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Assessment of Metal Levels in Fresh Milk from Cows Grazed around Challawa Industrial Estate of Kano, Nigeria

*Ogabiela E.E, Udiba U.U, Adesina O.B, Hammuel C, Ade-Ajayi F. A, Yebpella G.G, Mmereole U.J and Abdullahi M

National Research Institute for Chemical Technology, P.M.B 1052, Zaria, Nigeria.

ABSTRACT

Milk is a very important component of human diet. The presence of toxic element in milk may create significant health problems. In this study, metal levels of fresh cow milk were determined. Fresh milk samples were collected from 17 cows grazed around challawa industrial estate, Kano and 9 cows grazed on open fields in Zaria, Kaduna state. Metal levels were determined using Atomic Absorption Spectrophotometer after wet digestion. The result shows the mean concentration of Al, Mn, Ni, Cu, Cd, Pb, Cr, Fe, and Zn in samples from Kano to be 10.1318, 1.78824, 30.1329, 2.52, 1.63176, 5.50176, 17.5669, 50.8735, and 32.3876 respectively. The mean concentrations of these metals in samples from Zaria were 6.246667, 2.19, 20.97111, 2.13667, 0.98667, 7.09778, 15.683, 32.37778, and 55.21333 respectively. The concentrations were very high as the metal levels of both Kano and Zaria samples exceeded the WHO permissible limits. Except for Pb, Zn and Mn, metal levels of cow milk from Kano were higher than those obtained from Zaria, though the result indicated no statistically significant difference between them. The implication of the results obtained to public health was discussed.

KEY WORDS: Toxic elements, milk, permissible limits, public health, Kano.

INTRODUCTION

Milk is considered as nearly a complete food in that it's a good source of protein, fat, and major minerals [1]. Cow milk contains some major elements such as cadmium, potassium, phosphorous, and magnesium in addition to sodium, chlorine and a wide range of micro elements and even heavy metals. Increase in industrial and agricultural processes have resulted in increased concentration of metals in the air, water and soil. These metals are taken in by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters also accumulate such metals in their tissues and milk if lactating [2]. A large amount of these metals taken in by plants and animals subsequently find their way into the food chain. This ever increasing pollution has given rise to concern on the intake of harmful metals in humans. Metals enter the human body through inhalation, ingestion or absorption through the skin [3,4]. The intake through ingestion depends on food habit. Cow milk which is a very important food stuff consumed by man is one of the major sources [5]. It has been reported that the content of the main elements in milk are fairly constant and undergoes slide changes depending on lactation phase, quality of nutrition and environmental conditions mainly chemical pollutants [5, 6]. In recent times, the amount of metals in cow milk is widely studied, particularly in industrialized and polluted areas of the developed and the developing countries of the world since animals grazed freely on open fields are considered as bio-indicators of environmental pollution [7, 8].

Metal levels in uncontaminated milk is generally low, but by inhalation of polluted air, intake of contaminated feeds and absorption through the skin, many dangerous elements or compound such as metals and metalloid, accumulate along the food chain [9]. The toxicity of metals depends on a number of factors; the particular metal in question, dose absorbed and the age of the person concern. For instance, children are vulnerable to the effect of lead exposure because they absorb several times the percentage ingested compared to adults and because their brain is plastic and even brief exposure may influence developmental processes [10]. Lead, cadmium and mercury residue in milk are therefore of great concern because milk is largely consumed by infants and children.

Although metals are essential nutrients, have a variety of biochemical functions in all living organisms and important industrial uses, their potential toxicity to humans and animals is a source of concern. It is therefore necessary

***Corresponding Author:** Dr. Ogabiela E.E., National Research Institute for Chemical Technology, Zaria, Nigeria. Email: edwardogabiela@yahoo.com +2348033495955

to monitor and control their levels in consumed food. The measurement of metal levels is helpful not only in ascertaining risk to human health but also in the assessment of environmental quality [5, 9]. Many reports indicated heavy metals in milk and attributed the presence of these heavy metals in milk and dairy products to exposure of lactating cows to environmental pollution, consumption of contaminated feed stuffs and water as well as the production process. Cow milk is a good source of both micro and macro element in human diet and the location of the cows determine their concentration [6]. The levels of lead and cadmium in milk from cows grazed on open fields in Kaduna were reported to exceeded the permissible maximum daily intake (0.05 mg/kg body weight) recommended by WHO [11].

In Nigeria, however, references to the presence of metals in cow's milk have been found in literature, though the data are sparse. The aim of this study is to determine metal levels in milk from cows grazed around Challawa industrial estate, Kano, Nigeria, to evaluate the contribution of anthropogenic pollution to toxic metal levels using cows from a relatively non industrialized are of Zaria, Kaduna State, Nigeria as control.

MATERIALS AND METHODS

Sample Collection

Raw milk samples from 17 lactating cows grazed around Challawa industrial estate of Kano and 9 Lactating cows grazed on a relatively non industrialized area of Zaria Kaduna State Nigeria were collected in June 2009. The milking was done by nomads in the morning and stored in propylene tubes. They were preserved in coolers packed with ice blocks and transported immediately to the National Research Institute for Chemical Technology, Zaria, for analysis.

Sample Digestion

1ml of each sample was digested with 5ml of concentrated hydrochloric acid (HCl) and 5ml of concentrated perchloric acid ($HClO_4$), all analytical grades. The digested samples were quantitatively transferred into 50mls flask, made up to the mark with distilled water and stored in 50mls propylene bottles.

Elemental Analysis

Metal concentration in the digest was determined by Atomic Absorption Spectrophotometer (AAS) using Shedmazu AA6800 apparatus with graphite furnace and background correction. The flame condition and graphite furnace were optimized for maximum absorbance and linear response with the aspiration of known standard. The standards were prepared from 1000ppm stock solution.

RESULTS AND DISCUSSION

The results of elemental concentration of 17 samples of fresh milk from cows grazed around Challawa industrial estate, Kano and 9 samples of fresh milk from cows grazed on open fields in Zaria, Kaduna State are presented in table (1) and (2) respectively. The average concentration of Al, Mn, Nl, Cu, Cd, Pb, Cr, Fe, and Zn for both samples from Kano and Zaria are presented in table (3) while table (4) shows the average elemental concentration in comparison with other published works.

As indicated by data in table (1), the highest concentration of lead (1.264mg/l) in fresh cow milk was recorded in the samples from Kano. The lowest concentration of 0.055mg/l was also recorded in the samples from Kano. The average concentration of lead in fresh cow milk sample from Zaria was higher than that in milk samples from Kano (Fig.2). The difference was not statistically significant ($P›0.05$). The mean concentrations of lead in this study were higher than those reported in previous studies [5, 12, 13 and 14]. Similar lead levels in cow milk were reported in Kaduna, Nigeria [11], as shown in table IV, while higher lead levels were recorded in Egypt [15]. The average concentration of lead in milk samples from both Kano and Zaria exceeded the maximum permissible limit of lead for milk and dairy product taken as 0.02mg/kg [16]. European diet provides maximum potential for weekly intake of lead to be 4.31mg of lead per kg body weight [17]. This study therefore shows that lead is frequently found in cow milk not only in the areas of great industrial activities like Kano, but also in most small cities. This could be due to fodder contamination, climatic factors such as wind, use of Agro-chemicals and very importantly drinking water. Furthermore, these cows graze along rail lines, roadsides etc. lead which is a fuel additive could be emitted from the car exhaust to contaminate the environment.

Lead is one of the limited classes of element that can be described as purely toxic. Most other elements thought toxic at high concentration are actually required nutrient at lower levels. There is no exposure level below which lead appears to be safe. High level of lead is particularly of great concern especially due to the fact that milk and dairy products are consumed mostly by infants and children who are uniquely susceptible to the effect of lead. Lead absorption constitutes serious risk to public health. It induces reduced cognitive development and intellectual performance in children, increased blood pressure, and cardiovascular diseases in adult as well as liver and kidney dysfunctioning [15 and 18].

Cadmium is a cumulative toxic agent with half-life of several years and their burden of the body increases with age. Table 3 shows that the average concentration of cadmium in fresh milk sample from Kano was 0.163176mg/l with a maximum concentration of 0.361mg/l and minimum concentration of 0.03mg/l (table I), while the average concentration of cadmium in fresh milk samples from Zaria was 0.098667mg/l with a maximum value of 0.193mg/l and a minimum value of 0.038mg/l. The average concentration of cadmium in fresh milk from Kano was higher than that from Zaria. This could be as a result of anthropogenic addition through industrial discharges and emissions which may be particularly true of Nigeria where environmental laws are rarely enforced. The difference was not statistically significant ($p > 0.05$). The tolerable cadmium intake established by WHO is 60µg/day for adult women and 70µg/day for adult men. CE Regulations [16] put the permissible limit for cadmium in food at 0.075mg/g while the Egyptian standards put it at 0.05mg/g [19]. The presence of cadmium above the established limits suggests that there are toxicological risk in the consumption of cow milk from Kano and Zaria. A similar result of cadmium concentration in cow milk above the permissible limit was reported by Abdallah [15]. Lower levels were reported by Cortes, Farid, Lawal and semaghuil [5, 9,11,14,]. Cadmium and solutions of its compounds are toxic, chronic exposure can cause irreversible damage to the lungs and eventually, death. Eating food or drinking water with high cadmium concentration irritates the stomach causing vomiting and diarrhea. It accumulates in the kidney and liver causing kidney dysfunctioning and liver failure, in addition to being a teratogenic and carcinogenic agent [15, 20, and 21].

Table: I. Elemental concentration in fresh cow's milk from Challawa industrial area Kano, Nigeria

s/n	Al	Mn	Ni	Cu	Cd	Pb	Cr	Fe	Zn
1	1.99	0.222	1.559	0.057	0.088	0.769	0.0032	5.405	0.899
2	0.437	0.052	2.128	0.305	0.147	0.715	0.0021	3.937	1.266
3	1.487	0.209	2.113	0.351	0.118	0.055	0.0044	4.539	0.283
4	0.744	0.072	3.137	0.339	0.143	0.11	0.0791	7.589	0.308
5	1.181	0.3	6.329	0.362	0.361	0.747	3.513	13.173	0.172
6	1.137	0.105	0.092	0.181	0.155	0.879	1.23	2.52	1.16
7	0.918	0.261	4.355	0.147	0.172	0.198	0.439	5.026	1.049
8	2.099	0.547	1.622	0.155	0.277	0.407	3.162	6.185	0.741
9	0.519	0.059	0.368	0.26	0.118	1.264	0.264	3.751	0.358
10	1.487	0.105	3.026	0.294	0.097	0.242	1.318	2.477	0.832
11	0.11	0.285	1.919	0.123	0.118	0.495	1.439	2.321	0.364
12	1.181	0.072	3.912	0.384	0.03	0.22	1.845	5.971	0.507
13	1.705	0.235	10.316	0.165	0.302	0.659	1.581	17.496	0.569
14	1.181	0.254	1.393	0.215	0.072	0.813	1.054	3.1923	8.792
15	0.022	0.052	2.002	0.396	0.164	0.484	1.757	2.785	1.987
16	0.08	0.196	3.173	0.475	0.269	0.769	7.728	4.954	5.968
17	0.874	0.39	3.782	0.075	0.143	0.527	4.391	9.135	29.804

The average concentration of chromium in cow milk samples from Kano and Zaria in this study are given in table 3 as 1.756694mg/l and 1.5683mg/l respectively. The highest concentration was 7.728mg/l and the lowest concentration 0.001mg/l (table 1 and 2). Fig.2 shows that the average concentration of chromium in fresh cow milk samples from Kano was higher than that from Zaria. The difference however was not statistically significant ($p > 0.05$). These high chromium concentrations could be attributable to discharges released from tanneries in Challawa industrial estate, since chromium is one of the major discharges in tannery waste. Chromium is an important mineral the body must have to function properly. The body stores chromium in the blood and in the hair. It's responsible for stimulating the activities of insulin in the body and also help controls blood cholesterol levels. The acceptable daily intake of chromium is 50-200µg/day. Lower values of chromium in milk were reported in previous studies [5, 9, 13]. L0w level exposure chromium can cause skin irritation, and ulceration. Long term exposure can cause kidney and liver damage as well as circulatory and nerve tissue problems [22].

Table: II. Elemental concentrations in fresh cow's milk in Zaria, Nigeria

s/n	Al	Mn	Ni	Cu	Cd	Pb	Cr	Fe	Zn
18	1.181	0.261	1.863	0.068	0.084	0.55	5.794	4.367	42.677
19	1.356	0.189	2.334	0.351	0.147	1.044	1.581	2.864	0.296
20	0.809	0.313	2.888	0.001	0.193	0.934	3.952	4.081	1.653
21	1.268	0.098	2.5	0.271	0.038	0.649	2.2	5.355	2.545
22	0.066	0.105	0.867	0.158	0.038	1.209	0.527	1.776	1.513
23	0.001	0.163	2.417	0.373	0.051	0.868	0.0167	2.692	0.25
24	0.744	0.261	1.697	0.158	0.093	0.705	0.0211	2.377	0.481
25	0.022	0.248	2.334	0.249	0.093	0.11	0.001	2.363	0.035
26	0.175	0.333	1.974	0.294	0.151	0.319	0.0219	3.265	0.242

Table: III. Average elemental concentration in milk from Kano and Zaria

Element	Challawa industrial estate, Kano Mean ± SD	Zaria, Kaduna Mean ± SD
Aluminum (Al)	1.008941 ± 0.64	0.624667 ± 0.56
Manganese (Mn)	0.178824 ± 0.13	0.219 ± 0.09
Nickel (Ni)	3.013294 ± 2.42	2.097111 ± 0.59
Copper (Cu)	0.252 ± 0.123	0.213667 ± 0.23
Cadmium (Cd)	0.163176 ± 0.89	0.098667 ± 0.05
Lead (Pb)	0.550176 ± 0.32	0.709778 ± 0.35
Chromium (Cr)	1.756694 ± 2.01	1.5683 ± 2.08
Iron (Fe)	5.987412 ± 4.02	3.237778 ± 1.15
Zinc (Zn)	3.238765 ± 7.21	5.521333 ± 13.9

Recorded data from the present study indicates that copper was detected in fresh cow milk sample from Kano and Zaria with an average concentration of 0.252 mg/l and 0.213667 respectively. These results are higher than the findings recorded in previous studies [5, 9, 11, 15] as shown in table 4. There was no significant difference between the average concentrations of copper in cow milk samples obtained from Kano and Zaria, though the average concentration of copper in cow milk samples from Kano was higher than that from Zaria (Fg.2). The provisional tolerable daily intake (PTDI) for copper is 3mg [3]. The average concentrations of both samples from Kano and Zaria were quite high. Abnormal accumulation of copper in the tissue and blood causes Wilson disease. Most of the absorbed copper is stored in the liver and bone marrow. Acute exposure to copper causes vomiting, bloody diarrhea, hypertension, and cardiovascular collapse [15].

Table; IV. Comparison of the elemental concentration of fresh cow's milk in this study with published values

Metals	This study		Lawal *et al* (2006)	Semaghiul *et al* (2008)	Abdallah (2011)	Farid *et al* (2004)	Ostapezuk *et al* (1987)	Bulinski *et al* (1992)	Cortes *et al* (1994)
	Kano	Zaria							
	mg/L		mg/L	mg/L	mg/kg	µg/kg			
Aluminum	1.009	0.625	-	1.18	-	-	-	-	-
Manganese	0.179	0.219	-	0.08	-	-	-	-	-
Nickel	3.013	2.097	-	0.04	-	-	-	-	-
Copper	0.252	0.214	0.062±0.026	0.17	0.11±0.002	48.9±0.6	49.9	90.0	100.0
Cadmium	0.163	0.099	0.0257±0.127	0.004	0.41±0.05	4.7±0.2	0.1	15.0	1.0
Lead	0.550	0.710	0.5312±0.299	0.12	2.55±0.12	3.5±0.2	5.5	20.0	50.0
Chromium	1.757	1.568	-	0.04	-	31.4±0.4	-	38.0	-
Iron	5.987	3.238	-	0.72	-	-	-	-	-
Zinc	3.239	5.521	-	0.98	3.661±0.003	944.9±2.4	3730.0	3770.0	3000.0

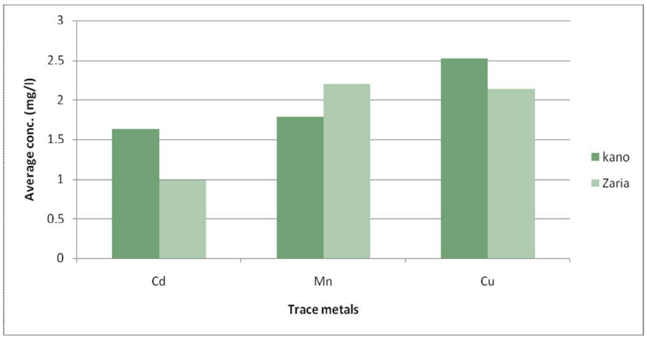

Fig. 1: Distribution of concentration of Cd, Mn, and Cr in fresh cow's milk from Kano and Zaria

The average concentration of zinc shown in table (3) was 3.238765mg/l for Kano and 5.52133mg/l for Zaria. The Provisional tolerable daily intake for Zinc is 60mg/l for an average adult of 60kg body weight [3, 23]. The concentration of zinc in fresh cow milk from Zaria was higher than that from Kano (Fig.3). The difference was not statistically significant ($P > 0.05$). Similar result was reported by Abdallah [15]. Lower values were recorded by Farid , Lawal , and Semaghuil in their studies [5, 9, 11,] as shown in table 4. Chronic zinc exposure results in anemia, leucopenia, gastrointestinal diseases and diarrhea.

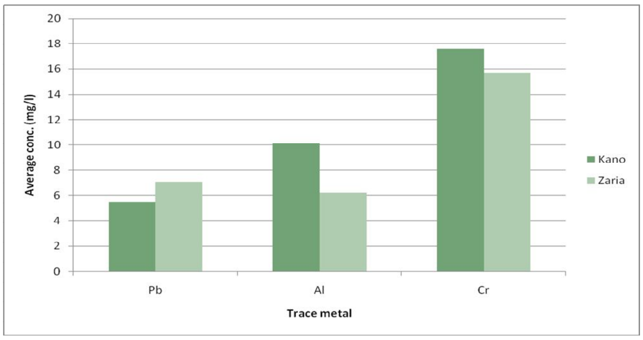

Fig. 2: Distribution 0f concentration of Pb, Al, and Cr in fresh cow's milk from Kano and Zaria

Manganese is an essential nutrient that is important for normal processes in the human body, though adverse effect have been reported at higher doses. Exposure to high concentration of manganese is associated with impaired neurological and neuromuscular control, mental and emotional disturbances (muscle stiffness, and lack of coordination). Exposure to very high doses result in impaired male fertility, birth defects, and impaired bone development [24]. The estimated safe and adequate Daily Dietary intake of manganese is 2-5 mg/day for adults and 2.5-25μg per kilogram body weight for infants [26]. In this study the average concentration of manganese in fresh cow milk samples from Kano and Zaria were found to be 0.178824mg/l and 0.219mg/l respectively (Table 3). Both the highest and the lowest concentration of manganese were recorded in milk samples from Kano (Table 1). The mean concentration of manganese in milk from Zaria was greater than that of milk samples from Kano (Fig.1). The difference was not statistically significant ($P > 0.05$).The level of manganese in fresh cow milk samples from Kano and Zaria were found to be within the Safe and Adequate Daily Dietary intake limit.

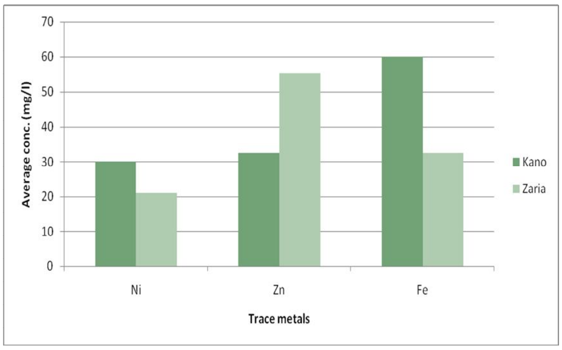

Fig.3: Distribution 0f concentration of Ni, Zn, and Fe in fresh cow's milk from Kano and Zaria

From table 3, the average concentration of Aluminum in fresh cow milk samples from Kano and Zaria are 1.008941 mg/l and 0.624667mg/l respectively. Samples from Kano had a higher mean concentration than that from Zaria (Fig.2). The difference was not statistically significant ($p > 0.05$). Aluminum is involved in the activities of a small number of enzymes in the body. Excess dose is linked with Alzhermers, a brain disorder that destroys brain cells causing memory loss, problems with thinking and general behavior. It is also linked with parkistan diseases, a degenerative disorder of the central nervous system that impairs motor skills and speech disorder [18]. The levels of Aluminum in fresh cow milk from Zaria and Kano were quite high and a source of serious concern. The maximum contaminant level (MCL) for Aluminum is given as 0.2mg/l [18].

Small amount of nickel is needed by the body to produce red blood cells. However, excess amount can become toxic. Long term exposure to nickel causes decrease body weight, skin Irritation, heart and liver damage [22]. In this study the average concentration of nickel in milk samples from Kano (3.013294mg/l) was found to be higher than that from Zaria (2.097111mg/l) fig. 3. The difference was not statistically significant ($P>0.05$). EPA does not presently regulate nickel levels. Though it accumulates in aquatic life, its presence is not magnified along the food chain [22]. The recommended daily allowance for iron is 15mg [25]. The average

Concentration of iron in cow milk from Kano and Zaria were found to be 5.987412mg/l and 3.237778 mg/l respectively (table 3). Liver, kidney and the cardiovascular system are the target organs for iron toxicity. The levels of iron in this study are within the recommended daily allowance. Consumption of cow milk from Kano and Zaria therefore pose no danger to iron toxicity.

CONCLUSION

This study indicates the contamination of fresh cow milk from cows, grazed around Challawa industrial areas of Kano and cows grazed on open fields in Zaria Kaduna state Nigeria by metals exhibiting a wide range of hazardous impact on human health. There was no significant difference in metal levels between the two set of samples. Metal levels recorded in the study were higher than those obtained from literature, especially studies carried out before 2004 as in table 4. These are mainly due to greater pollution of the environment (air, water, and soil). Further studies are therefore necessary to evaluate the levels of metals on a large number of samples from the region. Determination of metal levels in fodder and water fed to the animals are also necessary.

REFERENCES

1. Enb A. Abou Donia M.A., Abd-Rabou, Abou Arab and El-Senaity M.H, 2009. Chemical Composition of Raw Milk and Heavy Metals Behaviour During Processing of Milk Products, Global Veterinaria 3(3) 268-275.
2. Yahaya M. I., Ezor G. C., Musa Y. F and Muhamad S. Y, 2010. Analysis of heavy metals concentration in road side soils in Yauri, Nigeria. African journ. Of pure and applied chem.. vol 4(3).
3. Ogabiela E.E, Yebpella G.G, Ade-Ajayi A.F, Mmereole U.J, Ezeayanaso C, Okonkwo E.M, Ahola D.O, Udiba U.U, Mahmood A, and Gandu I, 2010. Determination of the level of some elements in edible oils sold in zaria, northern Nigeria. Global Journal of Pure and Applied Sciences vol.6 (3): 325-331.
4. Ahmed, W.M.S, 2002. Studies on heavy metal pollution farms in relation to production performance Ph.D thesis Fac.of Vet, Med.Zag University.
5. Farid S.M., Enani M.A. and Wajid S.A, 2004. Determination of trace elements in cow milk in Saudi Aribia, JKAU: Egn. Sci. Volume 15 (0-2): pp131-140.
6. Dobrzanski Z., R. Kalaeu, H. Gorecka, K. Cholnaka and Barkowalk , 2005. The content of micro elements and Trace Elements in Raw Milk from cows in Silesian Region. Polish Journal of Environmental Studies, Volume 14 (No.5) 685-689.
7. Korenekovg B.M., Skalicka, and P. Nai, 2002. Concentration of some heavy metals in cattle reared in the vicinity of metallurgic industry Vet. Archiv. 72, 254-267.
8. LI-Quang Φin, Xino – Ping Wang, Wei Li, Xing Tong and Wei-jun Tong, 2009. The minerals and heavy metals in cow's milk from China and Japan: Journal of Health Science 555(2); 300-305.
9. Semaghuil Binghila, Simona D, G. Stanciu, A. Soceanu, 2008. Determination of Major and MinorElements in milk through ICP-AESEnviromental Engineering and Management Journal. No 6,805-808.
10. Samara S. and Richard H, 2009. Heavy metal toxicity, emedicine Specialist > Emergency Medicine > Toxicology. New York.
11. Lawal A.O., Muhammed S.S. and Damisa D, 2006. Assessment of levels of Cu, Cd an Pb in secretion of mammary gland of cow grazed on open fields. Science World Journal Volume I. No.OI.
12. Ostapezuk P, Valenta P,Rutzel H, and Nurnberg H. W, 1987. Determination of Heavy metal in environmental sample, sc. of total environ, 60:1-16
13. Bulinski R, Bloniarz J, and Libelt B, 1992. Trace element content in in milk and some milk products.Bromat.chem. toskykol, :327-331
14. Cortes, E., Das, H , A.,Tarafdar D A. and Vasconcellos, B A, 1994. Toxic Heavy metals and other trace element in food stuff from 12 different countries. Biological trace Element Research,20;415-422
15. Abdallah M.I.M, 2011. Evaluation of some heavy metals residue in whole milk powder used at confectionary plant regarding the public health significance, Animal research institute, agricultural research center, Giza Egypt.
16. CE Regulation, 2001. CE Regulation no.2001/466 march 8, 2001, GUE L77/1 march 16 2001
17. GEMS/Food, 2000. Regional Diets prepared by Global Environmental monitoring system /food contamination monitoring and Assessment programme (GEMS/FOOD)
18. The Nigerian Guardian news paper (26/11/2009).Heavy metalscontamination of Lagos water. Pp 25
19. Egyptian Standard (2001)**:** Milk powder. Egyptian Organization for Standardization and Quality Control. E.S., No. 1648.
20. WHO (1973). Evaluation of certain food additives and contaminant: Hg,Pb,Cd. 16th Reoort of Expert committee, WHO Technical Report Series no51
21. WHO, 1980. Recommended Baseline in occupational Exposure to Heavy metals.Report of WHO study Group. Technical Report no 647
22. Lenntech, 2004. Water Treatment and Purification, Lenntech , Rotterdamseweg, Netherland
23 FAO/WHO, 1999. Expert committee on Food Additives summary and conclusions, 53rd meeting Rome
24. U.S.EPA (2007). Toxicity and exposure Assessment for Children's Health. *http://ww.epa.gov/teach/.*
25. Durdana R. H, Shahnaz I, and G. H, Shaikh, 2007. Assessment of the the levels of trace metals in commonly edible vegetable slocally available in the markets of Karachi city. Pak. J. Bot. 39(3).
26. Perveen F, Majid Mumtaz and T. H. Usmani,(2005), Estimation of metal content in various varieties of milk in Karachi city, Jour. Chem.,pak.vol.27 no 6.