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Determination of the levels of some selected metals in *ocimum lamiifolium* in Wolaita Zone, Southern Ethiopia

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FAAS;

Medicinal plants;

Herbal remedies;

Permissible limits

ABSTRACT

Medicinal plants have worldwide applications in the treatment of different types of human diseases. Among the medicinal plants of Ethiopia, *Ocimum lamiifolium* Hochst. Ex Benth (Damakese, in Amharic) is one of the well celebrated and most widely used home remedy for the treatment of a disease locally known as "Mitch" which is characterized by headache, fever, inflammation, joint pain, sweat, loss of appetite, etc. The aim of the present study on this medicinal plant was to determine the levels of heavy and trace metals in the leaves using the flame atomic absorption spectrometer (FAAS), which is nov AA model. The sampling technique used to carry out the analysis was purposive for the community in the selected area use the plant widely to treat different diseases. In addition, for each of three kebeles, selected from Duguna Fango District, three sites were selected to homogenize the samples. The concentrations or levels of heavy and trace metals, Cd, Co, Pb, Cr, Cu and Zn, in the leaves of the selected medicinal plant were found to be (in mg/L) 0.0489, 0.0579, 0.0936, 0.153, 0.214 and 0.847, respectively. The results revealed that the selected medicinal plant accumulated these metals at different concentration levels in different sites. The results also confirmed that the concentration levels of the metals in the leaves of the selected medicinal plant were not higher than the internationally accepted permissible limits. Thus, the results indicated thatthe medicinal plant under the study is safe for medicinal uses. Furthermore, monitoring such medicinal plants for heavy and trace metals concentrations is of great importance in protecting the community from the adverse effects of the heavy metals..

INTRODUCTION

Background of the study

Medicinal plants play an important role since prehistoric time as they are used in traditional medicine and also as home remedies. Environment, pollution, atmosphere, soil are some of the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Traditional medicines include herbal medicines composed of herbs, herbal materials, and finished herbal products, that contain as active ingredient parts of plants, or other plant materials, or combinations of all mentioned (WHO, 2005).

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Herbal medicines usually refer to plant-derived substances that occur in nature and are utilized with little or no industrial processing for treatment of illnesses (Tilburt and Kaptchuk, 2008). Various plant parts are used in the formulation of herbal medicines. These include, but not limited to, leaves, roots, barks, fruits and seeds. Because of their natural origin, herbal remedies are perceived by people who patronize them to be safer than western pharmaceutical products. The WHO reckons that over 80% of the population in Africa and other developing countries depend on herbal remedies for their healthcare needs (WHO, 2005). For most people in Africa, the high costs of western pharmaceuticals put modern health care services out of their reach and therefore heavily rely on herbal medicine and medicinal plants to meet their primary health care needs. In addition, western pharmaceuticals are most of the time inaccessible to most people in Africa and so herbal medicines have become one of the major options for treating various diseases (Debas *et al*., 2006).

When consumed beyond a certain threshold, lead (Pb) can increase blood pressure accompanied by debilitating effects to key organs such as the kidney and the brain. Cadmium (Cd) poisoning is associated with a number of respiratory disorders, renal failures and cardiovascular problems. Even though an essential mineral, an overdose of zinc (Zn) can cause fever, nausea and general weakness. Though iron deficiency causes anemia, too much iron is particularly dangerous in children and could cause gastrointestinal and skin problems (Baker, R. D *et al*., 2010).Therefore, it is necessary to measure and establish the levels of heavy and trace metals in the herbal plants as these elements when consumed at higher levels become toxic. Thus, the objective of the present study was to determine the levels of selected heavy and trace metals in the leaves of *Ocimum lamiifolium* plant using flame atomic absorption spectrophotometer.

Statement of the problem

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health care settings. However, mass media reports of adverse events tend to be sensational and give a negative impression regarding the use of herbal medicines in general rather than identifying the causes of these events.

Currently, the majority of adverse events related to the use of herbal medicines that are reported are attributable either to poor product quality or to improper use. In order to expand knowledge about genuine adverse reactions to herbal medicines, and to avoid wasting scarce resources for identifying and analyzing adverse events, events resulting from such situations will need to be reduced or eliminated.

Ocimum lamiifolium, among vital medicinal plants, is used to treat various ailments such as cough, headache, eye infections, abdominal colic, bloat, inflammation, joint pain, etc. Thus, it is used by most people in wolaita zone for the treatment of mentioned diseases. The level of heavy and trace metals in herbal medicines beyond the permissible limit is a matter of great concern to public safety all over the world (Khan *et al.*, 2008). The problem is more pronounced in the case of Ethiopia because the herbal medicines used by the society without realizing the concentration of toxic heavy metals as well as the trace metals. World Health

Organization (WHO) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium, and lead which amounts to 1.0 ppm, 0.3 ppm and 10 ppm, respectively (WHO, 2006). The widespread conception among the population that "natural" means "safe" and that drugs of natural origin are harmless and have no risk associated with their use, does not match reality. Some medicinal plants have inherent toxicity and herbal medicines, like any medicine, have side effects that can cause many diseases (Lanini et al., 2009). Thus, the current study focuses on the determination of the levels of heavy and trace metals in the leaves of *Ocimum Lamiifolium* that is grown in Duguna Fango district in order to protect the individuals from their adverse effects when used beyond the permissible limits.

Objectives of the study

The study was conducted with the objectives of determining the levels of selected heavy and trace metals (lead, cadmium, chromium, cobalt, copper, and zinc) in *Ocimum lamiifolium* using FAAS technique and comparing the levels of the mentioned metals present in the leaves of *Ocimum lamiifolium* with the permissible limits of WHO standard and other international standards.

Significance of the study

Society has increasing interest in the therapeutic benefits of herbal remedies. However, there is a wide spread misconception that natural herbs and plants are inherently safe. There is also insufficient information available on the safety of traditional herbs and their products. Therefore, this study helps provide important information on the levels of selected heavy and trace metals in *Ocimum lamiifolium* grown in the study area so that the society could be free of the potential health risks caused from the excessive uptake of the heavy and trace metals in the herbal medicines. On the other hand, the results of this study could be used as reference for other researchers who want conduct the similar studies on the same plant growing in different parts of the country.

Scope of the study

This study was restricted to the investigation of concentrations of the selected heavy and trace metals found in *Ocimum lamiifolium* grown in Duguna Fango District, Wolaita zone, Southern Ethiopia using the technique called spectroscopy specifically using the analytical instrument flame atomic absorption spectrophotometer. The metals Pb, Cd and Cr were selected for they are more toxic, and the metals Co, Cu and Zn were selected merely to represent trace elements. Furthermore, leaf part of the selected medicinal plant was taken to carry out the analysis for the society use this part of the plant to treat different diseases.

MATERIALS AND METHODS

Study Area

Description of the Study Area

The study was conducted in three kebeles (the smallest administrative unit in Ethiopia) selected from Duguna Fango Woreda of Wolaita zone, which is found in Southern Nations, Nationalities, and Peoples Regional (SNNPR) state. The area is located at 431Km south of Addis Ababa and 82 Km from Hawassa,

between 6°40' - 7°58'N latitude and 37°4' - 37°56'E longitude with a total land area of 46,660 hectares. Wolaita Zone has 16 woredas (districts) and 3 town administrations. The Wolaita people are one of the indigenous people of Ethiopia who have their own culture, tradition, political legacy and kingdom. The study area lies at an altitudinal range between 1000 – 2500 meter above sea level and have agro ecologies of dega (high land), woynadega (mid altitude) and kola (low land) with a mean annual temperature of 19.5°c and annual rainfall that varies from 750-1350mm according to the projected CSA final report of 2019.

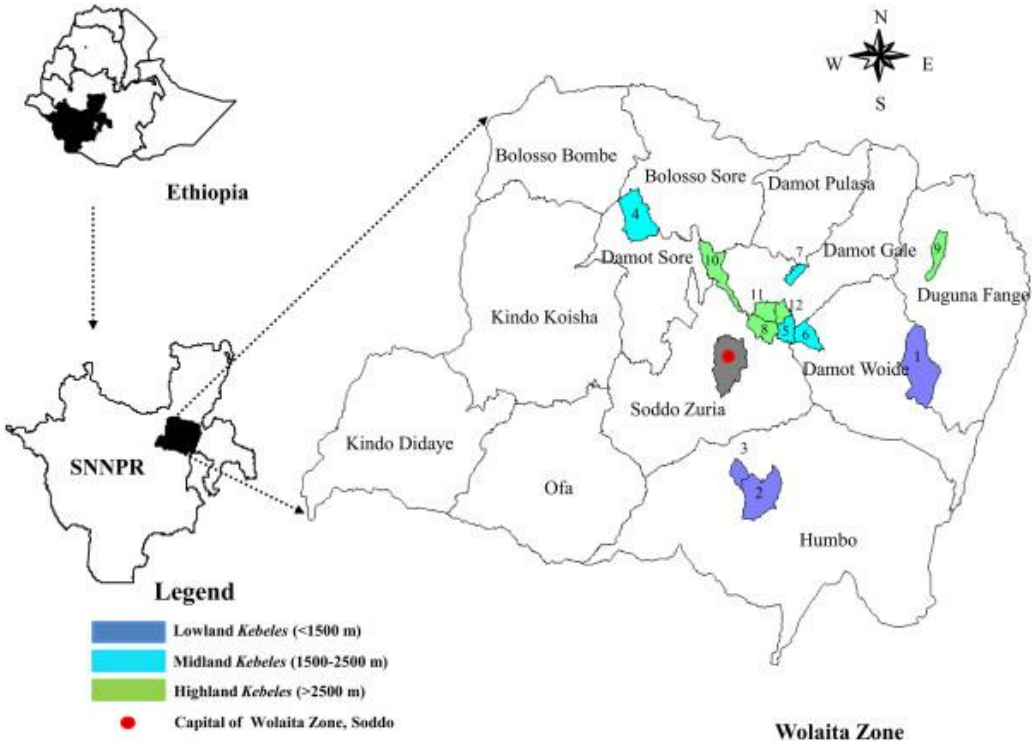

Figure 1: The map of Ethiopia, SNNPR and Wolaita zone (Adapted from Wikipedia)

Instrument and apparatus

Heavy and trace metals determination in the leaves of *Ocimum Lamiifolium* was done using atomic absorption spectroscopy (AAS). Flame atomic absorption spectrometer (FAAS) (Germany, novAA) is a suitable technique for determining metals at parts per million (ppm) concentration levels with good precision for many elements. FAAS offers air-acetylene and/or nitrous oxide flame atomizer. FAAS technique provides fast analysis of 10-15s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost. As indicated in Figure 3, a typical AAS consists of radiation (energy) source, atomization compartment, monochromator, detector and data readout system.

Stainless steel axe and Teflon (SSAT) knife were used to cut the plant pieces while air-circulating oven is for drying the samples placed on porcelain. Blending device, ceramic pestle and mortar, were used for grinding and homogenizing the samples. Digital analytical balance was used for weighing the samples. Round bottom flasks with grounded glass (100

mL) fitted with reflux condenser was employed in digesting the sample in microwave digester (Gallenhamp, England). Borosilicate volumetric flasks (50, 100 and 250 mL) were used during dilution of sample and preparation of metal standard solutions.

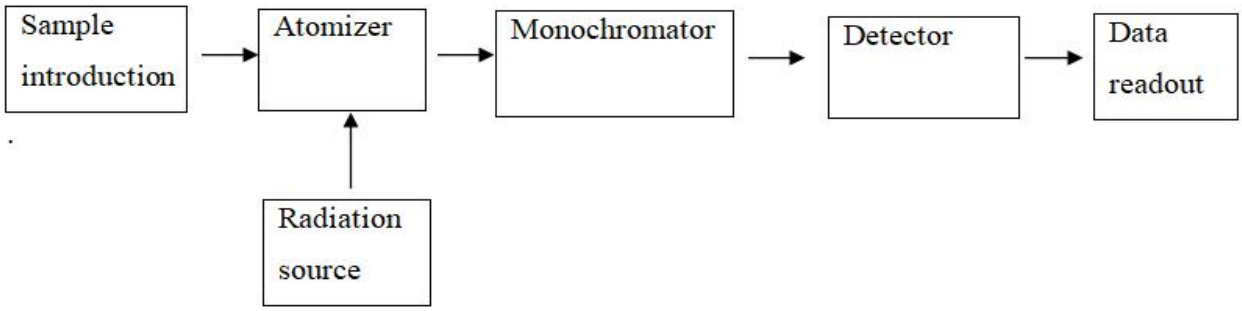

Figure 2: Basic components of atomic absorption spectrophotometer

Chemicals and reagents

Analytical grade chemicals were purchased from Sigma-Aldrich Company found in Germany. 69% nitric acid (HNO_3), 70% perchloric acid ($HClO_4$) and 30% hydrogen peroxide (H_2O_2) were used for digestion in microwave digester, while multi-element standard solution was used as a reference material. Stock standard solution for each metal cadmium (Cd), lead (Pb), zinc (Zn), cobalt (Co), chromium (Cr) and copper (Cu) with a concentration of 1000mg/L was used to prepare intermediate or working standard solutions of 10mg/L for the calibration standards of each metal. Deionized water was used throughout the study. All glass wares were soaked in 5% (v/v) HNO_3 overnight then rinsed with deionized water and dried using laboratory dryer prior to use.

Experimental work procedures

Sample collection and preparation

The samples of fresh leaves of *Ocimum Lamiifolium* were collected from three different kebeles, which are Aruse weyde, Edo mazegaja and Dendo Koysha, in Duguna Fango district from uncultivated fields. From each kebele, three sites were selected to collect the plant leaves in order to homogenize the sample. Samples were placed in plastic bags and labeled, and brought to the laboratory. Samples were washed with distilled water, and first air-dried at room temperature and oven was used for further drying and placed in dust free environment; then ground in to fine powder manually using a porcelain mortar and pestle and allowed to pass through a sieve of 0.5mm mesh size. The powdered samples were placed in plastic containers and stored in a cool dry cupboard prior to analysis. The plant species was collected from different localities based on its availability and knowledge of the societies regarding its medicinal values of the plant.

Optimization of the digestion procedure of samples

One of the basic requirements for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which leads to minimum reagent volume consumption, minimum digestion time,

minimum residue (clear solution), minimum digestion temperature and ease of simplicity. Optimization of the digestion procedure involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Thus, by observing the nature of the solution in different procedures, the solution with the optimum conditions as indicated in Table 1 was selected for suitable analysis using FAAS.

Sample digestion

One gram (1g) of each of the powdered samples were weighed on a digital analytical balance and transferred into a 250 mL beaker. An optimized amount of the mixture of 69% concentrated nitric acid (HNO_3), 70% perchloric acid ($HClO_4$) and 30% of hydrogen peroxide (H_2O_2) was added to each plant sample according to the procedure that was optimized. The digested solutions were allowed to cool for 30 minutes. To the cooled solutions, distilled deionized water was added to dissolve the precipitate that may be formed on cooling and gently swirled. The resulting solutions were filtered into a 50mL volumetric flask with a Watchman filter paper number 41 to remove any suspended and turbid matter. Subsequent rinsing of the filtrate with distilled deionized water was followed until the volume reached the mark. For each bulk sample, triplicate digestions were carried out. The digested and diluted sample solutions were kept in plastic sample bottles until analyzed by FAAS.

Chemical analysis

Instrument operating conditions

Intermediate standard solutions containing 10 mg/L were prepared from the atomic absorption spectroscopy standard stock solutions that contain 1000 mg/L. These intermediate standards were diluted with distilled or deionized water to obtain five working standards for each metal of interest. In this study, a total of six metals were analyzed using flame atomic absorption spectrometer equipped with deuterium arc background corrector and air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. AAS is a quantitative method that measures the concentration of the element by passing light in specific wave length emitted by a radiation source of a particular element through cloud of atoms from a sample. Atoms absorbed light from an energy source known as hollow cathode lamp (HCL). The reduction in the amount of light intensity reaching the detector was seen as a measure for the concentration of particular element in the original sample. A typical AA spectrometer consists of energy (light) source, sample compartment (atomizer), monochromator, detector, and a data process system. Three replicate determinations were carried out on each sample. Hollow cathode lamp for each metal operated at the manufacturer's recommended conditions was used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the six metals (Pb, Zn, Cu, Co, Cr and Cd) were analyzed by the absorption mode of the instrument.

Instrument Calibration

Calibration curves were prepared to determine the concentration of the metals in the sample

solution. The instrument was calibrated using five series of working standards. The working standard solution of each metal was prepared from the 10 mg/L intermediate standard solutions of their respective metals. Wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curve for each of the metals were identified (Table 4).

Method detection limits (MDL)

The method detection limit (MDL) is the minimum concentration that can be detected by the analytical method with a given certainty. It is also the smallest concentration or amount of an analyte that can be reliably shown to be present or measured under defined condition, and the limit of detection is the lowest concentration level that can be determined statistically different from a blank (with 95% confidence). The MDL/LOD is typically determined to be in the region where the signal-to-noise ratio is greater than 3, but not necessarily quantified as an exact value It can be calculated by multiplying the standard deviation of the reagent blank (Sblank) by three (MDL = 3 × Sblank) (Chen, 2007).

Method validation

Method validation is the process of providing that analytical method is acceptable for its intended purpose. Due to the absence of certified reference material for leaves and seeds sample in the laboratory, the efficiency of the optimized procedure was checked by adding known concentration of each metal in 1g of *Ocimum Lamiifolium* leaves' samples. The percentage of recovery is a crucial parameter for method validation. The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyte was calculated by:

$$\text{Percentage recovery} = \frac{\text{Cm in the spiked samples} - \text{Cm in the non-spiked samples}}{\text{Amount added}} \times 100\%$$

Where, Cm = Concentration of metal of interest (Adapted from: IJRPC (2014), 4(1), 202-216)

Statistical Analysis

All measurements were done in triplicates and expressed as mean ± standard deviations. Data was analyzed using analysis of variance (ANOVA) at level of 5% ($p \leq 0.05$) followed by least significant difference Post Hoctest in Microsoft Excel for the determination of statistical significance of a given metal across the samples, not within a given sample. Data was further manipulated with Origin Pro 2020b SrOH(1) for windows version software program.

RESULTS AND DISCUSSION

Optimization of working procedures

As indicated in Table 1 below, eight optimization procedures were used to get the optimum digestion conditions. The procedures used in steps one through four were not chosen as optimum conditions for they used maximum reagent volumes and the highest digestion temperature as well as long time even if some of the procedures gave clear and colourless solutions. In steps six up to eight, the volumes of reagents were relatively low, but they took place at relatively long times. Therefore, procedure five was chosen as optimum condition for the digestion, because it took place at relatively minimum reagent volumes, low digestion time to give clear and colorless

solution.

Table- 1: Methods tested during optimization of the digestion procedure for the samples of the leaves of *Ocimum Lamiifolium*.

No.	Wt. (g)	Volume of reagents (mL)			Max. Temp. (^oC)	Time (min)	Results
		HNO_3	$HClO_4$	Total			
1	1.0	4	3	7	200	60	Clear but turbid
2	1.0	5	2	7	200	60	Clear but yellowish
3	1.0	5	1	6	200	60	Clear but pale yellow
4	1.0	3	2	5	200	10	Clear and colourless
5	**1.0***	**3***	**2***	**5***	**150***	**20***	**Clear and colourless**
6	1.0	3	2	5	140	60	Clear and colourless
7	1.0	4	1	5	130	40	Clear and light yellow
8	1.0	3	2	5	140	30	Clear and light yellow

*Optimum digestion conditions

The results of the analytical recovery test

The validation of the method was tested by spiking the samples with a standard of known concentration of the analyte metals. As depicted in Table 2 below, the results indicated that the concentrations of elements determined are in agreement within the acceptable range for all metals, that is 80-120%. Hence, the digestion method was efficient because the values of the percentage recoveries lied within the acceptable range.

Table- 2: Analytical recovery results obtained for the validation of the optimized procedure of plant samples.

Metal	Concentration in non-spiked sample (mg/L)	Amount added (mg/L)	Concentration in spiked sample (mg/L)	Percentage recovery (%)
Cd	0.05	0.03	0.08±0.01	100±0.025
Co	0.06	0.04	0.095±0.01	87.5±0.028
Cr	0.15	0.14	0.28±0.007	92.9±0.078
Cu	0.21	0.19	0.39±0.01	94.7±0.11
Pb	0.09	0.08	0.17±0.007	100±0.049
Zn	0.85	0.82	1.68±0.02	101±0.49

Instrument operating conditions

The operating conditions for the instrument were prepared for each metal at an appropriate wave length, slit width, current and IDL (Table 3). Intermediate standard solutions of 10mg/L were prepared using 100 mL flask from stock standard solution that contained 1000 mg/L of soluble salts of $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Co(NO_3)_2$, $Zn(NO_3)_2$, $Cu(NO_3)_2$ and oxide of chromium for each metal of interest. MDL was calculated by multiplying the standard deviation of blank

solution by three (MDL = $3S_{blank}$).

Table- 3: Instrument operating conditions for the analysis of metals in the samples of selected plant.

Element	Wavelength (nm)	Slit width (nm)	Current (mA)	IDL* (mg/Kg)
Cd	228.80	0.70	2.00	0.0001
Zn	213.90	0.70	2.00	0.0001
Cu	324.80	0.70	2.50	0.0001
Co	240.70	0.20	5.00	0.00075
Cr	357.90	0.70	4.04	0.00005
Pb	283.3	0.7	2	0.003

*Instrument Detection Limit

Concentrations of working standard solutions and correlation coefficients of calibration Curves

Working standard solutions were prepared from intermediate standard solutions containing 10mg/L, which was prepared from stock standard solutions, by diluting with deionized water to obtain five working standards for each metal of interest as indicated in Table 4. The table also showed that the correlation coefficients of calibration curves of all six metals were closer to one. Thus, these results confirmed that there are strong linear relationships between two variables, which are absorbance and the concentrations of working standard solutions.

Table- 4: Concentrations of working standard solutions and correlation coefficients of the calibration curves for the analysis of plant samples.

Metal	Concentrations of working standard solution	Correlation coefficient
Cd	0.5, 1.0, 1.5, 2.0, 2.5	0.999
Co	0.1, 0.5, 1.0, 1.5, 2.0	0.9989
Cr	0.5, 1.0, 1.5, 2.0, 2.5	0.9988
Cu	0.1, 0.5, 1.0, 1.5, 2.0	0.9977
Pb	0.5, 1.0, 1.5, 2.0, 2.5	0.9959
Zn	0.5, 1.0, 1.5, 2, 2.5	0.9974

The determination of heavy and trace metals in *Ocimum Lamiifolium*

As indicated in Table 5, all six metals (Cd, Pb, Co, Cr, Cu, Zn) were detected in all samples of the selected medicinal plant with variable

concentrations in the sites. The results showed that the concentration of zinc was the highest and that of cadmium was the least of all the metals. Furthermore, the results showed that there were variable concentrations of each analyte metal in the selected sites.

Concentration trends of metals in Dendo koysha

As depicted in Table 5, zinc had the highest concentration among the metals and the metal with the next highest concentration was copper. The fact that zinc had the highest concentration among these metals was also true in other herbal medicinal plants based on different literatures (Baye and Haymete, 2010). But, cadmium and cobalt had the same concentration, which is 0.04 mg/L; similarly chromium and lead had the same concentration, that is, 0.10 mg/L.

Concentration trends of metals in Aruse weyde

The concentration of the metals in this sample site varied from 0.05 to 0.78 mg/L. The metal with the highest concentration and the one with the least concentration were zinc and cadmium, respectively. The concentrations of Co, Cr, Cu and Pb were 0.06, 0.18, 0.25, and 0.08, respectively (Table 5). The concentrations of the selected metals varied in different sites may be due to the difference in soil types, ecological locations, etc. (Ambaye and Mussa S., 2015).

Concentration trends of metals in Edo mazegaja

Based on the results given on Table 5, the concentrations of the metals varied from 0.05 to 0.54 mg/L. Though the concentrations differed from the other sample sites in this study, the metals with the highest and the least concentrations were zinc and cadmium, respectively. The concentration of Co, Cr, Cu and Pb were 0.07, 0.17, 0.19 and 0.10, respectively. Here, also the concentration differences of the selected metals from the other sites were observed due to the variation of different factors like ecological locations.

Comparison of the concentration of each metal among the sample sites and with different permissible limits

Cadmium

As indicated in Table 5, the concentration of cadmium ranges from 0.04 mg/L to 0.05 mg/L. This result showed that there was no such much difference in the concentration of Cd in all the sites selected. Other literatures showed that the concentration of cadmium in other herbal medicines varied between 0.0045 mg/L and 0.0091 mg/L; for instance, in champion leaf, its concentration was 0.0068 ppm (Baye and Haymete, 2010). However, the concentration of cadmium in the study area was well below the permissible limit set by WHO and other organizations. The permissible limit of cadmium in medicinal plants set by WHO, China and Thailand, was 0.3 mg/L, which is equivalent to 0.3 mg/L (FAO/WHO, 2006). The literatures suggested that it is safe for consumption if its level is less or equals to this permissible limit.

Cobalt

The concentration of cobalt in the studied medicinal plant ranges from 0.04 mg/L to 0.07 mg/L. This result indicated that there was variation in the concentration of cobalt in the sample sites. However, the difference in the concentration is not such much great. For herbal plants, the WHO/FAO has not set any regulation

limit for cobalt. But, according to Jabeen *et al*. (2010) the concentration of cobalt in different plant samples ranges from 0.18 to 0.4 mg/L 9. Thus, the obtained result of cobalt is not greater than these results.

Chromium

As depicted in Table 5, the concentration of chromium ranges from 0.10 mg/L to 0.18 mg/L. This range indicated that there was a concentration variation of chromium in different sites of the selected area. The research done on the heavy metal analysis of seven herbal medicines reported that the concentration of chromium ranges from 0.04 ppm to 0.20 ppm (Baye and Haymete, 2010). So, these values are comparable with the concentrations of chromium in the current study. The permissible limit for chromium in herbal medicinal plants has not been set by the WHO yet. However, 2.0 ppm was set by Canada as the permissible limit of chromium in raw medicinal plant. High intake of chromium is reported to have a toxic effect, causing skin rash, kidney and liver damage, cancer of the lungs and nose irritations (Khan *et al*., 2008).

Copper

As indicated in Table 5, the concentration of copper varied between 0.19 mg/L and 0.25mg/L. This result indicated that there is a concentration difference of the metal in different sites which may be related with different factors like soil type difference. The regulatory limits of the WHO/FAO have not been established yet for copper in herbal medicines. However, China and Singapore in 2008 set the permissible limits of 20 mg/Land 150 mg/L, respectively (Jabeen *et al.*, 2010). Thus, the concentration of copper in the studied medicinal plant was well below than these limits.

Lead

The results in Table 5 showed that the concentration of lead in the study area varied between 0.08 mg/L and 0.1 mg/L. From this, it is obvious that there was almost uniform pattern in the distribution of lead. The concentrations of lead varied between 0.02 mg/L and 0.09 mg/L in other medicinal herbs as indicated in the report of the Journal of Scientific and Engineering Research, 2016, 3(2). So, the result of lead in the present study was approximately equals to the above values. The WHO (2006), Malaysia, China and Thailand (2008) set the permissible limit for lead in medicinal herbs as 10 mg/L (Khan *et al*., 2008). So, the results of the study area showed that the concentration of lead was well below the permissible limit.

Zinc

Table 5 indicated that the concentration of zinc in the study area ranges from 0.54 mg/L to 1.21 mg/L. When compared to the variation in the concentration of other metals, the difference is great in the case of zinc as well as its concentration was high. The permissible limit for zinc in herbal medicines set by WHO/FAO is 50 mg/L. Though there is little information about its toxicity, consumption of zinc beyond the permissible limit may result in toxic effect on the immune system (Waheed and Fatima, 2013). The concentration of zinc in the studied medicinal plant was very much below than this value.

Table- 5: Mean concentrations of metals (mg/L) in the studied medicinal plant in the samples collected from different sites.

Metals	Concentrations of metals in three sites (mg/L) Mean±SD		
	Dendo Koysha	Aruse weyde	Edo Mazegaja
Cd	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Co	0.04 ± 0.01	0.06 ± 0.00	0.07 ± 0.01
Cr	0.10 ± 0.01	0.18 ± 0.03	0.17 ± 0.02
Cu	0.20 ± 0.02	0.25 ± 0.01	0.19 ± 0.00
Pb	0.10 ± 0.01	0.08 ± 0.02	0.10 ± 0.06
Zn	1.21 ± 0.13	0.78 ± 0.06	0.54 ± 0.06

CONCLUSION

The contamination of herbal medicines is due to the accumulation of the metals in different parts of the medicinal plants. Hence, the concentrations of the metals should be measured in order to be free of their toxic effects.

In the present study, the analysis of heavy and trace metals in the selected medicinal plant was made by using FAAS by following the optimized digestion method. The digestion method was optimized by changing the parameters until clear and colourless solution was obtained. The concentrations of all the selected metals in the medicinal plant, *Ocimum Lamiifolium*, were determined. The concentrations of Zn, Cu, Cr, Pb, Co and Cd are 0.847, 0.214, 0.153, 0.0936, 0.0579 and 0.0489, respectively.

In the studied area, the concentration of zinc was the highest of all the metals. However, its concentration was not above internationally accepted permissible limits. In the medicinal plant under study, the concentrations of some metals were below and that of others were nearly equal to internationally accepted permissible limits so that this showed the plant is safe for medicinal uses.

Furthermore, the efficiency of the digestion method was confirmed by percentage recoveries which were within accepted ranges, that is, 80 – 120%.

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