

## Demographic-Based Assessment Of Heavy Metals In The Breastmilk Of Selected Mothers In Zamboanga City

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### *Abstract*

Mother's milk is the best method of nutrition for infants. Breast milk also has an influence on the development of intestinal microflora and structural-functional maturity of mucous membranes and reduces the risk of allergies and autoimmune diseases. This study aimed to determine the levels of heavy metals such as  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$  in parts per million in the breast milk of nursing mothers, the significant difference if the levels of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Cd}^{2+}$  of the breast milk sample of mothers when grouped according to age, occupational status, number of children and tribal affiliations, and whether the level of heavy metals present exceeded the limit set by JECFA. This study used a descriptive design using the Kruskal-Wallis non-parametric test. Findings revealed that the level of concentrations of the heavy metals  $\text{Cu}^{2+}$  had the highest parts per million followed by  $\text{Pb}^{2+}$ , and  $\text{Cd}^{2+}$  respectively. Moreover, the levels of these heavy metals do not exceed JECFA. There was no significant difference in the level of heavy metals when nursing mothers were grouped categorically. In the light of foregoing findings and conclusions drawn from this study, it is safe to say that the breastmilk of the sampling mothers was not harmful to their children.

Keywords: Chemistry, heavy metals, breastmilk, mothers, Philippines.

### **Introduction**

Breastfeeding is the best method of nutrition for infants, The World Health Organization (WHO) recommends breastfeeding as the executive method of nutrition for newborns and infants at least up to 6 months of age, mother's milk is so important particularly to the newly born babies since the component of mother's milk, such as

lactoferrin, lysozyme and  $\alpha$ -lactalbumin, create a barrier protecting the baby against harmful environmental factors, enhancing the body's defense mechanisms and stimulating the development of the immune system.

Breast milk also has an influence on the development of intestinal microflora (Lonnerdal, (2003)) and structural and functional maturity of mucous membranes and reduces the risk of allergies and autoimmune diseases (Matson, Thrall, Rafti, Lingenhel, Puddington (2010). However, mother's milk can also be a source of cadmium (Cd) and Lead (Pb) for a baby. The content of these elements in breast milk reflects the level of environmental pollution and the mother's diet. The presence of these materials in food products has become a global problem (Katzining and Traver, 1995). In Poland, the highest content of Cd and Pb is found in food derived from plants including but not limited to vegetables, in particular, potatoes, and grains and cereals. Mothers who smoked tobacco create an additional source of toxic trace elements in the body, thus increasing the content of Cd and Pb in the breast milk. (Garcia-Esquinas, et al. (2011). Research by Ohrvik et al. revealed that Cd leads to a dysfunction of the mammary glands in mice, which is the reason for the development disorders among sucklings. Since 'heavy metals' is a poor scientific term, in the present study, the term 'toxic metals' is used with reference to Cd and Pb, and "essential metals" with reference to Copper (Cu) and zinc (Zn) (Burtis and Bruns, 2015).

As mentioned by Baldwin and Marshall (1999) metals and anions form an important but disparate group of agricultural, household, and industrial poisons that is common in many underdeveloped parts of the world. Chronic poisoning as a result of occupational or environmental exposure to heavy metals and pesticides can be a cause of ill-health in both adults and infants. The toxicity of metallic poisons may be influenced by the chemical nature of the compound ingested (valence state, solubility, inorganic or organic compound) and the route of administration.

Breast milk is scientifically known as important component for growth and development of infants. Many studies have been conducted to prove its benefits; however, recent discoveries of traces of lead and other toxic metals in breast milk of nursing mothers questioned its identified benefits.

### **Statements of the Problem**

This study aimed to determine the presence of heavy metals such as lead, cadmium, and copper in the breastmilk taken from some selected nursing mothers living in Zamboanga City.

Specifically, this study sought answers to the following questions:

1. What are the levels of heavy metals ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ) in parts per million (ppm) of the breastmilk of the selected nursing mothers in Zamboanga City?
2. Is there a significant difference in the levels of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Cd}^{2+}$  of the breast milk sample of nursing mothers when grouped according to age, occupational status, number of children and tribal affiliations?
3. Does the level of heavy metals in the collected breastmilk samples exceed the allowable level set by the Joint Expert Committee of Food Additives (JECFA, 2003).

The result of this study may benefit the following: Nursing mothers, for the result of this study can be an eye-opener to mothers in terms of the diet or kind of food they should take in while breastfeeding their infants or even during pregnancy. Furthermore, their awareness of the presence of lead, cadmium and copper in their milk may guide them as to its positive and negative effects to their infants. This also can guide the nursing mother as to the kind of activities or things where lead, cadmium, and copper can be present to appropriately avoid them. For the health institution, this study may serve as a scientific database for institutions to consider discussing lead contamination from occupational and non-occupational sources when initiating programs related to breastfeeding pregnancy.

This may be an avenue or starting point for a more thorough study on the components of breast milk that might harm the baby feeding only on mother's milk. The advantages and disadvantages of copper, lead and cadmium on developing babies or infants can also serve as input for the planning of infants' formula.

Moreover, the American Dietetic Association and the American Academy of Pediatrics, as well as Canadian organizations, strongly recommend breastfeeding of babies exclusively for the first four months and maybe until six months, and a combination of breastfeeding and infant food until one-year-old. The World Health Organization recommends breastfeeding for at least two years, supplemented with other foods (Ettinger, et al. 2004).

Recognizing the importance of breast milk for babies and mothers as well as the environmental concerns affecting nursing

mothers, the researcher felt the need to analyze or study the copper, lead and cadmium content of breastmilk taken from selected participants from Zamboanga City. If the breast milk is found affected by some amount of copper, lead, and cadmium from both occupational and non-occupational sources, it is necessary that a mother's milk must be subjected to a test on the presence of copper and other heavy metals such as lead and cadmium (Lawrence, 2011). Moreover, Lawrence (2011) emphasized that women who knew they have been exposed to specific chemicals or pollutants should consult with their physician regarding analysis of the contaminant of their breast milk to avoid any untoward effect to the infant. Since the researcher could not find any literature and empirical studies conducted locally or in Zamboanga City regarding metals ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ) contaminants in the breast milk of nursing mothers, this prompted the realization of this research.

Essential metals, such as Cu and Zn, are necessary to ensure the correct functioning of the body but, in excessive amounts, have a harmful effect. Cu is an element necessary for synthesizing hemoglobin, forming myelin sheaths in the nervous system, and for bone tissue formation processes. Zn is an important element at the fetal stage. A deficit of this element can lead to defects in the nervous system and cause retardation of the fetus' growth and development (Pediater, 2010).

In Poland, previous research concerning the content of Pb, Cd, Cu, and Zn in breastmilk was very scarce or its results were unavailable. No detailed evaluations of the effect of age and lifestyle of Polish women on the content of metallic elements in breast milk are available. That study aimed to evaluate the content of Cd, Pb, Cu, and Zn in their breast milk and to estimate the intake of these metals by breastfed infants.

Breastfeeding, a maternal-infant dyad, is an example of the interconnectedness of humans to their environment. A mother's body becomes the environment for her children throughout pregnancy and through breastfeeding. Over the past several decades, the public has grown increasingly aware that chemicals in the environment are entering our bodies and sometimes causing harm. Recently, this awareness has extended to concern about what effect(s) toxic contaminants have on breastfeeding women and their children (Nickerson, 2006).

The best source of micro and macronutrients for infants up to twelve months is human milk. Its benefits have been recognized worldwide not only for babies but as well as for nursing mothers.

However, despite all the empirical studies regarding its importance to infants, detectable amounts of copper ( $\text{Cu}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and cadmium ( $\text{Cd}^{2+}$ ) in the breast milk of nursing mothers had also been documented (Ettinger, et al., 2004).

For this study, the first metal contaminant considered for analysis in breast milk is copper ( $\text{Cu}^{2+}$ ). Though the body needs copper in a very minute amount, its presence in high concentration becomes toxic. Copper occurs naturally in many foods so deficiency related to it is rare. However, copper deficiency may occur for people using concentrated zinc supplements as well as premature infants feeding on cow's milk which is lower in copper content (Schlenker & Long, 2007).

Lead ( $\text{Pb}^{2+}$ ) remains among the most useful metal, despite its known health risks, because of its beneficial physical properties such as its malleability and ease to work with other metals. Other benefits of lead include the following: insulates well without rusting; mixes well to make long-lasting paint pigment; and it is strong and durable. Lead will be undesirable when its particles reach human, particularly infants. Human activities that serve as pathways of lead to the environment can be enumerated as follows: mining, smelting, refining, manufacturing, and recycling. Lead-contaminated soil and dust can find its way into the food and water supply. However, lead serves no useful purpose in the human body and may even damage the hematopoietic tissues, liver, nervous system, kidneys, gastrointestinal tract and reproductive system. Lead for nursing mothers and infants represents a major environmental hazard since it is present both in the air and water throughout the world (Katzining and Traver, 1995). Lead compounds and other heavy metals continue to be used as components of traditional medicines (Moor and Adler, 2000). Acute lead poisoning is relatively uncommon, however, its most symptomatic cases result from chronic ingestion, or inhalation of lead fumes or dusts during occupational exposure or used of lead-containing traditional medicines (Carton et al., 1987). Adam and Cooper (2015) stressed that extensive study demonstrated the harmful effect of child development, behavior and intelligence.

In addition to copper and lead, cadmium ( $\text{Cd}^{2+}$ ) is one of the most toxic heavy metals for humans to include nursing mothers. The main source of non-occupational exposure to cadmium includes smoking, air, and food and water contaminated by Cadmium (Nagatta et al., 2005). Moreover, ((Nagatta et al., 2005) herbal medicine is another source of cadmium contamination which might affect the mothers in some ways.

Nursing mothers or breastfeeding mothers require an ample quantity of nutritious food like vegetables, fruits and cereals. These agricultural crops might have grown near heavily traveled roads or industrial zones where copper, lead and cadmium can be acquired from exposure to these sources. Though the concerns with regards to environmental contaminants in human milk or breast milk are legitimate, the benefits that nursing mothers and babies can get from the process are very well-studied and well-established. The risks from environmental contaminants are still theoretical. With this information, it is best to continue with breastfeeding until sufficiently strong research data well prove its

The cycle of metals in the environment is linked with the food chain from the soil, plant, animal, then to man. Heavy metals or toxic metals are referred to  $Cd^{2+}$  and  $Pb^{2+}$ . The transfer of toxic metals to the higher link results in a cumulative increase in their content. Cd is primarily deposited in the liver and kidneys, but in infants, it poses the greatest hazard to their dynamically developing nervous system, also affecting bone formation processes, and is a significant carcinogen (Schlenker, E.D., & Long, S., 2007). Tests on rats demonstrated that Cd supplied with milk affects the serotonin level in the brain of a growing animal. The highest Cd accumulation levels are recorded during the first 3 years of human life. In children, it can inhibit intellectual development and causes anemia and rickets (Adam and Cooper, 2013). Significantly, a positive correlation between the concentration of autism has been observed. Neurological effects of Pb observed in children include motor skills disorder and behavioral problems. Exposure to this metal for several years after birth is particularly detrimental to the future intellectual potential of children. Children are exposed to the accumulation of Pb due to a slower excretion process and lower body weight and also because of reduced immunity.

### **Theoretical Framework**

This study was anchored on the knowledge and information which explained how food contaminants, specifically heavy metals, enter into the environment then into the food chain where human health might be affected from its exposure.

Metals are naturally present in the environment; however, its concentration varies from regions due to their properties and some influences of environmental factors. Of the 92 metals occurring in nature, approximately 30 metals and metalloids are potentially toxic to human including Copper (Cu), Cadmium (Cd), and Lead (Pb). These

metals are considered heavy metals because by definition their atomic masses are greater than 40 g/mole (Ming-Ho, 2005). Heavy metals enter the environment by natural and anthropogenic means. Although some people are exposed to these contaminants in their workplaces; however, for most people these heavy metals can be ingested or inhaled through their diets such as food and water. The contamination chain of heavy metals follows a cyclic order: industry, atmosphere, soil, water, foods, and human (agency for Toxic Substance and Disease Registry (ATSDR), 2008; Castro-Gonzales & Mendez-Armenta, 2008).

Lead (Pb) and cadmium (Cd) are widely spread into the environment. There has been no documentation to prove that these elements have beneficial effects in humans, as well as, there is no known homeostasis metabolism (Draghici, 2010). In fact, these metals are considered the most toxic for humans of which even exposure to such in low concentration have diverse effects such as but not limited to neurotoxic and carcinogenic actions (ATSDR, 2008). Specifically, for lead (Pb), food is one of the major sources of this element; others are air (primarily as lead dust from petrol) and drinking water. In humans, lead (Pb) ingestion may arise from eating lead-contaminated vegetation or animal foods. Furthermore, about 20 to 50% of inhaled, and 5 to 15% of ingested organic Pb is absorbed. Once lead is absorbed by the body, it will be distributed among blood, soft tissue, and mineralizing tissue. The bones and teeth of adults contain more than 95% of the total body burden of lead. Children are sensitive to lead contamination because of their more rapid growth rate and metabolism, with critical effects in the developing nervous system (ATSDR, 2008; Castro-Gonzales & Mendez-Armenta, 2008). The Joint FAO / World Health Organization Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for lead at 0.025 mg/kg body weight (JECFA, 2004).

The utilization of cadmium by humans is current, and tobacco smoke is one of the largest single sources of cadmium exposure in humans. Tobacco in all of its forms contains an appreciable amount of cadmium. This heavy metal is easily absorbed from the lungs that from the gastrointestinal tract, thus it significantly contributes to the total body weight (Figueroa, 2008; Ming-Ho, 2005). For non-smokers, food products can be the source of cadmium exposure, in food, only inorganic cadmium salts are present. Organic cadmiums are unstable; cadmium ions are readily absorbed by plants. It is taken up through the roots of plants to edible leaves, fruits and seeds. The core of the kernel is concentrated with cadmium. This metal is also accumulated

in animal milk and fatty tissues, as well as in seafoods such as mollusks and crustaceans (Figueras, 2008). Cadmium toxicity can affect the liver, kidney, lung, bones, placenta, brain and central nervous system (Castro-Gonzales & Mendez-Armenta, 2008). It can also affect the reproductive and immune system of the human body (Apostoli & Catalani, 2011; ATSDR, 2008). The Joint FAO /WHO has recommended 0.007 mg/kg body weight for cadmium (JECFA, 2004).

Copper as a metal is usually toxic when associated with other elements such as chromated copper arsenate (CCA). In this mixture, arsenic and copper act as insecticide, and fungicide, respectively. Copper is an element and a well-recognized essential nutrient for human health. It is present in a wide range of food such as beef/ calf liver, shrimp, nuts, avocados, and beans (ATSDR, 2004). Human contact with CCA is mainly due to environmental and/or occupational exposures. Skin exposures, ingestion and inhalation are the main routes of absorption of CCA. To sum up, heavy metals have been proved to be toxic to human and environmental health, thus human exposure to these metals should be closely monitored.

### Conceptual Framework

The conceptual paradigm of this study is shown in the figure below. The figure shows the relationship of the variables involved.



Figure 1. The Conceptual Framework Showing the Relationships of the Variables of the Study.

The figure above showed the flow of the investigation of the variable being considered (breast milk) and the processes involved in the determination of lead cadmium and copper in this study. As shown above, breast milk samples were collected from the selected mothers served as the independent variable the study.

The different laboratory experiments and procedures served as the process involved in the determination of lead, cadmium, and copper in collected breast milk samples, while the amount of lead, cadmium, and copper determined from the collected sample served as the dependent variable of the study. Furthermore, this study



considered the following terms and were defined operationally according to their use. Breast Milk or Human Milk is the perfect source of nutrition for infants. It contains appropriate amounts of carbohydrates, proteins and fats. It also provides digestive enzymes, minerals, vitamins, and hormones that all infants acquire. Breast milk contains valuable antibodies from the mother that can help the baby resist infections. Breast milk is a tailored-made food to meet the nutrient needs of the human infant (Boyle & Long, 2013). Its carbohydrate is lactose, and its fat provides a generous portion of the essential omega-6 fatty acid, linoleic acid, and its products. A nursing mother who consumes food rich in omega-3 fatty acids will pass these beneficial nutrients on to her infant through breast her milk. The kind of protein in breast milk is digestible and useful in the tissue growth of the baby. Furthermore, human milk contains fat-digesting enzymes that help ensure efficient fat absorption by the infant. It also transports immune factors, which protect an infant and informs its body about the outside environment (Sizer and Whitney, 2003).

Sizer and Whitney (2003), mentioned that breast milk protects the baby against infections to include antiviral and antibacterial agents as well as infection inhibitors. The best example of this health benefit is that breast milk interferes with the growth of bacteria that attack the infant's vulnerable digestive track linings. This is the reason why breast-fed babies are not prone to develop stomach and intestinal disorders that resulted in less vomiting and diarrhea than formula-fed babies. Research also indicated that breast milk contains not only antibodies against rotavirus but also a substance that inhibits the replication of the infective agent. Breast milk also protects infants from other forms of illness such as middle ear infections and respiratory-related illnesses. Breast milk produces colostrum, a pre-milk substance containing antibodies and white cells from the mother's blood. Certain factors in colostrum and breast milk factor the growth of probiotic bacteria in the infant's digestive tract, preventing other harmful bacteria from thriving there. Breast milk composition changes throughout lactation to meet the babies' dynamic development.

As mentioned by Brown (2008), breast milk contains a very low amount of vitamin D, so babies receiving human milk need to obtain it from exposure of their skin to sunlight or from supplements. Furthermore, breast milk contains essential and non-essential fats and other substances that promote optimal growth and development of the nervous system and eyes. According to Insel et al. 2010, breast milk provides more than nutrients, however, the health-promoting

factors in breast milk are difficult to replicate in any infant formula. Furthermore, breast milk has been shown to reduce the incidence of respiratory, gastrointestinal, and ear infections, allergies, diarrhea and bacterial meningitis.

With all the aforementioned benefits of breast milk to human infant, environmental contaminants, such as copper ( $\text{Cu}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and cadmium ( $\text{Cd}^{2+}$ ) transferred into breast milk. Scientifically, the mechanism for this is the environmental contaminants may be stored in a woman's fat or bone tissues. When the fat stores are later broken down for use in breastmilk or the calcium in bone is mobilized, the contaminants stored in the fat or bone may enter the breast milk. However, it is concluded that the benefits of breastfeeding outweigh the risks to infants from harmful substances in the environment (Brown, 2008).

Milk production varies widely among breastfeeding mothers, and a number of factors may influence a woman's lactation capacity. The range of milk volumes varies among mothers from 500mL/day to 1200 mL/day, with calculations of breast milk transfer to the infant gradually increasing from approximately 650mL/day at 1 month, to 770 mL/day at 3 months, to 800 mL/day at 6 months (Neville et al., 1998). As solid foods are introduced around 6 months. Milk production decreases to between 95 to 315 mL/day at 15 to 30 months (Hennart, Delgne-Desmoeck, Vis, & Robyn, 1981; Kent, Minodlas, Cox, Owens, & Hartman, 1999; Neville et al., 1991).

**Atomic Absorption Spectrophotometry.** This term refers to the technical and standard procedure used in the analysis of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Cd}^{2+}$  of the collected breast milk samples. The Breastmilk. Refers to the sample to be used in the analysis of Copper ( $\text{Cu}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and cadmium ( $\text{Cd}^{2+}$ ) level; **Copper ( $\text{Cu}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and cadmium ( $\text{Cd}^{2+}$ )** are the heavy metal ions tested for its concentration in breastmilk samples collected from nursing mothers determined using Atomic Absorption Spectrophotometry. The following are used to categorically classify the respondents according to **Age**. This refers to whether the respondent is a young mother or old. **Number of Children.** The number of children of the mother had during the collection for samples. **Occupational Status.** This identifies the respondent whether the mother is busy in her work, and Tribal Affiliations, refer to the nursing mother's tribe, which is either Samal, Zamboangueño, Bisaya and Tausug.

Trace elements have a significant role in the growth and development of babies, even if they are only required in small amounts. Mammary glands get traces of metals from the blood,

control its excretion, and regulate its transport from the membrane and, finally its excretion from the milk. The data for trace-elements content of human milk differ widely from region to region. These variations may be due in part to differences in sampling and analytical techniques rather than geographic variations.

## **METHODOLOGY**

This chapter contains the research method, site of the study, participants of the study, sampling design, data gathering procedure, research instruments, and the statistical tools to be used for the analysis of the data.

### **Research Design**

This study used descriptive design since it only described collective information from the laboratory analysis of breast milk. The purpose of descriptive research is to observe, describe, and document aspects of a situation as it naturally occurs (Polit & Hungler, 1999). Parahoo (1997) describes a research design as “a plan that describes how, when, and where data are to be collected and analyzed”. As mentioned by Polit et al. (2001) a research design is defined as “the researcher’s overall for answering the research question or testing the research hypothesis”. The breast milk samples collected were fresh from the nursing mothers housed in a Medical Center (not disclosed for confidentiality). The analysis of the collected milk samples followed standard procedures adopted from the Association of Official Analytical Chemists (AOAC) Method.

### **Research Participants**

The participants of the study were selected in reference to the variables being considered in this study such as age, occupational status, number of children, and tribal affiliations. The following respondents were qualified to the different criteria set by the researcher before conducting the study. According to the literature, the volume of milk produced by the nursing mother as they age in the feeding there was a decrease in the amount of milk produced. Thus, the researcher preferred respondents whose lactating age is between 1 and 6 months because according to Kent et al. (1999), lactation decreases significantly between 6 and 9 months, with only a small drop of milk production. The breasts return to their preconception size by 15 months of lactation. Furthermore, according to Neville et al. (1998) the range of breastmilk transfer to the infant gradually

increasing from approximately 650 ml/day at 1 month, to 770 ml/day at 3 months, to 800 ml/day at 6 months.

Table 1 below shows the summary of the participants in terms of age, occupational status, number of children and tribal affiliations. There were four Muslims; two Bisayans; and three Chavacanos nursing mothers who were participants in this study. These mothers' age range is 24 to 34 years, and the number of children is between 1 to 6. Most participants were non-working, while there were three professionals.

#### Participants of the study

Sample	Age	No. of Children	Tribal Affiliation	Occupational Status
1	34	1	Bisaya	Non-working
2	25	2	Bisaya	Non-working
3	24	4	Tausug	Non-working
4	32	3	Chavacano	Professional
5	30	2	Tausug	Non-working
6	28	1	Chavacano	Professional
7	28	3	Tausug	Non-working
8	30	5	Chavacano	Health Officer
9	32	6	Samal	Non-working

This study was conducted at Zamboanga City involving nursing mothers according to the four factors considered in this study including age, occupational status, number of children, and tribal affiliations.

This study is also delimited only on the determination of lead, cadmium and copper contamination in the collected breast milk samples using Pin AAcle 900F Atomic Absorption Spectrophotometry (AAS) with the lowest 0.0001 ppm will not be reported due to its dilution factor of the breastmilk samples which be multiplied by

0.001x100 ml. H<sub>2</sub>O/10g breastmilk samples was been considered in the analysis of the tree trace metals namely cadmium, copper and lead respectively. More consideration was focused on lead due to its higher molecular weight. There were only 9 collected bottles of breast milk which were properly sealed and stored for analysis

### **Sampling Design**

The purposive sampling design was utilized in the selection of nursing mothers since the study focused only to certain characteristics: Like age, occupational status, number of children, and tribal affiliations in a particular population to be considered as participants in the study.

### **Data Gathering Procedure**

This section id divided into the collection of breast milk from the participants and the analysis of metals from the collected sample.

### **Collection and Storage of Breastmilk**

Milk collection was done by hand or pump expression or a combination of both. It started by washing hands, pumping, and collection device carefully. Massage breasts and stimulate nipples prior to expressing 20 milliliters). Label the polystyrene containers with date of expression and name of mother (as respondent) and the number of children. Glass and hard plastic containers with tight lids are recommended. All containers should be food grade. Plastic bags designed for milk storage may be used, but it may be difficult to prevent spillage and puncture of these bags (Arnold, 2004). However, this study utilized the polystyrene plastic containers that were sealed immediately to avoid contamination. It was labeled with the date of expression, name of the mother (as respondent) and the number of children. The plastic lid of the container was then sealed with Teflon Tape to avoid spilling with a frozen gel pack on top in an insulated lunch bag. After the milk samples were properly sealed and packed for analysis.

### **Milk Storage**

- A. Refrigeration and Freezing. The refrigeration and freezing of human milk can be safely stored under appropriate conditions to ensure infants receive their mother's or banked milk under a variety of conditions (Lawrence, 1999; Ogundele, 2000). Recommendations for storage times and temperatures vary among sources. The Academy of Breastfeeding Medicine recommends storage at room temperature 16-29°C (60-85°F), for

3-4 hours as optimal; storage for 6-8 hours is acceptable under very clean condition. Storage in the refrigerator at  $\leq 4^{\circ}\text{C}$  ( $39^{\circ}\text{F}$ ) for 72 hours is recommended as optimal, with longer period of 5-8 days being acceptable under very clean conditions. In the freezer  $< -17^{\circ}\text{C}$  ( $0^{\circ}\text{F}$ ) for 6 months is recommended as optimal, with 12 months being acceptable (Academy of Breastfeeding Medicine, Protocol Committee, 2010). According to Human Milk Banking Association of North America (2011), freshly expressed milk can be stored at room temperature for  $\leq 6$  hours, in refrigerator for  $\leq 5$  days for a term infant and for  $\leq 8$  days for an older child, and in the freezer ideally for 3 months, optimally for  $\leq 6$  months, although 12 months is acceptable if in a deep freezer ( $-20^{\circ}\text{C}/-4^{\circ}\text{F}$ ). Previously frozen milk that has been thawed in the refrigerator but not warmed can be stored at room temperature for  $\leq 4$  hours and in refrigerator for  $\leq 24$  hours, but should not be frozen.

The containers for the samples of the breastmilk utilized the hard plastic polyethylene containers with tight lids and should be food grade. However, plastic bags designed for milk storage may be used. Prevent spillage and puncture of bags are recommended (Arnold, 2004).

### **Experimental Analysis of Metal Contaminants**

Atomic Absorption Spectrophotometry of Lead, Copper, and Cadmium in Foods, This method is applicable to the determination of Cu in a variety of foods by dry ashing and flame atomic absorption spectrophotometry (FAAS), and Cd and Pb by dry ashing and graphite furnace atomic absorption spectrophotometry (GFAAS). Moreover, it determines elements at concentration above approximately Pb (0.3), Cd (0.1), Cu (5) mg/kg.

Caution: Always gently add acid water.

Avoid environmental contamination by Pb. Store quartz crucible in 20%  $\text{HNO}_3$  and rinse with deionized water before use. When necessary, crucibles may be boiled with 20%  $\text{HNO}_3$  before use. Heat platinum crucibles until red hot and boil with 50% (v/v) HCl prior to use.

- A. Principle. Test portions are dried and then ashed at  $450^{\circ}\text{C}$  under a gradual increase ( $\leq 50^{\circ}\text{C}/\text{h}$ ) in temperature. 0.6M HCL (+1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1M  $\text{HNO}_3$  and the analytes are determined by flame and graphite furnace procedures.
- B. Apparatus. (a) Atomic Absorption spectrophotometer. With an air-acetylene burner or nitrous oxide-acetylene burner for flame

and graphite furnace for electrothermal determinations, with appropriate background (nanomistic) correction. (b) Lamp. A hollow cathode or electrode less discharges lamps for all elements determined. IR 250 W, fixed to a retort stand in a way that allow adjustment of the distance to a hot plate. (c). Furnace. Programmable or muffle furnace thermostat maintaining  $\pm 450^{\circ}\text{C} \pm 25^{\circ}\text{C}$ , If the muffle furnace is used, a separate pre-ashing device is required. (d) Hot plate, with heating control, to heat up to about  $300^{\circ}\text{C}$ . (e ) Ceramic plate, e.g., dessicator plate on a low stand, with a hole that fits the hot plate. (f) Glass cover, e.g., Crystallizing dish, 185mm diameter, 100 mm height to fit on a ceramic plate. (g) Wash bottles, "Scrubber", containing  $\text{H}_2\text{SO}_4$  for purification of air. (h) Quartz or platinum crucibles, 50-75 mL. (i) Polystyrene bottles, with leak-proof closures, 100 ml. Carefully clean and rinse all glasswares and plasticwares with  $\text{HNO}_3$ , furnace + 4500 ml deionized water. Wash first water and detergent. Rinse with tap water, followed by deionized water then with dilute acid. Finally rinse 4-5 times with deionized water.

- C. Reagents. Reagents should be at least analytical reagent grade preferably ultrapure (suprapur), or equivalent. [Note: commercially available standard solutions for AAS (e.g., BDH Chemicals Ltd. Poole, UK] may be used for all metals standard solutions. (a) Water, Redistilled or deionized, resistivity  $\geq 18 \text{ M}$ . (b) Hydrochloric acid. -6 M. Dilute 500 ml HCl (37 % w/w) with water to 1 Liter. (c) Nitric acid, 65% (w/w). (d) Nitric acid, 0.1M. Dilute 7 mL  $\text{HNO}_3$ , with water to 1 Liter. (e ) Lead standard solution, 1mg/mL. Dissolve 1.000g Pb in 7 mL  $\text{HNO}_3$  in 1 L volumetric flask. Dilute to volume with water. (f)Cadmium standard solution, 1mg/mL. Dissolve 1.000g Cd in 14 mL water + 7 mL  $\text{HNO}_3$ , in 1 L volumetric flask. Dilute to volume with water. (g) Copper standard solution, 1 mg/mL. Dissolve 1.000 g Cu in 7 mL  $\text{HNO}_3$ , in 1 L volumetric flask. Dilute to volume with water. (h) Working standard solutions, (1) For graphite furnace analysis. Dilute standard solutions, (e ) – (g), with 0.1M  $\text{HNO}_3$  to a range of standard that covers the linear range of the element to be determined . (2) for flame analysis, dilute standards, (e )-(h), with 0.1M  $\text{HNO}_3$ , to a range standards that covers the concentration of the element to be determined.
- D. Procedures. (a) Pretreatment. Homogenize product if necessary, using noncontaminating equipment, Check for leaching metals if the apparatus consists of metal parts. (b) Drying. In a crucible, weigh 10-20 g test portion nearest 0.01g. Dry in a drying oven, on

a water bath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace. (c) Ashing. (1) Ashing in a programmable furnace. Place dish in a furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C/h to 450°C. Let dish stand for at least 8 h overnight. Continue according to (d). (2) Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in B(d)-(h). Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200°C - 250°C and slowly raise temperature to 450 °C at a rate of no more than 50°C/h. Let stand for at least 8h overnight. Take crucible and let it cool. (d) Solution. Wet ash with 1-3 ml water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50°C-100°C/h) to 450°C. Proceed with ashing at 450°C for 1-2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product. Add 5 ml 6M HCl, C (b), to crucible ensuring that all ash comes in contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0-30.0mL., of 0.1M HNO<sub>3</sub>, C(d). Swirl crucible with care so that all ash comes in contact with acid. Cover with watch glass and let stand for 1-2h. Then stir solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blanks with each analytic batch. (e ) Atomic Absorption Spectrophotometry. Pb and Cd in foods generally require graphite furnace AAS and Cu in most foods can be determined by flame AAS. Wavelength, gas mixture/gas program and other instrumental parameters that are most appropriate for each metal are found in the manual on AOAC method.

This study was also delimited only on the determination of lead, cadmium and copper contamination in the collected breast milk samples using the Pin AAcle 900F Atomic Absorption Spectrophotometry (AAS) with the lowest 0.001 ppm will not be reported due to its dilution factor of the breast milk sample which be multiplied by 0.001x100 ml H<sub>2</sub>O/10g breastmilk samples was been considered in the analysis of the three trace metals namely cadmium,



copper and lead respectively. More consideration was focused on lead due to its higher molecular weight. There were only 9 collected bottles of breast milk which were properly sealed and stored for analysis.

### Statistical Tool

The statistical methods used in the analysis of the data collected were as follows:

The Kruskal-Wallis One-Way Analysis of Variance (named after William Kruskal and W.Allen Wallis). It was a non-parametric method which is used to test two or more samples that were independent and may have different sample sizes. The test did not assume the normal distribution of the residuals, unlike the analogous one-way analysis of variance.

### Ethical consideration

The researcher of the study asked a verbal permission of each breastfeeding mother-respondent as to their willingness to be part of the study. Their inclusion to this study as a respondent in all aspects and the data gathered from the analysis of trace metals from the samples given would be kept confidential and used for research purposes only.

### RESULTS AND DISCUSSION

Presented in this chapter are the analysis and interpretation of results of the data gathered.

- A. The levels of heavy metals like  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Cd}^{2+}$  found in the Breastmilk of the Nursing Mothers.

**Table 1**  
**Concentration of the heavy metals and the allowable limit set by JECFA**

Heavy Metals	Concentration of heavy metals (ppm)	Allowable limit (JECFA)	Inference
$\text{Cd}^{2+}$	0.0489	0.1000	Retain the null hypothesis
$\text{Cu}^{2+}$	0.1933 <sup>nr</sup>	nr	Not regulated
$\text{Pb}^{2+}$	< 0.1000	0.3000	Retain the null hypothesis

Note: (nr) not regulated by the FDA

The table above showed the level of concentrations of the heavy metals mentioned in the study. The  $\text{Cu}^{2+}$  has the highest parts per million (ppm) concentration followed by  $\text{Pb}^{2+}$  then,  $\text{Cd}^{2+}$ . Although  $\text{Cu}^{2+}$  has the highest ppm not regulated but a high intake or exposure to  $\text{Cu}^{2+}$  especially in environment or in drinking water or diet (from copper or brass cooking pots) may lead to copper related liver disease in babies and young children (Sethi et al. 1993; Spaziano et al. 2002). The main risk is associated with the copper fumes in smelting furnaces, which lead to respiratory illness and metal-fume fever. Copper related liver and respiratory disease has also been reported in vineyard workers who spray copper containing fungicides (Bordeaux mixture). Toxicity has also been reported following occupational exposure during the application and removal of anti-fouling paints in ship repair facilities.

Copper occurs naturally in many foods, so dietary deficiency is rare. Copper deficiency has developed in patients on total parental nutrition (TPN) that excluded copper, persons using concentrated zinc supplements that interfered with copper absorption, and premature infants fed only cow's milk, which is low in copper. (Schlenker and Long, 2007).

**A. Comparison of the Levels of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  were lower compared to the limit set by JECFA, 2003.**

In the table 1 above the readings of the concentration of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  were lower compared to the limit set by the Joint Expert Committee of Food Additives (JEFCA, 2003). While the concentration of the  $\text{Cu}^{2+}$  not regulated.

The results of this study revealed that the null hypotheses were retained. It simply showed that the breastmilk samples of the selected nursing mothers did not suppress the limit set by JEFCA.

**B. Comparison between the Levels of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Pb}^{2+}$  when they are Grouped according to Age, Occupational status, Number of children, and Tribal Affiliations.**

**a. Age group**

**Table 2.**

**Showing the mean of ppm of the metals and its significant value when the respondents were grouped according to age.**

AGE GROUP	p-Value	Inference
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Heavy Metals Concentration (ppm)	24-26	27-29	30-32	33-35	( $\alpha=0.05$ , 2-tailed)	
Cd <sup>2+</sup>	0.020	0.085	0.055	0.010	0.124	Retain the null hypothesis
Cu <sup>2+</sup>	0.230	0.145	0.215	0.130	0.710	Retain the null hypothesis
Pb <sup>2+</sup>	< 0.100	<0.100	<0.100	<0.100	1.000	Retain the null hypothesis

The table above showed the concentrations (in ppm) of the heavy metals when they were grouped according to age. It can be gleaned from the results that levels of Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> do not differ significantly. Therefore, the hypothesis which states that. "there is no significant difference in the level of the heavy metals when grouped according to age", is hereby accepted. This could mean that age does not give a significant contributions to the levels of Pb<sup>2+</sup>, Cu<sup>2+</sup>, and Cd<sup>2+</sup>. The traces of the heavy metals like Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> were found in the breastmilk of the nursing mothers. However, when they were compared to different age bracket it has no significant difference. Therefore, as the mothers grow older the levels of the heavy metals did not significantly change.

#### b. Occupational status

**Table 3.**

**Showing the mean of ppm of the metals and its significant value when they are grouped according to occupational status.**

Heavy Metals Concentration (ppm)	OCCUPATIONAL STATUS		p-Value		Inference
	Working	Non-working	Student	( $\alpha=0.05$ , 2-tailed)	
Cd <sup>2+</sup>	0.030	0.040	0.055	0.124	Retain the null hypothesis
Cu <sup>2+</sup>	0.147	0.176	0.420	0.710	Retain the null hypothesis

Pb <sup>2+</sup>	< 0.100	<0.100	<0.100	1.000	Retain the null hypothesis
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The above table showed the levels of heavy metals when the nursing mothers were grouped according to occupational status. The data showed that the breastmilk had no significant differences.

Therefore, the null hypothesis which states that, “there is no significant difference in the concentration of the heavy metals like Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> in the breastmilk of the nursing mothers when grouped according to their occupational status”, is hereby accepted. This very insignificant difference between the nursing mothers simply showed that the breastmilk of the mothers are not affected with the nature of their work. Even though the mothers were very busy in doing their work it cannot affect the levels of the heavy metals tested their breastmilk.

### c. Number of siblings

**Table 4**  
Showing the mean of ppm of the metals and the significant value when the mothers were grouped according to the number of children.

Heavy Metals	Number of Children						p-value ( $\alpha=0.05$ , 2-tailed)	Inference
	1	2	3	4	5	6		
Cd <sup>2+</sup>	0.050	0.015	0.060	0.030	0.080	0.080	0.600	Retain the null hypothesis
Cu <sup>2+</sup>	0.130	0.125	0.145	0.420	0.180	0.340	0.387	Retain the null hypothesis
Pb <sup>2+</sup>	< 0.100	<0.100	<0.100	< 0.100	<0.100	<0.100	1.000	Retain the null hypothesis

The table 4 above showed the concentrations of the heavy metals when the nursing mothers were grouped according to their number of siblings. The null hypothesis which states that, “there is no significant difference in the concentration of Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> when

the nursing mothers are grouped according to their number of siblings”, is hereby accepted.

Therefore, the results revealed that mothers who has one or more children can have more or less the same amount of heavy metal traces in their breastmilk.

**d. Tribal affiliations**

**Table 5**  
**Showing the mean of ppm of the metals and its significant value when the mothers were grouped according to their tribal affiliations.**

Heavy Metals Concentration (ppm)	Tribal Affiliations				p-Value (a=.05, 2-tailed)	Inference
	Bisaya	Chavacano	Tausug	Samal		
Cd <sup>2+</sup>	0.010	0.070	0.043	0.080	0.128	Retain the null hypothesis
Cu <sup>2+</sup>	0.085	0.147	0.263	0.340	0.122	Retain the null hypothesis
Pb <sup>2+</sup>	< 0.100	<0.100	<0.100	<0.100	1.000	Retain the null hypothesis

Table above showed that the concentration level of Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> in any tribal affiliations of the nursing mothers does not affect the amount of the traces of heavy metals. Therefore, results revealed that different customary practices at home cannot affect the breastmilk of the nursing mothers as to traces of heavy metals in this study.

**CONCLUSION AND RECOMMENDATIONS**

**Summary of Findings**

Based on the results of the following were the findings:

1. The heavy metal content of the breastmilk samples were as follows: the concentration of Cu<sup>2+</sup> is 0.1933 ppm, Cd<sup>2+</sup> is 0.0489 ppm, and Pb<sup>2+</sup> is <0.100 ppm.

2. The concentrations of the  $Cd^{2+}$  and  $Pb^{2+}$  were lower compared to the standard set by the JECFA (2003) while the concentration of  $Cu^{2+}$  was not regulated.

There were no significant differences in the level of concentrations of  $Cd^{2+}$ ,  $Pb^{2+}$  and  $Cu^{2+}$  when the respondents were grouped according to age, occupational status, number of siblings, and tribal affiliations. These findings support the result of the study conducted by Peadiatr (2010) which analyzed the concentrations of zinc and copper in infant formulas and human milk during prolonged lactation. Levels of these metals were examined in relation to selected parameters such as age, weight, height, education, and occupation of mothers. The findings concluded that there was no significant relationship between levels of these elements in human milk and the evaluated parameters. The study further revealed that the concentrations of zinc and copper in breast milk are lower than those reported in the literature. Copper deficiency increases free radicals and leads to the reduction of defense against oxidative stress.

### **Conclusion**

Based on the results of the study, the following conclusions were drawn:

1. The level of  $Cd^{2+}$ ,  $Pb^{2+}$  and  $Cu^{2+}$  heavy metals of the breastmilk sample of the nursing mothers are low compared to the allowable limit set by JECFA, 2003. Therefore, the breastmilk samples were acceptable by the WHO standard limit.
2. The trace of heavy metals like  $Cd^{2+}$ ,  $Pb^{2+}$  and  $Cu^{2+}$  in breastmilk is not so much affected even when the nursing mothers become older.
3. The occupational status of the nursing mothers did not affect the breast milk of the heavy metals of the respondents. Therefore, rich or poor mothers have the trace metals like  $Cd^{2+}$ ,  $Pb^{2+}$  and  $Cu^{2+}$  present in their breastmilk as the nursing mothers are significantly the same.
4. The number of children the nursing mothers have did not affect the traces of heavy metals in their breast milk. Since the traces of heavy metals in nursing mothers with one child are significantly the same as the trace of heavy metals for mothers with many children.
5. The tribal affiliations did not affect the traces of heavy metals in the breastmilk of the nursing mothers. Therefore, even if the customary practices at home were different, the level of concentration of the heavy metals was not affected.

## Recommendations

The researcher proposed the following recommendations:

1. Inductively Coupled Plasma-Atomic Absorption Spectrophotometry may be used in a study that can detect a very low detection level.
2. Future researchers who are planning to study similar heavy metals mentioned in this study may use different methods of digestion to validate the results of the recovery of the analytes in this study.

## REFERENCES

- Abadin, H.G., Hibbs, B.F., Pohl, H.R. (July-August, 1987). Toxicol Individual Health. Breastfeeding Exposure of Infants to Cadmium, Lead and Mercury: A Public Viewpoint. 13 (4), 495-517.
- Adam, N., Cooper, G. (2013). Clarke's Analytical Forensic Toxicology (2<sup>nd</sup> ed.). London, UK: Press p. 101.
- Arnold, L.D.W. (2004). Safe Storage of Expressed Breastmilk for the Healthy Infant and Child, East Sandwich, M.A: Health Education Associates.
- Baldwin, D.R., Marshall, W.J. (1999). Heavy metal poisoning and its laboratory investigation. *Ann Clin Biochem* 22:267-300.
- Bayly, G.R. et al. (1995). Lead poisoning from Asian traditional remedies in the West Midlands-report of a series of 5 cases. *Hum Exp Toxicol* 14:24-28.
- Bhandari, S., Bhandari, B. and Bhandari, L.M. (Accepted: August, 1982). Copper, Zinc, Magnesium and Cadmium Levels of Breast Milk of Indian Women. *Oxford Journal*, Vol. 77 (2), 201-203.
- Bjorklund, K.L., Vahter, M., Palm, B., Grander, M., Lignell, S., and Berglund, M. (2012). *Environmental Health*, 11:92. doi:10.1186/1476-069X-11-92. <http://www.ehjournal.net/content/11/1/92>. Received: 31 July 2012. Accepted: 10 December 2012, Published: 14 December 2012.
- Boyle, M.A. and Long, S. (2013). *Person Nutrition* (8<sup>th</sup> ed.) Warworth Cengage Learning.
- Brown, J.E. (2005). *Nutrition Now*. Thomson Learning Asia, p.11.
- Burtis, C.A., Bruns, D.E. (2015). *Tiets Fundamentals of Clinical Chemistry & Molecular Diagnostics* (7<sup>th</sup> ed.), Missouri: Elsevier Saunders, St. Louis, pp. 600-601.
- Carton, J.A., et al. (1987). Acute and sub-acute lead poisoning clinical findings and comparative study of diagnostic tests. *Arch Int Med* 147:697 – 703.
- Chang, R. (1991). *Chemistry* (6<sup>th</sup> ed.) USA: McGraw-Hill.
- Fisher, A.A., Le Couteur D.G. (2000). Lead poisoning from complementary and alternative medicine in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 69:687-689.

- Garcia-Esquinas, E., Perez-Gomez, B., Fernandez, M.A., Perez-Meixeira, A.M., Gil, E., De Paz, C., Iriso, A., Sanz, J.C., Astray, J., Cisneros, M., de Santos, A., Asenio, A., Gracia-Sagredo, J.M., J.F., Viogue, J., Pollan, M., Lopez-Abente, G., Gonzalez, M.J., Martinez, M., Bohigas, P.A., Pastor, R., Aragones, N. (September, 2011). Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and socio-demographic variables in Madrid (Spain). *Chemosphere* 85 (2): 268-76 Doi: 10.10161. Epub 2011 June 21.
- Gulliver, J.M. (1991). A fatal copper sulfate poisoning. *J Anal Toxicol* 15: 341-391.
- Insel, P., Turner, E.R., and Ross, D. (2010). *Discovering nutrition* (3<sup>rd</sup> ed.). USA: Jones and Barlett Publishers, LLC.
- Katzising, B.G. & Trevor, A.J. (1995) *Pharmacology* (4<sup>th</sup> ed.). USA. Prentice Hall International. pp 395-396.
- Lawrence, R.A. & Lawrence, R.M. (2011). *Breastfeeding: A guide for the medical profession* (7<sup>th</sup> ed.), St. Louis, MO:m Elsevier/Mosby, p.396.
- Mahdavi, R., Niknia, L., Pourghassem Gargani, B., and Ghaem Maghami, S.J. (July-August, 2011). Evaluation of Zinc, Copper and Iron Concentrations in Breast Milk of Mothers Belonging to Different Economic Classes and their Correlations with Infants Growth. *Journal of Shahid Sadoughi University of Medical Sciences and Health Services*, Vol. 19, 3(78), 281-291.
- Margolis, S. (2006). *John Hopkins Symptoms and Remedies*. Medletter Associates Inc.p. 725
- Mason, H.J. et al. (1991). Follow up of workers previously exposed to silver solder containing cadmium. *Occup Environ Med* 56:553-558.
- Needleman, H.I., Gatsonis C.A. (1990). Low level lead exposure and IQ of children. A meta-analysis of modern sciences. *JAMA* 263: 673-678.
- Nickerson, K. (2006). Environmental Contaminants in Breast Milk. *Journal Midwifery Women's Health*. 51(1) pp. 26-34.
- Norberg, M. (2204). Environmental exposure and preventive measures in Sweden and EU. *Biometals*17:589-592.
- Pediatr, J. (March, 2010). Zinc and Copper Concentrations in Human Milk and Infant Formulas, Revised. Nov. 15, 2008. Final Revision: May 16, 2009; Accepted: Oct. 15, 2009. Vol. 20 (no. 2), pp. 53-57.
- Polit, D.F., Hungler, B.P. (1999). *Nursing Research: Principles and Methis* (6<sup>th</sup> Ed.). Philadelphia, Lippincott.
- Republic Act No. 7272, March 24, 1992.  
[www.doh.gov.ph/zambo/index.htm](http://www.doh.gov.ph/zambo/index.htm)
- Schlenker, E.D., & Long, S. (2007). *Williams Essentials of Nutrition & Diet Therapy*. (9<sup>th</sup> ed.), St. Louis, MO: Elsevier/Mosby, pp. 171-172.
- Schiff, W.E., (2009). *Nutrition for Healthy Living*. New York: Mc-Graw Hill International Edition, p. 289.
- Sizer, F. & Whitney, E. (2003). *Nutrition Concepts and Controversies* (9<sup>th</sup> Ed.) Australia. Wadworth, Thomsom Learning.
- Smolin, G. (2008). *Nutrition*. USA: John Wiley & Sons, Inc.