ANALYSIS OF HEAVY METALS IN COW-MILK BASED INFANT FORMULA BY INDUCTIVELY COUPLED PLASMA - OPTICAL EMISSION SPECTROMETRY

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Abstract

Most of dairy products especially milk is very popular all over the world. It is consumed daily as it is considered as a nutritionally balanced food and is a major source of the nutrients for infants in the first six months of life. Infant milk formulas are intentionally manufactured to supply essential elements in the diets of newborns. These elements are necessary for biological process and play an important role in the development of infants. However, some of these essential elements become toxic when their concentrations exceed the permissible limit and this creates significant health effects to infants. In this study, the concentration of Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn) for seven different brands of infant formulas which are retailed in the Malaysian market were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). From the results obtained, Zn has the highest concentration in infant formulas followed by Cu, Pb, Ni and Cd. The concentration of Zn, Cu, Pb and Ni were from 0.3467 mg/kg to 0.9833 mg/kg, 0.1433 mg/kg to 0.2533 mg/kg, 0.0133 mg/kg to 0.0267 mg/kg and 0.0033 mg/kg to 0.0100 mg/kg, respectively for all seven different brands of the infant milk formulas. For Cd, the concentration was below the detection limit of the instrument. All heavy metals content in infant formulas were within the permissible limit. The obtained results were compared to an existing standard of permissible amounts of toxic heavy metals in infant milk formulas and with previous literatures.

Keyword: ICP-OES, infant milk formula, heavy metals

Introduction

Infant milk formula began to be used primarily as a substitute for human milk and was made available commercially in the late 1800s. At the beginning, home prepared milk formula was based on modification of whole cow's milk. However, in the early 1900s, the differences in the nutritional content of human and cow's milk were better understood which led to the modification of cow's milk that was more similar to the human milk compositions (Kuratko *et al*., 2013). Infant formula is the most essential dairy product because it contains the needed nutrients for growing infants and children (Pereira *et al*., 2013).

The infant milk formula is also known as powdered milk where the milk is prepared by drying the concentrated liquid milk to form dried milk. It is rated the second best after breast milk as it gives advantages for children in terms of growth and development of immune disorder, diabetes, food allergies and coronary heart disease (Bermejo *et al*., 2000). Three major types of infant formula available in the markets are cow milk-based formula, soy-based formula and protein hydrolysate formula. Protein hydrolysate formula contains protein that has been broken down into smaller size than those in cow's milk and soy-based formulas. Protein hydrolysate formula is manufactured for infants that are allergic to cow milk-based formulas or soy-based formulas. Soy-based formula may be an option for infants who are intolerant to animal proteins, lactose and carbohydrate found in cow milk-based formula. Soybean is known to have high level of aluminum (Al) and neutroxins (Kazi *et al*., 2009).

Cow milk-based formula is one of the most commonly used milk formulas. It is often advertised for its good balance of protein, carbohydrates, fats, lactose and minerals. In addition, the formula offers an advantage over human milk where it contains three to four times higher protein content (Raikos & Dassios, 2014). Two types of proteins that can be found in cow milk-based formulas are whey and casein with a ratio of around 60:40. These proteins make the cow milk-based formula easier to digest compared to other formulas. It is the most suitable formula for infants up to 12 months of age and they are available in both liquid and powdered form that needs to be mixed with water.

The trace elements are divided into essential metals and non-essential metals. The essential elements that can be found in infant milk formula are calcium (Ca), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na) and zinc (Zn). Even though these metals are essential, it can be toxic when taken in excess (Li *et al*., 2005). These elements are necessary in trace quantities for biological process, proper metabolic activities and development of the infants. Requirements for essential elements are more crucial during infancy and childhood period (Melø *et al*., 2008).

Arsenic (As), barium (Ba), cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), tin (Sn) and titanium (Ti) may be found in the infant formula. These non-essential elements do not have biological role and considered as non-nutritive. These elements are dangerous since they tend to bio-accumulate in biological organism faster than being excreted or metabolized (Lutfullah *et al*., 2014). It can directly affect infant growth, alter an organ development and function in which will cause adverse health effects later in life (Ljung *et al*., 2011). Moreover, the infants and young children are more at risk to exposure due to their greater intestinal absorption compared to adults.

The infant milk formula might be contaminated with various elements during processing, storage or packaging of the products. The information and data obtained regarding the level of essential and non-essential elements in infant formulas are considered during the manufacturing process. Moreover, the actual concentration of these elements should be determined in the marketed infant milk formula in order to ensure they do not exceed the permissible limit.

The purpose of this study is to determine the concentration of Cd, Cu, Ni, Pb and Zn in the seven different brands of cow milk-based formulas by using inductively coupled plasma optical emission spectrometry (ICP-OES) and to compare the results to the permissible content of heavy metals in infant milk formulas as set by World Health Organization (WHO), Joint FAO/WHO Expert Committee on Food Additive (JECFA) and also by the previous studies by other researchers.

Materials and Methods

Standard Solution Preparation

Dilution of stock solution (1000 mg/L) had been carried out to prepare standard solutions for selected heavy metals (Cd, Cu, Pb, Ni, Zn) in 100 mL volumetric flask. Deionized water was used to mark up the solution until the calibration mark of the volumetric flask. The series of prepared standard solution were 0.5, 1.5, 2.5, 3.5 and 4.5 mg/L, respectively. The formula used for stock and standard solutions dilution was:

$C_1V_1=C_2V_2$

C_1 : Concentration of stock solution (1000 mg/L)
V_1 : Volume of stock solution needed to make new solution
C_2 : Final concentration of new solution
V_2 : Final volume of new solution (100 mL)

Sample Collection
Seven different brands of cow milk-based formulas (dry powdered milk) for infants aged 0-12 month were bought from the nearby supermarkets in Jengka, Pahang. The infant milk samples were kept in their original packages and then were packed into a polyethylene bag. Each sample was labelled with S1, S2, S3, S4, S5, S6 and S7, respectively.

Sample Preparation
Approximately 2.5 g of each different brand of cow milk-based formula sample was weighed and placed in the beaker. 15 mL of concentrated HNO_3 and 5 mL of 10% H_2O_2 were then added into the beaker. The heating of mixture on the hotplate was performed until there were no brown fumes observed, followed by the cooling of solution at room temperature. The resulting solution was filtered by using filter paper and diluted by using deionized water until the calibration mark of 50 mL volumetric flask. The preparation of each sample was carried out in triplicates (Odhiambo *et al*., 2015).

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Analysis
The standard solutions and samples were introduced into the ICP as aerosol which was carried into the center plasma (superheat inert gas). The aerosol transformed into a solid by the plasma, vaporized the solid into gas and then separated individual molecules into atoms. The high temperature of plasma excited the atoms to emit light at a particular wavelength that corresponded to different element in the solution samples. The intensity of the emission then corresponded to the concentration of the element detected. **Table 1** shows the ICP-OES parameter conditions used in order to analyze various metals in the cow milk-based formula samples.

Statistical Analysis
The obtained data was analyzed by using Statistical Package for Social Sciences (SPSS version 21) software. The mean and standard deviation were calculated. The data were analyzed by one-way analysis of variance (ANOVA) to obtain any significant differences between each brand of infant milk formula samples. The Pearson's correlation analysis of heavy metals was also performed in order to analyze and deduce the relationship between the selected heavy metals.

Table 1 ICP-OES operating conditions for metals analysis

Parameter	Condition
Argon flow rate	
(L/min)	0.7
Nebulizer flow	12
Plasma flow	1
Auxiliary flow	1.2
RF power (kW)	5
Read time (s)	Synchronous vertical dual view
Viewing mode	(SVDV)
Viewing height(mm)	8
Pump speed (rpm)	12
Measurement	3
replicates	
Wavelength(nm)	
Cd	228.802
Cu	324.754
Ni	216.555
Pb	220.353
Zn	330.258

Results and Discussion

Calibration Curve

The intensity reading of all metals analyzed through standard solutions in various concentrations had been obtained using optimized operational and instrumental parameters. The constructed calibration graph from 0 to 4.5 mg/L Cu was represented by the linear regression equation of $y = 58555x + 1132$ with 0.9999 of correlation coefficient (R^2), as shown in **Figure 1**.

Meanwhile, the linear regression equation for Cd, Ni, Pb and Zn were $y = 27330x + 1212$, $y = 8017x + 295$, $y = 3460x + 141.7$ and $y = 412.1x + 42.29$, respectively. **Table 2** shows that the R^2 values lay between 0.99968 and 0.99989. The R^2 values had been approved for the acceptable fit of the obtained intensity against concentrations to the regression line as they were more than 0.9980 (Gary, 2004).

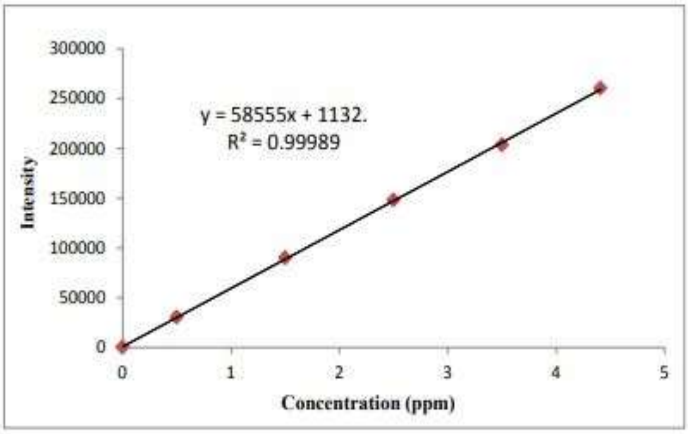

Figure 1 Linear curve of intensity against concentrations of Cu

Table 2 Regression coefficient for each metal analyzed by ICP-OES

Element	Regression Coefficient (R^2)
Cu	0.99989
Pb	0.99979
Ni	0.99975
Cd	0.99969
Zn	0.99968

Copper (Cu) Concentration in Infant Milk Formula

The concentration of Cu in the seven different brands of infant milk formula were shown in the **Figure 2**. The Cu concentration was from 0.1433 mg/kg to 0.2533 mg/kg. It shows that sample S7 has the highest concentration of Cu which was 0.2533 mg/kg, while sample S4 has the lowest concentration which was 0.1433 mg/kg. The second highest concentration of Cu was in sample S6 with 0.2133 mg/kg followed by S2 with 0.2033 mg/kg, S5 with 0.1833 mg/kg, S1 with 0.1767 mg/kg and S3 with 0.1633 mg/kg.

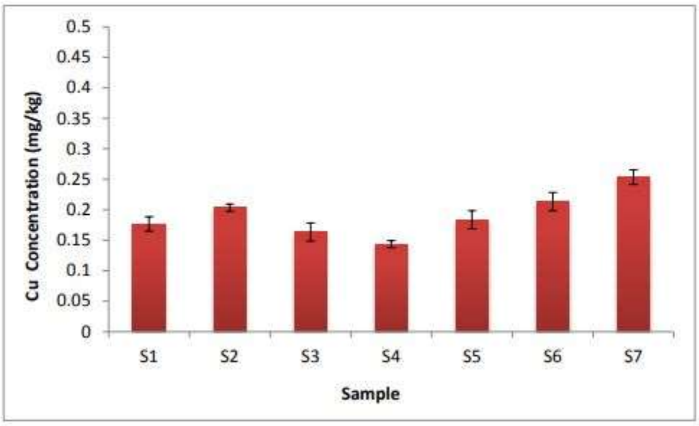

Figure 2 Average concentration of Cu in different brands of infant milk samples ± SD (n=3)

All infant milk formulas were in the range of the allowed Cu limit from 0.05 mg/kg to 0.50 mg/kg as set by JECFA. Jannat *et al.* (2009) had mentioned that a higher Cu content which was 4.436 mg/kg Cu in infant formulas was found in Tehran, Iran, compared to the present study. Pereira *et al*. (2013) had also mentioned a higher Cu content which ranged from 0.45 mg/kg to 0.72 mg/kg. The high Cu content in infant milk formulas was suggested because of its essentiality for infant growth. The Cu is vital in part of several proteins occupied in a variety biological process which is necessary to sustain life especially for infants (Romaῆa *et al*., 2011).

Zinc (Zn) Concentration in Infant Milk Formula

Figure 3 shows that the mean concentration of zinc (Zn) in the infant milk formulas ranged from 0.3467mg/kg to 0.9833 mg/kg. The highest concentration of Zn was 0.9833 mg/kg which was in sample S5. While, the lowest concentration of Zn was in sample S4 with 0.3467 mg/kg. The concentration of Zn was 0.9700 mg/kg for sample S1, 0.9433 mg/kg for sample S2, 0.8467 mg/kg for sample S3, 0.8600 mg/kg for sample S6 and 0.7933 mg/kg for sample S7. The content of Zn in the infant milk formula were within the permissible limit set by JECFA which is from 0.0300 mg/kg to 1.0000 mg/kg.

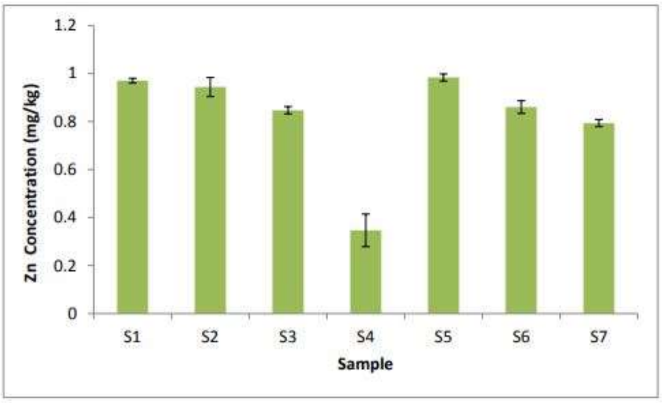

Figure 3 Average concentration of Zn in different brands of infant milk samples ± SD (n=3)

The concentration of Zn in this study is much lower than stated by Khaghani *et al*. (2010) who found 3.98 mg/kg Zn in infant milk samples collected from Tehran, Iran and by Ljung *et al*. (2011) who found average concentration of 4.80 mg/kg Zn in investigated samples. The result obtained from this study was closely similar to Zand *et al*. (2011), who found a Zn content of about 0.8000 mg/kg in all varieties of infant milks available in the United Kingdom market.

The content of Zn is the highest in infant milk formulas compared to other metals might be because it is one of essential elements for the normal growth and proper functioning of body especially for infants. In short, acceptable quantity of Zn is required in the infant milk formula in order to produce the same metabolic response as human milk feeding (Olu-Owolabi *et al*., 2007).

Lead (Pb) Concentration in Infant Milk Formula

Figure 4 shows that the range of Pb content was from 0.0133 mg/kg to 0.0267 mg/kg in the infant milk samples. The highest concentration of Pb was 0.0267 mg/kg in sample S6 and the lowest concentration of Pb was 0.0133 mg/kg in sample S2 and S3. The concentration of Pb was 0.0233 mg/kg for sample S4 and S7, 0.0200 mg/kg for sample S1 and 0.0167 mg/kg for sample S5.

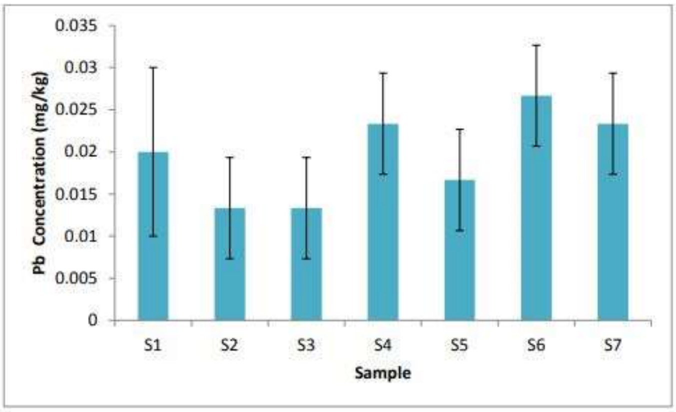

Figure 4 Average concentration of Pb in different brands of infant milk samples ± SD (n=3)

Sample S6 contained Pb of 0.0267 mg/kg which was slightly above the recommended permissible limit (0.025 mg/kg) by JECFA while the other sample lay within the permissible limit. The level of Pb in this study was lower than stated by Abdulkhaliq *et al*. (2012) who found a Pb level ranged from 0.48 mg/kg to 0.80 mg/kg in examined infant milk samples distributed in Zagazig City, Egypt. Moreover, higher Pb content was also found by Abdelkhalek *et al*. (2015) in milk samples for infant sold in Mansoura City, Egypt which was 0.12 mg/kg. Meanwhile, lower Pb level of 0.012 mg/kg was analyzed by Jalilian and Saber (2015).

The presence of Pb in infant milk formulas may be caused by the contamination of original cow's milk. This may happen due to the exposure of lactating cow to environmental pollution from heavy traffic or consumption of contaminated water and grass. Infant formulas also may be contaminated through its packaging from metallic Pb from Pb soldered cans (Salah *et al*., 2013).

Nickel (Ni) Concentration in Infant Milk Formula

The highest content of Ni was in sample S3 and S6 with 0.0100 mg/kg. The lowest concentration of Ni was in sample S1, S2 and S3 with 0.0033 mg/kg. The concentration of Ni in sample S4 and S7 were 0.0067 mg/kg, as shown in **Figure 5**. The Ni content in all samples were within the permissible limit which was 0.011 mg/kg as set by WHO. The level of Ni in this study was lower than stated by Kazi *et al*. (2010) who found the concentration of Ni from 0.124 mg/kg to 0.332 mg/kg in infant formulas retailed in Pakistan market and Khan *et al*. (2014) had found that the concentration of Ni was 0.1531 mg/kg in infant milk formulas which were sold in South Korea.

The concentrations of Ni in the present study were also lower than Lutfullah *et al*. (2014) who found 0.0202 mg/kg Ni in infant formulas which were available from the local markets in Peshawar, Pakistan. The presence of Ni in the infant milk formulas may be because of the usage of contaminated utensil and equipment in their preparation (Kent *et al*., 2015).

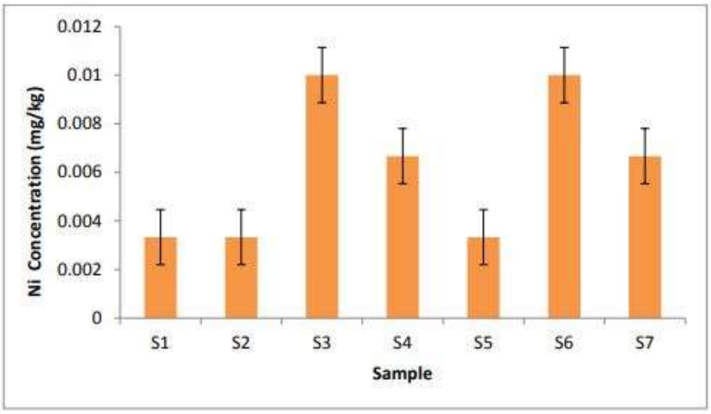

Figure 5 Average concentration of Ni in different brands of infant milk samples ± SD (n=3)

Cadmium (Cd) Concentration in Infant Milk Formula

The concentration of Cd was below the detection limit which indicates the samples have extremely small amount of Cd. Wadhwa *et al.* (2014) had also found the concentration of Cd in all infant formulas below the detection limit. In previous study by Winiarska-mieczan (2009), the concentration of Cd varied from 0.0004 mg/kg to 0.014 mg/ kg in infant formulas which were purchased in grocery shop in Lublin while Dhamo *et al*. (2014) has found Cd level at 0.000725 mg/kg for collected infant formulas from pharmacies in Tirana, Albania.

Correlation Coefficient Analysis

The Pearson's correlation analysis between Cu, Pb, Ni and Zn was shown in **Table 3**. The correlation coefficient with $r \leq 0.35$ indicated that correlations were weak, $0.36 \leq r \leq 0.67$ were represented as moderate relationship and $0.68 \leq r > 1.00$ were represented as strong correlation. Cu showed moderate positive correlations with Zn (r=0.384) and weak positive correlations with Pb (r =0.172) and Ni (r=0.052). The correlation might suggest that the selected heavy metals originated from common sources.

Table 3 Correlation coefficients between heavy metals

	Cu	**Pb**	**Ni**	**Zn**
Cu	1	0.172	0.052	0.384
Pb		1	-0.126	-0.269
Ni			1	-0.076
Zn				1

One-Way ANOVA

There were statistically significant differences of elements between the brands ($p < 0.005$). These differences could be attributed to the different manufacturing practices, quality of raw materials and packaging containers used (Odhiambo *et al*., 2015).

Conclusion

The content of Zn is the highest in infant formulas followed by Cu, Pb, Ni and Cd. The content of Zn, Cu, Pb and Ni were 0.3467mg/kg to 0.9833 mg/kg, 0.1433 mg/kg to 0.2533 mg/kg, 0.0133 mg/kg to 0.0267 mg/kg and 0.0033 mg/kg to 0.0100 mg/kg, respectively for all seven different brands of the infant milk formulas. For Cd, the content is below the detection limit of the instrument. All heavy metals content in tested infant milk formulas were within the permissible limit as set by JECFA.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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