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Comparative Study on The Determination of Some Major, Minor-Essential and Toxic Metals From Two Species of Cactus Fruit (*Opuntia Ficus Indica* and *Opuntia Stricta*) and Their Supporting Soil Samples Cultivated in Two Zones of Tigray, Northern Ethiopia

Research Article

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Abstract

A comparative study was carried out for the determination of some selected metals (K, Mg, Ca, Cu, Zn, Ni, Mn, Cd and Pb) in fruits of two cactus species (*Opuntia ficus indica* and *Opuntia stricta*) and supporting soil from two zones of Tigray region of northern Ethiopia. After digestion with microwave acid digestion, some selected metals were determined by Flame Atomic Absorption Spectrophotometer. The concentration of metals obtained for these fruits are found to be within the range of WHO and other international guideline limits. Levels of the metals concentration in this study is that, in all the fruit samples, K >Ca > Mg in concentration and the levels of trace metals are, Zn > Mn > Cu > Ni. Concentrations of Cd and Pb are below the method detection limit in all the fruit samples. The metals concentration between the two species (*Opuntia ficus indica* and *Opuntia stricta*) cultivated in the same zone are not significantly different ($p = 0.05$). In the comparison of metals concentration of same species cultivated in different zones, the concentrations of K, Ca and Zn in fruit and soil are significantly higher in Southeastern zone than Eastern zone. Concentrations of Mg in *Opuntia ficus indica* and soil are significantly higher in Southeastern zone than Eastern zone. The concentrations of Mn in fruit and soil are significantly higher in Eastern zone than Southeastern zone. Concentrations of Mg in *Opuntia stricta*, concentrations of Ni in both fruits and soil and concentrations of Cu in both fruits are not significantly different in these two zones, but soil of Southeastern zone is significantly higher in Cu concentration than Eastern zone.

Key words: Metal; *Opuntia ficus indica; Opuntia stricta*; Soil; Microwave Assisted Digestion; Flame Atomic Absorption Spectrophotometer.

Introduction

In Ethiopia different wild and cultivated plants have been used as a source of food, different parts of the plants such as leaf, steam, fruit and root are used especially by the rural population. These wild and cultivated plants contribute to improve local food security and people's income In addition to this these plants are good sources of vitamins, minerals, trace elements and proteins [1,2, 3].

In Tigray (northern Ethiopia) there are varieties of wild and cultivated plants used as a source of food especially by the rural population. Cactus fruits (*Opuntia ficus indica* and *Opuntia stricta*) are the major fruits that consumed directly by the people of the region. The cactus fruit or beles plays an important economic and cultural role, which is reflected by traditional songs, "Belesey Belesey awtsaekini hamley nehasey kisab zredieni sigemey" this is to mean that "Oh my beles you spare me this summer till barley has cheerfully come to rescue me". In this region it is estimated that, there is about 360, 000 hectare of cactus plant, of which about two thirds consists of spiny plants (*Opuntia ficus indica*), about half of the existing area of *Opuntias* was planted and the remainder has been invaded by the naturalized cactus [4, 5].

Utilization of cactus by human being was recorded in Mexico. Cactaceae family includes approximately 130 genera and 2,000 species. These Cacti are largely found in Mexico, United States, Madagascar, Australia, Sri Lanka and India and was introduced to North Africa in the 16th century [6, 7].

The species of *Opuntia* is the most abundant of the Cactaceae

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family, grown throughout the Americas, area of the Mediterranean, Europe, Asia, Africa and Australia. *Opuntia ficus-indica* and *Opuntia stricta* are predominantly found in Ethiopia [8, 9].

The fruits of *Opuntia* species contain significant amounts of ascorbic acid (vitamin C), lipid, protein, minerals, fiber and β carotenes (provitamin A). These fruits are also rich in free amino acids (proline, taurine, glutamine and serine), polysaccharides and polyphenolic compounds (quercetin, kaempferol, isorhamnetin and their derivatives) [10, 11, 12].

Materials and Methods

Site characterization

The cactus fruits and supporting soil samples of the study were collected from Ganta Afeshum and Saesie Tsaeda Emba woredas of Eastern zone and Enderta and Hintalo Wejirat woredas of Southeastern zone of Tigray region, northern Ethiopia. These four woredas were selected depending on the availability of the cultivated cactus plants.

Ganta Afeshum is topographically lies at an altitude between 1500 – 3500 meters above sea level. The mean annual rainfall of the woreda ranges from 582.5 – 775.5 in milliliters and the annual temperature ranges from 10 – 17.5^{0}C. Saesie Tsaeda Emba is topographically lies at an altitude between 2000 – 2500 meters above sea level. The mean annual rainfall of the woreda ranges from 500 - 700 in milliliters and the annual temperature ranges from 11 – 18^{0}C. Hintalo Wejirat is topographically lies at an altitude between 500 – 3500 meters above sea level. The mean annual rainfall of the woreda ranges from 500 – 905.7 in milliliters and the annual temperature ranges from 7.5 – 23.5^{0}C. Enderta is topographically it lies at an altitude between 1000 – 2500 meters above sea level. The mean annual rainfall of the woreda ranges from 500 – 715.9 in milliliters and the annual temperature ranges from 7.5 – 25.5^{0}C [13].

Sample collection and preparation

Fruit samples (*Opuntia ficus indica and Opuntia stricta*) and their supporting soil were collected from two woredas of each zone. Two kebeles were selected from each woreda and two sites were selected from each kebele. Therefore a total of eight kebeles of 16 sites were selected randomly from the four woredas. Accordingly ten cactus plants for each plant species were selected from each site; out of these a single fruit was taken from each sampled cactus plant using random selection method. Therefore a total of 320 fruit samples (160 *Opuntia ficus indica* and 160 *Opuntia stricta*) were collected from both zones.

Soil sample was collected from the place where the sampled cactus plants grow. About ¼ kg of soil sample was taken from each cactus plant, accordingly 2 kg soil sample from each zone, then a total of 4 kg soil sample was taken from the two zones.

The collected fruit samples were thoroughly washed with tap water and detergent solution and rinsed with distilled water to remove surface contaminants, and then were peeled with a stainless steel knife and the pulps were mixed by a mixer, the resulting fruit juices was dried at 900C for 72 hrs in the oven and the dried sample was powdered using pestle and mortar, the well ground sample was homogenized manually and stored in polyethylene bags until digestion. The collected soil sample was mixed gently and was dried with an open air until the weight of the soil was constant. The dried soil sample was ground using pestle and mortar; the well ground and homogenized soil sample was stored in polyethylene bags until digestion.

Sample digestion

All samples were digested by microwave acid digestion apparatus (BMS-1), exactly 0.3 gram of dried and homogenized fruit sample was digested by mixture of 7 mL of concentrated HNO_3 (68%) and 3 mL of H_2O_2 (30%). Exactly 0.5 gram of dried and homogenized soil sample was digested by mixture of 2.5 mL of concentrated HNO_3 (68%) and 7.5 mL of concentrated HCl (38%). For both the fruit and soil samples, all shacked samples were placed in the fume hood for 15 minutes prior to digestion. Then the predigested fruit sample and reagents in the digestion vessels were closed and heated on microwave oven. The digestion vessel was cooled to room temperature for about 20 minutes in a fume hood and then the cooled sample solutions were transferred to 10 mL volumetric flask and the volumes were filled with double distilled deionised water up to the mark. The digested samples were then kept in a refrigerator until analyzed by FAAS.

Results and Discussion

The concentrations of the nine elements in the digested and diluted solutions of cactus fruits and soil samples are shown in Table 1 and 2.

Levels of metals in cactus fruits of Eastern zone

As it is shown from Table 1 the concentration of K (3805.01 + 16.23 mg/kg) is higher followed by Ca (1159.71 + 7.68 mg/kg) and Mg (1044.33 + 10.70 mg/kg) in *Opuntia stricta* of Eastern zone . The levels of minor – essential metals also, Zn (13.89 + 2.28 mg/kg) is higher in concentration followed by Mn (8.41 + 0.07 mg/kg), Cu (2.97 + 0.40 mg/kg) and Ni (2.45 + 0.35 mg/kg) respectively. In case of *Opuntia ficus indica* of this zone, K (3696.27 + 19.91 mg/kg) is found in higher concentration followed by Ca (1173.04 + 15.67 mg/kg) and Mg (1003.37 + 11.34 mg/kg) and the levels of the minor – essential metals in this fruit is Zn (13.71 + 1.54 mg/kg) is in higher concentration followed by Mn (8.36 + 0.05 mg/kg), Cu (2.88 + 0.30 mg/kg) and Ni (2.45 + 0.98 mg/kg) respectively.

Levels of metals in cactus fruits of Southeastern zone

In these cactus fruits, the levels of the metals concentration are as follows, in *Opuntia ficus indica*, K (4524.11 + 18.28 mg/kg) is found in higher, Ca (1235.06 + 7.69 mg/kg) and Mg (1097.48 + 16.61 mg/kg) are found in the 2nd and 3rd levels respectively. The levels of the minor – essential metals are, Zn (18.99 + 0.08 mg/kg) is in higher concentration followed by Mn (7.24 + 0.06 mg/kg), Cu (3.09 + 0.04 mg/kg) and Ni (2.45 + 0.88 mg/kg) respectively. The levels of the metals in *Opuntia stricta*, major-essential metals are, K (4640.98 + 10.28 mg/kg) > Ca (1221.76 + 7.68 mg/kg) > Mg (1103.58 + 9.18 mg/kg). The levels of the minor- essentials are also Zn (18.45 + 0.57 mg/kg) > Mn (7. 45 + 0.23 mg/kg) > Cu (3.40 + 0.39 mg/kg) > Ni (2.54 + 0.46 mg/kg).

Table 1. Metals mean concentration (n=3, mg/kg dry weight) in cactus fruit samples.

No	Metal (mg/kg)	Sample			
		Eastern zone		Southeastern zone	
		Opuntia ficus indica	*Opuntia stricta*	*Opuntia ficus indica*	*Opuntia stricta*
1	K	3696.27 ± 19.91	3805.01 ± 16.23	4524.11 ± 18.28	4640.98 ± 10.28
2	Mg	1003.37 ± 11.34	1044.33 ± 10.70	1097.48 ± 16.61	1103.58 ± 9.18
3	Ca	1173.04 ± 15.67	1159.71 ±7.68	1235.06 ± 7.69	1221.76 ± 7.68
4	Cu	2.88 ± 0.30	2.97 ± 0.40	3.09 ± 0.04	3.40 ± 0.39
5	Zn	13.71 ± 1.54	13.89 ± 2.28	18.99 ± 0.08	18.45 ± 0.57
6	Ni	2.45 ± 0.98	2.45 ± 0.35	2.45 0.88	2.54 ± 0.46
7	Mn	8.36 ± 0.05	8.41 ± 0.07	7.24 ± 0.06	7.45 ± 0.23
8	Cd	ND	ND	ND	ND
9	Pb	ND	ND	ND	ND

ND = non detected

Table 2. Metals mean concentration (n=3, mg/kg dry weight) in soil samples.

No	Metal (mg/kg)	Sample	
		Eastern zone	Southeastern zone
1	K	5547.5 ± 12.79	5582.51 ± 9.37
2	Mg	4310.8 ± 11.35	4411.28 ± 15.13
3	Ca	3557.83 ± 6.77	3628.21 ± 6.78
4	Cu	35.84 ± 0.82	44.73 ± 0.81
5	Zn	47.22 ± 0.81	66.85 ± 0.78
6	Ni	32.04 ± 2.32	33.37 ± 2.30
7	Mn	83.84 ± 0.54	61.55 ± 0.83
8	Cd	ND	ND
9	Pb	7.26 ± 0.79	8.62 ± 0.79

ND = non detected

Table 3. Zonal comparison of metals (at 0.05 levels).

Metal (mg/kg)	sample	Eastern zone	Southeastern zone
	Opuntia ficus indica	3696.27[a]	4524.11[b]
K	*Opuntia stricta*	3805.01[a]	4640.98[b]
	Soil	5547.5[a]	5582.51[b]
	Opuntia ficus indica	1003.37[a]	1097.48[b]
Mg	*Opuntia stricta*	1044.33[a]	1103.58[a]
	Soil	4310.8[a]	4411.28[b]
	Opuntia ficus indica	1173.04[a]	1235.06[b]
Ca	*Opuntia stricta*	1159.71[a]	1221.76[b]
	Soil	3557.83[a]	3628.21[b]

Cu	*Opuntia ficus indica*	2.88[a]	3.09[a]
	Opuntia stricta	2.97[a]	3.4[a]
	Soil	35.84[a]	44.73[b]
Mn	*Opuntia ficus indica*	8.36[a]	7.24[b]
	Opuntia stricta	8.41[a]	7.45[b]
	Soil	83.84[a]	61.55[b]
Ni	*Opuntia ficus indica*	2.45[a]	2.45[a]
	Opuntia stricta	2.45[a]	2.54[a]
	Soil	32.04[a]	33.37[a]
Zn	*Opuntia ficus indica*	13.71[a]	18.99[b]
	Opuntia stricta	13.89[a]	18.45[b]
	Soil	47.22[a]	66.85[b]

a, a indicates not significantly difference and a, b indicates significantly difference

Table 4. Comparison of metals among different cactus species (at 0.05 levels).

Metal (mg/kg)	Zone	Sample	
		Opuntia ficus indica	*Opuntia stricta*
K	Eastern	3696.27[a]	3805.01[a]
	Southeastern	4524.11 [a]	4640.98[a]
Mg	Eastern	1003.37[a]	1044.33 [a]
	Southeastern	1097.48 [a]	1103.58[a]
Ca	Eastern	1173.04[a]	1159.71 [a]
	Southeastern	1235.06[a]	1221.76[a]
Cu	Eastern	2.88[a]	2.97[a]
	Southeastern	3.09 [a]	3.4 [a]
Mn	Eastern	8.36[a]	8.41[a]
	Southeastern	7.24[a]	7.45[a]
Ni	Eastern	2.45[a]	2.45[a]
	Southeastern	2.45[a]	2.54[a]
Zn	Eastern	13.71[a]	13.89[a]
	Southeastern	18.99[a]	18.45[a]

a, a indicates not significantly difference

Table 5. Comparison of present study with different international guidelines.

Metal	Name of organization	AI	UL	Present study			
		(mg/day)	(mg/day)	Eastern zone		Southeastern zone	
				Opuntia ficus indica (mg/day)	*Opuntia stricta* (mg/day)	*Opuntia ficus indica* (mg/day)	*Opuntia stricta* (mg/day)
K	WHO, 2012	Adults, 3510	--------	554.44	494.65	678.62	603.33
		Adults					
Mg	IOM, 1999	Women, 320	--------	150.51	135.76	164.62	143.46
		Men, 420					
		Age					
Ca	IOM, 1999	19 – 50, 1000	2500	175.96	150.76	185.26	158.83
		>50, 1200					
Cu	IOM, 2001	Adults, 0.9	10	0.43	0.39	0.46	0.44
Zn	IOM, 2001	Women, 8	40	2.06	1.81	2.85	2.4
		Men, 11					
Ni	IOM, 2001	---------	1	0.37	0.32	0.37	0.33
Mn	IOM, 2001	Women, 1.8	11	1.25	1.09	1.09	0.97
		Men, 2.3					

In all the fruits of these zones, the concentrations of Cd and Pb are below the method detection limit. In general this might be due to the concentration of the metals in soil, availability of the metals in the soil, soil nature for the solubility of the metals from parent materials and the plant nature for up taking and transferring of the metals from root to fruit part, mobility of these metals in the plant tissue. These cactus fruits (*Opuntia ficus indica and Opuntia stricta*) are good sources of the major and minor-essential metals.

Level of metals in soil of the two zones

Table 2 represents the level of the analyzed metals in soils of the two zones. The results of soil of Eastern zone show that, K (5547.5 + 12.79 mg/kg) is in higher concentration, Mg and Ca are found in larger amount next to K with values of 4310.8 + 11.35 mg/kg and 3557.83 + 6.77 mg/kg respectively. The levels of the minor-essential metals show that Mn (83.84 + 0.54 mg/kg) is found in large amount. Zn, Cu, Ni and Pb are found in large amounts next to Mn with respective value of 47.22 + 0.81 mg/kg, 35.84 + 0.82 mg/kg, 32.04 + 2.32 mg/kg and 7.26 + 0.79 respectively. The result of soil of Southeastern zone shows that, K (5582.51 + 9.37 mg/kg) is in higher concentration. Mg and Ca are found in large amount of 4411.28 + 15.13 mg/kg and 3628.21 + 6.78 mg/kg next to K respectively. The levels of minor-essential metals concentration in this soil is, Zn (66.85 + 0.78 mg/kg) is found in large amount. Mn, Cu, Ni and Pb are found in the next amounts of 61.54 + 0.83 mg/kg, 44.73 + 0.81 mg/kg, 33.37 + 2.30 and 8.62 + 0.79 mg/kg respectively.

Comparison level of metals in the same species of the two zones

At 0.05 levels, the concentrations of K and Ca in both the cactus fruits (*Opuntia ficus indica* and *Opuntia stricta*) and soil are significantly higher in Southeastern zone than Eastern zone. The concentrations of Mg in *Opuntia ficus indica* and soil of Southeastern zone are significantly higher than Eastern zone, but are not significantly difference in *Opuntia stricta* of the two zones. The concentrations of Mn are significantly higher in both the fruits (*Opuntia ficus indica* and *Opuntia stricta*) and soil of Eastern zone than Southeastern zone. Unlike Mn, the concentrations of Zn are significantly higher in both the fruit and soil of Southeastern zone than Eastern zone.

The concentrations of Ni are not significantly different in both the fruit and soil of these two zones. The concentrations of Cu in both the fruits are not significantly different in the two zones, but Cu concentration in soil of Southeastern zone is significantly higher than Eastern zone. From all metals in soil, only the metals that are bioavailable are transferred to the plants. These factors are widely different from one geographical location to another. Chemical compositions of plants which grow in different geographical location might be differing due these and other factors [14, 15, 16, 17].

This difference in concentration in both the fruits and soils might be due to the difference of soil nature of the two zones. Soil natures of Eastern and Southeastern zones are sand and clay type respectively. Clay soils have the smallest particles with numerous pore spaces between them and are highly effective holders of both water and nutrients because water and nutrients cannot leach easily, but sandy soils have just the opposite problem because the pore spaces between particles are too large they drain too well and water and nutrients are leached away easily. Therefore clay type soils contain relatively large amount of water and other nutrients than sand type soils [18].

Comparison level of metals between fruits of different species of the same zone

Concentrations of K, Mg, Ca, Cu, Mn, Ni and Zn between the two species of cactus fruits (*Opuntia ficus indica* and O*puntia stricta*)

which cultivates in the same zone are not significantly different (at 0.05 levels). In general the chemical composition of the two species are not significantly different, example the chemical composition of mucilage of the two cactus plants (*Opuntia ficus indica* and *Opuntia stricta*) cultivated in Tigray have relatively same amount of crude fiber content, fat content, bulk and tapped density, protein content and pH values. From this similarity in chemical composition of these species, their potential for metals uptake may not significantly different [19].

Comparison of present study with different international guidelines

Different international organizations have been developing regulatory frameworks and guidelines for metals level in the environment, drinking water and foodstuffs. Since these cactus fruits are consumed freshly, it is better to calculate the metals daily intake from these fruits. Accordingly the daily intake of metals through the consumption of cactus fruits was calculated based on the equation below.

Daily intake of metals (mg/day) = daily fruit consumption (kg/day, dry weight) X average metals concentration in fruit (mg/kg) [20].

As it is shown from Table 5, even though the metals daily intake from both the cactus fruits (*Opuntia ficus indica* and *Opuntia stricta*) of this study is below the adequate intake, the society of the area got another additional concentration from other daily intakes. This indicates that, the concentration of the major and minor-essential metals in fruits of these two cactus species (*Opuntia ficus indica* and *Opuntia stricta*) are in the safe side.

Conclusion

In the present work fruits of two cactus species (*Opuntia ficus indica* and *Opuntia stricta*) which cultivated in two zones of Tigray region along with supporting soil were analyzed for their contents of some major, minor-essential and toxic metals. In the present investigation the levels of major (K, Mg and Ca) and minor-essential metals (Mn, Cu, Ni and Zn) in fruits of cactus species and supporting soil from both sampling zones are found in appreciable amount but also within the safety limits. Whereas Toxic metals (Pb and Cd) are not detected in both fruit of cactus species but Pb is detected in supporting soil of both zones. These results indicates that fruit of cactus species are free from Cd and Pb metals toxicity so suitable for safe consumption although Pb is detected in both soils of cactus growing zones. Presence of Pb in supporting soil may be from the parent material of the soil nature or may be due to the usage of different pesticides for agricultural purpose around the cactus cultivation areas. The concentrations of K, Ca and Zn are significantly higher in both the fruits (*Opuntia ficus indica* and *Opuntia stricta*) and soils of Southeastern zone than Eastern zone. Mn is significantly higher concentration in both the fruits (*Opuntia ficus indica* and *Opuntia stricta*) and soil of Eastern zone than Southeastern zone. The concentrations of Ni in both the fruit samples (*Opuntia ficus indica* and *Opuntia stricta*) and soil samples are not significantly different in the two zones. In the comparison of metals concentration of different fruit species that cultivated in same geographical location (area), the concentration differences are not significant for all the metals. In the present work the concentration levels of some major, minor-essential and toxic metals in the fruit of cactus species was compared with different international guidelines (WHO and IOM) reported in literature. Although the metals concentration in the fruits are below the adequate intake the society of the area got additional amount from another daily intakes. Therefore concluded that fruits of *Opuntia ficus indica* and *Opuntia stricta* cultivated in the two zones of Tigray region are good source of essential metals but free from bioaccumulation of toxic metals like Pb and Cd so suitable for safe human consumption.

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