EVALUATION OF HEAVY METALS PROFILE IN DIFFERENT BRANDS OF INFANT FOOD NUTRITION

ABSTRACT

The concentrations of five essential metals in six selected (NAN, COWBELL, MY BOY, CERELAC, FRISO GOLD and NUTREND) infant formulae sold in Makurdi metropolis, were determined using Atomic Absorption Spectrophotometer. Data collected were subjected to Analysis of Variance using SPSS 24 statistical software package. Significant differences in means were separated using Duncan Multiple Range Test. There was significant difference in the levels of the trace metals in the different brands of infant formula milk. The results of the toxic trace metals shows that Cr has the highest concentration of 6.175 ±0.001 mg/kg (COWBELL), when compared to other toxic trace metals analysed. Milk based infant formula showed copper concentration ranged from 2.55±0.001– 6.357±0.001mg/kg which was higher than the provisional tolerable daily intake of copper is 3 mg (FAO/WHO, 1999). Chromium, Copper, Manganese and lead were detected in the six brands of infant formula milk but they were below the limits set by WHO. However, the intake levels of Cd in this study from these concentrations were below the WHO limits. These results validate and in some cases counter earlier reports on the composition of infant formula found in our local markets. Therefore, the concentrations of the studied elements were within safe limits recommended by National Industrial Standard and the Institute of Medicine, for foods but higher concentration of trace metals in some brands of infant formulae above the recommended limit poses a risk of bioaccumulation

INTRODUCTION

Infant formula as food supplements has a part to play in the diets of infants that is very important. In the sense that, they supply to the body minerals and vitamins which is required in a larger quantity. Since they are primarily derived from animals or plants, they are therefore mostly milk, soya or cereal-based. They almost have all the nutrient requirements that are in breast milk, although it is difficult to produce a formula equal in all respects to breast milk (Joseph *et al.*, 2011). Studies have reported contamination of infant formula milk by various substances such as nitrates, nitrites, aluminium, cadmium, mercury, nickel, lead and melamine (Ljung *et al.*, 2011). Water that is used to reconstitute the infant formula milk can also be a source of contaminants such as lead and nitrate ion (Ljung *et al.*, 2011). In 2008 melamine contamination of infant formula milk in China lead to deaths and illness to several infants. Other cases of recall of infant formula milk in developed world as a result of deficiency in necessary nutrients and contamination have also been reported (Marsha, 2010). In 2006 Mead Johnson Company recalled a batch of its infant formula milk due to contamination with metal particles (Nakashima *et al*., 2009). A study to determine the concentration of selected trace and toxic elements in breast milk and infant formula milk reported the concentrations in infant formula milk to be tenfold higher than in breast milk, thus confirming infant formula milk as an exposure route of toxic elements to infants (NDRC, 2005). Infants are at a critical point of their brain development and exposure to elements such as lead and cadmium pose severe health risk to the child and can lead to speech delay, hyperactivity, learning disabilities, attention deficit disorder and neurological deficits (Lanphear *et al*., 2008). The effect of metal poisoning on infants is compounded by the fact that even at low levels of exposure metals bioaccumilate in vital organs such as the kidney, an effect that persists in adulthood (Mielke *et al*., 1999). The relationship between nitrate, nitrite ions and infant methemoglobinemia, nausea and diarrhea has been well reviewed and appreciated, nitrates and nitrites may also cause endogenous formation of carcinogenic N-nitroso compounds (Salah, 2012). Chemical composition of infant formula milk also

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depends on the duration of its storage and therefore should be used as early as possible from the date of manufacture (Salah, 2012). The WHO / FAO have set in place provisional tolerable weekly intake limits for these ions and metals by infants (IOM, 2010). To determine the micronutrients concentrations of heavy metals (Cu, Mn, Co, Pb and Cd) in some selected infant formula sold in Makurdi metropolis.

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MATERIALS AND METHODS

Study Area

The study area (Figures 1) is Makurdi the capital of Benue State is located along River Benue with coordinates as 7^0.43'50"N and 8^0.32'10"E with an estimated population of over 500,000 as at 2007 (NPC 2006) and still growing. Average annual temperature 31^0C with SW wind at 10km/hr and relative humidity of 66% annually. The climate of Makurdi town is the tropical wet and dry type, Koppen's classification, with double maxima (Ayoade, 1983).

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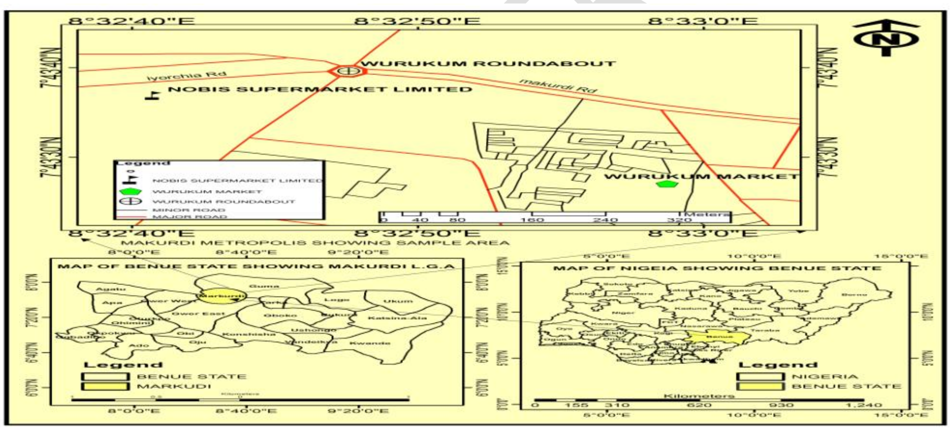

Figure 1. Map of Benue State showing the Sampling Points

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Sample collection

Six (6) different brands of infant formula samples were purchased from the major markets, in Makurdi Benue State, North Central Nigeria using a simple random technique in the two markets. The six different brands comprises of three (3) infant milk formulae and three (3) infant cereal formulae.

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Chemicals / Reagents

In the preparation of reagents, chemicals of analytical grade purity and distilled water were used. Petroleum ether (40 - 60), Nitric acid, Sulphuric acid, Hydrogen perioxide and distilled water. Standard solutions of Cu, Mn, Co, Pb and Cd having concentrations were prepared and Working standards / control were prepared from standards of each metal.

Apparatus

All glass wares were cleaned by immersion in 10% nitric acid in 24 hours, and then washed with detergent solution. They were rinsed with tap and deionized / distilled water before drying in the oven. Desiccators, porcelain crucible, filter paper, kjeldahl flask, kjeldahl distillation apparatus, conical flask, muffle furnace, soxhlet extractor, round bottom flasks, weighing balance, Heating mantle, Beakers, measuring cylinder, funnels, volumetric flask and Flame Atomic Absorption Spectrophotometer were clean with Devo-Clean® solution.

Digestion of Sample / Sample Preparation

The samples were digested following the procedure described by (Iyaka, 2007). Exactly 2.0g of each sample was weighed in a beaker and 15cm^3 HNO_3 was added. It was allowed to stand for 15min. The mixture was heated until the liquid reduced to 5cm^3. Then after cooling, 5 cm^3 HNO_3, 5 cm^3 H_2O_2 were added and the content was evaporated to 5cm^3. After cooling, the residual acid was eliminated by adding 10cm^3 distilled water and the mixture was boiled for 10 minutes. After cooling, the digested samples was poured into 25cm^3 volumetric flasks and was made up to the mark with distilled water.

Preparation of Reagents

I. 1000mg/ dm^3 chromium (Cr) solution

Anhydrous potassium dichromate ($K_2Cr_2O_7$) of 5.653g was weighed and dissolved in distilled water in a beaker containing $10cm^3$ of 10% nitric acid. The resulting solution was transferred into a $1000cm^3$ volumetric flask and was made up to 1000 cm^3 with distilled water in a volumetric flask giving 1000 ppm chromium standard solution.

II. 1000mg/ dm^3 Copper (Cu) solution

Copper nitrate ($Cu(NO_3)_2.3H_2O$) of 3.804g was weighed and dissolved in distilled water in a beaker containing $10cm^3$ of 10% nitric acid. The resulting solution was transferred into a $1000cm^3$ volumetric flask and was made up to $1000cm^3$ with distilled water in a volumetric flask giving 1000 ppm copper standard solution.

III. 1000mg/ dm^3 Lead (Pb) solution

Lead nitrate ($Pb(NO_3)_2$) of 1.598g was weighed and dissolved in distilled water in a beaker containing $10cm^3$ of 10% nitric acid. The resulting solution was transferred into a $1000cm^3$ volumetric flask and was made up to $1000cm^3$ with distilled water in a volumetric flask giving 1000 ppm Lead standard solution.

IV. 1000mg/ dm^3 Manganese (Mn) solution

Manganese sulphate monohydrate ($MnSO_4.H_2O$) of 3.076g was weighed and dissolved in distilled water in a beaker containing $10cm^3$ of 10% nitric acid. The resulting solution was transferred into a $1000cm^3$ volumetric flask and was made up to 1000 cm^3 with distilled water in a volumetric flask giving 1000 ppm Manganese standard solution.

V. 1000mg/dm^3 Cadmium solution

$Cd(NO_3)_2.4H_2O$ (2.744 g) was weighed and dissolved in 5.00cm^3 concentration HNO_3. The solution was made up to 1000 cm^3 with distilled water in a volumetric flask giving 1000ppm cadmium standard solution.

Preparation of Standard Solutions

Ten centimeter (10 cm^3) of 1000 mg/dm^3 was pipetted and was made up to the mark with distilled water in a 100 cm^3 volumetric flask. This gives rise to 100 mg/dm^3 of each metal (Cu, Mn, Co, Pb and Cd). From the resultant solution, 2, 4, 6, 8, and 10cm^3 was pipetted and made up to 100 cm^3 mark with distilled water, each with new concentration of 2, 4, 6, 8 and 10 mg/dm^3 respectively. The experiment was repeated using all the stock standard solutions.

Instrumentation

The digested samples were analyzed for the presence of Cu, Mn, Co, Pb and Cd using spectrometer. From each tin 2.5 g of infant formula milk was accurately weighed into a Kjeldahl flask, 15 ml of concentrated nitric acid and 5 ml of 10% hydrogen peroxide were added and the resulting solution heated until no more brown fumes were produced. The resulting matrix was filtered through Whatman paper no 1 into 50 ml volumetric flask and its volume topped up with deionized water to the mark. The measurements for Cu, Mn, Co, Pb and Cd were done in triplicates using computerized Varian Atomic absorption Specrometer (Model: AA-10). Blank samples were digested following the same procedures as the samples and run in the instrument. Their absorbance were recorded, the mean absorbance and standard deviations were determined and used for calculating limit of detection using Equation 3.2.

$$Limitof \ \det ection = \frac{3xs \tan darddeviat \ ionofblank \ readings}{Absorbance \ ofs \tan dardxMeana \ bsorbanceo \ fblanks}$$

Data Analysis

The results obtained in this study were subjected to analysis of variance (ANOVA) using Statistical Package for Social Science. For multiple comparisons of means across different infant formulae brand, ANOVA was used. In all the case p-values less than 95% confidence level (α = 0.05) were considered significantly different using Tukey test.

Result and Discussion

The effect of trace metals concentrations in the various infant formulae are shown in Table 1. There were significant different (P<0.05) across the selected infant formulae sample for chromium, copper, manganese, cobalt, cadmium, and lead respectively. The concentration of chromium (Figure 1) was significantly (P<0.05) higher in COWBELL (6.175mg/kg) and lowest in FRISOGOLD (0.70mg/kg). Copper had significantly (P<0.05) higher concentration in NAN (6.357mg/kg) while the least concentration was recorded in MYBOY (2.55mg/kg), though not detected in CERELAC, FRISOGOLD and NUTREND (Figure 1). Manganese had significantly (P<0.05) higher concentration in COWBELL (5.10mg/kg) while the least concentration (Figure 2) were observed in CERELAC, FRISOGOLD and NUTREND (1.275mg/kg). Cobalt had significantly (P<0.05) higher concentration in COWBELL (0.150mg/kg) while the least concentration was recorded in NAN (0.10mg/kg), though not detected in CERELAC, FRISOGOLD and NUTREND (Figure 3). Lead had significantly (P<0.05) higher concentration in NAN (37.263mg/kg) while the least concentration was recorded in COWBELL (0.38mg/kg; Figure 4), though the trace metals were not detected in MY BOY, CERELAC and FRISOGOLD (Figure 4.7). Cadmium (Figure 5) was detected in NAN (0.013mg/kg) while it was not detected in MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND.

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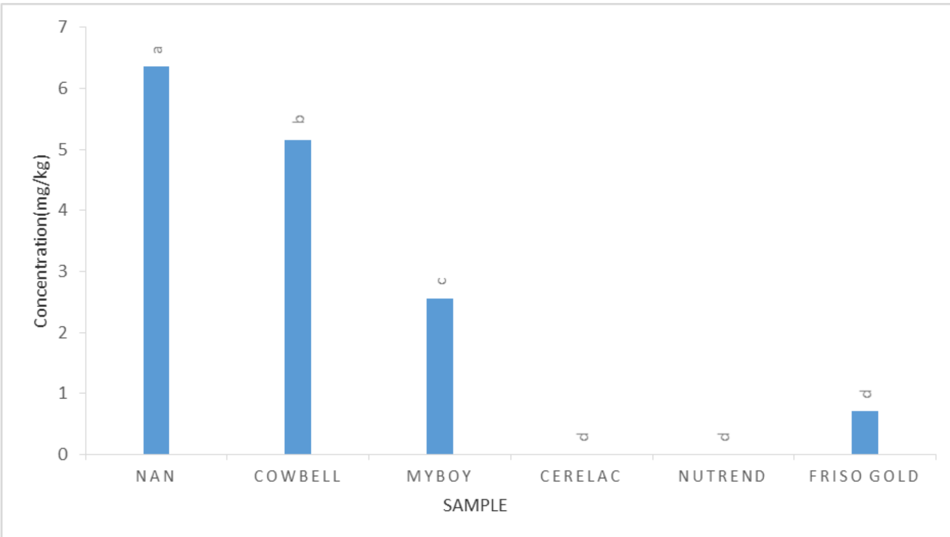

Figure 1: Concentration of Cu in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND infant formulae

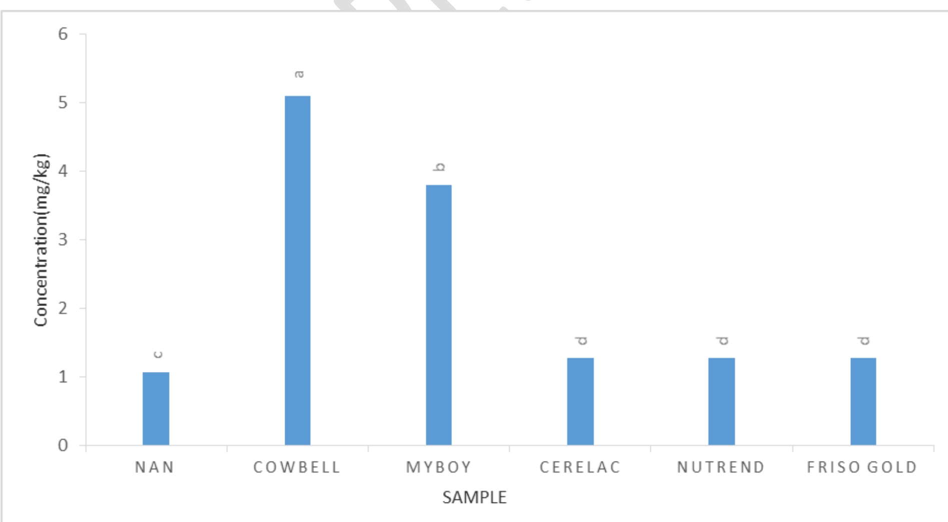

Figure 2: Concentration of Mn in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND infant formulae

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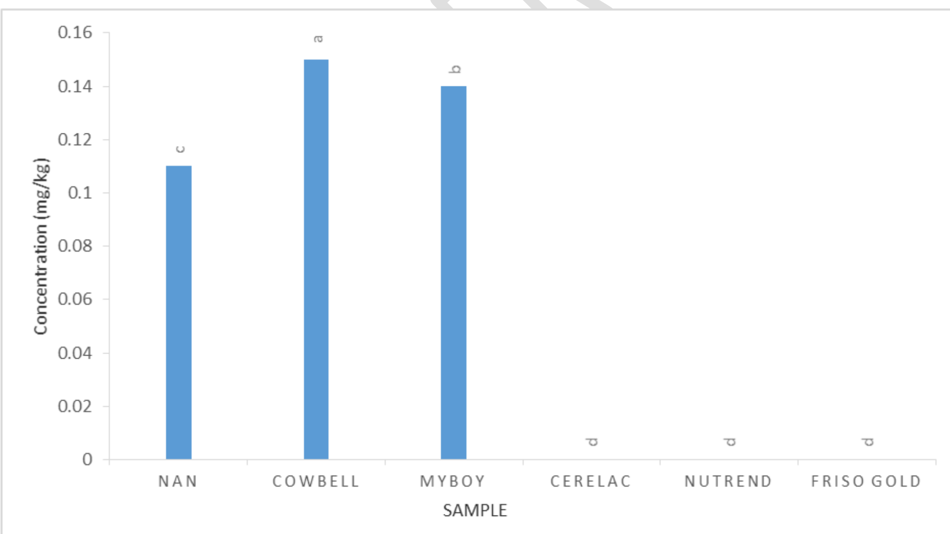

Figure 3: Concentration of Co in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND infant formulae

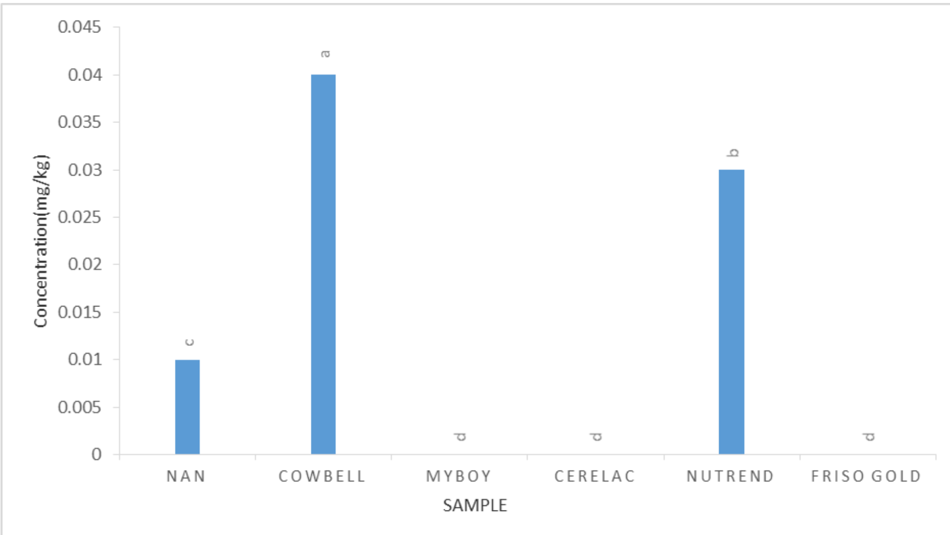

Figure 4: Concentration of Pb in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND selected infant formulae

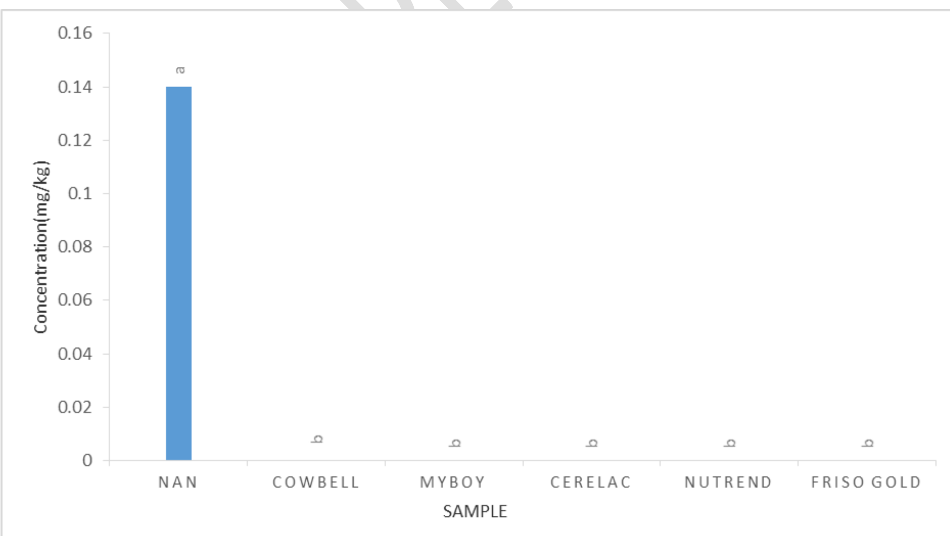

Figure 5: Concentration of Cd in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND infant formulae

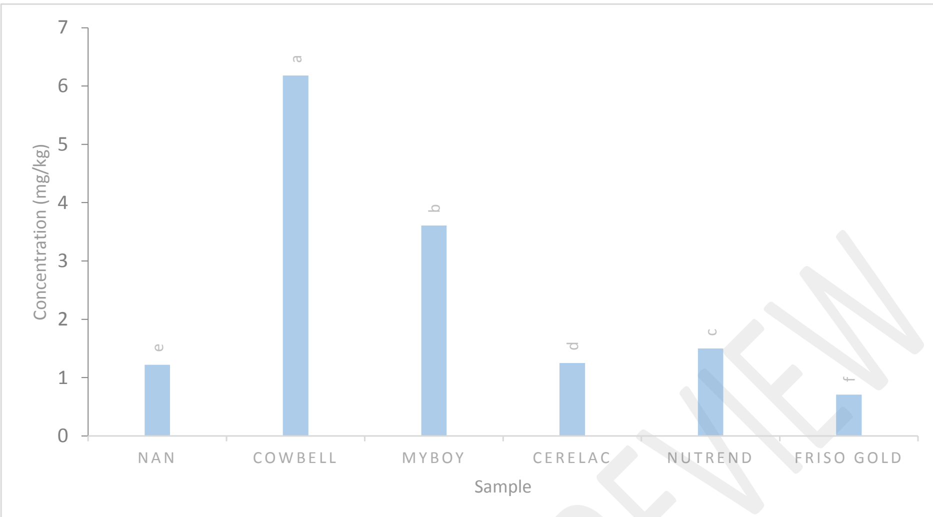

Figure 6 : Concentration of Cr in NAN, MYBOY, COWBELL, CERELAC, FRISOGOLD and NUTREND infant Formulae

Differences observed across the brands of infant formulae for the trace metals agrees with the reports of several authors (Buzallaf *et al*., 2004). Ayivoret *al*. (2011) investigated trace elements levels of some commercial baby cereals/formulas sold in several markets in Ghana. Variation observed in this study as compared to the work of Ayivor *et al*., (2011) could be linked to different methods for analysis of trace metals. Copper, manganese, cobalt, cadmium, lead were present in minor concentrations in most of the samples analyzed. The results of this study clearly depicts that the imported commercial baby cereals in the Nigerian market contained some trace metals which were within specified limits in international guidelines. Among various baby foods, milk formulae, along with certain bottled water and beverages have been identified as significant sources of ingested fluoride (Buzallaf *et al*., 2004). During manufacturing, infant dry cereals are processed in slurry and placed in a revolving drying drum. The water from the slurry evaporates and the fluoride from the water remains in the cereal. Some of the milk based baby foods were found within the limit and not exceeding the required upper limit set by

Comment [H9]: Ayiyor *et al.*

Comment [H10]: Please distinguish trace metals from heavy metals. Very important

Comment [H11]: What level of heavy qualifies to be termed as trace metal ?

WHO/UNICEF (1998). Their findings showed that all six types of baby foods were commercially prepared to fulfill the babies' need of nutrients as required by WHO/UNICEF (1998). It was also observed that there were presence of some nonessential elements (Cr, Cu, Mn, Co, Cd and Pb) though in small quantities but of concern to the researcher due to health reasons. It was concluded that the reasons for the variations in these trace metals could be attributed to the different manufacturing practices, quality of raw materials and packaging containers used by infant food manufacturers, especially cereal based food products for babies. Copper concentration was within the range reported by some authors (Ojo and Olabode, 2013) as depicted in. Milk based infant formula showed Copper concentration ranged from 2.56±0.07 – 6.36±0.07mg/kg and was not detected in the analysed Cereal based infant formula. Deficiency causes low white blood cell count and poor growth. Excessive intake can cause vomiting, nervous system disorder, Wilson's disease, liver and kidney damage, brain damage, intestinal discomfort, dizziness and head ache (Sullivian, 2008). Akan *et al.* (2010) reported that excessive accumulation of Cu in the liver may result in hepatitis. The concentration of manganese was detected among the six infant formulas analyzed. Manganese is a cofactor for a number of enzymatic reactions. Cowbell milk has the highest concentration followed by My Boy milk. The Cereal based infant formula (Cerelac, Friso Gold and Nutrend) have concentration of 1.28±0.01 mg/kg and NAN (1.08±0.07) has the lowest concentration. This work agrees with the report of Al khalifa and Ahmad (2010) who had similar trend with the pattern observed in this study. The concentration of manganese in this study was close to the manufacturer recommendation. Cobalt was detected in milk based infant formula (0.11±0.07 mg/kg in NAN, 0.15±0.01 mg/kg in Cowbell and 0.14±0.07 mg/kg in My Boy) as shown in Figure . It was not detected in Cereal based infant formula (Cerelac, Friso Gold and Nutrend). Cobalt levels in our milk-based samples were significantly different than those in the cereal based samples. This may be due to the various sources of the formulas and the manufacturing processes. The concentration of Pb was low which ranged from 0.01±0.00 -0.03±0.01ppm. The difference in the concentration of Pb among the brands can be attributed to the different manufacturing practices and quality of raw materials used (Khalifa and Ahmad, 2010). Lead was detected in three samples. NAN, Cowbell and Nutrend in Figure 4.7 gives the senario of the metal in the studied infant formulas. Although it is below WHO maximum value of 25µg/kg and was also detected by Ojo *et al.*, (2013). Lead is considered to be a toxic element mainly due to some neurological problems, renal dysfunctions, gastrointestinal tract problems, hypertension, and cancer for which there is evidence for animals and human (WHO/UNICEF, 1998). The most important group of species is the organolead compounds which are quient toxic. The more subsituted the organic chain the higher the the toxicity.Cadmium was not detected in all the brands of infant formula milk with the exception of NAN which had low concentrations. However, infant formula milk is a possible source of exposure of cadmium to infants since the water used to prepare the infant formula milk may be contaminated with cadmium (Khalifa and Ahmad, 2010).

CONCLUSIONS

The findings indicated that trace metals are present in adequate concentrations in all the selected commercial baby food samples which suggest that they have no adverse effect on the health status of the infants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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