INSTITUTE OF HEALTH SCIENCES

FACULTY OF PUBLIC HEALTH

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY

HEAVY METALS CONCENTRATION IN INFANT FORMULA FOOD COLLECTED FROM LOCAL MARKETS IN FICHE, OROMIA, ETHIOPIA

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RESEARCH PAPER SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY, FACULTY OF PUBLIC HEALTH, INSTITUTE OF HEALTH SCIENCE, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH

SEPTEMBER, 2022

JIMMA, ETHIOPI

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SEPTEMBER, 2022

JIMMA, ETHIOPIA

APPROVAL SHEET

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Solomon Amare entitled as: Heavy metals concentrations in infant formula food collected from local markets in Fiche, Oromia, Ethiopia: We recommended that it could be submitted as fulfilling the thesis requirement.

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As member of the board of examiners of the MSc. thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Solomon Amare and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental Health.

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External Examiner Signature Date

DECLARATION

I declared that this is an original work and has not been submitted in any previous application for a degree or professional qualification in any other institution. I confirm that due references have been provided on all supporting literature and contributor to any resources in this work has been appropriately acknowledged.

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ABSTRUCT

***Back ground:** - The majority of milk products in the world undergo processing procedures which is exposed to heavy metals contamination for infant formula products. Exposure to heavy metals can result in long-term effects on health of infants.*

Objective: To determine heavy metals concentration in powdered infant formula foods collected from local markets in Fiche town, Oromia, Ethiopia.

***Method and material**: Cross sectional study was implemented and 6 imported and 3 domestic formulae selected by simple random sampling technique from three purposively selected highest infant formulae suppliers in Fiche. Totally, 27 samples were analyzed to detect Cd and Pb by MP-AES and HGAAS for Hg from April 30-June 13/2022.*

***Result and Discussion**: The concentration of Cd in Cerifam (0.07 ± 0.0451) mg/kg < Faffa (0.11 ± 0.03) mg/kg < Bebelac (0.15 ± 0.05) mg/kg < Mother's choice (0.19 ± 0.03696) mg/kg < Liptomil (0.29 ± 0 .03) mg/kg < Aptomil (0.42 ± 0.01) mg/kg < S 26 (0.51 ± 0.11) mg/kg < Francelait (0.55 ± 0. 02) mg/kg < NAN (0.6 ± 0.03) mg/kg. Highest concentration of Cd detected in NAN infant formula and the lowest detected in Cerifam. The concentration of Hg in Aptomil (0.0147 ± 0.0031) mg/kg < NAN (0.0183 ± 0.0015) mg/kg < Francelait (0.0193 ± 0.0012) mg/kg < Faffa (0.029 ± 0.0012) mg/kg < Cerifam (0.032 ± 0.002) mg/kg < Liptomil (0.0353 ± 0.0042) mg/kg < S 26 (0.062 ± 0) < Bebelac (0.069 ± 0.0012) mg/kg < Mother's choice (0.0813 ± 0.0031) mg/kg. The highest concentration of Hg detected in Mother's choice while lowest detected in Aptomil. The concentration of Pb in S 26 (0.0666 ± 0.0115) mg/kg < Aptomil (0.0667 ± 0.0116) mg/kg = Liptomil (0.0667 ± 0.0116) mg/kg < Francelait (0.20 ± 0) mg/kg < NAN (0.2 ± 0.022) mg/kg <Cerifam (0.2 ± 0.0346) mg/kg < Bebelac (0.267 ± 0.0306) mg/kg < Faffa (0.4666 ± 0.4163) mg/kg < Mother's choice (0.533 ± 0.1155) mg/kg. Imported formulae contain higher Cd and Hg than domestic products but, higher concentration of Pb detected in domestic products. Based on formula source, higher concentration of Cd, Hg and Pb detected in plant and cow source than plant based powdered infant formulae.*

***Conclusion and Recommendation:** All studied infant formula brands didn't comply with WHO and FAO (Cd 0.05 and Pb 0.01) mg/kg and US EPA (Hg 0.002 mg/kg) standards, so it is compulsory to give attention and regulate contaminants in infant formula foods.*

Key words: Infant Formula, Cadmium, Mercury, Lead, Brand, Domestic, Imported

ABBREVIATIONS AND ACRONYMS

AAS-	Atomic Absorption Spectrometry
BMS -	Breast Milk Substitution
Cd-	Cadmium
CNS-	Central Nervous System
C^{o} -	Degree centigrade c
EFDA-	Ethiopian Food and Drug Administration
EU-	European Union
EDHS-	Ethiopian Demographic Health Survey
FAO-	Food and Agricultural Organization
FDA-	Food and Drug Administration
FMHACA-	Food Medicine and Healthcare Administration and Control Authority
FSIS-	Food Safety and Inspection Services
GDP-	Gross Domestic Production
HACCP-	Hazard Analysis and Critical Control Points
HGAAS-	Hydride Generated Atomic Absorption spectrometry
IQ-	Intelligence Quotient
JECFA-	Joint Expert Committee on Food Additives
Pb-	Lead
MP-AES -	Micro Plasma Atomic Emission Spectrometry
MDG-	Millennium Development Goal
nm-	Nano meter
NHANES-	National Health and Nutrition Examination Survey
ND-	No Date
NGO -	Non-Governmental Organization
SDG-	Sustainable Development Goal
US-	United States
US EPA-	United State of American Environmental Protection Agency
WHO-	World Health Organization

H_2O_2- Hydrogen per Oxide

Hg- Mercury

µg/L- Microgram per Liter

Mg/kg - Milligram per Kilogram

HNO_3- Nitric Acid

UNICEF- United Nation Children’s Fund, originally known as Unite Nations International Children's Emergency Fund

ACKNOWLEDGMENT

First and foremost, I would like to praise Almighty God with his mother St. Marry and with his saints for bestowing up on me health, strength, patience and protection throughout my study period. I express my deepest and heartfelt gratitude to my advisors Mulunesh Deti (MSc.) and Higemengist Astatkie (PhD. Assistant Professor) for their valuable suggestions, guidance and relevant comments starting from proposal writing to the preparation of this research report document.

My special thank goes to Jimma University Environmental Health Science and Technology Department, for their willingness for preparation of Letter for institutions that have heavy metal analysis instruments and support for provision of laboratory facilities. I would also like to offer thanks to Ethiopian Food and Drug Control Authority Food Laboratory service for allowing me to use their laboratory and provided with the necessary materials during sample preparation and digestion and also Ethiopian Construction Design and Supervision Work Corporation water quality Laboratory service for their cooperation for analysis of mercury concentration and all the necessary supports.

Next, I would like to thank Oromia Regional Health Bureau for sponsoring and giving an education chance to me and for North Shoa Zonal Health Office as well as to all my families for their supportive and suggestion in all my activities. Last but not least, my heartfelt gratitude goes to my colleagues, they deserve special, valuable and encouragement to me.

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CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Infant formula is a synthetic version of mother's breast milk belongs to dairy substitutes. The food which represented for special dietary use solely as a food for infants by reason for its simulation of human breast milk or its suitability as a complete or partial substitute for human breast milk. In 1867, Henri Nestlé created the first commercially sold infant formulae (Maslin and Venter, 2017).

The occurrence of chemical residues in infant's milk product is a matter of public health concern, since dairy products are widely consumed by infants and children throughout the world. Governments have responsibility for making regulations to protect consumers against harm arising from chemical in food (Thompson and Darwish, 2019).

The FAO of United Nations and WHO jointly established the Codex Aliment Arius Commission in 1963. Its main purpose is "protecting consumers' and ensuring fair practices in food trade." In 1976 the Commission health adopted a Codex Standard for infant formula. Infant formula prepared in accordance with applicable Codex Aliment Arius standards was a safe complementary food and a suitable breast milk substitute (Lee, 2019) .

In 2003, WHO and UNICEF published their Global Strategy for infant and young child feeding, which restated that "processed-food products for young children when sold or distributed, should meet applicable standards recommended by Codex Aliment Arius Commission", and also warned that "lack of breastfeeding, especially lack of exclusive breastfeeding during the first half-year of life are at risk (Obladen, 2014).

WHO estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (Prüss-Üstün and Corvalän, 2007). Some heavy metals are essential to maintain various biochemical and physiological functions in living organisms when in low concentrations; however, they become poisonous when they exceed certain permissible limit (Campbell-Lendrum and Corvalán, 2007).

Worldwide, technologies and related human activities have resulted in the introduction of numerous chemicals, directly or indirectly, into food supply. Only a few of these chemicals have been fully characterized in terms of their potential toxicities to animals and humans (Procurement,2015).

In Africa, today there is a shift from exclusive breastfeeding practice towards the introduction of formula feeding. The increasing incidence of bottle feeding in developing countries particularly in Africa reflects absorption of western way of life. This is due to the impacts of globalization, increasing availability of formula milk in supermarkets and promotion of formula milk by advertising through different media (Tawfik *et al.*, 2019).

Starting from 2016, Ethiopian government has implemented several directives such as Infant Formula and Follow-up Formula Directive No. 30/2016 and Food Advertisement Directive 33/2016 to encourage breastfeeding by restricting the promotion of formula feeding practice (Food , Medicine and Healthcare Administration and Control Authority of Ethiopia (FMHACA) Guidelines for Registration of Vaccines', 2018). However, the proportions of mothers who still breast-feed their children are considerably low, particularly among women living in urban centers of Ethiopian (Setegn *et al.*, 2012).

In Ethiopia, only 58% of infants less than 6 months were exclusively breastfed and formula feeding was 30% among the age of up to 1 month, it was 45% between two and 3 months and it increased to 68% in the infants from 4 to 5 months (Abebe *et al.*, 2019).

Breastfeeding is the best and richest mode of nutrition for infants during the early stage of life between 0 to 6 months, as it contains all the nutrients and immunological factors (Naser *et al.*, 2012). However, infant formulas provide a suitable alternative, especially when breastfeeding is not possible or not adequate (Pplied and State, 2021).

However, the safety of infant formula food was not given attention in Ethiopia. This study was conducted to determine heavy metal concentration of powdered formula food that mostly consumed by infants aged 0–12 months in Fiche town, Ethiopia. The finding of this study will provide relevant updated information regarding formula food safety which is helpful for policy makers and health sector stakeholders to develop appropriate regulatory strategies and interventions for those vulnerable groups.

1.2 Statement of the problem

The majority of milk produced in the world undergoes processing, during which a variety of technological procedures occurs. Throughout human's daily activity, heavy metals enter in our environment and contaminate food processing system due to their persistence ability for long period of time. Exposure to heavy metals during growth and development can result in long-term effects on health of infants. Most of these heavy metals especially Mercury, Lead and Cadmium do not degrade easily instead they accumulate in vital organs of our body (Naser *et al.*, 2012).

Infants are more sensitive than adults to food contaminants due to a higher rate of uptake by the gastrointestinal tract, an incompletely developed blood-brain barrier, an undeveloped detoxification system, and high food consumption relative to body mass. Heavy metals are contaminants which can accumulate in infant foods through food chain, during food processing or leakage from packaging materials.

Rapid and unorganized urban and industrial developments have contributed to the elevated levels of heavy metals in urban environments (Yeshiwas and Tadele, 2017).

The high content of toxic elements in baby foods may be due to the food additives or other ingredients, especially rice and vegetable (Warde-Farley *et al.*, 2010). Among heavy metals Hg, Pb and Cd need special attention due to their high toxic effect (Anyimah-ackah *et al.*, 2019). The USA congress report found that out of ten infant formula foods nine of them were highly contaminated with toxic heavy metals (Mcnicholas *et al.*, 2022).

Powdered infant formula milk manufacturing is one of the most difficult and high risk branches of food manufacturing requiring great care and attention at all stages. This is due to the sensitivity of the product and the complexity and cost of getting it right first time all the time (Shamim and Naz, 2006).

According to test results obtained by United State Economic and Consumer Policy Committee, commercial baby foods are tainted with significant levels of toxic heavy metals lead, cadmium, and mercury. According to this study, out of 168 studied formula foods 95% of formula foods heavy metals concentration was above maximum permissible limit of FAO (Report and Policy, 2021).

Use of infant formula has been cited for many increased health risks (Aytenfsu *et al.*, 2016)**.** Heavy metals get in to infants body mostly through ingestion thus; a high concentration couldn’t excrete from infants' body and accumulated in various organs, results in accumulation and causing critical disorders such as infertility, neurological and autism disorder, even death (Özbolat and Tuli, 2016). In 11 villages in Northern India formula feeding associated with higher morbidity and mortality compared to who were breastfed by their mother (Patel *et al.*, 2010).

Infant formula foods are the main source of heavy metals intake by this population due to contamination of raw materials used and during food processing (Zand *et al.*, 2012). The infant formula products contain many new and novel ingredients, and the legacy test methods are not always sufficient to measure nutrients accurately (Laboratories and Council, 2016). An infant's exposure to cadmium from soya infant formula is about 20 times higher than the amount usually found in breast milk (Palminger Hallén and Oskarsson, 1996).

There is a rising concern about the quality and safety of foods because contaminant sources of ingredients and adulteration of food for economic reasons necessitate the need for accurate, sensitive and precise analytical procedures for the presence of (Cd, Hg and Pb) toxic metals and are reported to be exceptionally toxic (Maslin and Venter, 2017). Studies in developing countries indicated that majority of infants in resource-rich settings consume infant formula in the first year of life, even where breastfeeding initiation rates are high (Setegn *et al.*, 2012)

In Ethiopia, study in imported powdered formula was conducted (Moges, 2014). Other study conducted (Mihretu, 2018) in imported powder milk products from local market but, both of them didn’t considered local powder formula products. The present study couldn’t found studies carried out in determining the levels of heavy metals in domestic products of powdered infant formula in Ethiopia, especially at the current study area. Usage of infant formulae in Fiche town is common especially, among government employee. Therefore, this study aims to determining Cd, Pb and Hg concentration in powdered infant formula and compare imported and domestic products.

1.3 Research questions

- ❖ What is the concentration of heavy metals in different types of infant formula brands?
- ❖ Is there a difference of heavy metal concentration between a compound of cow and plant and plant based formula products as well as between domestic products and imported products of infant formula brands?

1. 4 Significance of the study

A study conducted by international breast feeding journal in 2015 shows that, if all babies breastfed according to WHO recommends, over 800, 000 infant deaths would be avoided each year. BMS harm intellectual development of the baby to such an extent that it is possible to detect the impact on GDP for bottle-fed population (Maslin nd Venter, 2017).

Appropriate good manufacturing practice and fulfillment of HACCP system were required for the maximum safety manufacture (Barlowska *et al.* 2011).

At global level, high rates of breastfeeding were a cornerstone of infant and maternal nutrition programs. However, breastfeeding outcomes may be undermined if policies in donor countries and non-governmental organizations are dominated by 'mass production and market based approaches' to aid or trade (Afrasa and Gebretsadik, 2018).

Generally; this study might be used for:

- ✓ Health policy makers and implementers at different level to set appropriate standards
- ✓ The whole community to understanding the levels of cadmium, lead and mercury present in different powdered infant formula brands
- ✓ Powdered infant formula feeders such as infants that their mothers were HIV/AIDS carriers, loss their mothers by disasters and unable to feed human breast milk due to psychological disorder
- ✓ Used as a baseline for other researchers

CHAPTER TWO

2. LITRATURE REVIEW

2.1 General overview of infant formula food productio

Global milk production is dominated by five animal species: dairy cattle, buffalo, goats, sheep, and camels. According to FAO Statistical Databases (2010), the total world milk production accounted for 83.3% was cow milk, 13% buffalo milk, 2.2% goat milk, 1.3% sheep milk, and 0.2% was camel milk. The major cow milk producers worldwide are the European Union. The most important animals in world milk production are cattle. Cow milk is the most universal raw material for processing, which results in the broadest spectrum of manufactured products (Barłowska and Litwi., 2011).

Cow's milk-based baby formulas for infants up to six months of age are called stage 1 or starter formulas. From six months, parents choose stage 2 or follow-on formula. For babies under 12 months of age, cow's milk-based baby formula is recommended over formulas made from soybeans, goat's milk or low-lactose or lactose-free formula. But babies who can't have cow's milk-based formula might need special formula. Some babies can't have dairy-based products because of allergies or intolerances. Or sometimes parents might not want to use regular baby formula because of cultural, religious or other beliefs. In such like conditions, plant based infant formulas are needed (Ababa, 2018).

Heavy metals presence in infant food may be referred to many from contamination of original milk, that attributable to consumption of dairy animals to contaminated feed and water or exposure to massive environmental pollution, species diversity also during manufacture and packaging (Ibrahim, Saad and Hafiz., 2020).

The high content of heavy metals (Lead, Mercury and Cadmium), in some baby foods may be due to the food additives or other ingredients, especially rice and vegetable (Warde-Farley *et al.*, 2010). Toxic elements could bio-accumulate in vital organs even in low concentrations as due to renal immaturity of children (<12 months); hinders their elimination.

The infant mortality rate is a key population health indicator. A high infant mortality rate can reflect poor quality of care or lack of access to care. The sustainable development goal (SDG) target for child mortality aims to end, by 2030, preventable deaths of new born and children under 5 years of age with all countries aiming to reduce neonatal mortality to at least as low as 12 deaths per 1000 live births and under five mortality to at least as low as 25 deaths per 1000 live births (WHO, 2016).

Infant mortality contributed to more than 75% of all under five deaths globally. To reduce infant deaths, massive investment has been done including an access to improved basic health care, under five nutrition, personal hygiene and environmental sanitation and uptakes of breast feeding (Wardlaw *et al.*, 2014) .

The quality and safety regulations of infant formulas play a key role in global public health because large numbers of infants worldwide consume these products sometime during their first year of life either as sole source of food or through periodic use. Therefore, standards and regulations for the safety of infants are much stricter than regulations for other food products (Shamir and Ashwell, 2012).

Global population growth result in the growth of global infant formula consumption. Infant formula should be highly regulated by government agencies and all manufacturers must ensure adequate content to attend national and international quality criteria (Martin *et al.*, 2016). Infant formulas are found in the form of powder, which must be reconstituted with water for consumption, concentrated liquid which must also be mixed with water and ready for consumption (Yates *et al.*, 2016). These products must have nutritional security and adequate formula to support the growth and development of infants. Contaminants, toxins, and residues shall fulfill with the maximum levels and maximum residue limits established for these products (Ambrus and Yang, 2016).

Sub-Saharan Africa is the region with the highest under-five mortality and Ethiopia is one of five countries which contributed to more than half of the global under-five deaths in 2018. Ethiopia is one of 10 countries accounting for more than half of global neonatal deaths with 80,000 deaths each year. Complete, timely, and accurate data are essential to understand the causes and underlying risk factors of maternal and child morbidity and mortality. These data and the resulting knowledge generated are subsequently key to the development of effective interventions to improve health outcomes (Mesfin, 2021). BMS requirement may be temporary or longer term.

Temporary BMS indications: - include: during re-lactation; transition from mixed feeding to exclusive breastfeeding; short-term separation of infant and mother; short-term waiting period until donor human milk is available.

Long term BMS indications:- include: infant not breastfed pre-crisis; mother not wishing or unable to re-lactate, infant established on replacement feeding in the context of HIV, orphaned infant, infant whose mother is absent long-term, specific infant or maternal medical conditions, very ill mother, infant rejected by mother (Gribble, Hausman and Gribble, 2012).

2.2 Commercial prepared formula foods

The popularity of commercial baby food in recent decades has a major impact on what we assume babies need. Many major food companies have jumped on this profitable bandwagon, but it is important to not take for granted what parents are feeding their child in his or her earliest months and years. Manufacturers spend millions marketing infant formula, baby food and beverages for toddlers, assuring parents their products are nutritious and healthy. But many commercial baby foods aren't as healthy as we are led to believe. Many ingredients in commercial baby food are not only ultra-processed, but also contain contaminants with links to serious health problems. Infants and young children are particularly vulnerable to food contaminant chemicals because their immune systems are not developed enough to fight. That's why extra care should be taken when handling and preparing their food items (Salah and Mudawi, 2019).

2.3 Heavy metals

Heavy metals are generally referred as those metals which possess a specific density of more than 5 g/cm3 and adversely affect the environment and living organisms. Heavy metals include mercury (Hg), Lead (Pb), Chromium (Cr), Cadmium (Cd), Barium (Ba), Aluminum (Al) and Copper (Cu). There have been increasing environmental and public health concerns resulting from heavy metals contamination in recent times. Human exposure to these metals is also on the rise due to their growing applications in manufacturing, agriculture, homes and technology (Tepanosyan *et al.*, 2018). Though heavy metals exist naturally, human activities such as mining smelting, farming and manufacturing significantly contribute to their release into the environment (Patlolla *et al.*, 2012).

Contamination may also occur due to their corrosion, atmospheric deposition, and erosion of their ions, leaching, sediment re-surfacing (coating) and the evaporation of the metals from a contaminated water source into the soil and groundwater. Natural events such as volcanic explosions and weathering also play a role in its environmental contamination. For their high toxicity levels, mercury, lead, chromium, cadmium and barium pose an urgent challenge to public health. It is worth-knowing that these metals are capable of damaging the various human organs even at lower exposure degrees. This is either classified as confirmed or probable human carcinogens where their poisoning potency depends on diverse mechanics. It is noted that, each of them has exclusive characteristics and chemical properties that define its harmfulness. Some of these elements lack adequate explanation to their toxicological mechanisms and actions. There is therefore the need for a concise body of knowledge that can guide the implementation of countermeasures to mitigate health complications in children arising from heavy metals exposures (Balmer, 2018).

A comprehensive analysis of published data indicates that heavy metals such as arsenic cadmium, chromium, lead, and mercury, occur naturally. However, anthropogenic activities contribute significantly to environmental contamination. These metals are systemic toxicants known to induce adverse health effects in humans, including cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic and immunologic disorders, and various types of cancer. The main pathways of exposure are ingestion and inhalation. The severity of adverse health effects is related to the type of heavy metal and its chemical form, and is also time and dose-dependent.

Although the acute and chronic effects are known for some metals, little is known about the health impact of mixtures of toxic elements. Recent reports have pointed out that these toxic elements may interfere metabolically with nutritionally essential metals such as iron, calcium, copper, and zinc. However, the literature is scarce regarding the combined toxicity of heavy metals. Simultaneous exposure to multiple heavy metals may produce a toxic effect that is either additive, antagonistic or synergistic (Tchounwou *et al.*, 2012).

2.4 Toxicity of heavy metals

The toxicity of these metals has two main aspects: (a) the fact that they have no known metabolic function, but when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs; (b) the potential, particularly of the so-called heavy metals mercury, cadmium and lead accumulate in biological tissues, a process known as bioaccumulation. This occurs because these metals, once taken up into the body, is stored in particular organs, for example the liver or the kidney, and is excreted at a slow rate compared with its uptake. This process of bioaccumulation of metals occurs in all animals, such as fish and cattle as well as humans. It is therefore necessary to control the levels of these toxic metals in foodstuffs in order to protect human health (Series, 2009).

2.4.1 Lead

Lead poisoning is one of the most common and best-recognized childhood diseases of toxic environmental origin. Children around the world today are at risk of exposure to lead from multiple sources. Lead poisoning accounts for about 0.6% of the global burden of disease (WHO, 2009).

Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations. Lead has many different industrial, agricultural and domestic applications. It is currently used in the production of lead-acid batteries, ammunitions, metal products (solder and pipes), and devices to shield X-rays. An estimated 1.52 million metric tons of lead were used for various industrial applications in the United Stated in 2004. Of that amount, lead acid batteries production accounted for 83%, and the remaining usage covered a range of products such as ammunitions (3.5%), oxides for paint, glass, pigments and chemicals (2.6%), and sheet lead (1.7%) (Report, 2006).

In recent years, the industrial use of lead has been significantly reduced from paints and ceramic products, blocking, and pipe solder. Despite this progress, it has been reported that among 16.4 million United States homes with more than one child younger than 6 years per household, 25% of homes still had significant amounts of lead-contaminated deteriorated paint, dust, or adjacent bare soil (Jacobs *et al.,* 2002).

Lead in dust and soil often recontaminates cleaned houses and contributes to elevating blood lead concentrations in children who play on bare, contaminated soil. Today, the largest source of lead poisoning in children comes from dust and chips from deteriorating lead paint on interior surfaces. Children who live in homes with deteriorating lead paint can achieve blood lead concentrations of 20µg/dL or greater (Charney, Sayre and Coulter, 1980).

2.4.2 Cadmium

Cadmium from various manufacturing processes can contaminate natural resources. Typically found as a byproduct of lead, copper, and zinc extraction, it is also used to manufacture rechargeable batteries. Other uses of cadmium include the production of pigments, coatings, plastic stabilizers, and solar cells (Zhang *et al.*, 2016). When cadmium is released via this production and manufacturing processes, it can contaminate the surrounding environments. It can be carried through the atmosphere on the breathable-sized particles, and deposited in soil and water sources (Senarath *et al.*, 2010). In the soil, cadmium tends to bind to organic matter, allowing its uptake by the surrounding plant life. This becomes concerning when this uptake occurs in plants that are typically consumed such as grains, root crops, and leafy vegetables. As for water sources, cadmium only migrates to water when it is in its soluble form. Consumption of products from these contaminated sources may lead to adverse health effects that can have lifelong implications (Luo *et al.*, 2012).

Childhood exposures to cadmium may contribute to illnesses of the digestive and respiratory systems. Ingestions of a high dosage of cadmium may result in irritation of the digestive system, leading to nausea, vomiting, diarrhea, and even death in severe cases (Valko *et al.*, 2006).

Acute inhalation at high concentrations may lead to damage of the lungs and nasal cavities. When ingested or inhaled over a longer time period, cadmium begins to bio accumulate in the kidneys. For infants, this is especially problematic because there is evidence that cadmium absorption in the kidneys occurs at a higher rate in this age group when compared to adults. Long term exposures will eventually lead to impairment in the kidney’s ability to metabolize vitamin D (Luo *et al.*, 2012). This will lead to a loss in bone mass, which can eventually progress to osteoporosis.

Cadmium is considered a human carcinogen, long term exposures can also lead to cancer of the lungs, kidneys, or prostate glands (Balmer, 2018). Due to its tendency to bio accumulate and the harmful effects it can lead to, cadmium exposures (especially from food sources) should be mitigated starting from a young age. Cadmium (Cd) is cumulative toxic agent with biological half-life of several years and their burden of the body increase with age (Husien, 2020). In addition, Cd is highly teratogen and carcinogenic as well as results in pulmonary impairments.

2.4.3 Mercury

Mercury (Hg) is a toxic metal naturally found in the environment in inorganic, organic and elemental (Hg°) forms. Elemental mercury is used in chlorine gas production and in caustic soda for industrial use, as well as electrical equipment, lamps, thermometers, pressure gauges, barometers, and dental amalgams. Inorganic mercury occurs as salts of its divalent and monovalent cationic forms, mainly chlorine and sulfur (Poulin and Gibb, 2008). Mercury (Hg), the most toxic of all mercurial is methyl mercury as the irreversible CNS damage have been responsible for kidney impairments and postnatal poisoning via breast milk with infant symptoms are similar to those in adult (Grandjean, Jorgensen and Weihe, 1994).

2.5 The danger of toxic heavy metals to children's health

Children's exposure to toxic heavy metals causes permanent decreases in IQ, diminished future economic productivity, and increased risk of future criminal and antisocial behavior. Babies' developing brains are exceptionally sensitive to injury caused by toxic chemicals, and several developmental processes have been shown to be highly vulnerable to chemical toxicity (Hodson, 2004).

The fact that babies are small, have other developing organ systems, and absorb more of heavy metals than adults, exacerbates their risk from exposure to heavy metals. Exposure to heavy metals at this developmental stage can lead to "untreatable and frequently permanent" brain damage, which may result in "reduced intelligence, as expressed in terms of lost IQ points, or disruption in behavior. A recent study estimates that exposure to environmental chemicals, including lead, are associated with total IQ points loss in 25.5 million children (or roughly 1.57 lost IQ points per child) more than the total IQ losses associated with preterm birth brain tumors, and traumatic brain injury combined. For every one IQ point lost, it is estimated that a child's lifetime earning capacity will be decreased by $18,000.6 Well-known vectors of child exposure to toxic heavy metals include lead paint in old housing and water pollution from landfills (Barłowska, Szwajkowska and Litwi, 2016).

The American FDA has declared that inorganic lead, cadmium, and mercury are dangerous, particularly to infants and children. They have “no established health benefit” and “lead to illness, impairment, and in high doses, death.” According to FDA, even low levels of harmful metals from individual food source can sometimes add up to a level of concern.” FDA cautions that children are at the greatest risk of harm from toxic heavy metal exposure. Currently, there is no federal standard on, or warning to parents and caregivers about these toxins (Report and Policy, 2021a).

According to test results obtained by United State Economic and Consumer Policy Committee, commercial baby foods are tainted with significant levels of toxic heavy metals lead, cadmium, and mercury. These results are multiples higher than allowed under existing regulations for other products. The FDA has set the maximum allowable levels in bottled water at 5 ppb lead, and 5 ppb cadmium, and the Environmental Protection Agency has capped the allowable level of mercury in drinking water at 2 ppb. The test results of baby foods and their ingredients eclipse those levels: including results up to 177 times the lead level, up to 69 times the cadmium level, and up to 5 times the mercury level (Report and Policy, 2021b).

Nurture baby Food Company sold a finished baby food product that contained 10 ppb mercury, and happy baby food sold that contained 9.8 ppb. A level of 10 ppb is five times more than the EPA’s 2 ppb standard for drinking water. In total, Nurture sold 56 products that contained over 2 ppb mercury. The USA congress reported that out of ten infant formula foods nine of them were highly contaminated with toxic heavy metals and all users of these products were prone to those toxic heavy metals (Mcnicholas *et al.*, 2022).

Study conducted in Nigeria among children aged below 6 months showed that the proportion of infants who were given formula feeding was 83.6%, (Agho *et al.*, 2011). Other studies conducted in India, Nepal, and Bangladesh revealed that more than half of the mothers with a child aged below 1 year were formula -feeding their babies (Senarath *et al.*, 2010).

2.6 Conceptual framework

Environmental pollution has been a major area of concern worldwide. Industrial and agricultural processes have caused an increased concentration of toxicants like heavy metals in the environment and as a result being taken up by plants or animals into their systems which cause further distribution of toxicants to the environment (Ahmad *et al.*, 2002). This types of contamination can also be attributed to the manner of manufacturing dairy products where toxic substances have the possibility to be included during the food production process (Salah and Bayoumi, 2013).

In addition, baby food producers are adding ingredients into their products to increase nutritional content of infant formulae, through this process; toxic heavy metals enter in to powdered infant formula foods. Based on this; modification was done and the following assumption was developed by factors affecting the concentration of toxic heavy metals (Cd, Pb and Hg) concentrations in powdered infant formulae brands.

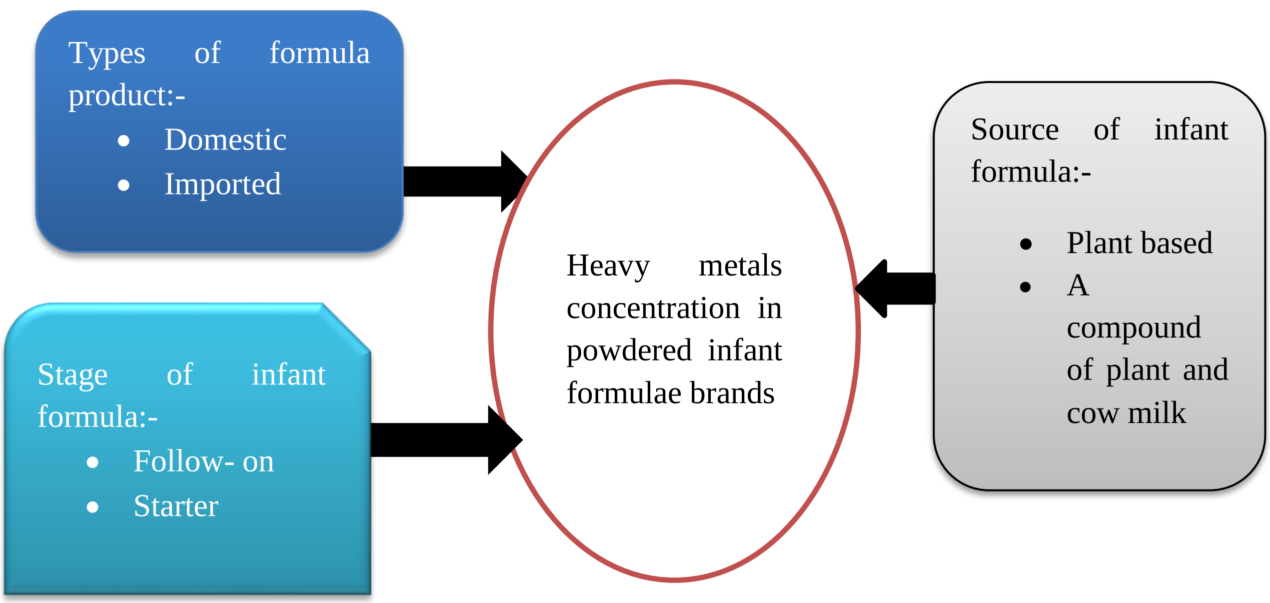

Figure 1 Conceptual framework shows interaction of variables modified from Emeritu (2002).

CHAPTER THREE

3. OBJECTIVES OF THE STUDY

3.1 General objective:

To determine selected Heavy metals concentration in powdered infant formula foods collected from local markets in Fiche town, Oromia, Ethiopia

3.2 Specific objectives:

1. To determine the levels of Pb, Hg and Cd in powdered infant formula foods
2. To compare heavy metals concentration in different powdered infant formula brands
3. To compare the levels of heavy metals concentration between domestic product and imported products of infant formula brands

CHAPTER FOUR

4. MATERIAL AND METHOD

4.1 Study area

The study was implemented in the central part of Ethiopia in Oromia Region, North Shoa Zone Fiche Town. The town located 114 kilometer away from Addis Ababa, the capital city of Ethiopia. According to 2021 North Shoa Zone Annual Health Report, the town has total population of 48,539 and total numbers of shop owners were 3484 (North Shoa Zone Trade Office Report, 2021). The numbers of pharmacies were two and the numbers of drug stores were 11. Officially known super markets were two. Therefore, the community uses infant formula from these 15 infant formula foods suppliers.

During vaccination follow up, parents brought their infants with bottle feeding to health facility especially; those whose mothers were government employee, used powdered infant formulae rather than maternal breast milk during their postnatal period.

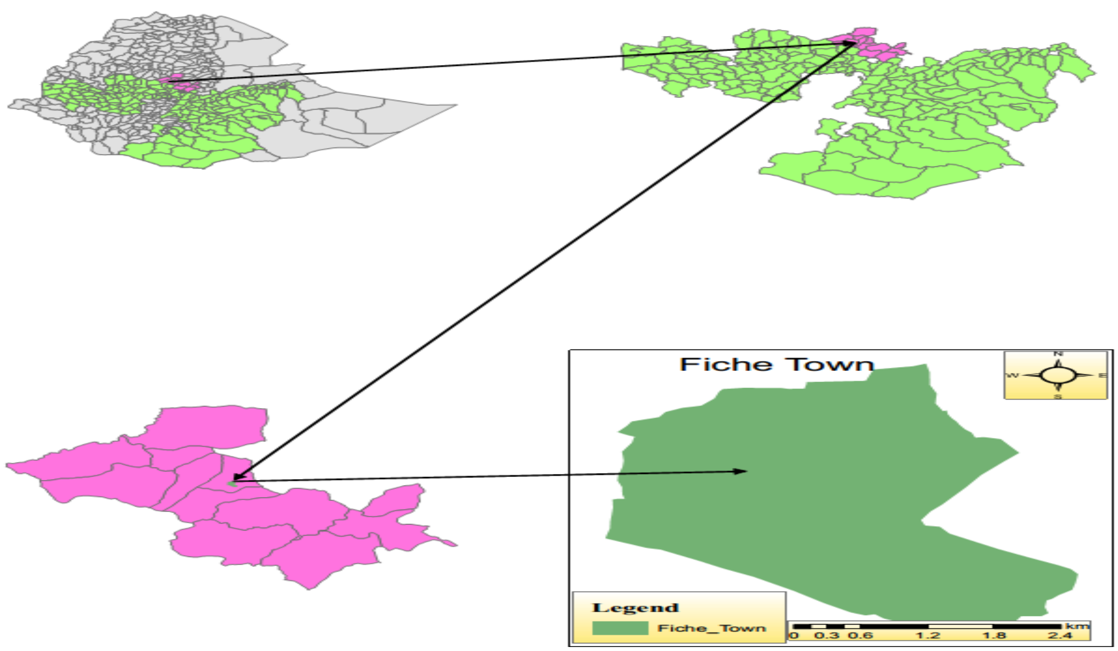

Figure 2: Location of the study area

4.2 Study design and period

Laboratory based cross sectional study design was implemented from April 30 / 2022 to June 13 / 2022 for six consecutive weeks.

4.3 Sampling and sample collection

From 15 powdered infant formula food suppliers, three of them were selected purposively due to their highest supply of powdered formula foods. During the study period, 12 types of imported and 6 types of domestic, a total of 18 powdered formulae were available in three powdered formula food suppliers. From a total of 18 powdered formula foods, nine ISO certified, (three domestic and six imported products) were selected by simple random sampling technique and purchased to detect concentration of Cd, Hg and Pb**.** From nine powdered formula foods, 27 samples (2.5 gram for each) were taken and samples were labeled for ease of identification. Then, transported to Addis Ababa, Ethiopian Food and Drug control Authority food laboratory for sample preparation and digestion process. Full information of collected samples were in table 1 as follows

Table 1 Powdered infant formula brands collected from local markets

Formula brand name	Sample code	Formula Product type	Source of infant formula	Age Classification of infant formula	Package	Labeled as ISO Certified
Aptomil	PIF –A	Imported	Cow + plant	Starter	Tin	Certified
Bebelac	PIF-B	Imported	Cow + plant	Follow on	Tin	Certified
Cerifam	PIF-C	Domestic	Plant	Follow on	Card board	Certified
Faffa	PIF-FA	Domestic	Cow + plant	Follow on	Card board	Certified
Francelait	PIF-F	Imported	Plant	Follow on	Tin	Certified
Liptomil	PIF-L	Imported	Cow + plant	Follow on	Tin	Certified
Mother's choice	PIF-M	Domestic	Cow + plant	Follow on	Card board	Certified
NAN	PIF-N	Imported	Cow + plant	Starter	Tin	Certified
S 26	PIF-S	Imported	Cow + plant	Starter	Tin	Certified

4.4 Inclusion and exclusion criteria

Infant formula which was powdered products, both plant and cow based, both imported and domestic products, well labeled and packed, formula starter and formula follow-on was considered for this study. Those formula food products that has been expired, without labeled and not packed (tired) were excluded.

4. 5 Study variables:-

- ❖ Independent variables:-
- ➢ Source of infant formula products (plant + cow milk and plant)
- ➢ Types of infant formula products (domestic and imported)
- ➢ Classification of formula brand by age (stage 1 and stage 2)
- ❖ Dependent variables:-
- ➢ Heavy metal (Cd, Hg, Pb) concentration in infant's formula food brands

4.6 Term definitions

Ethiopian Food, Medicine and Health Care Administration and Control Authority Directive on Infant Formula and Follow-up Formula March 2006 use the following definitions (EFMHAC, 2016)

- ❖ Infants: children under the age of twelve months
- ❖ Infant formulae: foodstuffs intended for particular nutritional use by infants during the first year of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding.
- ❖ Babies: cover subcategories of newborns, infants, and toddlers, or 0 months to 2 years.
- ❖ Toddlers: range from one year to about three years.
- ❖ Brand:-A category of products that are all made by a particular company and all have a particular name.
- ❖ Breast-milk substitution: - any food being marketed or otherwise presented as a partial or total replacement for human breast milk
- ❖ Complementary food:- any food formulated industrially as suitable or represented as suitable as an addition to breast milk, infant formula or follow up formula for infants
- ❖ Follow-on formula:- a milk or milk-like product of animal or vegetable origin formulated industrially in accordance with the appropriate standards for follow-up

formula and marketed or otherwise represented as suitable for feeding infants and young children from 6 month up to 2 years of age;

- ❖ Additives:- a substance, other than a typical ingredient, which is in accordance with appropriate standard or appropriately evaluated for safety and quality and is included in a product for a specific reason including colorant, stabilizer, sweetener, flavor ant, emulsifier, and preservative.
- ❖ Appropriate standard means a product standard set in the Ethiopian standard, if any or CODEX Alimentarious or other international standards.

4.7 Instrument used

The concentration of Hg was analyzed by Hydride generated Atomic Absorption spectrophotometry (Analytic Jena, HGAAS nov AA 400P; German)) which has high detection capability in ppb of metals concentration (Haeri *et al.* 2014).To determine concentration of Cd and Pb, Micro Plasma Atomic Emission Spectrophotometry, (Agilent Technology 4210 MP-AES) was used. MP-AES is a new instrumental technique for elemental determinations using atomic emission with microwave plasma excitation source using nitrogen-based plasma: runs on air. MP-AES uses Agilent's patented microwave waveguide technology. It uses nitrogen as the plasma gas & gives energetic plasma with a conventional torch. MP-AES has high sensitivity with detection limits down to sub ppb levels and is faster than conventional flame Atomic Absorption Spectrophotometry (FAAS) for a typical multi-element analysis, low running cost, safe, easy to use, better detection limits and greater linear dynamic range. Balance machine, oven, sample digester, volumetric flask, funnel, filter paper, digestion flask and distilled water were used during the process (Elmer, 1996).

Figure 3: MP-AES Instrument (Agilent Technologies, 2016)

4.8 Chemicals and reagents used for heavy metal determination

Reagents that were used for analysis of the selected metals were all analytical grade. 70 % HNO_3 and 5% H_2O_2 were used for the digestion of formula food samples, distilled water was used to wash apparatuses and for the dilution of the digested samples and standard solutions. The standard stock solutions containing 1000 mg/L in 10ml HNO_3 was taken to prepare intermediate standard solution of concentration 10 mg/L, where working calibration standard solutions were prepare from it (Duan *et al.*, 2003).

4.9 Wet digestion and preparation of powdered infant formulae samples

The wet digestion procedure was tested by varying reagent volume, digestion time and digestion temperature to develop a procedure that consume less reagent volume, clear solution, that requires minimum digestion time and minimum digestion temperature was applied. 2.5 g of dried powder sample was weighed on a digital analytical balance (Nazaret et.al, 2020). Then, samples were transferred into the digestion flask. 10 ml of HNO_3 (70%) was added to the sample. Then distilled water was added to the flask. The flasks were shacked properly to homogenize the solution. The powder formula digestion was done according to the method of Nigussie and Wendimu (2011).

The sample blank was prepared as same as the sample preparation except taking the sample on it. The flasks were left in the fume hood to digest the samples in 60 C^o for 24 hours. After 24 hour digestion, the flasks were placed on the heating mantle of the electric oven at 90 C^o for 60 min. Other volumetric flasks were prepared by washing with distilled water for filtration preparation. The funnels were washed with distilled water and placed on every flask and the filter paper was folded and placed on the flasks. 30ml of distilled water was added into the sample flasks to dilute the sample content. The flasks were shacked and rotated to mix the content properly. The diluted digested samples were filtered into 100 ml volumetric flasks. Another 30 ml of distilled water was added and the flasks were left to filter again.

The entire filtered samples in the flasks were labeled properly to identify one from the other. The sample flask was washed with distilled water and filtered into the flask to ensure that no element is left in the digestion flask. To ensure that no element is left on the filter paper, the filter paper was rinsed with distilled water. In the same way, the blank flasks were washed to ensure the complete transfer. After filtration, each of the funnels from the flasks was removed. The solution in the volumetric flask was checked and distilled water was added. The content was homogenized and a portion was taken for chemical analysis. Sample digestion and preparation was conducted at Addis Ababa, Ethiopian food and Drug control Authority food Laboratory. After labeling, 18 samples analysis for Cd and Pb was implemented at this site but due to lack of reagent, 9 samples for Hg analysis conducted in Ethiopian construction design and supervision Work Corporation water quality Laboratory, Addis Ababa, Ethiopia (Elmer, 1996)

4.10 Standard solution preparation

Every metal standard solution was prepared for calibration of the instrument for each element being determined on the same day as the analyses was perform due to possible deterioration of standard solution with time. All samples were prepared from chemicals of analytical grade with distilled water. 1 gram of metal Cadmium, mercury and Lead was dissolve in 2% HNO_3 solution and stock solution of 1000 mg/l of Cd, Hg and Pb, was prepared (Everaldo, 2019). Then 100 ml of working standards of each metal was prepared from these stock using micropipettes in 5ml of Nitric acid. Reagent blank was prepared in the same manners of sample preparation without sample to avoid reagents contamination.

4.11 Method validation procedures

Freshly prepared standard stock solutions was serially diluted and used to obtain calibration curves with linearity values. The correlation coefficient values were good indicator of the linearity for HGAAS and MP-AES instrument for precision and accuracy of results. Further, using recovery tests where samples with known amounts of standards prior to measurements was performed to confirm the accuracy of the instruments (Duan *et al.*, 2003).

4.12 Determination of calibration curve for heavy metals determination

Working standard solution for each heavy metal was prepared to draw calibration curve for determination of heavy metals from infant formula brand samples. The calibration curve and coefficient of determination (R^2) of heavy metals were prepared from the absorbance versus concentration of standard solution. The calibration curve equation was used to check the accuracy of the instrument, the concentration of heavy metals in the sample whither in the given range or not, and to cross check the concentration measured by the instruments.

4.13 Powdered infant formula sample analysis

From wet digestion result, determination of concentration of Pb and Cd were carried out with MP-AES while Hg carried out with HGAAS Spectrometer with their corresponding wave Length Cd 228.802 nm, Pb 405.781 nm and Hg 253.701 nm and method detection limit of (Cd 0.011, Hg 0.002 and Pb 0.03) ppm according (Elmer, 1996). Before analysis of the sample, instruments optimized using standard solutions of the metals to give maximum signal strength by adjusting the parameters such as wavelength, nebulizer flow, pump speed and lamp current for each element. Calibration curves were drawn using linear regression analysis of the concentrations of the standard solutions versus emission values. Sequence of working standard solutions was prepared by diluting the intermediate standard solution with distilled water. The same analytical procedure was employed for the determination of the elements in digested blank solutions and for the spiked samples. Triplicated measurements were engaged for each sample and the mean was plotted.

Concentration of the metal existing in the powder formulae was determined by reading their emission and comparing it with the corresponding standard calibration curve. Correlation coefficient between concentrations and emission of the metals were done (Agilent Technologies, 2016). The correlation coefficients of calibration curves for Cd (0.99976), Hg (0.9962) and Pb (0.99998). All heavy metals showed linear relations of the instrumental. The correlation coefficient > 0.995 showed that there is strong linear relationship between concentrations and emission intensity.

The solutions of heavy metals were aspirated into the plasma of the MP-AES instrument and the emission value of the metals was recorded. Both the proposed analytical methods HGAAS and MP-AS were perceived to exhibit good linearity, confirming its reliability for determining levels of the heavy metals in powdered formulae. Concentrations of each metal were determined from the emission versus concentration plotted calibration curves. For each 27 samples, triplicated determinations were performed and average results were reported.

4.14 Data quality control

All reagents and chemicals used were analytical grade. Thoroughly glassware was cleaned with hydrogen per-oxide and rinsed numerous times using distilled water before use. To assure and control the quality of data during sample analysis, series of events like cleaning of laboratory glassware and calibration of field equipment were performed before and just after measurements to ensure validity and accuracy of data. The reproducibility and accuracy of the analytical procedure were done by spiking and homogenizing three replicates of each samples. The triplicate of each sample was picked with three diverse concentrations of the metal of interest.

Analysis was done following standard procedures and each sample was triplicated. A thousand milligrams per liter standard solutions of each metal were prepared and used for calibration purposes. The analytical method accuracy was evaluated by drawing calibration curves and the simultaneous performance of analytical blanks. To assess the precision and accuracy of results, triplicated analysis was done and standard solution and blank solution was used for calibration and R^2 value > 0.995 (PREKIN, 2015).

4.15 Data analysis

Statistical package for social science (SPSS) version 26 and micro soft excel 2010 were used for data analysis. The data were presented in tables and descriptive statistics for all data were expressed as mean ± SD to evaluate the significance differences between concentration levels of each metal in nine types of powdered infant formula samples. The data set was tested for homogeneity of variance and for normal distribution. As the data were being normally distributed, accordingly; one way analysis of variance (ANOVA) parametric test was implemented to test the possible significance ($p < 0.05$) among mean values of heavy metals concentrations.

The heavy metals concentration result obtained from analysis of HGAAS and MP-AES in μg/L was changed to mg/kg by calculating using the following equation:

$\mathbf{C}(\text{mg/kg}) = \frac{\text{C AAS Reading (μg/L)}}{\text{sample weight(g)}} \times \text{V (ml)}$ (Oloyede,2008) (1) where:

C = final heavy metal concentration in (mg/kg)

C AAS Reading = Concentration of heavy metal obtained from AAS Reading

V (ml) = Final volume of digested milk sample solution (ml)

Sample weight = weight of measured sample during digestion (g)

By calculating using the above equation, the concentration of the studied heavy metals in each powdered formula sample was summarized in table 2. Finally, the result of the study was compared with maximum permissible limit of WHO and FAO and with other standards (Ibrahim and Hafiz, 2020).

4.16 Ethical consideration

Ethical approval and clearance obtained from Jimma University, institute of health science ethical committee. Then, Letters of support were obtained from the Environmental Health Science and Technology Department and permission also got from North Shoa Zone Health Office. Moreover, an informed verbal consent was received from each powdered infant formula food seller.

4.17 Result dissemination

The finding of this study submitted to Jimma University, Institute of Health Sciences, Faculty of Public Health and Department of Environmental Health Science and Technology. The result was presented during defense in partial fulfillment for the requirement of Masters of Science in Environmental Health. Finally, written document was submitted to Jimma University, Department of Environmental Health Sciences and Technology and to North Shoa Zone Health Office for further action and the benefits of the community to alleviate the identified gaps. Then, the results of this study finding also publish in peer reviewed journals.

CHAPTRE FIVE

5. RESULT

5.1 Determination of heavy metals in infant formula brands

In the present study, the concentration of three heavy metals (Cd, Hg and Pb) in nine different types of powdered infant formula brand samples was investigated by collecting from Fiche town, Ethiopia. As shown from table 2, in the present study, concentration of Cd, Pb and Hg was detected in all infant formula brand samples and the highest concentration of Cd detected in NAN infant formula brand samples and the lowest concentration of Cd among nine formula brand samples detected in Cerifam. Cd concentration in Cerifam (0.07 ± 0.0451) < Faffa (0.11 ± 0.03) < Bebelac (0.15 ± 0.05) < Mother's choice (0.19 ± 0.03696) < Liptomil (0. 29 ± 0 .03) < Aptomil (0.42 ± 0.01) < S 26 (0.51 ± 0.11) < Francelait (0.55 ± 0. 02) < NAN (0.6 ± 0.03).

The highest concentration of Hg was detected in Mother's choice infant formula brand samples while the lowest concentration of Hg was detected in Aptomil infant formula brand samples. The concentration of Hg in Aptomil (0.0147 ± 0.0031) < NAN (0.0183 ± 0.0015) < Francelait (0.0193 ± 0.0012) < Faffa (0.029 ± 0.0012) < Cerifam (0.032 ± 0.002) < Liptomil (0.0353 ± 0.0042) < S 26 (0.062 ± 0) < Bebelac (0.069 ± 0.0012) < Mother's choice (0.0813 ± 0.0031).

In the current study, the highest concentration of Pb was detected in Mother's choice infant formula brand samples while the lowest concentration of Pb was detected in S 26 infant formula brand sample. Pb metal concentration in S 26 (0.0666 ± 0.0115) < Aptomil (0.0667 ± 0.0116) = Liptomil (0.0667 ± 0.0116) < Francelait (0.20 ± 0) < NAN (0.2 ± 0.022) < Cerifam (0.2 ± 0.0346) < Bebelac (0.267 ± 0.0306) < Faffa (0.4666 ± 0.4163) < Mother's choice (0.533 ± 0.1155).

The concentration of all the studied heavy metals in each powdered formula food brand sample was summarized in table 2 as follows;

Table 2 Concentration of heavy metals in nine different types of infant formula brand samples expressed as mg/kg and with their maximum permissible limit standards

Name of formula Brand	Infant formula Product	Source of infant formula	Formula food classification by age	Conc. of Cd (mg/kg)	Conc. of Hg (mg/kg)	Conc. of Pb (mg/kg)
				Mean ± SD	Mean ± SD	Mean ± SD
Aptomil	Imported	Cow + plant	Starter	0.42 ± 0.01	0.0147 ± 0.0031	0.0667 ± 0.0116
Bebelac	Imported	Cow + plant	Follow on	0.15 ± 0.05	0.069 ± 0.0012	0.267 ± 0.0306
Cerifam	Domestic	Plant	Follow on	0.07 ± 0.0451	0.032 ± 0.002	0.2 ± 0.0346
Faffa	Domestic	Cow + plant	Follow on	0.11 ± 0.03	0.029 ± 0.0012	0.4666 ± 0.4163
Francela it	Imported	Plant	Follow on	0.55 ± 0. 02	0.0193 ± 0.0012	0.20 ± 0
Liptomil	Imported	Cow + plant	Follow on	0. 29 ± 0 .03	0.0353 ± 0.004	0.0667 ± 0.0116
Mother's choice	Domestic	Cow + plant	Follow on	0.19 ± 0.03696	0.0813±0.0031	0.533 ± 0.1155
NAN	Imported	Cow + plant	Starter	0.60 ± 0.03	.0183 ± 0.0015	0.2 ± 0.022
S 26	Imported	Cow + plant	Starter	0.51 ± 0.11	0.062 ± 0	0.0666 ± 0.0115
Overall (mean ± SD)				0.32 ± 0.042	0.040037 ± 0.0019	0.2296 ± 0.073
WHO& FAO standard 2021				0.05 mg/kg	-	0.01 mg/kg
US EPA 2019 (for drinking water) standard				-	0.002 mg/kg	-
European Union 2019 standard				0.02 mg/kg	-	0.001mg/kg(2021)

*SD = Standard deviation, Conc. = Concentration

5. 2 Correlation Analysis

The correlation matrix shows the relationship between heavy metals concentration of infant formulae brand samples as Cd with Pb (r = -0.526), Hg with Pb (r = 0.413), and Cd with Hg (r = -375). The computed statistical correlation results show that Hg has a moderate negative correlation with Cd (r = -0.375) and a moderately positive correlation with Pb (r = 0.413). Pb also has moderate negative correlation with Cd (r = -0.526) (Demissie and Foeniculum, 2010).

Table: 3 Correlation coefficient (r) matrixes for heavy metals concentration in powdered infant formula brand samples:

Parameters	**Pb**	**Hg**	**Cd**
Pb	1		
Hg	0.413	1	
Cd	-0.526**	-0.375	1

****Correlation is significant at 0.05 (2 tailed)**

5.3 Comparisons of heavy metals concentrations in powdered infant formula brand samples based on different circumstances:

5.3.1 Imported and domestic powdered infant formula samples

Table; 4 Comparisons of mean concentration of heavy metal in infant formula products

Types of infant formulae product	**Cd Conc.(mg/kg)**	**Hg Conc.(mg/kg**	**Pb conc. (mg/kg)**
Mean concentration for domestic products	0.1233	0.0264	0.4
SD for domestic products	0.0374	0.0021	0.19
Mean concentration for imported products	0.4183	0.0364	0.1445
SD For imported products	0.042	0.0018	0.0146

*SD = Standard deviation,

The concentration of Cd in domestic infant formula brand samples was 0.1233 ± 0.0374 while in imported infant formula brand samples was 0.4183 ± 0.042. This refers that Cd metal concentration in imported infant formula brand samples were higher than domestic infant formula brand samples. ANOVA test result shows the concentration of Cd show statistically significant difference between domestic and imported products of powdered infant formula brand samples ($p < 0.05$).

The mean concentration of Hg in imported product powdered infant formula brand samples was 0.0364 ± 0.0018 which is higher than domestic products of infant formula brand samples (0.0264 ± 0.0021). ANOVA test result shows that the concentration of Hg no statistically significant differences were observed between domestic and imported products of powdered infant formula brand samples ($p > 0.05$).

The mean concentration of Pb in domestic infant formula band samples (0.4 ± 0.19) was higher than imported infant formula brand samples concentration of Pb (0.1445 ± 0.0146). ANOVA test result of the concentration of Pb shows statistically significant difference between domestic and imported products of powdered infant formula brand samples ($p < 0.05$).

5.3.2 Heavy metals concentrations of infant formula based on classification of infant's age

Table: 5 Comparisons of starter and follow- on infant formulae samples

Age Classification of infant formula brands	**Cd concentration (mg/kg)**	**Hg concentration (mg/kg)**	**Pb concentration (mg/kg)**
Mean for starter formulae	0.361	0.032	0.111
SD for starter formulae	0.05	0.0015	0.015
Mean for follow- on	0.227	0.0443	0.289
SD for follow- on	0.035	0.0021	0.105

*SD = Standard Deviation of metal concentration

The concentration of Cd metal in stage 1 (starter) infant formula brand samples were 0.361 ± 0.05 which is greater than stage 2 (follow-on) infant formula brand samples Cd concentration (0.227 ± 0.035). Mercury metal concentration in stage 2 infant formula brand samples was 0.0443 ± 0.0021 which is greater than stage 1 infant formula brand samples mercury metal concentration (0.032 ± 0.0015).

Lead metal concentration in stage 1 infant formula brand samples was detected as 0.111 ± 0.015, which is less than Pb metal concentration detected in stage 2 infant formula brand samples (0.289 ± 0.105). ANOVA test result of the concentration of Pb show no statistically significant differences between stage 1 and stage 2 powdered infant formula brand samples at 95% confidence level ($p > 0.05$).

5. 3. 3 Comparison of heavy metal Concentration based on infant formula food sources

Table: 6 plant based and compound of cow milk and plant sources of infant formulae

Source of infant formulae	Cd conc.(mg/kg	Hg conc. (mg/kg)	Pb conc.(mg/kg)
Overall mean for plant + cow source	0.3243	0.0442	0.229
SD for plant + cow source	0.043	0.002	0.088
Overall mean for plant source	0.31	0.0257	0.2
SD for plant source	0.033	0.0016	0.0173

*SD = Standard Deviation, Conc. = Concentration

The concentration of Cd metal in plant based powdered infant formula brand samples was detected as 0.31± 0.033 which is slightly less than a compound of plant and cow based powdered infant formula brand samples which is detected as 0.3243 ± 0.043.

Mercury metal detected in plant based powdered infant formula brand sample as 0.0257 ± 0.0016 which is less than the concentration of mercury metal (0.0442 ± 0.002) detected from plant and cow based powdered infant formula brand samples. The mean concentration of Pb in plant based infant formula band samples (0.2 ± 0.0173) was slightly less than plant and cow product of infant formula brand samples concentration of Pb (0.229 ± 0.088). ANOVA test result of the concentration of Cd, Hg and Pb show not significant differences at 95% confidence level between plant sources and a compound of cow and plant sources of powdered infant formula brand samples ($p > 0.05$).

CHAPTER SIX

6. DISCUSSION

Environmental pollution has been a major area of concern worldwide. Industrial and agricultural processes have caused an increased concentration of toxicants like heavy metals in the environment and as a result being taken up by plants or animals into their systems which cause further distribution of toxicants to the food industries (Ahmad *et al.*, 2002). This types of contamination can also be attributed to the way of manufacturing dairy products where toxicants have the possibility to be included during the food production process (Salah and Bayoumi, 2013).

Naturally occurring toxic heavy metals might not the only problem causing dangerous levels of toxic heavy metals in infant foods; rather, food manufacturing producers are adding ingredients into their products, such as vitamin and mineral to increase nutritional content of infant formulae, through this process, toxic heavy metals inter in to infant formulae foods (Directory *et al.*, 2015).

The present study finding revealed that the mean concentration of Cd metal for nine infant formula brand samples was (0.32 ± 0.042) mg/kg and is above the permissible limit (0.05mg/kg) set by WHO and FAO standard (WHO and FAO, 2021).This study finding is in line with a study conducted in Egypt (A, M and EE, 2015) from 75 infant formula brand samples found that the concentration of Cd exceeds WHO and FAO standards. Other study conducted (de Castro *et al.*, 2010) referred that Cd metal concentration in powdered formulae food was in line with the present study finding (Gustin et.al, 2018).

The present study revealed that the concentration of Cd mean concentration in domestic infant formula brand samples was 0.1233 ± 0.0374 (mg/kg) while in imported infant formula brand samples was 0.4183 ± 0.042 (mg/kg). This referred that Cd mean concentration in imported infant formula brand samples was greater than domestic infant formula brand samples. The concentration of Cd metal in stage one (starter) infant formula brand samples was 0.361 ± 0.05 (mg/kg) which is greater than stage two (follow-on) infant formula brand samples (0.227 ± 0.035) mg/kg. One way ANOVA test result shows the concentration of Cd at 95% confidence interval was significantly different between imported and domestic as well as between stage one and stage two powdered infant formula brand samples ($p < 0.05$). The higher Cd concentration in stage 1 infant formulae might be due to that higher processing activity implemented to change raw materials into stage 1 to make suitable for digestion of gastrointestinal tract because infants less than six months of age have lower digestion capacity than infants greater than six months of age that can use stage 2 infant formula foods easily.

Due to scarcity of study on domestic powdered infant formula brands in Ethiopia, it is difficult to do comparison of results. But, the reason for higher concentration of Cd in imported products of powdered infant formula samples is due to that most imported infant formula products entered in Ethiopia were from developed countries that were highly industrious. Industrial products such as metal works in mines (process water), fertilizers, battery recycling plants and waste from electroplating plants release Cd metal in to the environment as a waste and this can affect the concentration of heavy metal by contaminating infant formulae food manufacturing process (Maslin and Venter, 2017). Cadmium used in the production of pigments, coatings, plastic stabilizers, and solar cells (Zhang *et al.*, 2016). When cadmium is released via this production and manufacturing processes, it can contaminate the surrounding environments. It can be carried through the atmosphere on the breathable-sized particles, and deposited in soil and water sources. In the soil, cadmium tends to bind to organic matter, allowing its uptake by the surrounding plant life and these organic matters are used as input for infant formula food manufacturing processing (Senarath *et al.*, 2010).

In terms of Cost of infant formulae, imported infant formula products are more expensive than domestic infant formula products. But, due to impact of westernization, most of our industrialized society uses imported powdered infant formulae by stigmatizing domestic infant formula products. On the other hand, this is appropriate chance for those who use domestic infant formula products hence saving their babies from higher concentration of Cd metal intake from imported powdered infant formula products indirectly.

Based on source of infant formulae, the present study revealed that the mean concentration of Cd metal in plant based powdered infant formula brand samples was detected as 0.31 ± 0.033 (mg/kg) which is slightly less than a compound of plant and cow based powdered infant formula brand samples which is detected as 0.3243 ± 0.043 (mg/kg). Plant based infant formulae exposed to cadmium only from one source but a compound of plant and cow exposed to cadmium metal from both plant and cow sources. The One way ANOVA test result also shows no significantly difference of Cd concentration was observed between plant sources and a compound of plant and cow sources ($p > 0.05$).

Studies revealed that preparation of infant formulae only from cow milk is not recommended for breast milk substitution because Cow's milk has high electrolyte and protein concentrations giving it a high renal solute load. Hence, most infant formula foods preparation was a compound of cow milk and plant source. Some babies might not able to have dairy-based infant formula products because of allergies or intolerances, or sometimes parents might not want to use regularly one types of infant formula type because of cultural, religious or other beliefs. In such conditions, plant based infant formulas products are appropriate choice (Larton, 2006).

The mean concentration of Pb in the present study for nine powdered infant formula brand samples found that (0.2296 ± 0.073) mg/kg and is above the permissible limit (0.001 mg/kg) set by European Union (Directorate of Health and Consumer Protection, 2019), WHO and FAO (2021) 0.01 mg/kg (table 2). In similar way, the study conducted from 30 infant formula samples by European community revealed that the concentration of Pb agree with the present study (Domínguez *et al.*, 2017).

A study conducted in Libya from 78 infant formula brand samples found that Pb concentration agree with the current study findings (Abdeljalil *et al.*, 2021). Other Previous study finding from three powdered infant formula brand samples found that the concentration of Lead disagree with current study finding (Admasu, 2005). The other study also conducted in Nigeria from five infant formula brand samples found that the mean concentration of lead in infant formula brand samples reported within WHO permissible limit which is different from the present study (Pplied and State, 2021)**.** This result discrepancy happens due to variation in species that make up infant formula food sources.

The concentration of lead in domestic powdered infant formula brand samples was 0.4± 0.19 mg/kg while in imported powdered infant formula brand samples was 0.1445 ± 0.0146 (mg/kg). One way ANOVA test result shows the concentration of Pb was significantly different between domestic and imported products of powdered infant formula brand samples ($p < 0.05$).

In the present study, the concentration of lead in plant based powdered infant formula samples was 0.2 ± 0.0173 (mg/kg).The variation was not too much with a compound of plant and cow milk sources of powdered infant formula brand samples (0.229 ± 0. 088) mg/kg. The concentration of Pb in stage 1 powdered infant formula brand samples was detected as 0.111 ± 0.015 (mg/kg), which is less than Pb concentration detected in stage 2 powdered infant formula brand samples (0.289 ± 0.105) mg/kg.

ANOVA test result revealed that no significant difference of Pb concentration was observed at 95% confidence interval between plant sources and a compound of cow milk and plant sources of powdered infant formulae as well as between stage 1 and stage two powdered formulae samples ($p > 0.05$). The other study conducted from three powdered formula samples revealed that the mean concentration of lead exceeded permissible limit of FAO and WHO (2021) and agree with the present study finding (Moges, 2014).

The present study concluded from one way ANOVA test result that, the variation of Pb concentration in infant formula foods was as a result of variation of powder infant formula products. Being stage 1 and stage 2 or being plant source or a compound of plant and cow milk source did not show significant difference ($p > 0.05$) in concentration of Pb but significant difference of Pb concentration in powdered infant formula was observed between imported and domestic products of powdered infant formula samples.

The higher concentration of Pb in domestic powdered infant formulae products may be due to contamination that can attributed to the manner of manufacturing dairy products where toxicants have the possibility to be come in during powdered infant formula production process.

Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations. Lead has many different industrial, agricultural and domestic applications. It is currently used in the production of lead-acid batteries, ammunitions, metal products (solder and pipes), and devices to shield X-rays (Salah and Bayoumi, 2013). The higher concentration of lead in domestic powdered infant formulae happened form poor management of industrial wastes which release lead as waste since this affect lead concentration in powder formulae production by contaminating manufacturing process.

High levels of lead exposure can seriously harm children's health and development, specially the brain and nervous system. Neurological effects from high levels of lead exposure during early childhood include learning disabilities, behavior difficulties, and retardation of physical growth, hearing problems and lowered IQ. Because lead can accumulate in the body, even low-level chronic exposure can be hazardous over time (Mensah and Obeng, 2009).

The mean concentration of Hg in nine infant formula brand samples was 0.04 ± 0.0032 (mg/kg) which is above the permissible limit (0.002 mg/kg) that set by US EPA standard (Report and Policy, 2021b).

The study conducted in Cairo (Polonorum and Ibrahim 2021) from 30 powdered infant formulas found that the concentration of Hg metal was in line with our present study finding. Other study conducted in Chicago State University revealed that Hg metal concentration from 132 infant formula brand samples also agree with the current study finding (Akonnor and Richter, 2021).

According to the present study, mercury detected in domestic infant formula was (0.0264 ± 0.0021) mg/kg and in imported powdered infant formula product was (0.0364 ± 0.0018) mg/kg, where indiscreet variation was observed. Based on source of infant formula, the present study found that Hg metal concentration was 0.04 ± 0.0016 (mg/kg) for plant source of powdered formula and 0.044 ± 0.002 (mg/kg) for cow and plant powdered formula brand samples.

Mercury metal mean concentration in stage 2 powdered infant formula brand samples was 0.0443 ± 0.0021 (mg/kg) which is greater than stage 1 powdered infant formulae mercury concentration (0.032 ± 0.0015) mg/kg. One way ANOVA test result confirmed that no significant difference of Hg concentration was observed between imported and domestic, plant source and a compound of cow and plant sources as well as between stage 1 and 2 powdered infant formula samples ($p > 0.05$). Pb and Hg concentration in stage 2 formula samples was higher than stage 1 while higher concentration of Cd was detected in stage 1 powdered infant formula brand samples.

Although the variation of Hg was insignificant, both imported and domestic powdered infant formula brand Hg concentration was above permissible limit (Table 2) and infant formula brands are tainted with unexpected concentration of Hg. When Hg concentrations accumulate in infant's vital organs, it can alter infant's health.

The toxicity of mercury depends on its chemical form. Inorganic mercury is mainly associated with renal damage, but methyl mercury crosses the placenta and blood-brain barriers to cause irreversible neuronal damage in the fetus and growing children. Study found that both organic and conventional mercury can be prone to heavy metal contamination (Hassauer *et al.*, 2012).

Generally, the variation in toxic metals contents in infant's powdered formula brand samples happened mainly due to species variation in source of product and contamination during handling and manufacturing procedures. Or the possible pollution of dairy powders with toxic metals through the equipment used, such as painting materials, inappropriate use of gasoline and qualities of pipe metals used, so, control during all steps of manufacturing is essential to improve the quality of the final product and to prevent contamination of powdered infant formulae foods (Mohamed 2015).

The USA FDA has declared that lead, cadmium, and mercury are dangerous, particularly to infants and children. They have no established health benefit and leads to illness, impairment, and in high doses, death. Therefore, even low levels of harmful metals from individual food source can sometimes add up to a level of concern. Hence cautions should be considered that infants and children are at the greatest risk of harm from toxic heavy metal exposure (Report and Policy, 2021a). The current study finding of heavy metals concentrations also can cause illness and impairment of infant's health since all studied powdered infant formula brands for cadmium, lead and mercury exceeds maximum permissible limit (table 2).

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATIONS

7. 1 Conclusions

The present study conducted in nine powdered infant formula brands by collecting from Fiche town to detect three heavy metals (Cd, Pb and Hg) concentrations. The optimized wet digestion procedure developed for powdered infant formulae were efficient for all metals. It was evaluated through the recovery experiment and a good percentage recovery was obtained for all metals identified and triplicated reading was taken from each powdered formula sample analysis.

The mean concentration of Cd and Hg in imported formula brand samples was higher than domestic products while Pb mean concentration of domestic products was higher than imported products of infant formula brand samples. On the other hand, the concentration of Cd, Hg and Pb metal in plant based powdered infant formula brand samples was slightly less than a compound of plant and cow milk based powdered infant formula brand samples.

The presence of these heavy metals in powder infant formulae might be due to uncontrolled environmental pollution which is expressed by an increased concentration of heavy metals in air, water, and soil subsequently; then these metals are taken up by plants and animals, then to take their way into formula products. This is expressed by this study finding since all nine infant formulae are contaminated with unexpected toxic metals concentration. In addition to this, heavy metals might be added to formula food during production as well as food processing activities. All three heavy metals have moderate correlation on computed Pearson correlation. The metal composition of dairy products depends not only on infant formula sources but also on types of infant formula products.

Therefore, EFDA should increase the supervision of powdered infant formulae products to avoid the risk of their adverse effect on the consumers' health. According to the present study, all studied infant formula brands are highly contaminated with unexpected levels of lead, Cadmium and mercury concentration since all three heavy metals exceeds maximum permissible limit.

7. 2 Recommendation

- **For government organs:-**
  - ✓ International organisms (FAO and WHO) with National Health policy makers cooperatively should have established more restrictive limits in heavy metal content of infant food where majority of infants all over the world received these types of products
  - ✓ The regulation of illegal toxicants in infant's foods should be a cooperative effort of FSIS, NGO's, FDA and EPA
  - ✓ Training access about good manufacturing practices for dairy producers
  - ✓ Application of HACCP measures before drying should be implemented
  - ✓ Access of routine analysis of imported formulae in national laboratories before reaching to the consumers
  - ✓ Inspire, support and promote exclusive breastfeeding around 6 months of infants age
- **For infant formulae manufacturers:-**
  - ✓ Manufacturers should voluntarily phase out toxic ingredients of formula food and limit feeding children's packaged appetizer food item
- **For powdered infant formulae consumers:-**
  - ✓ Parents should lessen heavy metals intake in their children's diet by feeding them a variety of foods with simple steps of processed items
  - ✓ Understand the role of exclusive breastfeeding around 6 months of infants age
  - ✓ Plant sources are better than a compound of plant and cow based powdered formulae sources, in conditions when exclusive breast feeding is impossible
  - ✓ Imported brands, which were more expensive than domestic infant formula brands, did not have significantly lower heavy metal content compared to domestic brands; therefore domestic infant formulas are preferred for infant when breast milk is impossible irrespective of their nutritional value
- **For researchers:-**
  - ✓ Further study to estimate the health risk level of heavy metals for infant's as well as compare nutritional level of imported and domestic powdered infant formula food products is needed.

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ANNEXES

Annex 1. Results of one way ANOVA

1.1 One way ANOVA result for types of infant formula food product

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Concentration of Cd	Between Groups	.176	1	.176	7.967	.026
	Within Groups	.155	7	.022		
	Total	.331	8			
Concentration of Hg	Between Groups	.000	1	.000	.370	.562
	Within Groups	.005	7	.001		
	Total	.005	8			
Concentration of of Pb	Between Groups	.130	1	.130	9.005	.020
	Within Groups	.101	7	.014		
	Total	.232	8			

1.2 One way ANOVA result for classification of infant formula food by age of infants

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Concentration of Cd	Between Groups	.161	1	.161	6.606	.037
	Within Groups	.170	7	.024		
	Total	.331	8			
Concentration of Hg	Between Groups	.000	1	.000	.496	.504
	Within Groups	.004	7	.001		
	Total	.005	8			
Concentration of of Pb	Between Groups	.063	1	.063	2.621	.150
	Within Groups	.169	7	.024		
	Total	.232	8			

1.3 One way AVOVA result for source of infant formula food products

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Concentration of of Pb	Between Groups	.001	1	.001	.030	.867
	Within Groups	.231	7	.033		
	Total	.232	8			
Concentration of Hg	Between Groups	.000	1	.000	.108	.752
	Within Groups	.005	7	.001		
	Total	.005	8			
Concentration of Cd	Between Groups	.071	1	.071	1.912	.209
	Within Groups	.260	7	.037		
	Total	.331	8			

Annex 2. Standard procedures for sample preparation and digestion

Sample preparation by wet digestion method for Analysis of heavy metal According to ('Analytical Methods for Atomic Absorption Spectroscopy', 1996) procedure:-

Instruments used were; Balance machine, oven, sample digester, volumetric flask, funnel, filter paper, digestion flask and distilled water

❖ **Precautions considered;**

- All glass wares were cleaned properly to avoid contamination
- Avoid heating test of solution to prevent bad explosion of chemicals
- Activities were done in clean and dust free space to avoid contamination

♠ **The procedure has four consecutive steps**:-

❖ Step 1: Flask preparation:-

- Clean flask with water to avoid contamination
- Add H_2O_2 and rinse flask
- Sock flask with 2% HNO_3
- Rinse flask with distilled water
- Dry the flask in oven
- Clean glass for 90 min

❖ Step 2. Sample preparation:-

- Clean, dry and label flask for sample and blank
- Transfer sample in to flask and add 10 ml of 70 % HNO_3 in to both flasks
- Step 3. Sample digestion:-
- Left flask in to fume hood for 12 hour ,then sample flask changed to brown color then shake it
- Heat flask on mantle of digester for both sampled and blank
- Turn on power at 120 c^o for 30 min
- Brown fume was formed due to HNO_3, then it remove gradually from both flasks
- after 30 min it heat digestion no brown color, refers 2.5 g sample left in flask
- Step 4 Dilute Sample :-
- Dilute sample with distilled water
- Wash funnel with distilled water
- Fold filter paper and place on funnel, then add drop of distilled water to wet
- Add 30 ml of distilled water in to sampled flask and shake it
- Filter diluted digest sample in to 100 ml flask
- Add 30 ml of distilled water and keep flask left to filter
- Label flask for both sampled flask and flask that contain blank sample
- Wash flask and to check complete transfer, do the same way for blank
- Drop distilled water on filter paper to check left elements
- After filtration remove funnel and samples were diluted along with the blank further to appropriate dilution since the metal concentration is higher than standards to make the calibration curve
- Then, samples are ready for heavy metal analysis.

Annex 3. Calibration curve for standard solution of Cd, Pb and Hg

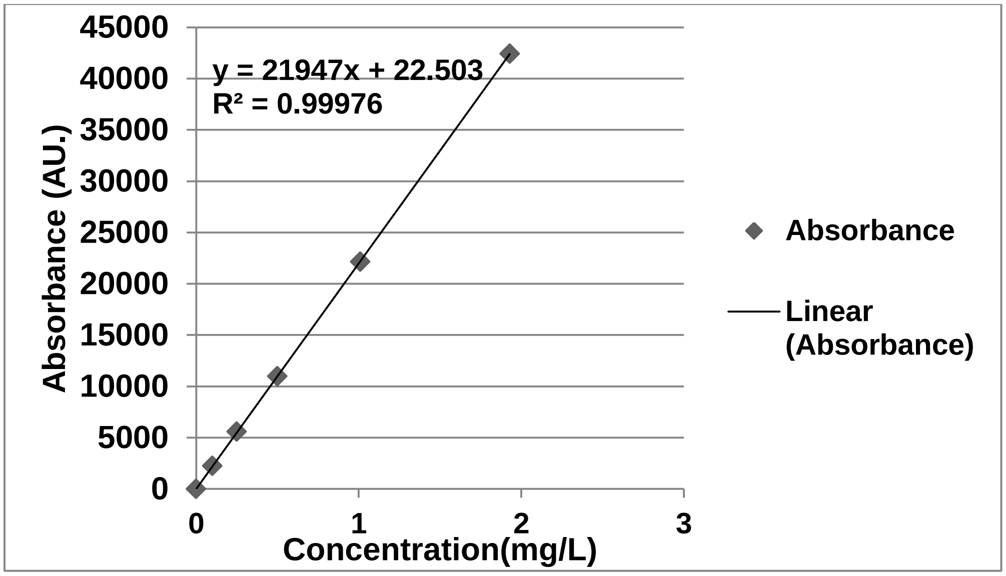

Figure 4 Calibration curve for standard solution of Cd using MP-AES

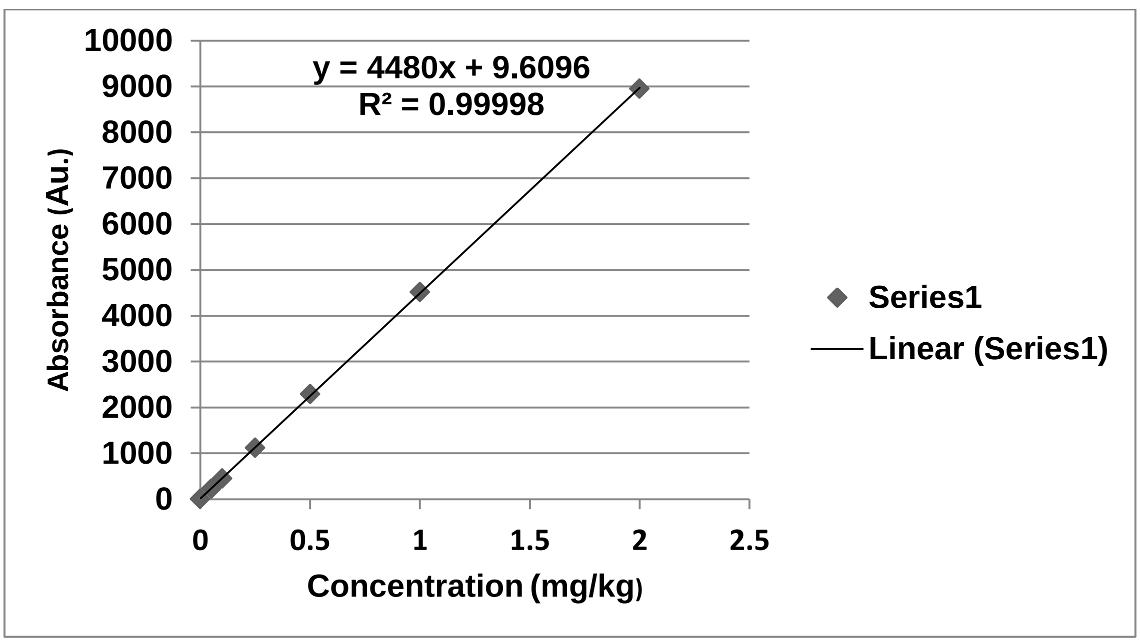

Figure 5 Calibration curve for standard solution of Pb using MP-AES

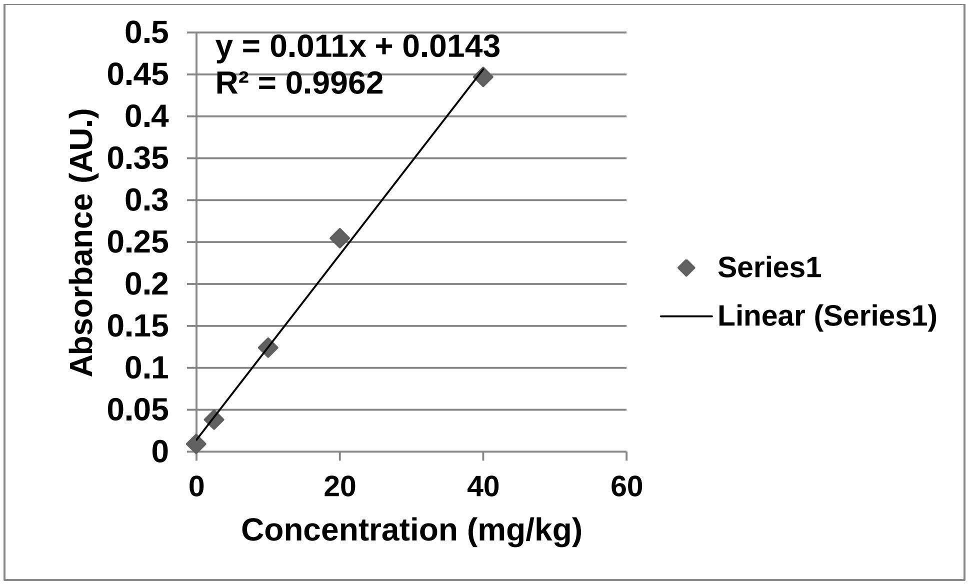

Figure 6 Calibration curve for standard solution of Hg using HGAAS

Annex 4 Nine powdered infant formulae collected from the study area: