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Profile of Major, Minor and Toxic Metals in Soil and Khat (*Catha edulis* Forsk) Cultivars in Ethiopia

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ABSTRACT

Thirteen khat (*Catha edulis* Forsk) varieties grown in different regional farms of Ethiopia and the respective soils were analyzed for their contents of major, minor and toxic metals (Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb) by flame atomic absorption spectrometry. Both the edible portion of khat leaves and the soils of the study farms showed similar accumulation patterns in their contents of the studied macro and micronutrients. Among the macronutrient metals, Ca was the most abundant element in the khat leaves (1.03-2.03 mg g^{-1}) followed by Mg (0.441-0.802 mg g^{-1}) on fresh weight basis. Fe was the predominant micronutrient heavy metal in the edible portion of khat leaves ranging between 37.2 and 90.3 mg kg^{-1} on fresh weight basis. Level of Zn (5.10-10.6 mg kg^{-1} on fresh weight basis) in the leaf tissue was found to be the second most abundant micronutrient next to Fe whereas concentrations of Mn, Ni, Cu and Co were relatively lower in khat samples. The toxic heavy metals Pb and Cd in the leaf tissues were present at levels too low to be detected by the analytical technique used in this study. The soils were found to be between weakly acidic to weakly basic (pH 5.28-7.34). Fe was the most abundant metal followed by Mg, Ca, Mn, Zn, Co, Ni and Cu, in the soils. Unlike the khat leaves, the soils were found to contain traces of the toxic metal, Cd (0.73-1.23 mg kg^{-1}). The levels of most of the metals determined in this study compared well with previous reports.

Key words: Ethiopia, khat, *Catha edulis* forsk, mineral, soil, macro-nutrients, micro-nutrients, toxic minerals

INTRODUCTION

The human body requires a number of minerals in order to maintain good health. A number of minerals essential to human nutrition are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment and can also accumulate metals such as Cd, Co and Ag which are of no known direct benefit to the plant (Tan, 1996; Grusak and Penna, 1999). It has been reported that traces of Cd and Pb can be detected in all plants and foodstuffs (Kabata-Pendias and Pendias, 2001). Thus plants are intermediate reservoirs through which trace elements from soil and partly from water and air, move to man and animal.

Micro-nutrients such as Cobalt (Co), Copper (Cu) and Manganese (Mn) are elements necessary for maintaining the life processes in plants and/or animals. The required amounts of trace elements are much lower than the required amounts of macronutrients such as Ca, Mg, K, N and P. For most essential trace elements a high intake causes toxicity. Heavy metals have come to the forefront dangerous substances and are considered as serious chemical health hazards for human and animals (NJF, 2005; Kabata-Pendias and Pendias, 2001; Ajasa *et al.*, 2004).

Absorption and accumulation of heavy metals in plants are influenced by many factors, including: Concentration of heavy metals in soil, composition and intensity of atmospheric deposition, including precipitations, phase of plant vegetation (Voutsa *et al.*, 1996; NJF, 2005; Kabata-Pendias, 2004; Anke, 2004; Begerow *et al.*, 2004; Nouri *et al.*, 2009). To all of these, can be added other sources generated by agricultural technologies such as: irrigation with wastewater, the administration of organic and mineral fertilizers with the load of heavy metals, or application of pesticides, which contain in their structure such chemical elements (Prabu, 2009; Hart *et al.*, 2005; Nwachukwu, 2008; Ramadan and Al-Ashkar, 2007).

The nature of soil is considered one of the most important factors that determine heavy metal content of food plants, probably because this is the binding and retention site for the toxicants (Kabata-Pendias, 2004; Nouri *et al.*, 2009; Anke, 2004; Itanna, 2002).

Khat *(Catha edulis* Forsk) is an evergreen shrub of the *Celastraceae* family. It is widely cultivated in Yemen and East Africa, where its fresh leaves are habitually chewed for their amphetamine-like effects. This many-centuries-old habit is practiced by millions of people and has been introduced to the western countries by immigrants (Elmi *et al.*, 1987; Abdulsalam *et al.*, 2002). Its cultivation extending from Southern Africa to the Arabian Peninsula more specifically in Yemen, Ethiopia, Kenya, Madagascar, Somalia, Tanzania and others as well (Al-Motarreb *et al.*, 2002; Abdulsalam *et al.*, 2002; Elmi *et al.*, 1987; Lemessa, 2001).

The most favored part of the plant is leaves, particularly the young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used. Khat is chewed for its stimulating property. This is due to the presence of the phenylalkylamines in the plant (Al-Motarreb *et al.*, 2002). In Ethiopia khat is grown in most parts of the country. There is an ever-growing demand both for domestic consumption and for the export market. Ethiopia is exporting khat to the neighboring and the Middle East countries and in recent years the market for khat has grown to Europe and America (Karlsson, 2006; Lemessa, 2001).

Now a day, chewing khat is a common practice among many individuals of all age levels of the country and its use is socially sanctioned and even prestigious (Belew *et al.*, 2000). Depending upon the type of khat, availability on the market and nature of the person, up to 500 g of fresh edible portion of the leaf can be chewed per day per individual. The amount of khat chewed may increase in certain occasions.

In Ethiopia, the plant is marketed under different names: Awadai, Kuto, Gelemso, Guragie, Wondo, Sebeta, Bahir Dar, Liyu, Chengie, Berdaye, Anferara, Colombia, Mokonisa, Debo, Gerbicho, etc. Of these, some of them are commonly available in the capital city, Addis Ababa and exported to the neighboring countries while the remaining are chewed by the local people around. Most names of khat are derived from the name of the place where the plant is growing. For example, Anferara khat is cultivated in Anferara.

Depending upon the geographical location, the variety of khat is enormous. Even within the same locality there are different varieties of khat. They differ in color, size and height of the leaves and size and height of the plant as a whole.

Khat requires well-drained field with pH range of 6.0 to 8.2. The optimal altitude and annual rainfall for its growth ranges from 1,500 to 2,100 m and 1,000 to 1,500 mm, respectively (Al-Motarreb *et al.*, 2002; Lemessa, 2001).

Now a day khat farmers are practicing use of paste control chemicals and fertilizers to protect the plant from pastes and to increase the yield of their product. In addition to this, they sometimes use traditional paste control mechanisms (Lemessa, 2001).

Because of expansion of khat market in all over the country and some parts of the world, farmers are trying to apply irrigation to flourish their yield and increase rate of production per year.

There is an extensive literature about khat, providing information about its history, chemistry and pharmacology and exploring the social, economic, medical, psychological and oral aspects of its use (Kalix, 1990; Griffiths *et al.*, 1997; Toennes *et al.*, 2003; Al-Motarreb *et al.*, 2002; Lemessa, 2001; Al-Hebshi and Skaug, 2005). Despite this extensive literature, studies that have investigated its mineral nutrients (both essential and non-essential) are much less than one may expect. Furthermore, the correlation between minerals in the soil and in the different khat cultivars is still not studied.

Thus, khat is becoming more popular all over the country and other parts of the world, having detail documents focusing on essential and non-essential minerals is very important to evaluate the total mineral intake of the individual who is using this plant regularly. Furthermore, analyzing the soil where khat is largely grown is very important to correlate accumulation of a particular metal in the plant and in the soil. This intern has an advantage to control the soil parameters and locate source of pollution based on the available data so as to take action.

One paper on Yemeni khat has been reported concerning trace metals concentration on khat (Matloob, 2003). Recently, we have also reported essential and non-essential mineral nutrients in some selected Ethiopian khat cultivars (Atlabachew *et al.*, 2010). As reported, elements such as Ca and Mg, are present at mg g^{-1} level, whereas elements such as Cr, Fe, Co, Cu, Zn, Mn, Pb and Cd are present at the level of a few mg kg^{-1}.

However, there is still paucity of information on the extent of essential and non-essential metals in khat and soil where the plant is cultivated as well as their toxicological implication. This study was therefore initiated to investigate selected mineral contents in the remaining khat varieties not analyzed so far with special emphasis on their toxicological implications and in the soil where these varieties are grown, i.e., to correlate the selected metals in khat varieties and in the soil where the plant is cultivated.

The study is expected to deliver preliminary data on the levels of metals in khat plants grown in Ethiopia and provide useful information for future studies which will be conducted on agronomy and physiology of the khat plant, fertilizer applications and nutritional, medical and toxicological effects in relation to the khat leaves grown in Ethiopia.

Therefore, in this study we have analyzed ten elements (Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb) content in edible portion of khat cultivars growing in different parts of Ethiopia and in soil where these varieties are cultivated. Thus, Yirba, Bole, Debo, Sebeta, Gerbicho, Gebeli, Anferara, Sike, Mokonisa, Chengie, Damile, Belechie and Basha type of khat grown in different parts of the country and the corresponding soils have been analyzed.

MATERIALS AND METHODS

Study site description: Khat leaf and soil samples were collected from different regions of the country. Namely: Oromiya region, Southern Nation Nationality Peoples' Region (SNNPR) and Amhara region. These regions are best known in khat cultivation for local consumption and export to the capital Addis Ababa, Ethiopia. Some of the khat varieties are also exported to the neighboring countries. Specific area of sampling site with respective trade name of khat variety analyzed is given in Table 1.

Table 1: Sampling sites with respective khat varieties

Sampling area	Region/province	Trade name of khat variety
Yirba	SNNPR	Yirba
Sike	SNNPR	Sike
Chengie	SNNPR	Chengie
Bole	SNNPR	Bole
Debo	Oromiya region/SNNPR	Debo
Damile	Oromiya region/SNNPR	Damile
Mokonisa	Oromiya region/SNNPR	Mokonisa
Sebeta	Oromiya region	Sebeta
Aleta-Wondo	SNNPR	Gerbicho
Bonga	SNNPR	Gebeli
Anferara	Oromiya region	Anferara/Dole
Wond Genet (Belechie)	SNNPR	Belechie
Wondo Genet (Basha)	Oromia region	Basha

SNNPR = Southern Nation Nationality People Region

Sample collection and preparation: For each variety of sample three to five nearby farming areas were selected. A total of 13 (about 1 kg each) bulk khat leaf samples (a bud near to the young shoot and five edible portions of leaves) were collected randomly from the selected farming areas. All the samples of khat and soils were collected between the months of January 2010 to March 2010. Then it was brought to Addis Ababa University, Science Faculty, Department of Chemistry for further treatments and analysis. The khat leaf samples were air-dried followed by in the oven at 45°C (until constant weight) and ground to fineness to pass through a 0.5 mm sieve and stored in polyethylene bags prior to analysis. At the spots where the khat leaf samples were plucked, a total of 13 (about 4 kg each) corresponding soil samples (0-20 cm depth) were collected, air-dried and ground to pass through a 2 mm sieve and stored in clean polyethylene bags prior to analysis.

Instrumentation: A stainless steel knife was used for marking and digging of the area for the collection of the soil samples. Both the plant and soil samples were ground using a ceramic mortar and pestle. All of the khat leaves and soil samples were weighed on a digital analytical balance. Round bottom flasks (100 mL) fitted with reflux condenser and Kjeldahl digestion block were used for the total digestion of all the samples. The concentrations of Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb in both the khat leaves and soil samples were determined by flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP, East Norwalk, USA) using an air-acetylene flame. A potentiometric digital pH meter (WTW Inolab pH/ION Level 2, Germany) was used to determine the pH of soil samples after stirring by a magnetic stirrer.

Reagents and chemicals: Concentrated HNO_3 (69-72%, Spectrosol, BDH, UK) and concentrated $HClO_4$ (70%, Fine Chem Industries Mumbai, India) were used for the digestion of the khat leaf samples. Concentrated HCl (36-38%, Hopkin and Williams, UK) and HNO_3 (69-72%, Spectrosol, BDH, UK) were used for the digestion of soil samples. Lanthanum nitrate hydrate (98%, Aldrich, USA) was used to avoid refractory interference (for realizing calcium and magnesium from their phosphates). Distilled de-ionized water was used through out the analysis.

CHEMICAL ANALYSIS OF SAMPLES

Analysis of khat leaves samples: Exactly 0.5 g of the dried and ground khat leaves sample was digested using 4 mL of 1:1 mixture of concentrated HNO_3 (69-72%) and concentrated $HClO_4$ (70%)

December 31, 2010under reflux. The digests were used to determine concentrations of Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb by atomic absorption spectroscopy (Atlabachew *et al.*, 2010).

Analysis of soil samples: Soil pH was measured potentiometrically by a pH meter in a suspension of a 1:1 soil:water mixture. About 10 g of air-dried soil (<2 mm) was weighed and transferred in to 100 mL beaker and 10 mL of water was added. The sample was stirred by a magnetic stirrer and the pH was measured after allowing the suspension to stand for 1 h at room temperature (Tan, 1996). Each determination was made in triplicates. Results of the analysis are given in Table 2.

1.0 g of soil was accurately weighed and transferred in to 100 mL round bottom flask and moistened with 1 mL of distilled de-ionized water. 10 mL of 1:3 mixture of conc. HNO_3 and HCl was added to the flask and kept for hours until it got stabilized. Then, digested on a Kjeldahl digestion block under reflux condenser for 2 h at 140°C. The digest was left to stand for 30 min to cool down to room temperature, then about 50 mL distilled deionized water was added to the flask, filtered through Whatman No. 41 filter paper in to a 100 mL volumetric flask and made up to the mark with rinsing the digestion flask. The solutions were used for the analysis of the total soil metal concentrations for Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb by flame atomic absorption spectrophotometer (AAS) (Kabata-Pendias and Pendias, 2001).

Recovery tests: To check the efficiency of the procedure, appropriate amount of stock solution of Ca, Mn, Zn, Co, Cu and Ni were spiked at once in to 0.5 g of khat sample and appropriate amount of the remaining metals in to another digestion flask containing 0.5 g of the same variety of khat sample. A recovery test was also performed for the soil samples using the same procedure. Each recovery test for both samples was performed in triplicate. The results of the measurement are presented in Tables 3 and 4. The results of percentage recoveries for the studied metal nutrients in khat leaves were all between 90 and 105% where as the values of percentage recoveries for the studied macro- and micronutrient and the toxic metals in the soil samples were within the range of 90-106% which are within the acceptable range.

Method detection limit: To determine method detection limit, replicate analyses for six blank samples were performed and the pooled standard deviation of the six reagent blanks was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. The method detection limits of each metal are given in Table 5. The method detection limits are generally comparable with that of the instrument for both khat leaves and soil samples. Furthermore the method detection limits for all the metals are low enough (<5 $\mu g\ g^{-1}$) to determine the metals at trace levels in both the khat leaves and soil samples. The only exception is Pb for which the method detection limits slightly higher (<10 $\mu g\ g^{-1}$).

Conversion factor determination: To report the result obtained (on dry weight basis) in terms of fresh weight basis, a conversion factor was calculated for all the khat varieties after drying a known fresh edible part of the plant. The results are present in Table 2. For all khat varieties, a conversion factor lies between 3.67-3.90, i.e., 3.67-3.90 g of fresh (wet) sample yielded 1 g of dry weight depending on the khat variety. The results given in Table 2 also indicate that the moisture contents of the khat varieties varied in the range 72.8-74.4%, i.e., roughly constant. In other words the chewable khat leaves are wet to nearly the same extent.

Table 2: Average value of soil pH and moisture content of khat varieties

Soil sampling sites	Soil pH (1:1 H_2O)	Khat varieties	% Moisture content of khat samples	Conversion factor
Anferara	5.28	Anferara	73.1	3.72
Aleta-Wondo	7.34	Gerbicho	73.9	3.83
Sebeta	6.60	Sebeta	73.1	3.72
Yirba	6.56	Yirba	73.2	3.87
Chengie	7.18	Chengie	73.1	3.72
Wondo-Basha	6.74	Basha	74.4	3.90
Mokonisa	6.12	Mokonisa	73.0	3.71
Debo	6.24	Debo	73.6	3.79
Damile	5.99	Damile	73.5	3.77
Wondo-Belechie	6.57	Belechie	73.4	3.76
Bole	6.59	Bole	74.3	3.89
Bonga	6.50	Bonga	73.3	3.75
Sike	6.51	Sike	72.8	3.67

Table 3: Recovery test results for khat leaf samples

Metal	Conc. in sample (μg g^{-1})[a]	Amount added (μg g^{-1})	Conc. in spiked sample (μg g^{-1})[a]	%Recovery[c]
Ca	6120.00	2000	7999.0±33	94±2
Mg	2710.00	1000	3634.0±29	92±3
Fe	121.00	40	158.0±2	93±5
Zn	29.10	15	42.7±1.3	91±8
Mn	14.30	8	21.5±0.8	90±9
Cu	13.80	8	22.2±0.6	105±8
Ni	9.30	5	13.9±0.2	91±3
Co	2.10	2	3.93±0.08	91±4

[a]Average value of 9 measurements (μg g^{-1}). [b]Values are mean±SD of triplicate readings of triplicate analyses. [c]Values are mean±SD of triplicate readings of triplicate.

Table 4: Recovery test results for soil samples

Metal	Conc. in sample (μg g^{-1})[a]	Amount added (μg g^{-1})	Conc. in spiked sample (μg g^{-1})[a]	% Recovery[c]
Ca	1040.00	500	1504.0±9	93±2
Mg	2030.00	1000	2995.0±39	97±4
Fe	20600.00	2300	22796.0±95	96±4
Zn	79.20	40	121.0±3	105±7
Mn	1920.00	600	2548.0±34	105±6
Cu	22.30	10	32.0±0.9	97±9
Ni	34.70	15	50.5±1.3	106±9
Co	12.70	5	17.4±0.3	94±7
Cd	1.20	1	2.33±0.06	91±6

[a]Average value of 9 measurements (μg g^{-1})[a]. [b]Values are mean±SD of triplicate readings of triplicate analyses. [c]Values are mean±SD of triplicate readings of triplicate.

Table 5: Method detection limits for khat leaf and soil sample (n = 6)

Metal	Ca	Mg	Cu	Zn	Mn	Ni	Fe	Co	Cr	Cd	Pb
MDL (μg g^{-1})[a] for khat leaf	3.1	2.6	2.1	0.85	1.1	4.2	4.6	1.0	5.1	0.79	7.5
MDL (μg g^{-1})[a] for soil	3.4	2.3	2.1	0.78	1.1	4.1	4.2	1.2	5.1	0.71	8.8

[a]Values are mean of 3 x standard deviation of seven blank determinations each measured three times. MDL: Method detection limit

RESULTS AND DISCUSSION

Level of metals in soil sample: Concentration of selected macro, micro and toxic elements in the selected khat farms are given in Table 6.

The values of pH of the soils for the selected fields of the thirteen khat growing farms are presented in Table 2. The soil pH of the farms was within the range of 5.28-7.34, which categorizes the soils under weakly acidic to weakly basic. Now a day farmers are practicing use of fertilizers to bloom their production (Lemessa, 2001). Thus one of the reasons for the slight acidity of soils of some of the farms might be due application of NPKS fertilizer.

Reports indicate that increasing rates of nitrogenous fertilizers generally increase soil acidity (Ishibashi *et al*., 2004). Soil pH is one of the most influential parameters controlling the conversion of metals from immobile solid-phase forms to more mobile and/or bioavailable solution-phase forms. As reported by many authors, the solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately pH 5.0 to 7.0) (Kabata-Pendias, 2004; Wang *et al*., 2006). The high pH values of soils could have accounted for a low transfer of metals from soil to plant.

The macro-, micro- and toxic metal contents analysed in the khat farm soils varied significantly from site to site. Among the macro-elements, Mg content of the soils was higher within a range of 1.13-2.20 mg g^{-1} followed by Ca (0.78-1.82 mg g^{-1}). Some Ethiopian khat producing soils of the farms are classed under the category of vertisols, kandic paleustalfs, hyperdystric acrisol, oxisols, ferralsols and plinthic alisols. Some of them are with clayey texture and dark reddish brown colour and others are with reddish colour (Solomon *et al*., 2001; Beyene, 1988; Atengo *et al*., 2006), which is indicative of the presence of excess amount of hematite (Fe_2O_3) (Tan, 1996). Soils with low pH contain high amounts of Fe and Al oxides (Hu *et al*., 2002). Thus, Fe is the predominant metal within the concentration range of 9.88-26.6 mg g^{-1} in these soils whereas Mn content is in the range of 0.70-1.77 mg g^{-1}. The concentration of Zn in the soil samples ranged from 49.9-131.3 mg kg^{-1}. Similarly, Co (12.7-38.1 mg kg^{-1}), Ni (8.27-41.7 mg kg^{-1}) and Cu (9.13-29.8 mg kg^{-1}) have been obtained in the analysed soil samples. On the other hand, cadmium was detected in most of the analyzed soils of farm lands except in Mokonisa, Debo and Damile farms. In the remaining soils of the farms, the level of the toxic heavy metal Cd ranges from 0.73 mg kg^{-1} for Sebeta farms and up to 1.20 mg kg^{-1} for soils of Chengie farms. The level of Pb, the other tested toxic metal, in the soils of all studied farms was found to be below the detection limit of the method used in this study.

Comparing the metal concentration in soil with guidelines for soils showed that all the metal concentration is below the guidelines for soils (Begerow *et al*., 2004; Kabata-Pendias and Pendias, 2001; Itanna, 2002; Environmental Agency, 2009). Looking at the table, most soils taken from the farms follow similar trend in metal accumulation. For the majority of the analyzed soils, the mean concentrations of the ten metals for this study were found to follow decreasing order:

$$Fe>Mg>Ca>Mn>Zn>Co>Ni>Cu>Cd>Pb$$

Comparing with some other parts of the world, major khat producing areas of Ethiopia are accumulated low concentration of heavy metals which implies that the investigated farm soils are more or less free from heavy metal contamination (Awode *et al*., 2008; Khairiah *et al*., 2009).

Statistical analysis (both one-way ANOVA and Pearson correlation matrices) have been conducted to see the correlation of metal accumulation in soils of the different sites and correlation between metals, respectively.

Table 6: Average metal concentrations in the soils of selected khat growing farms

	Metal concentration of soils (dry weight basis)			
Sampling site	Ca (mg g^{-1})	Mg (mg g^{-1})	Mn (mg g^{-1})	Fe (mg g^{-1})
Anferara	1.82±0.15	1.98±0.01	0.70±0.08	17.10±0.27
Aleta-Wondo	1.70±0.05	2.00±0.09	1.68±0.02	22.90±1.24
Sebeta	1.45±0.04	1.41±0.11	1.62±0.03	27.10±0.75
Yirba	1.33±0.10	2.20±0.06	1.57±0.05	26.60±0.31
Chengie	1.04±0.07	2.03±0.08	1.92±0.04	20.60±1.32
Wondo-Basha	1.09±0.04	1.42±0.10	1.44±0.05	14.20±0.11
Mokonisa	0.91±0.01	1.52±0.11	1.77±0.06	16.70±0.10
Debo	0.86±0.04	1.13±0.02	1.03±0.01	10.90±0.08
Damile	0.78±0.05	1.85±0.07	1.25±0.06	12.90±0.38
Wondo-Belechie	0.88±0.03	1.61±0.09	1.40±0.01	12.00±0.26
Bole	0.99±0.02	1.46±0.03	0.96±0.07	12.80±0.32
Bonga	1.00±0.02	1.24±0.02	0.89±0.04	9.85±0.16
Sike	0.90±0.01	1.55±0.06	1.42±0.03	14.10±0.61

	Metal concentration of soils (dry weight basis)					
	Zn	Co	Cu	Ni	Cd	Pb
Sampling site	(µg/g)					
Anferara	68.6±3.85	37.5±1.15	23.5±0.39	25.0±1.75	0.73±0.07	ND[a]
Aleta-Wondo	85.9±2.13	38.1±0.10	24.0±1.16	32.1±2.21	0.96±0.07	ND[a]
Sebeta	114.2±3.06	22.7±0.89	22.7±1.24	28.7±1.66	0.76±0.07	ND[a]
Yirba	131.3±4.31	30.0±1.67	25.9±0.30	41.7±0.93	1.23±0.20	ND[a]
Chengie	79.2±1.27	12.7±2.10	22.3±1.89	34.7±1.47	1.20±0.08	ND[a]
Wondo-Basha	63.9±1.43	34.8±1.21	15.2±1.54	23.8±1.53	0.94±0.08	ND[a]
Mokonisa	67.1±1.23	34.3±0.60	17.3±0.98	30.6±2.86	[a]ND	ND[a]
Debo	56.9±1.97	14.7±1.35	11.1±0.61	8.28±0.45	[a]ND	ND[a]
Damile	52.3±1.40	28.5±0.30	19.1±1.78	23.5±0.22	[a]ND	ND[a]
Wondo-Belechie	49.9±1.1	23.3±0.81	9.13±0.87	19.4±1.17	0.79±0.07	ND[a]
Bole	79.2±3.2	15.2±0.45	20.8±1.45	15.3±0.97	0.86±0.04	ND[a]
Bonga	84.3±4.0	21.3±0.59	16.0±0.87	18.6±0.64	0.92±0.05	ND[a]
Sike	91.1±4.65	31.2±1.38	14.3±0.31	28.5±1.71	0.78±0.06	ND[a]

[a]Below method detection limit

Level of metals in khat cultivars: The mean concentrations of metals found in the edible portion of different khat cultivar samples are presented in Table 7. Wide variations were observed in the accumulation of metals by the different variety of khat samples which represented different contributing (both natural and anthropogenic) factors.

The results of total contents of the studied nutrient and toxic metals in the thirteen clones of khat (*C. edulis* Forsk) variety show the ability of these clones to accumulate high amounts of both macro- and micronutrient elements. The most abundant metal among the macroelements analysed was Ca followed by Mg whereas Fe content of the khat leaves was the predominant among the tested micronutrient heavy metals followed by Zn, Mn, Cu, Ni and Co. On the other hand, the content of the toxic non-essential heavy metals, Cd and Pb in the analysed clones were found to be below the method detection limit.

Table 7: Average metal concentrations (X±SD, n = 9) of analyzed khat cultivars from each selected farms

	Metal concentration of khat varieties (dry weight basis)				
Khat variety	Ca (mg g^{-1})	Mg (mg g^{-1})	Mn (μg g^{-1})	Fe (μg g^{-1})	Zn (μg g^{-1})
Anferara	8.57±0.19	2.80±0.10	16.8±0.82	200±14.4	37.2±2.97
Gerbicho	7.98±0.12	3.07±0.06	18.2±0.72	325±13.5	40.6±1.71
Sebeta	8.45±0.05	2.82±0.04	22.7±0.90	336±27.0	28.6±1.62
Yirba	6.63±0.48	2.04±0.01	19.7±0.50	144±8.17	38.6±2.39
Chengie	6.12±0.08	2.71±0.20	14.3±0.49	121±10.6	29.1±1.99
Basha	5.03±0.16	1.97±0.01	23.9±0.82	213±28.6	26.0±2.0
Mokonisa	3.83±0.10	2.70±0.11	17.3±0.81	220±5.58	22.1±1.32
Debo	5.98±0.33	1.67±0.07	13.4±0.32	203±11.7	19.8±1.29
Damile	6.24±0.32	2.60±0.04	21.7±0.69	236±34.9	40.8±1.20
Belechie	5.12±0.17	2.11±0.10	24.8±1.27	171±9.13	33.2±2.11
Bole	6.34±0.50	2.19±0.08	17.2±0.69	120±6.69	31.1±1.45
Gebeli	5.57±0.31	1.86±0.05	12.3±0.17	195±6.63	25.9±1.42
Sike	6.23±0.10	2.51±0.11	11.4±0.51	209±13.5	18.7±1.78

	Metal concentration of khat varieties (dry weight basis)				
	Co	Cu	Ni	Cd	Pb
Khat variety	μg/g				
Anferara	1.80±0.17	13.90±1.12	11.90±0.48	ND[a]	ND[a]
Gerbicho	2.79±0.38	5.89±0.41	7.70±0.75	ND[a]	ND[a]
Sebeta	ND[a]	16.20±1.62	14.20±0.28	ND[a]	ND[a]
Yirba	1.90±0.15	9.70±0.94	7.10±0.23	ND[a]	ND[a]
Chengie	2.11±0.20	13.80±0.43	9.30±0.34	ND[a]	ND[a]
Basha	1.26±0.07	6.09±0.64	4.16±0.33	ND[a]	ND[a]
Mokonisa	2.45±0.23	4.81±0.48	8.59±0.80	ND[a]	ND[a]
Debo	2.50±0.17	6.01±0.16	6.81±0.67	ND[a]	ND[a]
Damile	2.70±0.25	14.80±0.21	4.71±0.38	ND[a]	ND[a]
Belechie	2.47±0.18	7.02±0.64	4.43±0.43	ND[a]	ND[a]
Bole	1.72±0.13	5.32±0.08	10.80±0.91	ND[a]	ND[a]
Gebeli	3.64±0.19	5.97±0.21	6.31±0.59	ND[a]	ND[a]
Sike	1.20±0.01	8.12±0.31	8.22±0.74	ND[a]	ND[a]

[a]Below method detection limit

It can be deduced from the levels of all the metals in the studied khat varieties of all the sampling sites, that the concentrations of the macro- and the micronutrient metals followed similar trend for most of the samples. In general, ranges of concentrations of the studied macronutrient and micronutrient metals could be arranged according to their levels on the khat plant varieties of all the sampling sites in the following order on dry weight basis: Ca (3850-8750 mg kg^{-1}) >mg (1670-3070 mg kg^{-1})>Fe (121-336 mg kg^{-1})>Zn (18.7-40.6 mg kg^{-1})>Mn (11.4-24.4 mg kg^{-1})>Cu (4.81-13.9 mg kg^{-1})>Ni (4.43-10.2 mg kg^{-1})>Co (ND-3.67 mg kg^{-1}). The same trend has been reported by Atlabachew *et al*. (2010) and Matloob (2003).

The higher levels of Ca and Mg in the studied khat plants is obvious since these nutrients are highly mobile in the plant tissue and are translocated from old leaves to young leaves (Marschner, 2002). Furthermore, broad range of Ca-bearing minerals in soil and water and usually abundant in ground water and surface water which can easily be absorbed by the plant.

The concentration of Fe, Zn and Mn were higher than the entire trace metals in the samples. Since the soil types of khat growing areas of Ethiopia are moderately acidic to slightly basic with the pH ranges from 5.28 to 7.34 (Table 2), the plant is expected to have a better accumulation of micronutrients like iron and zinc (Wang *et al.*, 2006; Kabata-Pendias and Pendias, 2001; Kabata-Pendias, 2004). Iron was the most abundant trace metal in all the khat varieties analysed ranging from 171 mg kg^{-1} in Belechie type khat up to 336 mg kg^{-1} in Sebeta type khat. This might be due to higher concentration of iron in the soil (Table 6). Furthermore, Dudai *et al.* (2006) reported that young shoot of khat contains appreciable amount of polyphenols. Thus it might facilitate Fe absorption through complexation. Higher concentration of manganese is expected because it is mostly accumulated in leaves of plants and also absorption of soluble Mn increases with decreasing soil pH (Ajasa *et al.*, 2004; Kabata-Pendias, 2004).

On the other hand, Cu content ranged between 4.81 mg kg^{-1} in Mokonisa type khat and 16.2 mg kg^{-1} in Sebeta type khat from Sebeta farm lands, whereas the concentration range of Zn was found to be 18.7 mg kg^{-1} in Sike type of khat up to 40.8 mg kg^{-1} in Damile type of khat cultivar from Damile farm lands. Previously we have reported comparable results in Cu and Zn concentration in khat cultivars from other locations (Atlabachew *et al.*, 2010). This result is also in agreement with other report from Yemen (Matloob, 2003).

Our results revealed that khat clones accumulate appreciable amount of Ni and to the lesser extent Co, ranging from 4.43-14.2 mg kg^{-1} and ND-3.64 mg kg^{-1} respectively, on dry weight basis which is more or less lies within the range of the previous report (Atlabachew *et al.*, 2010; Matloob, 2003).

As reported by Dushkenov *et al.* (1995) and Grusak and Penna (1999), Co is required by humans, but not by plants. Fortunately for humans, however, plants can acquire this element through non-specific influx processes using existing transporters localized to their roots. In fact, a wide range of plant's non-essential elements (both benign and detrimental) has been measured in plant tissues, with concentrations sometimes reaching dramatic levels if soil availability is high.

Matloob (2003), suggested the ability of khat plant to accumulate heavy metals like Cu and Zn, which are comparable to the present analysis. Fortunately, the concentrations of toxic heavy metals, Pb and Cd in the studied khat leaves cultivars were too low to be detected by the analytical technique used in this study. However, there is a report on the availability of these metals at lower levels in Yemeni khat leaves. Matloob (2003) has also reported level of Cd within a range of 0.007-0.018 mg kg^{-1}. On the other hand, level of Pb was reported within a range of 0.066-0.7 mg kg^{-1} (Matloob, 2003). In fact the concentration of the toxic heavy metals are expected to be very low in Ethiopian khat since these metals are related to environmental pollution caused by different industrial activities.

Comparing level of metals among the analysed khat varieties, it is impossible to have a common trend for the distribution of all the metals in all the khat varieties, i.e. some khat varieties accumulate relatively higher concentration of some of the metals while lower concentration for remaining metals relative to the other khat varieties analyzed. This might be due various contributing factors like nature of chemical and physical property of the soil, climatic condition of the region and application of fertilizers and pesticides. Furthermore, increase in population and industrialization results pollution to water, air and soil which in turn causes in unexpected concentration of trace metals in the plant. Particularly agricultural activities such as use of fertilizers, pesticides and irrigation with contaminated sewage are the major source of contaminations.

Comparing with other medicinal plant species, khat leaves accumulated comparable concentration for some of the selected metals for certain species, while incomparable concentration levels for some others were noticed (Ansari, *et al.*, 2004). However, khat leaves accumulated higher concentrations of most of the metals compared with some selected leafy vegetables (Aiwonegbe and Ikhuoria, 2007; Oboh *et al.*, 2009).

Pair wise statistical analysis of data was made to verify whether there was a significant difference in metal contents between the khat varieties analyzed. For the present study, the significance of variation within sample and between samples has been studied using one-way ANOVA and calculations were made using SPSS software.

For most of the studied khat cultivars, significant difference ($p<0.05$) at 95% confidence level was observed for the majority of the metals under investigation. While insignificant variation ($p>0.05$) at 95% confidence interval was seen among some khat varieties for some of the metals when pair wise comparison was made. For example no significant difference was observed between Debo, Mokonisa and Damile type khat varieties for most of the analyzed metals. Similarly no significant difference at 95% confidence level was observed for Basha and Belechie types of khat varieties and Sike, Chengie, Yirba and Bole type varieties for some of the mineral nutrients. Absence of significant difference might be due to the fact that the farm lands are found in the same province. For example, Basha and Belechie khat varieties are grown in Wondo-Genet province. Similarly, Debo, Mokonisa and Damile type khat varieties are grown in Sidama province while Chengie, Sike, Yirba and Bole type khat varities are grown in Awassa province. Thus, the farms within the same province might share comparable climatic conditions and soil properties. While existence of significant difference in metal contents among khat cultivars might be due to variations in the aforementioned factors.

Like that of the khat cultivars, more or less the same variations of metals content in the soils analysed have been observed.

Correlation of heavy metals in plant and soil: Correlation test was carried out between soil and plant for each analyzed metals to evaluate metals distribution in the soil and their availability and accumulation in the plant. Thus Pearson product moment correlation coefficient was calculated. Based on the results of r-value, the studied soils were found to correlate positively with the levels found in khat clones. The macronutrients and Cu from the trace elements were found to correlate more strongly than the rest of the nutrients analyzed.

Correlation test (Table 8) was also carried out between analyzed metals in soils and then between tested metals and pH in khat cultivars (Table 9). This test may answer some of the question on how the metals interact among themselves in plant and in soil.

Looking at Table 8, all the tested metals correlated each other positively except Ca and Mn which has negative correlation. Some of the metals have strong correlation while others have weak. Positive correlation in the case of tested metals normally indicates their mutual existence and enrichment in soil, whereas negative correlation indicates their competition to occupy the same sites in soil exchange base or lattice. This means that some of the metals may enriched together, whilst for Ca and Mn, the Ca content may increase if the Mn content decrease or vice versa.

Looking at Table, 9, Ni was negatively correlated with Fe and Mn. The same was true for Co between all the tested metals. Whilst the remaining tested metals correlated positively each other. The pH of soil has a significant negative relationship with Fe, Mn, Zn, Cu and Ni while weak positive relationship with Ca, Mg and Co has been observed. Negative correlation implies that decrease in soil pH go together with increase in the solubility of Zn, Fe, Mn and Cu in the soil and increase in their bioavailability to the plant.

Table 8: Correlation between major, minor and trace metals in the soil

Metals	Ca	Mg	Mn	Fe	Zn	Co	Cu	Ni
Ca	1.000							
Mg	0.434	1.000						
Mn	-0.034	0.322	1.000					
Fe	0.617	0.556	0.550	1.000				
Zn	0.439	0.284	0.294	0.729	1.000			
Co	0.460	0.349	0.074	0.165	0.036	1.000		
Cu	0.539	0.676	0.158	0.753	0.531	0.157	1.000	
Ni	0.379	0.675	0.689	0.734	0.558	0.501	0.580	1.000

Table 9: Correlation between pH, major, minor and trace metals in khat samples

	Ca	Mg	Mn	Fe	Zn	Co	Cu	Ni
Ca	1.000							
Mg	0.390	1.000						
Mn	-0.245	0.043	1.000					
Fe	0.218	0.426	0.429	1.000				
Zn	0.427	0.335	0.418	0.137	1.000			
Co	-0.268	-0.271	-0.214	-0.177	0.197	1.000		
Cu	0.127	0.426	0.443	0.296	0.294	-0.400	1.000	
Ni	0.55	0.50	-0.370	-0.02	0.205	-0.38	0.300	1.000
pH	0.134	0.020	-0.213	-0.063	-0.022	0.031	-0.424	-0.094

Table 10: Concentration range of tested metals in the khat cultivars on fresh weight basis elements (mg 100 g^{-1}) (wet weight basis)

Ca	Mg	Fe	Zn	Mn	Cu	Ni	Co	Cd	Pb
103-203	44.1-80.2	3.72-9.03	0.51-1.06	0.03-0.60	0.12-0.44	0.11-0.32	0.03-0.1	ND[a]	ND[a]

[a]Not detected

Since fresh khat is chewed for its stimulating property, the result in terms of dry weight basis is converted to fresh (wet) weight basis using a conversion factor of each cultivar given in Table 2. Furthermore, in most cases, the average quantity of khat chewed by Ethiopians ranges from 100 to 500 g daily. Table 10 presents the range of metal concentrations of the tested metals on fresh weight basis in the analyzed khat cultivars. Looking at the table, chewing khat contributes 103-203 mg 100 g^{-1} and 44.1-80.2 mg 100 g^{-1} fresh weight basis per day of Ca and Mg, respectively. Similarly chewing khat contributes 0.51-1.06 mg 100 g^{-1} and 0.12-0.44 mg 100 g^{-1} on fresh weight basis per day of Zn and Cu, respectively. As per the 1989 Recommended Daily Allowance (RDA) levels for Cu and Zn are respectively of 2-3 mg day^{-1} and 12-15 mg day^{-1} (National Research Council, 1989). These values do not pose a health risk if up to 100 g of the stated fresh khat are taken. Rather Zn from other source is required to satisfy the required amount. However, further increase in khat consumption may satisfy the maximum recommended RDA value of Cu and additional intake of Cu from other source might pose health impact.

In Addition, chewing 100 g of fresh khat contribute the following concentration range of Fe, Mn, Ni and Co: 3.7-9.03 mg 100 g^{-1}, 0.3-0.60 mg 100 g^{-1}, 0.11-0.32 mg 100 g^{-1} and 0.03-0.10 mg 100 g^{-1} on fresh weight basis per day, respectively. Still these results are below the RDA recommended limit and require additional sources of these metals to satisfy the individual need (National Research Council, 1989). Furthermore, chewing the aforementioned khat varieties contribute insignificant amount of the toxic heavy metals Cd and Pb.

Generally, based on the current status, chewing Ethiopian khat in addition to its stimulating property, it contribute appreciable amount of macro and trace metals for the daily requirements of the individuals and are free from toxic heavy metals. Particularly, khat could be good source of Fe and Zn for individuals who are chewing this plant regularly.

Result from the analysis of khat varieties for their selected nutrients supported the previous reports but it is the first time to investigate the correlation of metals accumulation in the khat varieties and soil samples. Thus the present study full filled the gap that existed between khat clones and their accumulation patterns of the selected metals from the soil. In addition, it is the first time to analyze soil samples from major khat cultivation farms. Hence, results of this investigation are a newly added data concerning major, minor and toxic metals in khat clones and soil samples of major Ethiopian khat grown farms. Thus, farmers will be benefited in controlling their soil property. Toxicological implication data is in agreement with the previous report and results of the present investigation are in support to the previous report.

CONCLUSION

This study determined levels of macro- and micronutrient and the toxic heavy metals (Cd and Pb) in edible portion of khat leaves of thirteen khat cultivars grown in different areas of Ethiopia and their respective soil samples using FAAS.

The results showed the ability of these plants to accumulate relatively higher amounts of Ca, Mg and Fe among the determined macro- and micronutrient metals, respectively. Heavy metals Cu and Co was found to be comparatively at lower levels in most of the analyzed khat cultivars. With respect to the khat varieties, the khat plants showed no significant difference in their pattern of accumulation of the studied metals. The studied metals content of all cultivars followed, generally, similar trend across the varieties that could be arranged in descending order:

$$Ca>Mg>Fe>Zn>Mn>Ni>Cu>Co$$

The levels of toxic heavy metals Cd and Pb in all clones were too low to be detected by the method used in this study. The results of the analysis showed that all the khat varieties analyzed contains appropriate concentration of essential major and minor metals and they could be source of mineral nutrients for those who are chewing khat regularly.

Correlation study revealed that accumulation of most of the metals in khat plants correlate negatively with the pH of the soil whereas there were positive correlation among studied metals in the khat cultivars. The ANOVA results suggested that there were significant variation in the level of some elements between the khat varieties which could be attributed to different factors such as age of the harvested khat, geographical and climatical variation, difference in physicochemical nature of the soil and different agricultural practices among khat cultivars. Whilst insignificant variations were observed among some khat cultivars for some of the metals investigated.

The soils of the study farms were found to contain high levels of Fe followed by Mg, Ca, Mn, Zn, Co, Ni and Cu. In all of the soils, level of Pb was below the method detection limit. However, unlike the khat leaves, the soils of all the farms were found to contain Cd at relatively lower levels except the farms of Mokonisa, Damile and Debo. In general, the levels of most of the metals in the studied soils were found to correlate positively with the levels found in the khat leaves.

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