to consumers at Mojo site. The most probable reason could farmland near to the road. Lead toxicity is known to cause musculo-skeletal, renal, ocular, neurological, immunological, reproductive and developmental effects [17].

Cadmium is highly toxic non-essential heavy metal and it does not have a role in biological process in living organisms. Thus, even in low concentration, cadmium could be harmful to living organisms [17]. Cadmium poisoning in man could lead to anaemia, renal damage, bone disorder and cancer of the lungs [18]. As can be seen from Table 2, the average concentrations of cadmium in onion bulb at Mojo, Meki and Ziway were 0.05, 0.03 and 0.06 mg/kg respectively. These result below permissible limits 0.2 mg/kg by FAO/WHO [12]. But, in onion leaf concentration of Cd was not detected.

Metals	Sites						FAO/ WHO (2001 mg/kg)
	Mojo		Meki		Ziway		
	Onion bulb	Onion leaf	Onion bulb	Onion leaf	Onion bulb	Onion leaf	
Cr	4.87 ± 0.50	ND	4.13 ± 0.12	ND	3.33 ± 0.12	ND	2.3
Cu	3.93 ± 0.61	8.73 ± 0.31	1.33 ± 0.50	1.93 ± 0.61	0.87 ± 0.12	1.20 ± 0.00	73.3
Zn	12.42 ± 0.29	45.20 ± 0.20	7.71 ± 0.61	31.20 ± 0.20	13.47 ± 0.24	92.27 ± 0.42	99.4
Pb	0.33 ± 0.12	ND	ND	ND	ND	ND	0.3
Cd	0.05 ± 0.02	ND	0.03 ± 0.01	ND	0.06 ± 0.02	ND	0.2
Mn	8.20 ± 0.20	63.33 ± 0.31	13.40 ± 0.20	63.73 ± 0.61	7.53 ± 0.42	60.07 ± 0.31	500
Fe	20.87 ± 0.64	1090.40 ± 0.72	24.33 ± 0.61	1836.47 ± 0.70	0.80 ± 0.20	764.33 ± 0.61	425.5

Table 2: Concentration of metal in onion bulb and onion leaf samples in mg/kg (mean ± SD).

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Manganese is essential element required for various biochemical processes. The kidney and liver are the main storage places for the manganese in the body. Mn is essential for the normal bone structure, reproduction and normal functioning of the central nervous system. Its deficiency causes reproductive failure in both male and female [16]. The average concentration of Mn in onion bulb and onion leaf at Mojo was 8.20 and 63.33, at Meki 13.40 and 63.73, at Ziway 7.53 and 60.07 mg/kg respectively. All the results were below the permissible limit of 500 mg/kg set by FAO/WHO [12]. When compared the concentration content between onion bulb and onion leaf, the onion leaf had higher concentration of Mn.

Iron is an essential element in man and plays a vital role in the formation of haemoglobin, oxygen and electron transport in human body [6]. Iron was found to have the highest concentration in all the onion leaf samples analyzed (Table 2). The FAO/WHO [19] maximum limit for Fe concentration in food is 425.5 mg/kg. The result obtained onion bulb at Mojo, Meki and Ziway were 20.87 mg/kg, 24.33 mg/kg and 0.80 mg/kg. In this study the concentration Fe was lower than the recommended limit. But, in onion leaf the concentration was exceeded the permissible limit. This might be due to translocation of Fe metals which is similar with Mn, Zn and Cu.

Generally, Mosiamisi and Sello [20] reported that concentrations of Cu, Zn and Ni in whole plant system (leaf+stem+root) is in decreasing order leaf>stem>root. Result of this study were in agreement with the finding of Stieger and Feller, [21] reported higher Fe and Mn contents can be detected in wheat shoots (Figure 1).

In this study Table 3 was used for the determination of bio-concentration factor and i.e., was on processing in other journal.

Metals	Sites			U.S.EPA 1993
	Mojo	Meki	Ziway	(mg/kg) Max. con.
Cr	39.13 ± 0.32	18.37 ± 0.15	7.03 ± 0.10	3000
Cu	35.88 ± 1.81	32.80 ± 0.33	19.25 ± 3.29	4300
Zn	145.66 ± 4.22	153.30 ± 5.75	92.40 ± 7.86	7500
Pb	0.833 ± 0.289	ND	ND	420
Cd	0.095 ± 0.005	0.083 ± 0.029	0.133 ± 0.029	85
Mn	1264.82 ± 0.635	1546.42 ± 0.317	789.43 ± 0.840	2000mg/kg
Fe	27,427.33 ± 3.21	25,922.67 ± 6.25	9,947.00 ± 3.50	1500 mg/kg [12]
ND: Not Detected				

Table 3: Concentration of metals in soil samples with (mean ± SD), n=3.

Metal	Sites		
	Mojo	Meki	Ziway
Cr	0.1243 ± 0.01185	0.2250 ± 0.00458	0.4739 ± 0.01272
Cu	0.1101 ± 0.02093	0.0407 ± 0.01554	0.0455 ± 0.00579
Zn	0.0853 ± 0.00311	0.0504 ± 0.00576	0.1467 ± 0.01577
Pb	0.3961 ± 0.00000	ND	ND
Cd	0.5263 ± 0.14826	0.3000 ± 0.10000	0.4667 ± 0.17638
Mn	0.0065 ± 0.00016	0.0087 ± 0.00013	0.0095 ± 0.00052
Fe	0.0008 ± 0.00002	0.0008 ± 0.00002	0.0001 ± 0.0000
ND: Not Detected			

Table 4: Bioconcentration factor (BCF) of metals between soil and onion bulb (mean ± SD), n=3.

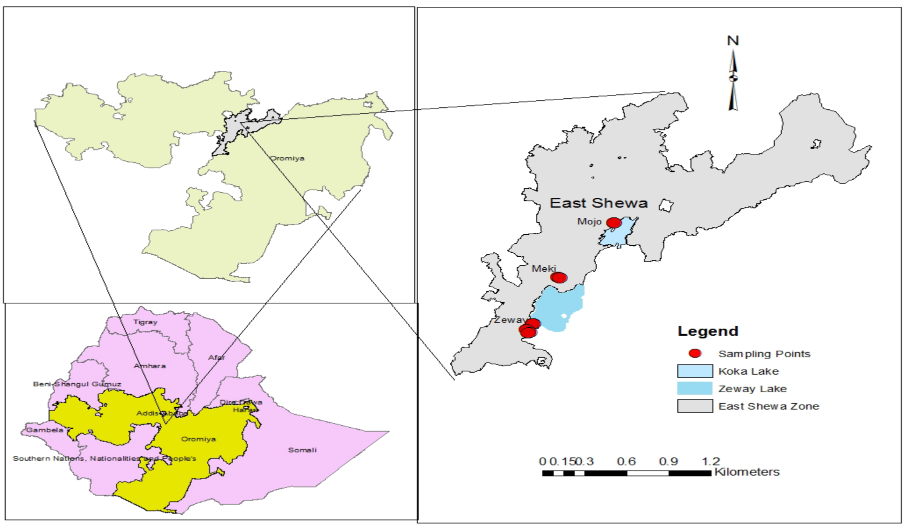

Figure 1: Location map of the study area.

Bioconcentration factor of metals between soil and onion bulb

Heavy metals have the capability to translocate from the soil to the edible parts of the food crop and can be determined by the bioaccumulation factor (BF) [11].

$$BF = \frac{Heavy\ metal\ concentration\ in\ the\ food\ crops\ edible\ parts}{Heavy\ metal\ concentration\ in\ the\ soil}$$

Where, BF: Bioconcentration Factor.

All the results were below 1, that indicating the bio-concentration factors was high in soil relative to onion bulb. On the other word; BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in onion bulb and therefore low uptake of heavy metals to vegetables. Several reports have dealt with BCF determinations for vegetables [7]. Among the different metals, Cd showed the maximum transfer factor value (Table 4), which ranged from 0.5263 (Mojo) to 0.3000 (Meki). Lokeshwari and Chandrappa [10] reported that Cd was retained less strongly by the soil and hence it is more mobile than other metals. Hence, among all elements, transfer factor of Cd was highest for assayed from the sampling sites, which showed that Cd is more mobile than the other metals.

Conclusion

In this study, onion bulb and onion leaf were analyzed for the concentration of heavy metals (Cu, Zn, Cr, Fe, Mn, Pb and Cd). The pattern concentration of metal in onion bulb at Mojo was in the order of (Fe>Zn>Mn>Cr>Cu>Pb>Cd), Meki (Fe>Mn>Zn>Cr>Cu>Cd) and Ziway (Zn>Mn>Cu>Fe>Cd). Out of the tested metals Cr concentration was found to be above the permissible limit collected from Mojo, Meki and Ziway sites and Pb also found above the permissible limit in Mojo sampling site in onion bulb; which indicates that consumption of onion from this area could be a health risk. Onion leaf had high concentration of Fe, Zn, Mn and Cu than onion bulb. But the concentration of Cr was the reverse; i.e., was high in onion bulb than onion leaf.

The result of bio-concentration factors indicates high heavy metal concentration in soil in relation to levels in onion bulb and therefore low uptake of heavy metals to onion.

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