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Quantitative Determination of the Heavy Metal Levels in the Wild Edible Plant Parts and their Corresponding Soils of the Central and Western Regions of the Oromia State, Ethiopia

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Abstract

This study was designed to assess the levels of selected metals (Pb, Cu, Zn, Cd, Ni, Cr and Ca) in wild edible plants and their corresponding soil samples by FAAS. A wet digestion procedure has been adopted to digest the plant and soil samples. The validity of the method was evaluated by spiking the sample with a standard of the selected metals. The levels of Pb, Cd, Ni, Cu and Cr were below detection limits in all the edible parts of the studied plants except in the root of *Eriosema cordifolium* where Ni and Cr were detected. Similarly, only Ni, Cu and Cr were detected in the soil samples of *Eriosema cordifolium* and *Physalis peruviana* plants. But, Zn, and Ca were detected in all the studied samples and varied in the range 31.3-157.5 and 24.4-6214.3 mg kg^{-1} in plants and 55.4-149.8 and 367.1-6032.3 mg kg^{-1} in soil samples, respectively. However, the value of selected metals in soils and plants sample were lower than the permissible limit recommended by European Union Standard and Joint FAO/WHO Expert Committee on Food Additives respectively with the exception of Zn in tuber of *Pachyeymbium sacculatium* plant.

Keywords: Wild edible plant; FAAS; Heavy metals; Accumulation; Uptake

Introduction

Wild edible plants (WEPs) refer to plant species that are neither cultivated nor domesticated, but are available from their wild natural habitat and used as sources of food. They play important role in maintaining food supplement as a means of survival during times of drought and famine [1]. Their uses have been overlooked though their consumption is still very common in rural areas of some countries including Ethiopia [2]. In Ethiopia, their occurrence and availability is mainly determined by the seasonal variation and their abundance increases during the rainy seasons but decreases during dry seasons [3].

Ethiopia is known for endemism of wild plant species which are used in different ways, including as medicine and food. The flora compromises approximately 6000 species of higher plants of which about 10% are endemic [4,5]. As a result, the country is known as a "biodiversity hot spot" and center of origin and diversification for a significant number of food plants [6,7]. Ethno-botanical studies conducted in Ethiopia have indicated that over 300 species of wild plants are gathered and consumed by the people [8,9]. The rural people traditionally harvest wide range of leafy vegetables, roots, tubers and fruits from wild plants because of their taste, cultural uses and as food supplements [10]. Consumption of such plants by children and sometimes adults is, thus, an integral part of the different cultures in the country [11]. Among the wild edible plants; root of *Eriosema cordifolium*, root of *Commiphora confusa Vollesen*, tuber of *Pachyeymbium sacculatium* and fruit of *Physalis peruviana* were selected for the study. Especially the root of *Eriosema cordifolium* and fruit of *Physalis peruviana* are highly consumed by children.

Plants get nutrient from environmental compartment (soil, water and air). But they are not perfectly selective only to essential nutrients; they may take up metals like heavy metals that are toxic even at low level. Soil is considered a critical environment as it accumulates pollutants (like heavy metals) that can be dispersed in it, both naturally and by various anthropogenic activities [12]. The total metal content in soil is not a good indicator of exposure or risk to plants or organisms since only a portion of the metal present in soil is potentially available for the plants. The dissolved and exchangeable forms of metals are available to the plants while the metals available as structural components of the lattices of soil minerals and insoluble precipitates with other soil components are potentially available in the longer term [13].

Metals present in the soil fractions vary in degree of mobility. Their bioavailability is regulated by soil properties (physical, chemical and biological processes) and interactions between them [14]. Changes in the chemical properties of the soils greatly affect concentration of free metals and result in changes in their availability for plants. With increasing pH, contents of organic matter and clay; solubility of most metals are decreased due to their increased tendencies for adsorption [12].

Plants readily assimilate elements through the roots. The direct contact between the plant root and soil allows most metals to enter the plant tissue through uptake of water and nutrients by plants, ion exchange at cell wall and other complicated metabolic mechanisms [15]. Once metals taken up by roots, they can either be stored by the roots or transported to other parts of the plant. So, plants take up metals and accumulate them in their edible and nonedible parts in quantities high enough to cause risks both to animals and human beings consuming these metal rich plants [16]. In particular, heavy metals pose a great health risks to all living organisms upon long term exposures [17-

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20]. Because of their non-biodegradability, long biological half-lives, potential to accumulate in different body parts and their solubility in water; the possibility of causing deleterious health effects is high [14]. Even at low concentration levels they have damaging effects to man and animals since there is no good mechanism for their elimination from the body [16]. They disrupt metabolic functions in two ways: the first is being accumulated in the body, they can disrupt functions in vital organs and glands such as the heart, brain, kidneys, bone, liver, etc. and secondly, they can displace the vital nutritional minerals from their original place, thereby, hindering their biological functions [14]. Because of this, they can pose serious risks to the consumers in terms of carcinogenesis, mutagenesis and teratogenesis and also lead a number of nervous, cardiovascular, renal, neurological impairment as well as bone diseases and several other health disorders by these two ways [21]. From the above, it is well understood that wild edible plants accumulate considerable quantities of essential and non-essential metal in their edible parts. As a result, these metals directly available to the consumers since the selected wild plants are consumed without undergo a process. Because of this, the consumers could suffer from metal toxicity and their normal body can be affected . Since such study are not get a serious attention in Ethiopia, it is very important to determine the level of the essential and toxic metals in the wild edible plants. Therefore, the proposed study will be focusing on the analysis of the concentrations levels of the accumulated heavy metals in the selected wild edible part of plants and in their corresponding soils. It is also hoped that findings of the study could be utilized as baseline information for the quality control.

Materials and Methods

Chemicals and reagents

All chemicals used in this study were of analytical grade reagents. Hydrochloric acid (36-38%) and nitric acid (69-72%) were obtained from Sigma Aldrich (Steinleim, Germany); perchloric acid (70%) was received from the Research Lab. Fine Chem. Industries (Mombai, India) and potassium chloride (99%) was purchased from Lamberk Chemicals pvt. Ltd (Ambala cantt, India) and the reference standards of the heavy metals under study were the products of Perkin Elmer (Boston, USA). The stock standard solutions, 1000 mg L^{-1}, were prepared from the nitrate salts of the metals. The working standard solutions of the selected metals were prepared freshly from the intermediated standard solutions (100 mg L^{-1}) which was obtained by diluting stock standard solutions.

Instruments

ZEEnit 700P model flame atomic absorption spectroscopy (Germany) equipped with deuterium ark background corrector, nebulizer, auto sampler and hollow cathode lamp was used for determination of the levels of selected metals. Other instruments including an oven (Germany) for drying of the samples; muffle furnace (Germany) for ashing of the soil samples in order to determine the organic content; ceramic mortar and pestle (USA) for grinding and homogenizing the samples; digital analytical balance, Adam Equipment (Jermany) for weighing samples; Kjeldahl (England) for digesting the samples; conductometer and pH meter (Romania) used for measuring electrical conductivity and pH of the soil samples were also utilized. All glassware used were first kept overnight in a 10% HNO_3 solution and then repeatedly washed with distilled-deionized water and dried in an oven for 24 h before use.

Description of the study area

The wild edible plants and their soil samples were collected from different localities of Oromia regional states, Ethiopia. Particularly from Jimma Rare and Yaya Gulale which are located in Horro Guduru Wellega and North Showa Zone of Oromia Regional state respectively. The geographical locations (latitude, longitude and elevation) of Sampling sites were 09°28'1.44" N, 37°26'2.34" E, 2293.50 m for Jimma Rare and 09°42'2.9" N, 38°03'4.2" E, 2760.00 m for Yaya Gulale . Their distance from Addis Ababa, i.e., capital city of Ethiopia is 245 km and 113 km respectively. *Eriosema cordifolium, Physalis peruviana* and their corresponding soil samples were collected from Jimma Rare while *Commiphora confusa Vollesen, Pachyeymbium sacculatium*, were collected from Yaya Gulale. Soil samples were collected from all the corresponding sites of the plant materials. The scientific name of these plants was authenticated by the National Herbarium, Department of Plant Biology and Diversity Management, Addis Ababa University. All the experiments were conducted in the Analytical Chemistry Research Laboratory of the Department of Chemistry, Addis Ababa University, Ethiopia.

Sample collection and preparation

Edible parts of the plants were collected manually; using vinyl gloves for protecting hands. The bruised portions were removed and the remaining samples packed in the polyethylene bags for transporting to the Analytical Laboratory. In the laboratory, collected plant samples were washed with tap water and then with double distilled water to eliminate adsorbed dust and particulate matters. The plant samples were then cut and chopped into small pieces using plastic knife in order to facilitate drying. The samples were then air-dried for five to six days and further dried in a hot air oven at 50-60°C for 24 h, to remove moisture and maintain constant mass. The dried samples were ground into powder using acid washed commercial mortar and pestle and then sieved to 0.425 mm mesh size. The sieved samples were finally stored in the polyethylene bags and kept in desiccators until the time of digestion.

Similarly, the soil samples were collected from the base of uprooted plant by auger and properly labeled and packed in polyethylene bags. Each soil sample was air dried at ambient temperature for three days and then, ground into powder using acid washed commercial mortar and pestle and sieved to 0.425 mm mesh. The sieved soil samples were stored in the polyethylene bags and placed in desiccators until the time of digestion.

Optimization of the digestion procedure

Different digestion procedure for the plant and soil samples were carried out using HNO_3, $HClO_4$ and HCl acid mixtures by varying volume of the acid mixture, digestion time and digestion temperature [22]. Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests. Based on this, the optimized parameter for digestion procedure were: 4 mL of 1:1 of HNO_3 and $HClO_4$ at 270°C for 3 h for wild edible plant parts and 5 mL of 3:1:1 of HNO_3, HCl and $HClO_4$ at 210 and 240°C for 2 h for the soil samples.

Digestion of the plant parts and soil samples

For each of the wild edible plant parts, 0.5 g of powdered and homogenized samples were weighed and transferred to 250 mL of round bottom flask. To this, 4 mL of 1:1 (v/v) of HNO_3 and $HClO_4$ was added and digested at 270°C for 3 h. The digested solutions were allowed to cool and 5 mL of distilled-deionized water were added to dissolve the precipitate formed on cooling and gently swirled and

filtered into a 50 mL volumetric flask through Wattman filter paper no. 42. The clear solution then diluted up to 50 mL with distilled-deionized water and analyzed by flame atomic absorption spectroscopy.

Digestion of the soil samples on the other hand was carried out as follows: 0.5 g of crushed, powdered, sieved and homogenized soil samples were weighed and transferred to a 250 mL round bottom flask. To this, 5 mL of 3:1:1 ratio of HNO_3, HCl and $HClO_4$ were added. The solutions were then digested at 210°C for the soil of *Commiphora confusa Vollesen* and *Pachyeymbium sacculatium* and at 240°C for the soil of *Eriosema cordifolium* and *Physalis peruviana* for 2 h. Then, the digested solutions were left to cool and 5 mL distilled-deionized water were added and gently swirled and filtered through Whatman filter paper no. 42. The filtrates collected in a 50 mL volumetric flask was filled to the mark with distilled-deionized water and analyzed by flame atomic absorption spectroscopy.

Performances of the analytical method

The detection limit is the lowest concentration or weight of analyte that can be measured at a specific confidence level. Near the detection limit, the signal generated approaches that from a blank and can be determined experimentally by running several blank samples to establish the mean and standard deviation of the blank. Accordingly, eight replicate blank samples were digested that have been utilized for the wild edible plant and the soil samples. Each of the blank samples was assayed for their heavy metal contents. The mean and standard deviations of the blanks were calculated to determine the method detection limit (MDL). It was then calculated using the following relation [22,23].

$$MDL=X_{Bl}+3SD_{Bl}$$

Where X_{Bl} is the mean concentration of the analyte in the digested blank solution and SD_{Bl} is the standard deviation of the replicate measurements of the analytes in the blank.

Validation of the analytical procedure

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. Because of the absence of certified reference material for the wild edible plants and their soil samples; validity of the digestion procedures were assured by spiking the samples with a standard solution of known concentration of the target analytes.

The spiked wild edible plant and their soil samples were digested in triplicate following the same procedure used for digestion of the plant parts and the soil samples. The resulting digest of the spiked samples were then analyzed for their respective metal contents using FAAS and percent recoveries were calculated both for the plant parts and the soil samples.

Characterization of the soil samples

The bioavailability of heavy metals in the plant parts depends on a number of physical and chemical factors in the soils. These include: pH, organic matter content and electrical conductivity.

Soil pH was measured in a suspension (1:2.5, *w/v*) of the soil and distilled water. 5 g of air dried soil (<0.425 mm) was weighed and transferred to a 100 mL beaker to which 12.5 mL distilled water was added. Then, the mixture was stirred and the pH was measured after allowing the suspension to stand for 10 min, at room temperature.

Electrical conductivity of the soil samples was measured in a suspension (1:2.5 w/v) of the soil and distilled water. 10 g of air dried soil (<0.425 mm) was weighed and transferred to a 100 mL beaker to which and 25 mL distilled water was added. The mixture was stirred and allowed to stand for 15 min at room temperature and the electrical conductivity was measured.

Soil organic matter content was determined using the method of loss on ignition. 5 g of the soil sample of the plant parts, which dried in oven at 100°C for 15 min, was accurately weighed in to a pre-weighed crucible. Then, the crucible, with soil, was placed in a muffle furnace and heated at 520°C for 3:30 h. The sample was then taken from the furnace and placed in desiccators to cool. Then, the sample was re-weighed and the percentage organic matter content was calculated.

Results and Discussion

Evaluation of the proposed method performance

The data qualities obtained from FAAS for metal analyzes are highly affected by the calibration curve. The calibration curves were prepared from a standards of known concentration; covering the concentration range expected in the sample. Then, the curves were established at four concentration levels corresponding to 0.25, 0.5, 0.75 and 1 ppm for Cd and Zn; 1, 2, 3 and 4 ppm for Cr, Pb and Ni; and 0.25, 0.5, 1 and 2 ppm for Cu and Ca. All the working standard of metals solution used for calibration curve exhibited good linearity with squared regression coefficients (r^2) ranged from 0.99281 to 0.99998. The value of MDL was shown in Tables 1 and 2. As observed, the values of the detection limit of the method obtained were compared with the instrument detection limit and found to have greater values in all case. It was also less than the lowest working standard solution but approach to the starting point of the linearity of the calibration curve. This confirms that the method was good and acceptable. The limit of detection of the wild edible plant was slightly less than the soil sample of the plant since different mixture of blank solution used.

Experimental procedure validation

The accuracy of the proposed digestion method was evaluated by determining the recoveries of the spiked analytes at various concentrations. The values were nearly quantitative and in the acceptable range for the wet digestion method as observed in Table 2. Similarly the precision was evaluated by applying the method to the spiked samples of the edible plants and soils. Each sample was prepared in triplicate under the same experimental condition and analyzed. Then, the results of precision expressed as relative standard deviations (%RSD) and are shown in Table 3. As can be seen, more or less acceptable precision (%RSD less than 10) was obtained.

Physiochemical parameters

Metal solubility tends to increase at lower pH and most of the mobility of metals is reduced with increasing soil pH because of the precipitation as insoluble hydroxides, carbonates and organic complexes [24]. Usually the intensity of root uptake of metal by plants decreases with increasing soil pH. Low soil pH value determines the activity of many metal ions in the water contained in the pores of the soil, affecting their bioavailability [25].

The value (mean ± standard deviation, n=3) of pH, electrically conductivity (mSm^{-1}) and organic matter (%) of the plant soil were: 5.86 ± 0.02, 0.09 ± 0.01 and 20.89 ± 0.39 for the soil of *Eriosema cordifolium,* 7.84 ± 0.04, 0.23 ± 0.01 and 4.98 ± 0.15 for the soil of *Pachyeymbium sacculatium*, 7.79 ± 0.03, 0.30 ± 0.01 and 7.20 ± 0.04 for the soil of *Commphora confusa Vollesen* and 6.00 ± 0.09, 0.04 ± 0.01 and 16.66 ±

Soil of :	Cd	Cr	Cu	Ni	Ca	Pb	Zn
Eriosema cordifolium	BDL	39.60 ± 0.02 RSD%, 5.05	1.50 ± 0.001 RSD%, 6.67	39.81 ± 0.042 RSD%,10.55	367.10 ± 0.36 RSD%, 9.81	BDL	85.50 ± 0.03 RSD%, 3.51
Pachyeymbium sacculatium	BDL	BDL	BDL	BDL	3614.70 ± 3.10 RSD%, 8.58	BDL	133.50 ± 0.01 RSD%, 0.97
Commiphora confusa Vollesen	BDL	BDL	BDL	BDL	6032.30 ± 0.09 RSD%, 0.15	BDL	55.40 ± 0.02 RSD%, 3.61
Physalis peruviana	BDL	34.19 ± 0.01 RSD%, 2.92	6.10 ± 0.01 RSD%, 10.39	42.12 ± 0.04 RSD%, 9.49	539.00 ± 0.33 RSD%, 6.12	BDL	149.80 ± 0.11 RSD%, 7.34
MDL(ppm)	0.231	0.795	0.207	0.806	0.205	0.881	0.206
IDL (ppm)	0.012	0.05	0.035	0.07	0.025	0.30	0.012

BDL - Below Detection Limit, RSD - Relative Standard Deviation, MDL - Method Detection Limit, IDL - Instrumental Detection Limit

Table 1: Concentration of heavy metals (mg kg^{-1}), (mean ± SD, n=3) in the respective soil of wild edible plants.

Wild edible plants	pH	Cd	Cr	Cu	Ni	Ca	Pb	Zn
Root of *Eriosema cordifolium*	6.06 ± 0.15 RSD%, 2.48	BDL	5.14 ± 0.40 RSD%, 3.89	BDL	23.87 ± 0.02 RSD%, 8.38	1288 ± 0.27 RSD%, 2.1	BDL	44.0 ± 0.02 RSD%, 4.55
Tuber of *Pachyeymbium sacculatium*	5.45 ± 0.23 RSD%, 4.22	BDL	BDL	BDL	BDL	6214 ± 0.21 RSD%, 0.34	BDL	157.5 ± 0.09 RSD%, 5.71
Root of *Commiphora confusa Vollesen*	6.52 ± 0.51	BDL	BDL	BDL	BDL	1447.0 ± 0.8	BDL	49.4 ± 0.02
	RSD%, 7.82					RSD%, 5.53		RSD%, 4.05
Fruit of *Physalis peruviana*	4.22 ± 0.25	BDL	BDL	BDL	BDL	24.4 ± 0.02	BDL	31.3 ± 0.03
	RSD%, 5.92					RSD%, 8.2		RSD%,9.58
MDL (ppm)		0.229	0.703	0.198	0.796	0.195	0.877	0.194
IDL (ppm)		0.012	0.05	0.035	0.07	0.025	0.30	0.012

Table 2: Concentration of heavy metals (mgkg^{-1}), (mean ± SD, n=3) in wild edible plants.

		Cd	Pb	Cr	Cu	Ni	Zn	Ca
Root of *Eriosema cordifolium*	%R (%RSD)	99.0 (5.2)	91.8 (5.0)	93.5 (10.2)	88.00 (9.45)	93.48 (6.15)	90.74 (8.40)	91.89 (5.21)
Tuber of *Pachyeymbium sacculatium*	%R (%RSD)	101.0 (6.3)	92.0 (6.3)	89.5 (7.4)	95.50 (7.35)	95.50 (5.30)	92.31 (7.40)	100.00 (8.42)
Root of *Commiphora confusa Vollesen*	%R (%RSD)	100.0 (5.2)	92.0 (5.1)	94.0 (10.4)	91.50 (8.01)	98.25 (2.40)	110.42 (6.15)	95.80 (6.15)
Fruit of *Physalis peruviana*	%R (%RSD)	101.0 (6.1)	92.0 (6.0)	105.5 (6.0)	99.00 (2.15)	96.50 (2.41)	108.57 (8.25)	97.76 (8.20)
Soil of *Eriosema cordifolium*	%R (%RSD)	101.0 (2.1)	91.0 (3.3)	99.2 (10.5)	89.08 (2.15)	95.00 (6.15)	87.50 (10.05)	102.15 (2.50)
Soil of *Pachyeymbium sacculatium*	%R (%RSD)	103.0 (5.1)	90.8 (5.5)	97.3 (10.12)	97.50 (2.15)	99.50 (6.15)	106.67 (9.45)	102.38 (6.30)
Soil of *Commiphora confusa Vollesen*	%R (%RSD)	101.0 (6.21)	91.25 (6.15)	108.50 (7.10)	97.00 (1.62)	100.75 (10.05)	106.25 (8.63)	93.75 (8.40)
Soil of *Physalis peruviana*	%R (%RSD)	100.0 (6.02)	91.0 (6.25)	97.37 (10.07)	92.39 (6.15)	92.86 (10.02)	109.09 (10.10)	93.33 (8.42)

Table 3: Recovery values (%RSD, n=3) of the proposed method for plant and soil sample.

0.54 for the soil of *Physalis peruviana* respectively. The soil of *Eriosema cordifolium* and *Physalis peruviana* were slightly acidic where as that of *Pachyeymbium sacculatium* and *Commphora confusa Vollesen* slightly basic. Hence, metals are more mobile in the soil of *Eriosema cordifolium* and *Physalis peruviana* than soil of *Pachyeymbium sacculatium* and *Commphora confusa Vollesen* plant species.

The effect of pH on the mobility of metallic elements in the soil is highly variable, depending on the content of organic matter. The higher the soil organic matter content, the higher the ability of that soil to retain metals within it. So based on the result, the metals become more retained in the soil of *Eriosema cordifolium* and *Physalis peruviana* when compared with the soil of *Pachyeymbium sacculatium* and *Commphora confusa Vollesen* plant species. Therefore, the bioavailability of metals in soil for the plant species becomes low when the organic content of the soil is high due to the adsorption reaction of metals on it.

Level of heavy metals in the soil of wild edible plants

Heavy metals may enter the human body through inhalation of dust, direct ingestion of soil and consumption of food plants grown on metal contaminated of soil [26].

The most important pathway through which human exposed to the toxic metals are soil-plant-human (food chain) and soil-human (incidental soil ingestion). Out of the two soil-to-plants transfer is the key components of human exposure to metals. Therefore, analyses of the level of metals in soil are important.

In this study the concentration of heavy metals in the respective soil of WEP vary from one to other. The concentration of Cd and Pb in all soil samples become below detection limit where as the concentration of Cr, Cu and Ni also below detection limit in the soil of *Pachyeymbium sacculatium* and *Commiphora confusa Vollesen* but detected in other

two soils of plants. Because the organic contents in the soil of *Eriosema cordifolium* and *Physalis peruviana* were high as compared with others. Hence, metals become more retained in the soil.

Metals like Cr, Cu, Ni, Zn and Ca were detected in both soil sample of *Eriosema cordifolium* and *Physalis peruviana* plants because both soil samples were collected from the same area and their physiochemical parameters more or less the same. Likewise, only Zn and Ca metals detected in soil of *Pachyeymbium sacculatium* and *Commiphora confusa Vollesen*. This confirms that the physiochemical parameters of the soil affect the availability of metals in the soil samples.

The concentration of Ca in the soil of *Pachyeymbium sacculatium* (3613.7 mg kg^{-1}) and *Commiphora confusa Vollesen* (6032.3 mg kg^{-1}) were high as compared with other. The reason is higher pH values of the soil which can result in greater retention of metals, lower solubility of metals and also resulting in decreased leaching effects of the soils metals.

Levels of heavy metals in wild edible plants

Even though, the poor bioavailability of heavy metals in soil, the plants may have high ability to accumulate them in their different parts [27]. So, analyses of wild edible plants were important to identify and measure the level of metals. Metal concentrations in wild edible plants were different among species. Metals like Zn and Ca were accumulate in all wild edible plants studied. The root of *Eriosema cordifolium* plant was potentially accumulates Ca (1287.6 mg kg^{-1}) which greater than its respective soils. Similarly, the tuber of *Pachyeymbium sacculatium* plant was accumulate both Ca (6214.3 mg kg^{-1}) and Zn (157.5 mg $7kg^{-1}$) that greater than its respective soils.

The root of *Eriosema cordifolium* and fruit of *Physalis peruviana* cannot accumulate Cu even if this metal available in their respective soils. Additional the fruit of *Physalis peruviana* plant species cannot accumulate Cr and Ni metals but the root of *Eriosema cordifolium* plant species accumulate. This may be because of the level of the metals in the plant which is not detected by FAAS, the bioavailability of these metals in soil, metal uptake ability of plants species and since plants absorb metals selectively.

Correlation coefficient of metals

A linear regression correlation test was performed to investigate correlations between metal concentrations in the wild edible plant and their respective soil samples. There is a perfect positive correlation when r=+1, no correlation when r=0, and a perfect negative correlation when r=-1. Hence, the closer the r values to 1 or -1, the stronger the relationship between the two variables.

There is a good correlation between the concentration of Ca and Zn (r=0.9942) in the fruit of *Physalis peruviana,* Cr and Ni (r=0.9907), Cr and Ca (r=0.9083), Ca and Ni (r=0.8430) in the root of *Eriosema cordifolium,* Cu and Ni (r=0.9992), Cu and Ca (r=0.8561), Ni and Ca (r=0.8761), Ni and Zn (r=-0.9919), Ca and Zn (r=-0.8077), Cu and Zn (r =-0.9962) in the soil of *Eriosema cordifolium,* Cr and Cu (r= 0.9661) in the soil of *Physalis peruviana,* Ca and Zn (r=0.9611) in the soil of *Commiphora confuse Vollesen*. But, it is not significant between Ca and Zn (r=0.1233), Zn and Ni (r=-0.4299), Zn and Cr(r= 0.3516) in the root of *Eriosema cordifolium*, Ca and Zn (r=-0.6952) in the tuber of *Pachyeymbium sacculatium,* Ca and Zn (r=0.5263) in the root *Commiphora confuse Vollesen,* Cr and Cu (r=-0.5499), Cr and Ni (r=-0.5162), Cr and Ca (r=-0.0393), Cr and Zn (r=0.6209) in the Soil of *Eriosema cordifolium,* Cr and Ni (r=0.6858), Cr and Ca (r=0.3345), Cr and Zn (r=-0.3962), Cu and Ni (r=0.4745), Cu and Ca (r=0.5666), Cu and Zn (r=-0.1456), Ni and Ca (r=-0.4565), Ni and Zn (r=-0.7896), Ca and Zn (r=0.7328) in the soil of *Physalis peruviana*, Ca and Zn (r=0.7436) in the soil of *Pachyeymbium sacculatium*.

Even if the concentration of metals is high in the soil of plants: it may be low or high in the wild edible plants and vice versa. Hence, analysis was made to test whether or not significant relation exists between the metals concentration in the soil sample of plants and that taken up by wild edible part of plants. The result indicates, there are good correlations of Zn level between: soil and root of *Eriosema cordifolium* (r=0.9999), soil and tuber of *Pachyeymbium sacculatium* (r=0.9487), soil and root of *Commiphora confusa Vollesen* (r=0.9989), soil and fruit of *Physalis peruviana* (r=0.9516). But the correlation of Ca concentration between soil and root of *Eriosema cordifolium* (r=-0.6747), soil and root of *Commiphora confusa Vollesen* (r=0.3156), soil and fruit of *Physalis peruviana* (r=0.4776) were not significant as such Zn except that of soil and tuber of *Pachyeymbium sacculatium* (r=-0.9244). The correlation of Cr concentration between soil and root of *Eriosema cordifolium* was also good (r=0.9907) but for that of Ni it is not significant (r=0.3246).

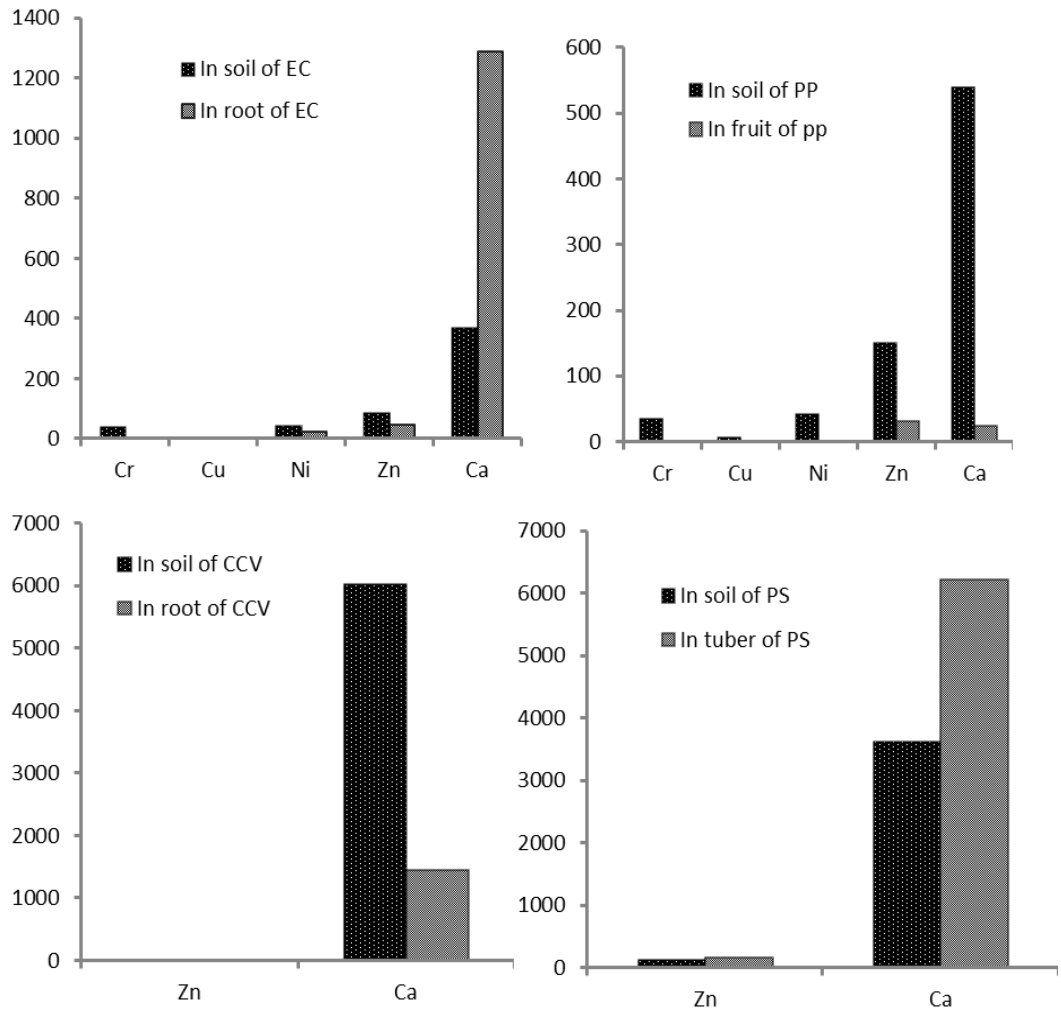
Trend of metals concentration in wep and their soil sample

Plants and their soil sample do not accumulate metals equally. The accumulation and distribution were depending up on the environmental factor [28]. The level of Cu and Ni were high in the soil of *Physalis peruviana* but low in the soil of *Eriosema cordifolium* plant where as the level of Cr was high in soil of *Eriosema cordifolium* but low in soil of *Physalis peruviana* plant. The level of Ca was highest in the soil samples of *Commiphora confusa Vollesen* but lowest in the soil sample of *Eriosema cordifolium*. Accordingly, the level of Zn was highest in the soil sample of *Physalis peruviana* but lowest in the soil sample *Commiphora confusa Vollesen.*

The tuber of *Pachyeymbium sacculatium* plant species had highest concentration of Ca and Zn metals, but the concentration of these two metals were lowest in the fruit of *Physalis peruviana* plant as compared with the rest. This may be the capability of plant species to uptake metals. Once metals taken up by roots, they can either be stored in the roots or exported to the other part of plants. Efficient transport of metals to other part of plants is an important aspect of plant metal accumulation. The transport of metals from root to other part of plants is come out primarily through xylem. Organic acids and amino acids have frequently been reported to be the potential metal chelators, which most likely facilitate metal translocation through xylem. Without being chelated by ligands, movement of metal cations from roots to shoot (other part of plants) is expected to be severely retarded as xylem cell walls have a high cation exchange capability [29]. This may be a reason for a smallest accumulation of metals in the fruit of *Physalis peruviana* plant species (Figure 1).

The relationship between the levels of selected metals in the wild edible plants and their respective soil was varied. Some of the selected wild edible plant species showed higher concentration of metals even if the level of metals low in the soil. The level of Zn and Ca was higher in the tuber of *Pachyeymbium sacculatium* than its respective soil. Similarly, the concentration Ca was also higher in the root of *Eriosema cordifolium* than its respective soil.

As observed from Figure 2, plant species differ widely in their ability to accumulate the metals. Ca metal highly accumulated in the root of *Eriosema cordifolium* plant where as the fruit of *Physalis peruviana* plant accumulate the lowest Ca and Zn metals as compared with the other plant species.



Where EC is *Eriosema cordifolium*; PP is *Physalis peruviana*; CCV is *Commiphora confusa Vollesen* and PS is *Pachyeymbium sacculatium*

Figure 1: Comparison of metals concentration between the WEP and their respective soil sample.

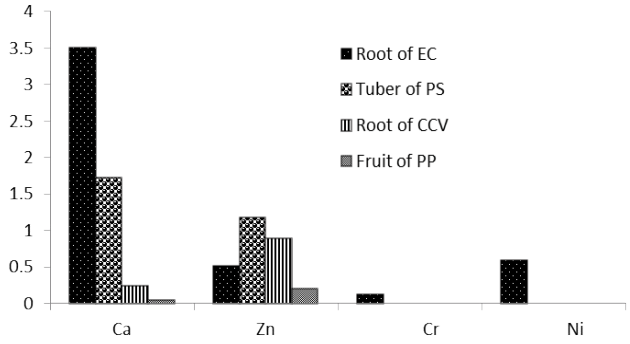


Figure 2: Translocation factors of metals in wild edible plants.

Comparison of metals level in soil sample with different standards

The European Union standards guide line for metals in soil were 100 mg kg^{-1} for Cr, 50 mg kg^{-1} for Ni, 100 mg kg^{-1} for Cu and Pb, 3 mg kg^{-1} for Cd and 300 mg kg^{-1} for Zn (EU, 2006). From this study, the soil concentration of metals was found in the range of: 39.81-42.12 mg kg^{-1} for Ni, 34.19-39.6 mg kg^{-1} for Cr, 1.5-6.1 mg kg^{-1} for Cu, 55.4-149.8 mg kg^{-1} for Zn and 367.1-6032.3 mg kg^{-1} for Ca but the level of Pb and Cd in the soil of plants were below detection limit of the FAAS. So, the analysis of metals in the soil sample of the plants shows that all the concentration of selected metals were lower than the permissible limit recommended by European Union Standards (EU, 2006).

Comparison of metals level in wild edible plant with different standards

The permissible limit recommended by FAO/WHO (2001) [30] were 73 mg kg^{-1} for Cu, 2.3 mg kg^{-1} for Cr, 67 mg kg^{-1} for Ni, 0.1 mg kg^{-1} for Cd, 0.3 mg kg^{-1} for Pb and 100 mg kg^{-1} for Zn in the edible plants. The level of Ni and Cr in the root of *Eriosema cordifolium* was 23.87 and 5.14 mg kg^{-1} respectively which lower than the permissible limit. The concentration of Zn in all wild edible plant were lower than the permissible limit except in the tuber of *Pachyeymbium sacculatium* plant species (157.5 mg kg^{-1}) which is above the FAO/WHO standard level. Similar the levels of metals like Cd, Pb, and Cu in all selected wild edible plant were found to be below the analytical method detection

limit, indicating that the selected wild edible plants pose no health risk from these heavy metals: Cd, Pb, and Cu.

Translocation factor of metals from soil to plant

The soil to plant transfer factor is one of the important parameters used to estimate the possible accumulation of metals. One approach to assess the bioavailability of metal to plants is to calculate the translocation factor of the plant. The translocation factor (TF) or mobilization ratio [31] is defined as follows:

$$TF = \frac{\text{Concentration of metals in plants parts}}{\text{Concentration of metals in the corresponding soil}}$$

The TF values quantify the relative differences in bioavailability of metals to plants and identify the efficiency of a plant species to accumulate a given metal [32] and it determines relative translocation of metals from soil to other parts (root and shoot) of the plant species [31].

The TF value of Ca for *Eriosema cordifolium* and *Pachyeymbium sacculatium* were 3.51 and 1.72 respective. The TF value of Zn for *Pachyeymbium aacculatium* was 1.18. This indicates that *Pachyeymbium sacculatium* species is a Ca and Zn metal accumulator, but *Eriosema cordifolium*. Rich species is the most accumulator of Ca metal. The rest wild edible plant species is not as such to accumulate these two metals.

Statistical analysis of the results

Pair wise statistical analyses of the results were made to verify whether there was a significant difference in the metal contents between the wild edible plants and their corresponding soil. For the study, the significance of variation has been studied using one-way ANOVA and calculations were made using microcal (TM) origin® version 6; copyright© 1991-1999 microcal software.inc.

There was a significant difference ($p<0.05$) at 95% confidence level for Zn and Ca contents between all the wild edible plants and their soil samples. Accordingly, significant different ($p<0.05$) at 95% confidence level for Ni content between the roots of *Eriosema cordifolium* and its soil samples were seen when pair wise comparison was made. However, insignificant variation ($p>0.05$) at 95% confidence level for Cr content between roots of *Eriosema cordifolium* and its soil was observed. Significant difference in metals concentration between the wild edible plants and their soil samples may be the level and bioavailability of metals in the soil and the accumulation ability of plants [33].

Conclusions

Analysis of the level of metals in soil and plant were important since human exposed to the soil contaminant through: soil-plant-human (food chain) and soil-human (incidental soil ingestion). The level of Cd and Pb in soil sample of the plants was below detection limits where as that of Cr, Cu, Ni and Zn lower than the permissible limit recommended by European Union Standard. Hence, the soil of wild edible plants is not contaminated with the selected metals and safer for the plants growth and animals.

On the basis of metal contents in the wild edible plant and corresponding soil, it may be concluded that all the selected wild edible plant containing metals had lower than recommended tolerable levels proposed by Joint FAO/WHO Expert Committee on Food Additives, with the exception of Zn which exhibited elevated uptake in tuber of *Pachyeymbium sacculatium* plant species. Therefore, the tuber of *Pachyeymbium sacculatium* plant may not safe from the hazardous effects of Zn. Generally, it is concluded that total concentrations and bioavailability of metals in soils as well as the ability of metal uptake are the main controls on their contents in plants.

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