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Assessment of metal contents in commercially available Ethiopian red pepper

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Abstract

The concentrations of major (Na, K, Ca) and trace (Fe, Co, Cu, Mn, Zn, Ni, Cr, Cd and Pb) metals were determined in Ethiopian red pepper samples collected from Alaba, Mareko and Addis Ababa open market (Merkato) using flame atomic absorption spectrometry after wet digestion. The metal concentrations in the samples were found to be K 2378–2486, Ca 161–222, Na 75.0–93.0, Fe 99.5–157, Cr 27.5–78.6, Zn 20.8–58.4, Mn 9.72–18.9, Ni 2.71–6.68, Cu 2.12–3.71, Co 1.02–2.24 and Cd 0.18–0.23 in $\mu g\ g^{-1}$, respectively. Pb was not detected in any of the pepper samples. The levels of metals K, Na and Ca in the red pepper samples from Alaba were found to be higher than those from Mareko and Addis Ababa open market. The levels of Co, Cu, Ni, Zn and Cd were found to be higher in samples from Mareko than in samples from Alaba and Addis Ababa open market pepper. The levels of Cr and Mn were higher in Addis Ababa open market pepper. The concentrations of metals in Ethiopian pepper are comparable to values reported in literature from different parts of the world. The trace metals levels in all the three samples were found to be lower than the World Health Organization (WHO) allowed limit. However, levels of Cd and Cr were above the WHO limit. The concentrations of metals in Ethiopian red pepper are comparable to values reported in the other spices in the literature. Furthermore, to correlate metal concentrations among each other metal levels in the red pepper using Pearson's correlation coefficient (r) was determined and except for few metals, most relationships showed either strong positive or negative linear relationships. From this relationship it can be understood that the presence of one metal in the plant influence the presence of the other.

Introduction

The genus *Capsicum* is a member of the Solanaceae family (Shaha *et al.*, 2013; Kim *et al.*, 2014). *Capsicum* contains approximately 2027 species, five of which are domesticated and cultivated in different parts of the world are *C. annuum, C. baccatum, C. chinense, C. frutescens,* and *C. pubescens.* Among the five species of cultivated *Capsicum, C. annuum* is the most well-known variety and it is the most commonly cultivated crops worldwide followed by *C. frutescens* (Sittiwong *et al.*, 2005; Nadeem *et al.*, 2011).

Red pepper is made from the ground fruit of a plant in the *Capsicum* species. The fruits, commonly known as "chilies" or "chili peppers," are fiery red or orange pods which rarely grow to more than 4 inches in length. The ground product ranges from orange red, to deep, dark red. The fruit is generally handpicked as it ripens, and then allowed to dry in the sun, although artificial drying is often employed in Europe and the United States. The fruit may be ground intact or after the removal of seeds, placenta parts, and stalks, increasing the fruit color and lowering the pungency (Wien, 1994). As a food colorant, red pepper has traditionally been in the form of paprika (ground powder), although today oleoresins are widely used. The red pepper is a vegetable known for its high antioxidant capacity (Arnnok *et al.*, 2012; Shaha *et al.*, 2013).

Chili pepper can be harvested at different fruit stages, depending on the final use. Fresh chili pods often are harvested at a physiologically immature stage. The dehydrated and mash industries use physiologically mature fruits, generally showing red color. The production of pepper for spice, vegetable, and other uses are increasing every year. It is estimated that it is annually cultivated on more than 1.5 million hectares, in several countries (Kouassi *et al.*, 2012).

Ethiopia has a variety of fruits, leafy vegetables, roots and tubers adaptable to specific locations and altitudes. The major producers of horticultural crops are small-scale farmers, production being mainly rain fed and few under irrigation. Shallot, garlic, potatoes and chilies are mainly produced under rain fed

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conditions (Yesus, 1992). The pepper species which are grown extensively in Ethiopia are *C. frutescens* and *C. annuum. C. frutescens* is the hot chili pepper, essential part of traditional cooking in most parts of the flora area, and cultivated throughout Ethiopia. The ripe fruits, which are always hot or very hot, are dried and powdered. The powder is used in the hot, mixed spice (known as Mitmita) and in the spicy sauce (known as Awazi), both are very frequently used in Ethiopian cooking. *C. annuum* fruits are used green as ingredients in cooked dishes and raw as a component in the fasting food (called Beyayenetu). The ripe fruits are dried and powdered and used as a component of the mixed spice (known as Berbere) (Hedberg *et al*., 2006).

Capsicum comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber and mineral elements. Many chili constituents are important for nutritional value, flavor, aroma, texture and color. Chilies are low in sodium and cholesterol free, rich in vitamins A and C which are important anti-oxidant, and are a good source of potassium, folic acid and vitamin E. Green *Capsicum* has the highest amount of vitamin C, which decreases with maturity. Fresh fruits may contain up to 340 mg per 100 g of vitamin C. The amount of vitamin C obtained from fresh green chili pepper is higher than that of citrus fruits and fresh red chili has more vitamin A than carrots. Two chemical groups of greatest interest are capsaicinoids and carotenoids (Sittiwong *et al*., 2005; Ozgur *et al.*, 2011; Arnnok *et al*., 2012). They are produced only in the fruit and are concentrated around the seeds and in the pericarp.

Due to various chemical composition of pepper, it is variously used for different purposes because of its nutritional value, flavor, aroma, texture, pungency and color in a wide assortment of foods, drugs, and cosmetics, while some are cultivated ornamentally, especially for their brightly glossy fruits with a wide range of colors, shape and sizes. It is also used as a source of pain relief for pharmaceutical use, and as a repellent. In many cases two or more of these properties are included in the same product; for example, paprika may be a source of color, pungency, and flavor, but color extracts have a very low content of capsaicin in contrast to the extracts used as flavoring agents (Tewksbury *et al.*, 2006; Akbas and Ozdemir, 2008; Sanatombi and Sharma, 2008).

Minerals are very important and essential ingredients of diet required for normal metabolic activities of body tissues. They are constituent of bones, teeth, blood, muscles, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals (Hanif *et al*., 2006; Akan *et al.*, 2009).

Heavy metals occur in all foods as natural or inherent components of plant and animal tissues and fluid and may also be present as a result of contamination from fertilizers and pesticides (Iwebue *et al*., 2008; Darko *et al*., 2014). Some of heavy metals are considered essential including Fe, Zn and Cu (Srividhya *et al*., 2011; Chowdhury *et al*., 2011) due to their involvement in certain physiological process. Elevated level of these has however been found to be toxic (Rapheal and Adebayo, 2011), while some metal ions such as Cd, Pb and Hg are non-essential metals which have toxic roles in biochemical reactions in our body at very low level (Soylak *et al.*, 2004). They are non-biodegradable and thermo-stable and thus readily accumulate to toxic levels (Umar and Salihu, 2014).

Vegetables take up elements by absorbing them from contaminated soils and waste water used for irrigating the vegetables as well as from deposits on different parts of the vegetables exposed to the air from polluted environment (Nenman *et al*., 2012). In addition, they could be contaminated from various species including trace metals as farmers wash them with waste water before bringing them to market (Divrikli *et al*., 2006). Heavy metals enter the human body through inhalation and ingestion, the latter being the main route of exposure to these elements in human population (Mohammed *et al*., 2013). Keeping in mind the frequent consumption of vegetables and fruits, it is necessary to analyze these food items to ensure the levels of these contaminants meet agreed international requirements.

Recently several studies have been conducted on the mineral contents of some vegetables, fruits, and spices cultivated in Ethiopia. These studies include levels of selected metals in the leaves of different species of thyme (Derbie and Chandravanshi, 2011), levels of essential and non-essential metals in *Rhamnus prinoides* (Gebre and Chandravanshi, 2012), levels of major and trace metals in onion (Kitata and Chandravanshi, 2012), levels of metals in vegetables (Weldegebriel *et al*., 2012), mineral contents of fruits of cactus pear (Aregahegn *et al*., 2013), assessment of potentially toxic elements in Swiss chard (Mekonnen *et al.*, 2014), levels of selected essential and non-essential metals in seeds of korarima (Mekassa and Chandravanshi, 2015), effect of cooking temperature on mineral content of yam and taro (Ayele *et al.*, 2015), levels of essential and non-essential metals in ginger (Wagesho and Chandravanshi, 2015), levels of major and trace elements in fennel fruits (Endalamaw and Chandravanshi, 2015), assessment of selected nutrients and toxic metals in citrus fruits (Yami *et al.*, 2016) and levels of fluoride in Ethiopian spices (Nigus and Chandravanshi, 2016).

Red pepper in Ethiopia is consumed in substantial amount as spice and also it is an important cash crop which has high value in export markets. However, no literature reports revealed the levels of metals in Ethiopian red pepper. The objectives of this study were (i) to determine the levels of Na, K, Ca, Mn, Cu, Fe, Zn, Cr, Co, Ni, Pb, and Cd metals in the Ethiopian red pepper using flame atomic absorption spectrophotometry (FAAS) and (ii) to compare the levels of these metals in the Ethiopian red pepper with the levels of metals in red pepper from the other part of the word.

Materials and Methods

Reagents and chemicals

Analytical reagent (AnalaR) grade chemicals and reagents and distilled-deionized water were used throughout the study. Nitric acid, HNO_3 (69–72%, Spectrosol, BDH, England) and perchloric acid, $HClO_4$ (70%, Aldrich, USA) were used for the digestion of pepper samples. Lanthanum chloride heptahydrate (BDH, England) was used to avoid refractory interferences (to release calcium and magnesium from their common phosphates). The standard stock solutions containing 1000 mg L^{-1}, in 2% HNO_3, of the metals Na, K, Ca, Mn, Cu, Fe, Zn, Cr, Co, Ni, Cd and Pb (Buck Scientific Graphic^{tm}) were used for preparation of calibration standards in the analysis of the samples in non-spiking and in the spiking experiments. Appropriate working standards were prepared for each of the metal solution by serial dilution of the intermediate solutions using distilled-deionized water.

Instrumentation and apparatus

A digital analytical balance (Mettler Toledo, Model AG 204, Swizerland) with a precision of ± 0.0001 g was used to weigh peppers samples. A drying oven (Digitheat, J. P. Selecta, Spain) was used to dry the pepper samples in order to powder red pepper samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample until analysis. A 250 mL round bottom flasks fitted with reflux condenser were used in Kjeldahl apparatus (Gallenkamp, England). Micropipettes (1–10 µL and 100–1000 µL) were used for measuring the volume of reagents and standards. Buck Scientific Model 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium arc background corrector was used for analysis of the metals (Na, K, Ca, Fe, Cu, Zn, Cr, Mn, Co, Ni, Cd, and Pb)) using air-acetylene flame.

Description of the study areas

Alaba Special Woreda is one of the special woredas in the South Nation Nationalities and People Regional State (SNNPR) and is part of the Southern Rift Valley of Ethiopia. Alaba is located at a latitude and longitude of $7^0$19' N and $38^0$04' E and 315 km away from Addis Ababa. The woreda is found in Woina-Dega climatic zone with the mean annual temperature ranging between 23–25^{o}C and the mean annual rainfall between 700-900 mm. The woreda located at 1554 to 2149 m above sea level, but most of the woreda is found at about 1800 m above sea level. Furthermore, it is known for the high quality red pepper production, which is mainly supplied to capital city Addis Ababa and other parts of the country.

Mareko is one of the woredas in the Southern Nations, Nationalities and Peoples' Regional State of Ethiopia. Mareko is about 250 km from the capital city, Addis Ababa. It is located at 8000' N and 38031' E. The climate is subtropical and the altitude of the area is 1700 m above sea level and pepper is one of the main crops in the woreda.

Merkato is the name for the large open air market place in the Addis Ketema district of Addis Ababa, Ethiopia. It is also the largest open air market in Africa, covering several square miles and employing several thousand people in different business entities. The primary merchandise passing through the Merkato is locally-grown agricultural products.

Sample collections and handling

For this study a total of three different pepper samples were collected from markets at Alaba Kulito (Alaba especial woreda administrative town), Koshe (Mareko woreda administrative town) in SNNPR and Merkato at Addis Ababa, capital city of Ethiopia. The two areas in SNNPR were chosen for this study mainly because they are the main pepper producers, highly demanded and also mostly marketed to the market in Addis Ababa and most parts of the country for public consumption. Merkato was chosen as study site, which is the place where red pepper grown in different parts of the country is easily available for marketing.

Half kilogram of the pepper sample was collected randomly from each bag with a total of 10 bags from different people from each site. The collected sample was pooled together and mixed well to have one bulk sample from each site. These were transported to the laboratory for processing and preservation. The sample size then reduced to about 500 g from the bulk. The pepper samples were thoroughly washed with tap water and thereafter with distilled water, sliced in nearly uniform size to facilitate drying of the pieces at the same rate and then dried in an oven at 105°C for 24 hours, until they are brittle and crisp. At this stage, no microorganism can grow and care was taken to avoid any source of contamination. The dried samples were homogenized by grinding into fine particles using clean acid washed mortar and pestle and stored in a clean plastic bag until digestion.

Sample digestion

In optimizing the digestion procedure, different procedures for the samples were tried and the one which consumed smaller reagent (acid) volume, shorter digestion time and a colorless solution with no residue was obtained was selected for the digestion of red pepper. A 0.5 g of ground samples (red peppers) was then weighed in to 250 mL round bottom flask. The sample was digested on a micro Kjeldahl digestion apparatus with 2 mL of concentrated nitric acid (69–72%) for 1 hour at 300°C and then cooled to room temperature. The contents were further digested with a 3 mL mixture of nitric acid and perchloric acid (1:2) at the same temperature for one and half hour until a colorless solution was obtained. The sample solution was allowed to cool for 10 min at room temperature, and then the solution was filtered with Whatman® (110 mm, dia) filter paper in to 50 mL volumetric flask. To use the portion of the samples remaining in the beaker, the beaker was rinsed with small portion of deionized-distilled water and then filtered in to the flask. Digestion of each sample and blank were carried out in triplicate. The samples were stored in 50 mL volumetric flasks and placed in a fridge to avoid any decomposition until analysis.

Method validation

In order to ascertain the reliability of the method for the analysis of the samples for major and trace metals, the validity (accuracy) of the analytical procedures used for sample analysis was tested by spiking known concentrations of standards to red pepper sample. For this purpose, a 10 µL of Cd, 250 µL of Ca, 100 µL of Na, 200 µL of K, 10 µL Ni and Mn from 100 mg L^{-1}, 30 µL Co and 50 µL of Cu from 10 mg L^{-1}, 50 µL Cr, 10 µL Zn and Fe from 1000 mg L^{-1} solutions were added to 0.50 g red pepper samples collected from Mareko as the procedures were used for the unspiked sample. The results of spiking measurements are given in Table 1. The mean percentage recoveries for all the metals are all within 100±10 percent. This indicates that the digestion procedure used was reliable and the recovery results were in good agreement with expected values. Therefore the method was used for the determination metals in the pepper samples.

Results and Discussion

Distribution pattern of metals in different red pepper samples

The levels of major and trace metals (Na, K, Ca, Fe, Cu, Zn, Cr, Mn, Co, Ni, Cd, and Pb) in the red pepper samples were determined by FAAS. All the pepper samples were found to contain these metals except Pb which was found below its detection limit. In all the three pepper samples studied, the major metals (K, Ca and Na) were accumulated in appreciable amounts. Of these metals, K was found at the leading followed by Ca. On the other hand, Fe was the most abundant trace metal within all the samples. Mean and standard deviation of each metal in pepper samples collected from three sites are presented in Table 2.

Levels of metals in Alaba pepper

The pepper from Alaba contains a more pronounced concentration of major metals (K, Ca and Na) than those trace metals being determined (Cu, Cd, Cr, Fe, Mn, Ni, Zn and Co) (Table 2). K had the highest value of 2486 µg g^{-1} followed by Ca which was found in appreciable amount 222 µg g^{-1}. Within the trace elements Fe was detected in a highest level (157 µg g^{-1}) followed by Cr (46.5 µg g^{-1}). The level of Co was generally low (1.04 µg g^{-1}) compared to any other metals studied in this sample. The level of toxic metal Cd was very low (0.36 µg g^{-1}) while Pb was not detected (Table 2). The concentration pattern of metals in this sample followed the order: Cd < Co < Cu < Ni < Mn < Zn < Cr < Na < Fe < Ca < K.

Levels of metals in Mareko pepper

The results of the analysis of red pepper in the sample from Mareko showed that the sample contained the highest level of K with concentration 2378 µg g^{-1} followed by Ca, 187 µg g^{-1} and Na, 80.5 µg g^{-1}. Of all the trace metals, Fe content was found highest (99.5 µg g^{-1}). The trend of accumulation of the metals concentrations in red pepper samples from Mareko was in the order: Cd < Co < Cu < Ni < Mn < Cr < Zn < Na< Fe < Ca < K. This trend suggests that peppers of Mareko have least concentration of Cd (0.648 µg/g) (Table 2).

Levels of metals in Merkato pepper

A pepper from Merkato contained the highest

Table 1. Recovery results obtained for validation of the optimized procedure after spiking with standard solutions

Metal	Concentration in sample ($\mu g\ g^{-1}$)[a]	Amount added ($\mu g\ g^{-1}$)	Amount found ($\mu g\ g^{-1}$)[a]	Recovery (%)[b]
Na	80.5±3	100	180±4	99.3±5
K	2378±12	200	2588±8	105±14
Ca	187±4	250	414±7	90.5±8
Fe	99.5±7.3	10	109±9	97.8±11
Cu	3.71±0.16	50	49.0±1.2	90.6±1.3
Zn	58.4±0.48	10	67.7±1.7	93±2
Cr	27.5±2.6	50	75.4±3.1	95.8±4.1
Mn	9.72±0.16	10	19.4±0.9	97.2±0.9
Co	2.24±0.137	30	30.3±2.4	93.4±2.4
Ni	6.68±0.29	10	17.5±0.8	108±1
Cd	0.23±0.019	10	10.7±0.3	105±1
Pb	ND[c]	-	ND[c]	-

[a]Concentration values are mean of three analyzed samples ± standard deviation
[b]Recovery values are mean ± standard deviation
[c]Concentration values of the studied metal below method detection limit

Table 2. Levels of metal (mean ± standard deviation) in red pepper samples

Metal	Alaba sample ($\mu g\ g^{-1}$)	Mareko sample ($\mu g\ g^{-1}$)	Merkato (Addis Ababa) sample ($\mu g\ g^{-1}$)	Mean of the three samples ($\mu g\ g^{-1}$)
Na	75.0±4.0	80.5±4.6	93.0±5.1	82.8
K	2486±15	2378±12	2418±11	2427
Ca	222±4.0	187±4.0	161±7.0	190
Fe	157±4.0	99.5±7.3	103±2.0	120
Cu	2.1±0.2	3.7±0.3	3.5±0.2	3.1
Zn	20.8±1.2	58.4±0.5	27.3 ±0.7	35.5
Cr	46.6±2.6	27.5±2.6	73.6±1.4	49.2
Mn	15.0±0.2	9.7±0.2	18.9±0.6	14.5
Co	1.0±0.1	2.2±0.1	1.0±0.1	1.4
Ni	2.7±0.1	6.7±0.3	2.8±0.1	4.1
Cd	0.2±0.01	0.21±0.01	0.23±0.02	0.21
Pb	ND	ND	ND	ND

ND = not detected (below detection limit)

level of K (241 $\mu g\ g^{-1}$). Fe was significantly higher concentration (103 $\mu g\ g^{-1}$) than the rest of trace metals followed by Cr (73.6 $\mu g\ g^{-1}$), while Cd was found to occur in least concentration 0.607 $\mu g\ g^{-1}$ (Table 2). The concentrations of the metals were ranked in the order: Cd < Co < Ni < Cu < Mn < Zn < Cr < Na < Fe < Ca < K.

Comparison of the levels of metals in the three pepper samples

All the pepper samples collected from the three study areas showed the presence of K, Na, Ca, Co, Cr, Cu, Fe, Zn, Mn, Ni and Cd, while Pb was found to be below detection limit in all the pepper samples. The major metal composition of pepper samples are given in Table 2, showing that the mineral concentrations in all the samples is comparatively varied and their concentration are more pronounced than those of the trace metals.

In all the studied metals K content was highest as compared to other metals, which indicates the characteristic of plant food. Its concentration ranges from 2486 to 2378 $\mu g\ g^{-1}$. Alaba provided maximum of 2486 $\mu g\ g^{-1}$ while its concentration was found minimum in Mareko (2378 $\mu g\ g^{-1}$). The order of K concentration is Alaba > Merkato > Mareko. The second abundant major metal found in pepper was Ca. Its mean concentration was found between 161

Table 3. Comparison of levels of metals (μg g^{-1}) in the Ethiopian red pepper with that reported in the rest of the world

Metal	Origin							
	Pakistan	Nigeria	Iran	Turkey	Canary Island	Korea	Netherlands	Ethiopia
Na	40.5	-	-	-	41.3-86.0	-	30	75.0-93.0
K	55.5	-	-	-	1200-2500	-	2540	2378-2486
Ca	30.5	-	-	-	123-231	-	130	161-222
Fe	35	-	414	234	2.2-4.6	-	-	99.5-157
Cu	1.7	2.41	24.1	4.2	0.3-0.8	8.45		2.1-3.71
Zn	4.2	5250	39.0	7.84	1.0-2.3	2.81	-	28.8-58.4
Cr	-	-	-	7.15	-	-	-	27.5-73.6
Mn	13	3140	28.6	11.9	0.3-0.8	-	-	9.72-18.9
Co	1.06	-	0.01	-	-	-	-	1.02-2.24
Ni	0.6	510	28.6	510	-	-	-	2.71-6.68
Cd	-	9	0.03	0.65	-	0.27	-	0.18-0.23
Pb	-	140	1.41	0.79	-	0.38	-	ND
Ref.	Ismail *et al.*, 2011	Rapheal *et al.*, 2011	Bigdeli *et al.*, 2008	Soylak *et al.*, 2004	Rubio *et al.*, 2002	Jung, 2008	Rubio *et al.*, 2002	This study

ND - Not detected

μg g^{-1} and 222 μg g^{-1}. The maximum amount was found in Alaba pepper (222 μg g^{-1}), while minimum amount was found in Merkato (161 μg g^{-1}). The total accumulation of Ca was in the order of: Alaba > Mareko > Merkato. With regard to the levels of Na in the three pepper samples, Merkato sample had significantly higher concentration than the rest of the samples, with the least concentration occurring in Alaba (75.0 μg g^{-1}). The trend of occurrence of Na in the samples followed the order: Merkato > Mareko > Alaba.

Of the trace metals, in all the pepper samples the concentration of Fe was relatively higher than any other trace element analyzed, even the concentration of Fe observed higher than Na in all the pepper samples. But its concentration varied within the pepper samples analyzed. Fe contents in the study were in the range of 99.5-157 μg g^{-1} (Table 2). The highest and the least concentration were observed in Alaba (157 μg g^{-1}) and in Mareko (99.5 μg g^{-1}), respectively.

The concentrations of Co were generally low in all the samples. The concentration pattern of Co followed the order Mareko > Alaba > Merkato. As can be seen in Table 2, the level of Co was generally low compared to other metals studied except Cd. The Ni contents in the samples were 2.71 μg g^{-1}, 6.68 μg g^{-1} and 2.81 μg g^{-1} in Alaba, Mareko and Merkato, respectively. Moreover, the lowest level of Ni was accumulated in Alaba pepper and the highest was in Mareko.

Zn was the second most accumulated trace metal next to Fe in Mareko pepper, but it is the third abundant metal next to Fe and Cr in Alaba and Merkato pepper. In this study, it was detected in the range of 20.8-58.4 μg g^{-1}, while the highest level of Zn was observed in the pepper of Mareko and the lowest level in the pepper from Alaba. The high concentration of Zn was probably because it is considered as essential micronutrients for plants growth and can easily taken up by plants. The Mn contents in the samples were found 15.0 μg g^{-1}, 9.72 μg g^{-1} and 18.9 μg g^{-1} in the pepper from Alaba, Mareko and Merkato, respectively. The order in the level of Mn follows the pattern Mareko < Alaba < Merkato.

Concerning to Cr, the concentration within the studied pepper samples was found in the range of 27.5-73.6 μg g^{-1}. These higher levels indicate that Cr is the second most abundant trace metal following Fe, with the exception in Mareko which is exceeded by Zn. The concentration of Cr metal determined from the samples of the study areas decreased in the order; Merkato> Alaba> Mareko. In the case of Cu, the mean concentrations in the pepper were found in the range of 2.12-3.71 μg g^{-1}. The highest was found in Mareko pepper (3.71 μg g^{-1}) while the lowest concentration observed in Alaba pepper (2.12 μg g^{-1}).

The level of toxic metal Cd found in this study was generally low compared to any metal examined in all the pepper samples. The three red pepper samples contained almost the same level of Cd. While the other toxic metal Pb was not detected in any of the three pepper samples.

In general, the results of this study revealed that the highest concentration of K, Ca and Fe were found in Alaba pepper, Co, Cu, Ni and Zn were highest in

Mareko pepper while Cr and Mn found maximum in Merkato pepper. The variations in concentration of metals in the samples were probably influenced by the site locations (i.e. the mineral content of the soil on which the plants grow).

Comparison of metal contents of Ethiopian pepper with results from other countries

There are some reports from different countries on the analysis of the metal contents of red pepper. It is important to compare the results obtained from the analysis of red pepper in Ethiopia with the literature values from other countries which helps to identify the differences in composition and if there exists a deviation from certain guide lines.

The level of K in Ethiopian pepper was found in the range of 2378–2486 µg g^{-1}. These values are found to be relatively higher than reported from Pakistan (Ismail *et al.*, 2011). The levels of K obtained in this study are lower than those recorded from Spain (Rubio *et al.*, 2002). However, K contents in the pepper are comparable with those reported from Netherlands and Canary Island (Rubio *et al.*, 2002). For Na, the determined levels in Ethiopian pepper are higher than reported by Rubio *et al*. (2002) and Ismail *et al*. (2011) (Table 3). The results obtained from Canary Island (Rubio *et al.*, 2002) are almost comparable with the results of this study. Similarly, the levels of Ca obtained in this study are higher than those recorded from Pakistan (Ismail *et al*., 2011), Netherlands and Spain (Rubio *et al*., 2002). But, it is comparable with the values of Canary Islands (Rubio *et al*., 2002).

As can be seen in Table 3, the level of Fe in this study are between 99.5 and 157 µg g^{-1} and are higher than those reported by Ismail *et al.* (2011), Nenman *et al*. (2012) and Rubio *et al.* (2002). However, the values were lower than results obtained from Iran (Bigdeli and Seilsepour, 2008) and Turkey (Soylak *et al*., 2004).

Zn is present in appreciable amount in the Ethiopian pepper samples (20.8–58.4 µg g^{-1}). These concentrations are higher when compared to literature reports from Canary Islands (Rubio *et al.*, 2002), Korea (Jung, 2008), Pakistan (Ismail *et al*., 2011) and Turkey (Soylak *et al*., 2004). Zn concentrations in samples studied are shown to be in a good agreement with the data obtained by Bigdeli and Seilsepour (2008). However, the level of Zn in Ethiopian pepper is much lower than the sample from Nigeria (Rapheal and Adebayo, 2011). When the levels of Mn obtained in this study compared with literature value, it is higher than with the results reported by Rubio *et al*. (2002) and lower than with the values obtained by Rapheal and Adebayo (2011) and Bigdeli and Seilsepour (2008). However, the levels of Mn are in agreement with the results obtained by Ismail *et al.* 2011) and Soylak *et al.* (2004).

As shown in Table 3, the levels of Ni obtained in this study are in agreement with the report of Nenman *et al*. (2012) and Soyalk *et al*. (2004). However, Ismail *et al*. (2011) and Rapheal and Adebayo (2011) reported the lowest and the highest Ni concentrations, respectively. On the other hand, Co concentration found in this study is higher than data reported by Bigdeli and Seilsepour (2008), but lower than that reported by Nenman *et al.* (2012). It is comparable with the results reported by Ismail *et al.* (2011). When comparing the results of this study with those cited in literature, the concentration of Cu found in this study is higher than value reported by Rubio *et al.* (2002). However, it is lower than with the values reported by Bigdeli and Seilsepour (2008), Jung (2008) and Rapheal and Adebayo (2011), while more or less it agrees with the results of Soyalk *et al.* (2004) and Ismail *et al*. (2011).

From the data given in Table 3, the level of Cr in pepper samples from Turkey (Soylak *et al.,* 2004) is much lower than that of Ethiopian samples. Regarding Cd, in most literature Cd was detected up to very low level. In the present study, the levels of Cd are lower than reports from Nigeria (Rapheal and Adebayo, 2011) and higher than from Iran (Bigdeli and Seilsepour, 2008). However, it is in a good agreement with the findings of Soyalk *et al*. (2004) and Jung (2008). Finally, Pb was detected in some studies. But, in the present study it is found below the detection limit.

As mentioned above, the results obtained in this study are comparable favorably with the findings of other researchers from other parts of the world. However, results those obtained from Canary Island are significantly lower in the contents of trace elements. In contrary, the reports of Rapheal and Adebayo (2011) showed extremely high levels of metals compared with the values of the present study. This difference in metals content may be ascribed to variation in composition of soil types, sample sites, sample treatment, the presence of agents which either increases or decreases the metal content.

Moreover, the levels of metals in Ethiopian red pepper are compared with the maximum limit given by WHO in the vegetables. On this basis Co, Cu, Fe, Mn, Ni and Zn in the Ethiopian pepper samples are found below the maximum limit of WHO, while the levels Cd and Cr are found above the limit set by WHO.

Table 4. Comparison of metal concentrations of red pepper and other spices in the literature

Metal	Concentration (mg kg^{-1}) in different types of spice sample						
	Red pepper	Thyme	Ginger	Korarima	Cardamom*	Fennel	Fenugreek
Ca	161-222	1239-2776	2000-2540	1794-2181	2719±35	20500-23000	15353-36771
K	2378-2486	—	—	—	—	—	6789-11517
Na	75-93	—	—	—	—	—	201-1559
Fe	99.5-157	728-2517	41.8-89.0	37.0-46.5	64.8±2.2	1140-1900	6041-18584
Cr	27.5-73.6	—	6.02-10.8	3.8-5.8	8.3±0.7	90.9-97.7	3-552
Ni	2.7-6.7	9.83-14.2	5.61-8.40	6.6-8.5	11.7±0.5	18.7-24.2	31-108
Zn	20.8-58.4	8.7-52	38.5-55.2	12-18	19.6±0.9	37.1-44.7	15-33
Mn	9.7-18.9	37.7-114	184-401	144-180	355.4±9.8	30.6-51.4	16-28
Cu	2.1-3.7	7.69-10.1	1.10-4.78	5.8- 8.3	9.5±1.0	23.9-103	ND-35
Co	1.0-2.2	2.59-4.50	2.04-7.58	2.0-2.3	2.6±0.2	26.2-70.8	4-15
Pb	ND	—	—	ND	ND	ND	615-1814
Cd	0.20-0.23	0.87-1.3	0.38-0.97	0.9-1.0	0.87±0.07	1.59-1.91	285-464
Ref.	This study	(Derbie and Chandravan-shi, 2011)	(Wagesho and Chandravan-shi, 2015)	(Mekassa and Chandrav-anshi, 2015)	(Mekassa and Chandravan-shi, 2015)	(Endalamaw and Chandravan-shi, 2015)	(Hagos and Chandrava-nshi, 2016)

*The values are mean ± SD
ND = Not detected
— = Not reported

Comparison of metal concentrations of red pepper with other spices

The concentration of metals determined in red pepper has been is compared with other spices studied in Ethiopia (Table 4). These spices are thyme, ginger, korarima, cardamom, fennel and fenugreek seed. The red pepper (161-222 mg kg^{-1}) contains lowest concentration of Ca while fenugreek seed (15353–36771 mg kg^{-1}) contains highest concentration of Ca. The ascending order of Ca concentration in these spices is as follows: red pepper < korarima < ginger < thyme < cardamom < fennel < fenugreek seed. The K concentration in the red pepper is (2378–2486 mg kg^{-1}) and in the fenugreek seed is (6789–11517 mg kg^{-1}) while its concentration is not reported in the other spices. The Na concentration in the red pepper is (75–93 mg kg^{-1}) and in the fenugreek seed is (201–1559 mg kg^{-1}) while its concentration is not reported in the other spices.

The Fe concentration in the red pepper (99.5-157 mg kg^{-1}) is in the middle of the range of Fe concentration in the spices. The highest Fe concentration is found in fenugreek seed (6041–18584 mg kg^{-1}) and the lowest in korarima (37–46.5 mg kg^{-1}). The ascending order in the concentration of Fe in these spices is as follows: korarima < cardamom < ginger < red pepper < fennel < thyme < fenugreek seed. Cr concentration in red pepper is (27.5-73.6 mg kg^{-1}) while its concentration is the highest (3–552 mg kg^{-1}) in fenugreek and the lowest in korarima (3.8–5.8 mg kg^{-1}), its concentration is not reported in thyme. The ascending order in the concentration of Cr in these spices is as follows: korarima < cardamom < ginger < red pepper < fennel < fenugreek seed. The lowest Ni concentration is recorded in the red pepper (2.7–6.7 mg kg^{-1}) while the highest concentration is in the fenugreek seed (31–108 mg kg^{-1}). The ascending order in the concentration of Ni in these spices is as follows: red pepper < ginger < korarima < cardamom < thyme < fennel < fenugreek seed. The Zn concentration in the red pepper is (20.8-58.4 mg kg^{-1}) while comparable Zn concentration is recorded in the ginger (38.5–55.2 mg kg^{-1}) and the lowest in the korarima (12–18 mg kg^{-1}). The Mn concentration in the red pepper (9.7–18.9 mg kg^{-1}) is the lowest while the highest concentration is in the ginger (184–401 mg kg^{-1}). The ascending order in the concentration of Mn in these spices is as follows: red pepper < fenugreek seed < fennel < thyme < korarima < cardamom < ginger. Lowest Cu concentration is in the red pepper (2.1–3.7 mg kg^{-1}) while the highest Cu concentration is in the fennel (23.9–103 mg kg^{-1}). The ascending order in the concentration of Cu in these spices is as follows: red pepper < ginger < korarima < cardamom < thyme < fenugreek < fennel. Co concentration is the lowest in red pepper (1.0–2.2 mg kg^{-1}) and the highest in fennel (26.2–70.8 mg kg^{-1}). The descending order in the concentration of Co

Table 5. Comparison of daily intake of metals from red pepper with recommended daily intake and tolerable upper limit of daily intake of metals (National Research Council, 1989; Food and Nutrition Board, 1997; NebGuide, 2009)

Metal	Daily intake from 20 g red pepper (mg/day)	Daily intake from 40 g red pepper (mg/day)	Recommended daily intake (mg/day)	Allowable upper limit (mg/day)
Na	1.66	3.31	1500	2300
K	48.5	97.1	4700	NE
Ca	3.80	7.60	1000	2500
Fe	2.40	4.80	18	45
Cu	0.062	0.124	2	10
Zn	0.710	1.42	0.150	40
Cr	0.984	1.97	0.120	0.120
Mn	0.290	0.580	2	11
Co	0.028	0.056	NE	0.25
Ni	0.082	0.162	NE	1
Cd	0.0042	0.0084	None	0.0714
Pb	ND	ND	None	None

NE = not established
ND = not detected

in these spices is as follows: red pepper < korarima < cardamom < thyme < ginger < fenugreek < fennel.

Pb is not detected in the red pepper. Cd concentration is lowest in the red pepper (0.20–0.23 mg kg^{-1}) among all the studied spices. The non-detectable Pb and negligible amount of Cd in the red pepper is good for the human health.

Daily intake of metals from red pepper

Daily intake of metals from red pepper has been calculated based on the assumption that an adult (Ethiopian) person consumes 20–40 g red pepper (Table 5). Ethiopian people consume relatively larger amount of red pepper than people from other countries. The metal contents of red pepper are given in Table 2. The daily intake of metals from red pepper, the recommended or adequate daily intake and the allowable upper limit of daily intake of metals are given in Table 5. The data in the table clearly show that the daily intake of most metals (Na, K, Ca, Fe, Cu, Mn, Co, and Ni) are well below the recommended daily intake while Zn exceeded the recommended daily intake but is well below the allowable upper limit of daily intake. Cr exceeded the recommended daily intake as well as the allowable upper limit of daily intake. This may result in harmful effect to the larger consumers of red pepper. Daily intake of toxic metal Cd from Ethiopian pepper is negligible and well below the allowable upper limit of daily intake. Pb is not detected in the Ethiopian red pepper and hence there is no toxicity results from red pepper consumption. Therefore, it can be concluded that Ethiopian red pepper is good source of essential metals and nearly free from toxic metals and hence safe for human consumption.

Statistical analysis of variance (ANOVA)

ANOVA is a powerful statistical technique which can be used to separate and estimate the different causes of variation. It can also be used in situations where there is more than one sources of random variation (Miller and Miller, 2005).

One-way ANOVA can be used when there is only one factor being considered and replicate data from changing the level of the factor are available. In the present study one-way ANOVA was used to compare the means between all the three pepper samples and the calculations were made using SPSS software.

The statistical analysis of levels of K showed that a significance difference ($p < 0.05$) between the means of pepper from Alaba and Merkato. Similarly, there is a significant difference between the means of K in pepper samples of Alaba and Mareko. However, samples from Merkato and Mareko showed no significance different.

Significant difference was found ($p < 0.05$) in the levels of Na between Alaba and Merkato peppers. Moreover, the pepper samples from Mareko and Merkato show a significant difference in Na levels. But, no significant difference was observed in means of Na within Alaba and Mareko pepper. In comparing the means of Ca in the three pepper samples at the 95% confident level, their means are significantly different. Of these samples, Alaba pepper has the highest while Mareko pepper has the lowest means. In similar manner, the results of one-way ANOVA also indicated that, there exist significant differences between the levels of Cr and Mn within all the three pepper samples. The analysis of the difference in the means of Co showed that significant differences were observed between pepper from Alaba and Mareko and between Mareko and Merkato. While within pair of means of Mareko and Merkato no significant differences were seen.

The ANOVA analysis of Fe indicated the existence of significant differences ($p < 0.05$) between means of Merkato and Alaba pepper and between Alaba and Mareko pepper, but no significant differences between Merkato and Mareko pepper were observed.

Concerning to the means of Ni and Zn, the means between Alaba and Mareko pepper and between Merkato and Mareko pepper have significant differences. But, no significant differences observed between the means of Ni and Zn in Alaba and Merkato pepper samples ($p > 0.05$).

Statistically, there were significant differences ($p < 0.05$) in the means of Cu in Alaba pepper with other pepper samples. However, no significant differences were observed in the Cu levels between Merkato and Mareko pepper ($p > 0.05$). There were significant differences between the mean of Cd of Alaba pepper with the means of Mareko and Merkato pepper, but no significant difference between Mareko and Merkato pepper.

To sum up, the results of one-way ANOVA revealed that means of Ca, Cr and Mn showed significant differences within all samples analyzed in this study. However, other metals show both significant and non-significant differences between their means.

Pearson correlation of metals in red pepper samples

To correlate the effect of one metal concentration on the concentration of the other metal in the red pepper sample, the Pearson correlation matrices using correlation coefficient (r) for the samples were used (Miller and Miller, 2005). It was found that there is high positive correlation of Na with (Cu, Cr, Mn), K with (Ca and Fe), Ca with Fe, Cu with (Zn, Co, Ni and Cd), Zn with (Co and Ni), Cr with (Mn and Cd) and Mn with Cd and moderate correlation of K with Mn; which may be arise from common anthropogenic or natural sources as well as from similarity in chemical properties. It was also found that there is high negative correlation of Na with (Ca and Fe), K with (Cu, Zn, Co and Ni), Ca with (Cu, Cr and Cd), Fe (Cu, Zn, Co, Ni and Cd), Zn with (Cr and Mn), Cr with (Co and Ni) and Mn with (Co and Ni); which indicate that the large absorption of the metal may affect the absorption of negatively correlated metals in the pepper plant. The other metals have showed weak negative or positive correlation indicating that the presence or absence of one metal affect in lesser extent to the other metals.

Conclusion

The study shows that the levels of K, Na, Ca, Co, Cr, Cu, Fe, Zn, Mn, Ni and Cd, in pepper samples were found in appreciable amount while Pb was found below detection limit. Among all the studied metals, K was observed in highest level. Similarly, Fe accumulated in highest level among the trace metals. However, Co and Cd were found at low concentration in all of the analyzed samples.

Concentrations of metals between samples were compared using one-way ANOVA, which indicated that there are significant differences in the levels of most metals between the three samples. The variation in the levels of metals between samples certified the difference in the composition of soils from which the pepper grown. The level of metals in Ethiopian pepper is found to be comparable with the results reported from different countries with little exception.

The results obtained in the present study were compared with international guidelines. All the metals determined are found below the levels allowed for vegetables by WHO, except Cr and Cd. The levels of Cd in all the three samples are slightly higher than 0.1 $\mu g\ g^{-1}$ recommended by WHO whereas Cr was found in higher amount than recommended by WHO.

Furthermore, to correlate metal concentrations among each other metal levels in the red pepper using Pearson's correlation coefficient (r) was determined and except for few relationships most relationships showed either strong positive or negative linear relationships. From this relationship it can be understood that the presence of one metal in the plant influence the presence of the other.

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