ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY

Determination of major and trace metals in infant formulas by microwave plasma atomic emission spectrometry.

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Addis Ababa, Ethiopia

Determination of major and trace metals in infant formulas by microwave plasma-atomic emission spectrometry.

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A thesis submitted to the department of chemistry presented in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

Addis Ababa University

Addis Ababa, Ethiopia

February, 2020

Declaration

I, declare that this thesis entitled "Determination of major and trace metals in infant formulas by microwave plasma-atomic emission spectrometry" is my own work done under the supervision of Dr. Weldegebriel Yohannes at the Chemistry Department of Addis Ababa University and I have not previously submitted it entirely or in part for obtaining any qualification at any other university.

Name: Addisu Gebremedhin Nadew Date _____________ Signature_________________

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This is to certify that the thesis prepared by Addisu Gebremedhin entitled “Determination of major and trace metals in infant formulas by microwave plasma-atomic emission spectrometry” and submitted in partial fulfillment of the requirments for the degree of Master of Science in Chemistry complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Approved by the examining committee

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LIST OF ABBREVIATIONS

UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization
MHz	Mega Hertz
RSD	Relative Standard Deviation
MDL	Method Detection Limit
RDA	Recommended Dietary Allowances
DRI	Dietary Reference Intakes
kcal	Kilocalorie
kj	Kilojoule
L_1	Liptomil infant formula
M_1	Mamilac infant formula
B_1	Bebelac infant formula
A_1	Anchor infant formula
Bl_1	Blank solution
MP-AES	Microwave Plasma -Atomic Emission Spectrometry
ANOVA	Analysis of variance

Abstract

Samples of infant formulas were collected from different supermarkets of Addis Ababa, Ethiopia. After preparation of samples, different digestion procedures were tested by varying reagent volumes, digestion time and temperature to develop an optimum digestion procedure. The optimal procedure required 1 hour and consumed 2 mL HNO_3 and 1 mL $HClO_4$ to completely digest 0.5 g of powdered infant formula samples. The accuracy of the optimized procedure was evaluated by analyzing spiked samples with standard solution. Recoveries of the spiked samples varied from 94 ± 0.05 % to 104 ± 0.4 % for infant formula samples. Concentrations of major and trace metals in the samples were analyzed by microwave plasma atomic emission spectrometry employing calibration curve. The observed average metals concentrations were (mean ± SD mg/kg). In Liptomil Na (714.67 ± 56.81), K (1872.33 ± 49.78), Ca (38550.13 ± 485.9), Al (70.93 ± 3.05), Cr (13.56 ± 0.67), Mn (13.72 ± 1.19), Fe (159.59 ± 59), Co (1.76 ± 0.14), Ni (0.38 ± 0.03), Cu (10.59 ± 0.38), Zn (135.12 ± 10.11). In Mamilac Na (739.74 ± 41.99), K (2081.93 ± 54.87), Ca (38258.72 ± 994.6), Al (43.58 ± 2.98), Cr (16.05 ± 1.23), Mn (14.12 ± 0.15), Fe (149.26 ± 5.61), Co (0.015 ± 0.01), Ni (0.56 ± 0.03), Cu (11.41 ± 0.25), Zn (121.22 ± 0.32). In Bebelac Na (691.12 ± 66.39), K (2572.92 ± 240.13), Ca (41963.82 ± 1941.29), Al (33.79 ± 2.19), Cr (16.39 ± 1.01), Mn (13.12 ± 0.68), Fe (142.32 ± 1 0.08), Co (2.52 ± 0.12), Ni (0.35 ±0.03), Cu (10.45 ± 0.52), Zn (98.22 ± 5.45). In Anchor Na (1163.73 ± 27.87), K (3605.39 ± 223.56), Ca (43373.41 ± 1302.23), Al (59.83 ± 4.45), Cr (15.33 ± 0.59), Mn (14.96 ± 0.69), Fe (187.68 ± 14.39), Co (2.51 ± 0.17), Ni (0.32 ± 0.03), Cu (8.86 ± 0.58), Zn (129.01 ± 9.14). This study showed that the metal contents varied with the brands of the infant formulas. The toxic metals Pb and Cd were not detected in all brands of infant formulas.The percentage relative standard deviations (% RSD) were less than 10% for all the analyzed elements. In addition, the observed results were also compared with the reported literature values analyzed from other parts of the world. The concentrations of most of the metals determined are found to be within the range of literature values.

Key words: Infant formulas, Microwave plasma- atomic emission spectrometry, Major and trace metals, wet digestion.

Chapter One: Introduction

1.1. Background

Milk is a white liquid produced by the mammary glands of mammals. All mammals, including humans produce milk to feed their offspring until they are ready for solid food. It contains valuable nutrients and offers a range of health benefits (Megan Ware, 2008). However, in some circumstances for example insufficient milk syndrome, breast feeding failure, social factors or for premature and low-birth weight infants, it is necessary to substitute or fortify breast-feeding with specially designed formulas (FDAGI, 2003).

Infant formulas are liquids or reconstituted powders given to infants. They serve as substitutes for human milk. Apart from breast milk, infant formulas have a special role to play in the diets of infants because they are the major source of nutrients for infants and a unique source of food during the first months of life (Ikem A, 2002).

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. 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.

On the other hand, due to the growing environmental pollution and contamination, it is also necessary to determine and monitor the levels of toxic metals in infant formula because they can significantly influence human health. Infant formulas are a product based on milk of cows or other animals or a mixture of other ingredients which have been proven to be suitable for infant feeding. The nutritional safety and adequacy of infant formula shall be scientifically demonstrated to support growth and development of infants. Therefore, it is necessary to study the concentration of major and trace metals present in infant formulas.

Breast milk composition varies with the age of the baby, time of day and point in the feeding thus adapting to the needs of the particular infant. Research continues to confirm the fact that breast milk is the optimal food source for developing babies.

Breast feeding completes the infant immune system. Breast-fed infants have lower rates of insulin dependent diabetes, respiratory illness, allergies, gastrointestinal illness and diarrhea which can be fatal in premature infants.

Breast-fed infants are also less likely than their formula-fed counterparts to die of Sudden Infant Death Syndrome. Studies indicate that breast feeding may offer some protection against multiple sclerosis (OPHA, 2004).

A number of published studies have examined the hypothesis that increases the risk for cancer in general or for a specific cancer or group of cancers (Davis, 2002). Studies have also suggested that breastfed infants have better cognitive development. This is most profound in the case of preterm infants.

Breast feeding also provides many benefits for the mother. Benefits include lower risk of ovarian cancer and osteoporosis and premenopausal breast cancer. High frequency breast feeding can serve also as a simple and free method of child spacing. One long used by traditional subsistence populations although used alone, breastfeeding is not a birth control method. In addition to these physical benefits, breast feeding brings with it a range of emotional benefits for both mother and child. The hormonal effects of breast feeding enhance the desire for infant - mother proximity and lower anxiety levels in mothers (OPHA, 2004). Mothers are sometimes worried about or have been discouraged from breast feeding because of concerns regarding toxins found in the body and often transferred to the baby through lactation (Walker, 2000).

Other reasons for mothers not to breast feed their babies may include the demands of work or school life circumstances, such as difficult home situations and social problems with breast feeding in public. HIV positive child bearing women are compelled to formula feed their children so as to minimize the risk of contamination of the virus (Gillard et al., 2001).

Optimal growth and development of infants can be guaranteed only when the intake of food and water provides the required doses of all essential elements and delivers only in effectual doses of toxic elements (Prohaska, 2000). It is estimated that proper infant feeding can prevent millions of deaths occurring from infantile gastroenteritis and malnutrition.

Milk is the fundamental food for infants. The most natural and best source is from breast feeding and this is greatly encouraged for the first 6 months of life and should be continued for as long as 2 years (Dorea, 2010). Certain situations may warrant a special diet for metabolic reasons or in view of some pressing factors, artificial feeding may have to be developed.

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 (Periera and Carrion, 2003). 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 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. Where as "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.

Infant formulas are a product based on milk of cows or other animals or a mixture of other ingredients which have been proven to be suitable for infant feeding. The nutritional safety and adequacy of infant formula shall be scientifically demonstrated to support growth and development of infants. Human milk is considered the best food for infants. It meets all their nutritional requirements and promotes infant health and development. Its composition is designed to provide the necessary energy and nutrients in appropriate amounts in a perfectly digestible and absorbable form (Prohaska et al, 2000). However, in some circumstances e.g. insufficient milk syndrome, breast feeding failure, social factors or for premature and low-birth weight infants, it is necessary to substitute breast-feeding with specially designed formulas (Pereira, 2003).

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. The very rapid rate of growth of healthy infants born at full term which doubles their weight in only 4-5 months after birth results in relatively high requirements of energy and nutrients per kilogram body weight. In addition to meeting maintenance requirements, infants must cover the energy and substrate needs for the synthesis and deposition of newly formed tissues. The resulting large metabolic requirements contrast with the limited capacity of young infants to compensate for an unbalanced nutrient supply due to small body stores of nutrients and immature homeostatic mechanisms (Thompkinson and Khrab, 2007).

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 the 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 (Periera and Carrion, 2003).

From the nutritional point of view metal contents of milk and dairy products can be grouped in to essential elements and non-essential elements such as iron, copper, zinc etc. at low doses or toxic elements such as lead and cadmium. The presence of lead and cadmium even in low concentrations is invaluable and leads to metabolic disorder with extremely series consequences. These metals are the cause of environmental pollution from sources like leaded petrol, industrial wastes and leaching of metal ions from soil in to lakes and rivers by acid rain. Diary animals ingest metals while grazing on the pasture and when fed on contaminated concentrate feeds. However in the cow, transfer of minerals to milk is highly variable (Maas et al., 2002).

Various industrial environmental contamination of soil, water, foods and plants with these metals cause their incorporation in to the food chain and impose a great threat to human and animal health such as weakness, heart failure, cancer and also affects the kidney (Billandzic et al., 2007). In general heavy metals are important sources of food contamination and health hazard the main threats to human health are associated with exposure to arsenic, cadmium, lead, copper and mercury. Sources of milk contamination include environmental and industrial pollution, agricultural practices, food processing and packaging. Absorption of heavy metals through food has been shown to have serious consequences on health and there by economic development associated with a decline in labor productivity as well as the direct costs of treating illness such as kidney disease, damage to nervous system, diminished intellectual capacity, heart disease, gastrointestinal disease, bone fracture, cancer and death (Jarup, 2003). The toxic metals in milk are of a particular concern because milk is largely consumed by infants and children and the determination of toxic metal levels in milk is particularly attended by international organizations.

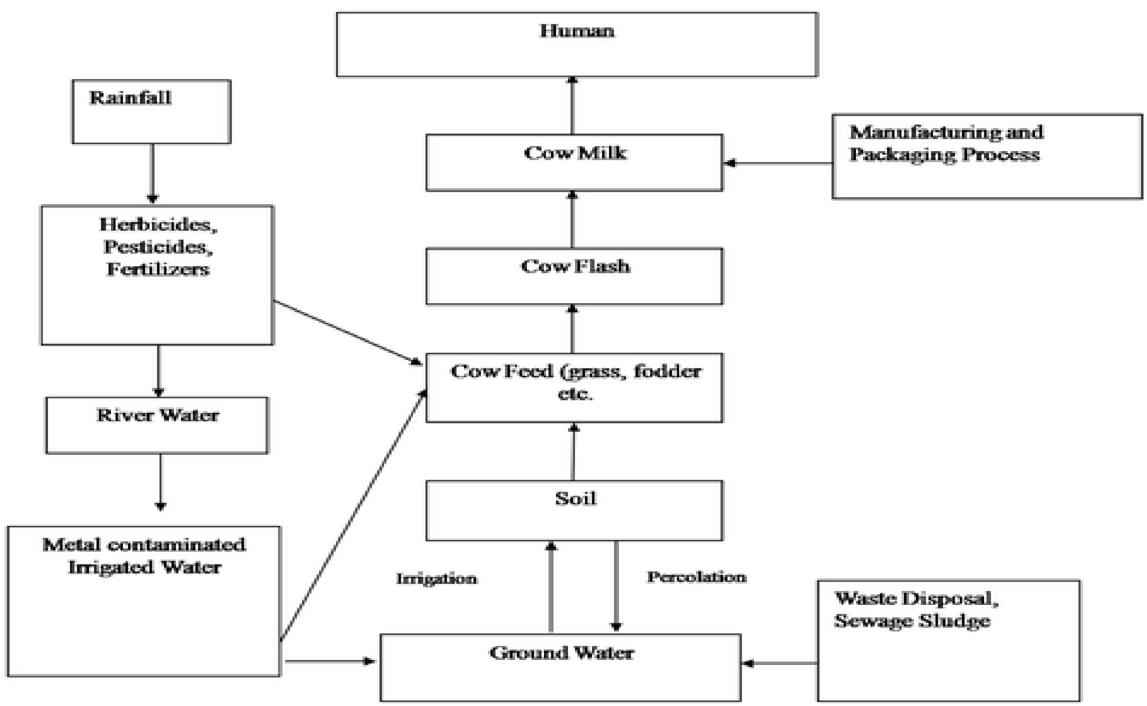


Figure 1 Sources of contamination

1.2. General Objective

The main objective of the research is to collect base line data on the levels of major and trace metals (Na, K, Ca, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in infant formulas commercially available in Addis Ababa.

1.3. Specific objectives

1. To identify suitable methods for the digestion of infant formula samples.
2. To identify major and trace metals found in infant formulas
3. To determine the concentration of major and trace metals in infant formulas by microwave plasma-atomic emission spectrometry.
4. To compare the metal content in infant formula with feeding tables supplied by infant formula manufacturers.
5. To compare the levels of major and trace metals in infant formulas with literature data.

1.4. Statements of the problem

Infant formulas have a special role to play in the diets of infants because they are the major source of nutrients for infants and a unique source of food during the first months of life (Ikem, 2002). They are handy for urban women and many mothers in industrialized countries choose to use commercially manufactured formula to feed their new born (Tripathi, 1999). Despite the benefits of infant formulas as a major source of food for infants, the presenceof contaminants, such as heavy metals, pesticides and polychlorinated biphenyls in infant formula may pose health risks to children. 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 (Ikem, 2002). So it is necessary to create awareness about infant formulas. Moreover, this study aimed to give information and determine the essential major, trace and toxic metals of infant formulas by using microwave plasma-atomic emission spectrometry.

1.5. Significance of the study

The output of this study has different benefits when viewed from different perspectives. The first is the determination of concentrations of essentials and trace metals in infant formulas that helps parents to balance metabolic activities within the children's. Thus, create awareness for the society in future life because the quantity and quality of nutrients supply during infancy has long term consequences on organ development and function. Moreover, alert governmental food controlling agencies so as to propose new strategies for controlling mechanisms and evaluate governmental policies and check the continuity of their strategies because special efforts are required to secure an adequate dietary composition for infants. The second is the identification of analytical method, formulation of analytical data and information on the chemistry of infant formulas that can be used as base line information for future research in various fields related to infant formulas.

Chapter two: Literature review

2.1. Human Breast Milk

Human breast milk contains carbohydrates, protein, fat, vitamins, minerals, digestive enzymes and hormones. In addition to these nutrients, it is rich in immune cells, including macrophages; stem cells, and numerous other bioactive molecules. Some of these bioactive molecules are protein-derived and lipid-derived while others are protein-derived and indigestible such as oligosaccharides.

2.1.1. Composition of Human Breast Milk

Human breast milk is a complex matrix with a general composition of 87% water, 3.8% fat, 1.0% protein, and 7% lactose. The fat and lactose, respectively, provide 50% and 40% of the total energy of the milk. However, the composition of human breast milk is dynamic and changes over time, adapting itself to the changing needs of the growing child. For instance, during each nursing session, the milk that is expressed first (foremilk) is thinner with a higher content of lactose, which satisfies a baby's thirst, and following the foremilk, hind milk, is creamier with a much higher content of fat for the baby's needs. Variations are also present with the stage of nursing (age of infant), maternal diet, maternal health, and environmental exposure.

During early lactation, the protein content in human milk ranges from 1.4–1.6 g/100 mL, after three to four months of lactation 0.8 – 1.0 g/100 mL and after six months 0.7 – 0.8 g/100 mL. The fat content varies significantly with maternal diet and is also positively related to weight gain during pregnancy. Remarkably, it has been observed that a mother's breast milk is almost always adequate in essential nutrients for her term infant's growth and development, even when her own nutrition is inadequate. Although the mean concentrations of protein, sodium, chloride and potassium in early preterm milk are adequate to meet the estimated requirements for preterm infants, specific nutritional supplementation is required for mother's milk delivered to preterm infants (Fomon et al., 2007).

2.1.2. Benefits of Breast-Feeding

Breast milk composition varies with the age of the baby, time of day and point in the feeding thus adapting to the needs of the particular infant. Research continues to confirm the fact that breast milk is the optimal food source for developing babies. Breast feeding completes the infant immune system. Breast fed infants are also less likely than their formula fed counterparts to die of Sudden Infant Death Syndrome (SIDS). A number of published studies have examined the hypothesis that artificial feeding increases the risk for cancer in general or for a specific cancer or group of cancers (Fomon et al., 2007).

Studies have also suggested that breastfed infants have better cognitive development. This is most profound in the case of preterm infants. Breastfeeding also provides many benefits for the mother. Benefits include lower risk of ovarian cancer and osteoporosis and premenopausal breast cancer.

High frequency breastfeeding can serve also as a simple and free method of child spacing; one long used by traditional subsistence populations, although used alone, breastfeeding is not a birth control method. In addition to these physical benefits, breastfeeding brings with it a range of emotional benefits for both mother and child. The hormonal effects of breast feeding enhance the desire for infant -mother proximity and lower anxiety levels in mothers.

Extensive and recent research shows that breast feeding of infants provides advantages with regard to general health, growth and development, while significantly decreasing the risk of a large number of acute and chronic diseases. Older children and adults who were breast fed are protected against different syndromes, such as diabetes mellitus, allergic disease, obesity, and hypertension (WHO, 2003). Infant formula is intended as an effective substitute for infant feeding (Stevens, 2009). Although production of an identical product to breast milk is not feasible, every effort has been taken to mimic the nutrition profile of human breast milk for normal infant growth and development. During the first six months of infant life, providing optimal nutrition is critical as the consequences of inadequate nutrition can be very severe.

2.2. Infant Formulas

Infant formula, baby formula or just formula (American English) or baby milk, infant milk or first milk (British English), is a manufactured food designed and marketed for feeding to babies and infants under 12 months of age, usually prepared for bottle-feeding or cup-feeding from powder (mixed with water) or liquid (with or without additional water). The U.S. Federal Food, Drug and Cosmetic Act defines infant formula as "a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk".

Manufacturers state that the composition of infant formula is designed to be roughly based on a human mother's milk at approximately one to three months postpartum; however, there are significant differences in the nutrient content of these products. The most commonly used infant formulas contain purified cow's milk, whey and casein as a protein source, a blend of vegetable oils as a fat source, lactose as a carbohydrate source, a vitamin-mineral mix, and other ingredients depending on the manufacturer. In addition, there are infant formulas using soy bean as a protein source in place of cow's milk (mostly in the United States and Great Britain) and formulas using protein hydrolyzed into its component amino acids for infants who are allergic to other proteins. A 2001 World Health Organization report found that infant formula prepared in accordance with applicable Codex Alimentary standards was a safe complementary food and a suitable breast milk substitute. In 2003, the WHO and UNICEF restated that "processed-food products for young children should, when sold or otherwise distributed, meet applicable standards recommended by the Codex Alimentary Commission", and also warned that "lack of breastfeeding and especially lack of exclusive breastfeeding during the first half-year of life are important risk factors for infant and childhood morbidity and mortality".

In particular, the use of infant formula in less economically developed countries is linked to poorer health outcomes because of the prevalence of unsanitary preparation conditions, including lack of clean water and lack of sanitizing equipment (WHO, 2003). A formula-fed child living in unclean conditions is between 6 and 25 times more likely to die of diarrhea and four times more likely to die of pneumonia than a breast fed child. Rarely, use of powdered infant formula has been associated with serious illness and even death due to infection with Cronobacter sakazakii and other microorganisms that can be introduced to powdered infant formula during its production. Although *C.* sakazakii can cause illness in all age groups, infants are believed to be at greatest risk of infection. Between 1958 and 2006, there have been several dozen reported cases of *E.* sakazakii infection worldwide.

Infant formula is intended as an effective substitute to breast milk and is formulated to mimic the nutritional composition of breast milk. The recently updated Food and Drug Administration rule on current good manufacturing practices for infant formula (WHO, 2003), requires among other things that formulas satisfy the quality factors of normal physical growth and a sufficient biological quality of protein component (adequate amounts of protein in a form that can be used by infants). Infant formula is only for the health of infants without unusual medical or dietary problems. The manufacturing process is highly regulated and monitored to meet national and international quality criteria.

2.3. Guidelines for Manufacturing of Infant Formula

Infant formulas must include proper amounts of water, carbohydrate, protein, fat, vitamins and minerals. The composition of infant formula is strictly regulated and each manufacturer must follow established guidelines set by government agencies. For instance, all the major components added to formula (protein, lipids, carbohydrates) have a range of minimum and maximum values for their effectiveness. These components must have established a history of safe use. The required range of each nutrient must be maintained throughout the shelf life of the product.

Infant formula prepared ready for consumption should contain no less than 60 kcal (250 kJ) and no more than 70 kcal (295 kJ) of energy per 100 mL) (WHO, 2003). Furthermore, product reformulation must be based on medical and nutritional findings. The committee of the "Evaluation of the addition of Ingredients New to Infant Formula" has recommended that "manufacturers must demonstrate that the formula containing the new ingredient is capable of sustaining physical growth and development over 120 days when formula is likely to be the sole source of infant nutrition" (IMNA, 2006).

In the United States, the Food and Drug Administration (FDA) defines that adding new ingredients to infant formula should have "reasonable certainty of no harm" as the safety standard (IMNA, 2006). The World Health Organization has noted that unmodified cow's milk should never be fed to infants and that unmodified goat's milk is also not recommended for infants. With the WHO guidelines, federal and local agencies of different countries control and monitor infant formula regulations including requirements for quality and manufacturing practices in their own countries. From a manufacturer's perspective, it is in their best interest to continuously improve their products to be as close as possible to human breast milk.

2.4. Classes of Infant Formula Products

There are three major classes of infant formulas: Cow-milk based formula, soy-based formula and specialized formula. They vary in nutrition, calories, taste, digestion, and cost. Specific kinds of formulas are available to meet a variety of needs. Some cow's milk substitutes are amino acid based or contain extensively hydrolyzed whey or casein proteins. Some are rice-based formula.

2.4.1. Cow Milk-Based Formula (Bovine Milk)

Bovine milk is the basis for most infant formula. However, bovine milk contains higher levels of fat, minerals and protein compared to human breast milk. Therefore, cow milk must be skimmed and diluted to more closely resemble human breast milk composition. Cow milk-based infant formula contains added vegetable oils, vitamins, minerals and iron for consumption by most healthy full term infants.

According to the American Academy of Pediatrics, children under one year of age should not be fed raw, unmodified or unpasteurized cow's milk as a replacement for human milk or infant formula. Additionally, unmodified milk does not provide enough vitamin E, iron or essential fatty acids. Moreover, infants' systems cannot handle the high levels of protein, sodium, and potassium of unmodified cow milk. Formulas with a protein content 2–2.5 g/100 mL and a protein/energy ratio < 3 g/100 kcal are used for normal infants, while with higher protein content (2.9 g/100 mL) and higher protein/energy ratio (3.5 g/100 kcal) are for a very low birth weight or preterm infants. Recent studies showed that high protein content in infant formula is associated with excess weight gain in infancy, which can lead to a 20% risk of obesity later in life (Michaelsen et al., 2014).

2.4.2. Soy-Based Formulas

Formulas made from soy proteins are effective options for infants with galactosemia or congenital lactase deficiency. They help with colic and milk allergies; however, infants who are allergic to cow's milk may also be allergic to soy milk. Soy products should not be used in infants under six months of age with food allergy. Because phytoestrogens are present in soy-based formula, the uses of soy-based formulas are limited by the concern of potential harm for the infant although this remains controversial (Adjent, 2016).

2.4.3. Specialized (Hypoallergenic) Formulas

Feeding extensively hydrolyzed cow's milk formula to children during the first 14 months of life can protect against the development of cow's milk allergy up to the age of four years. It has been suggested that this might be prevention and not just a postponement of the onset of symptoms but more research is needed to verify this. Cross-reactivity between certain cow milk and goat milk proteins has been reported suggesting that goat milk may not be a safe alternative to cow milk for many children with cow milk allergy. Occasionally, milk-allergic children may also develop a beef protein allergy. It should be noted that lactose, a sugar in milk that is commonly used ingredient in manufactured foods, may contain traces of casein and whey proteins and has been reported to cause adverse reactions in individuals sensitive to milk proteins (Renfrew, 2010). Food allergies are estimated to occur in 5 to 20 % of children. Allergy to cow's milk proteins is the most common food allergy in early childhood occurring in 2 to 3 % of infants within the first year of life.

2.5. Use of infant formula

In some cases, breast feeding is medically contra indicated. These include:

Mother's health: If the mother is extremely ill or has had certain kinds of breast surgery, which may have removed or disconnected all milk-producing parts of the breast. She is taking any kind of drug that could harm the baby, including both prescription drugs such as cytotoxic chemotherapy for cancer treatments as well as illicit drugs. One of the main global risks posed by breast milk specifically is the transmission of HIV and other infectious diseases. Breastfeeding by an HIV-infected mother poses a 5–20% chance of transmitting HIV to the baby. However, if a mother has HIV, she is more likely to transmit it to her child during the pregnancy or birth than during breastfeeding.

Baby is unable to breastfeed: The child has a birth defect or inborn error of metabolism such as Galactosemia that makes breast feeding difficult or impossible.

Baby is considered at risk for malnutrition: In certain circumstances infants may be at risk for malnutrition such as due to iron deficiency, vitamin deficiencies (e.g. vitamin D which may be less present in breast milk than needed at high latitudes where there is less sun exposure) or inadequate nutrition during transition to solid foods.

Personal preferences, beliefs, and experiences: The mother may dislike breast-feeding or think it is inconvenient. In addition, breastfeeding can be difficult for victims of rape or sexual abuse.

Absence of the mother: The child is adopted, orphaned, abandoned. The mother is separated from her child by being in prison or a mental hospital. The mother has left the child in the care of another person for an extended period of time such as while traveling or working abroad.

Food allergies: The mother eats foods that may provoke an allergic reaction in the infant.

Financial pressures: Maternity leave is unpaid, insufficient or lacking. The mother's employment interferes with breast feeding. Mothers who breast feed may experience a loss of earning power.

Societal structure: Breast feeding may be forbidden at the mother's job, school, place of worship or in other public places or the mother may feel that breast feeding in these places or around other people is immodest, unsanitary or inappropriate.

Social pressures: Family members such as mother's husband or boyfriend or friends or other members of society may encourage the use of infant formula. For example, they may believe that breastfeeding will decrease the mother's energy, health or attractiveness.

Lack of training and education: The mother lacks education and training from medical providers or community members.

Lactation insufficiency: The mother is unable to produce sufficient milk. In studies that do not account for lactation failure with obvious causes (such as use of formula and/or breast pumps), this affects around 2 to 5% of women. Alternatively, despite a healthy supply, the woman or her family may incorrectly believe that her breast milk is of low quality or in low supply. These women may choose infant formula either exclusively or as a supplement to breastfeeding.

Fear of exposure to environmental contaminants: Certain environmental pollutants such as polychlorinated biphenyls can bio- accumulate in the food chain and may be found in humans including mothers' breast milk. However, studies have shown that the greatest risk period for adverse effects from environmental exposures is prenatally. Other studies have further found that the levels of most persistent Organo halogen compounds in human milk decreased significantly over the past three decades and equally did their exposure through breastfeeding (AAP, 1997).

2.6. Growth of Breast-fed and Formula-fed Infants

It is well documented that the pattern of growth of formula fed infants differs from that of breast fed infants. 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.

2.7. Effect of Infant Formula on Stool Characteristics of 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 formulas 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 are 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 (Hyams, 1995).

2.8. Major and trace elements

Generally, infants fed formula made with tap water are at the highest risk from metals contaminating the water supply. Indeed some infant foods such as commercial infant formulas are deliberately fortified with essential elements such as zinc and copper to ensure that they provide infants' nutritional requirements for trace elements. Another important factor that must be borne in mind when assessing the exposure of infants to trace elements and heavy metals in their diets is that because infants grow and develop very rapidly in their first year of life, their energy requirements and hence their food consumption is on average much higher relative to their body weight than that of adults and older children (Saracoglu, 2007).

The intake of heavy metals as a result of consumption of contaminated milk and milk products has toxic effects depending on contamination and absorption levels. Symptoms of heavy metal toxicity include dizziness, nausea, vomiting, diarrhea, sleeping disorders and loss of appetite. Heavy metals have also been linked to cardiovascular disease, depressed growth, impaired fertility, nervous and immune system disorders, increased spontaneous abortions, and elevated death rate among infants (Yuzbasky et al., 2003).

2.8.1. Sodium

Sodium is required to maintain the water balance in the body regulate blood volume and ensure the proper functioning of cell membranes and other body tissues. Healthy, full-term infants consuming primarily breast milk or infant formula of standard dilution receive a relatively small amount of sodium but an amount adequate for growth. Estimated minimum requirements for infants are 100 to 200 mg/day. The sodium level in cow's milk is greater than that in breast milk and most infant formulas; however, cow's milk is not recommended for infants. Salt is not added to commercially prepare infant foods; however, salt is added to "junior" or "toddler" foods designed for children from 1 to 4 years old to improve their taste. These foods are not recommended for infants. The amount of sodium consumed by an infant on home prepared complementary foods reflects the cooking methods used in the home and the eating habits and cultural food.

2.8.2. Potassium

Potassium is one of the main blood mineral essential to both cellular and electrical function. Potassium is the primary positive ion found in the cells. Along with sodium, potassium regulates the water balance and the acid-base balance in the blood and tissues and plays a critical role in the transmission of electrical impulses in the heart.

2.8.3. Calcium

At birth about 99 % of the calcium in the body is part of the structural matrix of bone with the remainder being physiologically active as a free calcium pool with in cells and the extra cellular fluid. Within cells, calcium acts as a second messenger modulating the transmission of hormonal signals and regulating enzyme function. It is involved in blood coagulation, nerve conduction, muscle contraction and reproductive functions. Although net calcium absorption and calcium retention may be related to the calcium intake, there are a range of other factors either in the diet or related to metabolic regulation which influence the absorption around 30 to 50% of dietary calcium and retention of calcium (SCFE report, 2005). Hormonal factors such as calcitonin, parathyroid hormone and vitamin D play a clear role in this integrated function but the metabolic handling of other nutrients such as sodium, phosphorus, iron, zinc and magnesium are also known to exert an influence. In this regard the intake and metabolism of phosphorus is of particular importance as effective calcium retention requires that adequate phosphorus is available. For this reason the ratio of calcium to phosphorus within formula should be specified.

2.8.4. Aluminum

The toxicological aspects of orally consumed aluminum are less well defined. The element is poorly absorbed from the intestines. The small amounts absorbed from normal diets are excreted by healthy kidneys so that no trace element in human nutrition and health accumulation occurs. While there is no evidence that aluminum accumulates in the organism at these intakes**.** The risk of aluminum toxicity is greatly increased in persons with impaired kidney function**.** Aluminum interacts with a number of other elements including calcium, fluorine, iron, magnesium, phosphorus and strontium and when ingested in excess can reduce their absorption because of this property it has been used therapeutically to treat fluorosis and to reduce phosphorus absorption in uremic patients. By far the most important contribution to aluminum intake comes from anti-acid medications that can provide several grams of the metal per day. These amounts do interfere with the absorption of other elements. They also may lead to a gradual accumulation of aluminum in the skeleton. Locally increased concentrations of aluminum occur in the brain of patients with Alzheimer dementia but whether the metal has a causative role in the pathogenesis of this disease has not been established. It has been provisionally suggested that the tolerable weekly intake of aluminum may be approximately 7 mg/kg of body weight. In conclusion there is no known risk to healthy people from typical dietary intakes of aluminum. Risks arise only from the habitual consumption of gram quantities of aluminum anti-acids over long period of time.

2.8.5. Chromium

Chromium is an essential element which is involved in the metabolism of carbohydrates and which forms part of the glucose tolerance factor (GTF). As well as being associated with prevention of diabetes and cardiovascular disease. Chromium seems to be influential in metabolism of cholesterol and various proteins. Chromium can exist in various chemical valence states ranging from Cr^{3+} to Cr^{6+} among which more stable chemical forms are Cr^{3+} and Cr^{6+}.

Cr^{3+} is considered to be the trace element essential for proper functioning of living organisms. Cr^{6+} was reported to exert toxic effects on biological systems, where as Cr^{3+} is generally not transported over great distances because of its low solubility and tendency to be absorbed in the pH range typical for natural soils and water. Hexavalent cationic form is more available for living organisms than Cr^{3+} and plays a main role in removing this metal from water and soil systems. Its deficiency is characterized by disturbance in glucose, lipids and protein metabolism (Cabrera C., 2009).

2.8.6. Manganese

Manganese is an essential trace element known to be related particularly to reproductive function. It is involved in the formation of bone and in amino acid, cholesterol and carbohydrate metabolism. Manganese is a component of many enzymes. Glycosylated transferases are specifically activated by manganese. Manganese deficiency is associated in animals with growth retardation, impaired glucose tolerance and skeletal abnormalities, brain damage, teratogenicity and abnormal metabolism of carbohydrates and lipids. However, excessive manganese is toxic to humans. Relatively high doses of manganese cause mutations in mammalian cells, damage to DNA and chromosome aberrations and toxicity to the embryo and fetus.

2.8.7. Iron

Iron is essential for virtually every living organism. The dominating function of iron in the human body is as the oxygen binding core of hemoglobin, the red pigment of blood transporting oxygen from the lungs to all tissues. During the progress of iron deficiency, hemoglobin synthesis in the bone marrow is restricted resulting in anemia. Anemia caused by iron deficiency is called iron deficiency anemia, distinguishing this condition from other causes of anemia such as infection, inflammation, hematological disorders and other nutritional deficiencies. Human milk has unique properties making exclusive breast feeding a superior form of nourishment for infants during the first half of infancy. World health organization (WHO, 2003) estimated the world wide prevalence of anemia in children below 4 years of age to be 43% with a higher prevalence (51%) in developing regions as compared to (12%) in industrialized countries.

The term new born infant has a total body iron content averaging about 75 mg/kg body weight which can be compared with 55 mg/kg for an adult. Iron absorption and metabolism are influenced by interactions between iron and other dietary nutrients. Many components of the diet act to inhibit or enhance iron absorption; in a 6-month old infant ~ 0.5 mg iron/day is needed for expansion of the blood mass and 0.1 mg/day for growth of muscle and other tissues. Since iron requirements excluding growth would equal iron losses (0.15 mg/day), infant growth increases iron requirements 5- fold to approximately 0.75 mg/day (Oral lab manual, 1995).

2.8.8. Cobalt

Cobalt is an essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition. Cobalt can be taken into the body by eating food, drinking water or breathing air. Gastrointestinal absorption from food or water is the principal source of internally deposited cobalt in the general population. Cobalt is an essential element found in most body tissues with the highest concentration in the liver. Unlike other heavy metals, cobalt is safe for human consumption and up to 8 mg can be consumed on a daily basis without health hazard. Cobalt is usually considered non-toxic however, severe cardiac and some deaths in man resulted from consumption of large amounts of beer containing 1.2-1.5 mg/L cobalt. Inhalation and dermal exposure to cobalt in humans can result in sensitization. Bronchial asthma has been described in workers exposed to various forms of cobalt (Aziz et al, 2004).

2.8.9. Nickel

Nickel is a naturally occurring element found in a number of mineral ores including sulfides, oxides and silicates. It is present in the enzyme urease and as such is considered to be essential to plants and some domestic animals. Nickel related health effects such as renal, cardio vascular reproductive and immunological effects have been reported in animals. Its toxic effects are related to dermal, lung and nasal sinus cancers (Awofolul et al, 2005).

2.8.10. Copper

Copper is required as an essential dietary trace element. It is required for cellular metabolism in enzymatic and non-enzymatic systems. Copper acts as an important metallic activator of several enzymes (Tehran et al., 2006). Copper status is not easy to determine and the homeostatic mechanisms which control copper distribution and metabolism are incompletely understood. New borns have a high liver content of copper which is drawn upon for growth during the first six months of life. The copper content of human milk is not directly influenced by maternal dietary intake of copper and is generally higher than cows' milk. As cow milk presents a rather low concentration of copper whereas infant formula powdered milks are fortified with this element. In the 1980s the concentration of copper in infant formulas was reported to vary very widely with some milk having almost undetectable amounts. Copper interacts with other divalent cations such as iron and zinc for gastro intestinal absorption. Diets which contain excess copper have been reported to lead to toxicity and liver damage during childhood and the levels of copper in the tap water delivered through copper pipes has been reported to toxicity associated with levels in infant formula between 9 to 26.4 mg/L (SCFE report, 2005).

2.8.11. Zinc

Zinc is essential for growth and development. Zinc is a constituent of more than 200 metallic enzymes many of which regulate the metabolism of carbohydrates, lipids and protein. It plays a key role in the synthesis of genetic material and the regulation of gene expression as well as in cell division, epithelial integrity, cellular immunity and sexual maturation. Zinc also plays a clear role in the synthesis, storage and secretion of Insulin. The young infant has a high zinc requirement to support the very rapid growth of early infancy. Symptoms of zinc deficiency include impaired growth and altered cognition in children, loss of appetite and taste sensitivity, eye and skin lesions, diarrhea, susceptibility to infections, delayed healing of wounds and reproductive failure (Tirmizi et al., 2007). Prolonged use of high doses of zinc results in a reduction of copper absorption as it has been shown in patients with Wilson's disease. Iron supplementation could decrease zinc absorption. However, at levels present in food and at realistic supplementation levels, zinc absorption appears not be significantly affected by iron and copper (SCFE report, 2005)

2.8.12. Cadmium

Cadmium is a highly toxic metal with a natural occurrence in soil but also spread in the environment due to human activities. Exposure to cadmium occurs in many occupational settings as well as in the general environment especially in areas with industrial cadmium pollution or high natural cadmium content in the soil. Natural and anthropogenic sources such as industrial emissions, applications of fertilizers and sewage sludge from farming have led to the contamination of soils. The formulas are usually prepared by adding drinking water which could further increase the cadmium intake. Studies on adverse health effects due to cadmium exposure are mainly focused on exposure and risk in adults. Numerous studies have shown that chronic exposure leads to selective accumulation of cadmium in the liver and kidneys Neurotoxic effects like psychomotor disturbances, behavioral and cognitive disorders have been demonstrated in rat pups even after low-dose exposure to cadmium chloride and cadmium acetate during the gestation or lactation periods. Bone is a target organ of Cd toxicity and decreased bone density is found in the general population exposed to even low doses of Cd. The International Agency for Research on Cancer concluded that there was sufficient evidence to classify cadmium as a human carcinogen (Amina et al., 2003).

2.8.13. Lead

Lead occurs mostly in nature in the form of PbS and is used in accumulators, production of tetraethyl lead, ammunitions, solders and X-ray equipment’s (Tehran et al., 2006). Lead is a toxic metal which accumulates in the vital organs of man and animals. Recent studies indicate that low levels of lead exposure are correlated with irreversible fetal brain damage, hypertension, cardiovascular disease, kidney disfunction, impaired bone synthesis, impaired sperm production, and osteoporosis. Dissipation of lead in the soils, natural waters and atmosphere is due to deposition from emissions of vehicles that used leaded gasoline, discharge of industrial wastewaters, deterioration of lead-based paints and demolition or renovation of buildings with lead-based paint. From the atmosphere, waters and soils, the lead can arrive in the plants and then introduces in to the food chains of man and animals. Therefore the toxic effect of lead at low concentrations in the environment is a cause of increasing concern and explains the growing interest in determining its concentration in foodstuffs (Hussein et al., 2007).

2.9. Analytical Methods for the Determination of Metals in Infant Formulas

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 major and trace metals in infant formulas in order to maintain the health of infants. Many analytical methods including MP-AES for element determination in food materials require decomposition of the sample (Saracoglu et al., 2007).

2.9.1. Sample Decomposition

The organic fraction must be separated from the mineral fraction to avoid interference in the signal readout. Different sample treatments such as dry and wet digestion and decomposition with microwave heating in open and closed vessels have been used. Their efficiency is based on the time required and the completeness of the decomposition. Classical or traditional methods of solid sample digestion/dissolution are often referred to as wet decomposition, dry dissolution or acid digestion and involve the use of mineral acids and oxidizing agents such as hydrogen peroxide to affect the dissolution of the sample.

2.9.2. Dry Decomposition

Dry decomposition or dissolution often called “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 four to ten fold 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 placed in a muffle furnace at 800 to 1000 ^{o}C for as little as 15 min to as much as 8 hr 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 (Tehrani et al., 2006).

2.9.3. Wet Decomposition

Sample wet decomposition is a method of converting the components of a matrix into simple chemical forms. This decomposition is produced by supplying energy such as heat and by using a chemical reagent such as an acid or by a combination of the two methods. Where a reagent is used its nature will depend on that of the matrix. The amount of reagent used is dictated by the sample size, which in turn depends on the sensitivity of the method of determination.

However, the process of putting a material into solution is often the most critical step of the analytical process because there are many sources of potential errors that are partial decomposition of the analytes present or some type of contamination from the vessels of chemical products used. The majority of wet decomposition methods involve the use of some combination of oxidizing acids (HNO_3, hot concentrated $HClO_4$ and hot concentrated H_2SO_4) and non-oxidizing acids (HCl, HF, H_3PO_4, dilute H_2SO_4, dilute $HClO_4$) and hydrogen peroxide. All of these acids are corrosive in nature especially when hot and concentrated and should be handled with caution to avert injury and accidents. Concentrated acids with the requisite high degree of purity are available commercially but they can be purified further by sub-boiling distillation. Wet digestion has the advantage of being effective on both inorganic and organic materials. It often destroys or removes the sample matrix thus helping to reduce or eliminate some types of interference.

Wet decomposition or acid digestion involves the use of oxidizing agents (hydrogen peroxide) and mineral acids to affect the dissolution of a sample. Sulfuric acid is used as a dehydrating agent and has a high boiling point of around 300^{o}C 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. Aquaregia is widely used but again with chloride interferences.

2.9.4. Microwave Digestion

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% (Angel et al., 2007).

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 (Sneddon et al., 2006).

Chapter three: Experimental

3.1. Instrumentation and Apparatus

Analytical digital balance (SCIENTECH SA120, USA) was used to weigh the infant formula samples. Micropipette (DRAGONMED, 1-10 µL) was used for measuring different amounts of standard solutions. A 250 mL round bottomed flasks fitted with reflux condensers were used in kjeldahl apparatus (Gallen hamp) hot plate to digest the powdered infant formula samples. 50 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested samples until analysis.

3.2. Microwave Plasma-Atomic Emission Spectrometer (MP-AES).

As shown in figure 2, recently introduced Microwave Plasma-Atomic Emission Spectrometer (Agilent-4200, USA) is obvious interest in analytical chemistry. The merit of MP-AES equipment runs entirely on nitrogen gas, which means it reduce costs to carry out chemical analysis and increase the safety. MP-AES simultaneously measure very traces concentration of several elements. The gaseous reaction products are swept away by a flow of nitrogen in to MP-AES.

Figure 2 Microwave plasma- atomic emission spectrometer

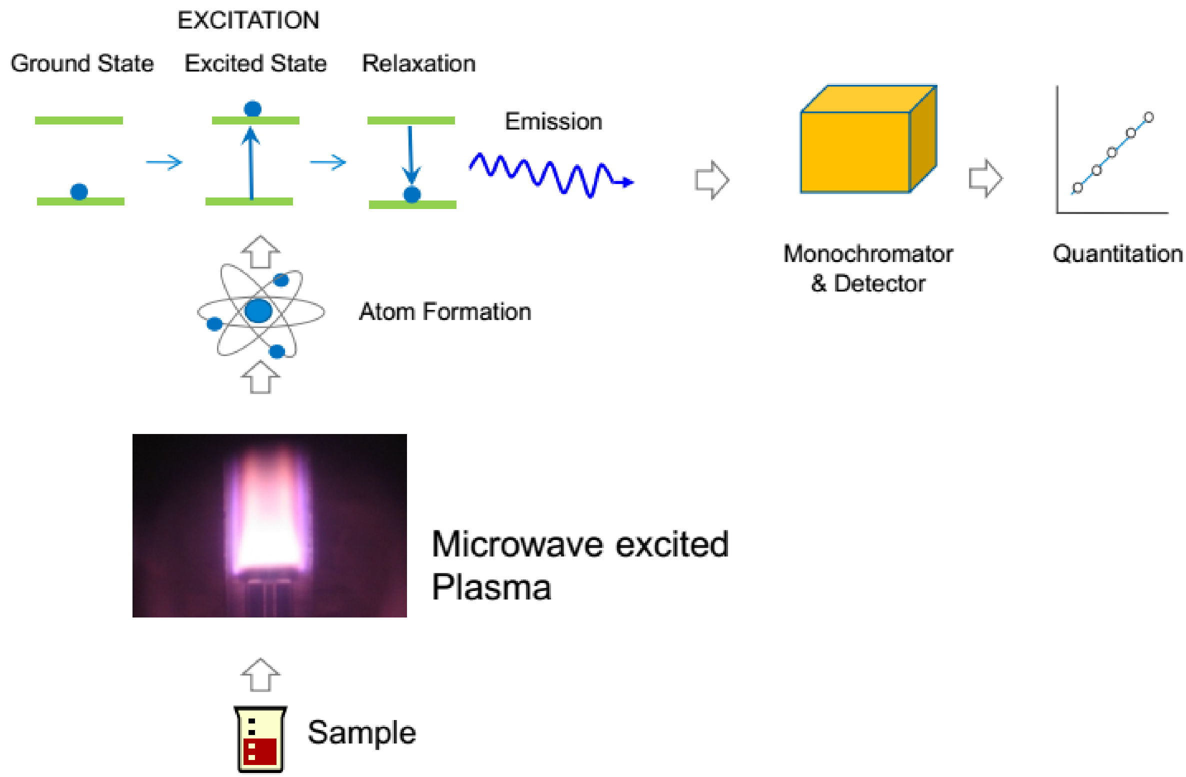


Figure 3 Microwave Plasma Emission Overview

In the MP-AES technique as shown in figure 3, high energy promotes the atoms in to exited electronic states that subsequently emit light when they return in to the ground electronic state. Each element emits light at a characteristic wave length which is isolated by a grating and detected via spectrometer. The wave length of the atomic spectral line provides the identity of the element (Helaluddin et al., 2016).

3.3. Reagents and Chemicals

Analytical grade chemicals and reagents were used in the analysis of samples of the infant formulas. 69-72% HNO_3 (Spectrosol, BDH, England), 70% $HClO_4$ (A.C.S. Aldrich UK, reagent) were used for the digestion of infant formula samples. Lanthanum nitrate hexa hydrate, La $(NO_3)_3.6H_2O$ (99% Aldrich, USA) was used to prevent the interference of phosphates in the measurement of Ca. A stock standard solution of 1000 mg/L, in 2% HNO_3 of the metals Mn, Fe, Zn, Cu and Cr (BUCK SCIENTIFIC PURO-GRAPHIC) were used for preparation of calibration standards and in the spiking experiments. Intermediate standard solutions of the metals were prepared by diluting the stock standard solutions to 10 mg/L and then the working standard solutions were prepared from the intermediate solutions before analysis. Deionized water was used throughout the experiment for rinsing the apparatus and diluting of sample solutions.

3.4 Sample Collection

Figure 4. Different Infant formulas

Four different brands of commercially available infant formulas as shown in figure 4 were used in this study. The four types of infant formulas used in the experiment were Liptomil, Mamilac, Bebelac and Anchor (Figure 4). Each infant formula was purchased from different supermarkets based on random sampling. For each of the infant formula brands, 50 g of the powder was taken and mixed to prepare the bulk samples. From each bulk sample, 0.5 g was used for digestion of the samples.

Table 1 Special characteristic of infant formulas. All samples were packaged in metal containers. Brand Sample Information: Follow-on Infant Milk Formulas (6-12 months).

Infant formula	Special characteristics
Liptomil (Switzerland)	Choline and taurine enriched.
Mamilac (Poland)	Minerals, vitamins and prebiotic.
Bebelac (France)	Vitamin D and Calcium enriched.
Anchor (New Zealand)	33 nutrients for growth and development.

3.5. Working Procedures

3.5.1. Cleaning Equipments

Equipments such as volumetric flasks, measuring cylinders and digestion flasks were washed with detergents and tap water then rinsed with deionized water, soaked in HNO_3 for 24 hours and again rinsed with deionized water.

3.5.2. Optimization of Digestion Procedure

Table 2 Optimization of reagent volume

Volume (mL)	Temperature (^{O}C)	Time (hr)	Result
V_1 = 5 mL HNO_3: 1 mL $HClO_4$	300	2	Light yellow
V_2 = 4 mL HNO_3: 2 mL $HClO_4$	300	2	Light yellow
V_3 = 3 mL HNO_3: 3 mL $HClO_4$	300	2	Clear with residue
V_4 = 2 mL HNO_3: 1 mL $HClO_4$	300	2	Clear &colorless
V_5 = 3 mL HNO_3: 2 mL $HClO_4$	300	2	Clear with residue
V_6 = 3 mL HNO_3: 1 mL $HClO_4$	300	2	Clear with residue

As shown in Table 2, V_4 = 2 mL HNO_3: 1 mL $HClO_4$ was preferred because the solution appeared clear and colorless at minimum volume level.

Table 3 Optimization of digestion temperature

Volume (mL)	Temperature (^{O}C)	Time (hr)	Result
V_1 = 2 mL HNO_3: 1 mL $HClO_4$	60	2	Light yellow
V_2 = 2 mL HNO_3: 1 mL $HClO_4$	90	2	Clear with residue
V_3 = 2 mL HNO_3: 1 mL $HClO_4$	120	2	Clear with residue
V_4 = 2 mL HNO_3: 1 mL $HClO_4$	150	2	Clear & colorless
V_5 = 2 mL HNO_3: 1 mL $HClO_4$	180	2	Clear & colorless
V_6 = 2 mL HNO_3: 1 mL $HClO_4$	210	2	Clear & colorless

As shown in Table 3, the result of digestion temperature 150, 180 and 210 ^{O}C are clear & colorless solutions. To minimize the resource, it is possible to reject higher temperatures. Therefore T_4 = 150 ^{O}C was preferred because the solution appeared clear and colorless at minimum temperature level.

Table 4. Optimization of digestion time

Volume (mL)	Temperature (^{O}C)	Time (min)	Result
V_1 = 2 mL HNO_3: 1 mL $HClO_4$	150	30	Light yellow
V_2 = 2 mL HNO_3: 1 mL $HClO_4$	150	45	Clear with residue
V_3 = 2 mL HNO_3: 1 mL $HClO_4$	150	60	Clear & colorless
V_4 = 2 mL HNO_3: 1 mL $HClO_4$	150	75	Clear & colorless
V_5 = 2 mL HNO_3: 1 mL $HClO_4$	150	90	Clear & colorless
V_6 = 2 mL HNO_3: 1 mL $HClO_4$	150	120	Clear & colorless

As shown in Table 4, the result of digestion time 60, 75, 90 min shows clear & colorless solutions. Similarly to minimize the resource, t_3 = 60 min or 1 hr was preferred because the solution appeared clear and colorless at minimum time level.

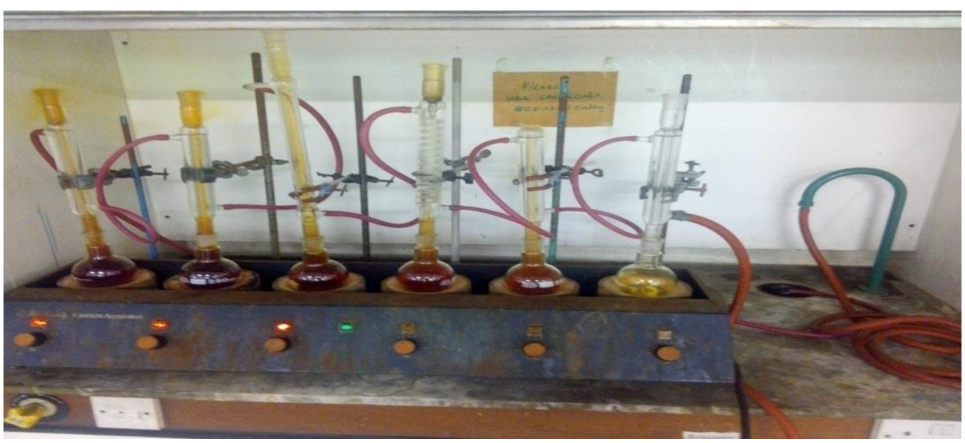


Figure 5 Optimization of digestion experiment

As shown in figure 5, a clear colorless sample solution that is suitable for the analysis using MP-AES was prepared. Different infant formula digestion procedures were assessed using HNO_3,

$HClO_4$ acid mixtures by varying parameters such as volume of the acid mixtures, digestion time and temperature. A 0.5 g of infant formula sample was weighed on a digital analytical balance and transferred quantitatively in to a 250 mL round bottomed flask. To this flask 2.0 mL of HNO_3 (62-72%) and 1.0 mL of $HClO_4$ (70%) were added to the sample and the mixture were digested on a Kjeldahl digestion apparatus by setting the temperature marked at 5 (150 ^{O}C) for 1 hr. The digestion gave a clear colorless solution and the digested sample was allowed to cool for 10 min and transferred quantitatively to a 50 mL volumetric flask and diluted with deionized water up to the mark. Each infant formula sample was digested in triplicate and hence a total of twelve digests were made for the samples of the infant formulas. Three reagent blank solutions were prepared by following the same digestion procedure as the sample. All the digested samples were stored in refrigerator until the levels of all the metals in the sample solutions were determined by MP-AES.

3.5.3. Calibration of Instruments

Calibration of an instrument or a piece of equipment involves making of a comparison of a measured quantity against reference value. Instrument calibration is particularly important and must be checked on a day-to-day or run-to-run basis. For example, to calibrate a spectro photometer response, select the appropriate reference material and measure the spectrometer response to it under the specified conditions and compare the measured value with the value quoted in the literature.

Calibration curves were prepared to determine the concentration of the metals in the sample solution. A series of working standard solutions were prepared from the 10 mg/L intermediate standard solutions of their respective metals which were prepared prior by taking 1 mL from the stock standard solutions containing 1000 mg/L in 2% $HNO_{3.}$ The intensity of working standard solutions was measured and the calibration curves for each of the analyzed metal (Na, K, Ca, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) were made by the operating conditions for MP-AES employed for each analyte as shown in Table 5.

Table 5 Operating conditions for MP-AES

Metal	Wavelength(nm)	Calibration Coefficient Limit	Instrument Detection Limit
Na	589.592	0.95	0.001
K	766.491	0.95	0.001
Ca	422.673	0.95	0.001
Al	396.152	0.95	0.001
Cr	427.48	0.95	0.001
Mn	259.372	0.95	0.001
Fe	371.993	0.95	0.001
Co	340.512	0.95	0.001
Ni	305.081	0.95	0.001
Cu	327.395	0.95	0.001
Zn	213.857	0.95	0.001
Cd	226.502	0.95	0.0001
Pb	283.305	0.95	0.0001

Chapter four: Results and Discussion

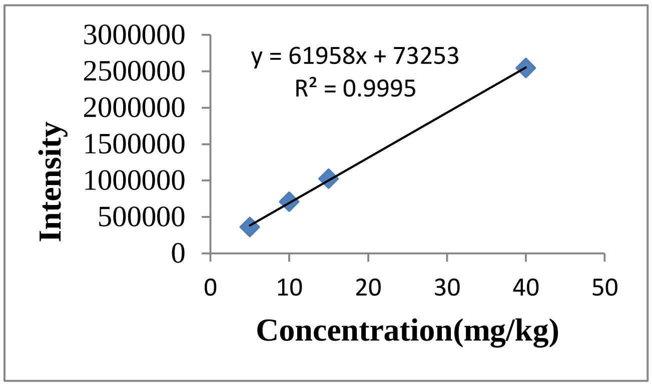
Following the optimized procedure, digestion was performed by taking 0.5g of infant formulas, weighed in triplicate using analytical digital balance and put in to 250 mL round bottom flask. Then 2 mL HNO_3 and 1 mL $HClO_4$ was added. The round bottom flask was fitted to a reflux condenser and heated on Kjeldahl apparatus hot plate for 1 hour at a temperature of 150 ^{O}C. The digest sample was allowed to cool for 10 min without separating the condenser and then further cooled to room temperature for 10 min by separating the condenser. The mixture was diluted with 20 mL of distilled deionized water and filtered with Whatman filter paper No.42 (Germany) in to 50 mL volumetric flask. The round bottom flask was further rinsed with 10 mL of distilled deionized water and the digested sample was kept in a refrigerator until analyzed by MP-AES.

Table 6 Working standard concentrations, intensity, correlation coefficient and equation of the calibration curves for determination of metals by MP-AES.

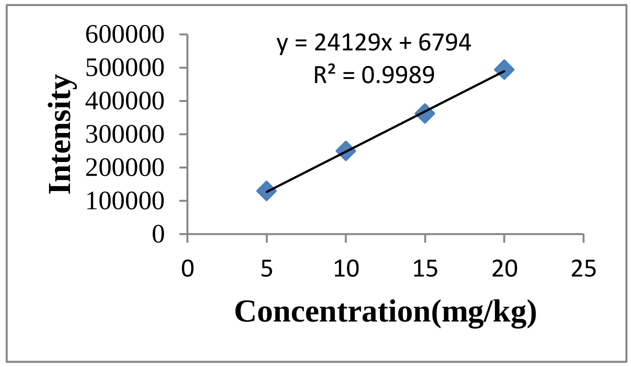
No	Metal	Working Standard Conc.(mg/kg)	Intensity	Correlation Coefficient	Equation of the calibration Curves
1	Na	5	356324	0.9995	y = 61958x + 73253
		10	706832		
		15	1023218		
		20	2543670		
2	K	5	129016	0.9989	y = 24129x + 6794
		10	249364		
		15	361437		
		20	493808		
3	Ca	5	355015	0.9983	y = 67343x + 21867
		10	712524		
		15	1008267		
		20	1378821		
4	Al	0.5	12563	0.9999	y = 24298x + 759
		1.0	25460		
		2.0	49355		
		4.0	97894		
5	Cr	0.5	10829	0.9997	y = 21960x - 184
		1.0	21166		
		2.0	44604		

		4.0	87372		
6	Mn	0.5	1614	0.9996	y = 2977x + 206
		1.0	3308		
		2.0	6114		
		4.0	12116		
7	Fe	5	25521	0.9986	y = 5627x - 3869
		10	50565		
		15	80420		
		20	109345		
8	Co	5	23931	0.9987	y = 5135x - 2873
		10	46970		
		15	73775		
		20	100577		
9	Ni	0.5	2361	0.9997	y = 4453x + 271
		1.0	4875		
		2.0	9189		
		4.0	18057		
10	Cu	0.5	16374	0.9997	y = 33562x + 525
		1.0	34571		
		2.0	68556		
		4.0	134316		
11	Zn	0.5	2496	0.9995	y = 4542x + 302
		1.0	4772		
		2.0	9627		
		4.0	18376		
12	Pb	0.5	837	0.9997	y = 1723x + 6
		1.0	1722		
		2.0	3517		
		4.0	6871		
13	Cd	0.5	408	0.9983	y = 661x + 119
		1.0	791		
		2.0	1494		
		4.0	2736		

4.1. The calibration curve of the metals.



A)



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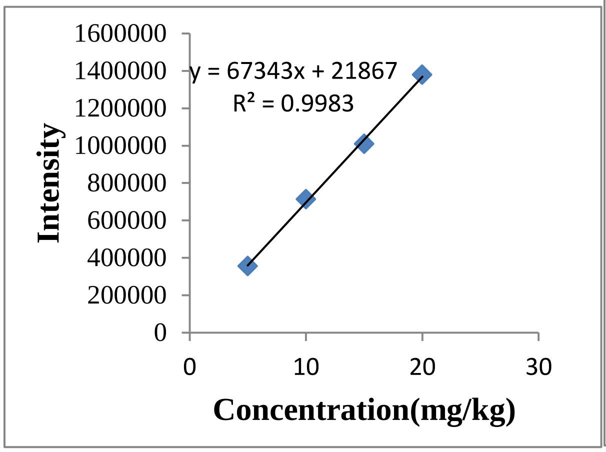
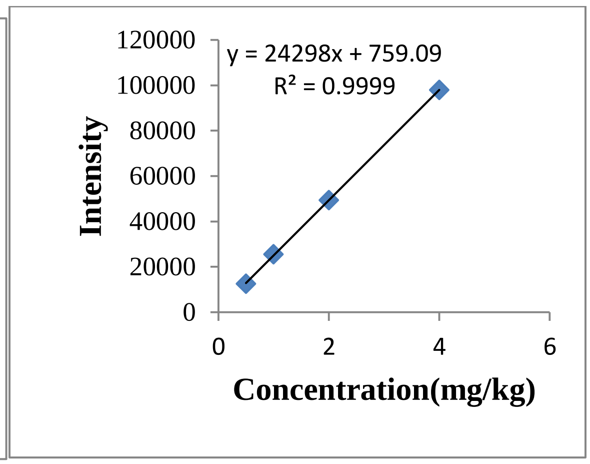
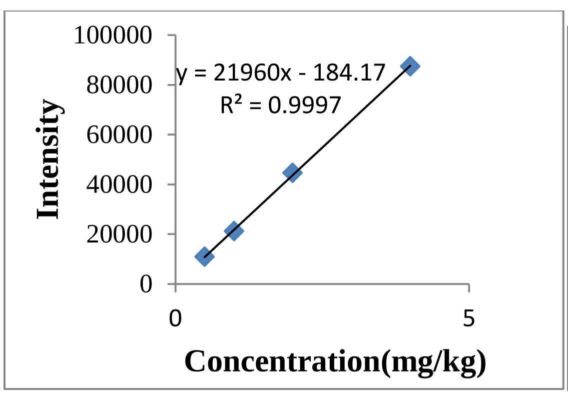


C)



D)



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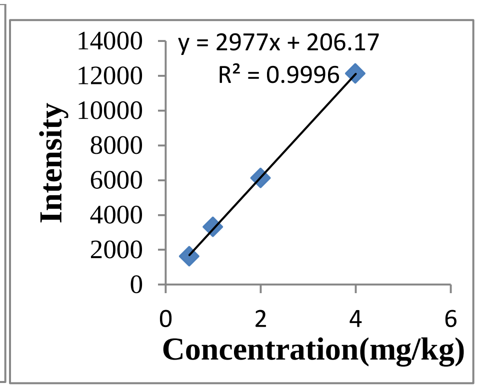

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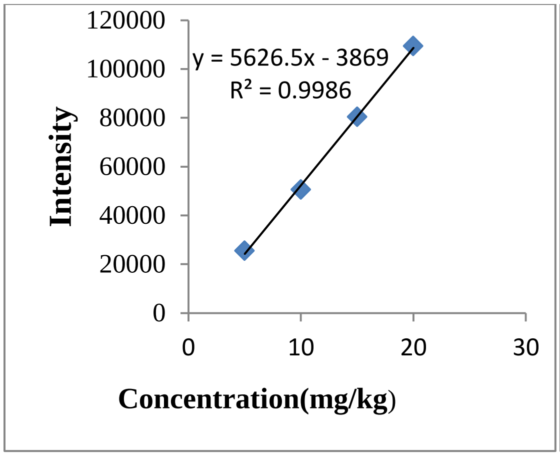
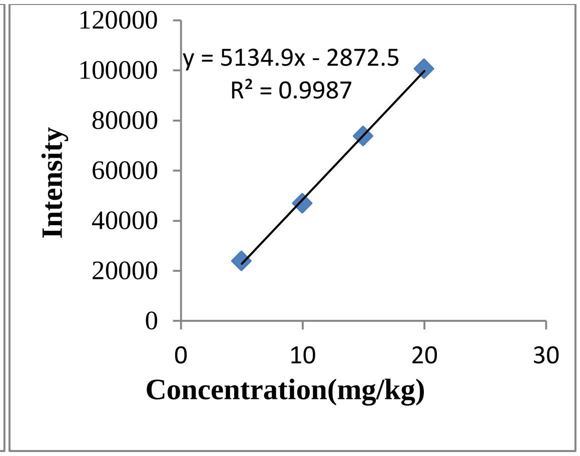
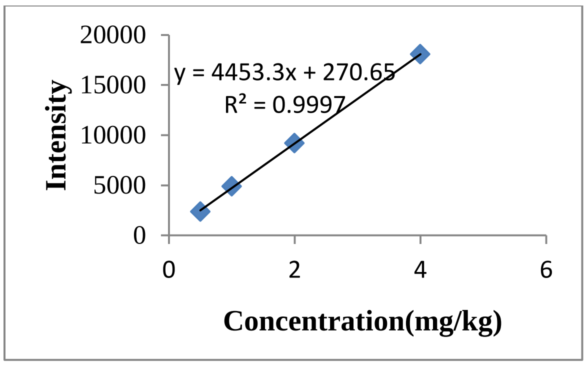
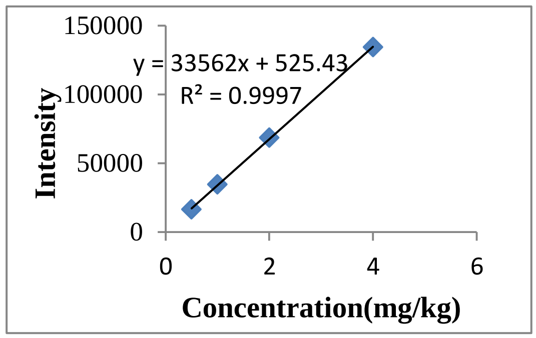

y = 5626.5x - 3869
R² = 0.9986
Intensity
Concentration(mg/kg)


y = 5134.9x - 2872.5
R² = 0.9987
Intensity
Concentration(mg/kg)

G)

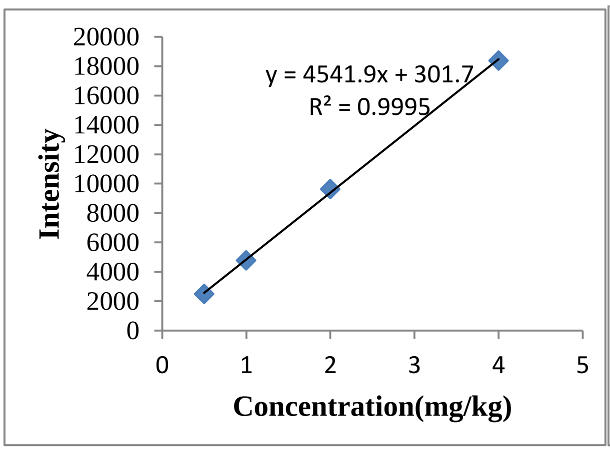
H)

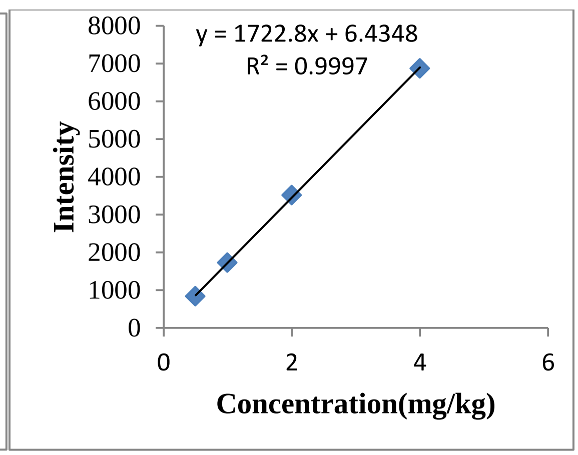

y = 4453.3x + 270.65
R² = 0.9997
Intensity
Concentration(mg/kg)


y = 33562x + 525.43
R² = 0.9997
Intensity
Concentration(mg/kg)

I)

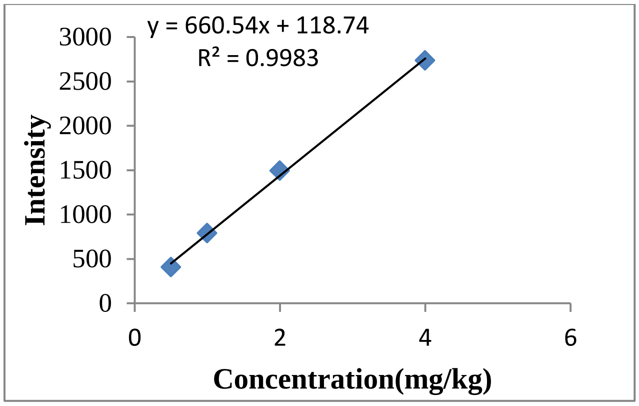
J)


y = 4541.9x + 301.7
R² = 0.9995
Intensity
Concentration(mg/kg)


y = 1722.8x + 6.4348
R² = 0.9997
Intensity
Concentration(mg/kg)

K)

L)



M)

Figure 6 Calibration curve for A) Sodium B) Potassium C) Calcium D) Aluminum E) Chromium F) Manganese G) Iron H) Cobalt I) Nickel J) Copper K) Zinc L) Cadmium M) Lead.

4.2. Method Validation

4.2.1. Precision

The precision of an analytical procedure expresses the closeness or agreement between a set of results. The precision of an analytical procedure is expressed by the variance, standard deviation of a series of measurements. In this study the precision of the results were evaluated by the standard deviation and percentage relative standard deviation of the results of four samples. These parameters are useful in estimating and reporting the probable size of indeterminate errors. The results of analysis were reported with the corresponding pooled standard deviation of 12 measurements and relative standard deviation. It can be seen that the values of percentage relative standard deviations (% RSD) are less than 10% for all the mean concentrations as summarized in Table 8. This shows the precision of the results obtained by this method is good.

4.2.2. Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured by the analytical method with a given confidence limit (Gazsco et al., 2001). In this work, after digestion of three blank solutions, triplicate readings were obtained for each sample. Then the pooled standard deviation of the three blank reagents was calculated. The method detection limit of each element was obtained by multiplying the standard deviation of the reagent blank by three (3σ blank, n =10), which is summarized in Table 7.

Table 7 Method detection limit for infant formula samples in (mg/kg).

Element	Na	K	Ca	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Pb	Cd
MDL (mg/kg)	3.09	2.12	3.21	0.72	0.06	0.21	1.29	0.06	0.03	0.003	1.02	BDL	BDL
IDL	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.0001	0.0001

In order to ascertain the reliability of the method for the analysis of the samples for major and trace metals, Certified Standard Reference Materials (CSRM) were not available for use in our laboratory or around instead the validity (accuracy) of the analytical procedures and efficiency of MP-AES used for sample treatment and analysis were tested by spiking experiment in a selected sample. From the stock solution (1000 mg/L) standard solutions, the following concentration of metals were taken. 25 µL of Fe, 27 µL of Zn, 3.5 µL of Cr, 3.5 µL of Mn and 3.5 µL of Cu from 1000 mg/L solutions were added to 0.5g infant formula samples. As the values given in Table 8, the results of the recoveries for all the metals in the analyzed samples were all found within 100 ± 10 percent. Therefore, the recovery test and reproducibility of the method were found satisfactory and indicates the digestion method used for sample preparation is precise and reliable.

$$\% \text{ Recovery} = \frac{(\text{Amount after spike} - \text{Amount before spike})}{\text{Amount Added}} \times 100$$

Table 8 Recovery results obtained for validation of the optimized procedure after spiking with standard solutions.

Element	Amount before spike(mg/kg)	Amount added (µL)	Amount after spike (mg/kg)	% Recovery
Fe	160 ± 15	25	186 ± 0.4	104 ± 0.4
Zn	135 ± 10	27	161 ± 0.23	96 ± 0.23
Mn	14 ± 1.2	3.5	17 ± 0.05	94 ± 0.05
Cr	14 ± 0.7	3.5	17 ± 0.01	98 ± 0.01
Cu	11 ± 0.4	3.5	14 ± 0.04	97 ± 0.04

Values are mean ± SD of triplicate readings of triplicate analyses.

4.3. Determination of the amount of Metals in Infant Formulas

The concentrations of 13 major and trace metals (Na, K, Ca, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb and Cd) in the infant formulas were determined by MP-AES. The analyzed metals except cadmium and lead which were below the method detection limit have been detected and quantified as given in Table 9. The most abundant among the metals is Ca followed by K and Na.

As it can be seen from Table 9 and Figure 19-21, quantitatively there is a variation in concentration of essential metals with in the infant formula brands. From the analyzed brands of infant formulas, Anchor has the highest concentration of major elements such as: Ca (43373.4), K (3605.4), Na (1163.7), Fe (187.7) and Mn (14.9) mg/kg. The lowest concentration of Ca (38258.7) mg/kg was found in Mamilac where as the lowest concentration of K (2572.9), Na (691.1), Fe (142.3), Mn (13.1) mg/kg were found in Bebelac. The remaining trace metals such as Zn (135.1), Al (70.9) mg/kg are highest concentration in Liptomil but these elements Zn (98.2) and Al (33.8) mg/kg were found lowest in Bebelac. Another group of trace metals Cu (11.4) and Ni (0.56) mg/kg were found highest in Mamilac but Cu (8.9) and Ni (0.32) mg/kg was lowest Anchor. On the other hand Cr (16.4) and Co (2.5) were highest in Bebelac but lowest concentration in Cr (13.6) in Liptomil and Co (0.02) in Mamilac.

The percentage relative standard deviations (% RSD) for Ca is in the range of 1.2 – 4.6%, for K 2.6-9.3%, for Na 2.3-9.6%, for Fe 3.7-9.4%, for Mn 1.1-8.6%, for Zn 5.5-7.6%, for Al 4.3-7.4%, for Cu 2.1-6.5%, for Ni 6.4-9.6%, for Cr 3.8-7.6%, for Co 4.5-7.9%. The percentage relative standard deviations (% RSD) were less than 10% for all the analyzed elements. These indicate that there is good precision in the measurements. As it can be seen from Table 12 and Figure 19-21, the pattern of concentration of elements in all brands (Anchor, Liptomil, Mamilac and Bebelac) was decreased as Ca > K > Na > Fe > Zn > Al > Cr > Mn > Cu > Co > Ni. The concentrations of Ca, K and Na are highest because they are major elements. From the trace metals Fe is the highest followed by Zn and the lowest was Ni.

Table 9 Concentration of metals in infant formula samples using MPAES (mg/kg)

Metal	Liptomin	%RSD	Mamilac	%RSD	Bebelac	%RSD	Anchor	%RSD
Na	715 ± 57	7.9	740 ±42	5.6	691 ± 66.4	9.6	1163.7± 28	2.3
K	1872 ± 49.8	2.6	2081 ± 155	7.4	2573 ± 240	9.3	3605 ± 223	6.2
Ca	38550 ± 486	1.2	38259 ± 995	2.5	41964 ± 191	4.6	43373 ± 12	3
Al	70.9 ± 3.1	4.3	43.6 ± 3	6.8	33.8 ± 2.2	6.4	59.8 ± 4.5	7.4
Cr	13.6 ± 0.7	4.9	16.1 ± 1.2	7.6	16.4 ± 1	6.1	15.3 ± 0.6	3.8
Mn	13.7 ± 1.2	8.6	14.1 ± 0.2	1.1	13.1 ± 0.7	5.1	15 ± 0.7	4.6
Fe	160 ± 15	9.4	149 ± 5.6	3.7	142 ± 10.1	7	188 ± 14	7.6
Co	1.8 ± 0.1	7.9	0.02 ± 0.1	4.5	2.5 ± 0.1	4.6	2.5 ± 0.2	6.6
Ni	0.4 ± 0.1	8.3	0.6 ± 0.1	6.4	0.4 ± 0.1	9.2	0.3 ± 0.1	9.6
Cu	10.6 ± 0.4	3.5	11.4 ± 0.3	2.1	10.5 ± 0.5	4.9	8.9 ± 0.6	6.5
Zn	135 ± 10	7.4	121 ± 0.3	7.6	98 ± 5.5	5.5	129 ± 9	7

4.4. Distribution of Metals in Infant Formula Samples

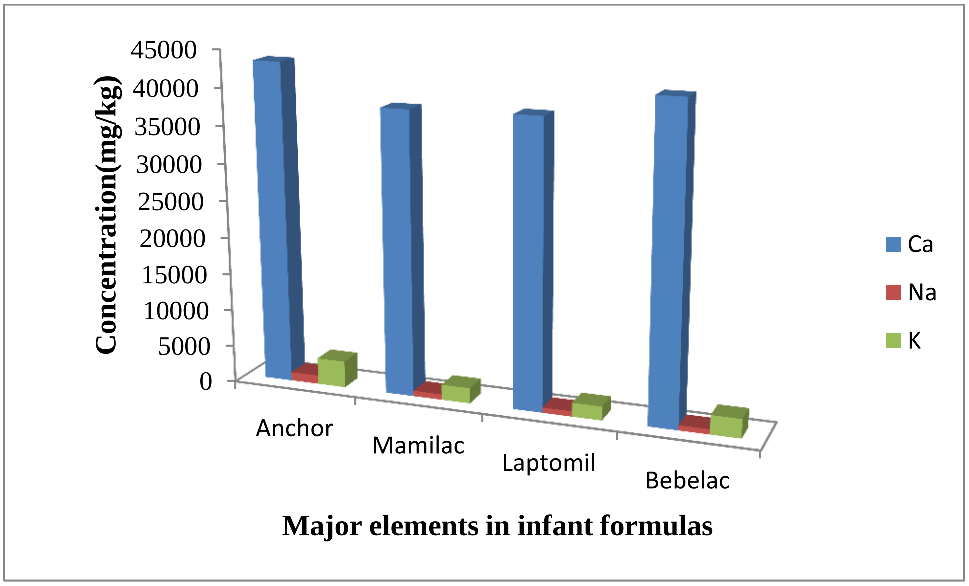


Figure 7 Graph concentrations of Ca, Na and K in different infant formula samples.

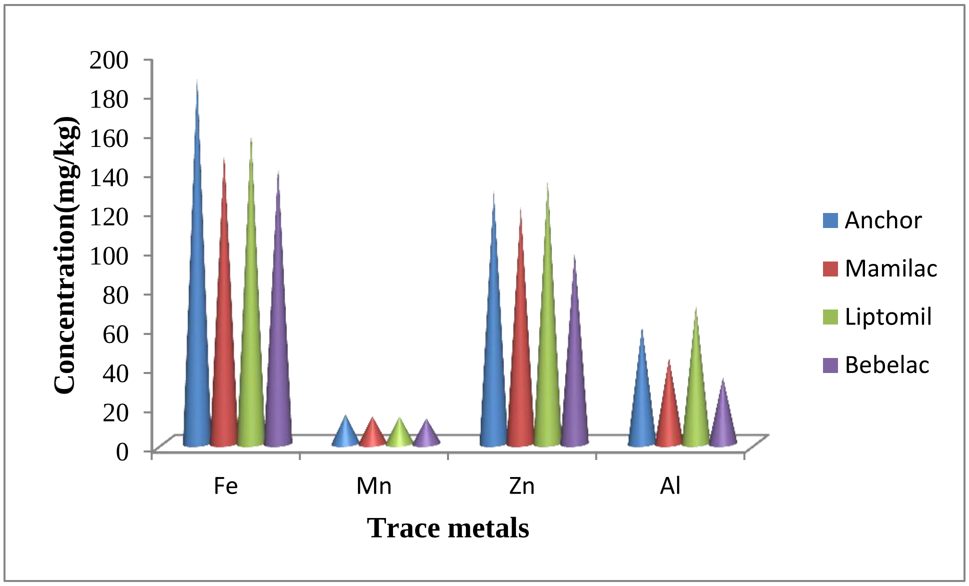


Figure 8 Graph concentrations of Fe, Mn, Zn, Al in different infant formula samples.

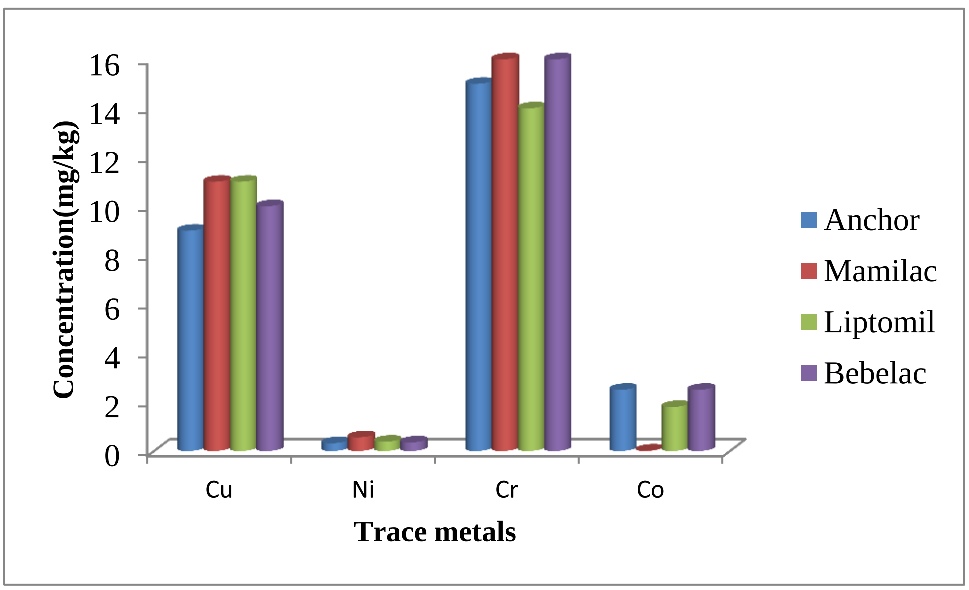


Figure 9 Graph concentration of Cu, Ni, Cr, Co in different infant formulas

4.5.Comparison of Observed Metals Concentrations with Feeding Tables Supplied by the Infant Formula Manufacturers

Table 10 Comparison of major and trace metals of the present study with the feeding tables supplied by infant formula manufacturers (mg/100g).

Metal	Liptomin (a) mg/100g	Liptomin (b) mg/100g	Mamilac (a) mg/100g	Mamilac (b) mg/100g	Bebelac (a) mg/100g	Bebelac (b) mg/100g	Anchor (a) mg/100g	Anchor (b) mg/100g
Na	71.5	200	74	170	69.1	184	116.4	290
K	187.2	600	208.2	610	257.3	810	360.5	1333
Ca	385.5	550	382.6	460	419.6	679	433.7	606
Al	7.1	NR	4.4	NR	3.4	NR	5.9	NR
Cr	1.4	NR	1.6	NR	1.6	NR	1.5	NR
Mn	1.4	0.7	1.4	0.6	1.3	0.05	1.5	0.15
Fe	16	8	14.9	8	14.2	6.9	18.7	8
Co	0.2	NR	0.01	NR	0.3	NR	0.2	NR
Ni	0.04	NR	0.06	NR	0.04	NR	0.03	NR
Cu	1.1	3.5	1.1	4.1	1.1	0.3	0.8.	0.4
Zn	13.5	6	12.1	4.2	9.8	4.5	12.9	7

(a) - Concentration of metals of present study.

(b) - Concentration of metals from infant formula feeding table.

NR – Not Reported.

Even though all the manufacturers recommended that breast milk is the best for babies, they suggested that preparing artificial feeding which substitutes breast milk is necessary for infants who cannot be fed at the breast, or should not receive breast milk or for whom breast milk is not available. It is believed that the infant formulas prepared by the manufacturers provide all the known essential nutrients for growth and development of infants. The comparison of major and trace metals in infant formula brands of this work with the feeding tables supplied by infant formula manufacturers is given in table 10. The mean concentrations of most metals in this study are comparable with the mean concentrations of metals given by the manufacturers in all brands. The concentrations of Na, K and Ca are less than the concentrations given by the manufacturers in all brands. The concentrations of Mn, Fe, and Zn are greater than the concentrations given by the manufacturers in all brands. The concentration of Cu is less than the feeding tables in Liptomil and Mamilac but greater than the feeding tables given by the manufacturers in Bebelac and Anchor. The rest metals Al, Cr, Co and Ni are not registered in the feeding tables so they are not compared. This difference may be arising due to the difference in digestion method and analytical method used for analysis.

4.6. Comparison of Observed Metals Concentration with Literature Values

Table-11 Comparison of metals concentrations of the present study with literature report (mg/100g).

Countries	Ca	Mn	Fe	Cu	Zn	Reference
*Ethiopia	3855-4337	1.4-1.5	14.2-18.8	0.9-1.1	9.8-13.5	Saracoglu et al , 2009
New Zealand	3670-4920	0.73-4.04	25.5-80.9	1.94-4.53	13.7-42.3	
Turkey	NR	0.31-3.29	1.02 - 67.5	1.48-2.63	21.9-29.8	
India	NR	NR	NR	1.11-3.16	9.37-34.59	

***** Present study in Ethiopia

Determination of major and trace metals in infant formulas has received considerable attention in order to save the health of infants. Essential metals may be toxic for infants when taken in excess and also some toxic metals found in infant formulas can affect the health of infants even at lower concentration. Therefore, different researchers have reported the concentration of metals in infant formulas. As it can beseen from Table 11, the concentration of Ca and Mn in present study fits with the reported value in New Zealand. In addition, the concentration of Mn in the present work was found in the range of the reported value of Turkey. The amount of Fe, Cu and Zn of the present work is found below the range of the reported value. The concentration of Fe in the present study is almost comparable with the reported value in Turkey but lower than the amount reported in New Zealand. The amount of Zn and Cu is lower than the concentration reported in New Zealand as well as in Turkey. The concentration of Zn in the present study is within the range concentration reported in India. The concentrations of Cu almost comparable with the values reported in India.

4.7. Statistical Analysis

Statistical methods and analysis are often used to communicate research findings to support hypothesis and give credibility to research methodology and conclusions. In analytical work two or more mean results are compared to check whether there is a significant difference or not.

The variation between the means of different samples can result from random and controlled sources of errors. Analysis of variance (ANOVA) is a collection of statistical models and their associated estimations procedures such as “variation” among and between group means in a sample. The variation comes from different sources such as experimental procedures or heterogeneity among samples. This variation can be estimated by a powerful statistical technique known as analysis of variance (Miller, 2010). In this research a one-way ANOVA and Pearson correlation coefficient were used to determine the source of variation between samples and to correlate the effect of one metal concentration on the other metal respectively.

4.7.1. Analysis of variance (ANOVA)

Table 12 Analysis of variance between infant formula samples at 95% confidence level

Metal	Na	K	Ca	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn
F_{cal}	23.2	33	2.4	3.7	0.3	1.6	3.6	22.9	5.8	1.7	5.4
F_{crit}	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
P_{value}	0.0003	0.0007	0.14	0.06	0.83	0.27	0.07	0.0003	0.02	0.24	0.02

The F statistics must be used in combination with the P value when deciding if the overall results are significant. The larger the F_{cal} or F_{crit} value the greater the relative variance among the group means. Performing a hypothesis test in statistics, a p-value helps to determine the significance of the results. The P value is a number between 0 and 1 and interpreted a small P value ($P \leq 0.05$) indicates a strong evidence against the null hypothesis. So it will have a significance difference (Lucia, 1986). In this study the variation in sample means was tested whether they have significance difference or not by using one-way ANOVA of SPSS version. Accordingly, there is no significant difference among the sample means for Ca, Al, Cr, Mn, Fe and Cu ($p \geq 0.05$). While there is a significant difference among the sample means for Na, K, Co, Ni and Zn ($p \leq 0.05$). This may be due to variations in factors other than experimental procedure. The source for this significant difference may be due to contamination, error in preparing and mixing ingredients during manufacturing of infant formulas.

4.7.2. Pearson Correlation coefficient of metals in infant formula samples

Pearson correlation is a number between -1 and 1 that indicates the extent to which two variables are linearly related. If the correlation coefficient approaches to positive one, there is a strong positive relationship between variables. If the correlation coefficient is -1, the two variables have a strong negative relationship. If there is no relationship between the two variables, the correlation coefficient equal to zero. In this research; to correlate the effect of one metal concentration to the other, the Pearson correlation matrices using correlation coefficient(r) for the sample was employed for metals determined in the sample and presented in table 13. The result of Pearson correlation shows that positive correlation are observed in Na with (Al, Co, Zn), K with (Cr), Ca with (Cr, Mn), Al with (Na, Co), Cr with (K, Ca, Ni, Cu), Mn with (Ca), Fe with (Co), Co with (Na, Al, Fe), Ni with (Cr), Cu with (Cr), Zn with (Na) were observed to have positive weak correlation. Weak correlation indicates that the presence or absence of one metal affects the other metal in a lesser extent. Positive strong correlation were observed in the metals Na with (K, Ca, Mn, Fe), K with (Na, Ca, Mn, Fe, Co), Ca with (Na, K, Fe, Co), Al with (Mn, Fe, Zn), Mn with (Na, K, Al, Fe, Zn), Fe with (Na, K, Ca, Al, Mn, Zn), Co with (K, Ca), Ni with (Cu), Cu with (Ni), Zn with (Al, Mn, Fe). This strong correlation may arise from common natural sources as well as from similarity in chemical properties.

While Na, K, Ca, Al, Mn, Fe, Co and Zn shows negative correlation. Na with (Cr, Ni, Cu), K with (Al, Ni, Cu), Ca with (Al, Ni, Cu, Zn), Al with (K, Ca, Cr, Ni, Cu), Cr with (Na, Al, Mn, Fe, Co, Zn), Mn with (Cr, Ni, Cu), Fe with (Cr, Ni, Cu), Co with (Cr, Ni, Cu, Zn), Ni with (Na, K, Ca, Al, Mn, Fe, Co, Zn), Cu with (Na, K, Ca, Al, Mn, Fe, Co, Zn), Zn with (K, Ca, Cr, Co, Ni, Cu). Zero correlation was observed in Mn with Co.

Table 13 Pearson correlation matrices for metals in infant formulas

	Na	*K*	*Ca*	*Al*	*Cr*	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*
Na	1										
K	0.898	1									
Ca	0.691	0.927	1								
Al	0.334	-0.028	-0.18	1							
Cr	-0.17	0.112	0.156	-0.957	1						
Mn	0.857	0.545	0.227	0.645	-0.426	1					
Fe	0.939	0.73	0.513	0.637	-0.495	0.928	1				
Co	0.374	0.583	0.792	0.127	-0.293	0	0.373	1			
Ni	-0.601	-0.677	-0.774	-0.39	0.484	-0.324	-0.651	-0.942	1		
Cu	-0.888	-0.901	-0.854	-0.347	0.316	-0.624	-0.863	-0.756	0.898	1	
Zn	0.389	-0.043	-0.308	0.929	-0.789	0.781	0.648	-0.186	-0.127	-0.227	1

5. Conclusion and Recommendation

In this study commercially available powdered infant formulas (Liptomil, Mamilac, Bebelac and Anchor) were analyzed for their contents of Na, K, Ca, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb and Cd effectively by using MP-AES. The optimized wet digestion method was found efficient for the metals and it was evaluated through the recovery experiment and a good percentage recovery was obtained for the metals identified. From the analyzed powdered infant formulas, Anchor has the highest content of major metals than other brands of infant formulas and Bebelac comparatively the lowest. Pb and Cd were below the method detection limit in all powdered infant formulas.

The concentrations of metals for this study were compared with metals concentrations with feeding tables supplied by the infant formula manufacturers. The concentrations of most of the metals determined are almost comparable with the feeding tables supplied by the infant formula manufacturers. In addition, the observed results were also compared with the reported literature values analyzed from other parts of the world. The concentrations of most of the metals determined are found to be within the range of literature values. Bottle fed infants consuming formulations with low or high levels of major and trace metals may suffer from nutritional excesses or deficiencies and consequent health problems. 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 major and trace metal contents of imported powdered infant formulas. But to have a complete and general information further research should focus on the composition of infant formulas.

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