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Spectroscopic analysis of heavy metals distribution in selected traditional medicinal plants and soil in Raya Azebo district, Northern Ethiopia

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Abstract

The use of traditional medicine is increasing dramatically worldwide. In Ethiopia, people largely depend on the use of traditional medicinal plants as herbal remedies. However, the effectiveness of medicinal plants is affected by the presence of heavy metals. Thus, this study aimed to evaluate the distribution of heavy metals in selected medicinal plant roots and the soils in which they were grown. A total of five root and soil samples in triplicate were used for analysis. A wet digestion procedure involving the use of a mixture of strong acids was used for the analysis of plant and soil samples. Based on the results, the concentrations of Zn (21.82mg/kg) and Fe (7.78mg/kg) were higher in Solanum incanum and Carissa spinarum plant samples, respectively, than in the other plants. The concentrations of Mn, Cu, and Pb ranged from 1.70 – 4.22mg/kg, 1.34 – 3.43mg/kg, and 0.14 – 0.34 mg/kg, respectively, but Cd was detected only in Carissa spinarum (0.15 mg/kg) and in Solanum incunm (0.21mg/kg). Regarding the contents of the metals in the soil samples, Zn (15.45 – 44.3 mg/kg) is the most dominant metal, followed by Mn (9.54 - 23.07mg/kg), Fe (7.58 – 12.68 mg/kg), Cu (3.16 - 12.55 mg/kg), and Pb (0.14 - 2.85 mg/kg) whereas Cd was detected only in Boyegararsa soil (0.21 mg/kg) and Warabaye (0.65 mg/kg) soil samples. The results indicated that the contents of the metals studied did not exceed the permissible limit for medicinal plants set by WHO/FAO. Further studies should be carried out on the bioavailability of toxic heavy metals in traditional herbal medicines.

Keywords: Heavy metals, Medicinal plant, Traditional medicines, Soil

Introduction

Traditional medicines are referred to as the total of all knowledge and practice, used in the diagnosis, prevention, and elimination of physical, mental, or social imbalances, and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in written form (WHO, 2001). Traditional medicine has always played a key role in world health and continues to be used to treat a vast array of conditions and complaints. Many countries in Africa, Asia and Latin America use traditional medicine as home remedies to meet primary healthcare needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries (Kassaye et al., 2006).

Ethiopia is believed to be home to diverse flora composition (Dawit and Ahadu, 1993). The plant species is estimated to be 6,500-7000 including medicinal plants. Medicinal plants have been used for various types of human and animal treatments in the country. According to Birhanu (2015) and Bekele and Reddy (2015), in Ethiopia, nearly 80% of the human population and 90% of livestock rely on traditional medicine.

In Ethiopia, the long history of using traditional medicinal plants for combating various ailments can be confirmed by referring to the medico religious manuscripts in the country (Kibebew, 2001). The past decade has seen a significant increase in traditional medicine due to its minimal side effects, ease of availability, and acceptability to the majority of the populace. Although the effectiveness of medicinal plants is mainly associated with their constituents such as essential oils, vitamins, and glycosides, prolonged intake can cause health problems due to the possible presence of heavy metals (Jabeen et al., 2010).

Medicinal plants that are grown in soils polluted with toxic and heavy metals take up such metals and accumulate them in their edible parts in quantities high enough to cause clinical problems both to animals and humans. Thus, metal mobility and plant availability are crucial when assessing the effect of soil contamination, plant metal uptake, and toxicity (Bhuiyan et al., 2011; Kebede et al., 2006). It is necessary to know the composition of essential and toxic elements of each medicinal plant and assess their therapeutic potential. Essential major and toxic elements in traditional medicinal plants have been analyzed by many researchers to strengthen their importance to human health and to determine the elemental compositions of medicinal plants

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using different analytical techniques from many countries all over the world (Subramanian, 2012; Lanini, 2009; WHO, 2004). Though there have been some efforts to analyze the metals in different plants and livestock product (e.g. Abezash and Alemu, 2022; Deribachew et al., 2015) elsewhere, there is lack of studies on the essential and non-essential elements in different types of herbal medicinal plants in the Raya Azebo district. Thus, the current study was conducted to determine the status of heavy metals (essential and non-essential elements) that can be accumulated in the medicinal plant species and their soils grown in different localities of the district to ensure individuals' health status.

Materials and methods

Description of the study area

The study was undertaken in the lowland plain of Raya Azebo district in southern Tigray, Northern Ethiopia. Raya Azebo, which covers an area of about 176,210 hectares, is one of the eight districts in the Southern Zone and is geographically located at 12o15′ and13o41′ north latitude and 39o54′ east longitude (ETHIO_GIS CSA, 2019; Gedif et al., 2014). The area has an altitude from 930 to 1800 m.a.s.l. It has three agro-ecological zones: highland shares 1.4%, midland shares 80%, and lowland shares 18.6% of the area, with a mean yearly rainfall of 400 – 700 mm and an average minimum and maximum temperature of 18°C and 25°C, respectively (Hailelassie et al., 2018; Hagos et al., 2016). The major soils available in the woreda are Vertisols (60.32%), Leptosols (35.87%), and Cambisols (3.76%). The pH of the soils is moderately to strongly alkaline (Yemane et al., 2020).

The southern zone of the Tigray region including Raya Azebo is known for the distribution of various important herbal medicines including *Carissa spinarum* (Agam), *Echinopske bericho* (dander or kebericho), *Verbascum sinaiticum* (Terneka or Dabakeded), *Verbena officinalis* (Atush or Atuch), and *Solanum incanum* (Engule or Embouy). Most of the individuals in the rural district of Raya Azebo consume herbal medicines regularly for the treatment of abdominal complaints such as digestion, stomach ache, dyspepsia, campy abdominal pain, amoeba, abdominal bloating, etc. (Araya et al., 2015; Birhane et al., 2011).

Apparatus and instruments

Stainless steel axe and Teflon knife were used to cut plant root samples, while an air-circulating oven (Ditheat, P. Selecta, Spain) was used for drying the samples. Blending pestle devices (Moulex, France), ceramic pestles, and mortar were used for grinding and homogenizing the samples. Digital analytical balance (Mettler Toledo, Model AG204, Switzerland) was used to weigh the samples. A muffle furnace (Nabertherm) was used for the dry-ashing process of soil samples. Round bottom flasks with grounded glass (100ml) fitted with a reflux condenser was employed to digest the root and soil samples on the Kjeldahl heating apparatus (Gallenhamp, England). A flame atomic absorption spectrophotometer (FAAS, novAA 400P, Germany) was used for metal content analysis.

Chemicals and reagents

The chemicals and reagents used in the analysis were all analytical grades. Concentrated HCl (38%) (Hopkins and Williams, UK), (69-72%) HNO_3 (Fine Chemical Industries, Mumbai, India), 30% H_2O_2 (Scharlaw Chemie. S. A), and 70% $HClO_4$ (Aldrich, UK) were used for the digestion of soil and plant samples.

Experimental procedures

Cleaning apparatus

All glassware was cleaned with detergent and hot water, rinsed several times with distilled water, and then soaked for 12 hours in a 10% nitric acid solution. Finally, rinsed with de-ionized water and dried in the oven at 105 ℃. Plastic containers and polyethylene bags were washed with tap water using detergent, rinsed with de-ionized water, soaked in about 10% (v/v) nitric acid for 24 hours, then rinsed with de-ionized water, dried in an oven, and kept dust-free until further use.

Plant and soil sample collection and preparation

Five plant species in triplicate were collected from five different localities, based on their availability and knowledge of the society regarding their medicinal values. Accordingly, medicinal plant root samples that are used as a cure for diseases of *Solanum incunm* from Werabaye, *Carissa spinarum* from Boye-Gararsa, *Verbascums inaiticum* from Chercher, *Echnopis kebericho* from Chekon, and *Verbena officinalis* from Embachara were collected, then packed into polyethylene plastic bags, labeled, and transported to a laboratory for further treatment. The roots of the stated plants were separated with stainless steel and Teflon knives,

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washed with distilled deionized water, and air dried and was put on acid-washed porcelain labeled according to the samples and dried in an air oven at 105^{o}C for 48 hours until they got brittle and crisp. Cooling to ambient temperature, the dried samples were ground into fine powder with a blending device, mortar, and pestle, and sieved (1 mm). The powdered sample was then placed in a pre-cleaned screw-capped polyethylene container and stored in desiccators with calcium chloride to keep at constant dry weight until digestion.

Soil samples from different points were collected in triplication from the five study sites on which the medicinal plants were grown. Using a soil auger soil samples were taken from 0-50 cm of the same plots where the medicinal plants were collected representing the plow layer and average root zone for nutrient uptake and heavy metals burden by plants (Nyangababo and Hamya, 1986). Fifty subsamples of the soil were collected and composited for one representative soil sample. A total of five composite soil samples; 1 kg from each leading site was collected. The soil samples taken from each site were separately labeled, transferred into air-tight polythene bags, and brought to laboratory analysis. To prepare soil samples for further laboratory analysis, larger particles and other debris were removed from the soil and then soil samples were air dried in a dry and dust free place at room temperature for 5 days, followed by oven drying until getting constant weights. The samples were then ground with a mortar and pestle to pass through a 2 mm sieve to remove coarse particles and homogenized. The dried, sieved, and homogenized soil samples were placed in polyethylene bags at ambient temperature until the time of digestion.

Digestion of plant samples

Obtaining an optimum condition for digestion is the basic requirement for sample preparation for analysis. An optimum digestion procedure was obtained following the standard analytical procedure as indicated by Huang et al. (2004) and Gupta et al. (2008). To digest plant samples, 0.5 g powdered root samples of the plants were weighed into the Teflon PFA vessels and digested for 2:30 hours at 200˚C with an added 6 ml mixture of concentrated HNO_3:$HClO_4$ (4:2 ml ratio) on a Kjeldahl digestion block. The resulting clear solution was filtered into 100 ml volumetric flasks through Whatman No. 42 filter paper and diluted with deionized water to mark and kept for further analysis. The same procedure was used to prepare the blank solution.

Digestion of soil samples

Air-dried and ground soil samples were sieved with 2 mm mesh size, 0.5g were placed in a block digester, and 6 ml of 3:2 ratio of HNO_3 to $HClO_4$ was added before adding 1 ml of concentrated H_2O_2 to prepare a clear and colorless sample solution that was suitable for the analysis using FAAS. The sample was swirled gently to homogenize, then fitted to a reflux condenser and digested continuously for 3:00 hours on the Kjeldahl digestion block. The temperature was adjusted within certain time intervals to give the maximum temperature of 150 °C, providing a clear, colorless solution. The digest was then cooled, and a few drops of water were added before filtering using Whatman No 42 filter paper. The filtrate was then diluted with deionized water to 100 ml and stored for further analysis (Hur et al., 2003).

Instrument operating conditions

Intermediate standard solutions were prepared from the atomic absorption spectroscopy standard stock solutions containing 1000 mg/L. These intermediate standards were diluted with distilled water to obtain working standards for each metal of interest. Parameters (burner and lamp alignment, slit width, and wavelength adjustment) were optimized for the maximum signal intensity of the instrument based on the instrument instruction (Table 1). Three replicate determinations were carried out on each plant and soil sample under FAAS conditions.

Table 1. FAAS Instrument operating condition for the metal analysis of plant root and soil samples

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	IDL (mg/kg)
Cd	228.9	0.7	4.00	0.005
Cu	324.8	0.5	4.00	0.02
Fe	248.3	0.2	5.00	0.03
Mn	279.5	0.2	5.00	0.001
Pb	283.2	0.7	5.00	0.10
Zn	213.9	0.7	5.00	0.005

Method validation

The validation of the method was done using recovery analysis of the elements and the analysis of standard solutions before and after spiking. The validity of the digestion procedure, precision,

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and accuracy of the method were assessed by spiking soil and plant root samples with the standard known concentration. The spiked and non-spiked plant root and soil samples were digested following the same procedure employed in the digestion of the respective samples and analyzed for available metals (Cu, Mn, Fe, Zn, Cd, Pb) in similar conditions (Table 4 and Table 5). Then the percentage recoveries of the analyte were calculated (Deribachew et al., 2015).

Measurement procedure of pH, EC, OC, TN, and available P in soils

The pH of the soil samples was measured in water suspension (1:2.5) soil-to-water ratio as described by (Jackson, 1967). Standard procedure of Jackson (1967) was also used to determine the electrical conductivity (EC) of soil samples. The organic carbon content of the soil samples was determined by the method of Walkley and Black (1934). The total N content in soil was determined using the Kjeldahl procedure of Gupta (2008) and available P was determined by the Olsen method (Olsen et al., 1954).

Statistical analysis

The digested root and soil samples were analyzed in triplicate and the data were presented as means ± standard deviations. Differences between treatment means were done by using an analysis of variance (ANOVA). For comparison of the means of the treatments, Fisher's least significant difference test was used at P = 0.05 significance level. A Pearson Correlation Coefficient was used to check associations of the same metal in the soil with plant roots and also to check whether the ions of one kind present in the soil, either facilitate or interfere with the uptake of the other kind of ions. All statistical works were done by SAS 9.1.3 window version software program (Miller and Miller, 2005; SAS Institute, 2002).

Results and discussion

Optimization of digestion procedure of plant and oil samples

The optimization of different conditions tested for the digestion procedure of 0.5 g plant samples were summarized in Table 2. The optimization procedure of the acid mixture of HNO_3:HNO_4 (4:2 ml), digestion temperature of 200˚C, and digestion time of 2:30 hours were found to be the optimal condition for the digestion of 0.5 g medicinal plant root samples.

Table 2. Optimization conditions of the digestion procedure for 0.5 g of root samples medicinal plant.

Trial	Reagents	Volume (ml)	Temp (℃)	Time (min)	Observation
1	HNO_3:$HClO_4$	2:1	120	60	Deep yellow
2	HNO_3:$HClO_4$	3:1	150	90	Deep yellow
3	HNO_3:$HClO_4$	3:2	180	100	Yellow solution
4	HNO_3:$HClO_4$	3:2	200	120	Yellow solution
5	HNO_3:$HClO_4$	5:2	210	170	Clear & colorless solution
6	HNO_3:$HClO_4$	4:2*	200*	150*	Colorless solution
7	HNO_3:$HClO_4$	6:1	240	170	Colorless solution
8	HNO_3:$HClO_4$	5:3	250	200	Colorless solution
9	HNO_3:$HClO_4$	6:2	270	210	Clear & colorless

* The optimum digestion conditions

Soil samples were digested using a mixture of (HNO_3 + $HClO_4$ + H_2O_2) as recommended by (Huret al., 2003). The optimum condition achieved for soil sample digestion was a reagent mixture of 6 ml acid (3:2 ratio of HNO_3 to $HClO_4$) and 1 ml H_2O_2, digestion temperature 150˚C, and digestion time of 3:00hours for 0.5 g soil sample (Table 3).

Table 3. Parameters optimized for the digestion of 0.5g of soil

Trial	Reagents	Volume (ml)	Temp (℃)	Time (hour)	Observation
1	HNO_3:$HClO_4$	5:10	180	4:30	Clear light yellow
2	HNO_3:$HClO_4$	4:10	180	4:30	Clear light yellow
3	HNO_3:$HClO_4$	5:2	210	4:35	Deep yellow
4	HNO_3:$HClO_4$	3:2	210	3:00	Deep yellow
5	HNO_3:H_2O_2	10:1	210	3:15	Clear yellow
6	HNO_3:$HClO_4$	4:10	240	4:00	Yellow
7	HNO_3:$HClO_4$	5:10	150	4:10	Clear light yellow
8	HNO_3:$HClO_4$:H_2O_2	3:2:1.5	150	3:00	Clear &colorless
9	HNO_3:$HClO_4$:H_2O_2	3:2:1*	150*	3:00 *	Clear & colorless

* The optimum digestion conditions

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Analytical method validation

As indicated in Tables 4 and 5, the percentage recovery for the root samples of the plant and soils lies in the range of 97.6% - 104.6%, this is within the acceptable range.

Table 4. Recovery test for the optimized procedure of heavy metals on root samples

Metals	Concentration in Sample (mg/kg)	Amount added (mg/kg)	Concentration In spiked sample(mg/kg)	Amount recovered(mg/kg)	% Recovery
Cd	0.15	5	5.12	4.97	99.4
Cu	3.43	10	13.21	9.78	97.8
Fe	7.78	15	23.24	15.46	103.06
Mn	2.22	10	11.98	9.76	97.6
Pb	0.34	2	4.33	1.97	99.5
Zn	18.98	7	26.15	7.17	102.4

Table 5. Recovery test for the optimized procedure of metals on soil samples

Metals	Concentration In Sample (mg/kg)	Amount added (mg/kg)	Concentration In spiked sample (mg/kg)	Amount recovered (mg/kg)	% Recovery
Cd	0.21	2	2.19	1.9	99.03
Cu	12.55	10	23.01	10.4	104.6
Fe	11.05	5	15.98	4.9	98.6
Mn	19.15	15	33.98	14.8	98.6
Pb	0.78	2	2.80	2.02	101
Zn	44.26	15	58.98	14.7	98.13

Evaluation of pH, EC, OC, TN, and available P of soil samples

The organic matter content and soil pH are among the major factors that greatly affect the bioavailability of macro and micronutrients and even toxic elements in the soils and their uptake

by plant roots (Xu et al., 2015; Brady and Weil, 2002). Recent studies have revealed that heavy metal accumulation and their toxicity are not dependent on the total heavy metal concentration, and heavy metal accumulation in plants tends to depend on the availabilities of the heavy metals in soils (Lee et al., 2015; Chen et al., 2014), which are generally influenced by the adsorption and desorption characteristics of the soil (Zhang et al., 2017; Monterroso et al., 2014). Another report by Scotti et al. (1999) indicated that the solubility and bioavailability of heavy metals (Zn, Cd, Ni, and Cu) are negatively correlated with soil pH because the soil pH affects solubility and speciation in soil solution (Zeng et al., 2011; Zhao et al., 2010). The pH and other physicochemical properties of soils that affect the availability of nutrients in the plant and nutrient accumulation in the soil were given in Table 6.

The pH of studied soil samples was found within the range of 6.81 to 7.99 in which a higher value was observed in SS5 and SS1 had a lower value of pH. The soil pH ranges from slightly acidic to moderately alkaline nature in the order of SS1, SS4, SS2, SS3, and SS5 respectively. The pH values obtained in this study were slightly higher than those reported by Alghobar and Suresha (2017) for soil samples irrigated with different water sources. ANOVA showed that the mean pH values of SS2, SS3, and SS5 were statistically significant ($p<0.05$) from SS1 and SS4.

Xu et al. (2015) reported that OM content in soil has been shown increased uptake of heavy metals (Pb and Hg) by the plant roots that determine the nutritional status of soil, and keep heavy metals in an exchangeable form and chelate with heavy metals to increase metal bioavailability (McCauley et al., 2009). The result of the percent organic carbon (OC) in the soil samples studied was in the order of SS2, SS3, SS1, SS5, and SS4 respectively. According to Charman and Roper (2007) report, the range of organic matter from 1.8 – 3% is moderate. The values obtained in this study were in the moderate range except in SS2 and SS3 which are below the moderate range.

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Table 6. Average values of soil pH, EC, OC, TN, and available P in five different types of soils

Soil Sample	pH	EC(dS/m)	OC (%)	Total N (%)	Available P
SS1	6.81	0.12	1.74	0.11	25.58
SS2	7.56	0.20	1.37	0.12	27.58
SS3	7.62	0.15	1.35	0.09	33.01
SS4	7.16	0.16	2.66	0.14	30.80
SS5	7.99	0.21	1.78	0.16	37.74

SS1 = Werabaye, SS2 = Boye-Gararsa, SS3 = Chercher, SS4 = Chekon, SS5 = Embachara, EC = Electrical conductivity, OC = Organic Carbon

The electrical conductivity (EC) obtained in this study ranged from 0.12 - 0.21 dS/m. According to Singaravel and Govindamsamy (2000), the EC value is rated as 0.5 dS/m for good soil. The data we have obtained from this study is much lower than the values reported by Alghobar and Suresha (2017) which have ranged between 172 – 297 µS/cm. Mekki andSayadi (2017) reported much higher EC values that range from 3800 – 4050 S/cm for soil samples saturated with phosphate processing wastewater. However, the EC results we investigated showed that the soils were below the recommended standard.

The total nitrogen (TN) of studied soil samples was found to be in the range of 0.09% - 0.16%. According to Landon (2014), the total nitrogen content of the soils was categorized as <0.1% as very low, 0.1 - 0.2%as low, 0.2 - 0.5% as a medium, 0.5 – 1% high, and >1% as very high. Accordingly, the TN content of the soil in the studied area was categorized as low. ANOVA showed that the mean TN concentration was statistically significant ($p<0.05$) (Table 6).

The mean concentration of phosphorus in studied soils ranged from 25.58 ± 0.07 mg/kg - 37.74 ± 0.31 mg/kg. The highest mean concentration obtained in SS5 and the lowest were recorded in SS1, respectively. One-way ANOVA showed that the mean concentration of phosphorus in the soil samples was statistically significant ($p<0.05$). Landon (1991) categorized the amount of extractable phosphorus from the soil as >50 mg/kg as high, 15 – 50 mg/kg as a medium, and < 15 mg/kg as low. Based on this classification, the available Phosphorus content of soils was

medium. According to Mishra et al. (2004), P in the Ethiopian soils possess different scenario wherein only a very low fraction of total P is available to plants.

Distribution of metals in root samples

The concentration of each element varies among root samples indicating that each plant varies in the ability to accumulate each element. The concentration of essential and nonessential metals (Pb, Zn, Cu, Cd, Fe, and Mn) investigated were expressed per dry weight as shown in Table 7 and Figure 2(a). The results showed that the samples had a variable composition of each metal with a wide concentration range. Among the investigated six essential and toxic metals, all were above the instrument detection limit (IDL) except cadmium (Cd) not detected in the roots of PS3, PS4 and PS5.

Table 7. Concentration (Mean ± SD, n=3 in mg/kg dry wt) of metals in plant root samples from five sample sites.

Plant Sample	Pb	Zn	Cd	Cu	Mn	Fe
PS1	0.24^{b}±0.03	21.82^{a}±0.61	0.21^{a}±0.01	2.58^{c}±0.03	4.22^{a}±0.10	4.84^{d}±0.12
PS2	0.34^{a}±0.02	18.98^{b}±0.14	0.15^{b}±0.01	3.43^{a}±0.02	2.22^{c}±0.10	7.78^{d}±0.09
PS3	0.14^{d}±0.03	16.47^{c}±0.06	ND	1.80^{d}±0.02	1.70^{d}±0.02	3.92^{e}±0.01
PS4	0.23^{bc}±0.03	8.85^{e}±0.07	ND	2.82^{b}±0.06	1.83^{d}±0.05	6.10^{b}±0.11
PS5	0.20^{c}±0.01	12.07^{d}±0.14	ND	1.34^{e}±0.02	2.43^{b}±0.05	5.28^{c}±0.04
CV	9.5	1.84	10.06	1.35	2.84	1.57
LSD	0.04	0.52	0.02	0.06	0.13	0.16

PS1= Solanumincunm, PS2 = Carissa spinarum, PS3 = Verbascum sinaiticum, PS4 = Echnopis kebericho, PS5 = Verbena officinalis, CV = Coefficient of Variation, LSD = Least Significant Difference, *Means with the same letter are not significant

Lead (Pb) is a non-essential trace element having functions neither in the human body nor in plants. Its exposure may adversely affect the blood, nervous, renal, skeletal muscular, reproductive, and cardiovascular systems, causing poor muscle coordination (Johnson, 1998). Pb 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 an amount of Pb above 0.01 mg/kg is detrimental to health, as it may result in possible neurological damage to fetuses, abortion, and

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other complications in children under three years (Asemave et al., 2012). As shown in Table 7, the Pb contents in the root samples were within the range of 0.14 ± 0.03 to 0.34 ± 0.02 mg/kg in PS3, PS5, PS4, PS1, and PS2 respectively. The lead content in the analyzed medicinal plants is below the permissible limit for medicinal plants of 2 mg/kg set by WHO (WHO, 1996). ANOVA showed that the mean concentration of Pb was statistically significant ($P > 0.05$) between PS2 and PS3 while the mean concentration of Pb among PS1, PS4, and PS5 was statistically not significant ($P < 0.05$). The study revealed that lead (Pb) concentration is not a matter of concern from the toxicity point of view for the observed medicinal herb as far as permissible level in medicinal plants is concerned.

The highest concentration of cadmium (Cd) in this study was recorded in PS1 (0.21 ± 0.01 mg/kg), followed by PS2 (0.15 ± 0.01 mg/kg). The remaining PS3, PS4, and PS5 have been detected below the limits of detection. The maximum allowable limit of Cd in medicinal plants recommended by WHO is 0.3 mg/kg (WHO/FAO, 2001), implying low Cd contamination was investigated in this study. Cd accumulates in the human body and damages mainly the kidneys and liver. Higher levels of Cd pose severe toxicological impacts on human health, and it gets into the human body through food ingestion, especially plant-based foodstuffs. The kidney, liver, and other internal organs, especially the renal tract, is the critical organ for intoxication after exposure to Cd (Martin et al., 2009).

Manganese (Mn) content in the plant root range from 1.70 ± 0.02mg/kg - 4.22 ± 0.10 mg/kg. The value (1.79 - 4.31 mg/kg) reported by Jasha et al. (2016) was almost comparable with the value obtained in this study. The highest amount of Mn (4.22 ± 0.10mg/kg) was reported in the root of PS1. The concentration of Mn in all medicinal plant root samples analyzed was below the permissible limit of 6.61 mg/kg (WHO, 1996). On an overall average basis, Mn distribution in the roots was in ascending order of PS3 < PS4 < PS2 < PS5 < PS1. Mn is a ubiquitous, essential element required for normal growth, development, and cellular homeostasis. Mn can be correlated with therapeutic properties against diabetic, cardiovascular diseases and have a role in neurodegenerative diseases (Aron et al., 2011). One-way ANOVA showed that the mean concentration of Mn in the roots of PS3 and PS4 was statistically not significant ($P>0.05$) while, the mean concentration of Mn in the roots of PS1, PS2, and PS5 was statistically significant ($P<0.05$).

As indicated in Table 7, the higher level of Zinc (Zn) was obtained in PS1 (21.82 ± 0.61 mg/kg) and the least amount investigated in the root part of PS4 (8.85 ± 0.07 mg/kg). Based on overall mean values, Zn distribution in the roots of the plants is in the order of PS4 < PS5 < PS3 < PS2 < PS1. FAO/WHO set the permissible limit for Zn in medicinal plants to be 50 mg/kg, while its intake in food is 11 mg/day (WHO, 2006; WHO, 2005). However, all the ventures exhibited low concentrations compared to the allowable limit of WHO. Zn is an essential component of many enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids and the metabolism of other micronutrients. It stabilizes the molecular structure of cellular components and membranes and contributes to maintaining cell and organ integrity. Its involvement in such fundamental activities probably accounts for the essentiality of Zn for all life forms. It plays a central role in the immune system, affecting several cellular and humoral immunity aspects. Zn is also an essential part of insulin, and it is known to improve insulin sensitivity in the management of diabetes (Sharma et al., 2018). ANOVA showed that the mean concentration of Zn was statistically significant ($P<0.05$) in all five root samples.

The mean concentration of iron (Fe) in the root samples ranged from 3.92±0.01mg/kg - 7.78±0.09mg/kg. On the overall mean basis, the distribution pattern of Fe in root samples was in ascending order of PS3 < PS1 < PS5 < PS4 < PS2. The variation in the distribution of the metal in plants might be due to geographical and geological differences in the soil as well as the use of different agrochemicals. The permissible limit set by FAO/WHO in edible plants was 20mg/kg (FAO/WHO, 1984). Comparing the level with the value obtained in this study, it was lower. ANOVA showed that the mean concentration of Fe in all plant root samples was statistically significant ($P<0.05$). Fe in the human body has three main functions. It is a part of hemoglobin and is responsible for oxygen transport, maintains a healthy immune system, and is a constituent of several enzymes responsible for energy production. Fe deficiency is probably the most common nutritional deficiency globally though it performs the most vital functions in the body. An estimate based on WHO criteria indicated that around 600-700 million people worldwide had marked iron deficiency anemia, especially in developing countries (De Maeyer et al., 1985).

Copper (Cu) is an essential micronutrient involved in several biological processes needed to sustain life. However, it can be toxic when present in excess (deRomañ et al., 2011)**.** The concentration of Cu ranges from 1.34±0.02mg/kg to 3.43±0.02mg/kg in the root sample of traditional medicinal plants (Table 7**)**. The distribution pattern was in the order of PS5 < PS3 <

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PS1 < PS4 < PS2. The results were below the permissible limit of 73.3mg/kg reported by (FAO/WHO, 2001). One-way ANOVA showed that the mean concentration of Cu in all the studied plant root samples was statistically significant (P<0.05).

Distribution of metals in supporting soil samples

As shown in Table 8, the soil samples contained an average Pb in the range of 0.14 ± 0.01 to 2.85 ± 0.05 mg/kg. The highest concentration of Pb was found at SS5 (2.85 mg/kg) followed by SS4 (1.18 mg/kg), SS1 (0.78 mg/kg), SS2 (0.77 mg/kg), and SS3 (0.14 mg/kg). The Pb levels obtained in all soil samples of this study were less than the values reported for Pb (10 mg/kg) (Sharma et al., 2018). The maximum permissible limit of Pb in agricultural soil sediments recommended is 100 mg/kg (FAO/WHO, 2001). This was much greater than the results of this study. ANOVA showed that the mean concentration of Pb in SS1and SS2 was statistically not significant (P>0.05) but it was statistically significant (P<0.05) among SS3, SS4, and SS5.

Table 8. Concentration (Mean ± SD, n=3 in mg/kg dry wt) of metals in soil samples of five sites.

Soil samples	Pb	Zn	Cd	Cu	Mn	Fe
SS1	0.78^{c}±0.06	44.30^{a}±0.12	0.21^{b}±0.01	12.55^{a} ±0.20	19.15^{b}±0.19	11.02^{b}±0.13
SS2	0.77^{c}±0.02	40.30^{b}±0.37	0.65^{a}±0.02	11.92^{b}±0.08	18.08^{c}±0.10	12.59^{a}±0.17
SS3	0.14^{d}±0.01	32.24^{c}±0.1	ND	3.16^{d}±0.11	9.54^{e}±0.31	7.58^{d}±0.07
SS4	1.18^{b}±0.01	15.45^{e}±0.22	ND	7.91^{c}±0.07	14.68^{d}±0.26	8.22^{c}±0.03
SS5	2.85^{a}±0.05	22.84^{d}±0.12	ND	2.55^{e}±0.05	23.07^{a}±0.11	12.68^{a}±0.13
CV	3.21	0.68	4.45	1.48	1.24	1.09
LSD	0.07	0.38	0.01	0.20	0.38	0.21

SS1 = Werabaye, SS2 = Boye-Gararsa, SS3 = Chercher, SS4 = Chekon, SS5 = Embachara, CV = Coefficient of Variation, LSD = Least Significant Difference, *Means with the same letter are not significant

The natural Zn range in soils is 10-300 mg/kg (Eddy et al., 2006). As shown in Table 8, the soil concentration of Zn was within the natural range between15.45mg/kg of SS4 and 44.30 mg/kg of SS1. The permissible limit of Zn in the soil set by WHO/FAO is 300 mg/kg (FAO/WHO, 2001). So, the concentration of Zn obtained in the soil samples of the current study was below the

allowable limit set by FAO/WHO. According to Odukoya et al. (2000), Zn is required in human nutrients for the body's normal functioning. The deficiency of Zn in man can lead to impaired growth, low energy balance, and low protein intake. In contrast, excessive Zn intake can lead to vomiting, dehydration, electrolyte imbalance, abdominal pain, and lack of muscular coordination. ANOVA showed that the mean concentration of Zn was statistically significant ($P<0.05$) among all soil samples of plant origin.

The levels of Cd in soil were 0.21 ± 0.01 mg/kg at SS1and 0.65 ± 0.01 mg/kg at SS2. The rest samples were below the method detection limit. Critical levels for Cd in soil are between 3-5 mg/kg (FAO/WHO, 2001). The Cd concentrations obtained in this study were lower than these values. ANOVA showed that the mean concentration of Cd was statistically significant ($P<0.05$) among SS1 and SS2 soil samples.

The Cu levels in soil ranged from 2.55 mg/kg of SS5 to 12.55 mg/kg of SS1 soil samples (Table 8). ANOVA showed that the mean concentration of Cu was statistically significant ($P<0.05$) among all soil samples of plant origin. The difference in Cu levels in the sampling sites could be attributed to their parent materials. Cu concentration levels reported in this study were below the permissible limits for 100 mg/kg (FAO/WHO, 2001), implying no Cu contamination in the soil.

The mean concentration range of Mn in soil samples was 9.54 ± 0.31 mg/kg to 23.07 ± 0.11 mg/kg of SS5 and SS3 soils, respectively. The value was below the permissible limit of 500 mg/kg set by (FAO/WHO, 2001). The levels of Mn obtained in the soil samples were much lower than the reference value of 2000 mg/kg reported by (Mahmood et al., 2014). One-way ANOVA explained that there is a statistically significant difference ($p<0.05$) in levels of Mn in all five soil samples under study.

As shown in Table 8, the results indicate that soil samples contained Fe in the range of 7.58 ± 0.07 mg/kg at SS3 and 12.68 ± 0.13 mg/kg at SS5. This is lower than the Fe content in the soils ranging between 73.62 mg/kg to 226.39 mg/Kg (Akubugwo et al., 2012). ANOVA showed that the mean concentration of Fe was not statistically significant ($P>0.05$) among SS2 and SS5 soil samples. In contrast, the difference was significant ($P<0.05$) among SS1, SS3, and SS4 soil samples. However, the average Fe content in SS2 and SS5 was statistically significant ($P<0.05$) from SS1, SS3, and SS4 soil samples.

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Pearson correlations of metals within plant samples

The high positive correlation between Cu with (Pb, Cd), Mn with Zn, and Fe with (Pb, Cu) indicates that the presence or absence of one metal affects the larger extent of the other (Table 9). The high negative correlation for Fe with (Zn & Mn) indicates that the large absorption of one metal may affect the absorption of the other metal in plant roots and the other metals have a weak positive correlation indicating that the presence or absence of one metal is lesser than the other.

Table 9. Pearson correlation of metals within plant samples

	Pb	Zn	Cd	Cu	Mn	Fe
Pb	1					
Zn	0.285	1				
Cd	0.633*	0.394	1			
Cu	0.799*	0.289	0.802*	1		
Mn	0.219	0.651**	0.588*	0.086	1	
Fe	0.876*	-0.035	0.439	0.756*	-0.153	1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

Pearson correlations of metals in plants and soils

Pearson's correlation coefficient analyzed the relationship between the contents of different elements in the soil and plant roots. The correlation analysis is a bivariant method that describes the relationship between two different parameters. A high correlation coefficient (near +1 or −1) means a good relationship between two variables. Its concentration around zero means no relationship between them at a significant level of 0.05%; it is firmly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show a moderate correlation between two different parameters (Sharma and Raju, 2013).

The results showed that the relationship among the elements Pb, Zn, Cd, and Cu, have negative correlations with Pb in soil, and Mn in soil with Cu negative correlations. Correlation analysis between metal concentrations in different fractions of soil and vegetables was performed at 95% and 99% confidence levels. Cd with Pb, Cu, Fe, and Cu with Pb, Cd, and Cu show a highly

positive correlation. Cu with Mn & Fe, Cd with Zn, and Fe with Pb show moderate correlation. There was no correlation observed between Pb & Mn and Fe &Cu, and the rest show a low correlation, as shown in Table10. A negative correlation indicated that higher concentrations of heavy metals were present in soils, but in comparison, much lower concentrations were found in the root of that soil.

Table 10. Pearson correlation of metals within plant and soil samples

	Pb in soil	Zn in soil	Cd in soil	Cu in soil	Mn in soil	Fe in soil
Pb in plant	-0.009	0.346	0.851**	0.770**	0.419	0.590*
Zn in plant	-0.498	0.995**	0.593*	0.583*	0.045	0.309
Cd in plant	-0.321	0.404	0.495	0.947**	0.160	0.132
Cu in plant	-0.485	0.338	0.760**	0.864**	-0.087	0.090
Mnin plant	0.034	0.620*	0.176	0.573*	0.505	0.409
Fein plant	0.102	0.043	0.769**	0.542*	0.340	0.514

*Correlation is significant at the 0.05 level (2-tailed);**Correlation is significant at the 0.01 level (2-tailed)

Davies and White (1981) reported that heavy metal concentrations are mostly higher in soils than in medicinal plants grown on the same soils. This indicates that only a small portion of soil metals is transferred to the vegetables, and the root acts as a barrier to the translocation of heavy metals within the plant. The concentrations of all essential and non-essential heavy metals were found to be greater in soil samples than in medicinal plants. This may reveal that the primary source of metal contents of plants is from their corresponding soil, which might be affected by environmental interferences like pesticides, fertilizers, and other additives used during farming.

Conclusion

The findings of this study showed that the trends in the distribution of heavy metals (both essential and non-essential metals) in the herbal medicinal plants display the following decreasing order Zn > Fe > Mn > Cu > Pb > Cd. However, for the soil samples, the levels of the metals investigated were in the order of predominance display Zn > Mn > Fe > Cu > Pb > Cd. The variation in the level of some elements between the plant varieties could be attributed to different factors such as the type and age of the plant, and the physicochemical nature of the soil. Heavy metals present in the analyzed medicinal herbs were within permissible limits of FAO &

WHO. Generally, most of the traditional medicinal herbs available in five selected sites of Raya Azebo district are safe for human consumption as far as levels are concerned. The data obtained in the present work will be useful in synthesizing new herbal drugs with various combinations of plants, which can be used to treat different diseases and establish their pharmaceutical alternative value for traditional healers and herbal remedy users in the study area.

Competing interests

The authors declare that they have no competing interests.

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