LEVEL OF HEAVY METALS (Cu,As,Cr,Pb and Cd) AND EXPOSURE RISKS IN INFANT FORMULA MILK BRANDS IN JIMMA

BY:

KAMIL KENO

A RESEARCH PAPER SUBMITTED TO JIMMA UNIVERSITY INSTITUTE OF HEALTH, FACULTY OF HEALTH SCIENCES, SCHOOL OF PHARMACY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN PHARMACEUTICALS QUALITY ASSURANCE AND REGULATOR AFFAIRS.

JUNE, 2022

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF PHARMACY

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ADVISORS: MAIN-ADVISOR: Mr. TESFAYE MOHAMED

CO-ADVISOR: YIMER MEKONEN

June, 2022

Jimma, Ethiopia

SUMMARY

Background: *Most infant formulas contain much higher concentrations of minerals and trace elements than those of breast milk. Infant formula milk Powdered milks are dried milk prepared by drying the concentrated liquid milks.*

Objective: *The main objective of the study was to determine and safety assessment of major chemical toxicants in infant formula milk brand in Jimma town.*

Method: *Samples of infant formulas was collected from different supermarkets. After preparation of samples, different digestion procedures were tested by varying reagent volumes, digestion time, temperature and amount of the sample to develop a procedure that consumes less regent volumes, short digestion time, low temperature of digestion and smaller mass of the sample. The optimal procedure was required 4:00 hours and consumed 3 ML HNO_3 and 2 ML $HClO_4$ to completely digest 0.5 g of powdered infant formula samples. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution.*

Results: *Based on the findings of this study from the analyzed brands of infant formulas, the most abundant toxic metal among the element is Cd followed by Cu and Cr. And also Cr and Cd not detected in Anchor but relatively highly identified in NAN which is 1.30 and 3.39 respectively. High concentration of Cu identified in Homilac followed by Anchor. Cu is the only toxic metals found in Anchor.*

Conclusion: *The findings of this study investigated the presence of cadmium, chromium and copper in the infant formulas and their estimated daily intakes were less than their respective safety limits. Furthermore, the health risk indices of both metals were below the threshold of 1 at mean exposure, which implies low health risks of these metals to the general infants upon consumption of the formula feeding.*

ACKNOWLEDGMENTS

I would like to thank my advisors Mr. Tesfaye Mohamed, and Mr.Yimer Mekonen for their guidance and advice in the preparation of this thesis starting from topic selection and modification. I am indebted to Jimma town Health Office for sponsoring me to attend the program and Jimma University for the resources provided in the preparation of this thesis.

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LIST OF ABBREVIATION AND ACRONYMS

AAP	American Academy of Pediatrics
AAS	Atomic Absorption Spectrometry
AF	Artificial Feeding
BE	Bebelac
eHF	extensively hydrolyzed formulas
ETAAS	Electro thermal Atomic Absorption Spectrometry
F	Breast Feeding
FAAS	Flame Atomic Absorption Spectrometry
FES	Flame Emission Spectrometry
FF	Iron Deficiency
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ICP-MS	Inductively Coupled Plasma Atomic Emission Spectrometry
IDA	Iron Deficiency Anemia
WHO	World Health Organization
IgE	Immunoglobulin E
MDL	Method Detection Limit
MHz	Mega Hertz
PCBs	Polychlorinated Biphenyls
PHF	partially hydrolyzed formulas
RSD	Relative Standard Deviation
SD	Standard Deviation
SIDS	Sudden Infant Death Syndrome

1. INTRODUCTION

1.1 Background of the Study

The importance of adequate practice on the nutrition of infant and young child is to achieve optimal health, growth, and development. Worldwide recommendations for infant and young child feeding according to the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) are exclusive breastfeeding up to 6 months old and from the age of 6 months the nutritionally adequate and safe complementary feeding with continued breastfeeding up to 2 years of age or more (1; 2). Although it is important to support breastfeeding due to its benefits to infants and young children, there are circumstances where human milk is not available, the mother is unable to breastfeed, or breastfeeding is not appropriate, for example, when the mother is taking medication that is contraindicated for breastfeeding or the mother is HIV positive. In these cases, infant formulas can be used as a suitable breast-milk substitute (3).

Moreover, although there is a clear consensus on the breastfeeding as a global public health recommendation because it uniquely offers the components for the highest standard of infant nutrition, mothers have the right to decide how to feed their children and their decisions may be linked to the information and conditions they have. It is important to point out that technological advances in the food industry have transformed infant feeding models over time, and although breastfeeding is able to satisfy the nutritional needs of most infants in the first 6 months of life, there is the early or inadequate use of milk formulas by some children (4). In accordance with UNICEF database, the exclusive breastfeeding global rate is only 41% of infants 0 to 5 months of age (5).

It is generally believed that breast-fed infants absorb adequate amounts of minerals and trace elements, whereas there is some concern about how well infants can utilize these nutrients from cow's milk formula and other infant diets. Therefore, most infant formulas contain much higher concentrations of minerals and trace elements than those of breast milk to ensure that formula fed infants receive the same nutritional benefits as breast fed infants in an optimal digestible and absorbable form [6]. Powdered milks are dried milk prepared by drying the concentrated liquid milks. These are stable and contain denatured proteins so that the curd is finer; they are available in a wide range to suit different ages. The amount of feeding milk depends on the variation in the

energy needs and mineral requirements of the children at different ages under various conditions [7]. The basic ingredient in most baby milk preparation is cow's milk, which has been modified to make it as similar to mother's milk as possible. Most infant formulas are derived from animals or plants and therefore are mostly milk-based or soy based formulations. Because of the nutritional properties of soy bean, diary-like products or infant formulas based on soybean are proposed as one of the most interesting alternatives for children who are allergic to animal proteins.

In addition to immediate consequences of infant feeding on growth, body composition, health and well-being, a number of recent studies have also provided indications that the quantity and quality of nutrient supply during infancy has important long-term consequences on organ development and function, health and disease risks as well as cognitive ability in later life. In consideration of the particular risk of infants to experience untoward effects by diets with providing either too low or too high supplies of specific substrates, and the fact that during the first months after birth usually one sole milk source must meet all the infant's dietary requirements, special efforts are required to secure an adequate dietary composition for infants [6, 8]. Thus, this study was attempted to determine and safety assessment of major chemical toxicants in infant formula milk brands in Jimma town.

Infant formulas milks are found in the form of (a) powder, which must be reconstituted with water for consumption, (b) concentrated liquid, which must also be mixed with water, and (c) ready for consumption (9). These products must have nutritional security and adequate formula to support the growth and development of infants. Infant formulas contain an essential composition of proteins, lipids, carbohydrates, vitamins, minerals, trace elements, and other substances such as choline, myo-inositol, l-carnitine, and optional ingredients. In addition, the essential composition may present food additives for use in foods for infants and children (10, 11). With the aim of becoming more and more analogous to breast milk, the composition of the infant formulas has been perfected both in terms of macro and micronutrients as well as in the incorporation of nutrients with biological functions like α-lacto albumin, long-chain polyunsaturated fatty acids, lactoferrin, beta-palmitate, nucleotides, prebiotics, and probiotics (12; 13).

1.2 Statement of the problem

Contaminants, toxins, and residues shall comply with the maximum levels and maximum residue limits established for these products (10, 11).Foods are sources of many nutrients, but can provide some chemical substances such as contaminants, food additives, pesticides, veterinary drugs, phytoestrogens, and so forth. The presence of the aforementioned substances in foods can negatively influence consumer health (14). WHO, through the International Program on Chemical Safety (IPCS), establishes the scientific basis for risk assessment to human health and the environment from exposure to chemicals to promote chemical safety and provide technical support for appropriate management? Chemical safety is achieved when the safety of human health and the environment are guaranteed (15). The risk of the presence of chemical substances in the diet will depend on the potential hazards and the associated risks to life and health resulting from exposure of the population to these substances over a given period (16). Because infants are at high risk for undesirable effects by ingestion of harmful substances through feeding, infant formula manufacturers must comply with rigorous regulations established to guarantee the safety of these products (17). Although a discussion of the microbiological risks is outside the scope of this article, it is important to highlight that because powdered formulae cannot be sterilized, several microorganisms, especially Enterobacter sakazakii and Salmonella, can also be present in infant formulas causing diseases in infants (18).

The optimum nutrition for newborns is breast milk. However, for the past two decades, approximately 67.0% of infants are not completely breastfed for the recommended six months [19]. In China, only 20.8% are completely breastfed for six months, 11.5% are breastfed for one year and 6.9% are breastfed for two years [20]. Infant formulas play a special role in infant nutrition because they can be used as a breast milk substitute [21]. Suitable infant formulas are preferred sources of nutrition for most infant when breast milk is absent or insufficient [22]. There are a variety of infant formulas in the Chinese market. In 2017, the import of infant formulas was substantial (296,014 t, $3.98 billion) [23].

According to the International Agency for Research on Cancer, arsenic (As), cadmium (Cd) and hexavalent chromium (Cr VI) are group 1 carcinogens (2012), inorganic lead (Pb) is a group 2A carcinogen (2006) [24]. Epidemiological and experimental evidence indicates that combination effects generated by multi-heavy metals might be quite different from that induced by the same

individual metal, because heavy metals at individual low acting concentrations can elicit higher toxicity on interactions [25.26, 27]. Pandya et al. [25] showed that under similar dosages, when Pb and Cd are present together, the toxic effect is antagonized by co-exposure due to possible competition among Pb and Cd for hepatic accumulation. Su et al. [28] found that combination of different heavy metals showed certain adverse effects on the hematologic, hepatic, renal and neurobehavioral function and could also disturb electrolyte and lipid balance in rats. This suggests that joint toxicity or interaction patterns among different heavy metals should be taken into consideration during the risk assessment for the exposure to multi-heavy metal simultaneously. The gut immune barrier is the first line of defense against any potentially harmful agents that have been ingested in food [29]. Contrary to the restricted macromolecular passage in adulthood, enhanced transfer takes place across the intestines during early life, due to the high endocytic capacity of the immature intestinal epithelial cells during the neonatal periods [30]. Young individuals are particularly sensitive to chemical contaminants due to their high exposure-body weight ratio [31], immunodeficiency and intestinal hypoplasia. Compared to adults, children have a greater risk to heavy metal exposure through milk consumption [32, 33]. Long-term exposure to these toxic elements may cause significant health problems.

The presence of some chemical contaminants and residues in these products may pose a potential health risk for the population of interest. Herein, this comprehensive review covers the occurrence, sources, and pathways of contamination by chemical contaminants and residues in infant formulas and their effects on infant health. Strategies to minimize infant formula contamination by those chemical agents are also included.

Based on the toxicity, frequency of occurrence and potential for human exposure, the Agency for Toxic Substances and Disease Registry developed a substance priority list. According to this list, As, Pb, Cd and Cr are the 1st, 2nd, 7th and 17th priority food contaminants, respectively [34]. In China, toxic elements in food have to be measured to ensure food safety [35]. Therefore, it is of utmost importance to measure the content of toxic elements in infant formulas [36, 37]. A previous study reported that certain milk powders and infant formulas are contaminated with toxic elements [36, 38]. Recently, toxic elements in infant formulas have been reported in Tanzania [36], Egypt [39], Nigeria, UK, USA [40] and Portugal [31]. Dietary exposure to toxic elements in adults and young individuals has been assessed in many countries [41–45]. In our

previous study, toxic elements in raw milk in China have been assessed [46]. However, the imported infant formulas have not been systematically investigated. Therefore, consider the scarcity, the aim of this study was to find answer for the following basic research questions (i) to what extent the toxic elements (heavy metals and arsenic) contaminated in infant formulas in Jimma town? (ii) Does these samples meet legal requirements? (iii) What likes the status when we compare the metal content in infant formula brands derived using feeding tables supplied by infant formula manufacturers? (iv) What likes the levels of toxic metals in infant formulas as compared with literature data? (v) How occur the exposure to toxic elements from infant formulas and what are the potential health risks to infants in Jimma town?

1.3. Significance of the Study

This work was supposed to be important to the regional & national health office regulatory bodies, to the stakeholders and to the community in general. It is expected that it will help achieve the following results;

It was of great importance to the zonal, regional, FMHACA & FMOH and regulatory bodies as it indicates the products status of their meet legal requirements. Thus, it was help as a decision tool on infant formula brands derived found in the market without legal requirement to take preventive measures as an improvement of Child health and also as a concern to reduce health impacts morbidity and mortality among children.

The research was developing an insight for the investigator on the case intensity and diagnosis procedure in detail and to enhance concern towards the case determination.

The study will also try to forward recommendations as possible solutions to the identified gaps in this study.

It was a source of inspiration for further research and narrows the gap of the increasing demand for the literature in the topic under consideration.

1.4 Scope of the study

The study was focused on determine and safety assessment of major chemical toxicants in infant formula milk brand in Jimma town. Thus, the study was confined infant formula milk brands which found in the market of Jimma town. The study was also delimit to determine and safety assessment of major chemical toxicants and was not considered identifying the essential chemicals from the products. In order to determine the concentrations of toxic metals in the samples it was analyzed by flame atomic absorption spectrometer (FAAS) was employed a four point external calibration curve.

2. LITERATURE REVIEW

Infants who cannot be fed at the breast, or should not receive breast milk, or for whom breast milk is not available, require breast milk substitutes of high quality for the first 4 to 6 months of life [6]. Standards for such breast milk substitutes, namely infant formulas manufactured from cows' milk or Soy, have been established in the European Union by the Infant Formulas Directive. According to the Infant Formulas Directive, "infant formulas" means foodstuffs intended for particular nutritional use by infants during the first four to six months of life and satisfying by themselves the nutritional requirements of this category of persons, or infant formulas means a breast-milk substitute specially manufactured to satisfy the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding. Whereas "follow-on formulas" means foodstuffs intended for particular nutritional use by infants aged over four months and young children and constituting the principal liquid element in a progressively diversified diet of this category of persons [47, 48].

Theoretically, infant formulas can be based on any appropriate blend of proteins, carbohydrates, fats, minerals and vitamins. However, infant milk products are based predominantly on cow's milk on the account of special properties of its proteins and lactose and its availability in Western countries. One of the major differences between cow's milk and human milk is the protein composition and content. Casein constitutes 80 % of the protein fraction in cow's milk and not more than 40 % in human milk. Thus, in most infant formulas cow's milk is enriched with whey or whey protein concentrate to attain usually a 40:60 casein/ whey protein ratio. The other major difference is the higher lactose content of human milk. In cow's milk based infant formulas the lactose content is increased by the addition of whey and/or lactose alone. In some cases, substitution or fortification of human milk with cow's milk based formulas is not a good choice as it can cause severe disturbances. Cow's milk allergy prevalence has been reported from 0.1 % to as high as 8 %, as all the major protein fractions of cow's milk are potentially antigenic and allergenic [8,9]. In some cases, formulas not based on cow's milk but made with soy protein or hydrolyzers of milk proteins are available.

Recently, and in the past, many authors have worked on infant formulations because of the need to maintain the good health of infants. Some research findings on infant formula report high aluminum content [8, 49], inadequate daily intakes of copper and zinc from infant formula

consumption [4, 18] and low selenium content in infant formulations [19, 20]. Home-prepared bottles from powdered cow's milk can have substantial addition of metals due to the water supply, which can further distance the metal profile from that found in human milk. Although the contribution of water used in home-prepared formulas is seldom reported for essential elements, there is evidence that toxic metals, like lead and mercury, can be substantially added during infant feeding, and depending on the water and utensils used during reconstitution of powdered formulas [4].

Despite the benefits of infant formulas as a major source of food for infants, the presence of contaminants, such as heavy metals, pesticides and polychlorinated biphenyls (PCBs) in infant formula may pose health risks to children. It has been reported that children are more susceptible to exposure because of their greater intestinal absorption than adults, and a lower threshold for adverse effects. These pollutants may arise from the raw materials used in production, poor quality production processes, adulteration of infant foods and bad practices by mothers as regards infant formulation preparation and handling [50].

It is well documented that the pattern of growth of formula-fed infants differs from that of breast-fed infants [19]. Breast-fed infants tend to gain less weight and usually are leaner than are formula-fed infants in the second half of the first year of life. This difference of growth pattern between breast- and formula-fed infants seems to be the result of differences of composition between the two diets, but may be also due to differences in infant self-regulation of energy intake. There is evidence that breast-fed infants self-regulate their energy intake at a lower level than do formula-fed infants.

In the United States approximately 50 % of all newborns, and 87 % of 3-month-old infants, are fed a commercial formula either as their sole source of nutrition or as a supplement to breast milk. It is common for many formula-fed infants to be switched from one formula to another either by their parents or physicians. Although the reasons for such frequent formula switching are sometimes elusive, most of the changes occur because of perceived abnormalities in stooling patterns (too much/too little, too hard/too loose) or reports that the infant is uncomfortable while consuming a specific formula. Although there may be considerable variability in the frequency with which infants pass their stools, the effects of various formulas on stool characteristics is

limited. Previous reports that have evaluated the impact of formula content on infant stool habits have been limited to cow's milk preparations with varying iron contents [51].

Infant formulas are very important for infants by substituting breast milk, which are the main source of minerals. Therefore it is necessary to determine the levels of essential and non-essential metals in infant formulas in order to maintain the health of infants.

A variety of analytical methods have been used for the elemental analysis in food and biological materials, including inductively coupled plasma mass spectrometry (ICP-MS) [3,5], inductively coupled plasma-atomic emission spectrometry (ICPAES) [60], polarized Zeeman atomic absorption spectrophotometry [52] and colorimetric [53]. The most frequently used analytical method to determine metals in foods and biological materials is atomic absorption spectrometry (AAS). This includes flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), electro thermal atomic absorption spectrometry (ETAAS), and flame emission spectrometry (FES) [54, 55]. Many analytical methods including AAS for element determination in food materials require decomposition of the sample [55].

Dry decomposition or dissolution, often call "fusion" or "dry ashing," is normally used for refractory materials or very difficult to digest samples such as geological materials, silicates, oxides, and alloys. Typically, a sample of less than 0.1 to as much as a few grams is mixed with a (typically) a four- to tenfold excess of the fusion reagent (most likely a metal alkaline hydroxides, carbonates, or borates). The most often favored is lithium metaborate. This occurs in a carbon or platinum crucible, which is then placed in a muffle furnace at 800 to 1000 0C for as little as 15 min to as much as 8 h and results in the formation of a molten salt. The melt is then added to a dilute solution of nitric acid and results in a solution for metal determination. This method has the advantage that it is almost universal and can be applied to just about every solid. Its disadvantages are (a) that volatile metals may be lost in the process, (b) dilution can cause a reduction in detection, (c) use of external reagents can increase blanks and, finally, (d) it can be time consuming and tedious [55, 54].

Wet decomposition or acid digestion involves the use of oxidizing agents (hydrogen peroxide) and mineral acids to affect the dissolution of a sample. Hydrochloric acid (HCl) should be avoided in GFAAS due to the well-known interference from chloride. Sulfuric acid is used as a

dehydrating agent and has a high boiling point of around 3000C, which will increase rate of decomposition of some specific samples. While per chloric acid is a strong oxidizing agent, it is extremely hazardous. Hydrofluoric acid (HF) is useful for dissolving silicates. Aqua regia is widely used, but again, chloride interferences are to be avoided in GFAAS work [56, 57].

Digestion with acids, which is necessary prior to instrumental proximate analysis, is time consuming and is usually the slowest step in the analysis. Recently, several researchers have proposed the use of ultrasonic radiation to accelerate digestion of dairy products. The use of microwave energy as a heat source in acid digestion has been used in the last few years on a variety of food matrices. A microwave digestion system can reduce sample preparation time for proximate analysis by 80 % [57].

Microwave digestion involves the use of 2450 MHz electromagnetic radiation to dissolve samples. The microwaves interact with the polar molecules and induce alignment of the molecular dipole moment with the microwave field. The field changes constantly; causing rotation of the molecules and intermolecular collisions will produce heat. Consequently, the rate of microwave digestion is dependent on the coupling efficiency of microwaves with digestion acids. Microwave technology is often recommended for safety considerations. They are also programmable and can accommodate large numbers of samples [58].

The amount of toxicity associated with factors such as pathway, amount of use, solubility, metal oxidation status, maintenance percentage, and duration of application, age, sex and the frequency of use, absorption rate and efficacy of excrement mechanisms [8]. Heavy metals by different types and methods enter the food chain. Sewage, waste generated from manufacturing activities, dust, and heavy metals in food, are common ways. Polluted soil also has a great impact. Therefore, considering the numerous complications and the possible presence of these metals in milk and its products is necessary to pay major attention to the presence of heavy metals in milk and its products as a high-consumption group in the food industry, which is the main objective of the article. Therefore, is used a lot of times in sentences which are sequential. Consider using different words. This sentence does not make sense. Rewrite. Factors are used a lot of times.

Milk and its products are very diverse and there are numerous elements that can be detected, many of them are essential and very primary. These metals are involved in decisive activities,

such as cofactors in enzymes. The amount of metals in non-contaminated milk is remarkably accurate, but their content may vary considerably through the production and packaging process. Also, metals that can contaminate cows and other environments, such as lead, cadmium, chrome, Nickel, and cobalt, can disrupt milk at different levels and cause serious problems [9]. Heavy metals are introduced into the plants by absorbing them through the roots [9-11]. Contaminated soils can pollute groundwater and aquifers with these agents. Therefore, the plant is contaminated and after plant ingestion, the plants entry in human body and animals. In a recent study, it has been shown that contamination is transmitted from the soil to the plant and therefore contaminated with water and plants. Consequently, their concentration in the body of their pets and their peripheral products, such as raw milk, increases and causes toxicity.

The toxic impacts of these factors depend on different factors, such as plant processes and the amount of raw materials used and some other factors which be mentioned later in current study [12,13]. The presence of heavy metals in dairy products may be due to contamination of the primary milk of the cow, which may be due to exposure to the environment or the consumption of food and water due to exposure to cow's livestock. In addition, raw milk may be contaminated during its production [14]. Cadmium, lead and mercury are very dangerous to humans and are considered as a major threat to food in terms of industrial use. Animals use metals when grazing in the pasture and feeding with contaminated concentrations. However, in the case of cows, the transfer of minerals to milk is very variable. Pollutants are transferred to the air as a result of various industrial activities. Pollution of various industrial environments in the soil, water, food and heavy metals causes them to join the food chain and create a great threat to human and animal health [15]. Toxins such as lead, and cadmium are typically airborne pollutants and are transported to the air due to various industrial activities.

The pollution of various industrial environments in the soil, water, food, and plants with these metals make them in the food chain. Lead and cadmium residues in milk and dairy products are of particular concerns because they are largely consumed by newborns and children. Food is the main source of lead and cadmium in the general population (90% of all cadmium in non-smokers), although inhalation can play an important role in very infected areas. Lead and cadmium are associated with carcinogenic elements and are associated with a number of diseases in the cardiovascular system, kidneys, nervous system, blood and skeletal system [15].

Increased environmental pollution has accelerated the problems of milk contamination and uncertainties about milk qualities (59). The worldwide milk contamination via environmental pollutants and xenobiotic compounds through cattle feeds like toxic metals, mycotoxin, dioxin and other pollutants are considered to have greater influence on public health (60). Uptake of these contaminated milk acts like an additional source of heavy metal exposure (61). The main sources of metal contamination to humans are industrial or domestic effluents, combustion, bushfires, decomposition of chemical fertilizers, pesticides etc. (62). Abdominal pain, hepatotoxicity, neurotoxicity, vomiting (63), decreasing of intelligence quotient (IQ) level, Alzheimer's disease, behavioral disorders (64), tissue injury, irritation of lungs, cancer (65) etc. could be generated due to over exposure of heavy metals. Besides heavy metals are no biodegradable in nature and become accumulated in the food chains via bio-transformation, bio-accumulation and bio magnifications (66). Complete elimination or prevention of chemical contaminants cannot be achieved from milk because the lipophilic contaminants will find its way into the persistent fat compounds from where heavy metals cannot be removed readily (67).

A contaminant is any substance not intentionally added to food or feed for animals reared for food production, and its presence is derived from the production, manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or feed, or as a result of environmental contamination. The toxic metabolites of certain micro fungi (mycotoxins) that are not intentionally added can also be defined as a contaminant (68). Residues are traces of pesticides or veterinary drugs applied during production and present in food products. Pesticide residues result from the use in agriculture, and the term includes any derivative of these substances. Veterinary drug residues can be present in food due to the use of these substances in food producing animals and include the parent compounds and/or their metabolites in edible portions of the animal product (69). Chemical safety of foods is not tested in clinical studies (70), but the establishment of the maximum acceptable levels for residues, contaminants, and toxins by regulatory agencies is based on a global risk analysis, and the maximum levels must ensure that the consumer is adequately protected (70, 71).

Maximum limits established for contaminants shall be based on sound scientific principles resulting in safe levels for the global consumer because the establishment of these standards allows the harmonization for international trade (68). The term "maximum residue limit" (MRL)

means the maximum concentration of a pesticide (mg/kg) permitted in food and animal feeds (69). The term “extraneous maximum residue limit” refers to a pesticide residue or a contaminant coming from environmental sources and not from direct or indirect use on the commodity. The purpose of establishing these limits is to ensure good agricultural practice and the use of minimum amount necessary to reach the control of pests. MRL is also used to refer to the maximum permissible concentration of a veterinary drug in products of animal origin expressed in mg/kg or μg/kg. The establishment of MRL values takes into account the Acceptable Daily Intake. The use of medicines in the veterinary clinic must follow the Good Practices in the Use of Veterinary Drugs, including the withdrawal period. MRL may be reduced to ensure good practices. Food that complies with the respective MRLs is intended to be toxicologically acceptable for the consumer (69).

The term Heavy Metals refer to any metallic element that has a relatively high density and is toxic or poisonous at low concentration (54). Heavy Metals are a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm3, or 5 times or more, greater than water (Syman and Huton,1986; Nriagu,1988; Hawks,1997) and also Heavy Metals are defined as those elements with a specific density at least five times the specific gravity of water, Heavy Metals include Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Iron (Fe) and Platinum group elements, Copper and Zinc are essential trace elements for living organisms at low concentration (<10mg/L). However, they become toxic at high concentration (>10mg/L). Most of these metal ion (Cd, Cu, Zn, Hg, As, Ag, Cr and Fe) can be released from the industries are in simple cationic forms (Volesky, 1995). The characteristics of heavy metals are described by Wang (2006). Toxicity that can last for a long time in nature. Heavy Metals cannot be degraded including bio treatment and are very toxic even at low concentration (1.0-10.0mg/L).

Heavy Metals are dangerous because they tend to bio-accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemicals concentration in the environment. Compound accumulate in living things any time they are taken up and stored faster than they are broken down (Metabolized) excreted (29).World Health Organization (WHO) has established levels of metals in foods above which, they should

not be consumed. For this reason the levels of trace metals in our food should be of much importance and concern to us.

Milk is considered as nearly a complete food in that it's a good source of protein, fat, and major minerals (29). Cow milk contains some major elements such as calcium, potassium, phosphorous, and magnesium in addition to sodium, chlorine and a wide range of micro elements and even heavy metals. Increase in industrial and agricultural processes have resulted in increased concentration of metals in the air, water and soil. These metals are taken in by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters also accumulate such metals in their tissues and milk if lactating (46). A large amount of these metals taken in by plants and animals subsequently find their way into the food chain. This ever increasing pollution has given rise to concern on the intake of harmful metals in humans. Metals enter the human body through inhalation, ingestion or absorption through the skin (62). The intake through ingestion depends on food habit. Cow milk which is a very important food stuff consumed by man is one of the major sources (59). It has been reported that the content of the main elements in milk are fairly constant and undergoes slide changes depending on lactation phase, quality of nutrition and environmental conditions mainly chemical pollutants (59,62).

Lead is rarely found as the free metal in nature, but it is present in several minerals, principally in Galena (PbS) the main source for lead production. It is also found as Anglesite (PbSO4) and Cerrusite (PbSO3). Lead is one of the most commonly used non-ferrous metals. It has many applications; its largest use is in making storage batteries, most of which are recycled. As a result of its resistance to corrosion and its malleability, it finds use in building constructions, storage tank lining and corrosive liquid containers. Other uses of the metal are for radiation shielding, ammunition, solder, cable sheathing and pipe work. Lead compounds are used as pigments in paints and ceramics, catalysis, antibacterial substances and wood preservatives.

Chromium is one of the known environmental toxic pollutants in the world. The main sources of chromium contamination are tanneries, steel industries and sewage sludge application and fly ash. Besides these, chromium plating and alloys in motor vehicles are considered to be a more probable sources of chromium (62) at an elevated concentration it could be toxic for plants and animals. Concentrations between 5-30 mg/kg are considered critical for plants and could cause

yield reductions. The problems that are associated with chromium exposure are skin rashes, upset stomach, ulcers, respiratory problems, weakened immune systems, kidney and liver damage, alteration of genetic material, lung cancer and ultimately death (44).

Among the different heavy metals, Chromium is a common and very toxic pollutant introduced into natural waters from a variety of industrial wastewaters. The two main forms of Chromium, (chromate and dichromate) pose significantly higher levels of toxicity than the other valence states (59). Chromium (Cr) below toxic limit balances blood sugar levels, regulates hunger, reduces cravings, protect DNA and RNA improves heart function, helps control fat and cholesterol levels in the blood (51).

Cadmium is known to be toxic for living organism even if it is present in low levels (51). Cadmium is obtained from the ore minerals Shalerite (ZnS, CdS) and Greenockite (CdS) (54).

Elemental analysis of the majority of organic and inorganic matrices requires the partial or total dissolution of the sample prior to instrumental analysis. Only a few direct methods allow the introduction of the samples without any preparation. In this case lack of reliable calibration is the major problem. On the other hand, sample preparation allows the separation and /or pre-concentration of analytics and makes possible the use of several determination methods (52).

Sample decomposition is useful for converting all the species in which a given element is present in such a way that it becomes present in one defined form eliminating interfering substances from the matrix and obtaining the element in a homogeneous and easily accessible matrix. The choice of decomposition techniques should take into account the objective of the final determination and factors such as the matrix composition, the elemental contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory (50). Different decomposition methods could be classified into dry ashing, wet digestion and microwave digestion (46).

Sample digestion processes prior to quantification of heavy metals includes closed or open digestion systems and the use of different combinations of acids, such as HNO3, HCl, HClO4, HF and others (58), as well as oxidants such as H2O2. Due to their chemical composition, degree of polymerization and the presence of molecules resistant to digestion, the recovery of heavy

metals in organic residues is subject to variation (62). Therefore, digestion methods must be chosen considering the residue and the recovery rate of the heavy metals investigated (58).

For the determination of essential and toxic trace metals in powder milks different spectrum chemical methods are used. However, FAAS in one of the most extensively used techniques for discriminating various elements with significant precision and accuracy. The main advantages of FAAS with atomization in a widespread air acetylene flame are low operational costs and reasonably good analytical performance (65). The possibility of only sequential analysis and the narrow ranges of linear response can be regarded as disadvantages of this method. Using FAAS for the determination of metals in powder milks the samples are usually mineralized in order to avoid possible matrix-related interference and organic matter (56). In this case, aqueous external standard solutions can be used for calibration (58). Otherwise, multiple linear regressions can be applied to minimize the effects of chemical interferences in the flame. This draw back can be alleviated by decreasing the concentration of organic compounds supplied to the nebulizers by using on line automatic dilutors or discrete sample introduction manifolds (i.e. flow injection) (49).

3. OBJECTIVES

3.1. General Objective

- To determine the level of heavy metals their by made aligned with the exposure risk factors reported in the literature in infant formula sampled collected for this study

3.2. Specific Objectives

- To determine the level of heavy metals and arsenic found in infant formula milk brands
- To assess exposure risk of the infant formula milk brands
- To compare the metal content in infant formula brands derived using feeding tables supplied by infant formula manufacturers.

4. METHODS AND MATERIALS

4.1. Equipment and Apparatus

Analytical digital balance was used to weigh the infant formula sample. Micropipette was used for measuring different amounts of acid mixtures and standard solutions. A 250 mL round bottomed flasks fitted with reflux condensers was used to digest the powdered infant formula samples. 25, 50 and 100 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. A refrigerator was used to keep the digested sample until analysis. Flame atomic absorption spectrometer equipped with deuterium ark background corrector was used for analysis of the metals (toxic metals) in the solutions of infant formulas (Co, As, Mn, Hg, Cr, Pb and Cd) using air acetylene flame.

4.2. Reagents and Chemicals

Analytical grade chemicals and reagents were used in the analysis of samples of the infant formulas. 69-72 % HNO3 70 % HClO4 was used for the digestion of infant formula samples. Lanthanum nitrate hydrate, La $(NO_3)_3.6H_2O$ (99 % Aldrich, USA) was used to prevent the interference of phosphates in the measurement of Ca and Mg. A stock standard solution of 1000 mg/ L, in 2 % HNO_3 of the metals Ca, Mg, Mn, Fe, Zn, Cu, Pb and Cd were used for preparation of calibration standards and in the spiking experiments. Intermediate standard solutions of the metals was prepared by diluting the stock standard solutions to 10 mg/L, and then the working standard solutions was prepared from the intermediate solutions before analysis. Deionized water was used throughout the experiment for rinsing the apparatus and diluting of sample solutions.

4.3. Sample collection

The infant formula samples used for the current study are collected/procured from the pharmacies/ supermarkets following the submission of the support later from Jimma University and providing information to the owner that the samples are intended for research purpose.

Six different brands of commercially available infant formulas milks from different manufacturers were randomly collected from pharmacies and supermarkets in Jimma town, Ethiopia, in 2021. The items were found in their original packages. The six types of infant formulas were used in the experiment includes NAN, Bebelac, momilac, honilac, nido and

anchor. The samples were maintained at -20 □C (refrigerated) prior to analysis. For each of the infant formula brands three containers were used. From each container 50 g of the infant formulas powder were taken and mixed to prepare the bulk samples. From each bulk sample 0.5 g was used for digestion of samples.

4. 4. Methods and procedures

4.4.1. Preparation of 1000ppm stock AAS standard solution for selected heavy metals (Cu, As, Hg, Pb, Cr and Cd)

The determination of a given metal concentration in the experimental solution was based on its respective calibration curve. In plotting the calibration curves for copper, Arsenic, mercury, lead, chromium and cadmium a stock solution of each metal ion of (1000ppm) was prepared by dissolving; 1.5980g of Pb (NO3)2, 7.6960g of Cr (NO3)2 .9H2O and 2.1032g of Cd (NO3)2 in deionized water and then diluting to 1 liter in a volumetric flask with deionized water, respectively. Blank solutions were prepared for the methods and for the standard working solution. To prepare 100ppm, 10ml of the standard Pb (NO3)2, Cr (NO3)2 .9H2O and Cd (NO3)2 stock solutions were pipetted and added in to 100 ml calibrated flasks finally diluted with deionized water and the solution was mixed thoroughly. The standard working solutions was prepared from 100ppm by pipetting it in to calibrated flasks and made up to volume with deionized water.

4.4.2. Preparation of series of standard solutions of the respective metal ion

The selected heavy metals were copper, Arsenic, mercury, lead, chromium and cadmium. Calibration curves for each of the selected metals were prepared using six standard solutions. The usual procedure in quantitative analysis method is to prepare a series of standard solutions over a concentration range suitable for the sample being analyzed i.e. such that the expected sample concentrations are within the range established by the standard. These standards prepared by dilution from 1000ppm stock solution were as follows: 0.5ppm, 1ppm, 1.5ppm, 2ppm and 2.5ppm for Lead, 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm,1.0ppm ,1.25 for Chromium and 0.2ppm 0.4ppm, 0.6ppm, 0.8ppm, 1.0ppm and 1.2ppm for Cadmium. Calibration curves were drawn for copper, Arsenic, mercury, lead, chromium and cadmium by plotting absorbance versus metal ion concentration.

4.4.3. Sample preparation

4.4.3.1. Digestion of samples

Exactly 0.5g of powdered milk with 5 ml Nitric acid was taken in triplicate in flasks fitted with a condenser and then heated at 210 ^{0}C. After cooling the mixture, 3 ml of Perchloric acid (70%) was added and heated again at 210 ^{0}C with occasional shaking until white fumes cease to evolve. The solution was cooled down in 25 ml measuring flask and A specified amount (10ml) of distilled water was added in the digested residue and filtered through Whatman filer paper No 1.Then the volume of the filtrate was made up to mark with deionized water.

4.4.3.2. Optimization for digestion procedure of powder milk samples

The basic requirements for sample preparation for analysis are to get an optimum condition for digestion. The optimum condition is the one which required minimum reagent volume consumption, minimum digestion time reflection and clear digestion solution, ease of simplicity and absence of undigested powder milk sample. In this study, to prepare a clear colorless sample solution that is suitable for the analysis using FAAS different digestions were carried out using HNO3 and HClO4 acid mixtures by varying parameters such as volume of the acid mixtures, digestion time and digestion temperature. Different conditions tested for optimization of digestion procedure for 0.5g of powder milk samples. From the optimization procedure the acid mixture of 5 ml of HNO3 (69-72%) and 3 ml of HClO4 (70%), digestion time of 2 hours and 30 minutes and digestion temperature of 210^{0}c were found to be the optimal conditions for digestion of 0.5g powder milk samples.

4.4.3.3. Digestion of powder milk samples

Applying the optimized conditions, 0.5g of dried, homogenized and representative of each brand of powder milk samples were transferred in to 100ml round bottom flask and mixed with 8 ml of a mixture of HNO3 (69-72%) and HClO4 (70%) with a volume ratio of 5:3 (v/v) and digested at 210 ^{0}c for 2 h and 30 minutes on a kjeldahl digestion apparatus fitted with reflex condenser. The digested sample was allowed to cool at room temperature. A specified amount of distilled water was added in to the digested residue and filtered through whatman filter paper No 1. The volume of the filtrate was made up to 100 ml using distilled water and the solution was further diluted 10 times before determinations of copper (Cu), Arsenic (As), mercury (Hg), lead (Pb), chromium

(Cr) and cadmium (Cd) using FAAS. The digestion was carried out in triplicate for both blank samples. Digestion of a reagent blank was also performed in parallel with powder milk samples keeping all digestion parameters the same.

4.4.4. Determination of metal content by AAS

4.4.4.1. Preparation of calibration curve

Stock solutions of (1000mg/l) of each metal ion was prepared by dissolving calculated amounts of each metal salt in 100ml of distilled water and diluted to 100ml. Six standard solutions was prepared by serial dilution of each stock solution. Calibration curves were prepared to determine the concentration of the metals in the sample solution. The instrument was calibrated using six series of working standards. The working standard solutions of each metal were prepared from the 10 mg/l intermediate standard solutions of their respective metals. Calibration curve for each metal ion to be analyzed was prepared by plotting the absorbance as a function of metal ion standard concentration.

4.4.4.2. Determination of metal contents of each digested sample

Concentration of the metal ions present in the sample was determined by reading their absorbance using AAS and comparing it on the respective standard calibration curve. Then copper (Cu), Arsenic (As), mercury (Hg), lead (Pb), chromium (Cr) and cadmium (Cd) were analyzed with FAAS (Buck scientific model 210GP) equipped with deuterium arc back ground corrector and standard air acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wave length adjustment) were optimized for minimum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hollow cathode lamps operated at the manufacturers recommended conditions were used at their respective primary source line.

The acetylene and air flow rates were managed to ensure suitable flame conditions. The metals were determined by absorption /concentration mode and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in digested blank solutions and for the spiked samples. The standard Atomic Absorption Condition was recorded in Table 1 below.

Standard AAS Condition for Cr, Cu, Cd, Pb, Hg and As

Metal	Wave length	Slit	Characteristics concentration	Linear range	Recommended Flame	Operation with high Sensitive Nebulizer Impact bead	Characteristics concentration With a N2O-C2H2 Flame
Cr	357.9	0.7	0.078mg/l	2.0 mg/l	Air-acetylene, Oxidizing (rich, yellow)	Will typically provide 2-3 sensitivity improvement	At357.9nm:0.31mg/L
Cu	324.8	0.5	0.15mg/ml	12.0mg/ml	Air-acetylene, Oxidizing (lean, blue)	Will typically provide 2-3 sensitivity improvement	At 324.8nm: 0.62mg/l
Cd	227.8	0.7	0.028mg/l	5.0 mg/l	Air-acetylene, Oxidizing (lean, blue)	Will typically provide 2-3 sensitivity improvement	At 228.8nm: 0.11mg/L
Pb	283.3	0.7	0.45mg/ml	20.0 mg/l	Air-acetylene, Oxidizing (lean, blue)	Will typically provide 2-3 sensitivity improvement	At 283.3nm: 2.7mg/L
Hg	314	0.5	0.021mg/ml	3.0mg/l	Air-acetylene, Oxidizing (rich, yellow)	Will typically provide 2-3 sensitivity improvement	At 314nm: 11.5mg/l
As	296	0.7	0.034mg/ml	1.5mg/l	Air-acetylene, Oxidizing (rich, yellow)	Will typically provide 2-3 sensitivity improvement	At 296nm: 10mg/ml

4.5. Flame Atomic Absorption Spectrophotometric Analysis

Lead, Chromium and Cadmium was determined by a procedure described by Khalid et al (1987). Before analysis, samples of same brands were thoroughly mixed to get homogeneous and representative samples. The digested samples were analyzed for copper (Cu), Arsenic (As), mercury (Hg), lead (Pb), chromium (Cr) and cadmium (Cd) using AAS. Concentration of each metal was obtained by comparing the observed absorbance on respective standard (calibration curve). Average concentration was taken for comparing with provisional tolerable weekly intake as stated by the food and agriculture organization/world health organization with joint expert committee on food additives (JECFA).

4.6. Method Validation

4.6.1. Precision and accuracy

Accuracy and precision are probably the most often quoted terms to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and to establish the probable limits of errors of these values (Kikuchi et al., 2002).The analyst will thus be concerned with the question of precision (repeatability of results), that is, the agreement between a set of results for the same quantity and also with accuracy, that is the difference between the measured value and the true value of the quantity, Which is determined (Dean, 1997). In this study, the precision of an analytical procedure is usually expressed as the variance, relative standard deviation and percentage relative standard deviation of a series of measurements (Matusiewicz and kopras, 1997). The precision of the results were evaluated by percentage relative standard deviation of the results of three samples (N=3) and triplicate readings for each sample giving a total of nine measurements for a given bulk sample. On the other hand, the accuracy and validity of the measurements were determined by analyzing spiked samples.

4.6.2. Method detection limit

Method detection limit is the smallest mass of analytic that can be distinguished from statistical fluctuations in the blank, which usually corresponds to the standard deviation of the blank solution times a constant. The limit of detection is most commonly defined as the amount of analytic that gives a signal equal to three times the standard deviation of a blank (36). In this study, after digestion of three blank solutions containing HNO3 and HClO4 three readings was taken for each blank and the standard deviation of these was calculated. The method detection limit of each element was obtained by multiplying the standard deviation of the reagent blank by three. As shown in Appendix-Table.

$MDL=3x\delta_{blank}$

Where δ_{blank} is standard deviation of the blank readings

4.6.3. Limit of quantitation (LOQ)

The lowest concentration level at which a measurement is quantitavely meaningful is called the limit of quantitation (LOQ).The LOQ is most often defined as 10 times the signal/noise ratio if the noise is approximated as the standard deviation of the blank ,the LOQ is 10xSD of the blank(53).In this study, LOQ was obtained from triplicate analysis nine reagents blanks Which were digested in the same digestion procedure for powder milk samples .The LOQ was calculated by multiplying pooled standard deviation of the reagent blank by 10 (LOQ =10xSDblank ,n=3)and the values for the elements was listed in Appendix table.

4.6.4. Validation of optimized procedures

Direct determination of the validity of the above optimized procedure towards analysis of the powder milk samples with respect to each of the selected metals (Cu, Hg, As, Pb, Cr and Cd) could not be made possible because of the absence of certified reference materials (CSRM) for these metals. Instead, spiking method on the above (same) digestion procedure was adopted for the same purpose. Accordingly, the efficiency of the optimized procedure was checked by adding different volumes of standard solutions containing 10 mg/L of each metal in to 0.5g powder milk. The spiked samples were then digested in the same manner as for original sample. Then the digests were transferred in to 50 ml volumetric flask and diluted to the mark with deionized water. Finally, the solutions were analyzed for metal concentration with FAAS. As used for the original samples, triplicate spiked samples were prepared and the readings were recorded. Recovery was calculated using the equation given below.

$$R = \frac{Cs - c}{s} x100$$

Where,

s= concentration equivalent of analytic added to the sample

Cs= metal content of the spiked sample

c = metal content of non-spiked sample

R = percent recover

4.7. Analysis of Metal contents of Digests

Atomic Absorption Spectroscopy was used to determine the concentration of digested samples after treatment through different conditions of the parameters. The analysis was done at working conditions of the Atomic Absorption Spectroscopy for Lead, Chromium, Arsenic, Mercury, Copper and Cadmium analysis as given in Appendix table.

4.8. Data Analysis

Data was analyzed using Microsoft Office Excel. The data were expressed in term of descriptive statistics while the figures were presented with Mean values as (Mean±SD). A p-value less than 0.05 were considered as Significant.

5. RESULT

5.1. Concentration of Metals in Powdered Infant Formulas

The levels of elements in six different milk-based infant formulas, targeted for infants aged 6 months to 1 year, are presented in Table 1. The concentrations of six elements (Cr, Cu, Hg, Pb, As and Cd) in the digested and diluted solutions of infant formulas were determined by flame atomic absorption spectrometer. Among the identified elements except mercury (Hg), Arsenic (As) and lead (Pb) which were below the method detection limit, all have been detected and quantified (Table 1).

Table 1: Mean concentration (X ± SD, N = 6, in μg/g) of infant formula samples.

Type of sample	**Concentration of Elements**					
	Cr	**Cu**	**Cd**	**Pb**	**Hg**	**As**
Momilac	0.37±0.02	1.01±0.03	1.65± 0.17	ND	ND	ND
Bebelac	1.11±0.12	1.85±0.24	2.01±0.34	ND	ND	ND
Honilac	0.74±0.14	2.86±0.17	2.29±0.41	ND	ND	ND
Ancor	ND	2.35±0.31	ND	ND	ND	ND
Nido	0.06±0.02	0.34±0.21	1.92±0.31	ND	ND	ND
NAN	1.30±0.13	2.02±0.16	3.39±0.28	ND	ND	ND

ND (Not Detected/ below Method Detection Limit)

As it can be seen from Table 1 and Figure 1, quantitatively there is a variation in concentration of toxic metals within the infant formula brands. Except for Hg, As and Pb, which were below the method detection limit in all brands, the selected elements were successfully determined. From the analyzed brands of infant formulas, the most abundant toxic metal among the element is Cd followed by Cu and Cr. And also Cr and Cd not detected in Anchor but relatively highly identified in NAN which is 1.30 and 3.39 respectively. High concentration of Cu identified in Homilac followed by Anchor. Cu is the only toxic metals found in Anchor (Figure 1).

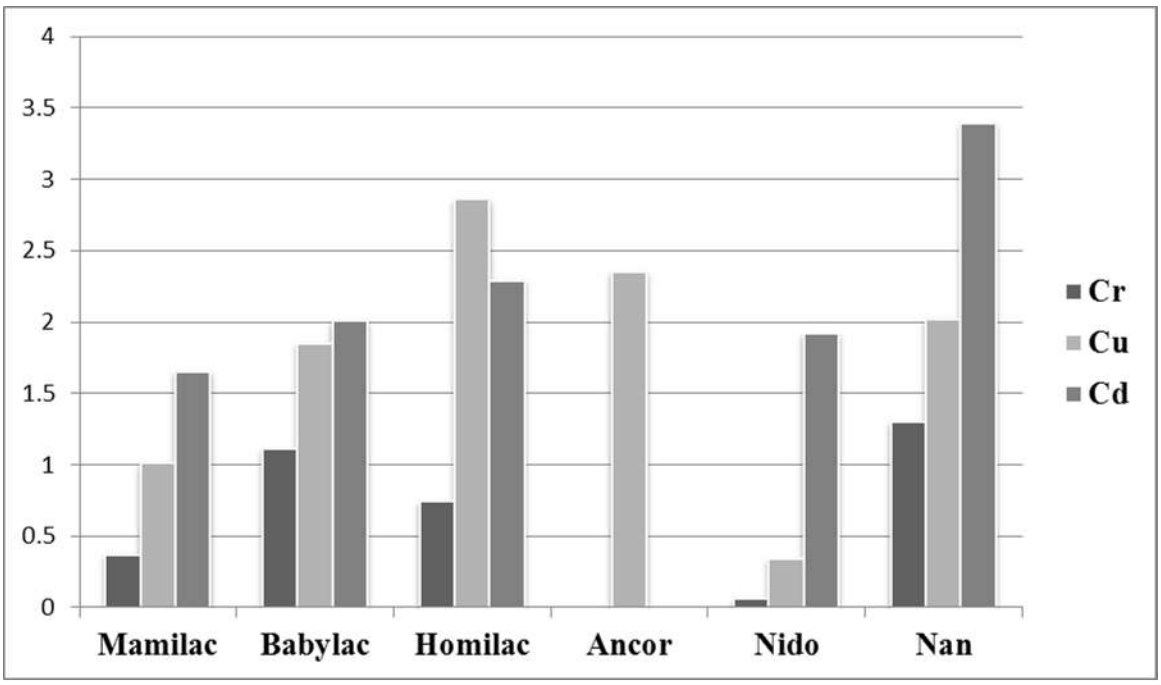

Figure 1. Average concentration of toxic metals (Cr, Cu & Cd) in powdered infant formulas

5.2. Instrumental Calibrations

The data qualities obtained from FAAS for metal analysis are highly affected by the calibration and standard solutions preparation procedures. The instrument was calibrated using a series of standard working solutions of each of the metals prepared freshly by appropriate dilution of the intermediate standard solutions. The intermediate standard solutions were prepared from stock solution of each metal. The concentrations of the intermediate standards, working standard solutions and values of correlation coefficients of the calibration graphs for the three metals of interest is presented in Table 3 and their calibration graphs were depicted in Appendix figure 1,2,3,4,5 and 6.

Table 2: Concentration Values of working Intermediate Standard solutions, Working Standard solutions and Correlation Coefficients of Calibration Graphs.

Metal	Concentration of intermediate Standard(mg/L)	Concentration of working standard(mg/L)	Correlation Coefficients of calibration curves (R2)
Lead	10	0.5, 1.0, 1.5, 2.0 and 2.5	0.999
Chromium	10	0.2, 0.4, 0.6, 0.8 , 1.25 and1.5	0.998
Cadmium	10	0.2, 0.4, 0.6, 0.8, 1.0 and 1.2	0.997
Copper	10	0.1,0.2,0.4,0.6,0.8 and 1.0	0.991
Arsenic	10	0.2, 0.4, 0.6, 0.8 , 1.25 and1.5	0.996
Mercury	10	0.2, 0.4, 0.6, 0.8 , 1.25 and1.5	0.995

5.3. Comparison of Observed Metals Concentrations with Reported Data in the Literature

Table 2 illustrates a comparison of metals concentrations in infant formulas investigated in this work with reported data in the literature. The levels of chromium in the samples in the present survey ranged from being not detected to 0.130mg/kg, which is not identified with the results obtained from other studies.

Table 3: A comparison of metals levels (mg/kg) in infant formulas with reported data in the literature.

Origin/market site	Types of Identified metals				Reference
	Cr	**Cu**	**Cd**	**Pb**	
Ethiopia	ND-0.00130	0.00034-0.0029	0.00165-0.0034	ND	This Study
Ethiopia			ND	ND–0.103	(44)
Ethiopia	-	-	ND	ND	(73)
Nigeria	-	-	0.05–0.4	0.08–0.23	(50)
Pakistan	-	-	0.0042–0.0123	0.0287–0.097	(61)
Iran			0.0403–0.058	0.03183–0.03185	(74)
Polish	-	0.20–1.00	-	-	(75)
China	0.0251–0.838	-	0.13–3.58	0.36–5.57	(46)

5.4. Exposure risk of the infant formula milk brands

Table 3 presents the EDI and HI values of both chromium and cadmium contents in the samples. The estimated daily intake values of chromium found in the baby formula ranged from 1.30 to 0.37 μg/kg bw/day and 0.94 to 0.24 μg/kg bw/day for infants during the formula feeding ages of 6 and 7–10 months, respectively. The value which indicated for this study, the daily intake of chromium was below the safety limit which is 1.9 μg/kg bw/day.

One-way analysis of variance revealed that there were significant differences between various brands of infant formula products for their Cr, Cd and Cu contents ($p < 0.05$). The mean levels of measured Cr and Cd were significantly higher in products N (NAN) which are 1.3 and 3.29, respectively, while infant formula H (homilac) had significantly higher concentration of cupper content.

Table 4: The estimated daily intake (EDI) values of metals from commercial infant formulas and the health risk index (HI).

Age in months	Metal	Safety Limit	Infant formula code, EDI						P-value	Mean EDI	HI
			M	B	H	A	Ni	N			
6	Cr*	1.9	0.37	1.11	0.74	-	0.06	1.30	0.019	0.5967	0.543
	Cd**	1.11	0.0017	0.002	0.023	-	0.02	0.034		0.0104	0.014
	Cu***	3.3	1.01	1.85	2.86	2.35	0.34	2.02		1.74	0.918
7–12	Cr	1.9	0.24	0.72	0.43	-	0.04	0.94		0.3950	0.377
	Cd	1.11	0.008	0.002	0.001	-	0.08	0.63		0.0067	0.011
	Cu	3.3	0.51	0.89	1.48	1.21	0.21	0.98		0.3950	0.4615

*∗Chromium level in μg/kg bw/day. ∗∗Cadium level in mg/kg bw/day. Cu***Copper level in mg/kg bw/day*

As indicated below on figure 2, the hazard index of Cu is high in both age category, which are 0.918 and 0.4615 for 6 month and 7-12months respectively. Whereas the lower hazard index indicated on Cd which are 00.014 and 0.011 for above indicated age category respectively.

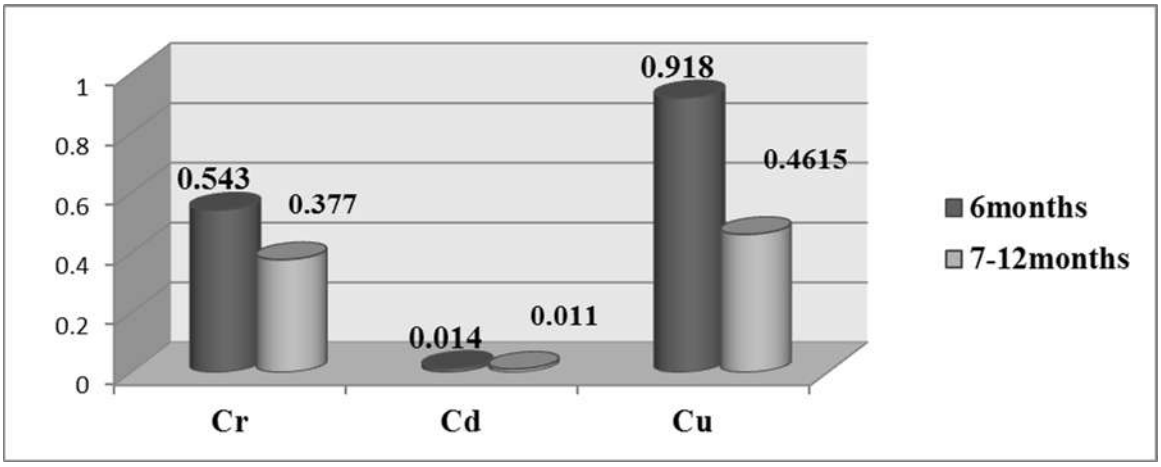

Figure 2: Hazard Index (HI) of toxic metals (Cr, Cu & Cd) for ages 6 months and 7-12 months.

6. DISCUSSION

In this study cadmium was detected in all brands of infant formulas except anchor the finding in this work which is in disagreement with the findings from the surveys done in Addis Ababa, Ethiopia (73), and Mekelle, Ethiopia (44) which were not detect cadimium, other studies in different origins investigated the presence of cadmium in formula feeding with slightly higher concentration from the findings of this study (50, 61, 74) as shown in Table 2. The difference of the contents in various studies might be due to the difference of the batch of the products on their production, and also various exposures to contamination during their production at the different study areas.

The levels of lead in the samples in the present survey ranged from being not detected in this study, while the study conducted in mekellle identified ranged from being not detected to 0.103mg/kg (44). Aguzue et al. (50) reported 0.08–0.23mg/kg of lead levels in infant formula in Abuja, Nigeria, whereas lower amounts, 0.00375–0.0249mg/kg, of lead were recorded in similar samples in Turkey (55). However, the study conducted in Addis Ababa, Ethiopia, revealed that the levels of lead in the powdered infant formulas were below the detection limit (73).

One-way analysis of variance revealed that there were significant differences between various brands of infant formula products for their Cr, Cd and Cu contents ($p < 0.05$). The mean levels of measured lead and zinc were significantly higher in products N and H, respectively, while infant formula H (homilac) had significantly higher concentration of cupper content.. Navarro-Blasco and Alvarez-Galindo (24) reported similar results that there were significant variations of lead concentrations across the infant formula brands. This difference could be attributed to the variation of raw materials used in manufacturing, production practices, finished products, and packaging containers used by infant formula manufacturers (25).

The EDI and HI values of both lead and zinc contents in the samples. The estimated daily intake values of chromium found in the baby formula ranged from 1.30 to 0.37 μg/kg bw/day and 0.94 to 0.24 μg/kg bw/day for infants during the formula feeding ages of 6 and 7–10 months, respectively. The value which indicated for this study, the daily intake of chromium was below the safety limit which is 1.9 μg/kg bw/day.

Due to some key physiological differences between infants and adults, infants are far more vulnerable to the environmental contaminants, thus to the increased doses of exposure. In infants, some protection mechanisms such as blood-brain barrier, plasma protein binding capacity, enzymatic elimination mechanisms in the liver and kidneys and immune system are underdeveloped (41–44). Infants are particularly sensitive to ingested contaminants due to larger specific surface area and efficient gastrointestinal absorption (21). Infant formulas represent the main or only source of nutrients for infants when breast milk is absent or insufficient. Therefore, the toxic element in infant formulas, which may constitute a risk factor for the babies, is highly significant. It is essential to ensure their quality and safety.

According to the findings of this study the hazard index of Cu is high in both age category, which are 0.918 and 0.4615 for 6 month and 7-12months respectively. Whereas the lower hazard index indicated on Cd which are 00.014 and 0.011 for above indicated age category respectively. The HI values were calculated from total THQ corresponding to each body weight at different age. The HI values are shown in Figure 1. The HI values were 0.055–0.192 for boys and 0.056–0.209 for girls. The HI value was higher for girls than boys, due to their lower body weight. The HI values were inversely associated with age and were lower than the established criteria 1. The non-carcinogenic risk values were in the safe range, indicating that exposure of As, Pb, Cr and Cd from infant formulas does not represent a health risk in China. However, food producers should try their best to reduce the levels of toxic elements in infant formula [16] considering the immunodeficiency and intestinal hypoplasia for young people.

7. CONCLUSION

The findings of this study investigated the presence of cadmium, chromium and copper in the infant formulas and their estimated daily intakes were less than their respective safety limits. Furthermore, the health risk indices of both metals were below the threshold of 1 at mean exposure, which implies low health risks of these metals to the general infants upon consumption of the formula feeding. However, regular assessment of infant formula products for toxic heavy metals is essential as infants are more sensitive population. Further study is necessary to estimate macro elements, trace elements and other toxic elements in infant formulas in Jimma, to compare nutritional values not only the potential risk.

Since infant formulas have a special role to play in the diets of infants, the nutritional safety and adequacy of infant formula should be scientifically demonstrated to support growth and development of infants. The present study will give some information about the toxic mineral contents of imported powdered infant formulas. But to have a complete and general information further research should focus on the composition of infant formulas.

8. RECOMMENDATION

In light of the findings and the conclusion drawn above, the following recommendations are forwarded.

- The efficiency of other extract ants like NHO3: H2O2, HNO3: H2S04, H2S04: HCLO4 should also be checked.
- This study might be repeated with GFAAS and ICP-OES to compare the metal contents of the selected powder milks.
- The concentration of heavy metals in similar powder milk samples from Jimma and other markets in the Southeastern part of Ethiopia that are not covered by this research should be determined.
- Monitoring of the levels of heavy metals in commercially available powder milk should be encouraged.

9. LIMITATION

- The limitation of these study was covers only few brands of infant formula and only from few supermarket or pharmacies.
- The study also focuses on the few batches of sample brands and only from Jimma town.

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Appendix of Tables

Table 1. Optimization for Reagent Volume

Trial No	Reagent Used	Reagent Volume(ml)	Temperature (^{0}C)	Digestion Time (h)	Observation
1	HNO_3:$HClO_4$	3:1	250	2:00	Deep Yellow
2	HNO_3:$HClO_4$	3:2	250	2:00	Deep Yellow
3	HNO_3:$HClO_4$	3:3	250	2:00	Yellow
4	HNO_3:$HClO_4$	4:1	250	2:00	Light Yellow
5	HNO_3:$HClO_4$	4:2	250	2:00	Light Yellow
6	HNO_3:$HClO_4$	4:3	250	2:00	Almost Clear
7	HNO_3:$HClO_4$	5:3*	250	2:00	Clear and Colorless
8	HNO_3:$HClO_4$	5:4	250	2:00	Clear and Colorless
9	HNO_3:$HClO_4$	5:5	250	2:00	Clear and Colorless

* The optimized condition for the given parameter

Table 2. Optimization for Temperature

Trial No	Reagent Used	Reagent Volume(ml)	Temperature (^{0}C)	Digestion Time (h)	Observation
1	HNO_3:$HClO_4$	5:3	80	2:00	Deep Yellow
2	HNO_3:$HClO_4$	5:3	100	2:00	Yellow
3	HNO_3:$HClO_4$	5:3	110	2:00	Light Yellow
4	HNO_3:$HClO_4$	5:3	140	2:00	Clear Yellow
5	HNO_3:$HClO_4$	5:3	180	2:00	Almost Clear
6	HNO_3:$HClO_4$	5:3	210*	2:00	Clear and Colorless
7	HNO_3:$HClO_4$	5:3	240	2:00	Clear and Colorless
8	HNO_3:$HClO_4$	5:3	270	2:00	Clear and Colorless
9	HNO_3:$HClO_4$	5:3	300	2:00	Clear and Colorless

* The optimized condition for the given parameter

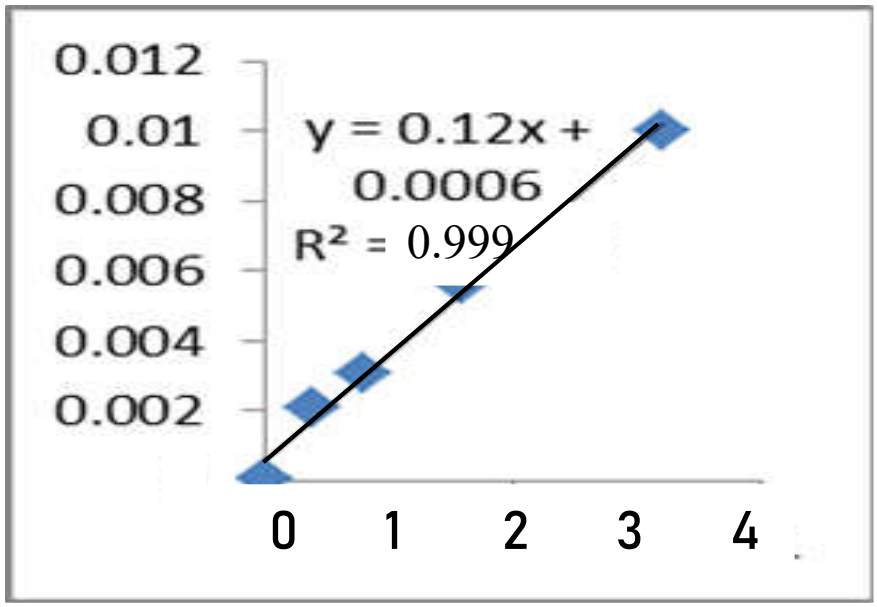
Table 3. Optimization for Digestion Time

Trial No	Reagent Used	Reagent Volume(ml)	Temperature (^{0}C)	Digestion Time (h)	Observation
1	HNO_3:$HClO_4$	5:3	210	1:00	Deep Yellow
2	HNO_3:$HClO_4$	5:3	210	1:30	Light Yellow
3	HNO_3:$HClO_4$	5:3	210	1:45	Yellow
4	HNO_3:$HClO_4$	5:3	210	2:00	Clear Yellow
5	HNO_3:$HClO_4$	5:3	210	2:15	Almost Clear
6	HNO_3:$HClO_4$	5:3	210	2:30*	Clear and Colorless
7	HNO_3:$HClO_4$	5:3	210	2:45	Clear and Colorless
8	HNO_3:$HClO_4$	5:3	210	2:50	Clear and Colorless
9	HNO_3:$HClO_4$	5:3	210	3:00	Clear and Colorless

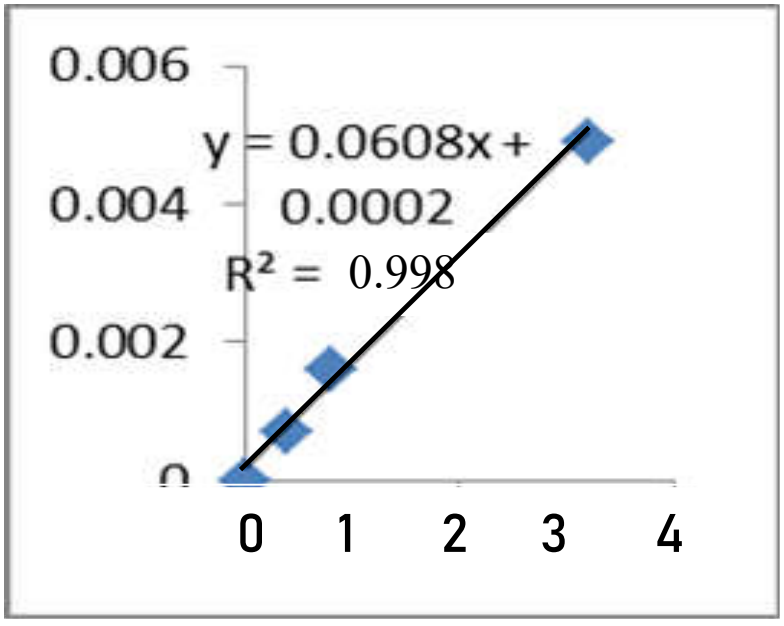
* The optimized condition for the given parameter

Appendix of Figures

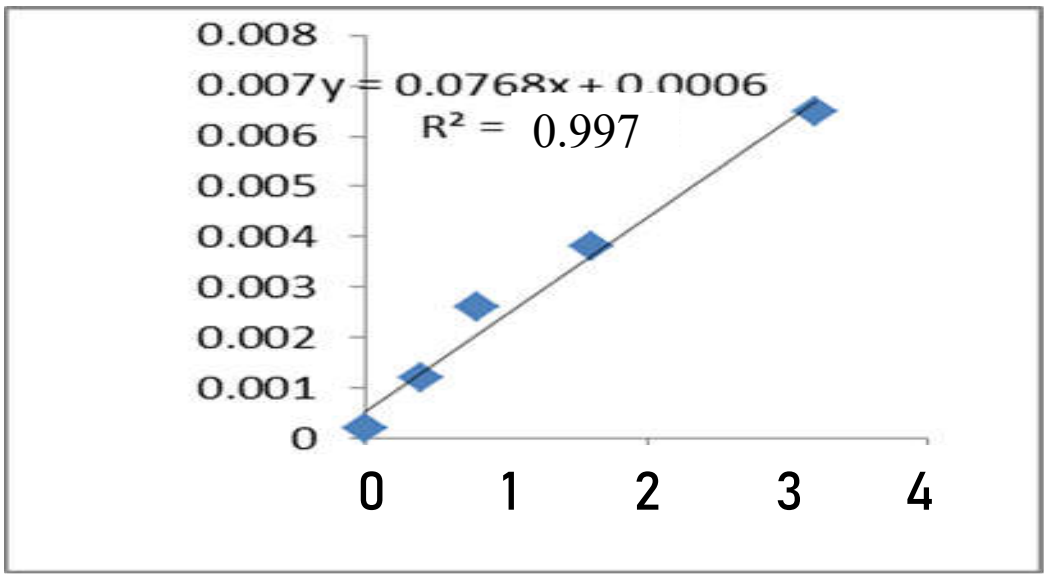
Calibration Curve for Chromium



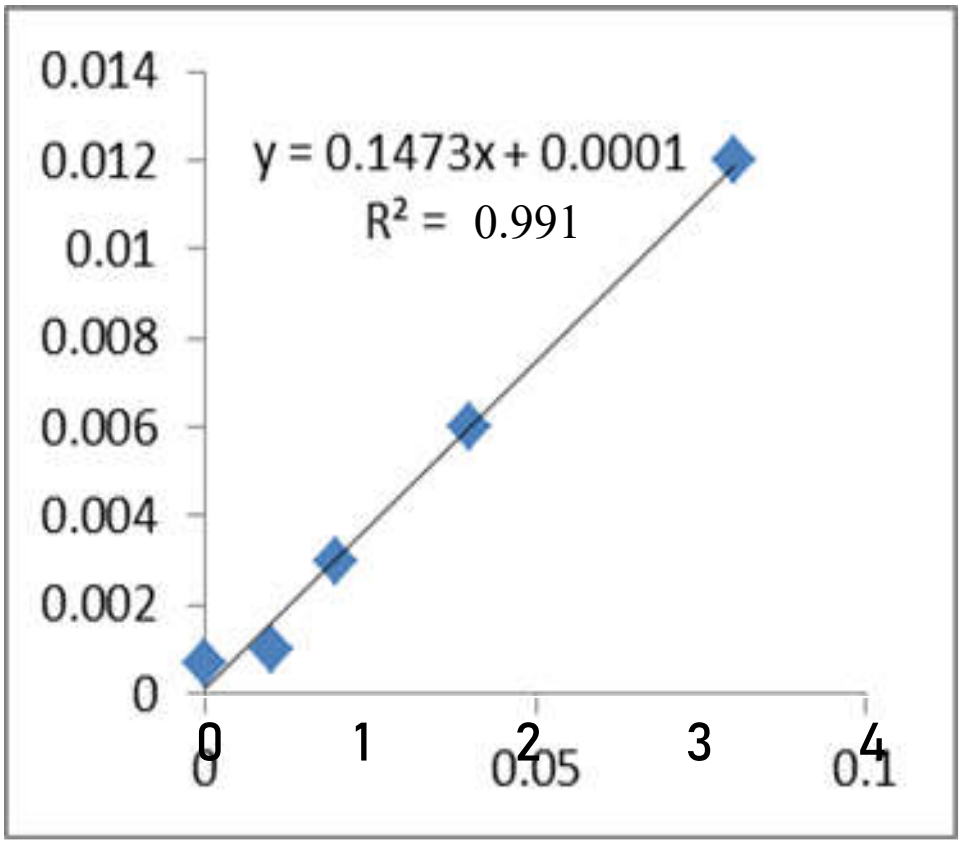
Calibration Curve for Copper



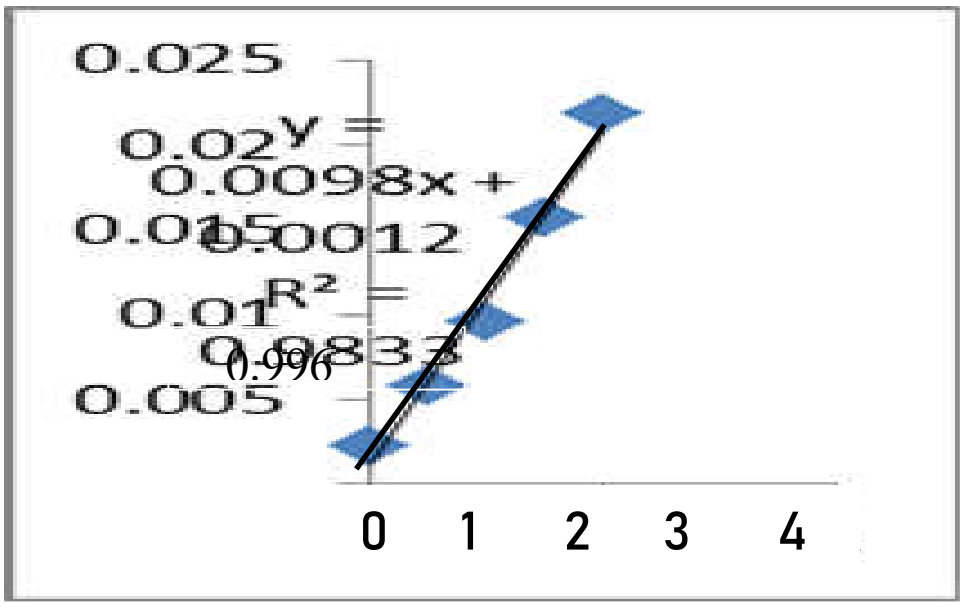
Calibration Curve for Cadmium



Calibration Curve for Lead



Calibration Curve for Mercury



Calibration Curve for Arsenic