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Determination of the Level of Traces Essential Metals (Cu, Fe & Zn) and Toxic Metals (Pb & Cd) from Nettle Leaves (Urticadioica L.) Grown from Gozamin Woreda

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Abstract: Nettle, Stinging Nettle (Common names) Urticadioca; Urticaurens (Scientific names) is considered by many to be a bothersome pest, but the nettle has been used since ancient times as a source of food, fiber, and medicinal preparations. They also called in commonly as nettle, weedy perennial plant of the nettle family (Urticaceae). It is known for its stinging leaves classified as eukaryote. Because they contain a true nucleus and membrane bound organelles. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 91.2% to 109%. The concentration determined (mg/100g dry weight) were in the ranges Fe (14.583 –14.7916), Cd (0–0.4076), Cu (0.4182–2.2962), Zn (13.432–18038) and Pb (0.001–0.3083). From these Fe has high concentration from trace metals. A statistical analysis of variance (ANOVA) at 95% confidence level indicated that there is significant difference in the levels of metals among the two samples means except Cd and Pb. The results indicates that Gozamin nettle leaves are good source of essential trace metals and free from the toxic metal. All the metals concentration in this study is slightly comparable with WHO guideline of vegetables.

Keywords: Edible Leaves, Minerals, Nutrients, Wet Digestion, FAAS, Toxicity

1. Introduction

Wild edible plants refer to species that are not mane cultivated, but they are available from their wild natural habitat and used as sources of food for living things [1]. Nettle (Urticadioica L.) is wild plant with leaves that have pointed edges, are covered in fine hairs and sting if you touch them [2]. It is also known as common nettle, stinging nettle or nettle leaf, or just a nettle or stinger, is a herbaceous perennial flowering plant in the family Urticaceae. Originally native to Europe, much of temperate Asia and Africa, it is now found worldwide [3]. The species have six sub classes, five of which have many trichomes which is ahollow stinging hair on the leaves and stems. It acts like auto injector needles, injecting toxic chemicals that produce a stinging sensation upon contact [4]. This perennial herb is 30 – 150 cm in height, lightly green in color, usually dioecious as shown in Figure 1.

In Ethiopia Uricadioica L is grown between altitudes of 1000 and 3600 m above sea level all over the country. It grows mostly in Dega and Woynadega but in kola is rare where it is mostly known by common names, ‘Samma’. In 1940’s, a request by the British was made for the collection of many tons of nettles, which were used for the extraction of green dye for camouflage. These belonging have been used direct mail in Germany as a food coloring agent for packed vegetables [5]. In ancient Egypt reports are found of the use of nettle infusion for the relief of arthritis and lumbago pains. Documentation or anecdotal reports of its use in this way have been found among the Ecuador Indians, ancient Romans, Canadians and American native tribes. Stinging nettle is originally from the colder regions of Ethiopia, today this herbaceous shrub grows all over the world. Stinging nettle grows well in nitrogen-rich soil, blooms between June and September of every year [6].

Figure 1. *Urticadioica L. plant.*

Some studies conducted in the northern highlands of Ethiopia on the nutritional importance of stinging nettle shows that the forage is rich in vitamins, proteins, minerals and has high value spatially in Gozamin. This nutritional status of the forage attracts researchers for further agronomic and animal evaluation [7]. Gozamin people use stinging nettle as food in the form of porridge with grinding barley.

Stinging nettle have a long history of use as nutritious diet in East Gojam specifically Gozamin. Most people in this place believed that foods prepared from Stinging nettle leaf are important for acting mother and peoples those are suffered by gastric. It has been recommended as an adjuvant treatment for rheumatic conditions, a nutritional tonic, and anti-anemic treatment, and has been suggested for the treatment of headache, eczema, skin care, edema, lower urinary tract infections, and allergies [8]. However, the level of essential trace (Cu, Zn and Fe) and toxic (Pb and Cd) metals have not been determined in. So, determining the levels of essential and toxic metals stinging nettle found in Gozamin Woreda is important in terms of knowing the mineral composition [7, 8]. This plant has sharp hairs that can irritate after sting when the plant is touched. It is one of the richest sources which contain a significant amount 2 of vitamins, such as A, C, D and K, as well as the minerals calcium, magnesium, zinc, copper and iron [9]. Stinging nettle mainly found in moist and partly shady places of the evergreen forest at height of 1000-3600meters from the sea level [10]. It is found in yards, fields, gardens, thickets, rich mixed swamps, next to buildings, roadsides, waste ground, logging clearings, shore and streamside groves [11]. It is native to Dega and Woynadega areas and has also been introduced to other areas of Gozamin.

There are a number of reports that address the role of nettle in human nutrition. Fatty acid and carotenoid content in leaf, stem, root and seed samples have been measured [12]. In terms of postharvest processing for long-term storage, microwave drying at 850 W was found to be the best for preservation of leaf color, energy consumption, and processing time [13]. Nettle also known as weed, are widely used as food in early spring. Its‘ young leaves are added to soups or salads as well as dried for winter use lately producers more and more are focusing on nettle as a common food in Ethiopia [14]. Besides iron (Fe) as the most interesting bio element, U. dioica *L* contains other metals. Potassium (K), magnesium (Mg), iron (Fe), and zinc (Zn) are involved in various enzymes and biomolecules. In the human body, they are in an ionized form as inorganic components, or in the form of inorganic components within the organic bimolecular [9, 15]. U. dioica *L* leaves, are eaten like spinach, prepared as cooked leaves, the leaves boiled or added to soups and sauce are eaten as famine food in many parts of the world [7, 11]. Leaves have been used, particularly in rural areas of Ethiopia spatially E/Gojam Gozamin Woreda. A sauce is prepared from young nettle leaves and barley powder and served with Injera. Stinging nettles are used as a wild source of vegetables in the Woreda.

2. Experimental Section

Gozamin Woreda is one of the 22 Woreda in East Gojam Zone and 151 Woreda in Amhara National Regional State [12]. The relative location of the Woreda is 300 km away from the capital city of the country, Addis Ababa and 260 km from Bahirdar, the regional capital city. This Woreda is found almost mid-way from Addis Ababa to Bahirdar. The geographical location of the Woreda is 100 36‘ 18 N and 370 55‘ 02 E. The Woreda is bounded by SenanWoreda in the North, Basoleben Woreda and Oromiya National Regional State in the South, Aneded and Debaytilatgen Woreda to the East and Machakle and Debereaelias Woreda in the West. The study area showing the study site is presented in Figure 2. Since the use of representative, number of samples from different geographical sources of nettle leaves has not been made so far. Thus, the ministry of agriculture, all food processing factories, environmental health and safety and others are recommended to use the results from the previous researches as a stepping ladder for further investigation and more elaborative mineral analysis and other related factors on the plant leaves.

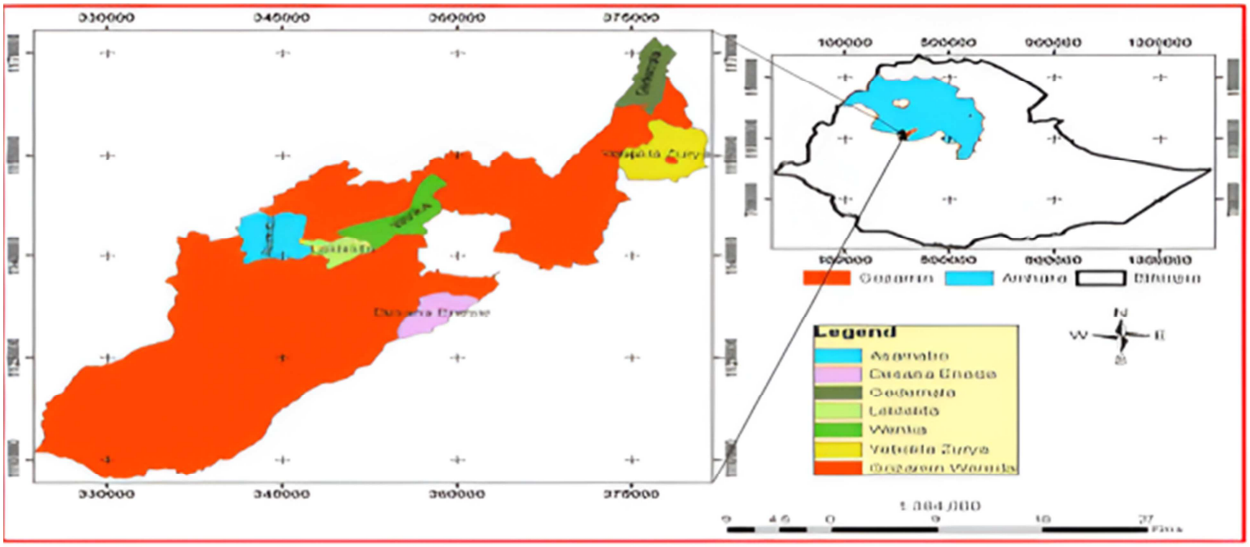

Figure 2. *Map of the Study area showing the sampling sites.*

2.1. Sample Collections

The most important step to analyze the sample is collecting the sample. Nine nettle samples i.e. two kinds Dega (Kegnabo) and Woynadega (Debremarkoszuria), which are grown in Gozamin Woreda, were brought from the agricultural land of different sites. The reasons for selecting Urticadioica among others; First, they are found everywhere in the Woreda, so they can be easily accessed by consumers. Secondly, it is used as food and traditional medicine mostly in this Woreda. Ten grams for each of a particular nettle leaves, which are from two locationaly different sites, were bought for the purpose of random sampling. Each sample was collected in a pre-cleaned polyethylene bag.

2.2. Sample Handling

The collected sample from the two sample sites (Kegnabo and around Debremarkos) were mixed to obtain a representative bulk sample (care has be taken to prevent stinging). Then the nettle leaf sample was washed with tap water followed by distilled water and dried with in an oven 80-110°C for half an hour, until it completely dried. The dried sample was grinded to fine particles using a clean acid washed mortar and pestle.

2.3. Apparatus and Instruments

A drying oven (G. P. O. box 58, Ambala catt-133 011 India) was used to dry the washed nettle leaves samples. Mortar and pestle was used to grind and powder the dried nettle leaves samples. A digital analytical balance (ESJ 210-4Ce) with ± 0.0001g precision was used to weigh nettle leaves powdered samples. Digested samples were kept in the refrigerator (Model: BXC-FW300) prior to characterization. Besides this, the researcher volumetric flask (50, 100, 250, 500, 1000 ml), conical flask (100 ml), beakers (100, 250 ml), fume hood (Model: FH1800), hot plate (Model: Laboratory Model), micropipette (Model: DRAGON MED 50-200µm), reagent bottles, spatula, graduated cylinder, filter funnel and thermometer were used during the laboratory work. Atomic Absorption Spectrophotometer (Model: 210VGP) was used for the analyte trace metals (Fe, Zn, Cu, Pb and Cd) using air-acetylene flame.

2.4. Reagents and Chemicals

Analytical grade chemicals, reagents, distilled and de ionized water were used throughout the study. All glass wares and plastic containers used were washed with detergent solution followed by soaking with 10% (v/v) HNO_3 and then rinsed with deionized water. Reagents that were used in the analysis were all analytical grade. HNO_3 (65%) and HCl (36%) were used for digestion of nettle leaf samples. Stock standard solutions containing 1000 mg/L, in 65% HNO_3, of the metals Fe, Zn, Cu, Pb and Cd (BUCK SCIENTIFIC PURO-GRAPHIC) were used for preparation of standards and spiking experiments. Deionized water was used for sample preparation, dilution and rinsing apparatuses prior to analysis and during the analysis throughout the experiment.

2.5. Preparation of Samples, Standards and Blanks

Nettle leave samples collected from Kegnabo and around Debremarkos were kept in a polyethylene bags. The spine and other light and heavy contaminants were removed from the leaf by massaging with ground using pieces of dry animal skin to ensure it is free from chaffs, dust and other impurities. The sample was washed in plastic bag using tape water many times until all dust is removed and then three times with deionized water. Then, to have constant mass and easily grind the washed nettle leaf sample was oven dried 25–113°C for half an hour prior to digestion so as to express the result in terms of dry mass basis. Drying at this temperature didn‘t affect the mineral content [13]. The dried nettle leaf sample was powdered using mortar and pestle until it feels smooth to touch. 0.25 g aliquot (three from each bulk sample) were taken for final digestion as shown in Figure 3.

Figure 3. *Sample preparation and process.*

2.6. Sample Digestion

For nettle leaf samples, in most cases, wet digestion was used for analysis by FAAS [14]. Different combinations of mineral acids have been employed for the decomposition of nettle leaf by wet digestion. A 0.5g of powdered Dega nettle leaf and 0.5g of Woynadega nettle leaf sample was taken and transferred to a 100ml conical flask. A freshly prepared 10ml HNO_3, HCl and H_2O_2, mixture in the ratio of 4:3:2 was added to this flask. The flask was shacked slightly to mix the acids with nettle‘s sample. The flask with the acid-sample mixture was then placed on the hot plate. Adjusting the temperature first to 30°C and slightly increasing up to 113°C. After 4 min deep red color appeared and then changed deep yellow after 7minutes. The mixture stayed to cool for 3minutes. Slightly addition of 2 ml HCl followed heating after 5minute light yellow color was appeared. The heating continued until the solution became colorless. After 18 minutes of digestion time the digested mixture was allowed to cool to room temperature for about 10 minute. Then after 10 ml of deionized water was added in to 100ml volumetric flask. The conical flask was rinsed with deionized water till the total volume reached to the 100mL mark. Blank solution was prepared following the same digestion procedure.

2.7. Preparation of Standard Solutions for FAAS

Standard solutions were prepared from analytical grade of metal salts treated with an appropriate reagent. Standard solution for each element was prepared from a stock solution by serial dilution. In this case, Standard solutions with a concentration of 1000ppm were diluted to obtain standard solutions of low concentration. Working standards were freshly prepared from stock solutions each time an analysis was carried out using dilution law. The absorbance obtained from FAAS instrument for each standard of a particular metal was used in drawing calibration curves. The reagent blank is a solution that contains all the same components (matrix) as the sample solution, but no known analyte materials. It identified the amount of the signal that is due to the reagents used in the preparation of the samples. This is prepared by mixing of each reagent without samples.

2.8. Determinations of Trace Metals in Nettle Leave Samples

In this study a total of five metals for each of leaves of Dega and Woynadega nettle leave samples were analyzed using FAAS with external calibration curve. For each metal three replicate determinations were carried out and it is tabulated in Table 1. Secondary standard solutions containing 10 mg/L were prepared in 100 ml volumetric flask from the atomic absorption spectroscopy (AAS) standard stock solutions that contained 1000 mg/L (BUCK SCIENTIFIC). Three working standards for each metal of interest were prepared from these secondary standards. These working standards were prepared freshly for each element from the secondary standards by appropriately diluting with deionized water for calibration purpose. Then Fe, Zn, Cu and Cd were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210VGP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength) were optimized for maximum signal intensity of the instrument. For each elements, their respective hallow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. The acetylene and air flow rates were managed to ensure suitable flame conditions. Three replicate determinations were carried out on each sample. The elements were determined by absorption/concentration mode and then, the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in the five digested blank solutions [15].

Table 1. *Instrument operating conditions for the determination of metals in nettle leaves samples using FAAS.*

Element	λ (nm)	SW (nm)	Lc (mA)	Energy (ev)	Instrumental detection limit (mg/L)	Flame type
Lead	217	0.7	3	0.407	0.006928	Air- acetylene
Iron	248. 3	0.2	3.5	0.003	0.9232	Air- acetylene
Zinc	213. 9	0.7	0.2	0.1296	0.2503	Air- acetylene
Copper	324.7	0.7	1.5	0.556	0.006245	Air- acetylene
Cadmium	228.9	0.7	2	0.621	0.003	Air- acetylene

λ =Wave length SW= slit width Lc=lamp current eV = electrovolt.

3. Results and Discussion

3.1. Method Validation

Due to the absence of certified reference material for leaves sample in the laboratory, the efficiency of the optimized procedure was checked by adding known concentration of each metal [16] in 0.25 g of nettle leaves samples. The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyte was calculated by:

$$\text{Recovery} = \frac{Cm\ in\ spiked\ sample - Cm\ in\ non-spiked\ sample}{amount\ add} \text{x}100$$

Where, C_m = Concentration of metal of interest as shown in Table 2. The results of percentage recoveries for the studied metal nutrients in both nettle leaves sample lie within the acceptable range 75-125%. Therefore, this verifies that the optimized digestion procedure was valid (good accuracy) for nettle leaves sample analysis.

Table 2. *Recovery test for the optimized procedure of nettle leaves sample.*

Metals	Amount added (mg/100g)	Concentration (mg/100g) (x)	% recovery (y)
Zn	6	13.0133±0.7037	118.666
Cd	6	0.2007±0.001215	79.1666
Pb	6	0.17467±0.2337	85.16666
Fe	6	14.868± 0.1030	108.333333
Cu	6	0.775 ± 0.2296303	98.5

(x)Values are mean ± SD of triplicate readings of triplicate analyses.
(y) Values are mean of triplicate percentage recovery values of triplicate analyses.

As it can be seen from Table 2, the mean percent recoveries for the studied all metals in the matrix spike sample ranged between 79.1666% and 118.666% for nettle leaf sample. The mean percentage recoveries for all analyte were within an acceptable range (75-125%), thus the laboratory performance for each analyte is in control. The concentration of each metal of the spiked sample was greater than the unspiked sample concentrations. This indicates that the method applied to determine essential trace and non-essential (toxic) metals in the two samples were valid [17].

3.2. Instrument Calibration

The qualities of results obtained for essential trace metals and toxic metals analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. Intermediate standard solutions of each metal containing 2 µg/100ml were prepared in 100 ml volumetric flask from the standard stock solutions that contained 0.25mg/250ml. The intermediate standards were diluted freshly with de-ionized water to obtain four working standards of each metal of interest for calibration purpose. The instrument was calibrated using five series of working standards. Concentrations of working standards and value of correlation coefficient obtained from Absorbance verses concentration calibration curve for each metal are tabulated in Table 3 and the calibration graph of each of metals of interest is shown in Figures 4 (a – e).

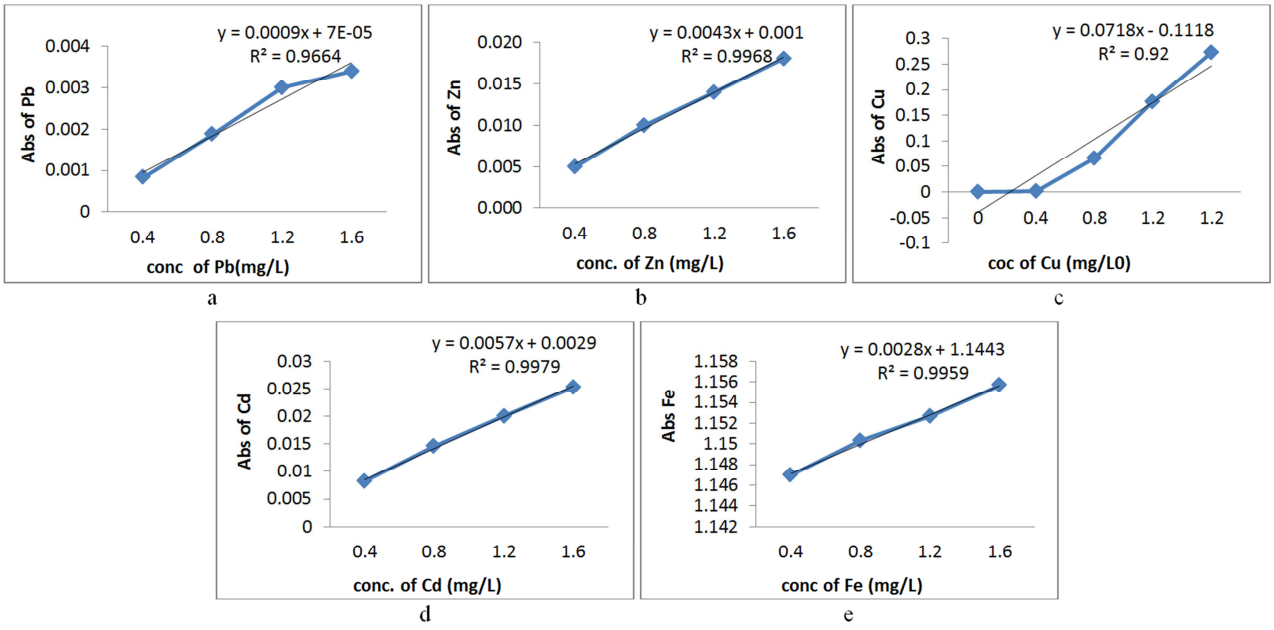

Figure 4. *a-e: Graphs of calibration standards.*

Table 3. *Series of working standards and correlation coefficients of the calibration curves for determination of metals in the nettle leaves varieties using FAAS.*

Metal	Concentration of standards solutions (mg/L)	Correlation coefficient Values (R^2)	R (correlation coefficient) square root value	Regression equation Y=mX+b or A=mC+ b
Fe	0.4, 0.8, 1.2, 1.6	0.9956	0.99979	A=0.00288x+1.1443
Cu	0.4, 0.8, 1.2, 1.6	0.9812	0.9905	A=0.0066x+0.00777
Zn	0.4, 0.8, 1.2, 1.6	0.9968	0.9984	A=0.0043x+0.001
Cd	0.4, 0.8, 1.2, 1.6	0.9979	0.9989	A=0.0057x+0.0029
Pb	0.4, 0.8, 1.2, 1.6	0.99664	0.9983	A=0.0009x+0.0.00007

A-absorbance, C- concentration (mg/ml).

3.3. Determination of the Concentration of Selected Trace Essential and Toxic Metals in Nettle Leaves

The levels of each metal in both samples were determined with FAAS after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. To determine the concentration of metals in the two different samples of same plant, a straight line calibration curve was drawn between all the known points of working standard solutions. Then, the unknown concentration of each metal in Dega and Woynadega was determined using the slope equation from the calibration graph, obtained using standards of known concentration in Microsoft Excel-2010 software. Triplicate determinations were carried out on each sample. The same analytical procedure was employed for the determination of elements in the digested blank solutions. The graph of calibration curves of each metals of interest are shown for plant samples in Figures 4 a-e above.

Among the analyzed metals Fe, Cu and Zn have relatively high concentration inboth samples. Whereas the concentration of Cd and Pb was found to be very low in Dega and Woynadega detected by instrument. The low level of the toxic metals might be an evidence for the absence of the use of some commercial fertilizers and herbicides for Nettle plant growth in the Dega and Woynadega sites. The concentration values of the metals and their corresponding SD are shown in Table 4. It was checked with t-test at 95% (p = 0.05) confidence level that for most quantified metals there is no significant difference in concentrations of the same metals from two sites.

$$CM = \frac{C.V.D}{MS} \text{ (dilution law)}$$

Where, CM = Concentration of metal (mg/100g);

C = the Concentration of metal read from the spectrophotometer (mg/l);

V = the volume of the solution (L) MS = the mass of the sample analyzed (gm);

D = the dilution factor (if any).

Table 4. *Average concentration (mean mg/100g ± SD, n = 3) and (%RSD) of metals in nettle leave.*

Metals	Dega		%RSD	Woynadega		%RSD
	Reading of unknown (y)	Calculated concentration (x)		Reading of unknown (y)	Calculated concentration (x)	
Zn	0.013	13.0133±0.7037	5.4	0.01767	17.27 ± 0.522	3.022
Cd	0.00318	0.2007±0.00122	6.05	0.003167	0.18364 ± 0.1062	57.83
Pb	0.000109	0.17467±0.2337	133.79	0.000078	0.17467± 0.2337	133.79
Fe	1.155	14.869± 0.1030	0.692	1.15257	11.8095± 5.179	6.93
Cu	0.10482	0.785 ± 0.22963	29.62	0.010123	0.776± 0.2962	29.93

y- reading of unknown from AAS, x- calculated concentration using Beers Lambert law (y=mx+b) Average of five determinations and their standard deviation.

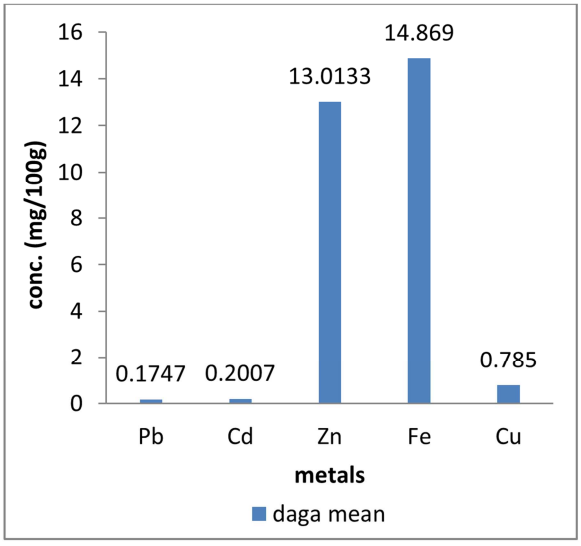

Figure 5. *Distribution of metal concentration in Dega nettle leaves.*

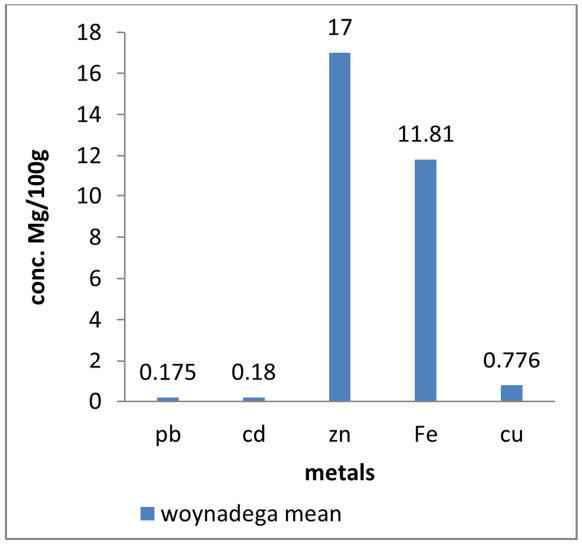

Figure 6. *Distribution of metal concentration in Woynadega nettle leaves.*

These results indicated that the mean concentration of Fe, Zn and Cu in the Dega nettle leaf samples, level of Fe, Zn and Cu in the Woynadega nettle leaves sample were found to be the highest among other toxic metals analyzed as shown figure 6 below, which suggests that the plant is rich in these micronutrients that can be considered as a potential human feed and the levels obtained are within the permissible limits of WHO [19]. From the figures 5 and 6 mean concentrations of toxic metals (Pb and Cd) in the nettle leaves sample were found to be very low. The mean Fe concentrations found in this study was from 14.77- 14.97mg/100g in Dega & 6.63-16.99mg/100g in Woynadega, which is in the value of the recommended standard ranged from 1.433-59.33mg/kg [20]. The level of trace essential and toxic elements in nettle leaf varies greatly due to the factors that affect mineral accumulation by plants. Including the geochemical characteristics of a soil and by the ability of plants to selectively accumulate some of these elements since bioavailability of the elements depends on the nature of their association with the constituents of a soil [21]. The variations of the metal concentrations in the leaf samples may be ascribed to nature of the leaf (i.e., the differences in physiological properties of metal uptake that varies from site to site, exposure surface area and plant age); the physical and chemical nature of the soil where the plant grew (cation exchange capacity, organic matter content and soil pH); atmospheric deposition of the metals (which may be influenced by innumerable environmental factors such as temperature, moisture and pH [22].

3.4. Accuracy and Precision

The precision of the results were evaluated by the standard deviation and relative standard deviation of the results of three replicate measurements (n=3). These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with the corresponding standard deviation of four measurements for a bulk sample and triplicate reading per sample and relative standard deviation [23]. The relative standard deviations of each metal in Dega Urticadioica leaf samples are Zn (0.054), Cd (0.061), Pb (1.338), Fe (0.00692) and Cu (0.2962). The relative standard deviations of each metal in Dega (from Figure 5) Urticadioica leaf samples are Zn (0.03), Cd (0.57), Pb (1.338), Fe (0.00693) and Cu (0.2963). This indicates that there is high precision between Fe, Cu and Pb metals of Dega and Woynadega nettle (from Figure 6) leaves. Whereas there is low precision between Zn and Cd of Dega and Woynadega nettle (*dioica L.*) leaves. It may arise from measurement or instrumental error.

3.5. Method Detection Limit

The method detection limit of each metal, six blank for each were digested and analyzed along with nettle leaves samples. Then the mean concentration of the blank and the standard deviation of the six blank samples were calculated for each metal. Finally, the detection limits were obtained by mean concentration of the blank plus three times of the standard deviation of the reagent blank. Triplicate analyses for six blank samples for all elements were performed and the pooled standard deviation of the four blank reagents with triplicate measurement was calculated as shown in Table 5, the method detection limit of each element is above the instrument detection limit. Method detection and Quantization limit (n = 5, MDL = 3Sblank and MQL=10Sblank in mg/100g) for all metals determined in nettle leaves sample.

Table 5. *Instrumental detection limit, method of detection and quantization.*

Metals	IDL (mg/l)	DLM (mg/100g)	MQL (mg/100g
Zn	0.006	12.09302	18.60465
Cd	0.004	0.746873	1.122054
Pb	0.028595	1.175706	1.192841
Fe	0.004	12.17368	12.67368
Cu	0.533988	0.837713	1.799557

DLM =detection limit, MQL= method of quantization, IDL=Instrumental detection limit.

3.6. Limits of Quantization

The quantification limit of each element was calculated as ten times the standard deviation of the blank [18]. As can be seen from the table, both the MDL and MQL are greater than the instrument DL, hence, the result of the analysis could be reliable. Limit of quantification is the lowest limit for precise quantitative measurements.

3.7. Comparison of Metals Within Nettle Leaves Samples

When the concentration of metals in the nettle leaves were compared the Woynadega nettle leaves has relatively higher concentration of Zn and Cu than Dega nettle leaves. But, Fe which is slightly higher in Dega nettle leaves. Whereas, both Dega and Woynadega nettle leaves have very low and relatively same Cd and Pb contents [Figure 7]. The pattern of concentration of elements in the two locational variety of nettle leaves studied decreases in the following order for Dega nettle leaves: Fe > Zn > Cu> Cd and Pb. For Woynadega nettle leaves Zn > Fe > Cu>Pb>Cd.

The small amount of Cu found in nettle leaves does not contradict with the requirement of the metal for proper functioning of the body, because this metal is required in small amount (Cu = 0.3 mg/day) as a constituent of vitamin B12 and Cu = 3.5 mg/day [24]. As can be seen from Figure 7 zinc is the most concentrated (17.3mg/100g) in Woynadega and Iron is the most concentrated heavy metals in all nettles with values 14.686mg/100g while Cd the lowest. From this, generally we can say that the iron content of nettle leaves is superior and it substitutes other grains like red tef, maize and others.

3.8. Comparison of Metal Contents of Nettle Leaves with Other Vegetables

Wild plants have since ancient times, played a very important role in human life; they have been used for food,

medicines, fiber and other purposes and also as fodder for domestic animals. Many plants have edible leaves. Most commonly known plants with edible leaves are cabbage, spinach, lettuce, coriander, sprouts, celery, basil, curry leaf etc. People sometimes use mixtures of two or more of these leaves with crops to enhance the nutritional values of their diets. Comparisons of the values for nettle leave with other vegetables are therefore very essential to know the dietary mineral intake of individuals who use vegetables in their diets. As shown table 6 the levels of mineral nutrients in edible leaves have been indicated by different researchers. From the table, it is possible to observe that the concentration range of some metals is higher in nettle leaves than other vegetables.

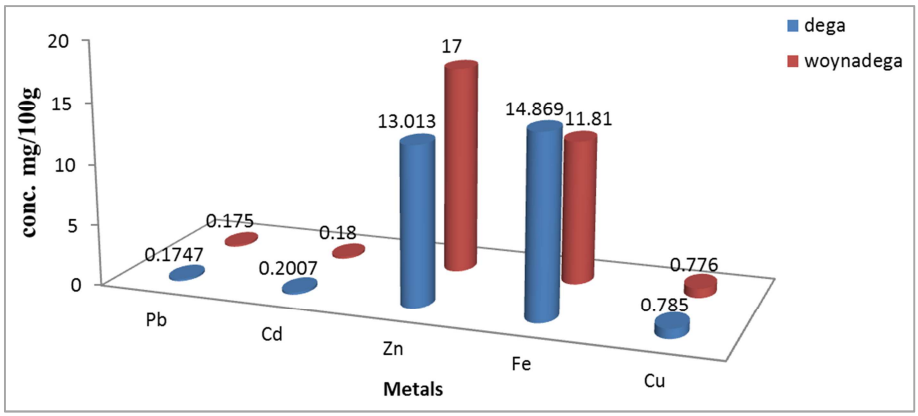

Figure 7. *Average concentrations for essential trace & toxic metals in two nettles leaves.*

Table 6. *Comparison of metal contents of nettle leaves with other vegetables (mg/kg).*

Elements	Pb	Cd	Zn	Fe	Cu	Reference
Nettle leaves	0.175±0.2336	0.2007±0.00122	15.2±1.778	14.686± 0.1030	0.6414± 0.2236	Present work
cabbage	0.005*	0.073±0.005	0.777±0.02	0.764±0.001	0.252±0.02	[8, 27]
Lettuce	0.0051±0.0001*	0.033±0.001*	1.893±0.05	150±0.04	0.273±0.02*	[25]
Spinach	2.25±0.09	0.035±0.001*	0.461±0.02	2.27±0.004	0.923±0.03	[26]
coriander	2.625±0.04	0.062±0.04	0.705±0.02	16.1±0.02	0.85±0.03	[27]
cauliflower	1.33±0.04	0.064±0.003	0.678±0.05		0.323±0.01	[28]

*values in mg/100g, others are in mg/kg.

Many authors reported the concentration of metals in cereal and vegetables. A lot of researchers have reported on the level of mineral nutrients in the major edible leaves types such as cabbage, spinach, lettuce, coriander, spinach, brussels sprouts, celery, basil, curry leaf [29]. From the table above, it is possible to see that the concentration range of some metals is higher in nettle leaves as compared to some vegetables. For example, the concentration range of Fe in nettle leaves, cabbage, lettuce, spinach, coriander are 14.687-14.787mg/100g, 0.763-0.765mg/100g, 149.006-150.004mg/kg, 2.269-2.274mg/kg and 16.09-16.12mg/100g respectively. This indicates nettle leaves have higher Fe concentration next to coriander. The concentration of Cu and Zn in nettle leaves is as good as spinach and coriander with its maximum of the range. Compared to all edible leaves have higher iron, copper and zinc content while cauliflower has the least amount of the mineral. However, Pb content of it is higher than nettle.

3.9. Comparison of Metal Levels of the Present Study with Literature Values

Comparison of analytical data with reference material is a common practice in analytical chemistry to validate the results. However, there is no standard reference material to do so and the determined results should be compared with the investigations made in other countries by other investigators. Different researches were being made by different researchers in different countries on nettle leaves, but in the Ethiopian case no detail studies were made on the levels of trace and toxic metal composition on nettle leaves. As it is seen from the tables below, the levels of micro-essential and toxic metals determined in this work were in good agreement with other studies done in other countries. The determination for levels of toxic metals was also carried out in this study and Cd was found to be below detection limit of the instrument. The level of Pb was found as 0.175–0.1980mg/100g. When it is compared with the values in the literature, it is lower than that mentioned by [30] in Nigeria (62.5–150 mg/kg), but higher than most others. The level of Cd was found to be 0.1805-0.2007mg/100g compared with [31] it is a little higher, but it is less than [32] work conducted on nettle plant.

Table 7. *Comparison of heavy metals (Cu, Zn, Fe) and toxic metals concentration (Cd &Pb), (mg/kg, dry weight basis) in nettle leaves samples with reported values.*

metals	Analyzed		Recommended Conc. (Literature synthesis)	Literature values	Country	Reference
	Dega	Woynadega				
Zn	15.2±1.778	16 ± 1.3578	15mg/100g-men and 12mg/100g-women	52±1. 5 19±2.5 4.83±1.25 99.4mg/kg	Poland Belgium Turkey AA (Ethiopia)	[26] [6] [29] [27]
Cd	0.201±0.0012	0.1836 ± 0.1030	0.02mg/kg	0.001±0.0 0.5±0.25 0.5mg/kg	University of Nis Nis Serbia	[28] [29]
Fe	14.686± 0.1039	14.686±0.1030	11.43±2.24 21.2±0.08 16.7±0.7	425.5mg/kg 8-11mg/100g	Turkey Washington AA (Ethiopia)	[33] [27]
Pb	0.17467±0.2337	0.005±0.000119	0.15mg/100g 0.3mg/kg	0.28±0.02 6.7±0.25	University of Nis Nis Serbia	[34]
Cu	0.644 ± 0.2336	0.776± 02.2962	73.7mg/kg	0.89±0.12 0.4±0.00 147±0.024	Turkey Gent, Belgium Poland	[35] [36] [37]

3.10. Statistical Analyses

In this study, samples were collected from east Gojam Zone two distinct areas of Gozamin Woreda, from randomly selected wild Urticadioicaleaves where they are commercially available. Each sample was mixed thoroughly and one representative bulk sample was taken for each two type. During this processes a number of random errors may be introduced in each aliquots and in each replicate measurements. Therefore, depending upon the type and nature of results at hand, a statistical method is used to check whether there is contribution from this random errors for difference in results of analysis or not.

Differences between the mean values of the various samples obtained were evaluated by student's dependent paired *t*-test. Linear regression statistical test and correlation analysis were performed for the calculation of the slope (*m*), and correlation coefficient (*R*) of the regression line. The number of degrees of freedom (Df) for finding the *t* value is $(N_1 + N_2) - 2$, where N_1 and N_2 are number of replicate measurements of sample 1 and sample 2 respectively [38].

$$T_{exp} = \frac{\mu 1 - \mu 2}{Sp}\sqrt{N} = \frac{\mu 1 - \mu 2}{Sp}\sqrt{\frac{N_1 N_2}{N_1 + N_2}}, \quad (1)$$

$$S_p \text{ is pooled standard deviation} = \sqrt{\frac{{S_1}^2(N_{1-1}) + {S_2}^2(N_{2-1})}{N_1 + N_2 - 2}} \quad (2)$$

$$Df = n_1 + n_2 - 2$$

Where: μ_1- mean of first sample, μ_2 –mean of second sample;

Df- degree of freedom of a sample mean;

T_{exp} -T calculated from the experiment;

N_1N_2 - replicate measurements of sample 1 and sample 2 respectively.

If the experimental T- calculated exceeds the critical value, then the means are significantly different and no significant difference if the opposite occurs. It is based on this fact that we say the means of the above mentioned metals are not significantly different.

Table 8. *Pair wise comparison between mean values of various nettle samples by student's t-test at the 95% confidence level.*

Metals	Comparison samples	Mean mg/100g	Df	T_{cal}	T_{cr}	Remark
Zn	Dega samples Woyna samples	13.0133 17.27	2 2	2.2874	2.13	There is significant difference among the sample means
Cd	Dega samples Woyna samples	0.2007 0.18364	2 2	0.199775	2.13	The means are not significantly different
Pb	Dega samples Woyna samples	0.17467 0.1768	2 2	0.2337	2.13	The means are not significantly different
Fe	Dega samples Woyna samples	14.689 11.8095	2 2	3.8398	2.13	There is significant difference among the sample means
Cu	Dega samples Woyna samples	0.785 0.775	2 2	0.041595	2.13	The means are not significantly different

SD = standard deviation, Df = degree of freedom, T_{cal}= T calculated, T_{cr}= T critical Metal.

From the table above variations were from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in wash away of agrochemicals from farm lands by natural phenomena (flood)). From the table one can see that, there is significant difference at 95% confidence level in mean concentrations of Zn and Fe in all the two sample forms and the means of Pb, Cd and Cu concentration in Dega and Woynadega. The source for this significant difference between sample means may be the difference in mineral compositions of the soil or pH of soil which predict the degree of mineral absorption by nettle

plants.

4. Conclusions

This study showed that the stinging nettle collected from the Gozamin Woreda can be considered as a source of valuable components. High content of minerals (Cu, Zn & Fe) determined in leaves is of great importance for the introduction of the stinging nettle in nourishment, as well as from medicinal and phototherapeutic point of view. The determined values for the quantities of minerals in the leaves of the stinging nettle are of significance, with indications of new directions for its utilization. The concentrations of three trace metals (Fe, Cu and Zn) and two toxic metals (Pb and Cd) in nettle leavesamples used as a food and traditional medicine in Kegnabo, Gedemala Kebele and around Debremarkos of Gozamin District in East Gojam have been determined using FAAS. The concentration level of metals in the samples are in the order of Zn (44%) > Fe (32%) > Cu (24%) > Cd (0%) and Pb (0%) nettle leaves from around Debremarkos, Fe (41%) > Zn (30) > Cu (27%) > Cd (2%) >Pb (0%) in the Dega nettle leaf (Gedemala and Kegnabo Kebele). The levels of three essential trace and two toxic elements were determined by FAAS in the two most common and Locational nettle varieties. A statistical analysis at 95% ($p = 0.05$) confidence level revealed that there is a significant difference in the mineral content of the nettle leaves. Although the data obtained is relatively small to draw authoritative conclusion about the mineral contents of nettle leaves, nevertheless, the present analysis on nettle leaves will give the base line for comparison for young researchers and good awareness for nettle plant leaf users and those who intend to use the vegetables.

Declarations

The authors declare that, it is original work and has not been published for any journal or any other conferences and that all sources of materials used in this work have been duly acknowledged.

Conflicts of Interest

There are no conflicts to declare.

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