Global Scientific JOURNALS

GSJ: Volume 8, Issue 1, January 2020, Online: ISSN 2320-9186

www.globalscientificjournal.com

Comparative Study of Heavy Metals Concentrations and Dietary Reference Intakes in Selected Foodstuffs

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Abstract

Heavy metals of selected foodstuffs (egg, banana, onion and milk) obtained from three different locations in Khartoum state, Sudan were determined and compared with dietary reference intake for both adult and children using Student's *t*-test. The foodstuffs samples were digested using dry ashing procedure and their minerals were determined by Atomic Emission spectrophotometer (ICPE). The results showed that intake of 100 gm per day of egg by adults or children significantly ($P < 0.05$ or $P < 0.01$) increased the level of Sc, Al, Cu, Hg, As, and Ba and exceeded the dietary reference intake in all locations. Intake of banana (100 gm per day) significantly ($P < 0.05$ or $P < 0.01$) increased the level of TI, I, Cr, Al, Pb, and Ba in all locations while intake of onion significantly ($P < 0.05$ or $P < 0.01$) increased the level of Sc, I, Cr, Se, Al, As, and Ba. However, intake of 100gm per day of milk had no adverse effect and increased ($P < 0.05$) the level of Sc, As and Ba only. It was observed that Ba is the predominant elements in all foodstuffs and locations and significantly ($P < 0.05$ or $P < 0.01$) exceeded the dietary reference intake.

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Keywords: heavy metals, dietary reference intakes, egg, banana, onion, milk

Introduction

The increasing demands for food and food safety has drawn the attention of researchers to the risks associated with consumption of contaminated foodstuffs i.e. pesticides, heavy metals and or toxins in fruits and vegetables (Radwan and Salama, 2006). Contaminants include micro-minerals (when higher than normal) and heavy metals. Microminerals, also known as trace elements or trace minerals, include those nutrients that are required in milligram or microgram quantities by organisms on a daily basis (Berdanier and Zempleni, 2009). The main function of micro-minerals, excluding boron and chloride, is to serve as constituents of prosthetic groups in metalloproteins and as activators of enzyme reactions and without trace elements the enzyme system in organisms would be an inert mass of proteins (Gupta, et la., 2008). Heavy metals are among the major contaminants of food supply and may be considered the most important problem to the environment and the problem is becoming more critical all over the world especially developing countries (Tegegne, 2015). Heavy metal contamination of foodstuffs may occur due to irrigation with contaminated water, addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting processes, storage and/or sale (Radwan and Salama, 2006). The intake of heavy metals in high levels can interact with some essential nutrients in the body, which in turn causes a decrease in body immunity, growth retardation, psychosocial dysfunctions, disabilities associated with malnutrition and a prevalence of gastrointestinal cancer (Turkdogan, et al., 2003). It is well known that plants take up metals by absorbing them from contaminated soils as well as from deposits on parts of the plants exposed to the air from polluted environments (Khairiah et al., 2004). Surveys have been carried out on trace element concentrations in infant foods, human milk, and powdered infant formula determining mineral elements in the digestion solutions (LaKind et al., 2004). Concentrations in animals may increase due to environmental contamination resulting in toxic elements in foodstuff (Coni et al., 1999). Infants and young children may absorb 50% of dietary toxic metals as compared with only 10% by adults (Krachler et al., 2000). Heavy metals are dangerous because they tend to bio-accumulate in a biological organism faster than being metabolized or excreted (Li et al., 2005). Some metals form stable covalent complexes and interact with macromolecules with affinity for organic binding (Silva et al., 2005), possessing damaging action at the molecular level. Most of the researches done are limited to study few heavy metals in foodstuffs. It is therefore necessary to widen the scope of the micro-minerals and heavy metal analysis to include more foods and provide adequate information. Therefore, this study was carried out to determine the levels of heavy metals in selected foodstuffs collected from different locations in Khartoum state, Sudan.

Materials and methods

Sample collection and preparation

A total of 36 samples (4 Foodstuffs X 3 locations X 3 samples) of foodstuffs namely, egg, banana, onion and milk and were purchased (3 kg, for each commodity from each location) from different locations (site A, Khartoum North; site B, Khartoum; and site C, Omdurman) in Khartoum State. For the analysis of banana and onion, only the edible portions were included, whereas bruised or rotten parts were removed. Sub-samples (1.5 kg, each) were taken at random from the composite sample (3 kg) and were first freeze dried for 24 h. The dried samples were powdered in a mixer grinder taking care not to overheat the sample.

Determination of heavy metals

Three powdered samples (0.5 g, each) with three replicates for each food item were accurately weighed and placed in crucibles and few drops of concentrated nitric acid were added to the solid as an ashing aid. Dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550 ^{o}C and then left to ash for 4 h. A blank solution was prepared at the same time with the sample. To determine metal concentration, the treated samples were injected into Atomic Emission spectrophotometer (ICPE), motel = 9000 (Shimaedzu, Japan) with the following condition: The ICPE. 9000 conditions used were Argon Gas (Ar), P (K Pa) = 533.50, plasma = 10.00, vacuum level (Pa) = 7.2, Direction = Axial, Auxiliary = 0.60, Nozzle Dest = Ro. The identification and quantification of each metal was determined using a standard solution of each.

Quality Assurance

The quality assurance of the method for the estimation of heavy metals in egg samples by ICPE has been done by calibrating the instrument with multi element calibration standard 2A (Lot No. 28 – 68 JB) obtained from Agilent Technologies. The method validity was further ascertained by spike recovery and replicate analysis.

Statistical analysis

Data were analyzed by independent student’s t-test with SPSS version 15.0 (Chicago, IL) and differences were considered statistically significant at either ** $P<0.01$ or * $P<0.05$.

Results and discussion

Table 1 shows the trace elements contents (mg/100gm DM) of egg collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI). The heavy metal content determined was based on dry weight of samples. The intake of heavy metals is calculated based on consumption of 100 gm egg per day per person. It was observed that the level of Sc, Al, Cu, Hg, As, and Ba significantly ($P < 0.05$ or $P < 0.01$) exceeded that of dietary reference intake when the consumer take 100 mg or more in all locations. The results showed that the level of Sc in egg obtained from all locations was ranged from 0.12 to 0.52 mg/100gm compared to that of dietary reference intake (0.001 mg/day). Cu content in all locations varied from 3.44 to 7.75 mg/100gm and significantly ($P < 0.01$) higher than that of adult and children dietary reference intake (1.5-2 mg/day) when the consumer takes ≤ 100 mg/day. Al content in all locations was found in the range of 13.58 - 18.81 mg/100gm and significantly ($P < 0.01$) higher than that of adult and children dietary reference intake (3.5-10 mg/day) when the consumer takes ≤ 100 mg/day. Also Hg, As and Ba in all locations gave results similar to the above elements. The level of other elements was found to be varied between locations and within the group of consumers (adults or children). Within the selected locations, the highest concentration of Al was observed in site B followed by site C and Si in site A followed by site C. Knowledge and assessment of the mineral content of eggs is very important for many reasons that are related to health and nutritional value of eggs. Therefore, monitoring and estimation of heavy metals is of great importance for nutritional, toxicological and environmental aspect (Abdulkhaliq et al., 2012). The heavy metals like Cr, Co, Cu, Fe, Mn and Zn are essential, which are so called micronutrients and are toxic when taken in above threshold levels. Whereas, the heavy metals especially Pb, Cd, Hg and As are non-essential elements and even toxic in trace levels (Siddiqui et al., 2011). The transfer of metals in eggs is likely to be takes place in the sequential order from soil to local feed to hen and to eggs. The main sources of getting the high levels of these micro metals are feeding behavior of hens, anthropogenic activities and environmental contamination. In general, Fakayode and Olu-Owolabi (2003) found strong positive correlations between the levels of metals in the feeds and the corresponding levels of metals in the eggs. Table 2 shows the trace elements contents (mg/100gm DM) of banana collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI). The intake of heavy metals is calculated based on consumption of 100 gm egg per day per person. It was observed that the level of TI, I, Cr, Al, Pb and Ba significantly ($P < 0.05$ or $P < 0.01$) exceeded that of dietary reference intake when the consumer take 100 mg or more banana in all locations. The results showed that the level of TI in banana obtained from all locations was ranged from 5.61 to 7.62 mg/100gm compared to that of dietary reference intake (5 mg/day). Iodine content in all locations varied from 0.49 to 0.92 mg/100gm and significantly ($P < 0.05$) higher than that of adult and children dietary reference intake (0.11-0.15 mg/day) when the consumer takes ≤ 100 mg/day. Cr content in all locations was found in the range of 0.30-0.34 mg/100gm and significantly ($P < 0.05$) higher than that of adult and children dietary reference intake (0.1-0.12 mg/day) when the consumer takes ≤ 100 mg/day. Moreover, Al, Pb and Ba in all locations gave results similar to the above elements. The level of other elements was found to be varied between locations and within the group of consumers (adults or children). Within the selected locations, the highest concentration among elements was Al and was observed in site C followed by site B and A. Table 3 shows the trace elements contents (mg/100gm DM) of fresh onion collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI). It was observed that the level of Sc, I, Cr, Se, Al, As and Ba significantly ($P < 0.05$ or $P < 0.01$) exceeded that of dietary reference intake when the consumer take 100 mg or more onion in all locations. The results showed that the level of Sc in onion was ranged from 0.127 to 0.143 mg/100gm in all locations compared to that of dietary reference intake (0.001 mg/day). Iodine content in all locations varied from 2.08 to 2.75 mg/100gm and significantly ($P < 0.01$) higher than that of adult and children dietary reference intake (0.11-0.15 mg/day) when the consumer takes ≤ 100 mg/day. Cr content in all locations was found in the range of 0.36-0.39 mg/100gm and significantly ($P < 0.05$) higher than that of adult and children dietary reference intake (0.1-0.12 mg/day) when the consumer takes ≤ 100 mg/day. In addition, Se, Al, As and Ba in all locations gave results similar to the above elements. The level of other elements was found to be varied between locations and within the group of consumers (adults or children). Within the selected locations, the highest concentration among elements was Al and was observed in site A followed by site C and B. Comparison with levels in fruit and vegetable in the present study revealed that the levels are generally comparable. Similar studies in fruits and vegetables were reported by many researchers (Dogheim et al., 2004; Karavoltsos et al., 2002; Parveen et al., 2003). Heavy metal depositions are associated with a wide range of sources such as small scale industries, brick kilns, vehicular emissions, re-suspended road dust and diesel generator sets.

Vegetable and fruits are often grown in polluted and degraded environmental conditions in the urban areas and are subjected to further pollution from vehicles and industries during marketing. Emissions of heavy metals from the industries and vehicles may be deposited on the vegetables and fruits surfaces during their production, transport and marketing. Al Jassir et al. (2005) have reported elevated levels of heavy metals in vegetables sold in the markets at Riyadh city in Saudi Arabia due to atmospheric deposition. Sharma et al. (2008) have reported that atmospheric deposition can significantly elevate the levels of heavy metals contamination in vegetables commonly sold in the markets in India. The prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardio-vascular, nervous, kidney and bone diseases (Agrawal et al., 2010). Some heavy metals such as Cu, Zn, Mn, Co and Mo act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As, and Cr act as carcinogens (Nagajyoti et al., 2010). The contamination of vegetables with heavy metals due to soil and atmospheric contamination poses a threat to its quality and safety. Dietary intake of heavy metals also poses risk to animals and human health. Heavy metals such as Cd and Pb have been shown to have carcinogenic effects (Nagajyoti et al., 2010). High concentrations of heavy metals (Cu, Cd and Pb) in fruits and vegetables were related to high prevalence of upper gastrointestinal cancer (Turkdogan et al., 2002). Table 4 shows the trace elements contents (mg/100gm DM) of milk collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI). It was observed that the level of Sc, As and Ba significantly ($P < 0.05$) exceeded that of dietary reference intake when the consumer take 100 mg or more milk in all locations. The results showed that the level of Sc in milk obtained from all locations was ranged from 0.125 to 0.226 mg/100gm compared to that of dietary reference intake (0.001 mg/day). Arsenic (As) content in all locations varied from 0.081 to 0.091 mg/100gm and significantly ($P < 0.05$) higher than that of adult and children dietary reference intake (0.002-0.003 mg/day) when the consumer takes ≤ 100 mg/day. Ba content in all locations was found in the range of 0.038-0.047 mg/100gm and significantly ($P < 0.05$) higher than that of adult and children dietary reference intake (0.001mg/day) when the consumer takes ≤ 100 mg/day. The content of trace elements in milk is determined by a variety of factors, including the content of a given element in soil, the content of energy in feed, the level of the organic components as fats, vitamins, protein, changes in absorption and retention of a given element, traffic along highway roads, fodder contamination, climatic factors and pesticide application. The important factors that influenced the occurrence of trace elements in milk, including toxic ones, are environmental pollution, mainly of anthropogenic origin as well as phenomena of inter element interactions. AbdulKhaliq et al. (2012) reported that Pb and Cd concentrations in milk and dairy products (except white cheese) exceeded the maximum allowed values. The elevated levels could be related to contamination during industry processing and environmental pollution.

Conclusion

It could be concluded that more than six heavy metals determined were found to exceed the dietary reference intake. Therefore, the consumption of average amounts (100 mg/day) of these foodstuffs including egg, banana, onion and to some extend milk pose a health risk for the consumer. The present study provides additional data on heavy metals pollution in the three locations in Khartoum State and also can help in risk assessment of consumer exposure to the expected heavy metal levels. It is therefore suggested that regular survey of heavy metals should be done on all food commodities in order to evaluate whether any health risks from heavy metal exposure do exist, to assure food safety and to protect the end user from food that might lead to health risk for both adults and children.

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Table 1. Trace elements contents (mg/100gm DM) of egg collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI).

Mineral	DRI$^{\Delta}$ (mg/day)		Site A			Site B			Site C		
	Adult	Children	Cal. Value (mg/100gm)	Mean difference Adult	Mean difference Children	Cal. Value (mg/100gm)	Mean difference Adult	Mean difference Children	Cal. Value (mg/100gm)	Mean difference Adult	Mean difference Children
Sc	0.001	0.001	0.52±0.04	0.52**	0.52**	0.12±0.012	0.119**	0.119**	0.14±0.07	0.139**	0.139**
Si	10	5	9.54±0.39	-1.46*	4.54**	7.46±0.352	-2.54**	2.46**	8.32±0.41	-1.68*	3.32**
Sr	0.05	1	0.40±0.021	0.35*	-0.6*	0.085±0.01	0.035	-0.915*	0.10±0.04	0.05*	0.900**-
Tl	5	5	1.35±0.042	-3.65**	-3.65**	0.31±0.023	-4.69**	-4.69**	0.32±0.01	4.68**	4.68**
Yb	N/A	N/A	0.21±0.082	N/A	N/A	0.03±0.002	N/A	N/A	0.07±0.005	N/A	N/A
Li	1.00	1.00	0.28±0.052	-0.72**	-0.72**	0.05±0.001	-0.95*	-0.95*	0.06±0.004	-0.94**	-0.94**
Co	0.04	0.05	0.013±0.003	-0.027*	-0.037*	0.01±0.002	-0.03	-0.04	0.01±0.004	0.03*	0.04*
Fe	15	8	7.34±0.57	-7.66**	-0.66*	10.17±0.37	-4.83**	2.17**	11.81±0.82	-3.19**	3.81**
Zn	11	8	4.58±0.47	-6.42**	-3.42**	5.23±0.64	-5.77**	-2.77**	4.40±0.16	-6.60**	-3.6**
Mn	5	2.5	0.59±0.04	-4.41**	-1.91*	1.11±0.03	-3.89**	-1.39**	1.14±0.07	-3.86**	-1.36**
Cu	2	1.5	3.44±0.97	1.44**	1.94*	7.75±0.34	5.75**	6.25**	7.76±0.68	5.76**	6.26**
Mo	0.10	0.07	0.05±0.006	-0.05	-0.02	0.05±0.004	-0.05	-0.02	0.05±0.003	-0.05	-0.02
I	0.15	0.11	0.09±0.01	-0.06	-0.02	0.07±0.003	-0.08	-0.04	0.09±0.01	-0.06	-0.02
Cr	0.12	0.10	1.26±0.04	1.14**	1.16**	0.29±0.08	0.17*	0.19*	0.31±0.02	0.19*	0.21*
Se	0.04	0.03	0.08±0.03	0.04	0.05	0.06±0.01	0.02	0.03	0.07±0.001	0.03	0.04
Al	10	3.5	13.58±0.21	3.58*	10.08**	18.83±0.54	8.83**	15.33**	16.17±0.69	6.17**	12.67**
Cd	0.002	0.001	0.004±0.001	0.002	0.003	0.004±0.00	0.002	0.003	0.004±0.00	0.002	0.003
Hg	0.002	0.001	0.01±0.004	0.008*	0.009*	0.01±0.00	0.008*	0.009*	0.01±0.001	0.008*	0.009*
As	0.003	0.002	0.091±0.002	0.088**	0.089**	0.09±0.01	0.087**	0.088**	0.09±0.001	0.087**	0.088**
Pb	0.10	0.10	0.04±0.001	-0.06*	-0.06*	0.04±0.00	-0.06*	-0.06*	0.04±0.003	-0.06*	-0.06*
Ni	0.85	0.35	0.02±0.003	-0.83**	-0.33**	0.037±0.005	-0.813**	-0.313**	0.02±0.003	-0.83**	-0.33**
Ba	0.001	0.001	0.22±0.013	0.219**	0.219**	0.032±0.003	0.031**	0.031**	0.07±0.004	0.069**	0.069**

Values are means of 6 samples ±SD. ** P<0.01, * P<0.05. Mean difference= calculated value- dietary reference intakes (DRI). N/A= not available. $^{\Delta}$FAO, WHO. "Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Geneva: Food and Agriculture Organization." *World Health Organization* (2004). Minerals needed in only small amounts, generally less than 20 milligrams per day, are called trace minerals (https://healthyeating.sfgate.com/list-trace-minerals-4893.html).

Table 2. Trace elements contents (mg/100gm DM) of banana collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI).

Mineral	DRI$^{\Delta}$ (mg/day)		Site A			Site B			Site C		
			Cal. Value	Mean difference		Cal. Value	Mean difference		Cal. Value	Mean difference	
	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children
Sc	0.001	0.001	0.12±0.005	0.119*	0.119*	0.101±0.03	0.10	0.10	0.125±0.023	0.124*	0.124*
Si	10	5	0.85±0.037	-9.15**	-4.15**	0.87±0.021	-9.13**	-4.13**	0.885±0.046	-9.115**	-4.115**
Sr	0.05	1	0.075±0.004	0.02	-0.93*	0.082±0.004	0.032*	-0.918**	0.085±0.005	0.035*	-0.915**
Tl	5	5	7.24±0.452	2.24**	2.24**	7.617±0.132	2.617**	2.617**	5.609±0.21	0.609*	0.609*
Yb	N/A	N/A	0.042±0.003	N/A	N/A	0.027±0.004	N/A	N/A	0.052±0.003	N/A	N/A
Li	1.00	1.00	0.037±0.001	-0.963**	-0.963**	0.0026±0.00	-0.997**	-0.997**	0.063±0.001	-0.937**	-0.937**
Co	0.04	0.05	0.015±0.00	-0.025	-0.035	0.015±0.001	-0.025	-0.035	0.016±0.001	-0.024	-0.034
Fe	15	8	4.20±0.123	-10.80**	-3.80**	5.492±0.28	-9.508**	-2.508**	2.722±0.132	-12.28**	-5.28**
Zn	11	8	4.69±0.432	-6.31**	-3.31**	3.417±0.36	-7.583**	-4.583**	2.95±0.235	-8.05**	-5.05**
Mn	5	2.5	1.61±0.04	-3.39**	-0.89*	1.732±0.32	-3.268**	-0.768*	1.67±0.212	-3.33**	-0.83*
Cu	2	1.5	0.74±0.05	-1.26**	-0.76*	0.64±0.88	-1.36**	-0.86*	8.51±0.421	6.51**	7.01**
Mo	0.10	0.07	0.057±0.006	-0.043	-0.013	0.058±0.005	-0.042	-0.012	0.067±0.002	-0.033	-0.003
I	0.15	0.11	0.92±0.094	0.77*	0.81*	0.49±0.086	0.34*	0.38*	0.72±0.121	0.57*	0.61*
Cr	0.12	0.10	0.302±0.021	0.182*	0.202*	0.342±0.004	0.222*	0.242*	0.33±0.0056	0.21*	0.23*
Se	0.04	0.03	0.106±0.002	0.066	0.076	0.106±0.006	0.066	0.076	0.107±0.001	0.067	0.077
Al	10	3.5	12.00±0.56	2.00**	8.5**	14.167±0.79	4.167**	10.667**	19.417±0.812	9.417**	15.917**
Cd	0.002	0.001	0.005±0.00	0.003	0.004	0.0048±0.00	0.0028	0.0038	0.006±0.001	0.004	0.005
Hg	0.002	0.002	0.01±0.00	0.008	0.008	0.0095±0.00	0.0075*	0.0075*	0.0107±0.002	0.0087	0.0087
As	0.003	0.002	0.08±0.004	0.077*	0.078*	0.081±0.001	0.078	0.079	0.0807±0.004	0.078*	0.079*
Pb	0.10	0.10	0.42±0.005	0.32*	0.32*	0.41±0.004	0.31*	0.31*	0.427±0.006	0.327*	0.327*
Ni	0.85	0.35	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*
Ba	0.001	0.001	0.06±0.012	0.059*	0.059*	0.022±0.003	0.021*	0.021*	0.044±0.002	0.043*	0.043*

Values are means of 6 samples ±SD. ** $P<0.01$, * $P<0.05$. Mean difference= calculated value- dietary reference intakes (DRI). N/A= not available. $^{\Delta}$FAO, WHO. "Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Geneva: Food and Agriculture Organization." World Health Organization (2004). Minerals needed in only small amounts, generally less than 20 milligrams per day, are called trace minerals (https://healthyeating.sfgate.com/list-trace-minerals-4893.html).

Table 3. Trace elements contents (mg/100gm DM) of onion collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI).

Mineral	DRI$^{\Delta}$ (mg/day)		Site A			Site B			Site C		
			Cal. Value	Mean difference		Cal. Value	Mean difference		Cal. Value	Mean difference	
	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children
Sc	0.001	0.001	0.143±0.012	0.142*	0.142*	0.127±0.012	0.126*	0.126*	0.135±0.021	0.134*	0.134*
Si	10	5	1.736±0.023	-8.264**	-3.264**	1.40±0.087	-8.60**	-3.60**	1.822±0.076	-8.178**	-3.178**
Sr	0.05	1	0.340±0.005	0.29*	-0.66*	0.36±0.003	0.31*	-0.64*	0.359±0.032	0.309*	-0.641*
Tl	5	5	3.731±0.32	-1.269**	-1.269**	3.036±0.064	-1.964**	-1.964**	3.580±0.0987	-1.42**	-1.42**
Yb	N/A	N/A	0.042±0.003	N/A	N/A	0.027±0.003	N/A	N/A	0.049±0.007	N/A	N/A
Li	1.00	1.00	0.0046±0.00	-0.995**	-0.995**	0.0037±0.00	-0.996**	-0.996**	0.0045±0.00	-0.995**	-0.995**
Co	0.04	0.05	0.0137±0.002	-0.0263	-0.0363	0.014±0.001	-0.026	-0.036	0.0136±0.001	-0.0264	-0.0364
Fe	15	8	0.826±0.082	-14.174**	-7.174**	0.629±0.07	-14.37**	-7.37**	0.844±0.041	-14.156**	-7.156**
Zn	11	8	0.49±0.078	-10.51**	-7.51**	0.38±0.021	-10.62**	-7.62**	0.52±0.046	-10.48**	-7.48**
Mn	5	2.5	0.287±0.014	-4.713**	-2.213**	0.33±0.003	-4.67**	-2.17**	0.329±0.025	-4.671**	-2.171**
Cu	2	1.5	0.322±0.073	-1.678**	-1.178**	0.214±0.06	-1.786**	-1.286**	0.418±0.087	-1.582**	-1.082**
Mo	0.10	0.07	0.056±0.002	-0.044	-0.014	0.057±0.002	-0.043	-0.013	0.056±0.006	-0.044	-0.014
I	0.15	0.11	2.75±0.023	2.60**	2.64**	2.33±0.083	2.18**	2.22**	2.08±0.023	1.93**	1.97**
Cr	0.12	0.10	0.391±0.03	0.271*	0.291*	0.363±0.021	0.243*	0.263*	0.384±0.043	0.264*	0.284*
Se	0.04	0.03	0.704±0.05	0.664*	0.674*	0.803±0.002	0.763*	0.773*	0.704±0.002	0.664*	0.674*
Al	10	3.5	18.75±0.79	8.75**	15.25**	13.33±0.876	3.33**	9.83**	14.17±0.473	4.17**	10.67**
Cd	0.002	0.001	0.004±0.00	0.002	0.003	0.004±0.00	0.002	0.003	0.004±0.00	0.002	0.003
Hg	0.002	0.002	0.0096±0.001	0.0076	0.0076	0.010±0.00	0.0076	0.0076	0.010±0.00	0.0075	0.0075
As	0.003	0.002	0.0818±0.003	0.0788*	0.0798*	0.083±0.003	0.080*	0.081*	0.081±0.003	0.078*	0.079*
Pb	0.10	0.10	0.0401±0.002	-0.0599	-0.0599	0.0402±0.006	-0.0598*	-0.0598*	0.040±0.004	-0.0598*	-0.0598*
Ni	0.85	0.35	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*
Ba	0.001	0.001	0.055±0.003	0.054**	0.054**	0.053±0.002	0.052*	0.052*	0.051±0.004	0.05*	0.05*

Values are means of 6 samples ±SD. ** $P<0.01$, * $P<0.05$. Mean difference= calculated value- dietary reference intakes (DRI). N/A= not available. $^{\Delta}$FAO, WHO. "Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Geneva: Food and Agriculture Organization." World Health Organization (2004). Minerals needed in only small amounts, generally less than 20 milligrams per day, are called trace minerals (https://healthyeating.sfgate.com/list-trace-minerals-4893.html).

Table 4. Trace elements contents (mg/100gm DM) of milk collected from different sites in Khartoum State and correlated with the dietary reference intakes (DRI).

Mineral	DRI$^{\Delta}$ (mg/day)		Site A			Site B			Site C		
			Cal. Value	Mean difference		Cal. Value	Mean difference		Cal. Value	Mean difference	
	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children	(mg/100gm)	Adult	Children
Sc	0.001	0.001	0.226±0.043	0.225*	0.225*	0.226±0.0364	0.225*	0.225*	0.125±0.008	0.124*	0.124*
Si	10	5	3.213±0.435	-6.787**	-1.787**	3.083±0.183	-6.917**	-1.917**	0.885±0.0437	-9.115**	-4.115**
Sr	0.05	1	0.143±0.056	0.093	-0.857**	0.207±0.043	0.157*	-0.793*	0.085±0.007	0.035	-0.915*
Ti	5	5	3.543±0.435	-1.457**	-1.457**	3.513±0.218	-1.487**	-1.487**	5.609±0.197	0.609*	0.609*
Yb	N/A	N/A	0.023±0.003	N/A	N/A	0.023±0.004	N/A	N/A	0.052±0.004	N/A	N/A
Li	1.00	1.00	0.0058±0.00	-0.994*	-0.994**	0.006±0.00	-0.994*	-0.994*	0.0596±0.008	-0.944*	-0.944*
Co	0.04	0.05	0.013±0.002	-0.027	-0.037*	0.013±0.002	-0.027	-0.037	0.0161±0.003	-0.0239	-0.039
Fe	15	8	0.85±0.324	-14.15**	-7.15**	0.68±0.042	-14.32**	-7.32**	0.6596±0.0643	-14.344**	-7.344**
Zn	11	8	0.472±0.165	-10.528**	-7.528**	0.372±0.012	-10.628**	-7.628**	0.35±0.032	-10.65**	-7.65**
Mn	5	2.5	0.212±0.045	-4.788**	-2.288**	0.145±0.032	-4.855**	-2.355**	0.671±0.0381	-4.329**	-1.829**
Cu	2	1.5	0.0729±0.013	-1.927**	-1.427**	0.095±0.014	-1.9045**	-1.4045**	0.071±0.002	-1.929**	-1.429**
Mo	0.10	0.07	0.023±0.001	-0.077	-0.047	0.012±0.001	-0.088	-0.058	0.013±0.006	-0.087	-0.057
I	0.15	0.11	0.018±0.002	-0.132	-0.092	0.014±0.001	-0.136*	-0.096	0.016±0.008	-0.134	-0.094
Cr	0.12	0.10	0.029±0.003	-0.091	-0.071	0.027±0.002	-0.093	-0.073	0.033±0.001	-0.087	-0.067
Se	0.04	0.03	0.002±0.00	-0.038*	-0.028**	0.0017±0.00	-0.0383	-0.0283*	0.002±0.000	-0.038*	-0.028*
Al	10	3.5	0.07±0.003	-9.93**	-3.43**	0.08±0.004	-9.92**	-3.42**	0.075±0.003	-9.925**	-3.425**
Cd	0.002	0.001	0.0038±0.00	0.0018*	0.0028*	0.0038±0.00	0.0018	0.0028	0.006±0.00	0.004	0.005
Hg	0.002	0.002	0.0099±0.00	0.0079	0.0079*	0.0099±0.00	0.0079	0.0079	0.011±0.002	0.009	0.009
As	0.003	0.002	0.091±0.004	0.088*	0.089*	0.091±0.003	0.0883*	0.0893*	0.081±0.006	0.078*	0.079*
Pb	0.10	0.10	0.038±0.002	-0.062	-0.062	0.038±0.001	-0.062	-0.062	0.043±0.005	-0.057	-0.057
Ni	0.85	0.35	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*	0.02±0.003	-0.83*	-0.33*
Ba	0.001	0.001	0.038±0.001	0.037*	0.037*	0.047±0.003	0.046*	0.046*	0.044±0.005	0.043*	0.043*

Values are means of 6 samples ±SD. ** $P<0.01$, * $P<0.05$. Mean difference= calculated value- dietary reference intakes (DRI). N/A= not available. $^{\Delta}$FAO, WHO. "Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Geneva: Food and Agriculture Organization." World Health Organization (2004). Minerals needed in only small amounts, generally less than 20 milligrams per day, are called trace minerals (https://healthyeating.sfgate.com/list-trace-minerals-4893.html).