Biol Trace Elem Res (2011) 141:317–328
DOI 10.1007/s12011-010-8732-z

Concentration Levels of Selected Metals in the Leaves of Different Species of Thyme (*T. schimperi* and *T. vulgaris*) Grown in Ethiopia

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Received: 24 January 2010 / Accepted: 11 May 2010 /
Published online: 25 May 2010

Abstract The contents of some selected metals Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd in different thyme leaf samples widely consumed in Ethiopia were determined by flame atomic absorption spectroscopy (FAAS) after acid digestion with 1:1 $HNO_3/HClO_4$ for 3 h at a temperature of 240°C by a Kjeldahl apparatus hot plate digester. The level of the nutrients in the four samples ranged from 1,239–2,517 μg/g, Ca; 1,524–1,786 μg/g, Mg; 728–2,517 μg/g, Fe; 37.7–114 μg/g, Mn; 2.59–4.3 μg/g, Co; 7.69–9.3 μg/g, Cu; 8.7–52 μg/g, Zn; and 9.83–14.2 μg/g, Ni; respectively. While the level of toxic metal Cd in the four samples ranged from 0.87–1.3 μg/g. The concentration of Ca was higher than the other metals in the three samples and Cd was the least of all the metals in the analyzed samples. The overall reproducibility of the method obtained from spiking experiment was within the range ±10%. This result will complement available data on food composition in Ethiopia.

Keywords Thyme leaves · Metals · Debresina · Wondogenet · Ethiopia · FAAS

Introduction

Thyme is native to the western Mediterranean area, especially the south of Italy, from where it spread to almost every region. This plant is cultivated in almost every country, as an aromatic for culinary uses (especially in the south of France, Spain, Morocco, and North America [1]).

The genus *Thymus* includes about 350 species worldwide and is widely distributed in temperate zones [2]. The two species, *Thymus schimperi* Ronniger and *Thymus serrulatus* Hochst.ex Benth are endemic to the Ethiopian highlands growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2,200–4,000 m altitudes. Both species are perennial herbs, woody at the base and 5–40 cm high.

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Thyme is mainly a temperate taxon and is uncommon in the African tropics. There are, however, two species, *T. schimperi* Ronniger and *T. serrulatus* Hochst.ex indigenous to Ethiopia [3, 4] while *Thymus vulgaris* has been recently introduced. *T. vulgaris* is a species, native to southern Europe, recently introduced into Ethiopia and cultivated in Wondo Genet by the Essential Oil Research Center [5]. *T. schimperi* is comparatively widespread in central, eastern and northern Ethiopia. It is locally known as Tosign. *T. serrulatus* has obovate to oblanceolate leaf-laminas with weakly crenate margins and is restricted to northern Ethiopia. It is locally known as Tesni or Thasne. Thymus species in Ethiopia are restricted to afromontane and afroalpine regions and are represented by two species *T. schimperi* and *T. serrulatus*. Bale, Shewa, Gonder, and Wollo are the major growing areas in Ethiopia [2, 3]. Wild thyme, used to make a tea, is harvested by people living close to the town of Dinsho and near Menz. It is dried, put in plastic bags and sold to travelers on buses [6].

The volatile oil from thyme was found to contain *p*-cymene, γ-terpine, carvacrol, rosmarinic acid, eugenol, and thymol [3, 5]. The volatile oil not only has carminative action, but also antiseptic, antimicrobial and antifungal activities [7]. In the Ethiopian traditional medicine the plant has many medicinal applications. Some of the reported applications are for the treatment of gonorrhea, cough, inflammation, spasm, thrombosis, urinary retention, mental illness, eye disease, toothache, stomach problems, leprosy, lung TB, acne, and ascaris [8, 9].

The fresh or dried leaves of both species are used locally as condiments in the preparation of chili powder, stew, bread and tea. Lemon thyme, *Thymus xcitriodorus*, is recommended for fish, for tea, and for salad dressings, or anywhere milder thyme is desired [3, 4].

Trace metals are minerals present in living tissues in small amounts. Some of them are known to be nutritionally essential, others may be essential, and the remainders are considered to be nonessential. Trace elements function primarily as catalysts in enzyme systems; some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. All trace elements are toxic if consumed at sufficiently high levels for long enough periods. For example, high levels of zinc can result in a deficiency of copper, another metal required by the body. The difference between toxic intakes and optimal intakes to meet physiological needs for essential trace elements is great for some elements but is much smaller for others [10, 11].

Heavy metals are stable elements (meaning they cannot be metabolized by the body) and bio-accumulative (passed up the food chain to humans). These include: mercury, nickel, lead, arsenic, cadmium, aluminum, platinum, and copper (the metallic form versus the ionic form required by the body). Most of the heavy metals have no function in the body and can be highly toxic [11]. Nutritionally essential metals may cause adverse health effects at some levels below or beyond the level required for optimum nutrition [12]. Injury to vegetation caused by trace metal has been well recognized because of many botanical and chemical investigations during the past 100 years. More than 60 elements in various parts of the human body have been detected. Among these, at least 25 elements are essential to human health and out of which, 14 are termed as trace elements [13].

Food is the major intake source of toxic trace elements by human beings. Vegetables, fish, meat, grains, soft drinks, condiments, etc. are used as staple part of food. Various studies investigated the concentration of major, minor, and trace elements from the different items of food. Vegetables [13–15], food condiments [16], water [17], bread [18], fish [19] are some among others. Little information has been reported on the levels of metal concentration of thymus species. Khan et al. [20] investigated seven macro-nutrients (Na, K, Ca, Mg, Al, Pb, and Cd) and five micro nutrients (Fe, Mn, Cu, Cr, and Ni) from *T. vulgaris* by atomic absorption spectrophotometer. No information has been reported yet on the level of the metal concentration on the two thymus species indigenous to Ethiopia and *T. vulgaris* cultivated now at Wondo Genet Essential Oil research Center, Ethiopia.

This research project was designed to investigate the levels of essential and non-essential metals (toxic) in the two thymus species *T. schimperi* Ronniger endemic to Ethiopia and *T. vulgaris* cultivated at Wondo Genet Essential Oil research Center which is endemic to Western Europe. The main objective was to determine the concentration of metals (Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd) in *T. schimperi* and *T. vulgaris* by flame atomic absorption spectroscopy and to compare the levels of the identified metals in the freshly prepared and market available thyme. It was also aimed to compare the levels of the selected metals in Ethiopian *T. vulgaris* with the levels of metals in *T. vulgaris* from the other part of the world.

Materials and Methods

Instrumentation and Apparatus

Ceramic pestle and mortar were used for grinding and homogenizing the dried thyme leaves; digital analytical balance (Mettler Toledo, Model AT250, Switzerland) with ±0.0001 g precision and oven (J.P.SELECT, Spain) were used for weighing and drying the samples, respectively. Quick-fit round bottom flasks (150 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the samples ready for analysis till determination. Micropipette (1–10 μL, 100–1,000 μL, Shanghai, China) was used for spiking of known concentration for recovery test. Buck Scientific Model 210VGP (East Norwalk, USA) Flame Atomic Absorption Spectrophotometer equipped with deuterium arc background corrector was used for analysis of the metals (Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd).

Chemicals, Reagents and Standard Solutions

For digestion of thyme leaf samples, 69–72% HNO_3 and 70% $HClO_4$ (Research—lab fine chem industries, Mumbai, India) were used. Stock standard solutions of the metals (Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd, 1,000 mg/L calibration standards Buck Scientific, prepared as nitrates for each element in 2% HNO_3) were used for the preparation of calibration curves for the determination of metals in the samples. Deionized water for preparation of standard solutions, dilution and for cleaning (rinsing) purpose was used. Lanthanum chloride hydrate (99.9%, Aldrich, USA) was used to avoid refractory interference for calcium and magnesium analysis from their phosphates.

Collection and Preparation of Samples

Thyme samples were collected from the growing fields of Tarmaber Woreda, Wondogenet Essential Oil Research Center, and Addis Ababa town. The type of species from Addis Ababa and Tarmaber Woreda was *T. schimperi* and from Wondogenet Essential Oil Research Center the species was *T. vulgaris*. Totally four kinds of samples were collected: (1) fresh leaves from a highland area which is about 10 km from Debresina town (*T. schimperi*), (2) processed dried thyme leaves from Debresina town (*T. schimperi*), (3) processed dried thyme leaves from Addis Ababa city (*T. schimperi*), and (4) fresh thyme leaves from the garden of Wondogenet Essential Oil Research Center (*T. vulgaris*).

The reason for the selection of sampling sites was based on the availability of the sample, proximity to the study area, proximity to the market center, and sampling cost. *T. vulgaris*, as stated earlier, was brought from Western Europe and was selected in order to relate and compare the mineral contents with the species growing in Ethiopia and with the same species in other countries. Debresina is found in the northern part of Ethiopia, 190 km from the capital, Addis Ababa. Wondogenet is in the southern part of Ethiopia and is 305 km of from Addis Ababa.

The sampling technique used in the present study was manual random sampling [21]. The leaves of fresh *T. schimperi* from Tarmaber woreda were collected from the growing area where ten bags were taken within the specific area with a difference of about 100 m between each site. A bulk sample was prepared by taking 50 g from each plastic bag. *T. vulgaris* from Wondogenet was sampled by taking randomly from five sites. All the leaves from the five sites were mixed and a bulk sample was prepared. The dried (processed) leaf samples were bought from Debresina and Addis Ababa supermarkets. In both places, five supermarkets were randomly selected and from each supermarket three bags were bought. For each type of sample a bulk sample of 500 g was prepared. The bulk sample was washed with tap water to remove dust material attached to the leaves of thyme. This was followed by washing with distilled and deionized water ten times in each case for removing trace metal contamination from tap water and dried in the oven at 75°C for 24 h. The sample was further powdered by making use of mortar and pestle, sieved (with 0.5 mm sieve), and kept in a desiccator prior to digestion. Finally, 0.5 g aliquot was taken from each sample for digestion and a solution for final metal determination was prepared.

Optimization of the Digestion Procedure

In this study, the thyme samples were made ready for the analysis after wet digestion using the Kjeldahl digester heating block. Hence, different digestion procedures were tested by varying the volume of reagent, digestion time, and temperature and reagent composition. Then nature of the final digests was examined, clear and colorless solution was selected and the procedure taken as an optimum. The selection considered clearness of the digests, less digestion time, low reagent volume, and low temperature.

Digestion of Samples

Applying the optimized procedure, 0.5 g of powdered thyme sample was weighed on analytical digital balance and placed in a 250 mL round bottom flask. To this, 3 mL of HNO_3 (69–72%) and 3 mL of $HClO_4$ (70%) was added. The round bottom flask was fitted to a reflux condenser and heated on a Kjeldahl apparatus hot plate for 3 h at a temperature of 240°C. The digest was allowed to cool for 10 min without dismantling the condenser and then further cooled to room temperature for 20 min by dismantling the condenser. The mixture then diluted with 20 mL of deionized water and filtered with Whatman filter paper (110 mm, diameter) into a 50 mL volumetric flask. The round bottom flask was further rinsed with 10 mL of deionized water and added to the filtrate. A 0.2% of lanthanum chloride was added to the mixture and the flask containing the filtrate was filled to the mark. For each sample the digestion was done in triplicate. Blank samples, a mixture of 3 mL of HNO_3 and 3 mL of $HClO_4$ were digested following the same procedure as the samples. Finally, the digests were kept in refrigerator until analysis.

Instrument Calibration and Method Detection Limit

Detection limit is defined as the minimum concentration that can be detected by the analytical method with a given certainty. This is often taken as the mean value of the blank plus three times its standard deviation [22]. In the present study, the detection limit of the method was estimated from the standard deviation obtained for each metal from the six blank samples. The detection limits for each metal were obtained by multiplying the standard deviation of the six blank solutions by three. The method detection limits of each metal are given in Table 1. The results clearly show that the calibration curves with good correlation coefficients and lower method detection limits were obtained during the analysis.

Evaluation of Analytical Method

To validate and assess the accuracy of the optimized procedure, spiking experiments were done and the recoveries were examined. This was done by adding a known concentration (1,000 mg/L) standard solution of each metal (Buck scientific standard solution) to known weight of the sample before digestion. The spiking was performed in three groups: (1) 400 μL of 1000 mg/L Mg were spiked in a flask containing 0.5 g sample, (2) 400 μL and 100 μL of 1000 mg/L Ca and Fe, respectively, were spiked in other flask with 0.5 g of the same type of sample, and (3) 20 μL of 1000 mg/L Mn, 70 μL of 10 mg/L Co, 20 μL of 100 mg/L Cu, 50 μL of 100 mg/L Zn, 20 μL of 100 mg/L Ni, and 20 μL of 10 mg/L Cd were spiked in the third group with the same amount and type of sample as in the earlier cases. Each group was done in triplicate. All the spiked and un-spiked samples were digested following the procedure described earlier in triplicates. Each sample was analyzed for their mineral contents. The percentage recoveries of each analyte metals are given in Table 2. The data clearly indicate that the recoveries are in the range between 92–103%. Hence, the method is of good accuracy.

Determination of Major, Minor and Trace Metals in the Samples

Ten milligrams per liter (intermediate standard solution) in 100 mL volumetric flask was prepared from by dilution of 1,000 mg/L stock solution of FAAS. From the 10 mg/L solution four working standard solutions were prepared at different concentration for each metal (Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd). The instrument was calibrated with working standards after the parameters (energy, slit width, lamp current, wavelength, and others) were adjusted to give maximum signal intensity. All the thyme and blank samples were analyzed for each metal using FAAS.

Table 1 Method Detection Limit of Metals in Thyme Leaf Samples (n=9)

Metal	MDL (μg/g)	Metal	MDL (μg/g)
Ca	8.4	Cu	0.5
Mg	10	Zn	1.8
Fe	3.4	Ni	1.1
Mn	6.9	Cd	1.0
Co	0.2		

MDL method detection limit

Table 2 Recovery Test Results for Analysis of Thyme Leaf Samples

Metal	Amount added (μg/0.5 g)	Amount found (μg/0.5 g)[a]	Recovery (%)[b]
Ca	400	383±15	95.8±4.0
Mg	400	386±19	96.5±4.8
Fe	100	96.0±8.0	96.0±8.3
Mn	20	19.1±1.5	95.4±7.6
Co	0.7	0.65±0.05	92.9±7.3
Cu	2	2.06±0.13	103±6.3
Zn	5	4.76±0.32	95.2±6.7
Ni	2	1.82±0.14	91.0±7.7
Cd	0.2	0.185±0.16	92.5±8.6

[a] Mean values (of the difference between spiked and unspiked samples) ± SD of triplicate readings of triplicate analyses

[b] Values are mean±SD of triplicate readings of triplicate analyses

Statistical Analysis

In analytical work, there may be two or more than two means to be compared to check whether there is a significant difference between them or not. For example, a variation between the means of different sample can be resulted from random and controlled sources of error. This kind of variation and the variation due to the difference in the original sample type can be separated and estimated by a powerful statistical technique known as analysis of variance (ANOVA) [23]. In this study, a one-way ANOVA and SPSS (SPSS 15.0 for windows, The Apache software foundation, 2000) software was used to know the variation between samples analyzed was significant or not for the mean concentrations of each metal in triplicate analysis.

Results and Discussion

Distribution of Macro- and Micro Nutrients in Different Thyme Samples

The concentration of nine metals (Ca, Mg, Fe, Mn, Co, Cu, Zn, Ni, and Cd) in some freshly prepared and commercially available Ethiopian thyme samples was determined by FAAS using four point external calibration curve. The results showed that the samples had variable composition of each analyte metals with wide concentration range (Table 3).

As can be seen from Table 3, Ca (2,776±130 μg/g) was observed to be of the highest concentration followed by Mg (1,786±13 μg/g). From the studied microelements, Fe (728± 58 μg/g) was found in a significant amount compared to Mn, Co, Cu, Zn, Ni, and Cd. Cd (1.3±0.08 μg/g) was the least of all the studied metals in fresh samples from Debresina. The level of metals in this thyme sample decreases in the order Ca > Mg > Fe > Mn > Zn > Ni > Cu > Co > Cd.

The study on the thyme sample collected from different supermarkets in Debresina showed incredibly a largest amount of Fe (2517±±24 μg/g) followed by Ca (1,980±81 μg/g) and Mg (1,739±14 μg/g). Cd (0.93±0.09 μg/g) was observed to be the least as in the case of fresh

Table 3 Mean Concentration (mean±SD, n=9, μg/g Dry Weight) of Major, Minor, and Trace Metals in Thyme Leaf Samples

Element	Concentration of metals (μg/g)							
	DF	% RSD	WF	% RSD	DM	% RSD	AAM	% RSD
Ca	2,776±130	4.7	1,239±102	8.2	1,980±81	4.1	2,066±110	5.3
Mg	1,786±93	5.2	1,524±77	5.1	1,739±70	4.0	1,735±90	5.2
Fe	728±58	8.0	1,103±71	6.4	2,517±94	3.7	1,521±75	4.9
Mn	114±4	3.5	112±8	7.1	37.7±2.6	6.9	43±2.9	6.7
Co	4.3±0.25	5.8	3.03±0.14	4.6	4.5±0.29	6.4	2.59±0.16	6.2
Cu	8.9±0.56	6.3	7.69±0.66	8.6	10.1±0.38	3.8	9.30±0.76	8.2
Zn	42±2.9	6.9	52±3.6	6.9	35.3±2.1	5.9	8.7±0.75	8.6
Ni	14.2±1.09	7.7	10.2±0.6	5.9	11.7±1.1	9.4	9.83±0.59	6
Cd	1.3±0.08	6.1	1.08±0.09	8.3	0.93±0.09	9.7	0.87±0.08	9.2

DF freshly prepared thyme samples near the town of Debresina, *WF* freshly prepared thyme sample from Wondogenet, *DM* thyme samples bought from town of Debresina, *AAM* thyme samples bought from Addis Ababa city

samples from Debresina. The order of metal concentration follows Fe > Ca > Mg > Mn > Zn > Ni > Cu > Co > Cd.

Ca (2,066±11 μg/g) was observed to have the highest concentration of all the metals studied in the powdered samples from Addis Ababa market followed by Mg (1,735±10 μg/g) and Fe (1,521±27 μg/g). As in the previous cases Cd (0.87±0.08 μg/g) was the one with the least concentration. The powdered samples from Addis Ababa market showed a larger amount of Cu (9.30±0.76 μg/g) which was less than Zn and Ni in the case of fresh and powdered samples from Debresina. The level of metals in decreasing order given by Ca > Mg > Fe > Mn > Cu > Ni > Zn > Co > Cd.

High amount of Mg (1,524±7 μg/g) was obtained from the freshly prepared *T. vulgaris* samples from Wondogenet. The order of the level of metals is Mg > Ca > Fe > Mn > Zn > Ni > Cu > Co > Cd.

Comparison of the Concentration of Micronutrients in Different Thyme Samples

All the results from the four samples showed a similar order of magnitude in the level of the concentration of metals except a slight change in powdered samples from Addis Ababa market where the concentration of Zn was less than Cu and Ni. The order of the level of the microelements in the other three samples is Fe > Mn > Zn > Ni > Cu > Co > Cd. The results showed a relatively high level of the essential elements Fe, Mn, and Zn where Fe exhibited the largest of the three.

The relatively high concentration of Fe reflects the normal composition expected of plant derived products, which most of plant foods and plant derived foods contain Fe in the form of metalloproteins, plant ferritins, Fe present in the sap, and Fe complexed to structural components or storage compounds predominantly as phytates. The incredibly higher concentration of Fe in thyme might be rationalized due to the presence of −OH functional group in the volatile oil constituents which can be readily complexed and hyper accumulated in the plant.

Comparison of the Level of Metals in *T. schimperi* Between Freshly Prepared and Market Available Samples

The concentration of most metals was found higher in the freshly prepared samples than those bought from market except little variation with the micronutrients. The level of metals in the fresh samples from Debresina was significantly (slightly in some cases) higher than the powdered samples from Debresina market and in powdered samples from Addis Ababa market. The macro and micronutrients contained within foods all show varying degrees of stability when foods are stored or processed. In the process of preparation of thyme leaves for usage, there may be a loss of some nutrients and enhancements of certain nutrients through contamination. It is difficult to point out clearly the exact reason for reduction of these nutrients in the processed thyme. The accidental (avoidable or unavoidable) losses of the nutrient during storage time and processing hopefully contribute a reasonable effect. Unlike the major nutrients there is a slight increment of some metals in market available thyme. As tried to point out in the above section, as there is nutrient loss during storage and processing, there may be contamination of the nutrients from outside source. The incredible increment of Fe concentration may be attributed to the fact that every processing utilities are made of iron and wood. Moreover there may be a contamination from dust particles during storage and when transported for sale. On the other hand it is difficult to trace the reasons for differences in the concentration of the metals in the market available with the freshly prepared samples because there is no controlled cultivation in which the leaves are brought to the market place from a specific area.

Comparison of the Level of Metal of Ethiopian Thyme with Other Reported Values

Earlier studies on different thymus species in different countries, in Ethiopia in particular, focused on the essential oil composition and biological aspects. Studies on the level of major, minor and trace metal composition of the plant was limited except in some researches.

Saeed Khan et al. [20] have determined the total metal content using FAAS from the leaves of dried *T. vulgaris* samples collected from the growing fields of Zhad near Sharjah (UAE). They observed that thyme sample contain: 463, 375, 1322, 96, 0.09, 25, 0.18, μg/g of Ca, Mg, Fe, Mn, Cu, Ni, and Cd, respectively. The results revealed that there is a good comparability among the metals Fe, Ni, and Cd and with the present study even though the concentrations in this study was expressed per dry weight of the powdered sample (Table 4).

Nnorom et al. [16] have also determined the metal content of dried thyme sample collected from a market in southeastern Nigeria using FAAS. They obtained an average concentration of Fe (255.70±231.01), Zn (37.03±15.66), and Cd (1.13±0.04) in micrograms per gram. The results were comparable with the present study except in the case of Fe which is higher in the present case (Table 4).

Harum Cifttci et al. [24] determined Ni concentration in thyme samples collected in Turkey. The result showed that the concentration of nickel in thyme sample to be 6.93± 0.38 mg/kg dry matter which was in a good comparability with the result obtained in this study (Table 4).

Comparison of the Concentration Level of some Selected Metals in Other Plants and Thyme

Many authors have reported the concentration of metals in different kinds of food items, drinks, and other materials in which human being used for their day-to-day

Table 4 Comparison of the Concentration of Macro- and Microelements in the Leaf of Thyme Sample with the Available Data in Literature

Metal	Reported values of metals (μg/g)			
	United Arab Emirates [26]	Nigeria [27]	Turkey [20]	This study
Ca	463	–	–	1,239–2,776
Mg	375	–	–	1,524–1,786
Fe	1,322	92.4–419.2	–	728–2,517
Mn	96	–	–	37.7–114
Cu	0.09	–	–	7.69–9.3
Zn	–	25.95–48.1	–	8.7–52
Ni	25	–	6.93±0.38	9.83–14.2
Cd	0.18	1.1–1.15	–	0.87–1.3

consumptions. Vegetables, medicinal plants spices and condiments are some of the concern areas in different parts of the world. Nnorom et al. [16], Khan et al. [20], Ozkutulu [25], Aiwonegbe [15], and Hashmi [13] are some of them who reported certain selected mineral nutrients.

Table 5 Comparison of Some of the Observed Metal Concentration in Thyme Leaves with Some Reported Values in Vegetables, Medicinal Plants, Spices, and Condiments

Food types	Metal concentration (μg/g)						Reference
	Fe	Zn	Ni	Mn	Cu	Cd	
Vegetables (leaf)							
Spinach	13.97±0.04	5.93±0.03	0.19±0.05			0.12±0.04	15
Cabbage	2.5±0.05	<0.01±0.00	0.37±005			ND	
Carrot	4.04±0.03	4.03±0.02	0.25±0.01			<0.01±0.00	
Mustard	16.3±0.03	5.4±0.03		2.6±0.01	3.3±0.01		13
Cabbage	17.7±0.03	3.8±0.02		1.7±0.01	1.1±0.00		
Spinach	32.3±0.05	6.1±0.03		1.8±0.01	2.9±0.01		
Medicinal plants							
Onion	256		12.8	76	0.1	0.06	20
Mint	568		7	72	0.08	0.34	
Coriander	348		3.44	77	0.08	0.28	
Spices and condiments							
Bouillon cubes	3.65–8.95	1.6–4.4				3.6–3.65	16
Chicken seasoning	11.05–32.7	3–3.7				3.9–5.05	
Curry powder	32.35–320.85	13.65–29.90				ND–1.8	
Beef seasoning	32.70–73.20	3.70–21.25				0.85–4.8	
Mixed spices	ND–50.60	3.40–22.55				0.80–4.9	
Black paper	374±7	11±0.6		191±5	11±0.05	0.206±0.0067	25
Ginger	34±1.1	5±0.9		73±3.4	3±0.8	0.072±0.0041	
Clove	52±4.6	6±0.5		355±0.7	4±1.0	0.013±0.0017	
Thyme							
	728–2517	8.7–52	9.83–14.2	37.7–114	7.69–9.3	0.87–1.3	This study

ND not detected

The concentration of some micronutrients studied by different researchers from different countries showed a varied concentration as can be seen from Table 5. The concentrations of Fe, Mn, Cu, Zn, Ni, Cd ranges from 2.5–32.3, 1.7–2.6, 1.1–3.3, <0.01–5.93, 0.19–0.37, and ND–0.12 in micrograms per gram, respectively, in different vegetables. The results obtained from thyme in this study are larger than those reported values for different kind of vegetables. Some of the spices and condiments studied and cited in this paper contain lesser amount of Cd (black pepper, ginger, and clove) and some others contain larger amount except curry powder (ND–1.8 μg/g) which is in comparable range. The Mn content of all the medicinal plants, spices and condiments are in a comparable range with thyme except a slight increase in the case of clove. On the other hand, Fe Cu, Zn, and Ni are found in a larger amount in thyme than the other kinds of food materials reported and cited as indicated in Table 5. The major discrepancy between the results reported in this study and those given elsewhere are those for Fe. The values are too high to be just contamination. The only real test for such values would be by analyzing the soil for high Fe concentrations to correlate fresh leaf and soil concentrations; this would possibly explain the high values found.

Generally, the concentration of metals as compared with the other kinds of food items studied earlier was quite larger in thyme species reported in this paper and by different authors [16, 20]. This may be attributed to the presence of polyphenols in the leaves of thyme. The polyphenols can be readily complexed with the metals so that they are accumulated in higher amount than in the other kind of food condiments, spices, medicinal plants, and vegetables.

Statistical Analysis

A one-way ANOVA revealed that for Cd, there was no significant difference ($p \geq 0.05$) at 95% confidence level between the mean concentrations of the four thyme samples (fresh

Table 6 One-way ANOVA Analysis Result at 95% Confidence Interval for a Pair Wise Investigation Between Thyme Varieties

Metals	Sample type with no significant difference ($p \geq 0.05$)	Sample type with significant difference ($p \geq 0.05$)
Ca	DM and AAM	Other pairs
Mg	DM and AAM	Other pairs
Fe	-	All
Mn	DM and AAM, DF and WF	DF and DM, AAM
		WF and DM, AAM
Co	WF and AAM, DF and DM	DF and WF, AAM
		WF and DM
		DM and AAM
Cu	DF and WF, DM, AAM	WF and DM, AAM
	DM and AAM	
Zn	DF and DM	Other pairs
Ni	WF and AAM, WF and DM	DF and WF, DM, AAM
		DM and AAM
Cd	All	–

DF freshly prepared thyme samples near the town of Debresina, *DM* thyme samples bought from town of Debresina, *AAM* thyme samples bought from Addis Ababa city, *WF* freshly prepared thyme sample from Wondogenet samples from Debresina, fresh samples from Wondogenet, powdered marketed samples from Debresina, and powdered marketed samples from Addis Ababa). The mean concentration of Ca, Mg, Mn, and Cu did not differ significantly ($p \geq 0.05$) for powdered marketed sample from Debresina compared with powdered marketed sample from Addis Ababa. The mean concentration of the four samples showed a significant difference ($p \leq 0.05$) for Fe at 95% confidence level. The samples taken from Debresina and Wondogenet (both freshly prepared) showed a significant difference ($p \leq 0.05$) for Ca, Mg, Fe, Co, Zn, and Ni at 95% confidence interval. The results of one-way ANOVA are summarized in Table 6.

The one way ANOVA showed a difference in concentration between different samples for some metals. This difference could be attributed to the soil type in which the plant grow, the geographical location, difference in processing of thyme and difference that can be resulted from way of transportation to the market places.

Conclusions

In this study the metal content of some freshly prepared and commercially available Ethiopian thyme has been investigated.

The level of metals obtained showed a comparable result with other reported values in some cases. The concentration of Fe investigated in this study was higher than the values reported by different authors cited in this paper. The concentration of the non-essential metal Cd, potentially toxic if it is above the recommended level, ranges from 0.87 ± 0.08 to 1.3 ± 0.08 µg/g. This concentration is above the WHO acceptable level (maximum 0.3 mg/kg) [5]. The Cd pollution may result from household materials like cosmetic containers, fossil fuels, car batteries, etc. Therefore, to use thyme for a day-to-day consumption, it has to be taken care in cultivation, processing, transporting and selling of thyme leaves to the consumer. Statistical analysis shows that there is a variation in the level of some metals between the different samples studied.

Acknowledgments The authors express their gratitude to the Department of Chemistry, Addis Ababa University, Ethiopia, for providing the laboratory facilities. Awraris Derbie is thankful to Department of Chemistry, Wolaita Sodo University, Ethiopia, for sponsoring his study.

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