American Journal of Applied and Industrial Chemistry
2018; 2(2): 8-14
http://www.sciencepublishinggroup.com/j/ajaic
doi: 10.11648/j.ajaic.20180202.11

Evaluation of Some Heavy Metals in Fresh Cow's Milk from Different Regions of Sudan

Mawia Hassan Elsaim[1, 2, *], Yahya Ali[1]

[1]Department of Chemistry, Faculty of Science and Technology, Abdulatif Alhamed University of Technology, Merowe, Sudan
[2]Department of Chemistry, College of Science, Beijing University of Chemical Technology, Beijing, China

Email address:
Maelsaimhu7@gmail.com (M. H. Elsaim), maelsaimhu@yahoo.com (M. H. Elsaim)
[*]Corresponding author

To cite this article:
Mawia Hassan Elsaim, Yahya Ali. Evaluation of Some Heavy Metals in Fresh Cow's Milk from Different Regions of Sudan. *American Journal of Applied and Industrial Chemistry*. Vol. 2, No. 2, 2018, pp. 8-14. doi: 10.11648/j.ajaic.20180202.11

Received: September 3, 2018; **Accepted**: September 14, 2018; **Published**: October 17, 2018

Abstract: Heavy metals considered as the most important contamination due to the industrialization and have the influence on its existence in milk and dairy products. Milk is a very important component of human diet. The presence of heavy metals in milk at high concentration may significant health problem. The present study is achieved to investigate the concentration of some heavy metals (Cu, Cr, Cd, Pb, and Zn) in fresh cow's milk samples available in north Sudan-Merowe using Atomic Absorption Spectrophotometer (AAS). The elements, Cd, Cr, and Pd were not detected in all the fresh cow's milk samples under study. There is no significant in the mean concentration of Cu and Zn between the fresh cow's milk samples of three farms where as that of Zn is significantly. Our results indicated that the mean concentration of Cu, in A 0.111, in B 0.180 and in C 0.130 mg\l respectively. However Zn in A 1.13, in B 1.42 and C 1.62 mg\l respectively. The study also focuses on prevalence of heavy metals to compared with ARD value and also with corresponding values of different countries available in literature. Our result of this paper it is so strong to meager the concentration of heavy metals and show an important.

Keywords: Atomic Absorption Spectrophotometer, Fresh Cow's Milk, Heavy Metals, Pollution, Merowe

1. Introduction

Heavy metals were persistent in contaminants environment that can cause serious environment and health hazards. They are released into the environmental from natural as well as an anthropogenic activates [1-4]. Some heavy metals like Cu, Cr and Zn are essential to maintain proper metabolic activity in living organisms, others like Pb and Cd are non-essential and have no biological role [5-7]. However, at high concentrations, even essential metals also cause toxicity to living organisms [8, 9]. Milk and its products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age people [10, 11]. Milk is excellent sources of calcium, vitamin, riboflavin, and phosphorus is a good source of protein, potassium, vitamin A, vitamin B-12 and niacin [12, 13]. Milk is contaminated with heavy metals such as zinc, lead, cadmium, selenium, sulphur, iodine and possibly even more dangerous arsenic and cyanide [2]. Due to the growing environmental pollution it is necessary to determine and monitor the levels of heavy metals in milk, because they can significantly influence human health [14-16] many reports indicate the presence of heavy metals in milk and other food products [4, 17, 18] they determination of trace inorganic constituents in milk is challenging task due to their complex emulsion like matrices and low concentration levels of the metal ions. Many digestion procedures to oxidize organic matrices of different samples have been reported in literature [19-21]. The acid digestion procedures are the most popular sample pre–treatment techniques for elemental determination in biological and environmental samples, and acid digestion induced by microwave energy is a well- established method [22, 23]. The determination of heavy metals can be performed by several instrumental techniques [24, 25] including indirect photometric chromatography, ion chromatography, flame atomic absorption spectrometry [26, 27, 28] furnace atomic absorption spectrometry [29]. Inductively coupled plasma optical emission spectrometry [6,30] potentiometric stripping [31] capillary zone electrophoresis [32] differential pulse anodic striping voltammetry [33] mid-infrared spectrometry [34] particle induced x-ray emission [35] and complex metric titration [36] in this study atomic absorption spectrophotometer was used [37, 38]. The aim of the present study is to determine the concentration of heavy metals namely Cr, Cd, Cu, Zn, and Pb in fresh cow's milk collected from three dairy farms of Merowe area the obtained mean elemental concentrations were compared with the corresponding values of different countries in the literature and the daily intake of these elements were also compared with the Recommended Dietary Allowance [RDA] values set by instrument was optimized to give maximum signal strength by adjusting the parameters such as wavelength, slit width, lamp current and sample energy for each element.

2. Martials and Methods

2.1. Sample Collection and Preparation

Fresh cow̓s milk form three grazing areas of Merowe metropolis was collected, packed in 50ml bottles and stored in deep freezer at a temperature of -20C^{o}. 20ml of fresh cow̓s milk sample was measured into beaker. This was digests by heating the content in the beaker to nearly dryness. After evaporation and cooling, 20ml of distilled. It was then transferred into 50ml sample bottle, labeled and heavy metals were determined using bulk scientific model VER 3.94 C model Atomic Absorption Spectrophotometer.

2.2. Data Assessment

Was collect and ordered all data in tables the concentration of some fresh cow's milk sample in ppm yielding positive results for the occurrence of microelements were transformed in to concentration in mg/l.

2.3 Reagents and Solution

The reagents which used of analytical grade (Merck). are: nitric acid 65% (RIEEL-DEHAEN AG), hydrogen peroxide 30% (Eurostar Scientific LTD), and concentrated hydrochloric acid. Double distilled water (deionized water) was used for the preparation of all solution. All samples were stored in plastic bottles.

2.4. Samples Collection

The three samples of fresh cow̓s milk of different area were collected from various location of Merowe north Sudan cites. The study was selected based on the dependence of farmers on lives stock production mainly from cow's is representative of the whole country as it is considered milk belt in Sudan fresh cow̓s milk samples were collected from their farms of Arrayan, Attakker and Egri in accordance with AOAC procedure.

2.5. Sample Preparation

To a 5ml aliquot of milk in a100ml volumetric flask, add 50ml of 24% (w/v) TCA and dilute to volume with deionized water shake the samples at 5-min intervals for 30min and filter. Transfer a 5-ml aliquot of the filtrate to a 50ml volumetric flask, add 1ml of 5% (w/v) lanthanum solution, and make to volume with deionized water.

Table 1. *Instrumental operating condition for determination of heavy metals in milk sample.*

Heavy Metals	Wave length (nm)	Slit Width (nm)	Lamp Current (mA)	Sample Energy (eV)
Cd	228.8	0.7	2.0	3.317
Cr	357.9	0.7	2.0	3.567
Cu	324.8	0.7	1.5	3.884
Pb	283.3	0.7	3.0	2.470
Zn	213.9	0.7	2.0	3.148

3. Results and Discussion

Heavy metals contents varies widely due to many factors such as difference between species, characteristic of manufacturing practices and possible contamination from equipment's during the process among the five elements analyzed, three sample (Cd, Cr and Pb) were not detected. This could be as a result of low pollution to these metals in the study areas.

3.1. Copper Concentration

Copper (Cu) concentrations obtained ranges between 0.111-0.180mg/l in this Study are below the accepted limit recommended dietary value of 1.5-3.0 mg/day. There is no significant differences.

Table 2. *The concentration of heavy metals in fresh cow̓s milk samples collected from Merowe.*

Cow milk concentration of Heavy metals (mg/kg)					
	Cd	Cr	Cu	Pb	Zn
A	ND	ND	1.11	ND	11.3
B	ND	ND	1.80	ND	14.2
C	ND	ND	1.30	ND	16.2

ND mean Not detected

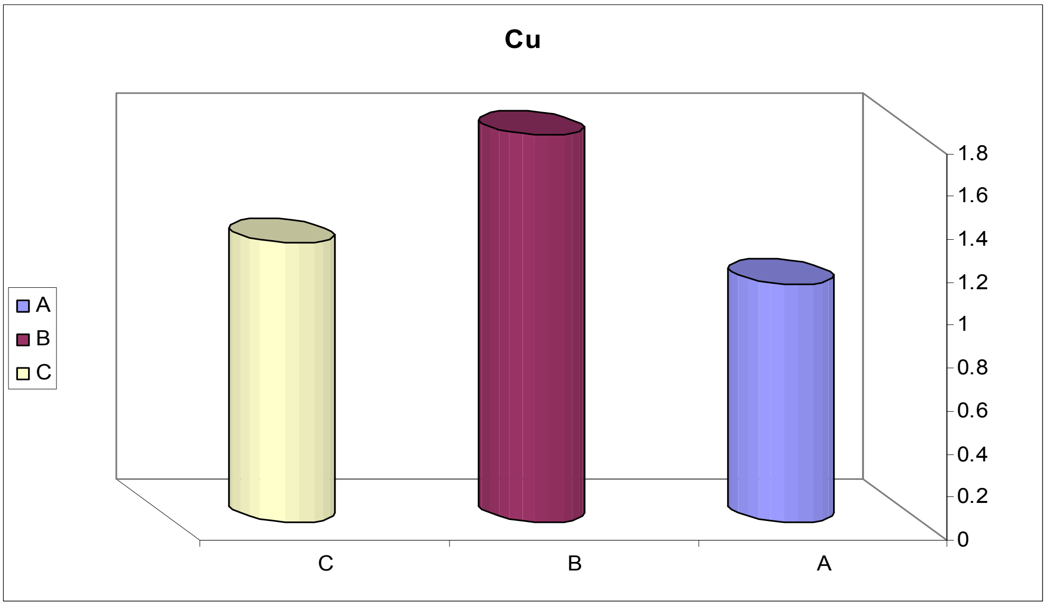

Figure 1. *Copper concentration (mg/g) of fresh cow's milk.*

3.2. Zinc Concentration

Has the least concentration 1.13mg/l and the highest concentration of 1.62mg/l both are below recommended dietary value of 12-15mg/day given by (NRC, 1989). Zinc plays an important role in the immune system but excessive absorption of zinc suppresses copper and iron absorption.

Among the five elements analyzed, three elements (Cr, Cd and Pb) were not detected this could be as a result of low pollution to these metals in the study areas of study. Many diverse biochemical roles of zinc have been identified. These include roles in enzyme function, nucleic acid metabolism, call signaling. Zinc is essential for physiological processes including development, lipid metabolism, brain and immune function [39]. It is also crucial for normal development and function of cells mediating nonspecific immunity [40].

Animal products except some such as milk are usually the best source of dietary zinc, in terms of both content and bioavailability. Because, diets in developing countries are predominantly based on plants and often high in phytates, which inhibit zinc absorption strongly, it can difficult for children in these countries to obtain their recommended intake of zinc from their usual diet [41]. In Merowe the consumption of zinc and other micronutrients is below the recommended dietary intake levels. A recent clinical trial revealed the importance of zinc supplementation to improve the growth.

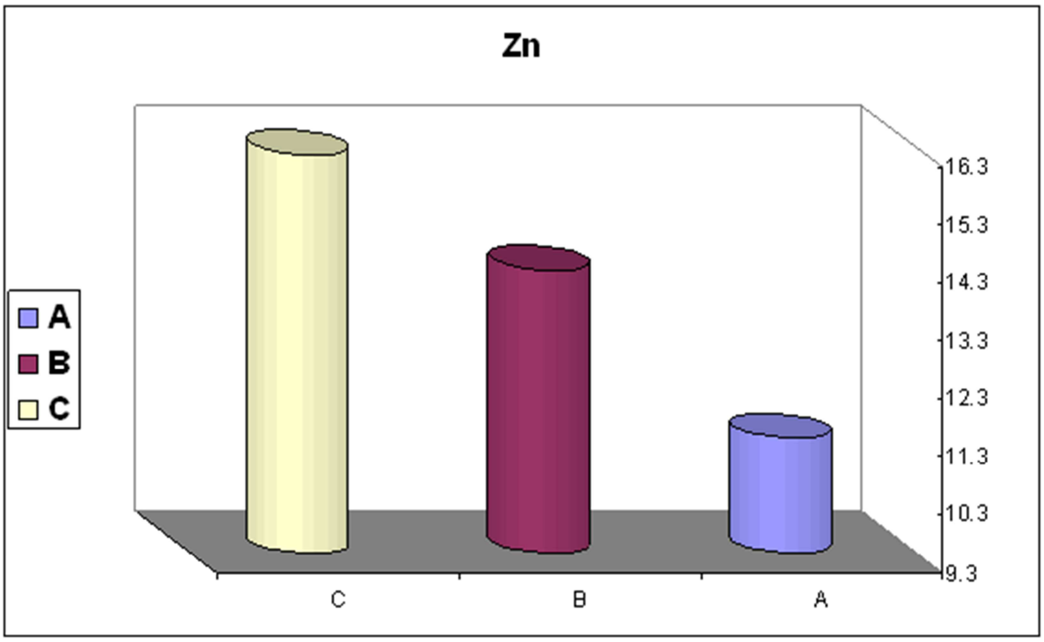

Figure 2. *Zinc concentration (mg/g) of fresh cow's milk.*

A zinc–deficient persons experience increased susceptibility to a variety of pathogens zinc is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells zinc deficiency also affects development of acquired immunity by preventing both the outgrowth and certain functions, of T lymphocytes and antibody production. The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiencies, which can dysregulate intracellular killing and phagocytosis [42].

It has been well established, both by studies in experimental animals and by human intervention trials, that zinc deficiency is growth-limiting. A deficiency of zinc in children causes manifestation of retardation in growth, related to decreased synthesis of growth hormone.

Among other endocrine disturbances attributed to the lack of zinc, shows insufficient development of gonad.

Zinc plays a role in certain sterility and male impotence.

It is also observed, in the regulation of thyroid abnormalities in individuals who have a rate of abnormally low plasma zinc.

Another consequence from lack of zinc is a geusia (decrease in taste and even the perception of abnormal taste) and reduction in the odor.

It is a symptom that often appears when receiving kidney dialysis and disappears after the administration of zinc [43].

3.3. Lead Concentration

Lead is toxic the blood and the nervous, urinary, gastric and genital systems [44]. Furthermore, it is also implicated in causing carcinogenesis and frontogenesis in experimental animals [10]. Lead exposure can cause anemia because it inhibits the body's ability to make hemoglobin. Several studies have also shown an association between lead exposure and increases in blood pressure. Lead readily crosses the placenta and there is evidence that exposure to high levels increases the risk of spontaneous abortion, miscarriage and stillbirth. There is also evidence of less strength that fetal lead espouser can increase the risk of reduced birth weight and premature birth. Human studies indicate the lead may have toxic effects on sperm starting at Pb B levels of around 40Mg|dL [45].

Children and fetuses are particularly more susceptible than adults, because their bodies are smaller, so the same dose of lead that an adult receives would have a greater effect on a child. Age-dependent differences in toxicological response can be due to differences in kinetics and be both quantitative and quantitative. Also the toxicological response can depend on the development of receptors to various chemicals which are developed at various stages. The brain is especially vulnerable during the brain growth spurt, which is the period when the brain undergoes several fundamental changes, like dendritic and axonal growth, synaptogenesis, rapid myelination, etc [46]. In children, even low levels exposure can result in reduced IQ, learning disabilities, attention deficit disorder behavioral problems, stunted growth, hyperactive behavior, impaired hearing, and kidney damage. The incidence of childhood cancer has increased, especially for acute lymphocytic leukemia, brain tumor and Wilmstumor. The last one is suspected to be in relation with paternal exposure to lead [47]. At high levels of exposure, a child may become mentally retarded, fall into a coma, and even die from lead poisoning. According to recent studies intellectual impairment in children has been seen at blood lead levels lower than 10 Mg/dL [48]. A level of concern is concentration above 25Mg/dL. Levels lower than 10Mg/dL [49].

3.4. Cadmium Concentration

Cadmium is considered to be one of the most toxic metals [10]. It is typical example of a cumulative poison. Prolonged increased uptake of cadmium interferes with the function of the kidneys and it is the target organ for damage and, when the concentration of cadmium in the renal cortex reaches approximately 200Mg\g, damage to the proximal renal tubules occurs and calcium, phosphorous, glucose, amino acids, and small peptides are lost in the urine (50). Once Accumulates in tissues it cannot be removed safely by chelation therapy wit out causing kidney damage [45].

3.5. Chromium Concentration

Major factors governing toxicity of chromium compounds are oxidation state and solubility. The more readily absorbed nature or ability of crossing biological membrane and its oxidation state accounts for greater toxicity of hexavalent chromium than the trivalent state. The toxicity of chromium within the cell may result from damage to cellular components during the hexavalent to trivalent chromium reduction process, by generation of free radicals, including DNA damage [57].

The effects of poisoning are evident within a matter of minutes in humans and animals and include haemorrhagicga stronger it is, heap to-cellular deficiency with icterus and disseminated intravascular coagulation syndrome, coma and eventually death [51].

Table 3. *Comparison of the elemental concentration (mg/kg) of fresh cow's milk in Sudan.*

Country	Cd	Cr	Cu	Pd	Zn	Reference
Japan	1.0	---	100.0	50.0	3000.0	[52]
Germany	0.1	----	49.9	5.5	3730.0	[53]
India	0.1	55.2	56.0	1.6	3100.0	[34, 54]
Spain	----	33.9	9.7	49.3	1419.3	[55]
Poland	15.0	38.0	90.0	20.0	3770.0	[56]
USA	9.7	29.1	19.4	34.0	2235.2	[57, 58]
Sudan	----	----	14.03	----	139	Present study

4. Conclusion

As mentioned, the importance of heaving heavy metals in milk and its products are very valuable today. Heavy metals can be trans-freed industrial sites to canals and rivers though direct discharge and runoff contaminated sites. In conclusion, in this present study the concentration of the heavy metals such as (Cd, Cr, and Pb) were not detected in the cow's of Merowe -Sudan even though they were reported in the cow's milk in several studies. The heavy metals Cu and Zn were detected in milk samples of Merowe zone in the all samples. The concentrations of Cu and Zn were 0.111 -0.130, 1.13 - 1.62 Mg/l, respectively in the milk samples of three area under study. The concentrations of heavy metals observed were comparable with some of the reported values in literature. The results obtained for detected elements in the present study were also compared with international daily intake guidelines of different international organizations for food and were found to be below the levels allowed. We suggest some project should be done to improve the operational efficiency and financial sustainability of provincial milk and dairy products and also improve water distribution system.

Acknowledgements

The author would like to thanks Dr. Nesreen Alrashid (University Abdulatif-Alhamad of Technology) for many useful suggestions for improvements of the article.

Conflicts of Interest

None of the authors have any conflict of interest associate with this study.

References

[1] Abduikhaliq, A., Swaileh, k. M., Hussein, R. M., Matani, M., 2012, Levels of metals (Cd, Pb, Cu and fe) in cow's milk, dairy products and hen's eggs from the west bank, Palestine., lnt Food Res J., 19 (3), 1089-1094.

[2] Nasr, l. N., Sallam, A. A. A., Abd El-khair, A. A., 2007, metals in fresh cow's milk at Gharbia governorate, Egypt., JAAl Sci., 7 (20), 3038-3044.

[3] Clarissa,. S. P. D. C, Andrea, F. A., Leandro, R. D. C., Jurandir, R. S. D., JezW. B. B., and Jose G. D., 2010, Toxic metals (Pb and Cd) and their respective antagonists (Ca and zn) in infant formulas and milk marketed in Brasilia, Brazil., lnt J Environ Res public Health., 7, 4062-4077.

[4] Sabiaa, S., D"Emiliob, M., Macchiatob, M., Anastasioc, a., Ragostad, M., Painoe, S., 2005, Metal Levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy., Environmental Research, 99, 48-5.

[5] Ayar, A. S. D., and Akin, N., 2009, the trace metal levels in milk and dairy products consumed in middle Anatolia-Turkey., Environ Monit Assess., 152, 1-12.

[6] Qin, L. Q., Wang, X. P., Li, W., Tong, W. J., 2009, The mineral and heavy metals in cow's milk from China and Japan., J Health Sci., 55 (2), 300-305.

[7] Khan, Z. I., Ahmad, K., Bayat, A., Mukhtar, M. K., Sher, M., 2013, Evaluation of lead concentration in pasture and milk; A possible risk for livestock and public health Pakistan J Zool., 45 (1), 79-84.

[8] Li, Y., McCrory, D. F., Powel, J. M., Saam, H., Jackson – Smith, D., 2005, A survey of selected heavy metal concentrations in Wisconsin Dairy Feeds. J Dairy Sci., 88., 2911-2922.

[9] Farid, S. M., Enani, M. A., Wajid, S. A., 2004, Determination of trace elements in cow's milk in Saudi Arabia., JKAU: Eng Sci., 15 (2), 131-140.

[10] Enb, A., Abou Donia, M. A., Abd -Rabou, N. S., Abou-Arab, A. A. K., El-Senaity, M. H., 2009, Chemical composition of raw milk and heavy metals behavior during processing of milk products., Global Veterinaria., 3 (3), 268-275.

[11] Asadi Dizaji, A., Eshaghi, A., AghajanzadehGolshani, A., Nazeradl, K., Y ari, A. A., Hoda, S., 2012, Evaluation and determination of toxic metals (Lead and Cadmium) in cow milk collected from East Azerbaijan, Iran., Eur J Exp Biol., 2 (1), 261-265.

[12] Ajai, A. I., Ochigbo, S. S., Ndamitso, M. M., Olaoluwajuwon, J., 2012, Proximate and mineral compositions of different raw cow's milks in Minna., Euro J ApplEngSci Res., 1 (1), 23-29.

[13] Anetta, L., Peter, M., Agnieszka, G., Jozef, G., 2012, Concentration of selected elements in raw and ultra heat treated com milk., J MicrobiolBiotechnol Food Sci., 2 (2), 795-802.

[14] Steijns, J. M., 2001, Milk ingredients as nutraceuticals., Int J Dairy Technol., 54, 81-88.

[15] Licata, P., Trombetta, D., Cristani, M., Giofre, F., Martino, D., Calo, M., Naccari, F., 2004, Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy., Environ Int., 30, 1-6.

[16] Singh, A., Sharma, R. K., Agrawal, M., Marshall, F. M., 2010, Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India., Food ChemToxicol., 48, 611-619.

[17] Soylak, M., Saracoglu, S., Tuzen, M., Mendil, D., 2005, Determination of trace metals in mushroom samples from kayseri, Turkey., Food Chem., 92, 649-652.

[18] Tuzen, M., Saracoglu, S., Soylak, M., 2008, Evaluation of trace element contents of powdered beverages from Turkey., J Food Nutr Res., 47, 120-124.

[19] Mingorance, M. D., 2002, Focused microwave-assisted digestion of vegetal materials for the determination of essential mineral nutrients., Anal Bioanal Chem., 373, 153-158.

[20] Arain, M. Kazi, T. G., Jamali, M. K., Afridi, H. I., Jalbani, N., Memon, A. R., 2007, Ultrasound-assisted pseudodigestion for toxic metals determination in fish muscles followed by electro thermal atomic absorption spectrophotometry: multivariate strategy., J AOAC Int., 90, 1118-1127.

[21] Shah, A, Q., Kazi, T. G., Arain, M. b., Jamali, M. K., Afridi, H, I., Jalbani, N., J. A., Sarfraz, R. A., Ansari, R, 2009, Comparison of electro thermal and hydride generation atomic absorption spectrometry for the determination of total arsenic in broiler chicken., food Chem., 113, 1351-1355.

[22] Jalbani, N., Kazi, T. G., Jamali, M. K., Arain, B. M., Afridi, H. I., Baloch, A., 2007, Evaluation of aluminum contents in different bakery foods by electrothermal atomic absorption spectrometer., J Food Compos Anal., 20, 226-231.

[23] Demirel, S., Tuzen, M., Saracoglu, S., Soyiak, M., 2008, Evaluation of various digestion procedures for trace element contents of some food materials., J Hazard Mater., 152, 1020-1026.

[24] Soyeurt, H., Bruwier, D., Romnee, J. M., Gengler, N., Bertozzi, C., Veselko, D., Dardenne, P., 2009, Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry., J Dairy Sci., 92, 2444-2454.

[25] Ehling, S., Tefera, S., Earl, R., Cole, S., 2010, Comparison of analytical methods to determine sodium content of low-sodium foods., J AOAC Int., 93, 628-637.

[26] Ogabiela E. E., Udiba, U. U., Adesina, O. B., Hammuel, C., Ade-Ajayi, F. A., Yebpella, G. G., Mmereole U. J., Abdullahi, M., 2011, Assessment of metal levels in fresh milk from cows grazed around Challawa Industrial Estate of Kano, Nigeria., J Basic ApplSci Res., 1 (7), 533-538.

[27] Abd-El Aal, S. F. A., A wad, E. I., Kamal, R. M. K. M., 2012, Prevalence of some trace and toxic elements in raw and sterilized cow's milk., J Am S ci., 8 (9), 753-761.

[28] Starka, K., Wojciechowska-Mazurek, M., Mania, M., Brulinska-Ostrowska, E., Biernat, U., Karlowski, K., 2011, Noxious elements in milk and milk products in Poland., polish J of Environ Stud., 20 (4), 1043-1051.

[29] Derakhshesh, A. M., and Rahimi, E., 2012, Determination of lead residue in raw cow milk from different regions of Iran by Flameless Atomic Absorption Spectrometry., Am-Eur J Toxicol Sci., 4 (1), 16-19.

[30] Pilarczyk, R., Wojcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., Tomza-Marciniak, A., 2013, Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm., Environ Monit Assess 185, 8383-8392.

[31] Munoz, E., and Palmero, S., 2004, Determination of heavy metals in milk by potentiometric stripping analysis using a home-made flow cell., Food Control., 15, 635-641.

[32] Masotti, F., Erba, D., De Noni, I., Pellegrino, L., 2012, Rapid determination of sodium in milk and milk products by capillary zone electrophoresis., J Dairy Sci., 95, 2812-2881.

[33] Tripathi, R. M., Raghunath, R., Sastry, V. N., Krishnamoorthy, T. M., 1999, Daily intake of heavy metals by infants through milk and milk products., Sci Total Environ., 227, 9.229-235.

[34] Sola-Larranaga, C., and Navarro-Blasco, I., 2009, Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain., Food Chem., 112, 189-196.

[35] Solis, C., Isaac-Olive, K., Mireles, A., Vidal-Hernandez., M., 2009, Determination of trace metals in cow' s milk from waste water irrigated areas in Central Mexico by chemical treatment coupled to PIXE., Microchem J., 91, 9-12.

[36] Hussain, Z., Nazir, A., Shafique, U., Salmen, M., 2010, Comparative study for the determination of metals in milk samples using flame-AAS and EDTA complexometric titration., J Sci RES,., 2 (1), 9-14.

[37] Sikiric, M., B rajenovic, N., Pavlovic, I., Havranek, J. L, plavljanic, N., 2003, Determination of metals in cow, s milk by flame atomic absorption spectrophotometry., Czech J Anim Sci., 48 (11), 481-486.

[38] Tajkarimi, M., M,. Ahmadifaghih, M., poursoltani, H., Salah Nejad, A., Motallebi, A, A., Mahdavi, H,. 2008, lead residue levels in raw milk from different regions of lran, nFood Control., 19, 495-498.

[39] Ackland and Michalczyk, 2006 Zinc deficiency and its inherited disorders –Areview. Genes and nutrition 1; 41-50.

[40] Soares, V. A., Kus, M. M., Peixoto, A. C., Carrocci, J. S., Salazar, R. F Filho, H. I (2009). Determination of nutritional and toxic elements in pasteurized bovine milk from Vale do Paraiba region (Brazil). Food Control, 21: 45-49.

[41] Allen, (1998) Zinc and micronutrient supplements for children. Am J CLinNutr., 68; 4495-4498.

[42] 42-Shankar A. H., and Prasad, A. S., 1998. Zine and immune function: the biology basis of altered resistance to infection. Am J Clin Nutr, 68: 447-463.

[43] Fahmida, U., Rumaawas, j., Utomo, B., patmonodewo, S., and Schultink, W., (2006). Zinc-iron, but not zinc-alone supplementation, `increased linear growth of stunted infants with low hemoglobin. Asia Pac J Clin Nutr., 16: 301-309.

[44] Zraly et al., 2008Z., Pisarikova B., TrekovaM and Navratilova M. (2007); Effect of humic acid on lead accumulation in chicken organs and muscles, acta, 77; 439-445.

[45] Spivey, M. R., (2004). Assessment of cadmium, lead and vanadium, status of large animals as related to the human food chain. Food and Drug Administration, Washing-Dc. Journal of Animal Science, 65: 1744-1752.

[46] Agneta, O, Palminger, 1., Sundberg, J., and petersson, K., (1998). Risk assessment inrelation to neonatal metal exposure, Analyst, 123; 19-23.

[47] Canfield, R. L., Henderson, C. R., Cory-Slechta, D. A., Cox, C., Jusko, T. A., and Lanphear, B. p., (2003). Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. New England Journal of Medicine, 384: 1517-1526.

[48] Annette, 1996 "Spivvey, 2004.

[49] Bielicka A,. Bojanowska, I,. andWisniewski. A., (2005). Two faces of chromium pollutant and bioelement, polish journal of environmental studies 14; 5-10.

[50] Sandra, M., Paul, A., Heema., S, and Sam (1999) Chromium in food, nutrition and health an.

[51] Cortes, E., Das, H., A., Tarafdar, D. A. and vasconcellos, B. A., Toxic heavy Metals and other Trace Elements in Foodstuff from 12 different countries. Biological Trace Element Research, 20; 415-422 (1994).

[52] Ostapczuk, P., Valenta, P., Rutzel, H., and Nurnberg, H. W., Determination of Heavy Metal in Environmental Samples. Sci. of Total Environ, 60: 1-16 (1987).

[53] Tripathi, R. M.; Raghunath, R. and Krishnamoorthy, T. M., Dietary intake of heavy metals in Bombay city, India, Sci. of Total Environ, 208: 49-159 (1997).

[54] Krishnamoorty. T. M, and tripathi, R. M., measurements and modeling in environmental pollution BARC news letterindia 179; 3-12 (1998)

[55] SEHUHMACHER, M., domingo, jl. andcorbella. j. dietary intake of copper. Chromium and zinc in Tarragona province spainsci of total environ., 132; 3-10 (1983).

[56] Bulinski, r., bloniarz, j. and libel, B., trace element content in milk and some milk products. bromate. chem toksykol., xxv; 327-331 (1992).

[57] Lopez., A., Collins., w. f. and Williams, H. L. essential elements in raw and pasteurized cow and goat milk, j. dairy sci., 68; 1878-1886 (1995).