Studies on the Concentration Levels of Heavy Metals in Soils Used for Cultivation of Garlic (*Allium sativum* L.) In Ambo Woreda, West Shoa Zone of Oromia, Ethiopia

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Abstract: The concentration levels of ten heavy metals (Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd) in soil used for cultivation of garlic were determined using ICP-OES. The samples were collected from four agricultural areas of Ambo Woreda (Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti) Kebeles, West Shoa Zone, Ethiopia. Wet digestion method using a mixture of 6 ml aquaregia and 1.5 ml H_2O_2 was used for digestion of the soil samples. The results obtained revealed that the concentrations of heavy metals in the soil samples in mg/kg dry weight were in the range of: Zn (74.29–112.2), Mn (101.05–125.67), Cu (14.91–24.55), Fe (12436–15638), Mo (1.90–2.58), Co (10.12–35.14), Ni (17.69–50.56), Cr (23.38–50.03), Pb (11.84–15.58) and Cd (1.63–1.92). The levels of all the metals analyzed were lower than the maximum permissible level in soils. The trend of the heavy metals concentration in the soils can be represented as: Fe > Mn > Zn > Cr > Ni > Co > Cu > Pb > Mo > Cd. The results of the ANOVA showed significant differences ($p < 0.05$) in the concentrations of the heavy metals Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr Pb and Cd among the analyzed soil samples while there was no significant difference ($p > 0.05$) in the concentrations of Mo. In general, the results reveal that the level of contamination of the soils by the heavy metals is not high at present and the soil is not polluted by toxic heavy metals (Pb and Cd). Therefore, the soils studied were not harmful for cultivation of garlic and other agricultural activities.

Key words: Heavy metals · Soil · Garlic · Wet digestion · ICP-OES

INTRODUCTION

Soil is generally loose material composed of weathered rock and other minerals and the partly decayed organic matter that covers large parts of earth [1]. Soil is a dynamic natural body developed as a result of pedogenic processes during weathering of rocks [2]. Soil is the natural medium for the growth of land plants. Soils are all unconsolidated material of the earth crust in which land plants can grow, if water and temperature are adequate, at least the minimum nutrients are available and toxic substances are in low concentration [3].

Soil is an important indispensable storehouse of water, air and nutrients that are essential for plant growth. For optimum plant growth the available nutrient elements and water must available in adequate amounts and suitable proportion, a term that is widely referred to as "soil fertility". To evaluate the soil fertility status of soil, there is a need to determine its physical, chemical and biological characteristics [4]. Soil serves as both a sink and a source for heavy metal contaminants in the terrestrial environment [5, 6]. Excessive accumulation of heavy metals in agricultural soils through the use of agrochemicals and by other sources may not only result in soil contamination but also lead to elevated heavy metal up-take by vegetables and thus affect food quality and safety [7].

Heavy metal is a member of a loosely defined subsets of element that exhibit metallic properties with relative high atomic weight, having specific gravities equal to or greater than 5.0 g/cm^3 [8]. Heavy metals are hazardous contaminants in food and the environment and they are non-biodegradable having long biological half-lives [7, 8]. The implications associated with heavy metal contamination are of great concern, particularly in agricultural production systems due to their increasing

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trends in human foods and environment [10]. The main source of metal accumulation in humans is plants and vegetables consumed by them, grown in polluted areas. Metal deposition in the environment is either due to natural processes or as a result of human activities. The factors responsible for soil contamination are extensive use of chemical fertilizers and pesticides, mining processes, industrialization, vehicular exhausts and several others [11].

Heavy metal pollution has a negative impact on plant, animal and human health because of the emissions from the vehicles on the highways and industrial organizations as well as wrong agricultural practices (fertilizer, pesticides, etc.). Evaluation of the soil pollution and to examine it in accordance with the acceptable heavy metal limit values is very important factor for the ecological function of the soil and sustainable agriculture [12]. Heavy metal contamination of soils has been a rising environmental problem affecting agricultural production, food quality and threatening human health. However, certain metals such as Fe, Zn, Mn and Cu are essential at low level, other metals like Cd, Cr, Pb and Ni are toxic and may pose a great danger to humans, plants and animals through the food chain [13, 14]. Heavy metals are variably entering an agro-ecosystem through both natural and anthropogenic processes. Normally soil inherits trace elements from its geological parent materials, whereas anthropogenic sources vary through the use of fertilizers, organic manures, industrial and municipal wastes, irrigation and wet and/or dry deposits [14].

Soil pollution due to heavy metals has got serious attention in developing countries due to peculiar nature of heavy metals [15]. Heavy metals have deleterious impacts on environment due to their persistent nature [16-18]. Some of the heavy metals are mobile in nature which move from soil to plants and underground water circulating through entire food chain indirectly affecting the health of human [15, 19, 20].

In the last decades, human activities have resulted in a continuous increase in the levels of toxic heavy metals in the environment [21]. Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to a chronic accumulation of trace metals in the human kidney and liver, causing disruption of numerous biochemical processes such as cardiovascular, nervous, kidney and bone diseases [6]. In many regions of Ethiopia only limited data are available on heavy metal concentrations in garlic growing soils. The aim of this study is to determine the concentrations of heavy metals Fe, Zn, Mn, Cu, Mo, Co, Ni, Cr, Pb and Cd in soil used for

cultivation of garlic in Ambo Woreda, Ethiopia using Inductively coupled plasma optical emission spectrometry and to evaluate their contamination status with respect to international guidelines.

MATERIALS AND METHODS

Study Area: This study was conducted in 4 locations (Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti) in Ambo Woreda, West Shoa Zone Oromia, which is about 112 km West of Addis Ababa, the capital city of Ethiopia. Ambo Woreda is located between latitude 8°59'N and longitude 37°51'E with an elevation of 2101 meters above sea level, The temperature ranges from 15°C-29°C with average temperature of 22°C (Figure 1).

Chemicals and Reagents: All chemicals and reagents were of analytical reagent grade (Uni Chem® Chemical Reagents, India): HNO_3 (65–68%), HCl (35–38%) and H_2O_2 (30%) were used for digestion of the soil samples. Li_2CO_3 (99%, Kiran Lighi, Laboratories, India) were used as a matrix for the method blank for soil samples. Stock standard solutions of concentration 1000 ppm of the metals to be analyzed were used to prepare calibration standard solutions and spiking standard solutions. Double distilled water (chemically pure with conductivity < 2.0 µS/cm) was used for dilution and preparation of reagents and standards solutions as well as for rinsing glassware and sample bottles.

Apparatus and Instruments: Polyethylene plastic bags were used for sample collection. Porcelain mortar and pestle were used during grinding of the samples. 0.5 mm mesh sieve was used to sieve the ground soil samples. Electronic analytical balance with an accuracy of ±0.0001 g (AA-200DS, Deriver Instrument Company, Germany) was used for weighing the soil samples. Digestive Furnace (Model: KDN-20C China) was used to digest the dried and powdered soil samples. Borosilicate volumetric flasks (50, 100 and 1000 ml) were used during preparation of sample and metal standard solutions. Micropipettes (Boeco, Germany) with adjustable volumes of 10- 100 ìL and 100-1000 ìL were used for the preparation of the standard solutions and samples. Whatman filter paper No. 41 was used to filter the digested samples. Capped glass bottles (50 ml) were used for storage of final digested samples. Inductively coupled plasma-optical emission spectrometry (Model Agilent 720, Australia) was used for the analysis of the heavy metals Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd in the soil samples.
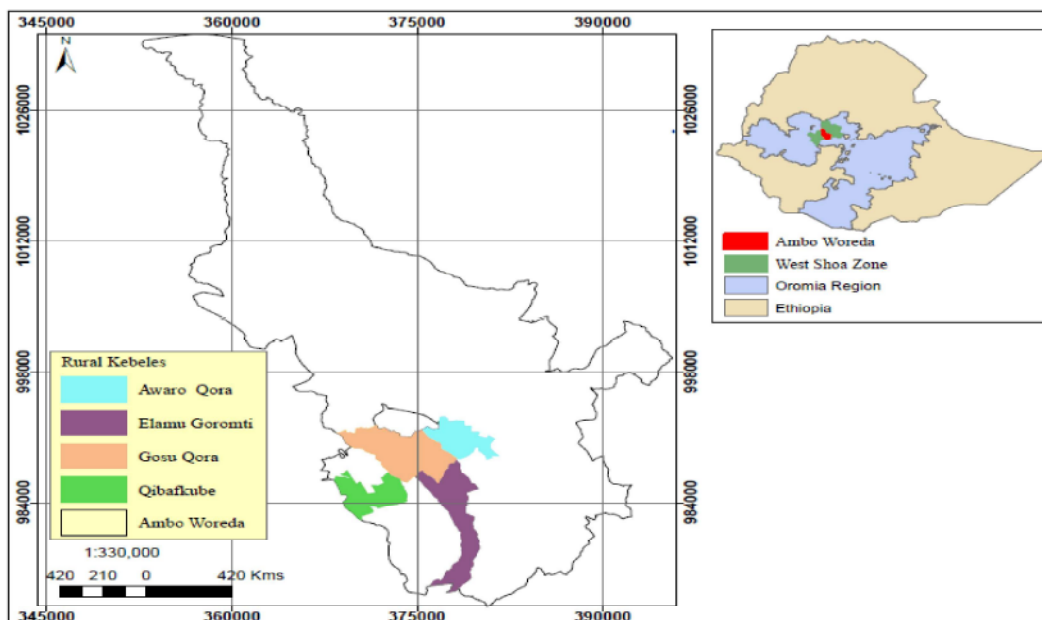
Fig. 1: Map of the study area

Apparatus Cleaning: The glassware and polyethylene containers used for analysis were washed with detergents and tap water. The glassware were then soaked in 20% HNO$_3$ solution overnight and rinsed several times with double distilled water. Then the apparatus were dried in oven (Model DHG-9070A, Shanghai, China) and kept in dust free place until analysis began.

Sample Collection: Soil samples were collected from four rural Kebeles of Ambo Woreda in the month of February, 2015. Five sub-sites were taken randomly from each of the main four sites. Top soil samples were collected at a depth of 0–15 cm by using soil auger. Five soil samples were taken from a particular sub-site. The collected sub-samples were then pooled together to form a composite sample of each sampling areas (about 1.0 kg of each composite sample from each area). Finally, the four soil bulk samples one from each stated areas were put in clean polyethylene bags, labeled and transported to the laboratory.

Sample Pretreatment: The soil samples were air-dried for a weak in the laboratory. The dried soil samples were ground with porcelain mortar and pestle and then sieved through a 0.5 mm mesh sieve, for analyzing total heavy metal content. The samples were stored in plastic containers until analysis.

Samples Digestion-Wet Ashing: 0.5 g of each soil samples were taken in to a digestive tubes containing a mixture of 6 ml aqua regia (4.5 ml HCl + 1.5 ml HNO$_3$) and 1.5 ml H$_2$O$_2$ in 4:1 v/v ratio. The mixture was digested in a digestive furnace (Model: KDN-20C, China) by setting the temperature first 150 °C for the first 1 hour, then increasing to 180 °C for the remaining 2 hours. The digest was allowed to cool for 10 minutes and filtered with Whatman filter paper No. 41 in to 50 ml volumetric flask. The digestive flask further rinsed with 10 ml double distilled water and added to the filtrate and the flask containing the filtrate was made up to the mark with double distilled water. The blank solutions were undergoing the same digestion procedure as that of the sample. Each sample was digested in triplicate and transferred into clean and dry plastic bottles, labeled and stored in refrigerator at 4 °C until analysis by ICP-OES.

Preparation of Calibration Standards: For calibration of the instruments, a series of five standard solutions were prepared by serial dilution of the stock standard solution (1000 ppm) of the metals to be analyzed. The prepared metal concentrations include: 0.2, 0.5, 1, 1.5 and 2 ppm of Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd.

Preparation of Spiking Standards: For the spiking processes of the soil samples, a mixture of standard solution containing 1 mg/L of each Zn, Mn, Cu, Fe, Mo,

Table 1: Wavelength of detection, calibration curve equation, correlation coefficient (R) of the calibration curves, limit of detection (LOD) and limit of quantification (LOQ) obtained for each element

Element	Wave length (nm)	Calibration equation	R	LOD (μg/g)	LOQ (μg/g)
Zn	206.2	Y = 4226.9x + 26.3	0.9999	0.3	1.2
Mn	294.9	Y =75560.8x + 76.3	0.9999	0.3	1.0
Cu	324.7	Y= 22876.2x + 180.1	0.9999	0.3	1.2
Fe	238.2	Y = 2068.4x + 18.7	0.9998	0.8	2.5
Mo	201.5	Y= 2295.5x + 24.8	0.9998	0.3	1.2
Co	236.4	Y= 2348.0x + 21.8	0.9999	0.6	1.9
Ni	221.6	Y= 2068.4x + 18.7	0.9999	0.6	1.9
Cr	206.5	Y= 4570.1x + 60.3	0.9999	0.5	1.6
Pb	283.3	Y= 1050.3x + 23.4	0.9999	0.3	1.2
Cd	214.4	Y= 1617.2x + 17.8	0.9997	0.1	0.3

Co, Ni, Cr, Pb and Cd was prepared by serial dilution from 1000 mg/L stock standard solution in to 100 ml volumetric flask and diluting to the mark with double distilled water.

Method Validation: The proposed method was validated by evaluating different parameters as linearity, matrix effect, limit of detection (LOD), limit of quantitation (LOQ), accuracy (in terms of recovery) and precision (in terms of repeatability) [22].

Accuracy and Precision: Accuracy and precision of the results was assessed by the analysis of matrix spike (MS) samples and laboratory control samples (LCS). Accuracy was evaluated through recovery studies of sample spikes. Precision was evaluated regarding repeatability by estimating the relative standard deviation (RSD) of the recovery percentage for each spiked level.

In this study, the recovery test was done by spiking a suitable known quantity of metal standard solution into a test portion of the sample. For doing so, each sample was spiked in triplicates at near mid-range calibration concentration (1.0 mg/L of Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd). The spiked and non-spiked samples were digested and analyzed using the same analytical procedure as the soil samples. The percentage recoveries of the analyte were calculated by using the following equation [23].

$$\% \text{ Recovery} = \frac{\text{conc. in spiked sample - conc. in unspiked sample}}{\text{actual spike conc.}} \times 100 \tag{1}$$

where, conc. = concentration of metal of interest.

The relative standard deviation for replicate analyses of the same sample were obtained as dividing the standard deviation by the mean value of the analytical data according to the following equation [23].

$$\%\text{RSD} = \frac{s}{\bar{x}} \times 100 \tag{2}$$

where, s = standard deviation of the replicate analysis and $\bar{x}$ is the mean of the replicate analysis.

Limit of Detection: The limit of detection (LOD) is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOD is calculated as:

$$\text{LOD} = 3S_a/b \tag{3}$$

where S_a is the standard deviation of the response; can be obtained by standard deviation of blank, response residual standard deviation of the regression line, or standard deviation of the y-intercept of the regression line and b is the slope of the calibration curve [24, 25].

In this study, the limit of detection was obtained from triplicate analysis of reagents blanks which were digested in the same digestion procedure as the actual samples. Table 1 shows the results of LOD for each analytes.

Limit of Quantification: The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy under the stated conditions of test [24, 25]. The LOQ is calculated as:

$$\text{LOQ} = 10S_a/b \tag{4}$$

where S_a is the standard deviation of the response and b is the slope of the calibration curve [24].

In this study, LOQ was obtained from triplicate analysis of reagents blanks which were digested in the same digestion procedure as the actual samples. Table 1 shows the results of LOQ for each analytes.

Method Blank: Method blank is an analyte-free sample carried through the analysis using the same reagents, glassware and instrumentation. Method blanks are used to identify and correct systematic errors due to impurities in the reagents, contamination in the glassware and instrumentation [26].

In this study, 0.5 g Li_2CO_3 was used as a matrix for the soil sample. The method blank solutions prepared from lithium carbonate were undergoing the same digestion procedure as that of the soil samples.

Laboratory Control Sample: The laboratory control sample (LCS) was analyzed in an identical manner as a sample and the results were used to assess accuracy and precision of the analytical method. In this work, three replicates of 0.5 g Li_2CO_3 were spiked with 1.0 mg/L of each of Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd. The spiked samples were digested like the soil samples including exposure to all glassware, digestion media, apparatus, solvents and reagents that are used with the soil samples. The percent LCS recoveries for each metal of interest were calculated using the following equation [27].

$$\% \, R = \frac{LCS\text{-}MB}{S} \times 100 \qquad (5)$$

where: % R = percent recovery, LCS = Laboratory Control Sample Results,
S = amount of spike added and MB = results of the method blank

Instrument Calibration: The instrument was calibrated using calibration blank and five series of working standard solutions of each metal to be analyzed by using 0.2, 0.5, 1, 1.5 and 2 ppm of Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd. The response curves for standard solutions were recorded and all the necessary graph and calculation were done and the results are presented in Table 1.

Determination of Heavy Metals in Soil Samples: The digested soil samples were analyzed for the heavy metal concentrations such as Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd using inductively coupled plasma-optical emission spectrometry (ICP-OES, Model Agilent 720, Australia). Final concentration of element in the samples was calculated as:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L) x V}}{w} \qquad (6)$$

Where: V is the final volume of the digested solution (50 ml) W is the weight of the sample (0.5 g).

Statistical Analysis: One-way analysis of variance (ANOVA) at P < 0.05 was used to determine statistically significant differences in the mean concentrations of heavy metals among groups of soil samples. A probability level of P < 0.05 was considered statistically significant. Pearson's correlation coefficient was used to determine the association between the heavy metals. All statistical analyses were done by SPSS version 16.0 software for windows.

RESULTS AND DISCUSSION

Method Validation Results
Calibration Curves, Limit of Detection and Limit of Quantification: Table 1 shows the results of the calibration curve parameters constructed for the studied soil samples. The table indicates the wavelength used for the ICP-OES elemental analysis, the calibration curve equation, the correlation coefficients, the limits of detection (LOD) and limits of quantification (LOQ) of the heavy metals analyzed.

The analytical curves presented good linearity with correlation coefficients (R) higher than 0.999 for all the heavy metals studied which were all greater than the acceptable limit (0.998) for the linearity of the regression line [28]. The high values of the correlation coefficients (*R*) obtained in Table 1 demonstrate good linear correlation of the absorbance with trace element concentrations indicating good calibration of the instrument.

From Table 1, the limit of detection (LOD) values for all the metals analyzed ranged from 0.1 µg/g for Cd to 0.8 µg/g for Fe and the limit of quantification (LOQ) values for all the metals analyzed ranged from 0.3 µg/g for Cd to 2.5 µg/g for Fe. The LOD and LOQ method obtained were low enough to detect the presence of metals of interest at trace levels in both samples.

Accuracy and Precision: The results of accuracy and precision were evaluated through recovery tests. Accuracy of the method was determined by matrix spike recovery studies and precision was expressed as relative standard deviation (RSD) of replicate results. The recovery values of the triplicate analysis of matrix spike soil samples were calculated using equation 1 and RSD values are were calculated using equation 2 and the results are showed in Table 2.

Table 2: Recovery and precision test results of metals for soil matrix spike sample

Metals	Conc. in sample (μg/g)	Amount added (μg/g)	Conc. in spiked sample (μg/g)	Recovery (%)	RSD (%)
Zn	96.12±2.85	100	189.4±3.70	93.28±1.48	1.59
Mn	113.56±1.37	100	208.6±4.26	95.04±3.27	3.44
Cu	19.59±0.69	100	119.2±2.73	99.61±6.13	6.15
Fe	12436±123.32	100	12528±81.14	92.00±7.18	7.80
Mo	2.34±0.32	100	93.7±1.42	91.36±2.65	2.90
Co	33.01±1.88	100	125.7±5.26	92.69±3.47	3.74
Ni	43.39±0.96	100	135.2±3.41	91.81±8.13	8.86
Cr	43.15±17.33	100	137±1.51	93.85±5.26	5.60
Pb	11.84±2.29	100	104.5±4.72	92.64±3.87	4.18
Cd	1.92±0.12	100	93.19±3.81	91.27±2.46	2.69

Table 3: Recovery and precision test results for the laboratory control samples

Metals	Amount added (μg/g)	Conc. in spiked Sample (μg/g)	Recovery (%)	RSD (%)
Zn	100	96.68±3.05	96.60±5.73	5.93
Mn	100	92.48±2.67	92.42±3.37	3.65
Cu	100	97.76±2.81	97.70±2.69	2.75
Fe	100	92.86±4.85	92.78±1.14	1.23
Mo	100	93.69±1.38	93.62±2.13	2.28
Co	100	94.53±1.58	94.48±6.28	6.65
Ni	100	90.32±2.14	90.26±4.50	4.99
Cr	100	92.43±1.83	92.36±1.28	1.38
Pb	100	91.86±3.42	91.78±4.06	4.42
Cd	100	93.49±2.91	93.42±5.12	5.48

As can be seen from Table 2, the percentage recovery of the metal analysis in the soil ranged between 91.27–99.61% and the RSD values ranged between 1.59–8.86%. The matrix spike recovery obtained in this study falls within the normal acceptable range of 90–110% for a good recovery study. The high percentage recovery obtained from the study validates the accuracy of the method and the reliability of the levels of metal concentration in this study. The RSD values of the samples were < 10%, indicating that that the proposed method was precise.

Laboratory Control Sample Results: Laboratory control sample recoveries and relative standard deviations (RSD) were calculated for the triplicate analysis of each analyte using equation 5 and 2 respectively. The corresponding results are summarized in Table 3.

As can be seen in Table 3, the percent recovery values of laboratory control sample (LCS) results lied in the range of 90.26% – 97.70% and the RSD values ranged from 1.23–6.65%. The percent recovery obtained in this study falls within the normal acceptable range of 90–110% for a good LCS recovery study and = 10% for RSD. These results showed that the analytical method possesses the required precision and accuracy.

Heavy Metals Concentration in Soil Samples: The mean concentrations of heavy metals in the soil samples are shown in Table 4. The data revealed that all the analyzed metals accumulated by the soil at different concentrations. Results are expressed as mean ± standard deviation of triplicate analyses (n=3).

Zinc (Zn): As can be seen from Table 4, the concentration of zinc was in the range of 74.29–112.2 mg/kg. The lowest zinc content was obtained in soil collected from Qibafkube site and the highest in soil collected from Awaro Qora site. The concentration of Zn in this study is much lower than the standard limit set by Ewers (1991) [29]. The high level of Zn in these agricultural sites could probably be due to heavy vegetable farming activities at these areas and high usage of various types of fertilizers and pesticides.

Manganese (Mn): From Table 4, the results of manganese concentrations were 113.56, 125.67, 101.05 and 106.08 mg/kg in sampling sites of Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti, respectively. The concentrations of manganese found in this study were lower than the FAO/WHO (2001) [30] and Pendias and pendias (1992) [31] recommended limit. The high Mn values in the study area indicated the high availability of Mn for plant uptake in the garlic farming areas.

Table 4: Mean concentrations of metals (mg/kg dry weight) of soil samples, mean±sd, n = 3

	Sample Sites				
	Awaro Qora	Gosu Qora	Qibafkube	Elamu Goromti	Max. Perm
Metals	Soil (mg/kg)	Soil (mg/kg)	Soil (mg/kg)	Soil (mg/kg)	L. in soil (mg/kg)
Zn	96.12±2.85	112.2±3.17	74.29±3.10	79.08±7.54	300[a]
Mn	113.56±1.37	125.67±2.04	101.05±0.68	106.08±1.49	2000[b,c]
Cu	19.59±0.69	24.55±1.32	16.73±0.81	14.91±1.11	100[a]
Fe	12436±123.32	14106±201.25	15638±241.69	13157±211.45	50000[b,c]
Mo	2.34±0.32	1.90±0.17	2.55±0.17	2.58±0.46	NA
Co	33.01±1.88	35.14±2.80	21.43±1.40	10.12±2.15	50[a]
Ni	43.39±0.96	50.56±0.21	26.99±1.29	17.69±0.23	50[a]
Cr	43.15±17.33	50.03±0.27	30.19±0.13	23.38±0.10	100[a]
Pb	11.84±2.29	13.76±3.62	15.58±4.12	12.44±3.11	100[a]
Cd	1.92±0.12	1.86±0.13	1.69±0.11	1.63±0.09	3[a]

Key: [a]Source: Ewers, 1991, [b]Source: FAO/WHO, 2001, [c]Source: Pendias and pendias, 1992 NA = Not available, sd = standard deviation

Copper (Cu): The concentration of copper (Table 4) in soil samples ranged between 14.91–24.55 mg/kg. The lowest concentration of copper was found in soil sample collected from Elamu Goromti site while the highest concentration in Gosu Qora soil. The content of Cu reported in this study was generally lower than the permissible levels set by Ewers [29] in soil.

Iron (Fe): The results in Table 4 reveal that the concentration of Fe was highest among the heavy metals analysed from all the sample sites and the levels obtained were found to be 12436 mg/kg in Awaro Qora soil, 14106 mg/kg in Gosu Qora soil, 15638 mg/kg in Qibafkube soil and 13157 mg/kg in Elamu Goromti soil. These results found were lower than the standard set by FAO/WHO [30] and Pendias and Pendias [31]. The high level of iron might be due its abundance because iron is the fourth most abundant element in the earth's crust.

Molybdenum (Mo): Results obtained showed that the level of molybdenum ranges from 1.90 - 2.58 mg/kg. The lowest concentration of molybdenum was found in soil sample collected from Gosu Qora site while the highest concentration in Elamu Goromti site (Table 4).

Cobalt (Co): The results revealed that the concentration levels of cobalt were 33.01, 35.14, 21.43 and 10.12 mg/kg in sample sites of Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti, respectively (Table 4). The levels of Co obtained in this study in all the sampling sites were lower than Ewers [29] recommended limit which is 50 mg/kg.

Nickel (Ni): As can be seen from Table 4, the highest concentration of nickel was found in Gosu Qora soil 50.56 mg/kg, followed by Awaro Qora soil 43.39 mg/kg,

Qibafkube soil 26.99 mg/kg and Elamu Goromti soil 17.69 mg/kg. The contents of nickel found in this study were lower than the Ewers [29] recommended limit.

Chromium (Cr): The concentration levels of chromium were 43.15, 50.03, 30.19 and 23.38 mg/kg in sample sites of Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti soils, respectively (Table 4). The levels of Cr obtained in this study in all the sampling sites were lower than Ewers [29] recommended limit which is 100 mg/kg.

Lead (Pb): In the studied soil samples, the results of Pb concentrations were 11.84, 13.76, 15.58 and 12.44 mg/kg in sample sites of Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti, respectively (Table 4). The relatively high levels of lead might have resulted from accumulation of lead through air pollution such as automobile exhaust fumes and from some pesticides, such as lead arsenates applied during cultivation. The values of Pb obtained in this study were lower than Ewers [29] recommended maximum limit that is 100 mg/kg.

Cadmium (Cd): As can be seen from Table 4, cadmium was the least abundant element in the soil samples studied. The cadmium content of soil in all the sampling sites were almost similar that is 1.92 mg/kg in Awaro Qora soil, 1.86 mg/kg in Gosu Qora soil, 1.69 mg/kg in Qibafkube soil and 1.63 mg/kg in Elamu Goromti soil. The high level of cadmium might be due to the use of cadmium-containing phosphate fertilizers and contamination from cadmium-containing dusts. The concentrations of cadmium obtained in this study were lower than Ewers [29] recommended maximum limit for soil that is 3 mg/kg. From this study also it was found that various agricultural activities carried out in the areas were not able to increase the Cd content in the soils.

Table 5: Correlation Coefficient (r) Matrix for Heavy Metals Concentration in Soil Samples

	Zn	Mn	Cu	Fe	Mo	Co	Ni	Cr	Pb
Zn	1								
Mn	0.992**	1							
Cu	0.951*	0.932	1						
Fe	-0.327	-0.320	-0.037	1					
Mo	-0.965*	-0.966*	-0.986*	0.074	1				
Co	0.822	0.749	0.891	-0.100	-0.807	1			
Ni	0.915	0.862	0.952*	-0.164	-0.898	0.982*	1		
Cr	0.930	0.882	0.964*	-0.162	-0.916	0.973*	0.999**	1	
Pb	-0.295	-0.295	0.003	0.998**	0.044	-0.041	-0.112	-0.111	1
Cd	0.800	0.721	0.776	-0.412	-0.697	0.942	0.927	0.914	-0.354

* r is significant at the 0.05 level (2-tailed) and ** r is significant at the 0.01 level (2-tailed)

The results of the heavy metals analyzed in the study areas showed that their concentration level is below the standard guide lines for maximum limit proposed for agricultural soil. Even though these heavy metal concentrations fell below the critical permissible concentration level in most heavy metals, it seems that their persistence in the soils of the study site may lead to increased uptake of these heavy metals by plants. One-way analysis of variance (ANOVA) was made at 95% confidence level. The results showed that there were significant differences ($p < 0.05$) in the concentrations of the heavy metals Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr Pb and Cd among the analyzed soil samples while there was no significant difference ($p > 0.05$) in the concentrations of Mo.

In general, the mean concentrations of heavy metals in soils collected from all sampling site decreased in the order of: Fe > Mn > Zn > Cr > Ni > Co > Cu > Pb > Mo > Cd. The levels of all the metals analyzed were lower than the maximum permissible level in soils.

Correlation Analysis: Pearson's correlation coefficient was used to examine the relationship between the various heavy metals in the soil samples from all the sample sites. Table 5 shows the correlation matrix of the relationship between heavy metals concentration of soil samples.

Rakesh and Raju [32] reported that high correlation coefficient (near +1 or -1) means a good relation between two variables and its concentration around zero means no relationship between them at a significant level of 0.05% level, it can be strongly correlated, if r > 0.7, whereas r values between 0.5 to 0.7 shows moderate correlation between two different parameters.

As can be seen from Table 5, the results of the correlation coefficients showed strong positive correlation between Zn with (Mn, Cu, Co, Ni, Cr and Cd), Mn with (Cu, Co, Ni, Cr and Cd), Cu with (Co, Ni, Cr and Cd), Fe with Pb, Co with (Ni, Cr and Cd) and Cr with Cd.

This strong positive correlation shows that the elements are closely associated, thus suggesting their common origin. The other elements have weak negative or positive correlation indicating that the presence or absence of one element affect in lesser extent to the other.

CONCLUSION

The concentration levels of of ten heavy metals (Zn, Mn, Cu, Fe, Mo, Co, Ni, Cr, Pb and Cd) in soil samples collected from Ambo Woreda (Awaro Qora, Gosu Qora, Qibafkube and Elamu Goromti) Kebeles, West Shoa Zone of Oromia, Ethiopia were determined by using Inductively coupled plasma- optical emission spectrometry (ICP-OES). Acid digestion method using a mixture of 6 ml aqua regia and 1.5 ml of H_2O_2 for digestion of soil sample was found to be efficient, precise and accurate for the metals analyzed and it was validated through recovery experiment and the recovery ranged from 90.27 to 99.61%, with RSD less than 10% for all analytes.

This study indicates that these soils serve as the potential source of the heavy metals in the environment and the concentration of all the studied heavy metals found in all kinds of soil samples were below the maximum recommended levels set by Ewers and FAO/WHO. The significance difference and increase in the elemental concentrations may be due to application of various types of pesticides and fertilizer in the vegetable farming areas.

In general, the results also show that the level of contamination of the soils by the metals is not high at present and the soil is not polluted by the heavy metals. Therefore, the soils studied were not harmful for the cultivation of garlic and other agricultural purposes, so there is no serious implication for health hazard. In conclusion, the present study will give brief information about the heavy metal contents of the soil and these

results may serve as a base line data for determination of mineral contents and physicochemical properties of the soil in the study area.

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