African Journal of Agricultural Research

Full Length Research Paper

Levels of some toxic heavy metals (Cr, Cd and Pb) in selected vegetables and soil around eastern industry zone, central Ethiopia

Dagne Bekele Bahiru[1*], Endale Teju[2], Tesfahun Kebede[2] and Negash Demissie[1]

[1]Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia.
[2]Chemistry Department, Faculty of Natural and Computational Science, Haramaya University, Haramaya, Ethiopia.

The purpose of this study was to determine the concentration of heavy metals (Cr, Cd and Pb) in vegetables and the soil contaminating levels as a result of irrigation using Flame Atomic Absorption Spectrophotometer (FAAS). The wet digestion and sequential fractionation extraction procedures were employed to solubilize the metals from the collected samples. The results obtained from this study showed overall concentration of heavy metals Cr, Cd and Pb respectively, in the range of (2.90-3.77), (2.20-3.68) and (4.60-5.50) (mg/Kg) in the edible parts of sampled vegetables. Whereas, concentrations (mg/Kg) of the metals in the soil samples were found to be in the ranges of 22.37-66.30, 27.93-45.33 and 18.82-64.87 for Cr, Cd and Pb, respectively. The modified Tessier sequential extraction procedure was used to fractionate the above three metals from the soil samples into five fractions. In this study the heavy metals were predominantly concentrated in residual fraction (F5); since lead was mainly associated with the organic matter bounded fraction (F4) (34.33-43.45%), it was found to be more bioavailable and mobile than the other investigated heavy metals. The concentrations of heavy metals (Cr, Cd and Pb) in the soil and vegetable samples were above the recommended limit of both WHO and FAO. But Pb for the soil samples was smaller than WHO and FAO recommended limit. Based on facts obtained from this study we suggests concerned official body (ies) to take the necessary precaution measures for cleaning the polluted area.

Key words: Heavy metals, sequential fractionation, eastern industry zone.

INTRODUCTION

Heavy metals are extremely persistent in the environment. They are non-biodegradable and non-thermo degradable and therefore readily accumulate to toxic levels.

Vegetables are rich sources of vitamins, minerals, and fibers and also have beneficial antioxidative effects. However, the intake of heavy metal contaminated fruits

and vegetables may pose a risk to human health; hence the heavy metal contamination of food is one of the most important aspects of food quality assurance (Radwan and Salama, 2006; Khan et al., 2008).

Unlike many other pollutants associated with the environments, metals are nonbiodegradable and can undergo biomagnifications in living tissues. Uptake and accumulation of heavy metals by plants is either via the roots and foliar surfaces (Sawidis et al., 2001). Some factors which affect metal uptake include soil pH, metal solubility, soil conductivity nature, stages of plant growth and plant species type (Ismail et al., 2005; Sharma et al., 2006).

Soil contamination with anthropogenic heavy metals, mainly from industrial activities, agricultural practices and atmospheric deposition, has received increasing attention in recent years. Heavy metal contamination in agricultural soils may lead to the disorder of soil functionality and retardation of plant growth, and influence human health through a contaminated food chain (Khan et al., 2008).

Dry-ashing and wet-digestion are the common methods of soil, plant and water sample digestion for elemental analysis. Dry-ashing methods are comparatively simpler and safe than wet-digestion methods but may introduce error due to volatilization, especially for arsenic (As), selenium (Se), cadmium (Cd) and mercury (Hg). In addition, dry ashing may be problematic with pyrolytic organic materials as they may resist thermal decomposition at temperatures of about 550°C and analyte reactions with the crucible material and sample contamination from combustion residues (Hoeing et al., 1998).

Wet-digestion methods are preferable because of the speed with which sample is processed. These techniques utilize strong inorganic acids ($HClO_4$, NHO_3, H_2SO_4 and HCl) and in some cases hydrogen peroxide (H_2O_2) to decompose the samples. Perchloric acid ($HClO_4$) was commonly used some time ago because of its strongly oxidizing ability but has largely been avoided because of handling issues, its capacity to react violently with organic compounds, and the possibility of explosion when dry. Additionally, the use of $HClO_4$ requires special ventilation equipment. Nitric acid (HNO_3) in combination with hydrogen peroxide (H_2O_2) is an effective substitute for $HClO_4$, with the benefit of increased safety (Enders and Lehmann, 2012).

Sequential selective extraction techniques are commonly used to fractionate the solid-phase forms of metals in soils. Many sequential extraction procedures have been developed, particularly for sediments or agricultural soils, and despite numerous criticisms, they remain very useful (Christian et al., 2002). The mobility and bioavailability of heavy metal depend absolutely on their speciation or chemical forms. These forms are determined by sequential extraction technique, this method gives vivid information about metal affinity to the soil components together with the strength to which they are bound to the soil matrix. Also heavy metal fractions can give detail about soil origin, biological and physicochemical availability, and their mode of occurrence, mobility and transportation of trace metals (Kotoky et al., 2003). Extraction procedures of soil are used both for the single-stage leaching and the sequential extraction. Among various methods of the sequential extraction of soil, Tessier's method is most often used both in case of soil samples, as well as sediments (Yoseph, 2015)

Some methods used in heavy metal analysis are AAS, EDXRF and ICP (Abolino et al., 2002). For analysis of various fractions obtained by sequential extraction, AAS, ICP-MS and ICP- AES and ICP-OES are used (Iwegbue, 2007). Also Milkessa (2013) used FAAS. ICP-MS and AAS are most preferred because they are not prone to polyatomic interferences and are less affected by matrix suppression (Harrison et al., 1981).The method used in the present study for analysis was AAS due to its availability. AAS is simple, sensitive and selective and has the advantage of being a fast method of analysis (Katz, 1984).

The aim of this study was to detect and determine the concentrations of toxic metals viz. Cr, Cd and Pb in samples of soils and selected vegetables from irrigation farms around Eastern Industry Zone, in which pesticide, fertilizer, and municipal and industrial sewage effluents are known to be discharged into surrounding irrigation farms. Cabbage, lettuce, and tomato were selected and most commonly-consumed edible vegetables which are cultivated by using effluent wastewater, due to lack of clean irrigation water. The study was necessary as a large number of people consume the produce and no research has been conducted to elucidate the extent of the problem in the area.

MATERIALS AND METHODS

Description of the study area

This study was conducted around Eastern Industrial Zone in Dukem, Ethiopia. Dukem Town was founded in 1914 and is one of the 18 special zones of the Oromia Regional State of Akaki Woreda which is located at 37 Km distance from Addis Ababa City. It is a town in central Ethiopia, to the South of Addis Ababa and 10 km to North West of Bishoftu Town. Its astronomical location is 08°45'25"-08°50'30" North Latitude and 38°51'55" 08°56'5" East Longitude (Abebe, 2012)(Figure 1).

The Eastern Industrial Zone (EIZ) of Ethiopia is located at 35 km southeast of Addis Ababa, and 680 Km from the port of Djibouti with 200 ha of land in Dukem. For Ethiopia, EIZ is the first and largest-scale industrial park. The Ministry of Industry of Ethiopia requires the EIZ to focus on Chinese companies in the area of textile, apparel, building materials (including east steel, cement factory), mechanical manufacturing, and agricultural processing. Currently, 26 Chinese firms are operational and producing different products for export markets having agreement with EIZ in all targeted areas. In addition to the present 26 manufacturing industries, more than 20 other manufacturing industries are about
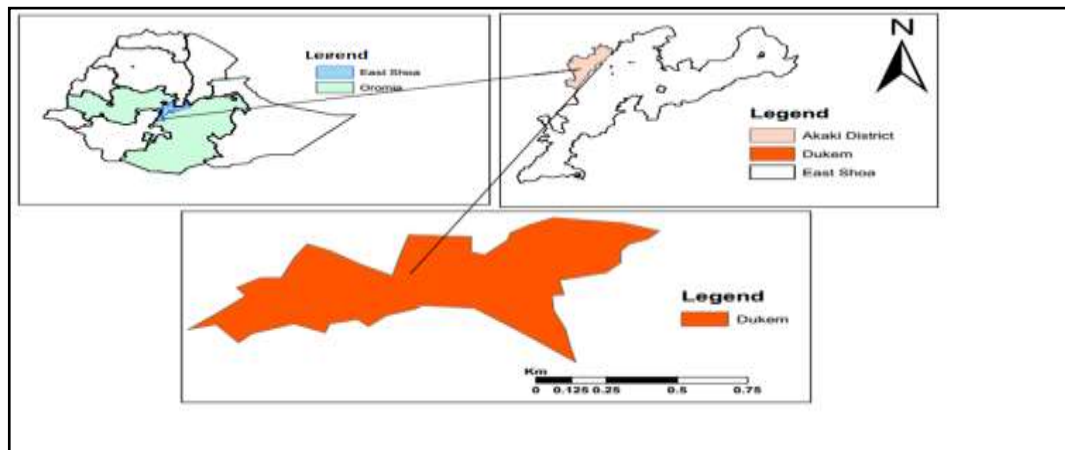
Figure 1. Location of study area.

to join the EIZ (Gebregeorgis, 2016). This implies that more municipal waste, gasses and wastewater from various industry of EIZ is discharged to the surrounding environment.

Chemicals, reagents and instruments

The instruments used for this study was FAAS, Agilent technology with model no. 210 for toxic heavy metal determination of vegetable and soil samples and a Mcroprossecer based PH-EC-TDS Meter; Model 1615 was used for the determination of soil pH and conductivity. The common laboratory apparatus which were used during the study include; different sized beakers, erlenmeyer flasks, funnels, volumetric flasks, block digester, fume hood, centrifuge, hydrometer, shaker, droppers, glass pipettes, spatula, measuring cylinders, plastic knife, vinyl gloves, steel less steel auger, stirrer, polyethylene bags, analytical balance, conical flasks and oven.

All the chemicals used were analytical reagent grade. Deionized water and distilled water were used for all preparation and dilution purposes throughout the study. Nitric acid, HNO_3 (69%), ammonium acetate (NH_4Ac) Sodium acetate (NaAc), potassium chloride (KCl), acetic acid (HAc), magnesium chloride ($MgCl_2$), hydroxide hydrochloride ($NH_2OH.HCl$), sulphuric acid, H_2SO_4 (98%) and hydrogen peroxide, H_2O_2 (30%) and hydrochloric acid (HCl) were used for digestion. Stock standard solutions of 1000 ppm were prepared for the selected heavy metals (Cr, Cd and Pb). All sample containers and glassware used in the present study were washed in detergent and soaked in 30% nitric acid for 2 h to leach out adsorbed metal ion. They were then rinsed in tap water followed by deionized water before drying in dust free area (APHA, 1999).

Sample collection and preparation

The soil, and vegetable samples were collected from vegetable samples were collected in February, 2017. About 1 kg edible part of cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa)* and tomato (*Lycopersicon esculentum* Miller). To this effect, three farmer farmlands were selected and three subsamples were taken for collecting representative edible parts of the vegetables. The collection was done manually. The representative reputable samples were thoroughly mixed to give a composite sample as representative fraction of the vegetables. The bruised or rotten portions were removed and the remaining samples were packed in polyethylene bags for transporting to the DZARC ANRL (Debre Zeit Agricultural Research Center Agricultural Chemistry lab). In the laboratory, the collected plant samples were washed with tap water and then with distilled water to eliminate adsorbed dust and particulate matters. The vegetable samples were cut and chopped into small pieces using plastic knife in order to facilitate drying. Accordingly, the samples were air-dried for six days and further dried in hot air oven at 50-60°C for 24 h, to remove moisture and maintain constant mass. The dried samples were ground into powder using acid washed commercial mortar and pestle and then sieved to 2 mm mesh size. The sieved samples were finally stored in polyethylene bags and kept in desiccators until the time of digestion.

Soil samples (about 1 kg) were collected from 0-20 cm depth from the site where the vegetables were grown (for each vegetable type) with an auger (Poggio et al., 2008) and the control soil sample was collected 2 km away from the study area. Then the samples were placed in clean polyethylene bags and transported to the DZARC ANRL for pretreatment and analysis. The composite soil samples were air-dried in a dry and dust-free place at room temperature (25°C) for 5 days, followed by oven drying to constant weights. The samples were then ground with a mortar and pestle to pass through a 2 mm sieve and homogenized. The dried, sieved, and homogenized soil samples were stored in clean and dry containers till digestion.

Digestion of soil and vegetable samples

The 0.5 g dried and homogenized soil samples were transferred in to 100 mL digestion flask in triplicate. In each of these flasks, 5 mL of deionized water and 30 mL of a mixture HNO_3 (69%) and 37% HCl with volume ratio of 5:1 were added. The sample dissolved in the acid mixture was digested in digestion hood (at 200°C) for 1 h and kept to cool. After adding 2 mL of H_2O_2 to the cold digestion mixture, the final, the mixture was filtered out through Whatman No. 42 filter paper to a 100 mL volumetric flask and finally diluted to the mark with distilled water (Loon, 1985). The varying filtrates obtained above were analyzed for the total content of each heavy metal by FAAS in Holeta Agricultural Research Center Chemistry Lab. The blank reagent was also digested following the same procedure as the soil sample.

A 0.5 g of homogenized powdered vegetables sample was placed in borosilicate digestion flask to which 10 mL of acid mixture containing HNO_3- HCl-H_2O_2 (8:1:1, v/v/v) ratio were added. The

mixture was heated at 120°C over 3 h on block digester. After digestion was completed, the clear and colorless solution was filtered out into 100 mL volumetric flask. Each digestion tube were rinsed with distilled water to collect any possible residue, and added to the volumetric flask and finally made up to volume with distilled water. All the dilute samples were stored in 100 mL plastic bottles (high density polyethylene) until analysis. Each vegetable sample was digested and analyzed in triplicate to confirm precision of the result. The blank solution was prepared by taking a mixture of 8 mL HNO_3, 1 mL HCl and 1 mL H_2O_2 and treating similarly as that of the sample (Street, 2008). The heavy metal concentrations were analyzed by FAAS in Holeta Agricultural Research Center Chemistry Lab.

Heavy metal fractionation in soil samples

The modified Tessier's procedure, Ma and Rao (1997) was used to determine operationally defined chemical species of the metals from soil. Five operationally defined fractions of the metals were removed by these sequential extractions. The SEP operationally groups heavy metals into the following five fractions:

(i) Soluble and exchangeable fraction (F1): The soluble and exchangeable metals from each of 2.5 g soil samples were extracted, into wide mouthed polypropylene bottle; 20 mL of 1 M $MgCl_2$ solution adjusted to pH of 7.0 were added. The bottles were shaken for 1 h at room temperature by an end-over end mechanical shaker. The extracts were separated from the solid residue by centrifugation (5000 rpm) for 15 min and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and kept for metal analysis.
(ii) The fraction bound to carbonates (F2): The carbonate bound metals in the residue left from the previous step were extracted with 20 mL of 1.0 M NaAc (CH_3COONa) solution adjusted to pH of 5.0 with HAc (CH_3COOH) by continuously shaking for 4 h at room temperature. It was then centrifuged for 15 min at 5000 rpm and filtered into 100 mL volumetric flask through Whatman No. 42 filter paper and kept for metal analysis.
(iii) The fraction bound to iron and manganese oxides (F3): Metals bound to iron and manganese oxides were extracted from the residue of the second extraction by shaking with 50 mL of 0.04 M $NH_2OH.HCl$/25 % HAc solution and placed in to a water bath for 5.5 h at 96°C, then centrifuged for 15 min at 5000 rpm and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and stored for metal analysis.
(iv) The fraction bound to organic matter (F4): Metals bound to organic matter were extracted by pouring 7.5 mL of a 0.02 M HNO_3 solution and 12.5 mL of a 30% H_2O_2 solution adjusted to a pH of 2.0 onto the residue from 3.6.3 and heated for 2 h in water bath at 85°C. After cooling, additional volume of 7.5 mL of 30% H_2O_2 solution adjusted to pH of 2.0 was added while maintaining continuous agitation and at a temperature of 85°C for another 3 h. These solutions were then cooled to room temperature. Then aliquot of 12.5 mL of 3.2 M NH_4Ac/ 20% HNO_3 solution was added and shaken for 30 min, then centrifuged for 15 min at 5000 rpm and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and stored for metal analysis.
(v) The fraction bound to soil matrix (residual fraction) (F5): The residues from 3.6.4, were quantitatively transferred into a digestion vessel and treated with aqua regia (7 mL of 10 M HCl and 2.3 mL of 15.8 M HNO_3). The temperature of the reaction mixture was slowly raised until reflux conditions and maintained for 2 h, centrifuged, at 5000 rpm for 15 min and then filtered through Whatman No. 42 filter paper into 100 mL volumetric flask. All dilutions were made to 100 mL with 2% (v/v) HNO_3. For each fraction a blank was subjected to the same procedure.

Method detection limit

Method detection limit is defined as the minimum concentration of analyte that can be measured. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank (Gezahegn, 2013). Three replicate blank samples were digested following the same procedures utilized for digesting the soil and vegetable samples. Each blank were assayed for its metal contents Cr, Cd and Pb by FAAS. The SD of the three replicate blanks was calculated to determine the MDL (David and Terry, 2008). Method detection limit (MDL) was then calculated according to the equation indicated below.

MDL = YB +3SD

Where: YB = Blank mean

Method validation

In present study due to the absence of certified reference materials for soil and vegetable samples in our laboratory, the validity of the digestion procedure, precision and accuracy of FAAS were assured by spiking soil and vegetable samples with standard of known concentration. The spiked and non-spiked vegetables and soil samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition. Then the percentage recoveries of the analytes were calculated by:

$$Recovery = \left(\frac{CM\ in\ the\ spik\ samples - CM\ in\ the\ non\ spik\ sample}{Amount\ added}\right) x\ 100$$

Where, CM = concentration of metal of interest.

Statistical analysis

The analyses of variance ANOVA were performed to examine the significance level of all parameters measured. Least Significant Difference (LSD) test was used for means comparison. The level of significance for means comparison was $p<0.05$. Methodological precision was therefore evaluated with standard deviation (SD).

Total metal concentrations in soil samples

FAO/WHO (2001) values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval. As shown in Table 1, heavy metals (Lead (Pb), and cadmium (Cd)) have no beneficial effects in humans, and there is no known homeostasis mechanism for them. They are generally considered the most toxic to humans and animals; the adverse human health effects associated with exposure to them, even at low concentrations, are diverse and include, but are not limited to, neurotoxic and carcinogenic actions (Vieira et al., 2011).

Chromium in soil samples

Chromium plays a vital role in the metabolism of

Table 1. Mean concentration of **Cr, Cd and Pb** of soil sample in wet digestion method (n = 3, ± SD mg/kg).

Sample code	Cr	Cd	Pb
Soil for tomato	50.50±0.53[c]	45.33±1.53[a]	63.00±2.26[a]
Soil for cabbage	66.30±2.46[a]	42.33±0.58[b]	64.87±0.45[a]
Soil for lettuce	62.23±2.35[b]	45.00±1.00[a]	63.33±3.58[a]
Control soil	22.37±0.31[d]	27.93±0.61[c]	18.82±0.08[b]
LSD	3.16	1.83	4.07
FAO/WHO	50	3	100
USEPA, 2002	150	3	300
EU, 2002	-	3	300

cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentration it is toxic and carcinogenic (Chishti et al., 2011). As shown in Table 1, the Cr contents in the soil samples were found to be within the range of 50.50±0.53 and 66.30±2.46 mg/kg. The highest and lowest contents of Cr occurred in the soils of cabbage and tomato, respectively. The WHO/FAO (2001) permissible limit of chromium in soil is 50 mg/kg. So, the concentration of chromium found in the three soil samples from lands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Major sources of Cr contamination include releases from electroplating processes and the disposal of Cr containing wastes (Smith et al., 1995).

Cadmium in soil samples

Cadmium is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes learning disabilities and hyperactivity in children (Hunt, 2003). As shown in Table 1, the experimental results showed that Cd concentration in soil samples occurred in the range of 42.33±0.58 and 45.33±1.53 mg/kg. The tomato soil observed to have the highest level (45.33±1.53 mg/kg) of Cd, while the cabbage originated soil had the smaller level (42.33±0.58 mg/kg) of Cd. Being a non-essential metal, it can be considered very toxic. So, the concentration of cadmium found in the three vegetables growing soil samples from lands irrigated with wastewater around the Eastern Industry might be harmful for human health. Comparison of cadmium level in the soil samples with that of the control soil sample (27.93±0.61 mg/kg) indicates that the higher levels found in all samples could possibly be attributed to the pesticide, fertilizer, and municipal and industrial sewage effluents are known to be discharged into surrounding irrigation farms. It is used in nickel cadmium batteries, PVC plastic and paint pigments. It can be found in soils because insecticides, fungicides sludge, and commercial fertilizers that use cadmium are used in agriculture (Okoro et al., 2012).

Lead in soil samples

Lead is one of the more persistent metals and is estimated to have a soil retention time of 150 to 5000 years (Sobolev and Begonia, 2008). It is a non-essential heavy metal. Pb causes oxidative stress and contributes to the pathogenesis of lead poisoning by disrupting the delicate antioxidant balance of the mammalian cells. High level accumulation of Pb in body causes anemia, colic, headache, brain damage, and central nervous system disorder (Rehman et al., 2013). As shown in Table 1, the soil samples contained Pb concentrations in the range of 63.00±2.26–64.87±0.45 mg/kg. The WHO/FAO (2001) permissible limit of lead in soil is 100 mg/kg. This is within ranges of soils studies by Premarathna et al. (2011) who reported a range of 15 to 311 mg/kg. However, Awokunmi et al. (2010) reported very high levels of lead in soils collected from various dumpsites ranging between 3500-6860 mg/kg. Aluko et al. (2003) also reported high values of lead in soil ranging from 1340-1693 mg/kg.

Lead has been known to have harmful health effects even at lower levels and there is no known safe exposure level. It is appropriate to note that exposure to amount of lead above 0.01 mg/kg is detrimental to health, as it may result in possible neurological damage to fetuses, abortion and other complications in children under three years (Asemave et al., 2012). So, the concentrations of lead found in all three soil samples collected from farmlands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Comparison of lead levels in the soil samples with that of the control soil sample (18.82±0.08 mg/kg) indicates that the higher levels obtained from all samples could possibly be attributed to the high levels of lead in the pesticide, fertilizer, and municipal and industrial sewage effluents are known to be applied into surrounding irrigation.

Metal concentrations in soil fractions

The metal distribution among specific forms varies widely based on the metal's chemical properties and soil

Table 2. Chemical fractionation of Cr, Cd and Pb (mg /kg) in soil samples from irrigated lands around the EIZ (n = 3, ± SD mg/kg).

Metal	Sample code	F1	F2	F3	F4	F5	F1+F2+F3+F4+F5	Wet-digestion
	Soil for tomato	0.61±0.03[c]	0.26±0.02[c]	0.96±0.04[c]	2.81±0.03[b]	32.27±1.65[b]	36.86±1.73	50.50±0.53
	Soil for cabbage	0.79±0.05[b]	0.44±0.02[a]	1.62±0.40[a]	4.23±0.23[a]	29.05±0.01[c]	36.13±0.71	66.30±2.46
Cr	Soil for lettuce	0.91±0.03[a]	0.37±0.03[b]	1.15±0.02[b]	4.46±0.05[a]	35.27±0.39[a]	42.52±0.52	62.23±2.35
	Control soil	0.19±0.01[d]	0.21±0.00[d]	0.21±0.00[d]	0.30±0.02[c]	20.35±0.09[d]	21.26±0.12	22.37±0.31
	LSD	0.05	0.04	0.07	0.26	1.73		
	Soil for tomato	0.41±0.01[a]	0.35±0.01[a]	0.48±0.02[b]	19.13±0.05[a]	18.84±0.02[a]	39.21±0.11	45.33±1.53
	Soil for cabbage	0.35±0.12[b]	0.20±0.02[b]	0.42±0.02[c]	18.85±0.02[b]	18.81±0.03[a]	38.63±0.21	42.33±0.58
Cd	Soil for lettuce	0.22±0.02[c]	0.13±0.01[c]	0.53±0.03[a]	18.85±0.00[b]	18.83±0.10[a]	38.56±0.16	45.00±1.00
	Control soil	0.14±0.01[d]	0.11±0.00[c]	0.09±0.00[d]	8.34±0.01[c]	18.13±0.14[b]	26.81±0.16	27.93±0.61
	LSD	0.03	0.03	0.03	0.06	0.19		
	Soil for tomato	12.27±0.42[a]	12.00±0.20[a]	7.87±0.31[c]	26.93±0.1[a]	13.36±0.04[a]	72.43±1.09	63.00±2.26
	Soil for cabbage	10.33±0.12[b]	10.40±0.60[b]	9.20±0.02[b]	24.87±0.1[b]	9.9±0.10[b]	64.70±0.96	64.87±0.45
Pb	Soil for lettuce	5.12±0.11[c]	6.47±0.31[c]	10.57±0.04[a]	23.87±1.2[b]	8.91±0.01[c]	54.94±1.76	63.33±3.58
	Control soil	0.32±0.02[d]	1.50±0.2[d]	0.70±0.30[d]	5.53±0.04[c]	8.06±0.04[d]	16.11±0.60	18.82±0.08
	LSD	0.46	0.63	0.36	1.34	0.10		

characteristics (Milkessa, 2013). The sequential extraction used in this study is useful to indirectly assess the potential mobility and bioavailability of heavy metals in the soils. The five chemical fractions are operationally defined by an extraction sequence that follows the order of decreasing solubility (Tessier et al., 1979).

Cr is mostly present in the residual fraction of all the samples (Table 2). The abundance of Cr in the residual phase is 20.35-35.27 mg/kg but in other geochemical phases was very low indicating that Cr was more stable in this environment than the other metals. Cd is obviously higher in abundance of the three elements in the last two fractions with compared to its concentration (Table 2). The abundance of Cd in the F4 and F5 were 8.34-19.13 mg/kg and 18.13-18.84 mg/kg respectively. The high proportion of the chemically reactive forms of Cd implies a high ecological risk (Zhang and Shan, 2008). Pb is obviously higher in abundance of the three elements in the F3 fraction Table 2. Soil OM has a large surface negative charge/ cation exchange capacity and elements such as Pb are observed to accumulate in the organic-rich, surface horizons (Zimdahl and Skogerboe, 1977).

Bioavailability and mobility factors of heavy metals

Assuming that bioavailability is related to solubility, then metal bioavailability decreases in the order: exchangeable (F1) > carbonate (F2) > Fe-Mn Oxide (F3)> organic (F4)> residual (F5). This order is just a generalization and offers only qualitative information about metal bioavailability. Based on the above information, one can further assume that metals in the nonresidual fractions are more bioavailable than metals associated with the residual fraction. The nonresidual fraction (NRF) is the sum of all fractions except the residual fraction (RF). The highest amounts of cadmium and lead were concentrated in the non-residual fraction but for chromium (89.80-82.95) and (79.62-83.78%) which was concentrated in the residual fraction (Figure 2).

The bioavailability factor was expressed as the ratio of the available concentration of a metal in soil to its total concentration. It shows the potentials of a particular metal from the soil matrix to enter the soil solution from which it can be absorbed by plants. Mobility factor was expressed as percentage of the Bioavailability factor (Kabata and Singh, 2001).

$$BF= \frac{F1+F2}{F1+F2+\cdots+F5}$$

$$MF= \frac{F1+F2}{F1+F2+\cdots+F5} \times 100$$

Table 3, shows the mobility, and bioavailability factors for all the sequential extractions steps. The high MF and BF values of soil Pb may be interpreted as symptoms of relatively high liability and biological availability of the metals in soil. Similar characteristics distribution patterns were observed for Cu Cd, Cr and Zn (Table 3).

Heavy metal concentration in vegetable samples

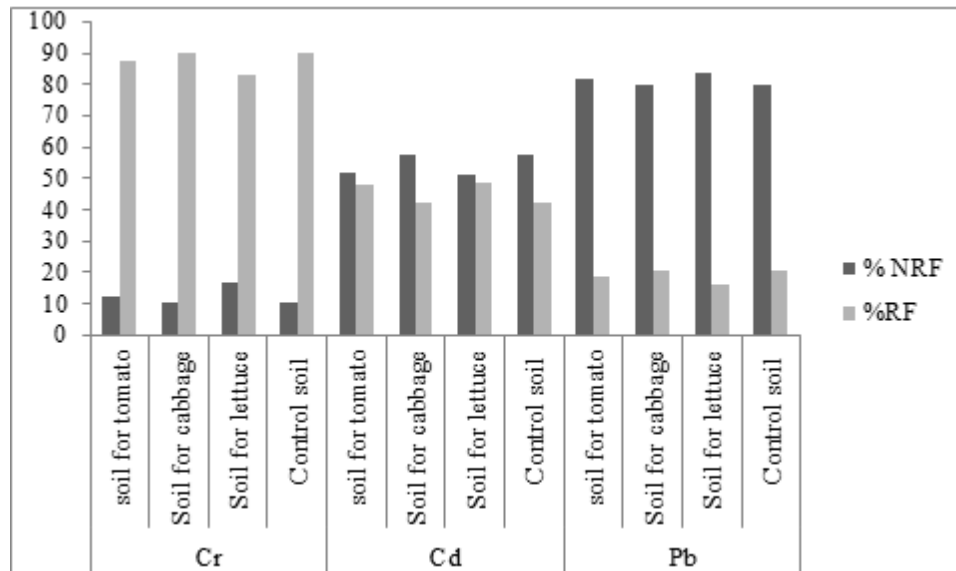Vegetables like cabbage (*Brassica oleracea* L.), lettuce

Figure 2. Chemical fractionation of heavy metals in (mg /kg) in soil sample collected from irrigated land around the EIZ (n = 3).

Table 3. The bioavailability and mobility Factor of Heavy Metals in soil sample fractionation (n = 3).

Elements	Sample code	F1	F2	Sum of F1 and F2	Sum of fraction	Bioavailability factor	Mobility factor
Cr	Soil for tomato	0.61	0.260	0.870	36.860	0.024	2.360
	Soil for cabbage	0.79	0.440	1.230	36.130	0.034	3.404
	Soil for lettuce	0.91	0.370	1.280	42.520	0.030	3.010
	Control soil	0.19	0.210	0.400	7.260	0.019	1.881
Cd	Soil for tomato	0.41	0.350	0.760	39.210	0.019	1.938
	Soil for cabbage	0.35	0.200	0.550	38.630	0.014	1.424
	Soil for lettuce	0.22	0.130	0.350	38.560	0.009	0.908
	Control soil	0.14	0.140	0.280	15.110	0.010	1.040
Pb	Soil for tomato	12.27	12.000	24.270	72.430	0.335	33.508
	Soil for cabbage	10.33	10.400	20.730	64.700	0.320	32.040
	Soil for lettuce	5.12	6.470	11.590	54.940	0.211	21.096
	Control soil	0.32	1.500	1.820	8.380	0.113	11.297

(*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Miller) were analyzed for total metals content. The level of heavy metals in vegetables varies by the ability of plants to selectively accumulate some of these elements. Bioavailability of the elements depends on the nature of their association with the constituents of a soil. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades (Harris, 1982; Gezahegn, 2013). The concentrations of, Cr, Cd and Pb in sample of vegetables (cabbage, lettuce and tomato) grown around EIZ irrigation farm land were presented in Table 4. From the study, it is revealed that most of the metals were accumulated to greater or lesser extents in the vegetable samples with compared to WHO standard as shown in Table 4.

The vegetables are consumed by the urban population of the city of Dukem and cities present near Dukem like Addis Ababa, Debre Zeit, etc. thus exposing the population to dangerous levels of heavy metals. The results presented demonstrate that there is a risk associated with consumption of vegetables grown on

Table 4. Mean concentration of Cr, Cd and Pb of vegetable samples in wet digestion method (means ± SD mg/kg), n=3.

Element	Vegetable			LSD	WHO (1999)	CMH (2005)	FAO (1985)
	Tomato	Cabbage	Lettuce				
Cr	2.97±0.21[b]	2.90±0.10[b]	3.77±0.12[a]	0.20	1.2	0.5-1.0	-
Cd	2.20±0.10[c]	3.20±0.10[b]	3.68±0.06[a]	0.22	0.2	0.05-0.2	0.01
Pb	4.60±0.10[b]	5.47±0.35[b]	5.50±0.40[a]	0.87	0.5	0.1-0.3	5.00

these irrigation land farm, with the vegetable still looking apparently healthy and growing well despite accumulating heavy metals to concentrations which substantially exceed maximum values considered safe for human consumption. The results of this study, heavy metal concentrations in vegetable samples were compared with WHO permissible values Source, WHO (1999), CMH: Chinese Ministry of Health. The means in the same row having different superscript letters are significantly different from each other at 5% confidence interval.

Distribution of chromium in vegetables

Exposure of human to chromium may occur through breathing, drinking, or eating food containing chromium or even through skin contact. Exposure to elevated levels chromium leads to skin irritation, ulceration, damage to circulatory and nerve tissues which cause health problems. However, daily uptake of it within a certain range of concentrations (up to 200 µg/day) by human beings and animals is considered to be essential for carbohydrate and lipid metabolism (Girmaye, 2012). In this study the chromium contents in vegetable samples were obtained to have ranged from 2.90±0.10-3.77±0.12 mg/kg and these result were higher than permissibility level set by WHO (1999) is 1.2 mg/kg (Table 4).

Distribution of cadmium in vegetables

The vegetable samples collected around EIZ irrigation farmlands contained Cd concentrations in the range of 2.20±0.10-3.68±0.12 mg/kg as shown in Table 4. The concentration of Cd was maximum (3.68±0.12 mg/kg) in lettuce sample and the minimum (2.20±0.10) was found in the tomato sample. According to WHO/FAO (1999) permissible level is 0.2 mg/kg. The high concentration of Cd in the vegetables might be due to the use of pesticide, fertilizer, and municipal and industrial sewage effluents. Applications of untreated industrial effluent build up concentration of metal into the soil (Chary et al., 2008). From the soil, metals can transfer to the vegetables and accumulate in the tissues of vegetables. Several compounds of Cadmium are used in chemical industries and in the manufacture of pesticides, herbicides used in agriculture (Ogundele et al., 2015). Cd is more soluble as

compared to other metals so, it can accumulate more into the vegetables tissues (Farid et al., 2015).

Distribution of lead in vegetables

Results show that the levels of lead in the vegetables studied had a range of 4.60±0.10 to 5.50±0.40 mg/kg as shown in Table 4. Data showed that in all vegetables, lead concentration is more than permitted level, so they are not suitable for consumption. Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human (Bigdeli and Seilsepour, 2008). In leafy vegetables the accumulation of airborne lead largely exceeds the soil borne part taken up via roots. Air borne lead is mainly accumulated at the leaf surface and can be removed to a larger extent by washing of the vegetables (Tyagi, 2014).

Comparison of heavy metals concentration from the current study with those reported on the literature

The heavy metal (Cr, Cd and Pb) levels in vegetables samples (tomato, cabbage and lettuce) from'fields irrigated with the Eastern Industry Zone were compared with different literature reported in Table 5.

SUMMARY AND CONCLUSION

As stated earlier, the major purpose of this study was to find out the level of heavy metals in soil, from three farmer farm and three subsamples from each farm for each edible part of the vegetables (tomato, cabbage and lettuce) were determined. The soil and vegetable samples were subjected to wet-digestion, sequential extraction and the concentration of heavy metals were determined via FAAS. The concentration of heavy metals in the soil display the following decreasing trend: Cr > Pb > Cd. These concentrations of heavy metals in soil samples were above the recommended level set by FAO/WHO (2001), EU (2002) and USEPA (2002) for

Table 5. Comparison of metal concentration in the vegetables with other reports in similar studies.

Vegetable	Source of heavy metals	Heavy metals in mg/k			References
		Cr	Cd	Pb	
Tomato	Agricultural activities and Industrial effluents	2.97	2.20	4.60	Present study
	Agricultural activities	0.34	0.11	5.25	Liu et al., 2006
	Wastewater	-	0.20	5.50	Mohod, 2015
	Wastewater	0.33	0.03	4.40	Khan et al., 2011
	Swage water	2.12	13.56	6.80	Perveen et al., 2012
Cabbage	Agricultural activities and Industrial effluents	2.90	3.20	5.47	Present study
	Swage water	1.20	16.71	48.00	Perveen et al., 2012
	Wastewater	0.57	0.22	0.31	Girmaye, 2012
	Wastewater	0.38	0.26	2.24	Khan et al., 2015
	Transport & Market	16.28	6.17	22.76	Dingkwoet et al., 2013
Lettuce	Agricultural activities and Industrial effluents	3.77	3.68	5.50	Present study
	Wastewater	1.86	0.36	0.53	Girmaye, 2012
	Swage water	2.20	15.25	2.20	Perveen et al., 2012
	Wastewater	0.41	0.51	1.52	Khan et al., 2015
	Transport & Market	11.07	-	37.81	Dingkwoet et al., 2013

irrigation soil. The concentration of heavy metals in the vegetable samples display the following decreasing trend: Cr > Pb > Cd. The study revealed that the concentrations of all metals in the vegetables were found to be above the safe limits set by different international organizations for consumption, posing a serious health hazard to humans. Therefore, regular monitoring of soils and vegetables are essential to prevent excessive build-up of the toxic heavy metals in food. Thus, the health risk and the extent of heavy metal contamination can be reduced. The soil–plant transfer factor (TF) decreased in the following order- TFPb > TFCd > TFCr. A sequential extraction procedure was used to fractionate Cr, Cd and Pb present in soils of tomato, cabbage and lettuce and reference (control) soils. The mobility and bioavailability of these metals were studied and a very high amount of these metals were concentrated at the residual, organic and Fe-Mn Oxide fractions. However, a very small concentration of these heavy metals was also found at the exchangeable and carbonate fractions. Mobility factor of, Cr, Cd and Pb in soil samples ranged from 1.881-3.404, 0.908-1.938 and 11.297-33.508, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abebe A (2012). Assessment of urban expansion in the case of Dukem town using remote sensing and GIS techniques. MSc thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Abolino O, Aceto M, Mentasticti E, Sarzanini C, Petrella F (2002). Assessments of metal availability in contaminated soil, by sequential extraction. Water Soil and Air Pollution 49:345-557.

Aluko OO, Sridha MKC, Oluwande PA (2003). Characterization of leachates from a municipal solid waste landfill site in Ibadan, Nigeria. Journal of Environmental Health Research 2:32-37.

American Public Health Association (APHA) (1999). American water works association, Water environment federation. Standard methods of the examination of water and wastewater, 20thed. New York. https://www.mwa.co.th/download/file_upload/SMWW_1000-3000.pdf

Asemave K, Ubwa ST, Anhwange BA, Gbaamende AG (2012). Comparative Evaluation of Some Metals in Palm Oil, Groundnut Oil and Soybean Oil from Nigeria. International Journal of Modern Organic Chemistry 1(1):28-35.

Awokunmi EE, Asaolu SS, Ipinmoroti KO (2010). Effect of leaching on heavy metals concentration of soil in some dumpsites. African Journal of Environmental Science and Technology 4(8):495-499.

Bigdeli M, Seilsepour M (2008). Investigation of metals accumulation in some vegetables Irrigated with wastewater in Shahre Rey-Iran and toxicological implications American-Eurasian, Journal of Agriculture and Environmental Science 4(1):86-92.

Chary NS, Kamala CT, Raj DSS (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol and Environ Safety 69:513-524.

Chinese Ministry of Health (CMH) (2005). Maximum Levels of Contaminants in Foods. CMH, Beijing, China. GB2762 _2005. https://gain.fas.usda.gov/.../Maximum%20Levels%20of%20Contaminants%20in%20F.

Chishti KA, Khan FA, Hassan SSM (2011). Estimation of heavy metals in the seeds of blue and white capitalism's of silybum marianum grown in various districts of Pakistan. Journal of Basic and Applied Science 7(1):45-49.

Christian G, Sylvaine T, Michel A (2002). Fractionation studies of trace elements in contaminated soils and sediments: A Review of sequential extraction procedures. Trends in Analytical Chemistry 21:451-467.

David AA, Terry P (2008). Limit of blank, limit of detection and limit of quantitation. Clinical Biochemist Review 29:49-52.

Dingkwoet DJ, So Danladi SM, Gabriel MS (2013). Comparative study of some heavy and trace metals in selected vegetables from four local government areas of Plateau State, Nigeria. Journal of Environmental Science, Toxicology and Food Technology 6(3):2319-

2399.

Enders A, Lehmann J (2012). Comparison of wet-digestion and dry-ashing methods for total elemental analysis of Biochar. Communications in Soil Science and Plant Analysis 43:1042–1052.

European Union (EU). 2002. Heavy metals in wastes, European Commission on Environment. Available at: http://ec.europa.eu/environment/waste/studies/pdf/heavy_metalsreport.pdf

Food and agriculture organization (FAO) (1985). Water Quality for Agriculture. Food and agriculture organization of the United Nations, Rome. Irrigation and drainage Paper 29:1.

FAO/WHO (2001). Food additives and contaminants. Joint codex alimentarius commission, FAO/WHO Food standards Program 34:745-50.

Farid G, Sarwar N, Saifullah Ahmad A, Ghafoor A, Rehman M (2015). Heavy metals (Cd, Ni and Pb) contamination of soils, plants and waters in madina town of Faisalabad Metropolitan and Preparation of Gis Based Maps. Advances in Crop Science and Technology 4(1):1-7.

Gebregeorgis AK (2016). Ethio-China economic relations: nature of China's foreign direct investment in Ethiopia. MSc thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Gezahegn L (2013). Chemical fractionation of selected metals in the soil of waste disposal sites of Dire Dawa Textile Factory and their contents in the sweet potato Leaves. MSc. Graduate research project, Haramaya University, Haramaya, Ethiopia.

Girmaye BR (2012). Heavy metal and microbial contaminants of some vegetables irrigated with wastewater in selected farms around Adama town, Ethiopia. MSc. Graduate project, Haramaya University, Haramaya, Ethiopia.

Harris DC (1982). Quantitative Chemical Analysis, 4th edition. W.H. Freeman and Company.

Harrison RM, Laxen DPH, Wilson SJ (1981). Environmental science and technology. Journal of Environment 38:25-32.

Hoeing M, Baeten H, Vanhentenrijk S, Vassileva E, Quevauviller PH (1998). Critical discussion on the need for an effective mineralization procedure for the analysis of plant material by atomic spectrometric methods. Analytica Chimica Acta 358:85-94.

Hunt JR (2003). Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. American Journal of Clinical Nutrition 78(3):6335-6395.

Ismail BS, Farihah K, Khairiah J (2005). Bioaccumulation of heavy metals in vegetables from selected agricultural areas. Bulletin of Environmental Contamination and Toxicology 74:320-327.

Iwegbue, C.M.A., (2007). Determination of trace metal concentrations in soil profiles of municipal waste dumps in Nigeria. Waste Management Resource, 25:585.

Kabata C, Singh BR (2001). Fractionation and mobility of copper, lead and zinc in soil profile in the vicinity of a copper smelter. Journal of Environmental Quality 30:485-492.

Katz SA (1984). Determination of heavy metals in sewage sludge. Environmental Chemistry 3:78-953.

Khan MJ, Jan, MT, Farhatullah Khan NU, Arif M, Perveen S, Alam S, Jan AU (2011). The Effect of using Wastewater for Tomato. Pakistan Journal of Botany 2:1033-1049.

Khan SA, Liu X, Shah BR, Fan W, Li W, Khan SB, Ahmad Z (2015). Metals uptake by wastewater irrigated vegetables and their daily dietary intake in Peshawar, Pakistan. Ecological Chemical Engineering Science 22(1):125-139.

Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008). Health risk of heavy metals in contaminated soils and food crops irrigated with waste water in Beijing, China. Environmental Pollution Series 152(3):686-692.

Kotoky P, Bora BJ, Baruah NK, Baruah J, Baruah P, Borah GC (2003). Chemical fractionation of heavy metals in soil around oil installation, Assam. Chemical Speciation and Bioavailability 15(4):115-125.

Liu WX, Li HH, Li SR, Wang YW (2006). Heavy metal accumulation of edible vegetable cultivated by people's of Republic of China. Bullet of Environmental Contamination and Toxicology 76:163-170.

Loon JC (1985). Selected methods of trace metal analysis biological and environmental samples. New York 5:3685-3689.

Ma LQ, Rao GN (1997). Chemical fractionation of cadmium, copper,

nickel, and zinc in contaminated soils. Journal of Environmental Quality 26(1):259-264.

Milkessa MA (2013). Chemical fractionation of selected heavy metals in the soils in the vicinity of waste water disposal sites in Dire Dawa Textile Factory. MSc project work, Haramaya University, Haramaya.

Mohod CV (2015). A review on the concentration of the heavy metals in vegetable samples like spinach and tomato grown near the area of Amba Nalla of Amravati City. International Journal of Innovative Research in Science, Engineering and Technology 4(5):2788-2792.

Ogundele DT, Adio AA, Oludele OE (2015). Heavy metal concentrations in plants and soil along heavy traffic roads in North Central Nigeria. Environmental Analytical Toxicology 5(6):1-5.

Okoro KH, Fatoki SO, Adekola AF, Ximba JB, Snyman GR (2012). A Review of Sequential Extraction Procedures for Heavy Metals Speciation in Soil and Sediments. Open Access Scientific Reports 1(3):1-9.

Perveen S, Samad A, Nazif W, Shah S (2012). Impact of sewage water on vegetables quality with respect to heavy metals in Peshawar Pakistan. Pakistan Journal of Botany 44(6):1923-1931.

Poggio M, Hepperle E, Marsan FA (2008). Metals pollutions and human bioaccessibility of topsoils in Grugliasco, Italy. Environmental Pollution 157:680-689.

Premarathna HM, Hettiarachchi GM, Indraratne SP (2011). Trace metal concentration in crops and soils collected from intensively cultivated areas of Sri Lanka. Pedologist 54(3):230-240.

Radwan MA, Salama AK (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. Journal of Food and Chemical Toxicology 44(8):1273-1278.

Rehman A, Ullah H, Khan RU, Ahmad I (2013). Population based study of heavy metals in medicinal plant Capparis decidua. International Journal of Pharmacy and Pharmaceutical Sciences 5(1):108-113.

Sawidis T, Chettri MK, Papaionnou A, Zachariadis G, Stratis J (2001). A study of metal distribution from lignite fuels using trees as biological monitors. Journal of Ecotoxicology and Environmental Safety 48(1):27-35.

Sharma RK, Agrawal M, Marshall FM (2006). Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. Environmental Contamination and Toxicology 77:311-318.

Smith A, Means JL, Chen A (1995). Remedial options for metals-contaminated sites, Lewis Publishers, Boca Raton, Fla, USA.

Sobolev D, Begonia MFT (2008). Effects of Heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. International Journal of Environmental Research in Public Health 5(5):451-459.

Street RA (2008). Heavy metals in South African medicinal plants research center for plant growth and development, PhD Dissertation, University of KwaZulu-Natal, Pietermaritzburg, South African.

Tessier A, Campbell PGC, Bisson M (1979). Sequential extraction procedure for the speciation of particulate traces metals. Analytical Chemistry 51(7):844-851.

Tyagi R (2014). Assessment of the uptake of toxic heavy metals on cultivation of vegetables of family Solanaceae in contaminated soil. PhD Graduate Thesis University of Kota, Kota, Rajasthan.

World Health Organization (WHO) (1999). Permissible limits of heavy metals in soil and plants (Geneva: World Health Organization), Switzerland.

U.S. Environmental Protection Agency (USEPA) (2002). PRG Tables. Preliminary Remediation Goals. Solid and Hazardous Waste Programs, Region 9, U.S. Environmental Protection Agency. [Online]. Available: http://www.epa.gov/Region9/waste/sfund/prg/s1_01.htm.

Yoseph Y (2015). Chemical fractionation of heavy metals in soil around Tinshu Akaki River Addis Ababa, Ethiopia. MSc. Graduate research project, Haramaya University, Haramaya, Ethiopia.

Zhang WJ, Jiang FB, Ou JF (2011). Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences 1(2):125-144.

Zimdahl RL, Skogerboe RK (1977). Behaviour of lead in soil. Environmental Science Technology 11:1202-1207.