Concentration of Pb, Sn and Fe Metals on Milk Products and Canned Fish in Gorontalo City

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Abstract. This study aims to determine the levels of Lead (Pb), Tin (Sn) and Iron (Fe) in milk products and canned fish at the markets around Gorontalo City. A total of 16 samples of infant formula and canned fish were analyzed using Atomic Absorption Spectroscopy (method. The calibration curves showed regression value $r=0.9997$ (Sn), $r=0.9965$ (Fe), $r=0.9995$ (Pb). LOD and LOQ value is Pb=0.09 and 0.11, Sn=1.81 and 2.23, Fe=0.07 and 0.13. The result showed that in infant formula concentration of Pb and Sn were lower than LOD value ppm and concentrations of Fe were obtained 6.91 ppm, 8.09 ppm, 11.07 ppm, 12.27 ppm, 6.79 ppm, 10.27 ppm, 5.96 ppm, 1.57 ppm. In canned fish concentration of Pb and Sn were lower than LOD value and Fe metal of 1-8 samples obtained 12.99 ppm, 8.24 ppm, 11.20 ppm, 15.67 ppm, 7.11 ppm, 11.56 ppm, 19.24 ppm, and 14.99 ppm. Dissensus that the concentration of Fe> Sn> Pb metal in infant formula and canned milk samples. Health Risk Analysis Index (HRI) value in infant formula and canned fish skin samples were <1, so can be assumed these products safe to consume infant formula.

1. Introduction

Milk is a bioactive substance that is an important source of nutrition for newborns, as a source of Ca that is very helpful in the growth and development as children and also helps reduce osteoporosis in the elderly. Although, milk is an ideal source of macro elements (Ca, K and P) and microelements (Cu, Fe, Zn,Se) addition amounts of contaminant metals might enter milk and dairy product reaching levels that are harmful to humans [1]. Based on Target Health Quotient (THQ) identification, cow’s milk is one source of food contaminated with Cd, Cr, Ar, Hg metals. The concentration of metals in cow’s milk tends to increase with the increasing breastfeeding age because the metals are bioaccumulated [2]. Hg, Cd and Sn at certain concentrations are very toxic to humans [3]. Cd, Cu, Fe, Pb, and Zn are heavy metals with high toxicity [4]. The data for estimated daily intake of heavy metals through milk showed that infants are most prone towards heavy metal toxicity due to the higher rate of milk consumption. The highest estimated daily intake of Pb concentration for male infants (1-2 year) mean weight 11.43(kg) milk intake (kg/day) 0.521 is 0.946 (μg /kg/day). For female infants (13 year) mean weight 9.52(kg)
milk intake (kg/day) 0.342 Pb concentrations 0.745(μg/kg/day) [3]. Pb, Cd, Zn, Cu and Fe concentration in milk and dairy products ranged from 0.044–0.751, 0.008–0.179, 0.888–18.316, 0.002–1.692 and 1.320–45.620 ppm respectively. The concentration of Pb in all samples exceeds the maximum limit (0.02 mg/kg) defined by the codex standard of the general standard for contaminants and toxins in food and feed [4]. Analysis of Sn> Cu> Pb in raw milk and dairy products was between 0.019, 0.02-0.25, and 0.007-0.02 mg/kg [2]. Pb and Cd concentrations were higher in powdered milk than liquid milk 0.022-0.057(μg/g), Fe = 3.2-12.91 (μg/g) [5].

Advances in industrialization have resulted in heavy metal contamination not only in milk and milk products [6][7] also during food processing and packaging such as canned fish products that pose health risks to consumers [2][8]. Solder is a source of Pb contamination in the canning process. Therefore, monitoring Pb concentration in canned fish is very important for human health. Metal Pb induces children's cognitive development and intellectual performance and increases blood pressure and cardiovascular disease in adults [9]. Elevated concentration of Fe metal due to damaged packaging and the presence of air which accelerates the oxidation reaction of Fe in cans. The quality of canned fish, the shelf life of canned fish, pH, coating quality and storage area affect the level of contaminants in fish [10][11]. The metal concentration (μg/g wet weight) of the average tuna fish samples was Cr=2.66, Cu=0.92, Fe=54.68, Mn=0.33 and Ni=0.22 lower than the Maximum Limit defined by WHO [11][12]. In canned tuna, Pb±0.011-0.089μg/g [12] ± 2.50 μg/g concentration compared to superior sardines from United States Environmental Protection Agency (USEPA) limits [13].

Industry and agricultural are common terms in the food chain. Increased agricultural production can be used in animal feed production, but also in dairy farming. Non-compliance and inappropriate use of technology, promising these chemicals as contaminants of agricultural and livestock product [14]. Wastewater and industrial water gradually increase the toxic metal in the soil environment and by the plants transferred to the food chain and its negative effects on human health such as kidney, nervous system damage, reduced IQ, heart, gastrointestinal, cancer and mortality [13]. Toxicology and environmental studies have encouraged the researchers to change non-existent elements. According to the database Rapid Alert System for Food and Feed (RASF) (1980-2016), heavy metals become the 4th category of hazardous material for health after mycotoxins, pathogenic -microorganism and pesticide residues. The majority of RASFF notifications on heavy metals occurred in the following product types: food 73%, food contact material 23% and feed 4%. In general, the highest number of notifications were related to Hg (36%), Cd (27%), Cr (14%), Pb (9%), Ar (6%) and Ni (4%). Notifications focused mainly on fish and fish products (38%) and food contact materials (28%) [9].

The health Risk Index (HRI<1) is a safe boundary for heavy metal polluters and is not at risk for health (adult/children) in consuming canned fish [11]. The consistency of heavy metals in dairy products and canned fish should be routinely monitored for consumers health, for nutritional, toxicological and environmental purposes. Processing and packing of milk may lead to an increase in metal concentration, this can be prevented by careful handling and treatment of raw materials [15].

2. Data and Method
2.1 Sample Preparation
Total of 16 samples were collected and analyzed for heavy metals Pb, Sn and Fe concentration. Samples consist of 8 powder/liquid baby milk products (A) and 8 canned fish (B). The analysis performed using AAS AA900F PerkinElmer and conducted at Industrial Research and Standardization Center of Manado Laboratory and Chemical Laboratory UNG
2.2 Determination of Fe, Pb, Sn in food by Graphite Furnace Atomic Absorption Spectrofotometer.

2.2.1 Sample digestion and preparation
Product was analyzed using dry ashing method. For the beginning product was homogenized and weighed 10-20g test portion \( w \) in a crucible nearest to 0.01g. Dried in oven at 105°C for 3 hours. Then dish placed in muffle furnace at 450°C for 8 hours. 1 ml of \( \text{HNO}_3 \) added to crucible and swirled with care so that all ash comes into contact with acid. Then solution in crucible transferred to 50 ml flask \( v \) through whatman filter paper and wash with aquabidest [5]. Then analyzed using AAS AA7000 Shimadzu at optimal conditions [8].

![Table 1: Optimal Condition for F-AAS](image)

| Lamp (nm) | (secd) | Slit (mA) | Fl-Support |
|-----------|--------|-----------|------------|
| Fe BGC-D2 | 248.3  | 7         | 0.2        | Acetylene-Air |
| Pb BGC-D2 | 283.3  | 7         | 0.5        | Acetylene-Air |
| Sn BGC-D2 | 224.6  | 7         | 0.5        | Acetylene-N\(_2\)O |

2.2.2 Calibration curve and measurement of metal concentrations
To the determine concentration of Pb, Sn and Fe linear type of calibration curve obtain from standard solutions of each metals were used. Preparation of standard solutions of Pb, Sn, Fe from dilution of Pb, Sn, Fe 1000 ppm stock solution, with a concentration range i.e. 0, 10, 20, 40 ppm (Sn), 0, 5, 10, 20 ppm (Pb) and 0.0, 0.2, 0.4, 0.8 ppm (Fe). A blank and standards solution were run in FAAS and three points of calibration curves were established. Each standard solution was measured three times and the mean of the value was plotted. The concentrations of each heavy metal were determined by interpolation from the calibration curves. The measurement of the standard form produces linear regression \( r=0.9997 \) (Sn), \( r=0.9995 \) (Pb) and \( r=0.9965 \) (Fe). The value of \( r=0.999 \) indicates that there is a strong linear relationship between rotational concentration and absorbance [16].

![Figure 1: The calibration curve Sn](image)

![Figure 2: The calibration curve Pb](image)

![Figure 3: The calibration curve Fe](image)
Analysis the Limits of Detection (LOD) and Limits of Quantification (LOQ). The value of LOD is given by the calculation:

\[
LOD = \bar{x}_{bl} + k.S_{bl}
\]

where: \( \bar{x}_{bl} \) is the mean of the 7 times blank determinations, \( k \) is the numerical factor chosen according to the confidence level desired and number of blank readings taken (\( k \) for LOD = 3), and \( S_{bl} \) is the sample standard deviation of the blank determinations. And the value of LOQ is given by the calculation:

\[
LOQ = \bar{x}_{bl} + k.S_{bl}
\]

where: \( \bar{x}_{bl} \) is the mean of the 7 times blank determinations, \( k \) is the numerical factor chosen according to the confidence level desired and number of blank readings taken (\( k \) for LOQ = 10), and \( S_{bl} \) is the sample standard deviation of the blank determinations [17].

| Metal | \( \bar{x}_{bl}(\text{ppm}) \) | \( S_{bl} \) | LOD\(_{\text{(ppm)}} \) | LOQ\(_{\text{(ppm)}} \) |
|-------|-------------------------------|------------|----------------|----------------|
| Pb    | 0.0768                        | 0.0034     | 0.0870         | 0.1108         |
| Sn    | 1.6310                        | 0.0598     | 1.8104         | 2.2290         |
| Fe    | 0.0451                        | 0.0084     | 0.0703         | 0.1291         |

Result of metal concentration calculated by using the following formula:

\[
\text{Concentration of metal (ppm)} = \frac{(R_v w)}{w}
\]

where: \( R \) is reading concentration of metal in AAS (ppm), \( v \) is final volume of sample (ml) and \( w \) is sample weight (gr).

2.2.3 Statistical Analysis

One-way ANOVA and Tukey’s test were used to determine whether Pb concentrations varied significantly between 14 (A & B) specimen, with probability values less than 0.05 (\( p<0.05 \)) considered statistically significant. The statistical calculations were done using SPSS 23rd version [10].

2.3 Potential Health Risk Assessment

To calculate the potential daily health risk assessment for the Intake of Metal (DIM), the average is calculated according to Eq 4 [13][15][18]

\[
DIM = \frac{C_{metal} x C_{factor} x D_{food intake}}{B}
\]

where is \( C_{metal} \) concentration of heavy metals in the sample (\( \mu g/ g \)), \( C_{factor} \), \( D_{food} \) ie the average intake/day, \( B \) (average BB) of adults/children. The conversion factor (0.05) is used to convert the wet weight to dry weight. A total of 160 respondents were 80 men/women (40-48 years old) canned fish consumers and 80 children (1-3 years) who consumed milk. The Health Risk Index (HRI) for the local population is calculated according to Eq 5 [13][15][18]

\[
HRI = \frac{DIM}{RfD}
\]

DIM and RfD are daily intake of metals and metal reference standards (mg/kg/day) according to (SNI 01-7387-2009) Sn = 200 mg/kg, Pb = 0.3 mg / kg, Fe = 30 mg / kg. Provisions for the value of HRI <1 is assumed that the population is not exposed to heavy metals.
Total (THRI) can be calculated according to Eq. 6 [18]

\[
THRI = HRI(\text{toxicant } 1) + HRI(\text{toxicant } 2) + \ldots + HRI(\text{toxicant } n)
\]  

(6)

3. Result and Discussion

A total of 160 respondents and distributed in 16 samples each of 8 milk samples (code A) and 8 canned fish (code B) samples with different brands and a sample of 10 respondents. About 80 men/women (40-48 years), average BB (60-75 kg) as consumers of canned fish samples. A total of 80 first boys/girls (1-3 years), average BB (8-17 kg) as consumers of milk samples. All respondents in this study were Gorontalo City residents and the data were obtained by conducting interviews and documentation directly. Concentration of Pb, Sn and Fe in samples were presented in Table 3.

| Sample Code | Measured value (mg/L) | Pb   | Sn   | Fe   |
|-------------|-----------------------|------|------|------|
| A1/B1       | < 0.09                | < 1.8| 6.91 | 12.99|
| A2/B2       | < 0.09                | < 1.8| 8.09 | 8.24 |
| A3/B3       | < 0.09                | < 1.8| 11.07| 11.2 |
| A4/B4       | < 0.09                | < 1.8| 12.3 | 15.67|
| A5/B5       | < 0.09                | < 1.8| 6.79 | 7.11 |
| A6/B6       | < 0.09                | < 1.8| 10.3 | 11.56|
| A7/B7       | < 0.09                | < 1.8| 5.96 | 19.24|
| A8/B8       | < 0.09                | < 1.8| 1.57 | 14.99|

Based on the data in Table 3, the milk product sample (A) and canned fish (B) contain Sn <1.8 (mg/L) and Pb <0.09 (mg/L). This metal concentration is within the maximum limits of Sn and Pb, which is 0.020 μg/g in milk and dairy products [13]. On the other hand, Fe concentration was significantly higher and varied according to milk brand with the order (mg/L) 12.3, 11.07, 10.3, 8.09, 6.91, 6.79, 6.79, 1.57 or (A4> A3> A6> A2> A1> A5> A7> A8) and canned fish types in the order (mg/L) 19.24, 15.67, 14.99, 12.99, 11.56, 8.24, 7.11 or (B7> B4> B8> B1> B6> B3> B2> B5). Due to the addition of iron from the company to the product [18]. Based on SPSS data for samples A1-A8 and B1-B8 with a probability value of 0.05 (p<0.05) for Fe metal there was a significant difference in samples of milk products and canned fish.

Milk is an important nutrient for humans, the presence of high concentrations of heavy metals can cause health problems [16]. According to ATSDR, FAO/WHO, FDA, European Union (EU), and WHO maximum values of Fe in canned fish (100.0 (μg/g) [10] and 8.04-48.18 (μg/g.wt) [15]. Fish is a major source of Fe for children/adults and as essential minerals needed in biological systems and human physiology. On the other hand, fish processing become canned fish have the possibility to accumulate metals in the muscles which can cause health risks. The source of this contamination can be from the equipment, packaging quality and coating of inert substances, place and duration of storage, pH and oxygen content, type of packaging material affects the quality of canned fish [6][15]. Milk as a primary nutrient for children, besides containing important minerals also has the potential for toxicity in the presence of Cd and Pb metals. This contamination is a result of waste disposal and the use of low-quality mineral supplements in animal feed/vegetables [4][10][16] also as a result of the oxidation process [16]. Fe can represent a problem in dairy technology because of its catalytic effect on oxidation of lipids with development of unpleasant smells, bounding preferably proteins and membrane lipoproteins of milk fatty globule [14]. Some results of Fe metal in milk powder is significantly higher than the milk brand with the following references: Fe> Pb> Cu> Cd [1]. Pb concentration exceeded the EC and USEPA recommended limits (0.2 μg/g): in powdered milk 0.93 μg/kg 0.791 μg/kg [19] metals Fe 0.365 - 10.688 μg/g and Pb metals (0.168 - 1,394 μg/g) [16]. In canned fish 2.50 μg/kg [18] 2.08 μg/g and 1.33μg/g
Estimated value of Pb metal intake in frozen fish/shrimp/canned fish (± 7-12 μg/day) where 250 μg/day is equivalent to 1,750 μg/week/kg BB [15].

| Table 4. Concentrations of metals (μg/g) in milk and canned fish from various countries |
|---------------------------------------------|------------------|--|--|--|---|---|---|---|
| Study                                      | Country/milk type   | Pb          | Sn          | Fe     |
|---------------------------------------------|--------------------|-------------|-------------|--------|
| SNI 01-7387-2009                            | Indonesia/metal intake | 0.3 mg/kg | 200 mg/kg  | 30 mg/kg |
| Abdulkhalilq et al.,2012                    | Palestina/cow’s milk, dairy products | ND-0.93 (μg/g) | - | 3.2-12.9 ppb |
| Mahalakshmi et al.,2012                     | Canadian-India/canned fish | 0.01-0.08 μg/g | - | - |
| Siddique.,2014                              | Pakistan/canned fish  | 2.95 μg/g  | - | - |
| Hosseini et al.,2015                        | Iran/canned tuna fish | 0.28 - 1.59 ppb | - | - |
| Younus.,2016                                | Pakistan/raw milk | <0.05 ppm | - | - |
| Ahsan, et al.,2017                          | Bangladesh/milk of cow ppm | 0.1 0.1-0.7 ppm | 2.8 – 11.9 ppm |
| Sobhanardakani et al.,2017                   | Iran/canned fish | 0.75 ± 0.65, (μg/g) | 0.1 | - |
| Ghafari.,2017                               | Hamadan city/milk | 21.75 (μg kg-1 ) | - | - |
| Sobhanardakani et al.,2018                   | Iran/canned Fish | - | - | 54.6 ppb |

Based on the data in Table 4, the results of the study explain that Fe is very high in samples of milk and canned fish. Monitoring the concentration of heavy metals in food is very important because of the increased discharge of pollutants into the environment, especially automatic marine ecosystems of various types of fish exposed to large amounts of heavy metals during their lifetime [18].

| Table 5. Daily intakes of metals (DIM) and health risk index (HRI) for individual heavy metal caused by the milk product |
|---------------------------------------------------------------|------------------|---|---|---|---|---|---|---|---|
| Sample                                                       | A1              | A2 | A3 | A4 | A5 | A6 | A7 | A8 |
| Cfighter                                                     | 0.4             | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Average BB                                                   | 12.56           | 11.00 | 10.22 | 8.56 | 12.76 | 11.56 | 10.63 | 9.7 |
| intakeday/BB                                                  | 12.94           | 13.89 | 12.17 | 9.78 | 8.11 | 9.33 | 7.44 | 12.38 |
| Daily Intake of Metal (DIM), The Health Risk Index (HRI) and The Total HRI (THRI)/Sample |
| Sn DIM                                                        | 4.5716          | 5.8041 | 3.7706 | 3.3984 | 4.5596 | 5.4900 | 3.2695 | 3.9727 |
| HRI                                                          | 0.0183          | 0.0232 | 0.0151 | 0.0136 | 0.0182 | 0.0220 | 0.0131 | 0.0159 |
| Pb DIM                                                       | 0.0003          | 0.0003 | 0.0002 | 0.0002 | 0.0003 | 0.0003 | 0.0002 | 0.0002 |
| HRI                                                          | 0.0134          | 0.0171 | 0.0111 | 0.0100 | 0.0134 | 0.0161 | 0.0096 | 0.0117 |
| Fe DIM                                                       | 2.8476          | 4.0862 | 5.2729 | 5.6075 | 1.7262 | 3.3155 | 1.6686 | 0.8015 |
| HRI                                                          | 0.2848          | 0.4086 | 0.5273 | 0.5608 | 0.1726 | 0.3316 | 0.1669 | 0.0802 |
| THRI                                                         | 0.1055          | 0.1496 | 0.1845 | 0.1948 | 0.0681 | 0.1232 | 0.0632 | 0.0359 |
Table 6. Daily intakes of metals (DIM) and health risk index (HRI) for individual heavy metal caused by canned fish

| Sample | B1     | B2     | B3     | B4     | B5     | B6     | B7     | B8     |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Cfaktor| 0.05   | 0.05   | 0.05   | 0.05   | 0.05   | 0.05   | 0.05   | 0.05   |
| Average BB intake/day/BB (gr/kg) | 64.09 | 68.55  | 64.91  | 69.27  | 61.91  | 59.82  | 65.27  | 69.55  |

Based on data in Tables 5 and 6, HRI values for Fe, Pb and Sn both in dairy products and canned fish <1. HRI values were within the safe limits (HRI < 1) and there is no potential health risk for adults and children via consumption of canned fish [10][18]. In recommendations if the HRI > 1 indicates carcinogenic risk for consumers. The high concentration of metals in the daily intake consumed will be harmful to health [15]. Recommended by FAO/WHO the maximum permissible limits for Pb in milk are 0.02-0.1mg/ml. The maximum permissible limit for milk established by (European Union, 2006) is 0.1mg/ml. Metal poisoning is caused by the presence of metal integrity exceeding the recommended limits. Metal poisoning through milk is a more serious problem than other foods because milk is consumed by many parents and babies [6]. Iron is an essential mineral needed in human biological and physiological systems, iron deficiency causes anemia and there is a decrease in physical work, the excess of this substance is also the cause of organ failure [6][13].

Metal as an important nutrient plays a role in the body's metabolic process which is an activator of several enzymes that regulate the balance of biochemical reactions such as Fe, Cu, Zn and Mn to protect the body from the negative effects of free radicals. The recommendation for the need for iron Fe in elderly men and women is 10000 μg/day (National Academy of Medicine, 2001). Metal content is the amount of metal in the sample and the average intake/day is the quantity of food consumed/day [13].

In this study, the health risk analysis phase includes hazard identification, exposure assessment, toxicity assessment and risk characterization [19]. In accordance (SNI 01-7387-2009) recommendations for the need for metal intake for the body for Sn(200mg/kg) Pb(0.3mg/kg) and Fe (30) mg/kg. The results of the study can be concluded that the average intake of Fe/day metal in both milk and canned fish indicates there is no carcinogenic risk for consumers in Gorontalo City.

4. Conclusions
The purpose of this study was to determine the concentration of Sn, Pb and Fe metals in samples of milk and canned fish products. The results showed that there was no carcinogenic risk for consumers of canned milk and fish in Gorontalo City. Regular monitoring of milk quality is very important because metal can function as a bio-indicator that can affect the quality and nutritional value of milk so that it poses a danger to human health.
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**Acknowledgements**

We would like to convey our sincere appreciation with deep gratitude to Mrs. Nurain Thomas and Mr. Taupik for data collecting, Mr. Hendrik for data analyzing and Mr. Doly Silaban for Laboratory report interpretation in this study.