URBAN RAW MILK STUDIES: ASPECTS OF SELECTED MINERAL ELEMENTS INVESTIGATIONS OF RAW MILK SAMPLES OBTAINED FROM SOME CITIES IN SOUTHERN NIGERIA

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Abstract Investigations of Cu, Cd, Co, Zn, Cr, Ca, Fe and Pb in raw cow milk samples were carried out in this study. Samples of raw cow milk used were in part, obtained from Benin City, Uromi and Auchi all in Edo State; then from Agbor, Asaba and Warri, the latter three cities are in Delta State. Both states are located in the southern part of Nigeria. Samples collections were carried out during the dry season and sterile plastic containers were used. The collected raw milk samples were homogenized and subsequently, subjected to wet digestion using concentrated tri-acid (HNO_3-H_2SO_4-$HClO_4$) as digestion mixture. The respective digests were thereafter, spectrophotometrically examined for their Cu, Cd, Co, Zn, Cr, Ca, Fe and Pb contents, using atomic absorption spectrophotometer (Buck Scientific Model 410). Additionally, data obtained were statistically analysed, using International Business Machine (IBM) statistical package for social sciences (SPSS). With respect to the analysed digests of samples collected from studied cities in Edo State, findings indicate the following mean total levels of occurrence for the examined mineral elements: Ca (120.274mg/kg); Zn (2.888mg/kg); Cu (0.102mg/kg); Fe (0.680mg/kg); Cr (0.060mg/kg); Co (0.083mg/kg); Pb (0.014mg/kg) and Cd (0.048mg/kg). The corresponding mean total of the examined mineral elements in digests of samples obtained from Delta State were: Ca (119.797mg/kg); Zn (2.817mg/kg); Cu (0.102mg/kg); Fe (0.606mg/kg); Cr (0.063mg/kg); Co (0.078mg/kg); Pb (0.013mg/kg) and Cd (0.048mg/kg). Variations were noted to occur in the respective values of the examined mineral elements. The observed variations were found to be statistically significant ($P<0.05$) with respect to the different examined mineral elements, but not with sampling locations. It is nutritionally desirable that the examined dietary relevant mineral elements occurred in the studied milk samples. It is however of concern, that Pb and Cd both of which are highly toxicologically relevant, also occurred in all the milk samples investigated. There is need to put in place, measures that will prevent further increase in the levels of these toxicologically relevant mineral elements in milk obtained from the studied locations.
Keywords: Digestion, Dumpsites, Investigations, Milk, Nutritional, Spectrophotometer, Toxicology.

INTRODUCTION

In countries where free animal grazing is practiced as it is the situation in Nigeria, there are high chances of these animals feeding on contaminated diets. This is particularly so, as the reared animals are often times left to feed on forages along road sides and those growing in wastes dumpsites. The concern here is that forages grown along road sides and wastes dumpsites could probably be contaminated by vehicular emission, pollutants from untreated and improperly disposed domestic and industrial wastes; as well as those from farmlands. Worthy of note also, is the practice whereby some animals that are reared, are left by their handlers to wonder into farms and graze on crops, even when the animal handlers lack history of the use of plant-protective agents (PPA) including herbicides, insecticides and fungicides, as well as the use of fertilizers on the respective farms. It is imperative to emphasize that these plant-protective agents contain toxic substances including heavy metals, that could find their way into the milk collected from the animals, that are allowed to graze on the contaminated plants.

The factors that affect the compositional chemistry of milk are numerous and dynamic in nature. Considering these factors vis-á-vis the increasing trend in trade globalization and the advent of illnesses of serious global concern such as COVID-19 and others, whose holistic understanding of their nature and treatments are still in-search, it is pertinent that routine analysis of food substances for the presence of nutritional and toxicologically relevant substances be carried out. According to Dibie and Ukhun (2018), dietary sources are significant means of human exposure to various mineral elements. Obviously, some of the ingested mineral elements due to foods consumptions are nutritionally desirable; while some others are of serious toxicological concern.

In this work, samples of raw milk obtained from some cities in southern Nigeria were used as study materials. Milk, as a sample for study is of utmost importance, as according to Choodamani *et al*. (2018), infants and the elderly which constitute the vulnerable age group consume more milk and dairy products. It should however be emphasized that milk consumption cuts across all ages, the difference is the quantity consumed at different ages of human life. The differences in the quantity of milk consumed with respect to different ages varies from country to country and even within the same country, variations exist among individuals.

The concern of this study was to examine the levels of calcium (Ca), iron (Fe), copper (Cu), cobalt (Co), zinc (Zn), chromium (Cr), cadmium (Cd) and lead (Pb) in raw milk samples obtained from some cities in southern Nigeria. Elsewhere, some authors (Ogabiela *et al*., 2011; Fatima *et al*., 2011; Abdelkhalek *et al*., 2015; Ahmad *et al*., 2017; and Zarah *et al*., 2018) had variously examined the levels of some mineral elements in milk. However, little or no work has been done with raw milk samples found in the cities, wherein samples used in this work were obtained. The safety of dairy products decreases with increasing concentration of toxic compounds and environmental pollutants, especially heavy metals (Rezaei *et al*., 2014). Southern Nigeria has been experiencing increasing urbanization and associated activities. Additionally, as it is in all parts of Nigeria, free animal grazing has continued unabated in southern Nigeria. Therefore, this work is imperative as it would provide information that will fill the identified gap in knowledge.

Investigations of the levels of Ca, Zn, Fe, Co, Cu, Cr, Cd and Pb in the studied raw milk samples would be carried out using the atomic absorption spectrophotometry, after they would have been subjected to wet digestion. Additionally, statistical analyses both aspects of descriptive statistical evaluation of data and statistical evaluation of the relation between variables (ANOVA) would be carried out. International Business Machine (IBM), Statistical Package for Social Sciences (SPSS) would be used in statistical evaluation of data.

MATERIALS AND METHODS

Sample Collection

Samples of unprocessed cow milk used in this study were obtained from some cities in Edo State viz: Benin City, Auchi and Uromi; as well as selected cities in Delta State viz; Asaba, Agbor and Warri. Edo and Delta States are situated in the southern region of Nigeria. Collections of raw cow milk samples were carried out during the dry season. Sterile plastic containers were used for the collection of the raw cow milk samples. Upon collection of the raw cow milk samples in the sterile plastic containers, the containers were corked tightly and thereafter, placed in cooler containing ice block. Subsequently, the cooler and its contents were immediately transported to the laboratory, where the samples were analyzed for the examined mineral elements.

Investigations of the collected raw cow milk samples for the examined mineral elements, were carried out within twenty four hours of the samples arrival in the laboratory. Prior to samples pre-treatment and further analysis, they were stored in refrigerator, wherein a temperature of 4^0C was maintained.

Sample Digestion

Samples digestion was carried out in triplicates. The glassware used were thoroughly washed, then, further cleaned using freshly prepared 10% HNO_3 solution and thereafter, cleaned with deionised distilled water. Prior to the actual digestion, the samples were homogenized. Digestion type was wet digestion which entailed the use of concentrated tri-acid (HNO_3-H_2S0_4-$HClO_4$) as digestion mixture. This is an adaptation of the procedure described by Estefan *et al*. (2013). Preparation of the tri-acid mixture (HNO_3-H_2SO_4-$HClO_4$) used in this work, entailed thorough mixing of concentrated HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:1:4 (Estefan *et al*., 2013). The prepared mixture was subsequently allowed to cool.

The digestion entailed steps consisting of the addition of 20ml of concentrated H_2SO_4 to measured 10ml of homogenized raw cow milk contained in a 300ml digestion flask; then, the flask containing a glass funnel placed in its neck was thereafter, placed on the surface of a thermostatically controlled hot plate. After which, the temperature of the hot plate was increased gradually to a temperature of 145^0C. Heating was maintained at this temperature for a period of 30mins. Thereafter, the heating was discontinued, and the flask was subsequently allowed to cool to room temperature. After cooling, 10ml of the prepared tri-acid mixture was added to the content of the digestion flask, followed by swirling of the flask. Subsequently, the digestion flask and its contents were again, heated. The latter heat treatment of the digestion flask and its contents was again, carried out with the aid of a thermostatically controlled hot plate, whose temperature was gradually raised to 200^0C. Thereafter, heating was continued at this temperature for a further 1 hour. During this period, a clear solution was obtained.

On completion of the digestion, the digestion flask and its content were allowed to cool to room temperature. This was followed by the transfer of the content of the flask to a 100ml volumetric flask and subsequently, made up to the 100ml mark using deionised distilled water. During the makeup, intermittently, the 100ml volumetric flask was thoroughly shaken to ensure that its contents were properly mixed. For each batch of digest, reagent blank that was subjected to similar treatments as carried out with the sample in the digestion process and subsequent make up stage, using deionised distilled water was prepared.

Measurement

The measurements of the levels of Cu, Cd, Co, Zn, Cr, Ca, Fe and Pb of the different diluted samples digests, were carried out with the aid of atomic absorption spectrophotometer (Buck Scientific Model 410). The atomic absorption spectrophotometer used in this study was operated in accordance with the guidelines specified for it by its manufacturer. Prior to the analysis of the examined mineral elements in the various diluted samples digests, series of suitable standards of the various investigated mineral elements were measured, using the atomic absorption spectrophotometer earlier mentioned. The respective values obtained for the standards of each of the examined mineral elements were subsequently, used in producing the corresponding calibration curves.

Quality Control

Chemicals: Analytical grade chemicals were used in this study.

Measurements: Replicate measurements of at least three determinations were done for each of the readings taken.

Limit of Detection (LOD): Investigation of the limit of detection was in accordance with the method described by Ismail *et al*. (2017). Notably, in line with the procedure used for milk samples digestions, replicate blank digestions were carried out. Thereafter, the levels of the investigated mineral elements in the blank digests were determined. The same atomic absorption spectrophotometer (Buck Scientific Model 210 VGP) was used to quantify the levels of Ca, Zn, Fe, Co, Cu, Cr, Pb and Cd in all the blank and sample digests. In all situations, similar equipment operating conditions were maintained. The values of the limit of detections for the examined individual mineral elements in this work were recorded.

The percentage recovery for the various examined mineral elements in this work was obtained in accordance with the method described by Ismail *et al*. (2017). The procedure entailed spiking known concentrations of the respective standard solutions of the examined mineral elements with known amount of pure compounds of the separately examined mineral elements. Subsequently, both the spiked and non spiked samples were investigated in line with the procedures described in this work, for the examined mineral elements. Using the data generated, the percentage recovery for the investigated mineral elements in the studied milk samples was calculated, in accordance with the equation below. Triplicate determinations were carried out and the final concentration used was the average of the replicate measurements made.

The equation described by Ismail *et al*. (2017) as stated below (equation 1), was used to calculate the percentage recovery with respect to the spiked milk samples.

$$\% \text{ Recovery} = \frac{\text{Conc. in spiked milk} - \text{Conc in non spiked milk}}{\text{Spiked amount}} \times 100 \quad \text{---- eqn(1)}$$

Data Analysis

Arithmetic means and standard deviation which represent descriptive statistical evaluation of data; as well as analysis of variance (ANOVA) were the statistical analysis carried out with the data generated in this work. One-way analysis of variance was carried out. Confidence interval was at $P<0.05$. International Business machine (IBM) statistical package for social sciences (SPSS) was used in statistical evaluation of data.

RESULTS AND DISCUSSION

Results obtained with respect to the investigations of the levels of Ca, Zn, Cu, Fe, Cr, Co, Pb and Cd in raw cow milk samples collected from some cities in Edo state, Nigeria, are presented in Table I below.

Additionally, findings from the determinations of the levels of the aforementioned mineral elements in raw cow milk samples obtained from some cities in Delta State, Nigeria, are presented in Table 2, also below.

Table 1

Selected Mineral Elements Levels (mg/kg) in Raw Cow Milk Samples Obtained From Some Cities in Edo State.

S/N	Mineral Elements	Cities and Customers																					
		Benin City							Auchi							Uromi							
		C_1	C_2	C_3	C_4	C_5	Mean	Range	C_1	C_2	C_3	C_4	C_5	Mean	Range	C_1	C_2	C_3	C_4	C_5	Mean	Range	Mean Total
1	Ca	124.601 ± 18.116	115.091 ± 21.621	119.218 ± 12.015	118.655 ± 9.438	125.019 ± 22.005	120.517	115.091 - 125.019	116.811 ± 13.416	122.944 ± 16.541	120.161 ± 4.969	118.415 ± 21.628	117.553 ± 12.753	119.177	116.811 - 122.944	121.941 ± 25.171	114.136 ± 8.438	119.811 ± 15.529	124.715 ± 19.120	117.884 ± 12.313	119.697	114.136 - 124.715	119.797
2	Zn	2.868 ± 0.511	3.015± 0.914	2.733± 0.186	3.112± 0.449	2.911± 0.358	2.928	2.733- 3.112	2.685± 0.517	2.927± 0.844	2.466± 0.655	3.007± 0.284	2.806± 0.914	2.778	2.466- 3.007	2.558± 0.106	2.956± 0.721	2.498± 0.475	3.019± 0.106	2.688± 0.322	2.744	2.466- 3.019	2.817
3	Cu	0.113± 0.011	0.126± 0.028	0.098± 0.016	0.109± 0.012	0.085± 0.031	0.106	0.085- 0.126	0.094± 0.023	0.107± 0.014	0.114± 0.021	0.124± 0.019	0.119± 0.008	0.112	0.085- 0.124	0.081± 0.024	0.093± 0.015	0.079± 0.008	0.098± 0.027	0.087± 0.011	0.088	0.079- 0.098	0.102
4	Fe	0.628± 0.105	0.711± 0.093	0.537± 0.116	0.708± 0.121	0.469± 0.152	0.611	0.469- 0.711	0.718± 0.046	0.655± 0.119	0.592± 0.125	0.624± 0.087	0.548± 0.095	0.627	0.469- 0.718	0.519± 0.113	0.622± 0.091	0.497± 0.124	0.648± 0.073	0.617± 0.117	0.581	0.469- 0.648	0.606

5	Cr	0.085± 0.032	0.059± 0.007	0.063± 0.018	0.049± 0.008	0.068± 0.021	0.065	0.049- 0.085	0.077± 0.025	0.083± 0.004	0.063± 0.018	0.053± 0.007	0.071± 0.015	0.069	0.049- 0.083	0.044± 0.019	0.058± 0.006	0.062± 0.009	0.049± 0.014	0.058± 0.011	0.054	0.044- 0.062	0.063
6	Co	0.105± 0.031	0.091± 0.016	0.084± 0.009	0.096± 0.005	0.064± 0.012	0.088	0.064- 0.105	0.093± 0.017	0.106± 0.009	0.086± 0.025	0.069± 0.014	0.057± 0.006	0.082	0.057- 0.106	0.062± 0.013	0.069± 0.011	0.058± 0.025	0.071± 0.018	0.065± 0.009	0.065	0.057- 0.071	0.078
7	Pb	0.012± 0.004	0.019± 0.008	0.014± 0.005	0.011± 0.007	0.022± 0.003	0.016	0.011- 0.022	0.018± 0.006	0.013± 0.009	0.009± 0.004	0.011± 0.005	0.015± 0.007	0.013	0.009- 0.018	0.010± 0.003	0.008± 0.005	0.012± 0.007	0.014± 0.006	0.011± 0.002	0.011	0.008- 0.014	0.013
8	Cd	0.071± 0.028	0.049± 0.013	0.028± 0.009	0.053± 0.017	0.043± 0.011	0.049	0.028- 0.071	0.059± 0.021	0.064± 0.015	0.068± 0.011	0.042± 0.008	0.025± 0.006	0.052	0.025- 0.068	0.047± 0.011	0.035± 0.006	0.038± 0.004	0.051± 0.002	0.049± 0.005	0.044	0.025- 0.051	0.048

C_1 = Customer (1); C_2 = Customer (2); C_3 = Customer (3); C_4 = Customer (4); C_5 = Customer (5)

Table 2

Selected Mineral Elements Levels (mg/kg) in Raw Cow Milk Samples Obtained From Some Cities in Delta State.

S/N	Mineral Elements	Cities and Customers			
		Asaba	Agbor	Warri	

		C_1	C_2	C_3	C_4	C_5	Mean	Range	C_1	C_2	C_3	C_4	C_5	Mean	Range	C_1	C_2	C_3	C_4	C_5	Mean	Range	Mean Total
1	Ca	120.985 ± 5.119	118.604 ± 12.611	124.552 ± 18.019	121.341 ± 10.884	119.648 ± 14.917	121.026	118.604 - 124.557	122.918 ± 7.822	118.004 ± 10.614	121.522 ± 3.861	117.941 ± 12.145	121.016 ± 6.589	120.280	117.941 - 122.918	119.314 ± 11.625	118.358 ± 5.009	119.758 ± 8.902	120.664 ± 13.001	119.486 ± 3.395	119.516	118.358 - 120.664	120.274
2	Zn	2.594 ± 0.308	3.017± 0.462	2.881± 0.115	2.942± 0.685	2.868± 0.236	2.860	2.594- 3.017	2.918± 0.724	2.895± 0.417	3.019± 0.144	3.004± 0.586	2.794± 0.098	2.926	2.794- 3.019	2.804± 0.266	2.935± 0.652	2.881± 0.153	3.114± 0.081	2.658± 0.427	2.878	2.658- 3.114	2.888
3	Cu	0.093± 0.025	0.106± 0.065	0.073± 0.019	0.104± 0.072	0.095± 0.054	0.094	0.073- 0.106	0.083± 0.011	0.077± 0.038	0.098± 0.009	0.101± 0.042	0.091± 0.064	0.090	0.077- 0.101	0.138± 0.066	0.112± 0.035	0.125± 0.018	0.099± 0.044	0.136± 0.078	0.122	0.099- 0.138	0.102
4	Fe	0.682± 0.244	0.602± 0.098	0.715± 0.131	0.693± 0.052	0.708± 0.211	0.680	0.602- 0.715	0.702± 0.311	0.641± 0.094	0.685± 0.201	0.693± 0.118	0.673± 0.082	0.679	0.641- 0.702	0.643± 0.244	0.698± 0.105	0.711± 0.318	0.676± 0.119	0.681± 0.074	0.682	0.643- 0.711	0.680
5	Cr	0.061± 0.009	0.052± 0.011	0.065± 0.028	0.048± 0.011	0.064± 0.007	0.058	0.048- 0.065	0.038± 0.019	0.046± 0.008	0.036± 0.012	0.058± 0.009	0.061± 0.017	0.048	0.036- 0.061	0.085± 0.026	0.071± 0.014	0.081± 0.021	0.069± 0.009	0.063± 0.011	0.074	0.063- 0.085	0.060
6	Co	0.099± 0.035	0.068± 0.021	0.086± 0.014	0.091± 0.028	0.055± 0.019	0.080	0.055- 0.099	0.079± 0.024	0.061± 0.016	0.069± 0.031	0.058± 0.019	0.073± 0.008	0.068	0.058- 0.079	0.114± 0.066	0.108± 0.021	0.096± 0.045	0.103± 0.015	0.084± 0.033	0.101	0.084- 0.114	0.083
7	Pb	0.018± 0.006	0.011± 0.004	0.015± 0.008	0.023± 0.009	0.019± 0.002	0.017	0.011- 0.019	0.011± 0.004	0.009± 0.003	0.008± 0.002	0.012± 0.001	0.011± 0.006	0.010	0.008- 0.012	0.018± 0.005	0.012± 0.008	0.016± 0.003	0.013± 0.004	0.015± 0.006	0.015	0.012- 0.018	0.014
8	Cd	0.051± 0.013	0.068± 0.011	0.037± 0.008	0.042± 0.015	0.048± 0.009	0.049	0.037- 0.068	0.041± 0.005	0.028± 0.001	0.036± 0.007	0.022± 0.009	0.049± 0.003	0.035	0.022- 0.049	0.057± 0.013	0.073± 0.015	0.036± 0.009	0.069± 0.008	0.071± 0.011	0.061	0.022- 0.073	0.048

C_1 = Customer (1); C_2 = Customer (2); C_3 = Customer (3); C_4 = Customer (4); C_5 = Customer (5)

It is discernible from results presented in Tables 1 and 2 that, the examined mineral elements were present in all the milk samples studied. While it is nutritionally desirable that the examined dietary relevant minerals occurred in the studied milk samples, it is however of concern, that the examined toxicologically relevant Cd and Pb also occurred in all the investigated milk samples. Equally discernible from the results presented in Tables 1 and 2 is the apparent variations in the respective values of the examined mineral elements. The observed variations were statistically significant ($P<0.05$) with respect to the various examined mineral elements, but not with sampling locations. Furthermore, statistical analyses did not reveal statistical significance ($P<0.05$) among respective mean values obtained for each of the examined minerals in the studied milk samples purchased from different customers within the same location. Similar handling conditions including feeding habits, medications and time of arrival of the cow to the respective studied, could even if partly, be ascribed to the remark made above.

In Table 1 and also in Table 2, findings further indicate that of all the examined mineral elements, calcium occurred highest. Though mean total value of 119.797mg/kg was obtained for Ca in the three studied locations in Edo State, 120.517mg/kg; 119.177mg/kg and 119.697mg/kg were the respective mean Ca values obtained in milk samples from Benin City, Auchi and Uromi respectively. In milk samples obtained from studied cities in Delta State, the mean total value obtained for Ca was 120.274mg/kg (Table 2). This is slightly higher than the mean total Ca value recorded for milk samples obtained in studied cities in Edo State. The health of the lactating cows, species, age, medications and feeding habits could have been responsible for the reported results. For the respective sampled locations in Delta State, the mean Ca level in milk samples collected from them were 121.026mg/kg (Asaba); 120.280mg/kg (Agbor) and 119.516mg/kg (Warri). Lean (2006) noted that milk is particularly rich in Ca. This is consistent with the results obtained in this work. Calcium is a dietary relevant mineral element, which as posited by Lean (2006), is needed to build bones and teeth.

Next in abundance to Ca among the examined mineral elements in this work is Zn. The mean total value of Zn in milk samples obtained from the sampled locations in Edo State was 2.817mg/kg (Table 1). It is additionally discernible from Table 1, that in milk samples obtained from Benin City, Auchi and Uromi, the respective mean Zn levels in them were 2.928mg/kg, 2.778mg/kg and 2.744mg/kg. Though species, age, health status, medications and feeding habits could have contributed to the levels of Zn in milk samples obtained from the sampled cities in Edo State, variations in Zn levels in milk samples obtained from the different studied cities, could even if partly, be also ascribed to differences in level of urbanization, industrialization and vehicular emissions. These are causative factors of environmental pollution, which perhaps increased release of Zn into the environment, with resultant increase in the level of Zn both in the foliage and water consumed by the various lactating cows. Apparently, Benin City is a more urbanized and industrialized city than Auchi and Uromi. In Benin City, there are also more vehicles compared to the number of vehicles separately found in Auchi or Uromi. The high level of vehicles found in Benin City seemed to have resulted in greater vehicular emission in the city. Auchi and Uromi by estimation, are on the same level of urbanization and possibly, experience close amount of vehicular emission. However, in Auchi and her environ, high solid mineral minning activities are taking place. Supposedly, the activities of the miners could cause environmental pollutions, aspect of which could have been the release of mineral elements into the environment. Suggestively, therefore, the observed variations in the levels of the examined mineral elements in milk samples obtained from Auchi, compared to those in milk samples obtained from Uromi, could be due to solid mineral minning activities in Auchi and her environ, which obviously are absent in Uromi and her surroundings.

The total mean value of Zn in milk samples obtained from the sampled cities in Delta State was 2.888mg/kg. Though closed, the mean value of Zn obtained in milk samples collected from sampled cities in Delta State, is higher than the corresponding mean total Zn level, in milk samples obtained from the sampled cities in Edo State. Variations in levels of urbanization, agricultural activities and general harmful environmental practices in both states, are implicated as factors responsible for the noted observation. Considering the milk samples collected from the studied cities in Delta State, it is discernible from the results presented in Table 2, that samples obtained from Asaba had mean Zn value of 2.860mg/kg. On the other hand, those obtained from Agbor and Warri, had mean Zn values of 2.926mg/kg and 2.818mg/kg respectively. With respect to urbanization, Asaba and Warri are more urbanized than Agbor. Furthermore, Warri and her environ, are homes to many petroleum industries involved in crude oil business activities. Agbor, on the other hand, is a fast growing urban center, with industrialization emerging geometrically. Additionally, agricultural practices with inherent use of agro-chemicals are more in Agbor, than in Asaba and Warri. It would appear therefore, that the aforementioned factors are fast contributing to the release of Zn and some other metals into Agbor environment, as evident in the results presented in Table 2.

Zinc is a constituent of some enzymes including carbonic anhydrase (Deman, 1999). Additionally, Belitz *et al*. (2009) posited that Zn and those other divalent metal ions, activate enzymes such as depeptidases, alkaline phosphatase, lecithinase and enolase. Zinc therefore, is nutritionally desirable. The world presently, is faced with growing bio-engineering practices. Among developing nations in particular, there could be abuses. Consequently, investigation of traditional and non-traditional food sources for their dietary relevant mineral elements level is imperative.

Results presented in Table 1, also indicated that mean total level of Cu in milk samples collected from Benin City, Auchi and Uromi, all in Edo State, was 0.102mg/kg. With respect to individual cities in Edo State where milk samples were collected, mean value for Cu in Benin City was 0.106mg/kg; in Auchi it was 0.112mg/kg and in Uromi, mean value of Cu was 0.088mg/kg. The explanations adduced for the trend of results obtained for Zn levels in milk samples collected from the studied cities in Edo State, are also applicable here. Results presented in Table 2, indicated that in Delta State, the mean total value for Cu was 0.102mg/kg. In Asaba, Agbor and Warri, the respective mean values obtained for Cu were 0.014mg/kg, 0.090mg/kg and 0.122mg/kg. Factors such as urbanization and industrialization, especially activities relating to crude oil exploration, exploitation and refining which is characteristic of the Warri environment, seem to be contributing to the release of Cu into the environment, as results indicate. Worthy of note is that, some agicides used in treating water facilities including swimming pools, are copper compounds. If these are indiscriminately released into the environment, they would contribute to elevated level of copper in the environment. Remarkably, Warri has many water facilities used by the various industries and these water facilities are constantly treated.

Some authors (Harris, 2001; Osredkar and Susta, 2011) earlier remarked that Cu is needed for skin and blood vessel's strength, as well as being essential for the production of myelin, hemoglobin and normal functioning of the enzyme systems. However, according to Storelli *et al*. (2007) and Barn *et al*. (2014) excessive intake of Cu may lead to immunity disorder, dermatitis, impaired nervous system, as well as gastrointestinal and neurological problems. IDF (1979) proposed maximum limit of 0.01µg/g for Cu in milk. On the other hand, a range varying between 0.1-0.9µg/g was proposed by Puls (1994) for Cu in milk. Interestingly, Cu levels in milk samples used in this study, were either below the range or within the range bracket proposed by Puls (1994). In Egypt, Malhat *et al*. (2012) reported Cu level in milk samples to be 1.451µg/g; while Bilandžić *et al*. (2011) reported Cu level in milk samples obtained in Croatia to be 0.914µg/g. The various Cu levels in milk samples reported in this study are lower than those of the aforementioned authors. Such factors as urbanization, industrialization and degree of exposure of cows to contaminated fields could be responsible for the different levels of Cu in milk, reported for the different regions.

It is additionally discernible from results presented in Table 1, that mean total level of Fe in milk samples obtained from the studied cities in Edo State was 0.606mg/kg. In Benin City, mean level of Fe in milk was 0.611mg/kg; in Auchi it was 0.627mg/kg; and in Uromi it was 0.581mg/kg. The highest level of Fe in milk samples collected from Auchi is again, ascribed to the solid mineral mining activities in Auchi and its environment. The indiscriminate dumping of minning wastes and minned solid minerals before collection by buyers, appeared to be adversely affecting Auchi environment. In Table 2, results indicate that the mean total level of Fe in milk samples obtained from Delta State was 0.680mg/kg. Mean Fe level in milk collected from Asaba was 0.680mg/kg, in Agbor, the value of 0.679mg/kg was obtained as the mean Fe level in milk samples investigated. Samples of milk collected from Warri had mean Fe value of 0.682mg/kg. Clearly, very minimal variations exist in the Fe levels of milk samples obtained from the different cities in Delta State. Iron is present in the hemoglobin (blood) and myoglobin (muscle tissue) pigments, as well as present in a number of enzymes including peroxidase, catalase hydroxylases and flavine enzymes (Belitz *et al*., 2009).

Chromium in milk samples obtained from Edo State as results indicate (Table 1), had a mean total value of 0.063mg/kg. With respect to the studied locations in Edo State, it is discernible from results (Table 1), that the respective mean values of Cr in the analysed milk samples obtained from Benin City, Auchi and Uromi were 0.065mg/kg, 0.069mg/kg and 0.054mg/kg respectively. The observed variations in mean Cr levels in the milk samples obtained from the different studied locations are ascribed to differences in the level of urbanization, industrialization and general wastes generation and handling factors. These, it would appear, created variations in pollution indices in the different environments, which apparently, contributed to the respective reported Cr levels. In partiular, the minning activities going on in Auchi, as well as other anthropogenic activities seemed to have contributed to the release of Cr into Auchi environment, which clearly, resulted in high levels of Cr in the diet and drinks of the lactating cows from which the milk samples were collected. This is evidently so, considering the fact that of the three studied locations, Uromi is probably on the same level of urbanization as Auchi, yet, variations exist in the results obtained. Furthermore, though Benin City is more urbanized than Auchi, the mean Cr level in milk samples obtained from Auchi was higher than that of milk samples obtained from Benin City. It is pertinent to mention that the solid minerals minning activites in Auchi and its environment, which are absent in Benin City and Uromi, would have contributed to the variations in Cr levels in milk samples obtained from the three cities in Edo State.

The mean total level of Cr obtained for milk samples studied in Delta State was 0.060mg/kg. This is slightly lower than the values obtained for samples collected from Edo State. Variations in the sum total of the factors that contribute to the release of Cr into the environment are responsible for this. It is particularly important to note that of the six studied locations in both states, the highest mean level of 0.074mg/kg was obtained for milk samples collected from Warri, even when the overall mean total of Cr level in milk samples collected from Delta State was lower than that of Edo State. The low mean value of Cr in milk samples collected from Asaba and Agbor obviously, contributed to lowering the mean total value of Cr in milk samples collected from Delta State. High industrial activities in Warri and its environ, which are predominantly petroleum industry related, appeared to have contributed to the release of Cr into Warri environment. This it would appear, contaminated the diets and drinks of the lactating cows from which the milk samples were obtained.

Results presented in Table 1 additionally, indicate that mean total value of 0.078mg/kg was obtained for Co in milk sample collected from studied cities in Edo State. The highest mean value of 0.088mg/kg was obtained for Co in milk samples collected from Benin City. Milk samples obtained from Auchi had mean Co level of 0.082mg/kg; while the mean Co level of 0.065mg/kg was obtained for milk samples collected from Uromi. It is also discernible from Table 2, that in Delta State, mean total value of 0.083mg/kg was obtained for Co in the investigated milk samples. With respect to Co values in milk samples obtained from Delta State as further discernible in Table 2, findings indicate that the highest mean Co value was recorded in milk samples collected from Warri. This was followed by mean Co value of milk samples obtained from Asaba, then followed by that of the mean value of Co in milk samples obtained from Agbor. The differences in the levels of urbanization, industrialization and environmental pollution would even if partly, account for the pattern of results obtained. In India, Palva *et al.* (2008) reported mean value of 0.19µg/g for Co in milk samples. The respective mean values obtained for Co in the studied Nigerian states in this work are lower than the values reported by Patra *et al.* (2008). Differences in the levels of pollution in the different studied locations could be responsible for this. However, compared to mean value of 0.006µg/g reported for Co in milk samples obtained from Korea by Khan *et al.* (2013) and 0.005µg/g reported by Rey-Crespo *et al.* (2013) for Co in milk samples obtained from Spain, the values we obtained are higher.

Cobalt, when present in excess, could disturb the reproductive system and the thyroid glands (Nordberg *et al*., 2007). Additionally, with respect to IARC (1991) classification, Co is implicated as a probable carcinogenic substance. It is imperative that these facts be considered in quality assessment of milk, especially when the most vulnerable age groups that is, adults and infants consume lot of milk.

The mineral element Pb occurred in all the studied milk samples. In Edo State, mean total Pb level of 0.013mg/kg was obtained in the studied milk samples. Milk samples obtained in Benin City had mean level of 0.016mg/kg for Pb; For Auchi, the mean level of Pb in the studied milk samples was 0.013mg/kg and in Uromi, the mean level of Pb in the studied milk samples was 0.011mg/kg. Clearly, location appeared to influence the mean level of Pb in the investigated milk samples collected from the studied cities in Edo State. The mean total Pb value obtained for milk samples collected from the studied cities in Delta State was 0.014mg/kg. This value is higher than that of Edo State. Though, Pb occurrence in the environment is most often ascribed to pollution, it is interesting to note that in Delta State, even when Warri is more industrialised than Asaba, the mean level of 0.017mg/kg recorded for Pb in milk samples obtained from Asaba, was higher than the corresponding Pb mean level of 0.015mg/kg obtained for milk samples collected from Warri. Instructively, Asaba shares common boundary with the highly industrialised and urbanized city of Onitsha in Anambra State; and truly, environmental pollution is trans boundary. Thus, Pb emissions from various sources in Onitsha could be contributing to Pb availability in Asaba environment, with the effect that foliages and water bodies in Asaba are contaminated with high Pb values. Findings from this work is suggestive of this, even if partly.

Lead is one of the most toxic heavy metals (Swarup *et al.*, 2005). The concern over Pb occurrence in the studied milk samples can be viewed from this perspective. In cow milk found in Egypt, Malhat *et al.* (2012) reported lead value of 4.404µg/g. In this work, our findings indicate that the mean total value of Pb in milk samples obtained from the studied locations in Edo State was 0.013mg/kg (0.013µg/g). The corresponding mean total value of Pb in milk samples obtained from the studied locations in Delta State was 0.014mg/kg (0.014µg/g). Evidently, the levels of Pb in milk samples studied in this work were lower than the value reported for Pb in milk samples studied by Malhat *et al.* (2012) in Egypt. Differences in the levels of urbanization, industrialization and subsequent environmental pollutions in the various studied cities. Furthermore, the levels of Pb contamination in the feed, water and drugs the lactating cows were exposed to in the various regions wherein the milk samples were obtained, are responsible even if partly, to the various results reported by the different researchers.

It is pertinent to also note that in Egypt, Sayed *et al.* (2011) reported mean level of Pb in milk samples studied to be 0.327µg/g. Again, comparing the respective mean total values reported for Pb in milk samples studied in this work, the values we obtained were lower than that of Sayed *et al.* (2011). Interesting observations emerged when the finding of Malhat *et al.* (2012) for Pb in milk samples obtained from Egypt, is compared with that of Sayed *et al.* (2011) for Pb in milk samples also obtained from Egypt. The variations in the mean level of Pb in milk samples studied by both authors, which appeared progressive with time, is consistent with the opinion of Swarup *et al.* (2005); that the level of Pb in milk and milk products is increasing day by day.

The respective mean total values of Pb in milk samples studied in this work, were noted to be higher than the 0.012µg/g for Pb in cow milk samples examined by Najarnezhad and Akbarabadi (2013) in Iran, as well as Pb level of 0.004µg/g in milk samples in Korea, as reported by Khan *et al.* (2014). Regional control measures put in place to control Pb contamination of animal feeds, as well as levels of industrialisations and urbanizations will significantly influence the level of Pb in milk samples. These factors are therefore identified, as been contributors to the various Pb levels in milk samples obtained from the different regions.

With respect to Codex Alimentarius Commission (2011) report, the maximum permissible limit for Pb in milk is 0.02µg/g. It is interesting to note therefore, that the mean total value of 0.013µg/g obtained for Pb in milk samples collected from studied cities in Edo State and the corresponding mean total value of 0.014µg/g obtained for Pb in the investigated milk samples obtained from studied cities in Delta State, are lower than the Codex Alimentarius Commission (2011) maximum permissible limit for Pb in milk. Putting in place stringent control measures that will ensure reduction of Pb contamination in milk samples in the studied locations, is imperative, if the desirable effects of our finding is to be long lasting.

It is further discernible from the results presented in Table 1, that in Edo State, the mean total value obtained for Cd in milk samples studied was 0.048mg/kg. Also, in Delta State, results (Table 2) indicate that the mean total value obtained for Cd in milk samples studied was 0.048mg/kg. However, variations exist with respect to the mean Cd level in milk samples obtained from the different studied locations. The trend of results was similar to those of the earlier discussed mineral elements that were examined in this study. Therefore, the earlier adduced explanations are also relevant with respect to the various reported Cd values in the studied cow milk samples.

In bovine milk samples obtained from Egypt, Erib *et al.* (2009) reported mean Cd value of 0.086µg/g. Additionally, in the study carried out by Ogabiela *et al.* (2011), mean Cd level in milk samples obtained from cows grazed around Challawa industrial estate of Kano (northern Nigeria) was noted to be 0.131µg/g. The mean total value of 0.048mg/kg (0.048µg/g) reported for Cd in milk samples studied in this work, is lower than the corresponding values reported by the aforementioned authors. Worthy of note, is the apparent difference in the value of Cd in milk samples reported by Ogabiela *et al.* (2011) and the value we obtained for Cd in milk samples studied in this work. In particular, both works investigated milk samples obtained from Nigeria. Suffice it to mention that Ogabiela *et al.* (2011) examined Cd in milk samples obtained from cows grazed around Challawa industrial estate of Kano (northern Nigeria). In this work, the milk samples used were obtained from animal markets located away from industrial centers. Thus, sampling locations even within the same country appeared relevant in the overall level of Cd in milk samples.

The report of P̂ilarezyk *et al.* (2013) showed that in milk obtained from Poland, Cd occurred at a level of 0.004µg/g. Additionally, Bilanžić *et al.* (2011) noted that in milk sample obtained from Croatia, Cd occurred at a level of 0.003µg/g. Apparently, the reported value of 0.048µg/g for Cd in milk samples studied in this work, is higher than those reported for milk samples obtained from both Poland and Croatia. The apparent low values of Cd in milk samples found in Poland and Croatia obviously, suggest very low if any, Cd toxicity due to milk consumptions in Poland and Croatia.

CONCLUSION

In this work, findings indicated variations in the levels of occurrences of the examined mineral elements in the studied milk samples. With respect to the levels of occurrence of individual mineral elements examined, variations were also noted, which tended to be related to the different sampling locations. The high toxicologically relevant Pb and Cd occurred in all the studied milk samples. It is suggested that appropriate control measures should be put in place, to check the escalation of the levels of the toxicologically relevant mineral elements in milk obtained from the studied locations.

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