ASSESSMENT OF NUTRITIONAL VALUE AND HEAVY METAL CONTAMINATION IN BABY FORMULAS IN BANGLADESH

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Roll No. : 0117/05

Registration No.: 425

Session: 2017-2018

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

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June 2019

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Dedicated

to

My Beloved Family

Acknowledgements

At the very beginning, the author would like to express her deepest gratitude to the Almighty God for giving her the ability and the strength to finish the task successfully within the scheduled time.

I pay heartily gratitude to **Professor Dr. Goutam Buddha Das**, Vice-Chancellor, Chattogram Veterinary and Animal Sciences University (CVASU) for giving special opportunity and providing such research facilities.

The author would like to express her heartiest gratitude to her supervisor **Md. A. Halim**, Professor, Department of Agricultural Economics and Social Sciences, Chattogram Veterinary and Animal Sciences University, due to his valuable suggestions and strict guidance made it possible to prepare a well-organized report.

The author also expresses her sincere appreciation and profound regards to the co-supervisor **Md. Altaf Hossain**, Assistant Professor, Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University for his scholastic guidance, valuable advice, radical investigation and constructive criticism in all phases of this study.

At the same time the author would like to express her gratitude, appreciation, regards and indebtedness to Professor, **Dr. Jannatara Khatun,** Dean, Faculty of Food science & Technology, Chittagong Veterinary & Animal Science University, for her initiation, guidance, constant inspiration and valuable suggestions.

I like to give special thank to the authority of Bangladesh Council of Scientific Industrial Research (BCSIR) for providing laboratory facilities. I sincerely thank to the Research and Extension of CVASU for giving me a research grant to accomplish my research work.

Finally, the Author also gratefully acknowledge with profound respect to beloved parents who have helped and supported her all way in whole life as friend.

The Author

June 2019

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List of Abbreviation

Abbreviation	Elaboration
%	Percentage
mg	Milligram
Kg	Kilogram
lb	Pound
CIF	Commercial Infant Formula
HIF	Homemade Infant Formula
PTWI	Provisional Tolerable Weekly Intake
RDA	Recommended Daily Allowance
JECFA	Joint FAO/WHO Expert Committee on Food Additives
WHO	World Health Organization
CF	Complementary Food
AGE	Advanced Glycation End
CR	Consumer Report
ATSDR	Agency for Toxic Substances and Disease Registry
IPHN	Institute of Public Health and Butrition
IFM	Infant Formula Milk
UNICEF	United Nations International Children's Emergency Fund
DNA	Deoxyribonucleic acid
AOAC	Association of Official Analytical Chemists
NRDC	Natural Resources Defense Council
AAS	Atomic Absorption Spectrophotometer
Kcal	Kilocalorie
KJ	Kilo joule
MAL	Maximum Allowed Limit
EER	Estimated Energy Requirement
ND	No Detectable
CHO	Carbohydrate
ANOVA	Analysis of variance
PVC	Polyvinyl chloride
USA	United State of America

Abstract

Infant formula is a synthetic version of mothers' milk belongs to a class of materials known as dairy substitutes. Although the World Health Organization recommends breastfeeding as the best feeding choice, infant formulas remain an alternative to breast-milk and plays an important role in infant's diet. The composition of most infant formulas has been increasingly altered to create a product which attempts to be as similar to breast milk as possible. The present study was carried out to evaluate the proximate composition, heavy and trace elements in five commercial infant formulas collected from Chattogram and in five homemade infant formulas. The proximate composition was determined by AOAC standard methods and metals wre quantified by Atomic Absorption Spectrophotometer. The moisture and carbohydrate content of both commercial and homemade formulas were within the standard level. However, the average protein content of the commercial infant formula (CIF) (15.43%) was considerably higher than that of homemade infant formula (HIF) (6.21%) and is also well below the recommended level for protein in infant food (12-20%). Also, the average fat content of the CIF (14.11 %) significantly exceeded the level found in HIF (1.59 %) and the set recommended level for fat in infant food (29.3- 40.0 %). The ash content was higher (3.03%) in CIF than HIF (1.90%), but both of formulas fiber content not exceeds the standard maximum value of 5%. The actual lead (Pb) content in all samples except RP (0.08 mg/kg) were higher the maximum allowed limit, 0.08 mg/kg. Another heavy metal Cadmium (Cd) average value was 0.03mg/kg in CIF and within the permissible limits 0.05mg/kg where HIF contained higher amount of Cd 0.06 mg/kg. Furthermore, average concentration of Chromium (Cr) in commercial infant formula sample was at higher concentration (0.144 mg/kg) than in homemade formula at 0.112 mg/kg where both are within the permissible limits 0.10-1.0 mg/kg. The concentrations of copper (Cu) found in this study were below the maximum allowed limits (1.5–2.5 mg/kg) and no health hazard is expected from copper in the consumption of these brands of infant foods. However, the zinc (Zn) contents in both of formulas fulfill the standard level (3.33 mg/kg) but iron (Fe) content of HIF was lower (1.64mg/kg) than standard value (2.9 mg/L) where the CIF contained 3.64 mg/kg.

Keywords: Infant formula, Heavy metal, Trace element, Proximate composition

Chapter-1: Introduction

1.1. Baby formula food:

Baby food is any soft, easily consumed food other than breast milk or infant formula that is made specifically for human babies between six months to two years old. The food comes in many varieties and flavors that are purchased ready-made from producers. Or it may be table food eaten by the family that has been mashed or otherwise broken down as a global public health recommendation, the World Health organization recommends that infants should be exclusively breastfed for the first six months of life to achieve optimal growth, development and health. Most six-month-old infants are physiologically and developmentally ready for new foods, textures and modes of feeding (WHO 2011). Experts advising the World Health Assembly have provided evidence that introducing solids earlier than six months increases babies' chances of illness, without improving growth.

The World Health Organization (WHO) currently recommends exclusively breastfeeding infants for the first 6 months of life and followed by introduction of adequate complementary foods (CF). This recommendation is for infants living in developing and developed countries. Although there is nearly universal agreement that breast milk alone is the optimal first food, the age range in which solids should be introduced is less clear, leading to “weanling’s dilemma”.(Qasem et al., 2015)

The complementary feeding period accompanies a critical window of vulnerability. During this time period, failure to grow is a significant concern (Shrimpton et al., 2011). Micronutrient deficiencies can also occur during this period, mostly because infants have higher nutrient demands relative to increased energy requirements. Deficiencies of certain micronutrients such as iron result in potentially irreversible negative effects on brain development and other detrimental psychological outcomes (Lozoff et al., 2006). There is general, but not universal, agreement that the iron stores of infants start to deplete at about 6 months of age, leaving the infants at high risk of iron deficiency and iron deficiency anemia. This is especially true among exclusively breastfed infants (Meinzen et al., 2006).

One of the health concerns associated with the introduction of solid foods before six months is iron deficiency. The early introduction of complementary foods may satisfy the hunger of the infant, resulting in less frequent breastfeeding and ultimately less milk production in the mother. Because iron absorption from human milk is depressed when the milk is in contact with other foods in the proximal small bowel, early use of complementary foods may increase the risk of iron depletion and anemia.

Baby foods are either a soft, liquid paste or an easily chewed food since babies lack developed muscles and teeth to effectively chew. Babies typically move to consuming baby food once nursing or formula is not sufficient for the child's appetite. Babies do not need to have teeth to transition to eating solid foods. Teeth, however, normally do begin to show up at this age. Care should be taken with certain foods that pose a choking hazard, such as undercooked vegetables, grapes, or food that may contain bones. Babies begin eating liquid style baby food consisting of pureed vegetables and fruits (Stephen et al., 2012).

When breast milk is no longer enough to meet the nutritional needs of the infant, complementary foods should be added to the diet of the child. The transition from exclusive breastfeeding to family foods, referred to as complementary feeding, typically covers the period from 6 to 18-24 months of age, and is a very vulnerable period. It is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children less than five years of age world-wide. WHO estimates that 2 out of 5 children are stunted in low-income countries.

Complementary feeding should be timely, meaning that all infants should start receiving foods in addition to breast milk from 6 months onwards. It should be adequate, meaning that the complementary foods should be given in amounts, frequency, and consistency and using a variety of foods to cover the nutritional needs of the growing child while maintaining breastfeeding.

Foods should be prepared and given in a safe manner, meaning that measures are taken to minimize the risk of contamination with pathogens. And they should be given in a way that is appropriate, meaning that foods are of appropriate texture for the age of the child and applying responsive feeding following the principles of psycho-social care.

The adequacy of complementary feeding (adequacy in short for timely, adequate, safe and appropriate) not only depends on the availability of a variety of foods in the household, but also on the feeding practices of caregivers. Feeding young infants requires active care and stimulation, where the caregiver is responsive to the child clues for hunger and also encourages the child to eat. This is also referred to as active or responsive feeding.

WHO recommends that infants start receiving complementary foods at 6 months of age in addition to breast milk, initially 2-3 times a day between 6-8 months, increasing to 3-4 times daily between 9-11 months and 12-24 months with additional nutritious snacks offered 1-2 times per day, as desired. Inappropriate feeding practices are often a greater determinant of inadequate intakes than the availability of foods in the households (WHO, 2005).

1.2. Types of baby formula food

1.2.1. Homemade baby formula food:

It is less expensive than commercial baby foods. Homemade food is appropriate only when the family has a sufficient and varied diet, as well as access to refrigeration and basic sanitation. It is important to follow proper sanitation methods when preparing homemade baby food such as washing and rinsing vegetables or fruit, as well as the cooking and packaging materials that will be used. Homemade food requires more preparation time than opening a jar or box of ready-to-eat commercial baby food. Food may need to be minced or pureed for young babies, or cooked separately without the salt, intense spices, or sugar that the family chooses to eat. Avocados and bananas are foods that can be easily mashed and are high in vitamins and nutrients, making them ideal starter foods for an infant 6 months in age or older. (Butte et al., 2010).

1.2.2. Commercial baby food or formulated food.

The popularity of commercial baby food in recent decades has had a major impact on what we assume babies need. Many major food companies have jumped on this lucrative bandwagon, but it is important to not take for granted what you are feeding your 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-borne illness because their immune systems are not developed enough to fight off infections. That's why extra care should be taken when handling and preparing their food and formula (Samour and King, 2013)**.**

1.3. Nutritional Quality of Commercial Baby Food

Many store-bought baby foods now contain organic ingredients, but closer inspection proves they are still not as healthy as we might think. Infant formulas are typically processed under high heat, leading to the formation of advanced glycation end (A.G.E.) products, which are sugar molecules that attach to and damage proteins in the body.

Studies prove that infant formulas processed with high heat may have 100 times more A.G.E.s than breast milk, showing why infants switched from breast milk to commercial formula within the first year of life have double the levels of A.G.E.s found in people with diabetes (Science Daily, 2011).

In fact, some even contain shocking ingredients for babies, such as added sugar, which isn’t something little palates and bodies need. Several generations have now been fed baby food, and we have become heavy sugar consumers. This has led to epidemic proportions of diabetes and obesity, even among young children.

Baby foods also include industrial vegetable oils, corn syrup, preservatives, emulsifiers and refined flours. Even natural flavors are usually made up of complex compounds designed by companies to create flavor addiction.

Commercial children and toddler foods have long been criticized for their lack of nutrition, high sugar content and sodium levels. Another study reviewed 79 mixed grains and fruit baby food where 41 contained added sugar. Of those, 35 had 35% or more of their calories attributed to sugar (Cogswell et al., 2015)

When they reviewed toddler dinners, they found 72% had high sodium level. They also found 32% of toddler dinners and most cereal bars, fruit products, infant/toddler snacks, juices and baby and toddler desserts contained added sugar.

In inorganic baby foods, traces of pesticides are also present, which increase health risks. A test conducted by the Environmental Working Group, found up to 16 pesticides in eight leading baby-food brands, including three probable human carcinogens, eight neurotoxins, five pesticides "that disrupt normal functioning of the hormone system, and five categorized as oral toxicity category one, the most toxic designation." While these levels are considered below federal standards for adults, they do not specifically incorporate protection for the immature bodies of infants or young children.

Shockingly, many baby foods are not tested for heavy metals. Ellipse Analytics, an accredited laboratory in Denver, Colorado, and the nonprofit Clean Label Project tested 700 baby-to-early-toddler foods for toxins. The results revealed 35 percent of formula samples tested positive for some level of lead.

1.4. Contamination of commercial baby foods

The presence of contaminants such as metals in infant formulas may pose health risks for children2. Therefore, cumulative exposure from such formulas should not exceed the provisional tolerable weekly intake (PTWI) for toxic metals as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the recommended daily allowance (RDA) for essential trace elements. When the RDA cannot be determined, adequate intake can be used as a recommended intake value. Cadmium (Cd), lead (Pb) and chromium (Cr) occur naturally in the environment, and the general population is exposed to these toxic metals from multiple sources, but the main exposure is via food. After ingestion, approximately 5–10% of Cd. (Liung k et al.2001).

1.4.1. Lead

Lead is the most common of the heavy elements. Lead poisoning can cause health problems, especially in the case of children. In this research, heavy metals of high concern for human health were measured in several baby-food products; lead was determined using atomic absorption spectrophotometer.

Food can be contaminated by naturally occurring lead in the soil as well as by lead from sources such as atmospheric fall out or water used for cooking. The total intakes and uptakes of lead from all sources are 29.5 and 12.5 mg/d, respectively, for children and 63.7 and 6.7 mg/d, respectively, for adults in urban areas (WHO, 1987). The relative contribution of water to average intake is estimated to be 9.8% and 11.3% for children and adults, respectively. The total intake of lead from three of the four major sources air, food, and dust appears to have dropped significantly since the mid-1980sas a result of regulatory and voluntary actions to control lead from air (via gasoline) and food (via cans).

Lead has been found in drinking water, those certain kinds of food. But a problem—or that these elements (and others, such as lead and cadmium), commonly known as "heavy metals," are also in many other foods. This includes foods made just for babies and toddlers, such as popular snacks, cereals, prepared entrées, and packaged fruits and vegetables. Over time, exposure to heavy metals can harm the health of adults and children. One of the biggest worries: cognitive development in very young children. "Babies and toddlers are particularly vulnerable due to their smaller size and developing brains and organ systems," food safety research and testing at Consumer Reports. "They also absorb more of the heavy metals that get into their bodies than adults do." That's why CR's food safety team analyzed 50 nationally distributed packaged foods made for babies and toddlers, checking for cadmium, lead, chromium, and inorganic arsenic, the type most harmful (EFSA 2009)

1.4.2. Cadmium

Cadmium is a byproduct of the mining and smelting of lead and zinc and is number 7 on ATSDR's "Top 20 list." It is used in nickel cadmium batteries, PVC plastics, and paint pigments. It can be found in soil because insecticides, fungicides, sludge, and commercial fertilizers that use cadmium in agriculture. Cadmium may be found in reservoirs containing shellfish. Cigarettes also contain cadmium. Lesser-known sources of exposure are dental alloys, electroplating, motor oil and exhaust. Inhalation accounts for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Target organs are the liver, placenta, kidneys, lungs, brain and bones (Zandn et al., 2011).

1.4.3. Chromium

Chromium (Cr) is one of the world's most strategic and critical materials having a wide range of uses in the metals and chemical industries. Cr alloys enhance metal resistance to impact, corrosion, and oxidation. Cr is used in stainless steel and non iron alloy production for plating metals, development of pigments, leather processing, and production of catalysts, surface treatments, and in refractoriness. Human exposure- Dermal exposure to chromium may also occur during the use of consumer products that contain chromium, such as fertilizer, wood treated with copper dichromate or chromate copper arsenate and leather tanned with chromic sulfate. In addition, people who reside in the vicinity of chromium waste disposal sites and chromium manufacturing and processing plants have a greater probability of elevated chromium exposure (Pellerin and Booker, 2000).

Exposure to chromium for occupational groups can be two orders of magnitude higher than the exposure to the general population (Hemminki and Vainio 1984).In baby the concentration of chromium is very negligible so it's not harmful for child.

1.4.4. Copper

Copper is an essential element which adds in infant formula. Copper deficiency accompanied by failures in collagen, elastin reticulation and problems in tissue, especially in arteries. its excess can cause to diarrhea, nausea, vomiting, anemia. International organization such as UNICEF 1999, emphasize on control and assessment of babies food products by purpose of health.

1.4.5. Iron

Iron Discussion of iron toxicity in this protocol is limited to ingested or environmental exposure. Iron does not appear on the ATSDR's "Top 20 List," but it is a heavy metal of concern, particularly because ingesting dietary iron supplement may acutely poison young children (five to nine 30-mg iron tablets for a 30-lb child). Ingestion accounts for most of the toxic effects of iron because iron is absorbed rapidly in the gastrointestinal tract. The corrosive nature of iron seems to further increasing the absorption. Most overdoses appear to be the result of children mistaking red-coated ferrous sulfate tablets or adult multivitamin preparations for candy. (Fatalities from overdoses have decreased significantly with the introduction of childproof packaging. In recent years, blister packaging and the requirement that containers with 250 mg or more of iron have child-proof bottle caps have helped reduce accidental ingestion and overdose of iron tablets by children). Other sources of iron are drinking water, iron pipes, and cookware. Target organs are the liver, cardiovascular system, and kidneys (Liung et al., 2011).

1.4.6. Zinc

Zinc is a vital nutrient that is essential for growth and development. Not only that, a lack of zinc has been associated with impaired cognitive development which makes it all the more crucial to add it to every child's diet. Zinc is a trace mineral that over 70 digestive enzymes rely on for regulating digestion and metabolism in the body. Children with zinc deficiencies experience growth defects and abnormalities which makes this a vital nutrient in every diet. It is also essential for the proper growth and development of reproductive organs in kids. According to The Office of Dietary Supplements, these are the following zinc dosages for children-

Ages: 4 to 8 – 5 mg/day

Ages: 9 to 13 – 9mg/day

Ages: 14 to 18 – 11mg/day

Teenage girls between the ages of 14 to 18 require only 9mg of Zinc a day.

Teenagers are highly recommended to not consume more than 34 milligrams of zinc a day which is the tolerable upper intake level, since they may experience various harmful side effects.

Women empowerment being the subject of the concern among the policy makers of developing countries like Bangladesh, the integration of women into the development process and therefore their participation in economic activities along with men, have been gaining importance in many national development plans. Empowerment facilitates women to access resources such as food, land, income and other forms of wealth, and social resources such as knowledge, power, prestige within the family and community. In Bangladesh women empowerment and job opportunity for women increase day by day as the country's development progress. So most of mothers are engaged with their corporate life and passed a busy schedule that's why they have little time to feed their children and now they are fully depended on commercially formulated baby food items. Besides commercial baby foods are less nutritive but major concern is the contamination with heavy metals like Pb, Cd, Cr, and some trace elements like Cu, Fe, and Zn. These heavy metals and trace elements are detrimental to child development and growth and the accurate level of these elements in baby foods are not established in Bangladesh. So assessment of these heavy metals and trace elements in different types of baby food has great importance for child health and development.

1.5. Objectives

- To investigate the levels of metals such as cadmium, lead, chromium and trace heavy metals such as copper, iron, zinc in various brand of infant formulas.
- To compare the heavy metals load and nutritional value between brand and homemade infant formula.

Chapter 2: Review of literature

Adequate nutrition during infancy and early childhood is fundamental to the development of each child's full human potential. It is well recognized that the period from birth to two years of age is a "critical window" for the promotion of optimal growth, health and behavioral development. Longitudinal studies have consistently shown that this is the peak age for growth faltering, deficiencies of certain micronutrients, and common childhood illnesses such as diarrhea. After a child reaches two years of age, it is very difficult to reverse stunting that has occurred earlier (Martorell et al., 1994). The immediate consequences of poor nutrition during these formative years include significant morbidity and mortality and delayed mental and motor development. In the long term, early nutritional deficits are linked to impairments in intellectual performance, work capacity, reproductive outcomes and overall health during adolescence and adulthood. Thus, the cycle of malnutrition continues, as the malnourished girl child faces greater odds of giving birth to a malnourished, low-birth-weight infant when she grows up. Poor infant feeding practices, coupled with high rates of infectious diseases, are the principal proximate causes of malnutrition during the first two years of life. For this reason, it is essential to ensure that caregivers are provided with appropriate guidance regarding optimal feeding of infants and young children.

Inappropriate feeding practices of infant and young children are the most serious obstacles to maintaining adequate nutritional status and contribute to levels of malnutrition in Bangladesh that are amongst the highest in the world. Poverty, malnutrition and disease are interlinked with each other. Malnutrition in children is the consequence of a range of factors, which are often related to poor food quality, insufficient food intake, severe and repeated infectious diseases; or frequently it involves some combination of the three (Deonis et al., 1993).

According to Institute of Public Health and Nutrition (IPHN), Bangladesh (2007), almost one-half of children under five years are underweight and 42% are stunted. Only 42% of infants aged less than six months are exclusively breastfed and almost one-third (29%) of children aged 6 to 9 months do not receive any solid or semi-solid foods. The most common complementary foods include khichuri, bhaat dal, suji and muri (IPHN, 2007).

The introduction of supplementation in terms of weaning foods prepared from easily available and low cost ingredients is of vital importance to meet the requirements of the growing children (Saeeda et al., 2009). In most developing countries, commercial weaning foods of excellent quality either imported or locally produced are generally 10 to 15 times higher than the cost of the common staple foods due to sophisticate processing, expensive packing, extensive promotion and solid profit margins (Bahlol et al., 2007).

The World Health Organization (WHO), international and national health agencies recommend exclusive breastfeeding for the first six months of infancy (CDC, 2006; WHO, 2015). Under unavoidable circumstances, safe and nutritious alternatives such as infant formula milk (IFM), dairy milk and sweetened liquids should be employed. Circumstances that divert breastfeeding include, but are not limited to death of the mother, risk of mother to child transmission of diseases, absence of the mother from the infant for an extended period, personal preferences and beliefs and societal pressure (Lawrence, 2004; WHO, 2004; Mamiro et al., 2005; CDC, 2006).

Infant formula milk is the only product which is considered nutritionally acceptable for infants under the age of one year (Gian et al., 2009). According to Natural Resources Defense Council (NRDC), the infant formula industry is an $8 billion per year business (NRDC, 2005). Across the globe, huge advertising budgets are spent to convince women that it is better and more convenient to bottle-feed their babies. Infant formula milk (IFM) is categorized by age brackets: 0-6 months, 7-12 months and beyond 1 year. In Kenya, more than seven brands of IFM are imported into the market. Kenya is a signatory to all global conventions with a commitment to promote, protect and support infant and young children feeding practices (KEBS, 2006; Komen, 2009).

Metal pollution as a result of increasing industrialization has penetrated into all sectors of the food industry and as such pose fears for IFM (Gian et al., 2009). Labeling on the package, however, does not indicate the minimal levels of the elements present. As a matter of fact, elements and ions may find route in foods as a result of processing, packaging, farming activities and industrial emission (Khalifa and Ahmad, 2010; Ljung et al., 2011).

Food and Agricultural Organization (FAO) of WHO has set provisional tolerable weekly intake limits for metal ions by infants but their poisoning effect even at low levels of exposure cannot be overemphasized and especially that they can bio-accumulate in vital body organs to persist in adulthood (Ljung et al., 2011).

The Kenya Bureau of Standards (KEBs) in Kenya has set standards for the levels of elements in not only IFM but all food products among other commodities (KEBS, 2006). Studies have reported contamination of IFM by various substances such as nitrates, nitrites, aluminium (Al), cadmium (Cd), mercury (Hg), nickel (Ni), lead (Pb) and melamine (Gian et al., 2009; Khalifa and Ahmad, 2010; Ljung et al., 2011).

In 2008, melamine contamination of IFM in China led to deaths and illness of several infants (Nakashima et al., 2009). China reported an estimated 300,000 victims with approximately 54,000 babies being hospitalized and six infants dying from kidney stones and other kidney damage (Branigan, 2008).

Infants are the population group most vulnerable to the toxic effects of heavy metals due to the higher absorption of metals by the gastrointestinal tract, faster metabolic processes, an incompletely developed detoxification system, and higher food consumption in relation to body weight. Additionally, the undeveloped blood-brain barrier allows elements noxious to infant health (primarily lead and mercury compounds) to accumulate in the brain, causing dysfunction of the central nervous system. Exposure to heavy metals during growth and development can result in long-term effects on the health of children. Infant foods are the main source of heavy metals intake by this population, primarily due to contamination of raw materials used and rarely by food processing itself (Trumbo et al, 2001).

Heavy metals are found naturally in the earth, and become concentrated as a result of human caused activities. Metals occur naturally in our environment, but rarely at toxic levels especially in the earth’s crust where they contribute to the balance of the planet. Common sources are from mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilizers; paints; treated woods; aging water supply infrastructure; and micro plastics floating in the world's oceans.

Table 2.1: Physical and Chemical properties of heavy metal

Physical properties	
Density	Usually higher
Hardness	Most are quite hard
Thermal expantivity	Mostly lower
Melting point	Low to very high
Tensile strength	Mostly higher
Chemical properties	
Periodic table location	Nearly all found in group 3 through 16
Abundance in Earth's crust	Less abundant
Main occurrence (or source)	Lithophilesor chalcophiles (Au is a siderophile)
Reactivity	Less reactive
Sulfides	Extremely insoluble
Hydroxides	Generally insoluble
Salts	Mostly form colored solutions in water
Complexes	Mostly colored

Lead is a metal which has been associated with human activities for the last 6000 years. In ancient civilizations, uses of lead included the manufacture of kitchen utensils, trays, and other decorative articles. However, lead is also toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract. The main sources of lead exposure are paints, water, food, dust, soil, kitchen utensils, and leaded gasoline. The majority of cases of lead poisoning are due to oral ingestion and absorption through the gut. Lead poisoning in adults occurs more frequently during exposure in the workplace and primarily involves the central nervous system. Symptoms of hemopoietic system involvement include microcytic, hypochromic anemia with basophilic stippling of the erythrocytes. Hyperactivity, anorexia, decreased play activity, low intelligence quotient, and poor school performance have been observed in children with high lead levels. Lead crosses the placenta during pregnancy and has been associated with intrauterine death, prematurity and low birth weight. In 1991, the Centers for Disease Control and Prevention in the USA redefined elevated blood lead levels as those > or = 10 microg/dl and recommended a new set of guidelines for the treatment of lead levels > or =15 microg/dl (Papanikolaou et al., 2005).

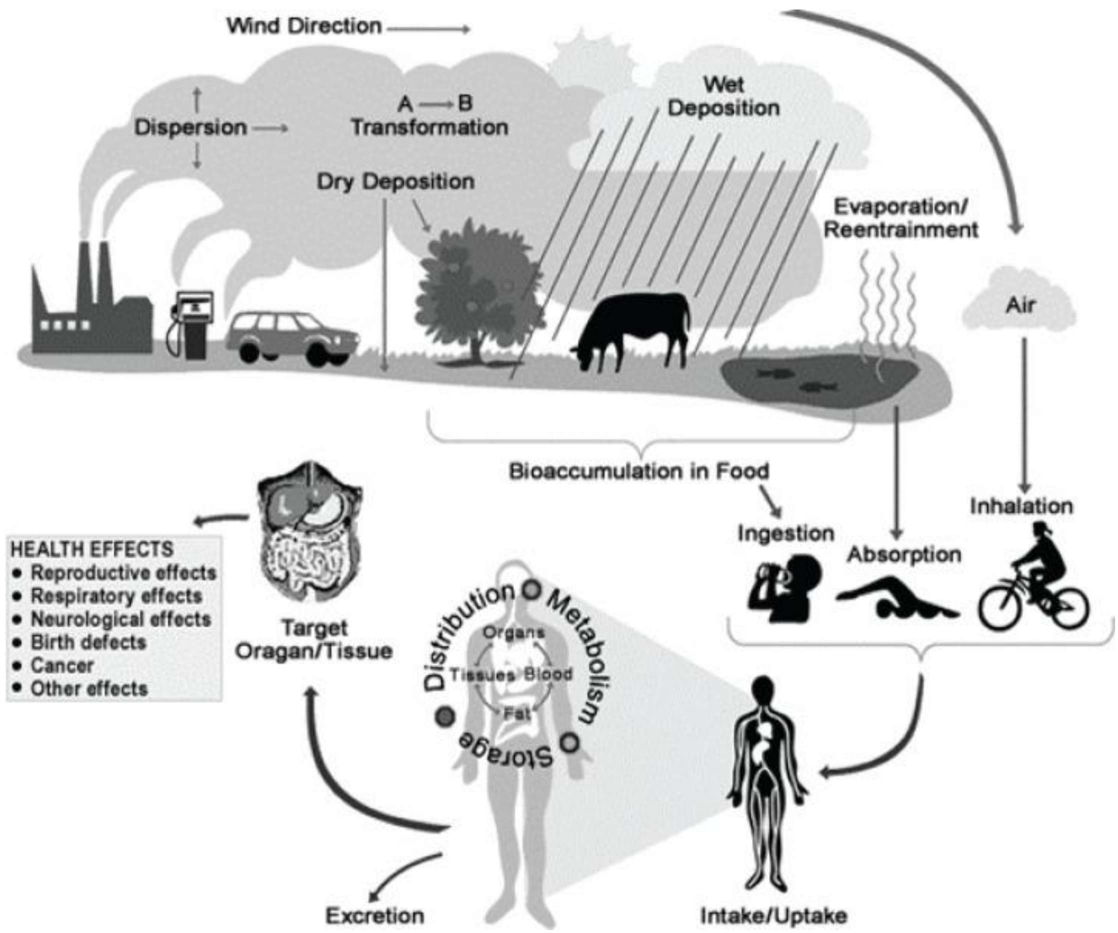

Figure 2.1: Illustration how people are exposed to chemicals in the environment and the effect of such chemicals on human health.

Persons living in lead contaminated areas had a significantly higher prevalence of renal dysfunction, bone mineral loss, hypertension and urinary stones than those living in non-contaminated areas. This health effects due to environmental cadmium exposure lead to prevalence's of diabetes, hypercholesterolemia, and hyper-triglyceridemia were not associated with cadmium exposure (Swaddiwudhipong et al., 2015).

However, because lead is a persistent metal, it is still present in the environment – in water, brass plumbing fixtures, soil, dust, and imported products manufactured with lead. Diagnosis of lead toxicity has traditionally been based on significantly elevated blood lead levels. However, data now implicates low-level exposures and blood lead levels previously considered normal as causative factors in cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, and renal impairment. Chelation is the conventional recommendation in the case of blood levels associated with acute toxicity and encephalopathic damage. Issues surrounding the assessment of body lead burden and the consequences of low-level environmental exposure are critical in the treatment of chronic disease related to lead toxicity. (Anttila and Sallmen, 1995).

Cadmium is a heavy metal that occurs as a natural constituent in earth's crust along with Copper, Lead, Nickel and Zinc. Cadmium is vastly used in batteries, coating, plating, alloys etc. in various industries. Humans are commonly exposed to cadmium by inhalation and ingestion. Cadmium enters in air and bind to small particles where it can combine with water or soil causing contamination of fish, plants and animals in nanoform. Spills at hazardous waste sites and improper waste disposal can cause cadmium leakages in nearby habitats. Foodstuffs like liver, mushrooms, shellfish, mussel, cocoa powder and dried seaweed are cadmium rich increasing the concentration in human bodies. The bioaccumulation of cadmium in human body and in food chain leads to acute and chronic intoxications due to bio-magnification. Health effects includes diarrhea, stomach pains, Bone fracture, Reproductive failure and possibly even infertility, damage to the central nervous

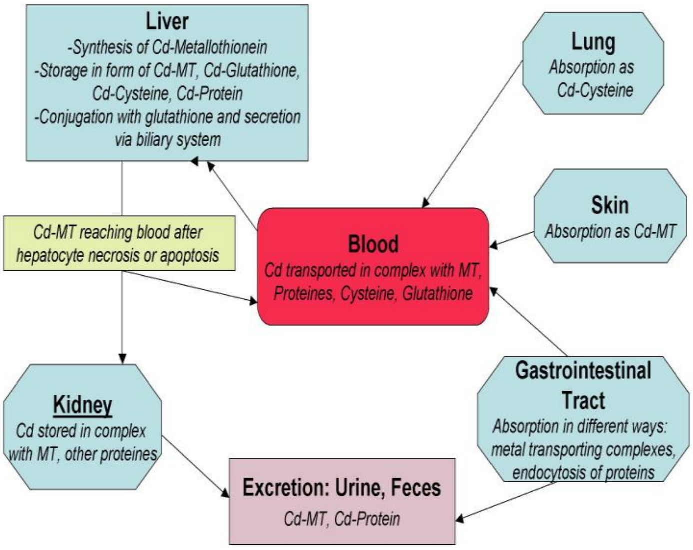

Figure 2.2: Metabolism, storage and excretion of cadmium in human body

system and immune system, psychological disorders, etc. Cadmium can also cause the transformation of normal epithelial cells into carcinogenic cells by inhibiting the biosynthesis of protein. Cadmium waste streams from the industries end up in soil which can pollute both soil and surface water. The organic matter in the soil absorbs cadmium increasing the risk of survival of various plants and also increases the uptake of this toxic metal in food. (Sharma et al., 2015).

Chromium is a toxic heavy metal which is found in environment in different oxidation states ranging from -2 to +6. But the most stable forms are trivalent and hexavalent chromium. Trivalent chromium has poor absorption inside the cell as compared to hexavalent chromium. The most common exposure routes of chromium to humans are ingestion, dermal contact and inhalation. The primary health hazards caused by chromium are bronchial asthma, lung and nasal ulcers and cancers, skin allergies, reproductive and developmental problems and this chromium is carcinogenic in nature. When taken in excess it may cause death also (Shekhawat et al., 2015).

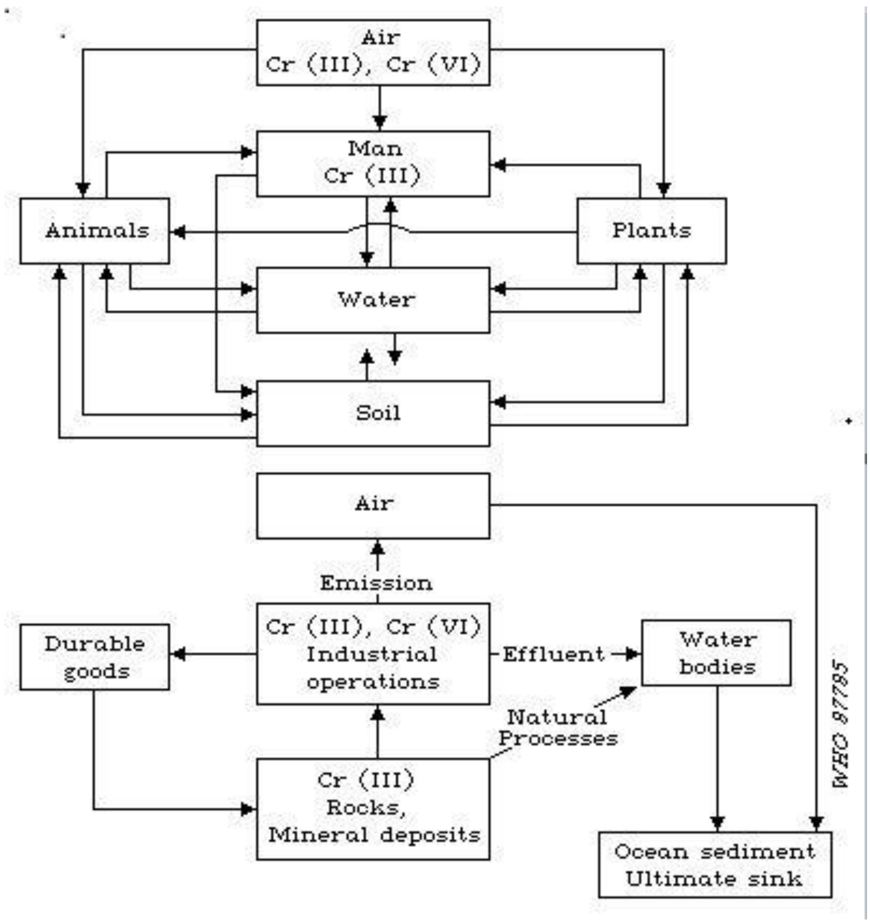

Figure-2.3: Environmental cycling of Chromium

Chromium is widely used in medical and dental implants, appliances and tools, where sufficient content s of this chemical element can provide a protective corrosion-resistant oxide on the alloy surface. At low concentrations chromium is used for medical purposes, and it is also involved in natural human lipid and protein metabolism. However, at sufficiently high concentrations particularly hexavalent chromium is toxic and carcinogenic. The healthy risks can be expected regardless whether the chromium originates from external sources such as polluted drinking water or internally from corroding dental appliances (Achmad et al., 2017).

Table 2.2 Health risk due to heavy metals (Lead, Cadmium and Chromium)

Metal	Effects
Lead (Pb)	• Decreased performance in nervous system • weakness in fingers,wrists, or ankles • small increases in blood pressure; anemia • damage the brain and kidneys and ultimately cause death. • In pregnant women, high levels of exposure to lead may cause miscarriage. • High level exposure in men can damage the organs responsible for sperm production.
Cadmium(Cd)	• Acute inhalation of cadmium oxide fumes-irritation of respiratory system • High exposure to cadmium oxide fume chemical pneumonitis and edema in the lungs, which can be fatal • Cadmium is human carcinogen causing lung cancer
Chromium (Cr)	• Breathing high levels can cause irritation to the lining of the nose; nose ulcers; runny nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing. • Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted.

Copper is an essential mineral for human health and at the same time can be toxic, depending upon the amounts ingested. Copper is associated with bone health, immune function and increased frequency of infections, cardiovascular risk and alterations in cholesterol metabolism. Its metabolism is tightly intertwined with other microminerals and its deficiency is known to impair iron mobilisation, resulting in secondary iron deficiency. A pressing challenge in modern nutrition is to define both the copper dose and regimen of administration for safe human consumption; this is a difficult task because our knowledge about the limits of safe copper exposure (homeostasis), the consequences of moderate excess copper exposure and the indicators to detect early adverse effects are not well established (Araya, 2007).

Exposure of humans to copper occurs primarily from the consumption of food and drinking water. The relative copper intake from food versus water depends on geographical location; generally, about 20–25% of copper intake comes from drinking water. Georgopoulos et al. (2001) conducted a review of issues that affect the ability to assess and quantify human exposures to copper from various environmental media with a primary consideration being exposure to copper from potable water supplies.

It is well-known that deficiency or over exposure to various elements has noticeable effects on human health. The effect of an element is determined by several characteristics, including absorption, metabolism, and degree of interaction with physiological processes. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport. However, as iron can form free radicals, its concentration in body tissues must be tightly regulated because in excessive amounts, it can lead to tissue damage. Disorders of iron metabolism are among the most common diseases of humans and encompass a broad spectrum of diseases with diverse clinical manifestations, ranging from anemia to iron overload, and possibly to neurodegenerative diseases (Abbaspour et.al., 2014).

The importance of micronutrients in health and nutrition is undisputable, and among them, zinc is an essential element whose significance to health is increasingly appreciated and whose deficiency may play an important role in the appearance of diseases. Zinc is one of the most important trace elements in the organism, with three major biological roles, as catalyst, structural, and regulatory ion. Zinc-binding motifs are found in many proteins encoded by the human genome physiologically, and free zinc is mainly regulated at the single-cell level. Zinc has critical effect in homeostasis, in immune function, in oxidative stress, in apoptosis, and in aging, and significant disorders of great public health interest are associated with zinc deficiency. In many chronic diseases, including atherosclerosis, several malignancies, neurological disorders, autoimmune diseases, aging, age-related degenerative diseases, and Wilson's disease, the concurrent zinc deficiency may complicate the clinical features, affect adversely immunological status, increase oxidative stress, and lead to the generation of inflammatory cytokines. In these diseases, oxidative stress and chronic inflammation may play important causative roles (Chasapis et al., 2012).

Compared to several other metal ions with similar chemical properties, zinc is relatively harmless. Only exposure to high doses has toxic effects, making acute zinc intoxication a rare event. In addition to acute intoxication, long-term, high-dose zinc supplementation interferes with the uptake of copper. Hence, many of its toxic effects are in fact due to copper deficiency. While systemic homeostasis and efficient regulatory mechanisms on the cellular level generally prevent the uptake of cytotoxic doses of exogenous zinc, endogenous zinc plays a significant role in cytotoxic events in single cells. Here, zinc influences apoptosis by acting on several molecular regulators of programmed cell death, including caspases and proteins from the Bcl and Bax families. One organ where zinc is prominently involved in cell death is the brain, and cytotoxicity in consequence of ischemia or trauma involves the accumulation of free zinc. Rather than being a toxic metal ion, zinc is an essential trace element. Whereas intoxication by excessive exposure is rare, zinc deficiency is widespread and has a detrimental impact on growth, neuronal development, and immunity, and in severe cases its consequences are lethal. Zinc deficiency caused by malnutrition and foods with low bioavailability, aging, certain diseases, or deregulated homeostasis is a far more common risk to human health than intoxication (Plum et al., 2014).

Chapter-3: Materials and Methods

3.1 Study area and study period

Commercial infant formulas and homemade formula were collected from different super shop of Chattogram division in Bangladesh. Analysis was conducted in Department of Applied Food Science and Nutrition, Chittagong Veterinary and Animal Sciences University and Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh. The study period was six months from January, 2019 to July, 2019.

3.2 Apparatus

The amount of (Pb, Cd and Cr) heavy metal and trace heavy metals (Cu, Fe and Zn) was measured by Atomic Absorption Spectrophotometer (AAS) Model: ZEE nit 700P, Germany (Shahriar et al., 2014). All laboratory works were performed at Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh.

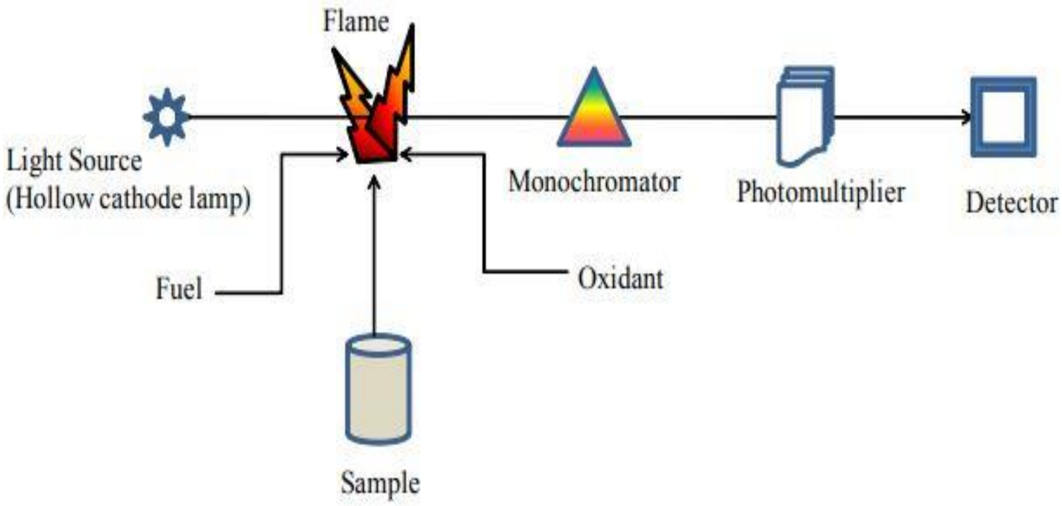

Figure-3.1: Schematic diagram showing the principle and working of atomic absorption spectroscopy (AAS)

3.3 Chemicals and reagents

All chemicals and reagents were of analytical grade. The chemicals, tartaric acid, CH_3COONa, $Pb(NO_3)_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Zn(NO_3)_2$ and HNO_3 in analytical grades and were purchased from Merck Company Germany. The metal stock solution (1 g/l) was prepared in 0.005 molar HNO_3. The acetate buffer (0.2 M, pH = 4.7) containing 0.2 M tartaric acid, which serve as a supporting electrolyte.

3.4 Sample collection

Five different brands of infant formulas (LA, CE, OT, JH, NM) were collected from supermarkets in Chattogram city, Bangladesh, and five homemade formulas (HP, RP, SA, SP, LM) were prepared at laboratory between January and April 2019. The infant formulas were the most widely available in the market, which are intended for consumption from six months until one year. All products were milk-based types and packed in metal containers. Samples were coded for ease of identification.

3.5 Sample digestion and Analysis

All samples were digested in duplicate following the procedures described in the literature (Cruz et al., 2009). Five grams from each milk-based sample was placed in different crucibles and heated in a maple furnace at 550° C for 3 hours to vaporize all other constituents and leave the heavy metals as a pure ash. The ash was cooled to room temperature before being dissolved in a 5 ml solution of nitric acid (70%) to compound with the heavy metals, if present. The solution was subsequently heated and evaporated to half its volume using a hot plate. The resulting solution was then poured into a volumetric flask and topped up to 25 ml with distilled deionized water. The digested samples were analyzed using flame atomic absorption spectrophotometer (AAS, Model: ZEE nit 700P, Germany).

3.6 Standard preparation

Every metal standard solution was prepared for calibration the instrument for each element being determined on the same day as the analyses were performed due to possible deterioration of standard with time. All samples were prepared from chemicals of analytical grade with distilled water. 1gm of metal Cadmium, Copper and Lead were dissolved in HNO_3 solution and 1 g of Copper, Iron and Zinc were dissolved in HCl solution. 2.8289 g $K_2Cr_2O_7$ (=1g Chromium) was dissolved in water and made up to 1 liter in volumetric flask with distilled water, thus stock solution of 1000 mg/l of Cd, Cu, Pb, Fe, Zn, Cr were prepared

(Cantle, 1982). Then 100 ml of 0.1, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/l of working standards of each metal except iron were prepared from these stock using micropipettes in 5ml of 2N nitric acid. 100 ml of 2.0, 2.5, 5.0, 10.0 and 20.0 mg/l of working standards of iron metal were prepared from iron stock solution. Reagent blank was prepared in the same manners of sample preparation without sample to avoid reagents contamination.

3.7 Method validation procedures

Freshly prepared standard stock solutions were serially diluted and used to obtain calibration curves with linearity values. The correlation coefficient values were a good indicator of the linearity for AAS instrument for precision and accuracy of results (Duan et al., 2003). Further, using recovery tests where samples were repeatedly spiked with known amounts of standards prior to measurements were performed to confirm the accuracy of the instruments (Duan et al., 2003). The percentage recoveries obtained were good, falling between 98.1-99.9%.

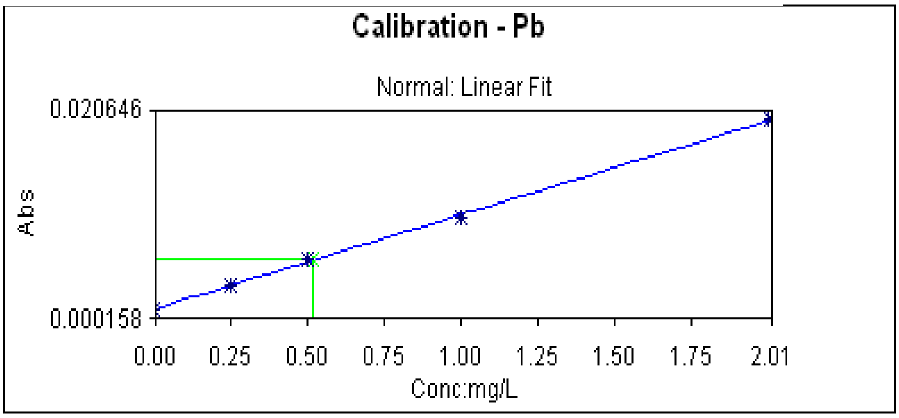

Figure-3.2: Calibration curve of lead standard solutions

3.8 Statistical analysis

Data were analyzed with SPSS 17.0 for windows. Descriptive analysis was performed by using percentages, mean and standard deviation for different variables. Finally one -way ANOVA was used to compare the level of heavy metal residues in different infant formula of Bangladesh. The level of significance was set ≤ 0.05.

Chapter 4: Results

The results obtained for the various parameters viz., proximate composition of commercial and homemade infant formula, heavy metals and trace elements content in infant formula are given in detail under this section.

4.1 Proximate composition of commercial infant formula(CIF)

Proximate composition such as moisture, ash, protein, crude fat crude fiber and energy was determined in five commercial infant formulas where samples were taken from super shop market of Chattogram city those were ready-to cook and obtained results are presented in table 4.1

Table-4.1: Nutritional value of commercial infant formula

Food ID	Moisture (%)	Ash (%)	CHO (%)	Protein (%)	Fat (%)	Fiber (%)	Energy (Kcal/100g)
LA	4.17 ± 0.3^{bc}	2.07 ± 0.3^{a}	61.40 ± 0.2^{b}	14.03 ± 0.2^{b}	18.40 ± 0.2^{d}	0	468 ± 1.0^{d}
CE	4.53 ± 0.2^{c}	2.50 ± 0.2^{a}	63.63 ± 0.2^{c}	13.87 ± 0.2^{b}	10.40 ± 0.1^{c}	1.57 ± 0.2^{a}	418 ± 1.0^{c}
OT	4.20 ± 0.1^{bc}	3.40 ± 0.2^{b}	68.60 ± 0.4^{d}	11.57 ± 0.2^{a}	8.50 ± 0.1^{b}	9.93 ± 0.2^{b}	403 ± 2.0^{b}
JH	3.23 ± 0.2^{a}	2.47 ± 0.2^{a}	72.47 ± 0.1^{e}	15.17 ± 0.3^{c}	4.63 ± 0.2^{a}	1.37 ± 0.1^{a}	338 ± 1.5^{a}
NM	3.67 ± 0.2^{ab}	4.70 ± 0.2^{c}	39.63 ± 0.2^{a}	22.50 ± 0.2^{d}	28.63 ± 0.2^{e}	0	506 ± 2.1^{e}

*Values are means ± standard deviation of three triplicate determination. Means within a column (for each variable) marked with different superscripts are significantly different ($P< 0.05$); CHO = Carbohydrate.

Food energy is defined as the energy released from carbohydrates, fats, proteins, and other organic compounds. When the three major calorigenic nutrients (carbohydrates, fats, and proteins) in a food are burnt entirely with sufficient amounts of oxygen, it releases energy or food calories that are expressed in kilojoules (kJ) or kilocalories (kcal). Food energy was measured by a bomb calorimeter based on the heat of combustion and the percentages of energy come from average mean value of commercial infant formulas are presented in figure 4.1

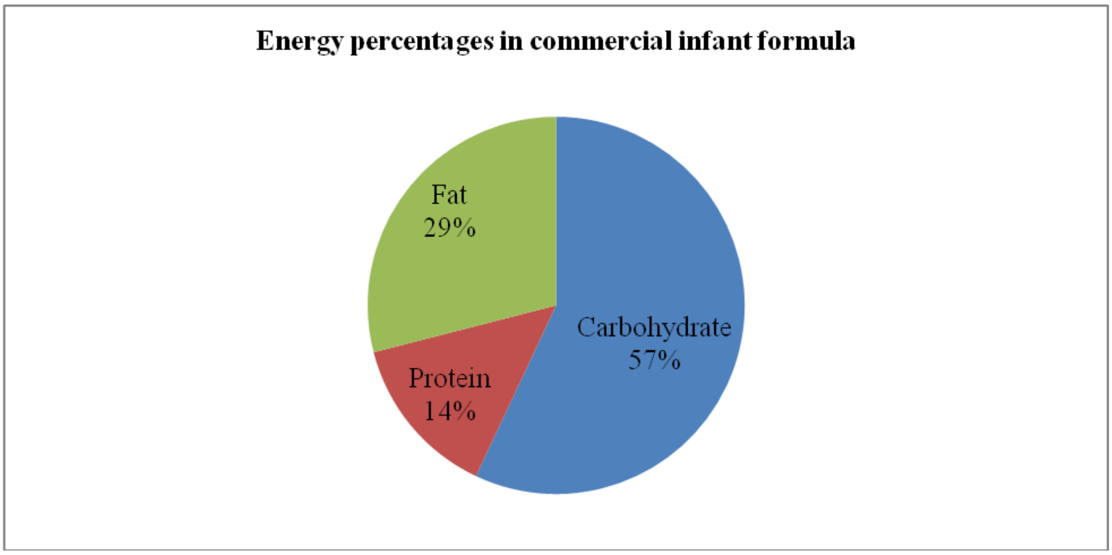

Figure: Energy percentages come from three major macronutrient of Commercial infant formula

4.2 Proximate composition of homemade infant formula (HIF)

On the other hand, five homemade infant formulas were prepared at applied food science and nutrition laboratory, CVASU where all samples were ready-to-eat and the proximate analysis such as Carbohydrate, protein, fat; ash, fiber and energy were estimated at BCSIR, Chattogram.

Table-4.2: Nutritional value of Homemade infant formula (Dry basis)

Food ID	CHO (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Energy (Kcal/100g)
HP	77.79 ± 0.2^{c}	7.53 ± 0.2^{c}	2.43 ± 0.3^{c}	8.67 ± 0.2^{d}	1.10 ± 0.2^{c}	363 ± 1.0^{bc}
RP	80.40 ± 0.1^{c}	6.27 ± 0.2^{c}	1.36 ± 0.2^{b}	6.63 ± 0.2^{c}	0.98 ± 0.01^{bc}	365 ± 1.5^{c}
SA	97.30 ± 2.3^{d}	1.66 ± 0.1^{a}	0.06 ± 0.01^{a}	0.48 ± 0.01^{a}	1.56 ± 0.02^{d}	369 ± 1.0^{d}
SP	73.33 ± 0.2^{b}	12.2 ± 0.3^{e}	0.82 ± 0.6^{ab}	3.66 ± 0.2^{b}	0.78 ± 0.01^{ab}	361 ± 1.5^{b}
LM	4.73 ± 0.2^{a}	3.40 ± 0.1^{b}	3.26 ± 0.1^{d}	0	0.57 ± 0.01^{a}	62 ± 1.0^{a}

*Values are means ± standard deviation of three triplicate determination. Means within a column (for each variable) marked with different superscripts are significantly different ($P< 0.05$); CHO = Carbohydrate.

The percentages of energy come from carbohydrate; protein and fat of average mean value of homemade infant formula are 87%, 8% and 5% respectively presented in figure 4.2

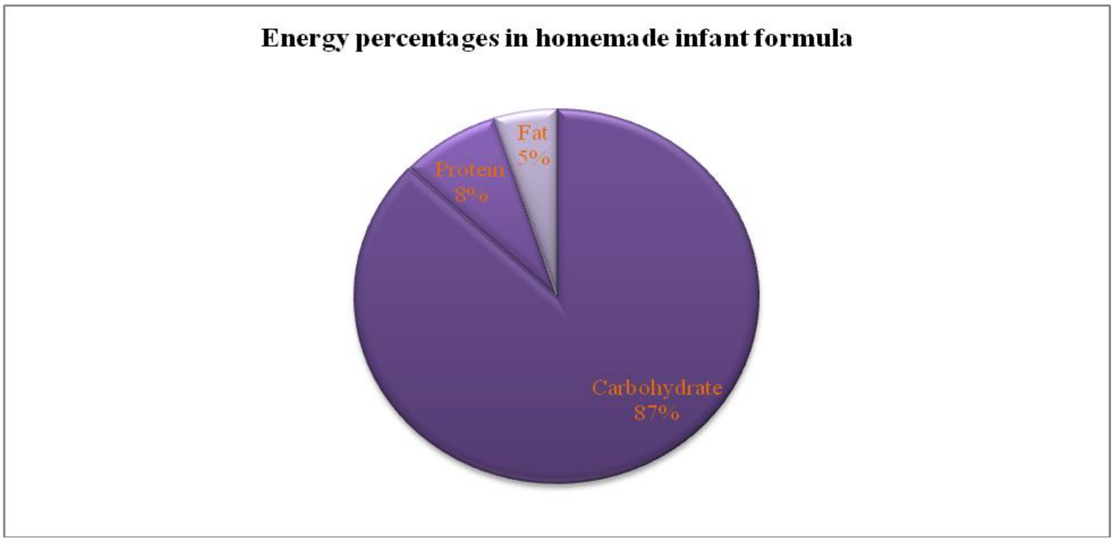

Figure 2: Energy percentages come from three major macronutrient of homemade infant formula

4.3 Instrumental conditions of Atomic Absorption Spectrometer

The detection limits for each metal were calculated as double the standard deviation of a series of measurements of a solution, the concentration of which is distinctly detectable above, but close to blank absorbance measurement (US-EPA, 1983). The values for Cd, Pb, Cu and Zn are presented in Table 4.3

Table 4.3: Standard conditions used in determination of different elements and their detection limits using Atomic Absorption Spectrometer.

Elements	Wavelength (nm)	Slit width (nm)	Burner height Mm	Fuel flow L/min	Detection limit (ppm)
Lead	217.0	0.5	7.1	1.1	0.001
Cadmium	228.8	0.5	7.0	1.2	0.002
Chromium	357.9	0.5	7.0	4.2	0.001
Copper	324.7	0.5	7.0	1.1	0.001
Iron	248.3	0.2	7.0	0.9	0.002
Zinc	213.9	0.2	7.0	1.2	0.002

4.4 Concentrations of Heavy metals and trace elements in Commercial infant formula foods

The concentrations of the three heavy metals Pb, Cd and Cr as well as the three trace elements Cu, Fe and Zn in five commercial infant formulas from the Chattogram super market analyzed as dry weight basis are presented in Table 4.4

Table 4.4: Concentrations of Lead, cadmium, Chromium, Copper, Iron and zinc in Commercial infant formula foods.

Food items	Mean concentrations in $mg.kg^{-1}$ dry weight ± S.E.					
	Pb	Cd	Cr	Cu	Fe	Zn
LA	$0.17±0.07^{a}$	ND	$0.14±0.06^{b}$	$0.65±0.04^{b}$	$6.03±0.15^{e}$	$5.03±0.02^{c}$
CE	$0.17±0.06^{a}$	$0.05±0.01^{bc}$	$0.16±0.07^{b}$	$0.63±0.26^{b}$	$2.73±0.41^{c}$	$2.29±0.26^{a}$
OT	$0.40±0.18^{c}$	$0.03±0.01^{b}$	$0.33±0.13^{c}$	$0.86±0.38^{d}$	$2.66±0.23^{b}$	$2.77±0.25^{a}$
JH	$0.19±0.01^{ab}$	ND	ND	$0.71±0.08^{c}$	$5.23±0.25^{d}$	$6.53±0.35^{d}$
NM	$0.20±0.01^{b}$	$0.06±0.01^{c}$	$0.09±0.01^{a}$	$0.55±0.04^{a}$	$1.55±0.03^{a}$	$2.54±0.05^{b}$

*Values are means ± standard deviation of three replicate analyses. Means within a column (for each variable) marked with different superscript letters are significantly different at ($p< 0.05$); ND- Not detected; (One way ANOVA, α =0.05 Tukey test).

4.5 Concentrations of Heavy metals and trace elements in homemade infant formula foods

However, homemade infant formula foods contain less amount of heavy metals but contain higher amount of trace elements and the concentration of metals are presented in table 4.4

Table 4.5: Concentrations of Lead, cadmium, Chromium, Copper, Iron and zinc in Homemade infant formula foods.

Food items	Mean concentrations in $mg.kg^{-1}$ dry weight ± S.E.					
	Pb	Cd	Cr	Cu	Fe	Zn
HP	$0.14±0.02^{b}$	ND	$0.06±0.02^{b}$	$0.52±0.01^{d}$	$2.27±0.03^{e}$	$2.45±0.13^{b}$
RP	$0.08±0.02^{a}$	ND	$0.03±0.02^{ab}$	$0.56±0.02^{d}$	$1.94±0.01^{d}$	$2.47±0.35^{b}$
SA	$0.14±0.02^{b}$	$0.18±0.02^{b}$	ND	$0.37±0.02^{c}$	$1.22±0.07^{c}$	$6.67±0.15^{c}$
SP	$0.13±0.01^{b}$	$0.14±0.01^{a}$	$0.23±0.01^{c}$	$0.14±0.01^{a}$	$0.56±0.05^{b}$	$1.30±0.05^{a}$
LM	ND	ND	$0.24±0.02^{c}$	$0.27±0.02^{b}$	$0.29±0.01^{a}$	$2.58±0.48^{b}$

*Values are means ± standard deviation of three replicate analyses. Means within a column (for each variable) marked with different letters are significantly different at ($p< 0.05$); ND- Not detected; (One way ANOVA, α =0.05 Tukey test).

4.6 Comparison of Heavy metals and trace elements in infant formula foods

The result showed that the heavy metals and trace elements content of commercial infant formulas were higher than homemade formula except cadmium.

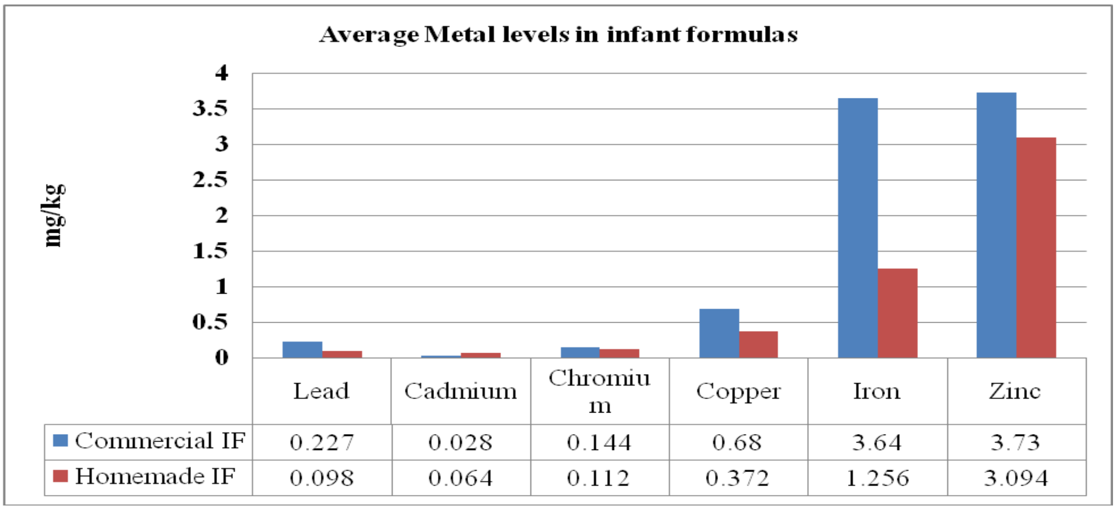

Figure 4.3 A comparison of average metals level in infant formulas

4.7 Permissible limit of heavy metals and trace elements in baby food

Heavy metals are naturally present in the environment in varying amounts, depending on the soil composition of the area. In addition, humans have locally increased the environmental levels e.g., through pollution. The uptake of heavy metals by crops and animals from the environment varies with species, and the local environmental levels also play a role in the resulting heavy metal content in raw agricultural commodities. Legislative limits have been set to maximum permissible levels of heavy metals in many foods.

Table 4.6 Permissible limit of heavy metals and trace elements in baby food

Metal	Maximum Allowed Limit (MAL) in infant formula (mg/kg)
Pb	0.08 (Vitosevic, 2007)
Cd	0.05 (Walker, 1988).
Cr	0.10-1.0 (CAC., 2003)
Cu	1.5–2.5 (Vitosevic, 2007)
Fe	Minimum 2.9 (WHO/FAO-CAC, 1981).
Zn	7-8 (Vitosevic, 2007)

Chapter 5: Discussion

The study was conducted with the aim of determination of nutritional value and assessment of concentration of heavy metal and also trace elements in commercial infant formula compared with homemade infant formulas. Samples on some selected commercial Infant Formulae were analysed in the laboratory for proximate composition and the data were subjected to Analysis of Variance to rank the significant differences in means. There were significantly different ($P<0.05$) across the selected infant formulae sample for moisture, ash, crude protein, crude fate, crude fiber and carbohydrates. All the important findings of this study have been discussed in this section.

The present study revealed that moisture contents of commercial infant formulas ranged from 3.23 to 4.53 % with significant differences among samples. Moreover, the actual moisture contents were within the values declared on their labels. Another study comparing moisture contents of infant formulas between developed and developing countries reported moisture contents (3.66% and 2.62%; respectively) (Olu-Owolabi et al., 2007). Moisture contents were below the maximum level recommended by Codex standard for infant formulas (5%) (WHO/FAO-CAC, 1981).

The higher carbohydrate level in human milk plays a significant role in infant nutrition. Human breast milk contains both digestible and indigestible carbohydrates, while infant formulae, only digestible carbohydrates are required and regulated (Thompkinson and Kharb, 2007). The present study revealed that carbohydrate contents of commercial and homemade infant formulas ranged from 39.63 % to 72.47% and 4.73% to 97.30% respectively. The average mean carbohydrate contents of commercial and homemade formulas are 61.15 and 66.71% where homemade formula contains higher amount of carbohydrate than commercial formulas. The actual carbohydrate contents were higher than that declared by the manufacturer for all analyzed commercial formulas. Another study comparing carbohydrate contents of infant formulas between developed and developing countries reported more or less similar (58.42% in formulas collected from developing countries and 68.0% in formulas from developed countries (Olu-Owolabi et al., 2007). Carbohydrate contents of infant formulas set in Codex Alimentarius range between 9 and 14.0 g/100 kcal (about 60.0 to 93.3 %) (WHO/FAO-CAC, 1981).

During infancy, high amount of protein is required because it is essential for normal growth, body development and tissue repair. The present study showed that protein contents differed significantly among most of the examined formulas and the average mean of protein content in commercial and homemade formulas are 15.43% and 6.21% respectively. Moreover, most of commercial formulas protein content fulfills the CAC standard where all most all the homemade formulas content were lower than standard value except SP sample. Another study reported protein contents of 11.63% in formulas collected from developing countries while they were 12.14% in formulas collected from developed countries (Olu-Owolabi et al., 2007). Protein contents of infant formulas set in Codex Alimentarius range between 1.8 and 3.0 g/100 kcal (about 12.0 to 20 %) (WHO/FAO-CAC, 1981).

Dietary lipids are indispensable for normal growth and development. Besides energy, they provide fat-soluble vitamins and are necessary for efficient absorption of fat-soluble vitamins, carotenoids, and cholesterol (Carey and Hernell, 1992). The present study showed that fat contents of commercial and homemade were 14.11% and 1.59%. The actual fat contents were lower than standard value in all formulas except NM which contained about 28.63%. A wider range was reported by another study (3.86 and 29.83%) (Olu-Owolabi et al., 2007). Anothe study found that higher fat contents of infant formulas ranged from 18.70 to 26.70 % (Kotb et al., 2016). Fat contents of infant formulas set in Codex Alimentarius range between 4.4 and 6.0 g/100 kcal (about 29.3 to 40.0 %) (WHO/FAO-CAC, 1981).

The present study revealed that the average ash content of commercial and homemade formulas was 3.03% and 1.90% respectively. The ash contents among commercial formulas were higher than homemade. Another study reported more or less similar range (2.04% to 3.09%) (Olu-Owolabi et al., 2007).

The crude fiber of OT was the highest with (9.93%) and no amount of crude fiber was detected in LA, NM and LM. The average crude fiber content in the different commercial brands of infant baby foods was 2.57%. This value is higher to the values reported for infant formula baby foods sold in Makurdi metropolis, Nigera 1.65% (Onuorah et al., 2019). However, homemade infant formula contained average 3.8% fiber which is higher and not exceeds the standard maximum value of 5% based on (SON., 1988) recommendations. The low fiber content in this study may be due to the fact that dehulled raw materials were used in the formulation. Low fiber influences nutrient availability positively while high fiber lowers plasma cholesterol levels (SON., 1988). LA, NM and LM were not detected; this is due to milk dissolution properties and might not need in the food of infants below six months.

Infants need energy from food for activity, growth, and normal development. Energy comes from foods containing carbohydrate, protein, or fat. The number of kilocalories needed per unit of a person's body weight expresses energy needs. The average energy content of commercial infant formula was 427 Kcal/ 100g which were higher than homemade infant formula 304Kcal/100g. The energy of NM was the highest with (506 Kcal/100g) and the lowest found in LM 62Kcal/100ml. The Institute of Medicine Food and Nutrition Board (2000) has determined that the Estimated Energy Requirements (EER) for infants should balance energy expenditure at a level of physical activity consistent with normal development and allow for deposition of tissues at a rate consistent with health and the estimated energy requirement for 6 to 12 months baby is 645-844 Kcal/day.

The differences in concentrations of metals in the various types of infant foods are significant (p<05). The result of elemental analysis indicates that the order of abundance of metals in the infant food follow Zn > Fe> Cu> Pb> Cr> Cd. As shown in Fig.4.3, the commercial formula products showed higher concentrations of zinc, copper, iron, lead and chromium than the homemade infant formulas.

Te levels of lead in the samples in the present survey ranged from being not detected to 0.40 mg/kg, which is consistent with the results obtained from other studies (Johnson and Samson, 2013), Another study reported 0.08–0.23 mg/kg of lead levels in infant formula in Abuja, Nigeria, (Aguzue et al., 2014), whereas lower amounts, 0.00375–0.0249 mg/kg, of lead were recorded in similar samples in Turkey (Sipahi et al., 2014). However, the study conducted in Addis Ababa, Ethiopia, revealed that the levels of lead in the powdered infant formulas were below the detection limit (Adanech. 2008). The actual lead contents in all samples except RP were higher the Maximum Allowed Limit; 0.08 mg/kg (Vitosevic, 2007)

The concentration of cadmium ranged between not detected to 0.18 mg/kg. The highest concentration of cadmium was found in brand SA. Within the same type of infant formula, the differences observed are significant (p<05). The levels of Cd in all samples were within the permissible limits 0.05mg/kg (Walker, 1988). The examined samples have cadmium contents below the permissible limit. The concentrations of cadmium in this survey were higher than levels reported in the literature (Olu-Owolabi et al. 2007).

The average concentration of Cr in commercial infant formula sample was at higher concentration (0.144 mg/kg) than in homemade formula at 0.112 mg/kg. There was significant ($P>0.05$) difference among Cr concentration in commercial and homemade infant formulas. This study exceed the Chromium levels ranging from 0.002 to 0.069 mg/ kg have been reported in baby food in Turkey (Saracoglu et al. 2006).The levels of Cr in all samples are within the permissible limits 0.10-1.0 mg/kg (CAC., 2003)

The concentrations of copper in this study varied considerably amongst the various types of infant foods analysed. The mean concentrations of copper in these infant foods ranged from 0.14 mg/kg to 0.86 mg/kg. The highest concentration of copper was observed in commercial infant formula OT. Apart from these brands, other types of infant formulas showed appreciable differences in the contents of copper within a particular brand. The concentrations of copper found in this study were below the maximum allowed limits and no health hazard is expected from copper in the consumption of these brands of infant foods. The levels of copper reported in this study are in agreement with copper levels found in human milk 0.07 mg/L (Silvestre et al. 2000). Similar levels of copper have been reported for infant foods in 0.1 mg/kg to 2.5 mg/kg (Vitosevic et al. 2007). Another study reported concentrations of copper in infant foods in India of 1.2–3.2 mg /kg (Tripathi et al. (1999). The concentrations of copper recorded in this survey are lower than values reported by these researchers.

With optimal reserves of iron in the newborn, its absorption from breast milk is sufficient to cover the daily requirements. At about 4–5 months of age, demand of tissues for iron increases so formulas should be supplemented with iron. Significant iron deficiency in infants may lead to impaired cell-mediated immunity, while an excess may lead to serious liver damage (Sola and Navarro, 2006). The present study showed that iron contents ranged from 0.29 mg/kg in LM homemade formula to 6.03 mg/kg in LA commercial formulas. The highest iron content in LA formulas may be attributed to their fortification with iron. Other studies reported wider iron ranges (4.3 and 26.6 µg/g) (Iwegbuea et al., 2010), and (1.02–67.5 µg/g) (Saracoglu et al., 2006).

Codex Alimentarius sets minimum iron content in infant formulas of 0.45 mg/100 kcal (about 2.9 mg/L) (WHO/FAO-CAC, 1981).The average iron content in commercial infant formulas were higher iron 3.64 mg/kg , on the other hand average iron content of homemade formulas were 1.26 mg/kg which is lower than standard level.

Zinc is required for the synthesis of protein and nucleic acid metabolism. For infants, the recommended daily Zn intake is 5.0 mg/day. The concentration of Zn in mother's milk is about 2 mg/kg and decreases over time with lactation to reach 20 % of its initial concentration after 3 months (Molska et al., 2014). The present study showed that zinc contents ranged from 1.30 to 6.67 mg/kg in SP and SA formulas respectively with significant variations in its contents among samples collected from commercial and homemade formulas ($P \leq 0.05$. An excess of zinc can lead to microcytic anemia or neutropenia and reduce the concentration of iron in the body. Additionally, zinc competes with magnesium at the absorption level in the intestines and in the structural parts of the bone (Molska et al., 2014). Another study reported that Zn contents of various brands of infant formulas ranged between 1.72 and 7.81 µg/g (Iwegbuea et al., 2010). Codex Alimentarius sets minimum zinc content in infant formulas of 5.0 mg/100 kcal (about 3.33 mg/kg) (WHO/FAO-CAC, 1981).

Chapter 5: Conclusions

The present study includes the investigation of the proximate composition and heavy metals in commercial and homemade infant formulas of Chattogram City Corporation. The Moisture and carbohydrate contents in all samples were within the standard level recommended by Codex standard for infant formulas. The average protein content of commercial infant formula fulfills the set limit, but in homemade formula the levels were far below the standard level. The fiber contents of homemade infant formula were higher than commercial formula. Overall, the estimated nutrients are higher amount in commercial formula than homemade formula due to fortification. So during homemade infant formula preparation the mother should include the entire nutrients in proper ratio so that it could reach the standard level.

The concentrations of toxic and essential elements in infant formulas were above the permissible levels for toxic elements, except for chromium and below the recommended desired levels for essential metals. However, there is a need to fortify these food materials with essential elements such as Fe, Zn and Cu in order to meet the nutritional needs of growing children. It is important to mitigate health risks by imposing a set of maximum permissible levels for all toxic elements in infant foods into the applicable legislation, especially in foodstuffs that characterize higher toxic metals contamination. Manufacturers of infant foods should ensure the quality of their products by selecting raw materials.

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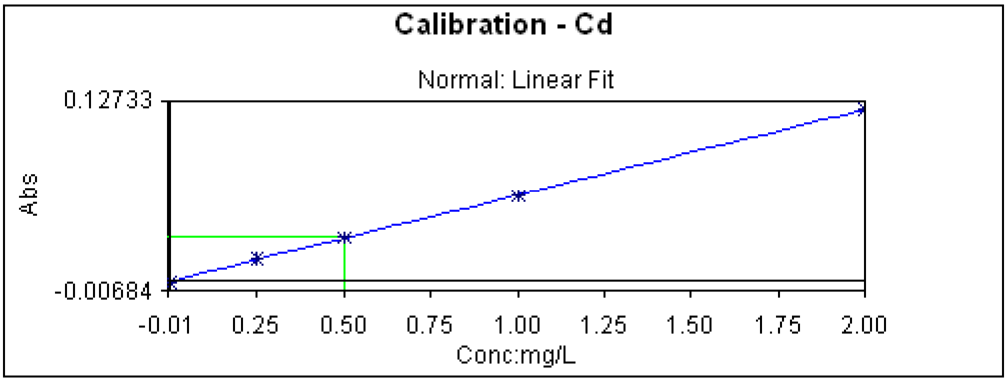
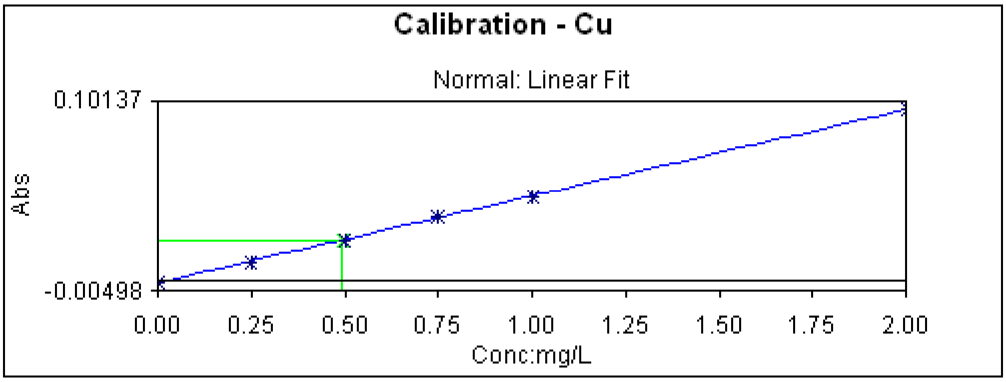
Appendix-I: Different commercial and homemade infant formula taken for this study

Sl. No	Commercial Formula Id with brand name	Homemade Formula Id with brand name
1	LA= Lactogen	HP= Hotchpotch
2	CE= Cerelac	RP= Rice Puree
3	OT= Oats	SA= Sago Puree
4	JH= Junior Horlicks	SP= Suji Puree
5	NM= Nido Milk	LM= Liquid Milk

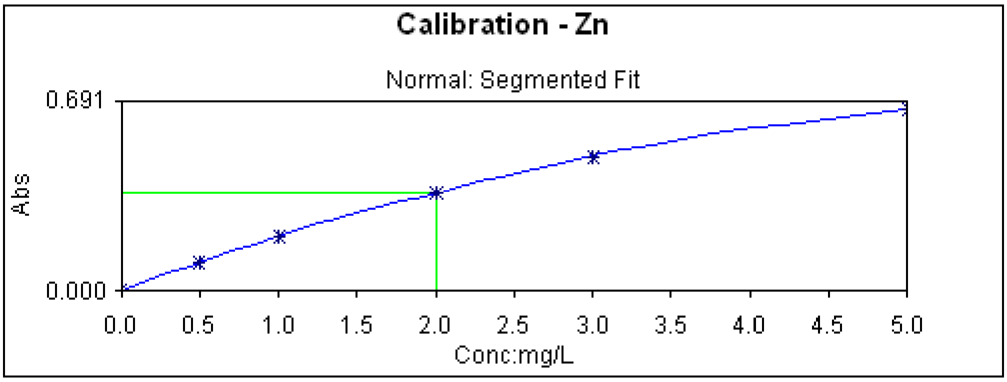
Appendix-II: Typical Standard calibration curve



Standard calibration chromatogram for Cadmium concentration



Standard calibration chromatogram for Copper concentration



Standard calibration chromatogram Zinc concentration

Appendix-III: Analytical activities in Laboratory

Oats (OT) Sample filtering

Sample Preparation

Sample running in Atomic Absorption Spectrophotometer

Brief Biography

Mrs. Tahmina chowdhury passed the Secondary School Certificate (SSC) Examination in 2008 and then Higher Secondary Certificate (HSC) Examination in 2010. Tahmina chowdhury obtained her B.Sc. (Hons.) in Food Science & Technology in 2016 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science & Technology, Chattogram Veterinary and Animal Sciences University, CVASU, Bangladesh. She has immense interest to improve child nutritional status across the world.