CONCENTRATION AND INTAKE OF MACRO, TRACE AND TOXIC ELEMENTS BY INFANTS THROUGH MILK AND BABY FOODS IN MOROGORO MUNICIPALITY, TANZANIA

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ABSTRACT

Excessive or too little minerals pose health effects for infants. Since the infancy period is the most critical time with respect to nutrition, there is a need to know the actual concentration of trace and toxic elements in their foods during this stage. This study aimed to determine the levels of calcium, potassium, zinc, copper, lead and cadmium in human-, cow's- and tinned baby- milk and canned baby foods marketed in Morogoro Municipal and intake of these mineral elements by infants. Human milk was collected from 41 mothers at different postpartum periods, cow milk was bought from dairy farms and infant formulas were purchased from shops. Atomic Absorption Spectrometry flame mode was used to analyze 97 samples. Intake of macro and micro elements were assessed for compliance with Recommended Daily Intake by World Health Organization. Mean elemental concentration in the seven days postpartum was higher and lowest in the 9-months postpartum. Human-, cow- and tinned baby- milk showed significant differences ($p < 0.05$) in all elements except for lead. Concentrations in different brands of baby foods were significantly different in each element except Zn and Cd ($p > 0.05$). Mineral content in human milk was found to be below recommendations. Cow milk had high Ca and K and tinned baby milk had high K and Cu. Calcium in baby foods matched recommendations, K was high, Zn and Cu were absent. Pb and Cd were below permissible levels. There was a relationship between K concentration in human milk with maternal age and marginally with wheat consumption ($p = 0.050$). This study concludes that concentration of toxic elements in milk and tinned baby foods in Morogoro Municipal are low hence safe for infant consumption. Conversely, tinned baby foods should be fortified with Zn and Cu to ensure infants consume enough for their growth and development.

DECLARATION

I, MARIA NGILISHO, here by declare to the SENATE of Sokoine University of Agriculture that this dissertation is the product of my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
FAO	Food and Agriculture Organization
IOCCC	International Obfuscated C Code Contest
NIMR	National Institute for Medical Research
PO – PSM	President's Office – Public Service Management
RCH	Reproductive and Child Health
RDI	Recommended Daily Intake
SUA	Sokoine University of Agriculture
UNICEF	United Nations Children's Fund
URT	United Republic of Tanzania
WHO	World Health Organization
Ca	Calcium
K	Potassium
Zn	Zinc
Cu	Copper
Pb	Lead
Cd	Cadmium

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Milk is a complex, bioactive substance meant to promote growth and development of the infant mammal and is also the fundamental food for infants. Each mammal's milk is naturally designed to meet the nutritional as well as growth and developmental requirements of its descendant which are very different from one species to another. The most natural and best source for all mammals 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 in human beings. Human milk is also a source of immune agent which can, among other functions, hold intestinal disease in check - almost as important as nutrition itself (Castle and Watkins, 1989; Mohan and Stump, 2000).

Despite mothers being consistently reminded about the necessity to breastfeed their infants, certain situations (hypogalactia, inverted nipple, nipple tenderness, medical conditions and unavoidable pressing factors) prohibit breastfeeding hence the need for artificial feeding may have to be resorted to. For elite urban women, the ready - made milk preparations from the market have become handy for infant feeding. Raw milk as it comes from the cow is the natural substitute to human milk for infant feeding. The amount of feeding milk depends on the variation in the energy needs and mineral requirements of the children under various conditions. It is estimated that proper infant feeding can prevent millions of deaths occurring from infantile gastroenteritis and malnutrition (Tripathi *et al.*, 1997; Cruz *et al.*, 2009).

Among the nutrients in milk, calcium (Ca) has been well recognized by researchers and the public because of their importance in children and osteoporosis in old people. In fact, milk is an ideal source of macro-elements, such as calcium (Ca), potassium (K) and phosphorus (P). Moreover, microelements and even heavy (toxic) metals can also be found in milk (Lampert, 1984; NDRC, 2005 and Yamawaki *et al.*, 2005). Micro-elements, also called trace elements such as chromium (Cr), iron (Fe), copper (Cu), and zinc (Zn) are known to be essential for normal growth and have a variety of biochemical functions in all living organisms. There is now growing evidence on the importance of trace elements in human nutrition, and there are reports suggesting that trace elements deficiencies can lead to impaired growth during infancy and childhood (Castle and Watkins, 1989; Mohan and Stump, 2000). While Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from elements-to-elements (Tripathi *et al.*, 1997; Yamawaki *et al.*, 2005; Ramamurthy and Thillaivelavan, 2005).

Since the neonatal period is one of the most critical time with respect to nutrition, there is need to know the actual intakes of trace elements by infants during the first year of life. On the other hand, heavy metals such as arsenic (As), cadmium (Cd) and lead (Pb) are harmful and have no beneficial effects on human. Intake of heavy metals by the human body via ingestion depends upon food habits. It is well established that Pb and Cd are toxic and children are at higher risk and more sensitive to these metals than the adults (Simsek *et al.*, 2000; NDRC, 2001; Gundacker and Zodl, 2002).

In Tanzania and particularly in Morogoro, studies of macro-elements, micro-element and heavy metals have been done on a variety of environments (Chove *et al.*, 2006; Giliba *et al.*, 2011; Mashauri and Mayo, 1990). The aim of the present study was to determine the concentrations of elements, namely Ca, K, Cu, Zn, Pb and Cd in different types of milk and baby foods found in Morogoro municipality. The daily intake of the elements by infants through different types of milk and baby foods marketed in the municipality will also be presented in this study and assessed for compliance with WHO recommended standard levels in foods.

1.2 Statement of the Problem

The modern world is saturated with chemical pollutants due to past and current uses of man-made chemicals and mined metals which have led to the widespread contamination of air, water and soil (NDRC, 2005). Chemical pollutants in the environment can enter people's bodies hence human have become reservoirs for these substances. The chemicals attack various parts of the body, including the liver, the brain, blood and breast milk. Some of the chemicals are short-lived, leaving the body quickly; others can stay for years. Among the chemicals that can invade breast milk, are metal such as heavy (toxic) metals which include lead and cadmium. Heavy metals enter human body through inhalation and ingestion (via food and drinking water). As minerals and trace elements, some heavy metals such as chromium, manganese, copper, and zinc are essential to life as they maintain the metabolism of the human body (Kiliç, 2009), however, they could be toxic when their concentrations exceeds limits of safe exposure (Reilly, 1991; Skurikhin, 1993).

In addition to toxicity problems, Cu and Zn deficiency may also be experienced, therefore, as it has been said by Iyengar (1989), knowledge of heavy metal contents in crops is important for the identification of adequate, sub-adequate and marginal intake levels for humans and animals, so that diseases related to trace element deficiency can be defeated. Large number of ailments comprising anaemia, depressed growth, dermatitis, dwarfism, electrolyte-imbalance, gastro-intestinal and neurological disorders, lethargy and nausea has been associated in humans with Cu and Zn deficiency, as well as with toxicity due to excessive intake (Somer, 1974; Graham, 1976; Prasad, 1976; Ward, 1995).

Exposure to hazardous substances during critical periods of infant development can disrupt the signals of normal development and lead to health problems later in life. Once in the body, heavy metals compete with and displace essential minerals such as calcium, magnesium, copper and zinc hence interfere with organ system function. Toxic metals may lead to a decline in the mental, cognitive, and physical health of the individual. A higher level of heavy metal toxicity in children is in the autistic spectrum. The evidence suggests that some children in the autistic spectrum build up more of these toxic metals because of an inability to excrete them. When the brain becomes lodged with heavy metals, it does not function normally, causing autistic symptoms and learning disorders (NRDC, 2005).

1.3 Justification

The world-wide contamination of milk with undesirable substances via animal feeds, heavy metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health. Recent report according to Semaghiul *et al.* (2008) indicate that good quality measurements are essential to control and often play a vital role in maintaining products and process quality, both in manufacturing, trade and in research. Milk products are very important human nutrients and their consumption has increased in recent years (Jigam *et al.*, 2011). Clinical interests in trace metal analyses for the diagnosis and prognosis of diseases and nutritional status have been stimulated by increasing awareness of the prevalence of poisonings from occupational, environmental and nutritional exposures to toxic metals (Ayodele and Hassan, 2001). It is also recognized that measurements of milk trace metal levels could provide a prognostic index (Lawal *et al.*, 2006). As reported by Semaghiul *et al.* (2008), many dangerous elements or compounds, such as metals and metalloids, accumulate along the food chain. Furthermore, their concentrations in the environment grow with the increase of urban, agricultural, and industrial emissions. The almost ubiquitous presence of some metal pollutants, especially cadmium and lead, facilitates their entry into the food chain and thus increases the possibility of them having toxic effects on humans and animals. Although heavy metals have industrial uses, their potential toxicity for people and animals is the object of several studies. For some elements the effects are accumulative and it is necessary to control their level in consumed food.

As heavy metals, zinc, copper, lead and cadmium residues in milk are of particular concern because milk is largely consumed by infants and children (Licata *et al.*, 2004; Caggiano *et al.*, 2005; Zheng *et al.*, 2007; Tajkarimi *et al.*, 2008). Heavy metals are responsible for many pernicious effects on human health as saturnisme (lead contamination), immune-depression and skin diseases (zinc and copper contamination) (Llobet *et al.*, 2003) and cadmium has been implicated in osteoporosis with impaired general health, lung and kidney damage (Vidovic *et al.*, 2003). Long term chronic exposure to cadmium has been associated with anemia, anosmia, osteomalacia and cardiovascular diseases especially hypertension (Ayodele *et al.*, 1999). Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage and even death (Abdulaziz and Mohammed, 1997). Heavy metal pollution is a result of increasing industrialization throughout the world, which has penetrated into all sectors of the food industry; and because of that, the World Health Organization (WHO) classifies heavy metals as one of the risks people are exposed to through food (Cruz *et al.*, 2009).

The findings from the study conducted by Bala *et al.* (2008) imply that consumption of the polluted water by animals or human beings could be hazardous to their health. Soil contaminated by these effluents will produce unhealthy food as heavy metals can enter the food chain and thus be consumed by human beings. For instance, the mean values of lead from the polluted areas, which was found to be above the maximum permissible level, could exert toxic effects on human beings if consumed from the water or irrigated agricultural products from the sites.

Although metals and other pollutants are passed on into breast milk in accordance with the environmental contamination and diet of the mothers (Grandjean *et al.*, 1995) the advantage of breastfeeding outweigh the risks under normal conditions. Thus, breastfeeding should be encouraged (Abadin *et al.*, 1997; American Academy of Pediatrics, 1997). Nevertheless, accurate data should be collected about pollutants in breast milk to counsel mothers optimally. Thus, with increasing modern agricultural practices, industrial pollutants in the environment, animal feeds and use of sewage sludge in agriculture is increasing (Kira and Maihara, 2007; Semaghiul *et al.*, 2008), the study of minerals; trace elements and heavy metal is quite imperative in human nutrition. For instance, the toxic metal content of milk and dairy products is due to several factors – in particular – environmental conditions. As a result of soil, air and water pollution by heavy metals that exposes man and grazing animals to health risks and therefore requires urgent attention, it becomes necessary to determine and monitor the levels of toxic metals in milk, because they can significantly influence the human health. Existence of manufacturing industries around town (Morogoro) and metallurgical factory contribute to water, air and soil pollution. Since the neonatal period is one of the most critical with respect to nutrition, there is need to know the actual intakes of elements by infants through milk and infant formulas.

1.4 Objectives of the Study

1.4.1 General objective

The general objective of this study is to determine the levels and intake of the macro-, trace and toxic elements by infants through milk (human, fresh cow, tinned) and baby foods marketed in Morogoro Municipality.

1.4.2 Specific objectives

i) Determine the concentration of macro elements (Ca and K), trace elements (Cu and Zn) and heavy (toxic) metals (Cd and Pb) in human milk, fresh cow milk, tinned baby milk and baby foods.

ii) Estimate the intake of Ca, K, Cu, Zn, Cd and Pb by infants through milk and baby foods.

iii) Investigate dietary and non-dietary factors related to presence of macro elements (Ca and K), trace elements (Cu and Zn) and toxic metals (Cd and Pb) in human milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Elemental Concentration in Milk and Baby Foods

Breast milk is recommended as the sole source of infant nutrition for the first 6 months of life. However, less than 35% of the world's infants are exclusively breast fed at this age (WHO, 2009). While less than 40% of 6 month old infants are exclusively breast fed in developing countries (WHO, 2009), only 13% of US infants and 3% of European infants are exclusively breast fed at this age (CDC, 2010; Freeman *et al.*, 2000). Substitutes to breast milk include other milks, sweetened liquids and solid foods in the developing world (Marriott *et al.*, 2007), while infant formula is the most common substitute in the US and in Europe for infants younger than 4 months (Freeman *et al.*, 2000; Grummer-Strawn *et al.*, 2008). In addition to formula, a number of commercial infant foods are available, intended for consumption before the age of 6 months. Approximately two thirds of European infants are reportedly fed some solid foods at 4 months of age (Freeman *et al.*, 2000).

For example; Sweden has one of the highest rates of breast feeding in Europe with 12% exclusively breast fed at 6 months, but breast feeding has declined in recent years and the use of substitutes is increasing (Socialstyrelsen, 2009, 2010). Recent research on formula composition has focused mainly on protein and energy content and a few nutrients and vitamins (Agostoni and Domellof, 2005). Most essential trace elements present in infant formula have received very little attention. For example, for eight out of the eleven essential elements regulated in formula, the data required for a science-based risk assessment of infant exposure is currently lacking (Codex Alimentarius Commission, 2007). Moreover, infant formula and foods may hold toxic elements as a result of their natural presence in raw materials used, from contamination, or from food processing. For example, rice-based food products intended for children were recently reported to contain concentrations of arsenic above what is considered safe (Meharg *et al.*, 2008). Women and infants face challenging health issues in their communities where there are environmental risks. Many chemicals can be transferred from the body stores and from blood into the breast milk of a lactating mother. Despite the attention focused on environmentally persistent organo-chlorine compounds in human milk, levels of toxic metals in milk are also of significance (Wappelhorst *et al.*, 2002; Koizumi *et al.*, 2008). Literature examining these contexts and the processes through which health is affected are relatively limited (Stout *et al.*, 2009).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed through metabolic processes. As human activities increase, especially with the application of modern technologies, pollution and contamination of food chain has become inevitable. Many reports indicate the presence of heavy metals in milk, and often it is needed to assess the levels of heavy metals in food. Lead, cadmium and mercury residues in milk are of particular concern because milk is largely consumed by infants and children (Caggiano *et al.*, 2005; Licata *et al.*, 2004; Tajkarimi *et al.*, 2008; Zheng *et al.*, 2007) and infants, particularly in the 6-12 months age group, are vulnerable to infection due to their immature immune system. This is also the time when they are weaned from a pure breast milk diet to one with solid foods. Infant formula milk is one of the primary foods infants ingest during this period. The determination of these heavy metals levels in milk is particularly attended by international organizations (Codex Alimentarius Commission, 2003).

A study by Conti (1997) found that packaging materials have been found to be source of heavy metals found in food stuffs. Other studies by Rabinowitz *et al.* (1985) and Hellen *et al.* (1995) found higher blood lead levels in formula-fed infants than in breast-fed infants. This may be a result of contaminated formula cans or formula prepared using tap water with high lead levels. Lead levels in blood and breast milk correlate closely with areas where lead is still used in gasoline, with the highest levels in areas with heavy traffic. Mothers in countries where lead is still used in gasoline, and mothers living near lead smelters have higher levels of lead in their breast milk due to community contamination (Oskarsson *et al.*, 1995). Much of the lead in breast milk does not come from the mothers' exposure during lactation; it comes from lead stored in the mothers' bones. During pregnancy and lactation, a woman's body extracts calcium from her own bones to provide calcium for her child's bone development; as a result, lead stored in the mother's bones also enters the blood and breast milk during pregnancy and lactation, posing an exposure risk to the fetus (Moline *et al.*, 2000). Cadmium levels in breast milk are significantly associated with cigarette smoking. The study by Radisch (1987) showed a direct relationship between the number of cigarettes a mother smokes per day and the level of cadmium in her breast milk.

2.2 Human Health Effects of Toxic Elements

Heavy metal poisoning is now so common that it is literally impossible to avoid it. Even newborn babies have been shown to have heavy metals as soon as they emerge from their mother's womb, as well as receiving mercury from breastfeeding (Tsuchiya *et al.*, 1984; Ong *et al.*, 1993; Truska *et al.*, 1989 and Yang *et al.*, 1997). Many studies have estimated that more than 20 per cent of U.S. children have had their health or learning significantly and adversely affected by toxic metals, such as mercury, lead, and cadmium (ATSDR/EPA 1997; U.S. EPA 1995, 1996). Georgious (2007) suggested that more than 50 per cent of learning difficulties and cognitive disturbances in these children were probably due to prenatal or postnatal exposure to toxic metals. Furthermore, toxic metals have been documented to be reproductive and developmental toxins, causing birth defects and damaging foetal development, as well as causing neurological effects, developmental delays, learning disabilities, depression, and behavioral abnormalities in many otherwise normal-appearing children (Bonithon-Kopp *et al.*, 1986; Needleman, 1995; Casdorph 1995; Atchison, 1988).

Lead can trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications can arise through the regular consumption of foodstuffs only slightly contaminated with lead. Lead is a typical cumulative poison and the danger of chronic intoxications is the greater problem (International Obfuscated C Code Contest (IOCCC), 1996). The nervous system is the principal target organ for lead, although lead can adversely affect most organs in the body. The nervous system of the fetus and young children is particularly vulnerable to lead because of its rapid growth during this time. Many studies have demonstrated neurobehavioral impairment in children even at low lead exposure levels (Rice, 1996; Tripathi *et al.*, 1999). A decrease of intelligent quotient (IQ) in children was associated with an increase in blood lead (Schwartz, 1994) and tooth lead (McMichael *et al.*, 1994). Basically, as a result of their comparatively high affinity for proteins, the lead ions consumed bond with the haemoglobin (red blood pigment) and the plasma protein of the blood. This leads to inhibition of the synthesis of red blood cells and thus of the vital transport of oxygen. If the bonding capacity here is exceeded, lead passes into the bone-marrow, liver and kidneys. Such intoxication leads to:

- Encephalopathies in the central nervous system (CNS);
- Disturbances in kidney and liver functions progressing as far as necrosis;
- Damage to the reproductive organs;
- Anaemia and many metabolic deficiency symptoms.

Some of the injurious processes are still not properly understood. Particularly dangerous to all forms of life are the organic lead compounds. They cause injuries to mental development such as reduction of intelligence, growth disturbances and spasticity. The foodstuffs which contribute most to the consumption of lead are vegetables, fruits, drinking water, beverages and cereal products (IOCCC, 1996).

The kidneys are the major targets of cadmium toxicity following oral exposure. A specific indicator of cadmium–induced renal effects is tubular proteinuria. An increase in urine cadmium correlates well with low molecular weight proteins in urine. Other effects include disturbances in calcium metabolism, hypercalciuria, formation of renal stones (Hayano *et al.*, 1996) and hypertension during pregnancy (Kosanovic *et al.*, 2002). Cadmium is also particularly concentrated is in the liver, the blood-forming organs and the lungs. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme inhibitions. It is now known that the Itai-itai sickness in Japan (with bone damage) is a result of the regular consumption of highly contaminated rice. Cadmium, like lead, is a cumulative poison, i.e., the danger lies primarily in the regular consumption of foodstuffs with low contamination. The decisive point is whether absorption of the existing cadmium actually takes place. This is, firstly, dependent upon the composition of the diet as a whole and secondly, on the bio-availability of the cadmium compound present. Present contamination of foodstuffs and acceptable maximum values among the foodstuffs which present a problem are offal, crustaceans and shellfish and some fungi. Overall, however, vegetables are of greater importance for human cadmium contamination. Rice and wheat contain 10-150 µg /kg; meat, fish and fruit between 1 and 50µg/kg. The cadmium content of milk products is very low (IOCCC, 1996).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was carried out in Morogoro Municipality situated on the foot slopes of the Uluguru Mountains about 200 kilometres west of Dar es Salaam (Fig. 1). It is the regional headquarters of Morogoro region with a population of 228 863 people (URT, 2005) in the ratio of 50.3 % women (115 224) and 49.7 % men (113 639). Economically people in this area engage in small-scale commercial activities, some practice urban farming as well as livestock keeping and others are employed to provide services in different sectors.

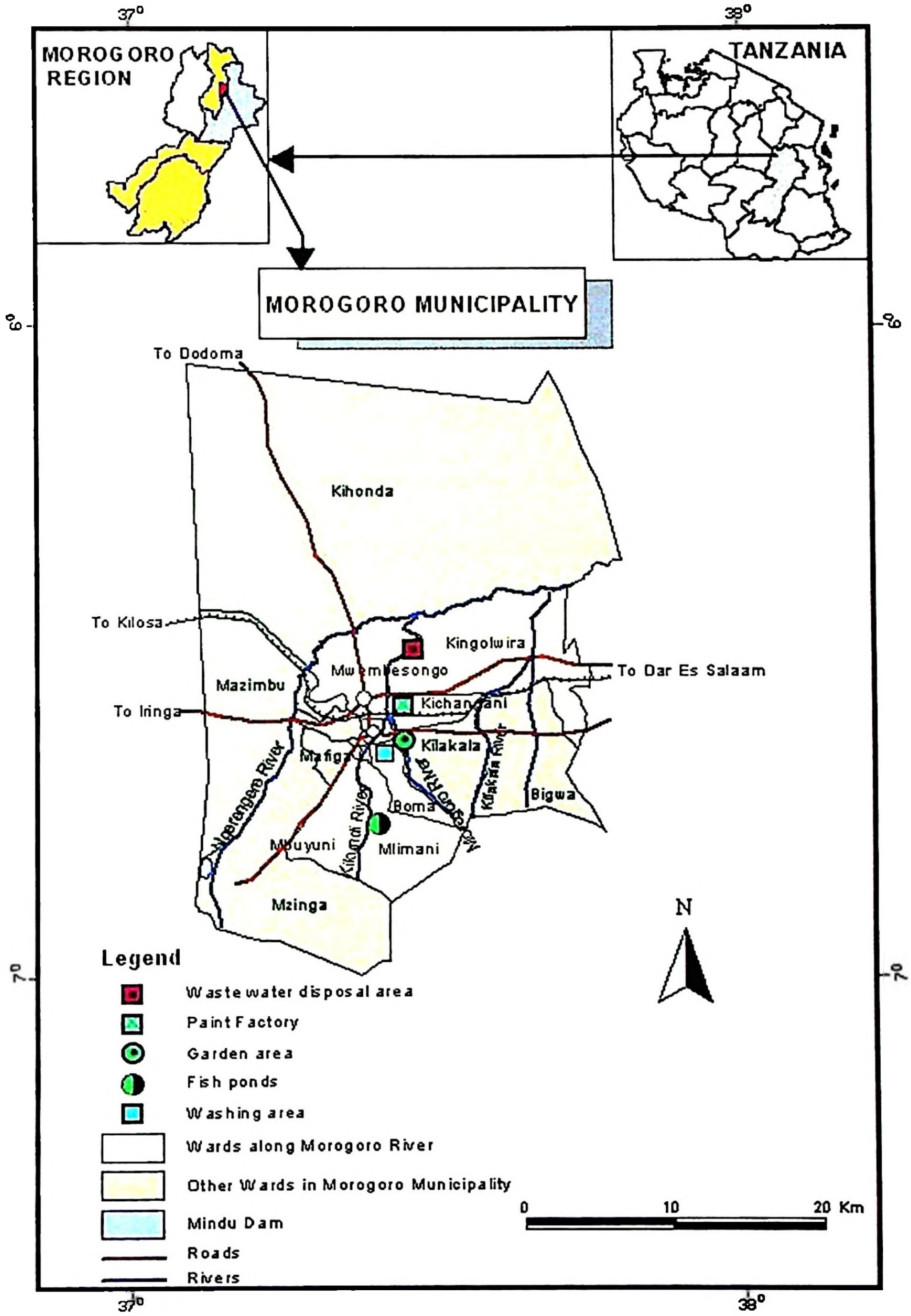

Figure 1: Map showing geographical location of the study area

3.2 Study Design

A longitudinal study design was used to collect fresh cow milk and infant formula (tinned baby milk and baby foods) samples to obtain factual information to ascertain the pattern of change on levels and daily dietary intake of the macro, essential and toxic elements by infants through consumption of fresh cow milk, tinned baby milk and baby foods. The collection was done once a month in four consecutive months starting from November, 2010 to February, 2011. A cross-sectional survey (collection of samples and data only once) was conducted between February and March 2011 to collect human milk samples and baseline data on levels and daily dietary intake of toxic/ heavy metals consumed by infants through human milk,

3.3 Sampling Protocol

Sampling in this study was purposive; targeting lactating mothers of infants of seven days, three months, six months and nine months; areas with mass milk production thus entail selling to general public and brands that are commonly available in markets around Morogoro municipality.

3.4 Inclusion and Exclusion Criteria

3.4.1 Human milk samples

Human milk samples were collected from the mothers who were in good healthy based on physical observation, pregnancy history from their Reproductive and Child Health (RCH) clinic cards, mothers with uncomplicated pregnancies and had delivered a single full term infant (39 ± 2 weeks), without any chronic disease (diabetes, high blood pressure, HIV/AIDS, sickle cell), have not been sick for the past two weeks, with anthropometric measurements and Body Mass Index (BMI) in the normal range of 18.5 – 24.9 (WHO, 2004) and have not be in any supplements. Mothers with complicated pregnancies and had delivered pre-term babies or twins or those with chronic diseases, have been sick recently, with anthropometric measurements and BMI above or below normal and who are using supplements were excluded from the study.

3.4.2 Consent to participate in the research

Selected mothers were educated about the purpose of the study, the study procedures and benefits of the study. They were allowed to decide and willingly give their consent to participate in the study. After their consent, weight in kilograms and height in centimeters measurements were taken to make possible to calculate BMI. The study participants were given consent document to read and sign, for those who could neither read nor write the researcher read the document content to them and asked for their consent and signed by a thumb print (Appendix 1).

3.4.3 Cow milk samples

Fresh cow milk samples were collected from local dairy farms/collection centres where it is available in plenty and the general public including mothers with infants had access to.

3.4.4 Infant formula samples

Tinned baby milk (SMA, S-26 (1), Nestle NAN (1), Lactogen) and canned baby foods (Nestle cerelac stage 1 (wheat), Nestle cerelac stage 2 (banana), Nestle cerelac stage 3 (chocolate), Heinz Farley's Rusk (banana) – stage 1 and Heinz Farley's Rusk (original) - for all ages) samples were bought from shops based on different brands commonly available and suitable for consumption at different stages of infant development as stated on the label. It was made sure that they were originally contained and distributed in tin or foil; and not expired. Four different batch numbers were bought for each brand to ensure that tins or foils from the different production batches are picked.

3.5 Sample Collection

3.5.1 Human milk samples

Human milk samples were collected from 41 lactating mothers at Mafiga Health Centre Morogoro. Mothers who live in the municipality and suburban areas of the municipality were recruited while attending RCH. Mothers of children with age of 7-days, 3-months, 6-months and 9-months were targeted. Mothers were educated about the study and its purpose. Those who were willing to participate in the study were given consent form to sign. 30ml of milk from each mother was expressed. Breast Pump and Feeding Sets CAMERA® model 21233 (made in Taiwan) (shown in Fig. 2) were used to express and collect the milk samples. A nurse was involved in sample collection. Sample were kept in clean sterilized plastic containers and were transported in ice-packed cool box and stored at -20 °C in the freeze until analysis was carried out. Also a structured questionnaire was used to capture some demographic, socio-economic, dietary intake and non-dietary factors information related to presence of macro elements (Ca and K), trace elements (Cu and Zn) as well as toxic metals (Cd and Pb) in human milk. The questionnaire was administered in Kiswahili (appendix 2b) which is the national language in Tanzania; the English version of it is attached as appendix 2a.

Figure 2: Samples of human milk and human milk pumping sets

3.5.2 Cow milk samples

Samples of fresh cow milk were bought once a month for four consecutive months starting from November, 2010 to February, 2011 from local dairy farms within the municipality namely Magercza, Magadu, Bigwa, Kihonda and Wami. The liquid samples (human and cow milk) were stored in sterilized plastic containers, transported in a cool box with ice packs and kept in the freezer at -20 °C until the analysis was carried out.

3.5.3 Infant formula samples

Nine brands of commercially readily available tinned baby milk and baby food (infant formulas) were purchased from local markets within the town every one month from November, 2010 to February, 2011; batch numbers were taken into consideration to avoid analyzing products of the same batch. The commercially available infant formula purchased included SMA, S-26 (1), Nestle NAN 1, Lactogen; and Nestle cerelac stage 1 (wheat), Nestle cerelac stage 2 (banana), Nestle cerelac stage 3 (chocolate), Heinz Farley's Rusk (banana) – stage 1 and Heinz Farley's Rusk (original) - for all ages.

3.6 Laboratory Analysis of Sample

3.6.1 Sample preparation for Calcium, Potassium, Zinc and Copper

A weight of 0.5 g for dry (5ml for liquid) homogenized sample was measured into Kjeldahl flask; 30ml HNO_3-$HClO_4$ (2:1 v/v) was added to the flask along with 4 glass boiling beads. Samples were left overnight in acid. Two reagent blanks were carried out through the entire procedure along with samples. Each Kjeldahl flask was placed on Kjeldahl block set at low temperature; gentle heating was continued until HNO_3 and H_2O have been driven off, and samples were contained in $HClO_4$. At this point effervescent reaction occurred between samples and $HClO_4$. After reaction of samples with $HClO_4$ was completed, high heating of samples was applied for 2hours. The flasks were removed from the Kjeldahl block and left to cool. Each digested sample was then transferred to 50ml volumetric flask and diluted to the mark with distilled water. The precipitate that occurred was shaken and left to settle overnight and then analyzed by method 984.27 (AOAC, 1990).

3.6.2 Sample preparation for Lead and Cadmium

A weight of 0.5 g for dry (5 ml for liquid) homogenized sample was weighed; 5 ml of K_2SO_4 ash solution was added and mixed thoroughly. 5 ml of distilled H_2O was added to ensure sample and ash are well mixed. The solution was covered with glass cover and dried at 120 ^{0}C in the oven overnight until thoroughly dried. The vessel was placed in a cold furnace and a temperature was set at 500 ^{0}C. This temperature was maintained overnight. The vessel was removed from the furnace and cooled; the ash was white and essentially carbon-free. The side of vessel was washed down with 5ml of distilled water, and then 2.0 ml of HNO_3 was added and stirred using stirring rod to break up solid particles, and dried thoroughly on hot plate at low setting, then increasing hot plate setting to medium for 30 minutes to ensure dryness.

The vessel was returned into the furnace at 500 ^{0}C for 30 minutes, and then cooled. A volume of 1 ml HNO_3 was added followed by addition of 5 ml H_2O to the vessel, swirled to dissolve the content, and left to stand for 5 minutes; then warmed gently on hot plate at 90 ^{0}C for 5 minutes to dissolve the remained residue. The solution was cooled and a sample was transferred to 50ml volumetric flask with the aid of distilled water, diluted to the mark with distilled water and mixed well; and left to stand to allow any precipitate to settle. A clear supernatant formed was used for determination by AAS.

3.7 Instrumentation, Reagents and Glass Wares

Atomic absorption spectrophotometer (UNICAM 939 Model) was used for the determination of calcium, potassium, zinc, copper, lead and cadmium. The maximum absorbance was obtained by adjusting the cathode lamps at the operation conditions shown in Table 1. Atomic absorption spectroscopic standard solutions for Ca and K was purchased from BDH Spectrosol Products; for Zn, Pb and Cd standard solutions were bought from Scharlau Chemie - S.A.; while standard solution for Cu was purchased from SIGMA Products. Working standard solutions were prepared by diluting the stock solution; Nitric acid, K_2SO_4 ashing solution, electrolyte solution (1.7 M in HOAc) and nitrogen pre-purified. Glasswares used were conical flasks, volumetric flasks, watch glass, pipette, measuring cylinder, ashing vessels, controllable hot plate, Kjeldah block and Kjeldah flask. Distilled water was used where required.

Table 1: Instrumental conditions for AAS flame mode

Parameters	Values					
	Ca	**K**	**Zn**	**Cu**	**Pb**	**Cd**
Wave length (nm)	422.7	766.5	213.9	324.8	217.0	228.8
Lamp current (%)	100	100	75	90	75	75
Fuel flow (l/min)	1.4	1.2	1.2	1.1	1.1	1.2
Acceptable fit	0.995	0.995	0.995	0.995	0.995	0.995

3.8 Calibration Curves

Standards were set at highest sensitivity. On reading each corresponding lamp and wavelength were set at zero. Zeroing was done after setting on flame and setting the wavelength (Ca: Ca lamp, λ=422nm; K: K lamp, λ=766nm; Zn: Zn lamp, 213nm; Cu: Cu lamp, λ=324nm; Cd: Cd, λ=326nm and Pb: Pb, λ=405nm).

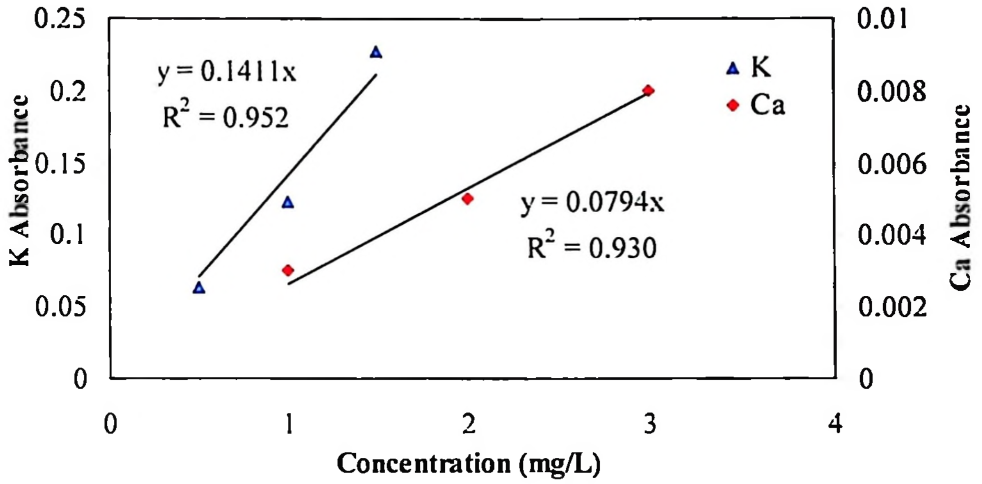

Figure 3: Concentration versus absorbance calibration curves for Ca and K

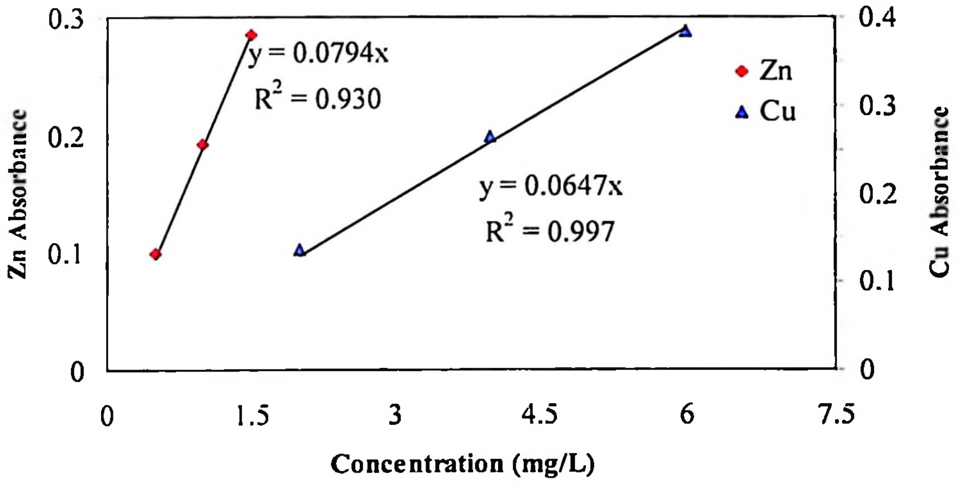

Figure 4: Concentration versus absorbance calibration curves for Zn and Cu

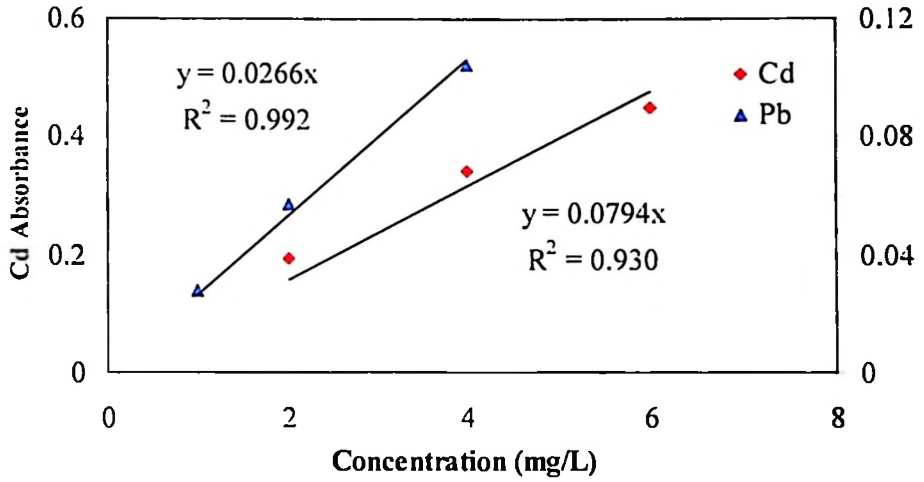

Figure 5: Concentration versus absorbance calibration curves for Pb and Cd

3.9 Elemental Determination

The concentration of calcium, potassium, zinc, copper, lead and cadmium were analyzed and determined by atomic absorption spectrophotometer (UNICAM 939 Model). 5 ml aliquot (injection volume) of each milk and baby food sample obtained after wet digestion was injected into the flame detector of the AAS with the help of an auto-sampler, and the elemental concentration was read from the output of the printer connected to the computer. Duplicate samples were analyzed for each element and the average was recorded. The concentrations of Ca, K, Zn, Cu, Pb and Cd were determined for each sample of human milk, fresh cow milk, tinned baby milk and baby foods. A total of 41 samples for human milk, 20 fresh cow milk samples and 9 samples of infant formula were analyzed in this study.

3.10 Quality Control

The reliability of the method for estimation of Ca, K, Zn, Cu, Pb and Cd concentration in milk and baby food samples by AAS technique was checked by analyzing standard reference milk sample (A-11) obtained from the International Atomic Energy Agency (IAEA). Measurements were taken several times and the average result agreed within ± 7% of the certified values. Standard milk and food samples were used as control samples.

3.11 Statistics

Statistical Package for Social Sciences version 12 was employed in the analysis of data. One-way Analysis of Variance (ANOVA) was used to compare the mean concentration of elements within and between groups. Further analysis was performed by Post hock multiple comparison tests to get homogenous subset results. A value of $p < 0.05$ was accepted as statistically significant.

3.12 Ethical Consideration

Formal permission to conduct the study was granted by the office of the Deputy Vice Chancellor (DVC) - Sokoine University of Agriculture, District Medical Officer (DMO) - Morogoro Municipal Council and the administration of Mafiga Health Centre - Morogoro. Authority to collect samples from human subjects was sought from and approved by National Institute for Medical Research (NIMR), Ethical clearance number NIMR/HQ/R.8a/vol.IX/1065 (Appendix 3).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Concentrations of Macro Elements, Trace Elements and Toxic Metals in Milk Samples

Milk is an important source of dietary minerals. However, the content of macro, micro and sometimes toxic elements in milk depends upon factors such as dietary habits, the contents of these elements in the soil and foodstuffs and food processing procedures which varies considerably among and within locations. Both, too low and too high content of these macro as well as micro elements can cause adverse health effects in humans; knowledge about dietary mineral elements in food is important. As for toxic elements, these enter the human body mainly by ingestion and inhalation.

4.1.1 Human milk samples

The results of this study for six elements in human milk samples (n = 41) is given in Table 2. The mean, standard deviation, minimum and maximum values of the samples collected are presented in the form of postpartum period, that is, 7 days, 3 months, 6 months and 9 months. The average mean concentration of elements (for the four postpartum periods) from the results of this study showed that, Ca was 24.91 g/L in the milk. K was 7.65 g/L, Zn was 0.0033 g/L, Cu as micro element was below detection limit; and Pb and Cd as toxic elements were also below detection limit. It is observed that mean elemental concentration in the seven days postpartum group (early postpartum period) is higher compared to 3-months, 6-months and 9-months postpartum, meaning that, there is an apparent decline in the mean levels as the stage of postpartum progresses. This declination in mineral elements concentrations in human milk as the postpartum period progresses has also been reported by other authors (Arnaud and Favier, 1995; Burguera *et al.*, 1988; Casey *et al.*, 1985; Ohtake and Tamura, 1993).

Table 2: Mean and standard deviation of elemental concentration (g/L) in human milk samples

Element		Postpartum period				p-value
		7 days	3 months	6 months	9 months	
Ca	Mean	27.320	23.869	22.831	25.605	0.506
	SD	9.702	7.905	6.551	14.769	
	Max	12.994	14.579	10.774	14.896	
	Min	43.433	39.153	32.653	68.324	
K	Mean	5.017	4.302	4.605	4.403	0.000
	SD	0.533	0.383	0.247	0.456	
	Max	4.103	3.834	4.103	3.346	
	Min	5.631	4.832	4.999	5.007	
Zn	Mean	0.006	0.002	0.003	0.002	0.000
	SD	0.002	0.001	0.002	0.001	
	Max	0.003	0.001	0.001	0.001	
	Min	0.007	0.004	0.007	0.002	
Cu	Mean	BDL	BDL	BDL	BDL	0.028
	SD	0.001	0.002	0.001	0.001	
	Max	BDL	BDL	BDL	BDL	
	Min	0.002	0.007	0.003	0.003	
Pb	Mean	BDL	BDL	BDL	BDL	0.440
	SD	0.001	0.002	0.003	0.002	
	Max	BDL	BDL	BDL	BDL	
	Min	BDL	0.0001	0.006	0.00007	
Cd	Mean	BDL	BDL	BDL	BDL	0.367
	SD	0.0001	0.0003	0.0003	0.0003	
	Max	BDL	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	BDL	

BDL: Below detection limit

4.1.2 Fresh cow milk samples

Table 3 presents the results of the study for six mineral elements: Ca, K, Zn, Cu, Pb and Cd in 20 fresh cow milk samples from local dairies located in different areas in Morogoro town. Statistical tests proved that, Ca concentration in fresh cow milk from the four locations sampled was not statistically different ($p = 0.11$); but further analysis showed that milk from Magereza was significantly different in mean Ca concentration ($p < 0.05$) from milk from Magadu and Kihonda but statistically similar with milk from Bigwa and Wami ($p > 0.05$); milk from Magadu and Kihonda had no statistical difference in mean Ca concentration ($p > 0.05$). K, Zn, Cu, Pb and Cd concentrations from the different locations each proved to be statistically different by ANOVA tests ($p < 0.05$); however, further analysis proved otherwise as follows; K concentration in milk from Magadu, Magereza and Kihonda was statistically similar while these three were statistically different from Wami and Bigwa milk; again there is significant statistical difference between Wami and Bigwa milk. Mean Zn concentration in fresh cow milk from Magereza and Bigwa were statistically the same but the two are statistically different from Wami, Magadu and Kihonda milk. Cu concentration in milk from the five locations (Magadu, Wami, Magereza, Bigwa and Kihonda) was significantly different.

Despite Pb and Cd being below detection limits; statistics has proved that there is no significant different in Pb concentration in milk from Magereza and Magadu; Magadu, Bigwa and Kihonda; Bigwa, Kihonda and Wami; however, there exists a significant difference in Pb concentration between milk from Magereza, Bigwa, Kihonda and Wami; Magadu, Kihonda and Wami. For Cd, Kihonda, Wami and Bigwa; Bigwa and Magadu; Magadu and Magereza; are statistically similar.

Dobrzański *et al.* (2005) studied the contents of 38 micro- and trace elements in raw milk of cows in the Silesian region, Poland; they found that the location of cows has a significant impact on the contents of many micro- and trace elements in milk, this means that, nutritional as well as toxic element concentrations in cow's milk vary widely due to constitutional (animal breed and lactation period) and environmental conditions related to geographic factors (climate, season, and soil contamination) and especially dietary composition of animal feed and manufacturing practices of final products (Santos, 2004).

Table 3: Mean and standard deviation of elemental concentration (g/L) in fresh cow milk from local diaries in Morogoro Municipality

Element		Dairy farm					p-value
		Magereza	Magadu	Bigwa	Kihonda	Wami	
Ca	Mean	61.884	74.567	67.849	76.945	73.140	0.112
	SD	0.140	0.420	10.482	0.589	0.701	
	Max	61.785	74.270	60.437	76.529	72.645	
	Min	61.983	74.864	75.261	77.361	73.636	
K	Mean	6.283	6.297	5.934	6.251	5.767	0.000
	SD	0.024	0.039	0.023	0.036	0.010	
	Max	6.266	6.269	5.917	6.225	5.761	
	Min	6.300	6.324	5.950	6.276	5.774	
Zn	Mean	0.006	0.006	0.006	0.006	0.006	0.000
	SD	0.00005	0.00002	0.000	0.000	0.00004	
	Max	0.006	0.006	0.006	0.006	0.006	
	Min	0.006	0.006	0.006	0.006	0.006	
Cu	Mean	0.002	0.005	0.000	BDL	0.005	0.000
	SD	0.0001	0.00006	0.000	0.000	0.00006	
	Max	0.002	0.005	0.000	BDL	0.005	
	Min	0.002	0.005	0.000	BDL	0.005	
Pb	Mean	BDL	BDL	BDL	BDL	BDL	0.015
	SD	0.000	0.000	0.003	0.0003	0.0001	
	Max	BDL	BDL	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	BDL	BDL	
Cd	Mean	BDL	BDL	BDL	BDL	BDL	0.011
	SD	0.00004	0.000	0.000	0.000	0.00004	
	Max	BDL	BDL	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	BDL	BDL	

BDL: Below detection limit

4.1.3 Tinned baby milk samples/Commercial infant milk formula

The mean concentrations of Ca, K, Zn, Cu, Pb and Cd determined in 16 different tinned baby milk such as SMA (1), NAN (1), S-26 (1) and Lactogen (1) were found to vary with values of 403.92 – 185.038 (Ca), 27.606 – 27.29 (K), 0.071 – 0.053 (Zn), 0.176 – BDL (Cu), 0.0009 – BDL (Pb) and 0.027 – BDL (Cd) g/L in different tinned baby milk brands respectively as shown in Table 4. With regard to elemental concentration in tinned baby milk samples analyzed, ANOVA statistical checks has showed that mean elemental concentration for Ca, K, Zn, Cu and Cd between the different brands is significantly different ($p < 0.05$) except Pb with $p = 0.12$. Advanced tests established that: mean Ca concentration is statistically different between SMA, Lactogen and S-26; NAN, Lactogen and S-26; but NAN and S-26 are statistically the same.

K concentration between S-26 and Lactogen and between SMA and NAN are statistically similar; but, S-26 and Lactogen are statistically different from SMA and NAN. Mean Zn concentration is statistically different between Lactogen, SMA and S-26; Lactogen, SMA and NAN; but NAN and S-26 are statistically the same. Mean Cu concentration in the four brands assessed was statistically significantly different; mean Pb concentration was significantly different between NAN and Lactogen; Lactogen and S-26; Lactogen and SMA; and significantly similar between NAN, SMA and S-26; and SMA and S-26. Besides being below detection limit, mean Cd concentration was statistically significantly similar between SMA and S-26; NAN and Lactogen; but, significantly different between SMA and NAN; SMA and Lactoge; S-26 and NAN; and S-26 and Lactogen. While human milk is superior for the newborn infant, milk substitutes play a necessary role in infant nutrition when breast feeding is not possible, desirable or sufficient. The search for human milk substitutes has been conducted since the Stone Age, since then; there have been progressive attempts to bring the composition of these formulations closer to human milk. Presently, research is concentrating on those substances in human milk which serve other than traditional nutritional roles. The final aim is not necessarily to mimic the composition of human milk in every respect, but to achieve physiological effects as in breast fed infants (Goedhart and Bindels, 1994).

Table 4: Elemental concentration (g/L) in tinned baby milk present in the market in Morogoro Municipality

Element		**Brand name**				**p-value**
		SMA	**NAN**	**S-26**	**Lactogen**	
Ca	**Mean**	403.924	185.038	188.960	225.347	0.000
	SD	4.199	1.399	1.118	0.0002	
	Max	400.955	184.049	188.170	225.347	
	Min	406.893	186.027	189.751	225.347	
K	**Mean**	27.578	27.606	27.292	27.302	0.038
	SD	0.055	0.123	0.103	0.034	
	Max	27.539	27.519	27.219	27.278	
	Min	27.617	27.693	27.365	27.326	
Zn	**Mean**	0.064	0.053	0.054	0.071	0.000
	SD	0.000	0.001	0.001	0.0004	
	Max	0.064	0.053	0.054	0.071	
	Min	0.064	0.054	0.055	0.072	
Cu	**Mean**	0.051	0.143	0.177	BDL	0.000
	SD	0.001	0.000	0.002	0.000	
	Max	0.051	0.143	0.175	BDL	
	Min	0.052	0.143	0.178	BDL	
Pb	**Mean**	BDL	BDL	BDL	0.001	0.116
	SD	0.004	0.000	0.001	0.001	
	Max	BDL	BDL	BDL	0.000	
	Min	BDL	BDL	BDL	0.002	
Cd	**Mean**	BDL	BDL	BDL	BDL	0.049
	SD	0.000	0.000	0.004	0.0004	
	Max	BDL	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	BDL	

BDL: Below detection limit

4.1.4 Concentration of elements in human milk, fresh cow milk and tinned baby milk

The distribution of concentrations of six mineral elements in human milk, fresh cow milk and tinned baby milk is shown in Table 5. The mean concentrations of Ca, K and Zn in human milk are lower than the corresponding values in fresh cow milk and in tinned baby milk while the mean concentrations of the same elements in tinned baby milk are higher than the corresponding values in fresh cow milk and human milk. Statistical examination of the different types of milk in this study has revealed that, there is a statistical significant difference in mean individual element concentration for Ca, K, Zn Cu and Cd when the different types of milk are compared ($p < 0.05$). Pb concentration had a p-value of 0.11 meaning that there is no significant difference in mean Pb concentration in the different types of milk that were investigated. Mean Cu concentration in human milk and cow milk (statistically similar) had a significant difference with tinned baby milk. Mean Pb concentrations were statistically similar between tinned baby milk and human milk; human milk and fresh cow's milk; yet there was a statistical significant difference between tinned baby milk and cow milk. No statistical significant difference was observed in mean Cd concentration between cow's milk and human milk; however, the two were significantly different from tinned baby milk.

Qualitative analyses have been made for many elements in human milk. Among minerals of principle interest includes calcium and potassium. Calcium in human milk varies greatly in concentration in different women, although the level of secretion by one woman is relatively constant. Many workers have reported a range of calcium content between 19 and 40 mg/100 ml (Kon and Mawson, 1950). There is less potassium in human milk than in cow's milk, the range being 48– 65 mg/100 ml (Macy, 1949). Cu is not found in human milk but is concentrated in tinned baby milk than in fresh cow's milk. On the other hand, Pb and Cd concentrations are all below detection limits. Pb levels in breast milk are normally lower than in milk-based infant formulas (Jansen, 1991). However, Schumann (1990) reported that heavy metal concentrations in tinned baby milk were usually in the same order of magnitude. Reconstitution of infant formulas with contaminated tap water can result in much higher Pb concentrations (Pescheck *et.al.*, 1990).

Nevertheless, tap water Pb concentration clearly contributed to the relatively high concentration of Pb in infant formulas compared to that in breast milk or cow's milk. As it is observed, Ca in fresh cow's milk is highly concentrated than in human milk but less concentrated in tinned baby milk. Compared with human milk, fresh cow's milk is relatively low in copper and iron. Because the synthesis of red blood cells depends on these two nutrients (copper and iron), consuming only fresh cow's milk for a prolonged period of time can lead to anemia in infant. In addition, fresh cow's milk contain too much calcium and phosphorous for the baby's kidneys to handle (Chen, 2011). There is a variation in nutrient content of human milk; the variation is shown to be related to diet, season, lactation stage, and the individual mother (Chen, 2011). Supplementation of the mother with copper and iron increases the amount of these nutrients in breast milk. Calcium can vary by two- to three-fold among individuals. Likewise, fresh cow milk shows variations with seasons and animal feeds. It tends to have more nutrient content in winter and early spring when the early milk colostrum is produced than in summer when the milk production has run its course (Chen, 2011).

Table 5: Mean elemental concentration (g/L) and associated standard deviation in different milk samples in Morogoro Municipality

Element		Milk sample			p-value
		Human milk	Cow milk	Tinned milk	
Ca	Mean	24.965	70.877	250.817	4.11E – 47
	SD	10.165	6.684	95.997	
	Max	10.774	60.437	184.049	
	Min	68.324	77.361	406.893	
K	Mean	4.599	6.106	27.445	1.6E – 103
	SD	0.495	0.229	0.171	
	Max	3.346	5.761	27.219	
	Min	5.631	6.324	27.693	
Zn	Mean	0.003	0.006	0.061	1.44E – 73
	SD	0.002	0.000	0.008	
	Max	0.001	0.006	0.053	
	Min	0.007	0.006	0.072	
Cu	Mean	0.000	0.002	0.091	1.54E – 19
	SD	0.002	0.002	0.078	
	Max	BDL	BDL	BDL	
	Min	0.007	0.005	0.178	
Pb	Mean	BDL	BDL	BDL	0.11
	SD	0.002	0.000	0.003	
	Max	BDL	BDL	BDL	
	Min	0.006	BDL	0.002	
Cd	Mean	BDL	BDL	BDL	2.76E – 81
	SD	0.000	0.000	0.002	
	Max	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	

BDL: Below detection limit

4.2 Concentration of Macro Elements, Trace Elements and Toxic Metals in Baby Foods

Concentration (g/kg) of macro-elements, trace elements and heavy metals in 20 samples of baby foods chosen among the five brands are displayed in table 6. The mean concentrations in g/kg of Ca, K, Zn, Cu, Pb and Cd determined in different baby foods including Cerelac (1), Cerelac (2), Cerelac (3), Heinz (banana) and Heinz (original) were found to vary with values of 281.441 – 163.543 (Ca), 28.791 – 6.423 (K), 0.028 -0.010 (Zn), 0.045 – BDL (Cu), 0.003 – BDL (Pb) and BDL (Cd) in different baby food brands. As it is observed, Ca has higher concentration in Cerelac brands than in Heinz brands and the same with K and Zn. Mean Ca, K, Cu and Pb were statistically different between the five brands sampled ($p < 0.05$) while Zn and Cd were not statistically different between the brands with p-values 0.06 and 0.68 respectively. Yet, further analysis proved that mean Ca concentration between Cerelac 1 and Cerelac 3 showed a significant statistical similarity while Ca concentration in these two brands had significant statistical differences with Cerelac 2, Heinz-banana and Heinz-original. Mean K and Cu concentration are all significantly different between the different brands. For Zn, Heinz-banana, Heinz-original and Cerelac 3; Cerelac 1 and Cerelac 2; and Heinz-original and Cerelac 3 were statistically similar except that there was a statistical significant difference between Heinz-banana and Cerelac 2; Heinz-banana and Cerelac 1; Heinz-original and Cerelac 2; Heinz-original and Cerelac 1; Cerelac 3 and Cerelac 2; and Cerelac 3 and Cerelac 1. Mean Pb concentration was significantly different between Cerelac 1 and Cerelac 2; Cerelac 1 and Cerelac 3; Cerelac 1 and Heinz-banana; Cerelac 1 and Heinz-original; however, there was no statistical significant differences between Cerelac 2 and Heinz-original. Mean Cd concentration did not show any statistical significant differences, even so, Cd concentration was below detection limit.

A study by Ikem *et al.* (2002) on infant formula samples sold in Nigeria, UK and USA analyzed for various essential elements and non-essential elements, found that soy-based powder infant formulas generally had higher element levels than milk-based powder formulas. Some brands also had low nutritional contents when compared with the recommended dietary allowances (RDAs) and dietary reference intakes (DRIs) for use in North America.

Table 6: Concentration (g/kg) of macro-elements, trace elements and heavy metals in baby foods (infant food formula)

Element		Brand name					p-value
		Cerelac 1	Cerelac 2	Cerelac 3	Heinz (banana)	Heinz (original)	
Ca	Mean	206.744	281.441	205.246	179.476	163.543	0.000
	SD	0.559	2.797	0.003	1.403	0.556	
	Max	206.348	279.463	205.244	178.484	163.149	
	Min	207.139	283.419	205.249	180.468	163.936	
K	Mean	26.003	27.966	28.791	8.031	6.423	0.000
	SD	0.075	0.110	0.287	0.027	0.007	
	Max	25.950	27.888	28.588	8.012	6.418	
	Min	26.056	28.043	28.994	8.051	6.428	
Zn	Mean	0.028	0.028	0.028	0.010	0.018	0.065
	SD	0.0002	0.000002	0.000002	0.001	0.011	
	Max	0.028	0.028	0.028	0.010	0.010	
	Min	0.029	0.028	0.028	0.010	0.026	
Cu	Mean	0.028	0.033	0.045	BDL	BDL	0.000
	SD	0.001	0.002	0.001	0.000005	0.001	
	Max	0.027	0.032	0.045	BDL	BDL	
	Min	0.028	0.034	0.046	BDL	BDL	
Pb	Mean	0.003	BDL	BDL	BDL	BDL	0.000
	SD	0.001	0.000	0.001	0.001	0.001	
	Max	0.002	BDL	BDL	BDL	BDL	
	Min	0.004	BDL	BDL	0.000	BDL	
Cd	Mean	BDL	BDL	BDL	BDL	BDL	0.677
	SD	0.0004	0.001	0.001	0.005	0.004	
	Max	BDL	BDL	BDL	BDL	BDL	
	Min	BDL	BDL	BDL	BDL	BDL	

BDL: Below detection limit

4.3 Comparison of Elemental Concentration in Human Milk, Fresh Cow Milk, Tinned Baby Milk and Baby Foods with Other Studies

There are wide variations in the published data for the elemental concentrations of human milk of different countries. These variations may be partly due to differences in sampling for both; food sample selection and participants and analytical techniques rather than to geographic variation. Some of the results are recorded in Table 7 for comparison with the values obtained in this study. Compared to Nigeria and Egypt, the concentration of Ca and K in Tanzanian Mothers milk is found to be highest; Zn concentration is the same for Nigeria, Egypt and Tanzania; and the same in India and Sudan. There is very low Cu in Tanzanian Mothers' milk involved in this study but mothers in Egypt, Sudan and India have Cu concentration about half the amount found in Nigerian mothers. Pb concentration levels in Swedish and Indian mothers are statistically similar but lower than those for mothers in Sudan. Cd levels reported from Sweden and India are of the same concentration. There is also a wide disparity in the published data for the elemental concentrations of fresh cow milk of different countries as shown in Table 7.

The Ca and K concentrations found in Tanzania cows milk are higher than those found in Egypt but Zn concentration is alike though it differs with (lower than) that of Germany, India and Nigeria. Cu concentration is the same in Germany and India, Egypt has higher levels of Cu than in the present study, but Nigeria has the lowest compared to all. The Pb and Cd concentration in cow milk compares well in Germany and India and is higher than in Nigeria. In Tanzania the levels found were below detection limit. Tinned baby milk as well as baby foods also showed variations in elemental concentration, furthermore, data on Ca and K concentration were missing from the referral studies cited and it does not imply that tinned baby milk and baby food formula were specifically prepared for Tanzania; they are also available in the other countries including the ones mentioned in this study. Comparison between Zn concentration for tinned baby milk in studies conducted in Iran, India and Tanzania (this study) showed variations; they were found to be higher in samples used in this particular study, followed by a study conducted in India and a study done in lastly Iran; in tinned baby foods mean Zn levels were the same for present study samples and India. The Cu concentration was higher in tinned baby milk sold in Tanzania where as in Iran and India concentrations were about the same.

Table 7: Comparison of elemental concentration in milk and baby foods samples with other studies

Type of sample	Country	Unit	Ca	K	Zn	Cu	Pb	Cd	Reference
Human milk	Sweden	µg/l	-	-	-	-	0.7	0.06	Hallen *et al.* (1995)
	India	µg/l	-	-	1772	195	1.9	0.09	Tripathi *et al.* (1999)
	Sudan	µg/l	-	-	1300	177	2.6	-	Abusamra (1995)
	Nigeria	ppm	6.26	1.6	2.95	0.34	-	-	Belewu *et al.* (2002)
	Egypt	mg/100g	119.9	147.02	0.38	0.017	-	-	Soliman, (2005)
	Tanzania	g/L	24.965	4.599	0.003	0.000	BDL	BDL	Present study (2012)
Fresh cow milk	Germany	µg/l	-	-	3730	40.3	1.8	0.1	Ostapczuk *et al.* (1987)
	India	µg/l	-	-	3177	43.2	1.7	0.07	Tripathi *et al.* (1999)
	Nigeria	mg/L	-	-	0.25 - 0.4	0.56 - 0.59	0.16 - 0.63		Jigam *et al.* (2011)
	Egypt	mg/100g	32.36	51.77	0.165	0.05	-	-	Soliman, (2005)
	Tanzania	g/L	70.877	6.106	0.006	0.002	BDL	BDL	Present study (2012)
Tinned baby milk	Iran	mg/L	-	-	3.98 ± 0.3	0.53 ± 0.2	-	-	Khagani *et al.* (2010)
	India	µg/Kg	-	-	34592	1691	47.9	0.61	Tripathi *et al.* (1999)
	Tanzania	g/L	250.817	27.445	0.061	0.091	BDL	BDL	Present study (2012)
Baby food	India	µg/Kg	-	-	23894	2812	77.7	11.3	Tripathi *et al.* (1999)
	Tanzania	g/Kg	207.290	19.443	0.021	0.017	BDL	BDL	Present study (2012)

- : No available data. BDL: Below detection limit

4.4 Intake of Macro Elements, Trace Elements and Toxic Metals by Infants Through Milk and Baby Foods

4.4.1 Intake of macro elements, trace elements and toxic metals by infants through human milk

It is estimated that on average a lactation mother produces 800 g of milk per day (Sievers *et al.*, 2002); therefore, mean breast milk concentrations of Ca, K, Zn, Cu, Pb and Cd from this study were used together with an average daily intake of 800 g per day of human milk for the exclusively breast fed infant as provided by Sievers *et al.* (2002) to calculate daily intake of the afore said elements; in comparison, an Indian mother on average produces 700 ml of milk in 24 hours during the first year of lactation which almost matches with Sievers *et al.* (2002) findings. Again, Gupte (1978) and Sievers *et al.* (2002) has indicated an amount of 702 g daily intake for the exclusively breast fed infant. With this regard, daily intakes in g/L of Ca, K and Zn were found to be 19.972, 3.679 and 0.0024. Human milk, though designed to meet the nutritional requirements for the infant, was not reaching the standards set by the WHO; the disparity could be influenced by factors like, nutritional and income. Cu, Pb and Cd were seemed to be missing from human milk as they were below detection limit. Breast milk concentrations at 7 days; 3, 6 and 9 months postpartum were assumed to relatively have no significant difference in concentrations as concentrations in breast milk vary little after the first month of lactation.

Although World Health Organization (WHO), United Nations Children's Fund (UNICEF) and Food and Agriculture Organization (FAO) recommend exclusive breast feeding up to 6 months of age, parents do not always follow the set guidelines. Globally, it is estimated that 85% of mothers do not comply with these recommendations (Synnott et al., 2007). Briefel *et al.* (2004) reported that around 30% of US infants were introduced to infant cereals or pureed food before the age of 4 months, and only 6% after 6 months. The mean age of introducing cereals to infants was 4.2 months. In Sweden, the National Food Board recommends the introduction of taste portions (0.5–1 teaspoon) at around 4–6 months of age, but Swedish parent internet forums reveal that many infants receive full food portions at this age (Ljung *et al.*, In press).

Khagani (2010) reported that zinc intake by infants from breast milk is inadequate during the weaning period, especially if weaning foods are introduced at an early stage. Similarly, copper deficiency can occur because of infants' inability to use absorbed copper rather than a dietary insufficiency of the element. The bioavailability of essential elements to infants depends solely on the trace-element content of the breast milk, length of breast feeding and physiological factors such as nutrient absorption and nutrient supplementation of the mother (Al-Awadi and Srikumar, 2000). Though the daily dietary allowances (Vijaya, 1993) of Zn and Cu for infants are not satisfied with breast milk, the anti-infective factors present in significant amounts in mother's milk even at the end of one year of lactation protect the child from common infections and the production of these factors are not influenced by the nutritional status of the mother. These qualities of breast milk are of prime importance in an infant's defense against infection.

Decrease of zinc in body causes growth stoppage (Rivera, 1998) and its reduction damages immune system (Magnus *et al.*, 2004; Zhang *et al.*, 2008). The incidence of cytomegalovirus infection is related to Zn deficiency (Schumann *et al.*, 2002). Cu deficiency increases the free radicals and leads to reduction of protection against oxidative stress (Schneider *et al.*, 2007). Reduction of Zn and Cu in infant are associated with iron deficiency and leads to several complications (Ebringer *et al.*, 2008; Leotsinidis *et al.*, 2005).

In human milk most of the Zn and Cu is bound to casein and serum albumin respectively. It is noteworthy that no existing reports describing zinc deficiency in full term infants fed with human milk, although calculated zinc intake of infants from human milk is less than 50% of the RDA (Feely *et al.*, 1983). Copper deficiency has not been observed in preterm and term infants fed with human milk. Copper is a recent addition to many infant formulas and little is known about its bioavailability (Feely *et al.*, 1983). So far no significant effects of supplementation on human milk metals have been observed in literature (Silmara *et al.*, 2006 and Picciano *et al.*, 1998).In the present study, no statistically significant differences were found between mean Zn and Cu concentrations in breast milk and maternal age. This was in contrast to findings of Picciano *et al.* (1998) and Henkin *et al.* (1975), who found higher concentrations of zinc and copper in human milk, and in addition, they reported that milk from older mothers (>30 years) was higher in zinc and copper content than that from younger mothers (20 to 30 years) (Henkin *et al.*, 1975). Given the lower bioavailability of zinc from tinned baby milk compared with human milk, the recommended dietary allowance may be marginal. A greater quantity of Zn is required in tinned baby milk to produce the same metabolic response as with human milk feeding because of differences in bioavailability. Citric acid and picolinic acid have been proposed as possible ligands in human milk although several reports have suggested that proteins in the formula inhibited Zn absorption. The high iron content of some milk formulas may also reduce Zn absorption; formula-fed infants have a lower zinc status than breast-fed infants (Feely *et al.*, 1983). However, literature concerning various factors affecting metal concentrations in breast milk is in general controversial (Henkin *et al.*, 1975).

4.4.2 Intake of macro elements, trace elements and toxic metals by infants through fresh cow milk

Intake of elements through fresh cow milk was calculated as concentrations of elements per a litre of fresh milk since it is not possible to establish exactly the amount of ingested elements per day; this may result from income disparities (which determines how much milk to buy) among other factors; therefore, daily intakes in g/L would be: 70.877 g/L/day Ca, 6.106 g/L/day K, 0.006 g/L/day Zn and 0.002 g/L/day Cu with the assumption that a litre of milk is consumed per day. From the aforesaid daily intakes, Ca intake through cow milk was high; K, Zn and Cu were less than the recommended levels. Pb and Cd concentration, -0.002 g/L and -0.0016 g/L respectively were below the provisional tolerable daily intake (PTDI) of 3.0×10^{-7} g/kg_{bw}/day for Pb and 1.0×10^{-6} Cd g/kg_{bw}/day and hence it was not possible to estimate their daily intakes. Consumption of cow milk in particular is associated with beneficial health effects beyond its pure nutritional value. Several reports indicated that dairy products could serve as vehicles for other functional ingredients, such as phytosterols (as cholesterol replacement), fatty acids (as omega-3 acids) and various kinds of probiotic bacteria (Mattila-Sandholm *et al.*, 2002).

Mean Cu concentration in different types of samples from this study are in line with those reported by other authors that had independently worked on cow milk (Tripathi *et al.*, 1999; Kira and Maihara, 2007; Kondyli *et al.*, 2007). Doull's (2000) said the low Cu levels in milk could be due to Zn contained in food that interferes with the Cu absorption system, explaining the presence of low levels of this metal in milk. Previous studies showed that cow milk from rural areas often contained Cu concentration less than 0.39 mg/L (Licata *et al.*, 2004). However, the plausible reason for this may be due to the proximity to industrial and traffic areas which increases significantly the Cu concentration in cow milk. Among the heavy metals studied, Zn for instance, is such a critical element in human health of which, a minor deficiency is detrimental to health. Cu is needed for proteins involved in growth, nerve function and energy release (Institute of Medicine, 2001). It is vital for the formation of some important proteins. It is a critical functional component of a number of essential enzymes, known as cuproenzymes. Cu is stored in appreciable amounts in the liver. It also has anti-oxidant properties and involved in the regulation of gene expression, on the other hand, Zn deficiency is characterized by growth retardation, loss of appetite and impaired immune function. Reports have shown that Zn deficiency causes hair loss (in most cases), diarrhoea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions (Ryan-Harshman and Aldoori, 2005).

A study conducted in Niger State in Nigeria by Jigam (2011) observed Pb value to be higher than those reported in literature (Caggiano *et al.*, 2005; Tajkarimi *et al.*, 2008) and lower than those reported by Licata *et al.* (2004). The obtained values were not within the permissible concentrations and this could arise from run offs emanating from petroleum product pollution by heavy duty trucks and related sources. Pb is very toxic. It is a potent neurotoxin and has a cumulative effect on vital organs (Licata *et al.*, 2004). The obtained results show how this metal is ever more frequently found in milk samples in regions with rural agrarian settings without industrial activity. The presence of Pb in milk samples from such areas could also be due to other factors such as transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds, and the use of pesticide compounds. One of the most important sources of lead contamination in milk is water, especially in more contaminated areas (Codex, 2003) so water testing should be one of the important topics for future studies. Therefore, it is necessary to monitor this metal over time for better clarification of its presence in milk from the studied areas.

4.4.3 Intake of macro elements, trace elements and toxic metals by infants through tinned baby milk

The daily intake of these metals through tinned baby milk is computed for infants less than 6 months. Manufacturer's guidelines are taken as a basis for computation and the recommendation reference guideline used was WHO/FAO (1993). Table 8 presents daily mean consumption of macro, micro and toxic elements by infants less than six months from different kinds of tinned baby milk. Ca intake in all brands was at an average of 3.26 g/day which is far below recommended intake of 21 g/day; K intake was 4.79 g/day which was above a desired intake level of 0.4 g/day. Zn intake from NAN (1) was 0.4 g/day while the recommendation is 0.2 g/day. The rest of the brand, that is, SMA (1), S-26 (1) and Lactogen (1) provide insufficient daily intake of Zn.

Recommended daily intake for Cu is 0.002 g/day; however, intake of Cu from different brands was diverse; daily intake of Cu from Lactogen was 0.004 g, SMA (1) had a daily intake of 2.56 g/day, S-26 (1) had a daily intake of 4.03 g/day and NAN (1) had a daily intake of 0.0004 g/day. None of the brands had detectable concentrations of toxic elements Pb and Cd. Although feeding patterns differ between tinned baby milk; daily intake of macro elements had no significant difference between brands, Ca daily intake was low and K daily intake was high. Daily intake of micro element Zn was sufficient in NAN (1) but in the rest of the brands the daily intake was low with no significant difference between brands; the daily intake of micro element Cu from Lactogen (1) was also sufficient. Fluctuations for daily Cu intake were observed in SMA (1) and S-26 (1) having higher intakes yet significantly different between the two; and NAN (1) having lower intake.

Table 8: Daily intake of Ca, K, Zn, Cu, Pb and Cd by infants less than six months from different tinned baby milk

Type of tinned baby milk	Consumption (g/day)					
	Macro elements		Micro elements		Toxic elements	
	Ca	K	Zn	Cu	Pb	Cd
SMA (1)	3.26	5.04	0.05	2.56	-	-
NAN (1)	3.15	4.53	0.4	0.0004	-	-
S-26 (1)	3.02	4.68	0.04	4.03	-	-
Lactogen (1)	3.61	4.92	0.06	0.004	-	-

- : No information available.

4.4.4 Intake of macro elements, trace elements and toxic elements by infants through baby foods

Intake of selected elements by infants through different baby foods was calculated on the basis of feeding instructions given by the manufacturers. Table 9 shows daily intake of elements by infants older than six months from different baby foods selected for this study. Ca and K were the only macro elements observed to be taken daily by infants older than 6 months, as it is seen, amount ingested daily varies with brand. WHO/FAO (1993) recommends daily intake of 27 g and 0.7 g of Ca and K respectively for infants aged seven to twelve months. Cerelac brands were far below the recommended standards in daily Ca intake while Heinz brands had high Ca daily intake. It seems these baby foods were fortified with Ca and K only. Zn, Cu, Pb and Cd were absent. As micro minerals, Zn and Cu were not mentioned from the product's nutritional information; therefore, commercial baby foods should be fortified with Zn and Cu due to their biological value and function. Pb and Cd are indeed toxic metals and are not expected to be found; if found it is a result of contamination during processing or through packaging materials.

Table 9: Daily intake of elements by infants older than six months from different baby foods

Type of baby food	**Consumption (g/day)**					
	Macro elements		**Micro elements**		**Toxic elements**	
	Ca	**K**	**Zn**	**Cu**	**Pb**	**Cd**
Nestle cerelac stage 1 (wheat)	16	21.2	-	-	-	-
Nestle cerelac stage 2 (banana)	17.8	22	-	-	-	-
Nestle cerelac stage 3 (chocolate)	13.2	18.3	-	-	-	-
Heinz Farley's Rusk (banana)	55	12	-	-	-	-
Heinz Farley's Rusk (original)	39	-	-	-	-	-

- : No information available

4.5 Socio-demographic Data

Some basic socio-demographic characteristics of the respondents are displayed in Table 10. The study population consisted of 41 respondents. The age of the respondents varied from 19 – 44 years with a mean ± standard deviation age of 26 ± 5.02 years. The majority of them (73%) fell under 21 – 30 years age category. No statistical significant differences were found between mean Ca, Zn, Cu, Pb and Cd concentrations in human milk with respect to maternal age ($p > 0.05$), this result matches with the results of the study by Feeley (1983) that no significant correlations were found between maternal age on the Zn and Cu contents in human milk; but K concentration showed significant differences ($p = 0.008$) between human milk and their maternal age.

Weight diversity ranged between 38.7 and 71.8 kg; mean ± standard deviation weight was 57.56 ± 7.27 kg. Respondents below 50 kg were 90%. No correlation was observed between mean Ca, K, Zn, Cu, Pb and Cd concentrations in human milk and maternal weight ($p > 0.05$).

The population expressed four categories of occupation, about half of them 54% were housewives, 22% were small scale businesswomen and 17% were salary workers of whom only one had a professional job (a doctor) and Farmers were 7%. From the occupational point of view it can be said that, the large portion of the interviewees had a low educational level; and in fact, some of them could neither read nor write. No statistical significant differences was found between mean Ca, K, Zn, Cu, Pb and Cd concentrations in human milk versus mother's occupation ($p > 0.05$).

Study respondents were from nine (9) different residential areas within Morogoro Municipality. Respondents resided in Chamwino were 32%; this is a high density area, 24% were in Mafiga (medium density area) where as 12% were from Kasanga (low density area). Attendance from Chamwino and Mafiga could be explained by these areas being located in the neighborhood of Mafiga Health Centre; Kasanga is a bit distant but Mafiga is the nearest Health Centre around. Manzese (7%), Misufini (7%), Kiwanja cha ndege (5%), Masika (3%) and Mawenzi (3%) are all medium density areas and Kihonda (7%) is a low density area (also known to be an industrial area); low attendance from this areas is probably due to the fact that there are other health centres nearer than Mafiga. Statistically, there were no significant differences between mean Ca, K, Zn, Cu, Pb and Cd concentrations in human milk and maternal area of residence ($p > 0.05$).

Table 10: Socio-demographic characteristics of the respondents

Characteristics	Respondents	
	Frequency	Percentage
Age (years)		
< 20	5	12
21 - 30	30	73
31 - 40	5	12
> 41	1	3
Weight (in kg)		
< 50	37	90
51-60	2	5
> 60	2	5
Occupation		
House wife	22	54
Farmer	3	7
Small scale business	9	22
Salary worker (waged)	7	17
Area of residence		
Chamwino	13	32
Kasanga	5	12
Mafiga	10	24
Manzese	3	7
Kihonda	3	7
Misufini	3	7
Kiwanja cha Ndege	2	5
Masika	1	3
Mawenzi	1	3

4.6 Dietary and Non-dietary Factors Related to Presence of Metals (Ca, K, Zn, Cu, Pb and Cd) in Human Milk

Several factors have been known to influence the presence and concentration of minerals in animals' body fluids including milk in human being. These factors encompass both the dietary as well as non-dietary factors. The dietary contribution for toxic metal intake has been studied (Food Survey Information Sheets (FSIS) 48/04, 2000; Santos *et al.*, 2004).

4. 6. 1 Vegetable consumption

Table 11 presents mean ± standard deviation concentration (g/L) of macro (Ca and K) and micro (Zn and Cu) elements in milk from healthy lactating mothers in Morogoro Municipality in relation to vegetable consumption. No statistical significant differences were found between mean Ca, K, Zn and Cu concentrations in human milk and vegetable consumption ($p > 0.05$). Vegetables are important in rational nutrition; they form an indispensable constituent of diet. Their nutritive significance is their richness in minerals and vitamins which are essential in the maintenance of human health (Caunii, 2010). They also form part of the daily diets in many households forming an important source of vitamins and minerals required for human health, they are usually consumed in relatively small amounts as side-dish or relish with the staple foods while fruits are palatable, and are often taken in large quantities (Thomson and Kelly, 1990; Iyaka, 2007).

Kumar *et al.* (2007) says that, there are 35 metals that concerned us because of occupational or residential exposure; 23 of these are the heavy elements or "heavy metals". Distribution of heavy metals in plant body depends upon availability and concentration of heavy metals as well as particular plant species and its population (Punz and Seighardt, 1973). Many researchers have shown that some common vegetables are capable of accumulating high levels of metals from the soil (García *et al.*, 1981; Khan and Frankland, 1983; Xiong, 1998; Cobb *et al.*, 2000). Certain species of *Brassica* (Cabbage) are hyper accumulators of heavy metals into the edible tissues of plant (Xiong, 1998). Many people can be at risk of adverse health effects from consuming common market vegetables cultivated in contaminated soil. In India, a similar kind of study was undertaken by Somasundaram *et al.* (2003) on heavy metal content of plant samples of sewage-irrigated area of Coimbatore district and leafy vegetables were found with very high levels of heavy metal contamination including Cd, Zn, Cu, Mn and Pb. Another similar research was carried out in Delhi and its surrounding regions on `Vegetables eating up vegetarians' found the presence of deadly heavy metals in vegetable samples collected from across the capital (Somasundaram, 2003).

Table 11: Concentration (g/L) of elements (Ca, K, Zn and Cu) in milk from healthy lactating mothers as influenced by vegetables consumed frequently

Characteristic	Respondents		Ca		K		Zn		Cu	
	N	%	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Vegetable consumption				0.283		0.568		0.909		0.910
Amaranthus	33	80	25.19 ± 7.27		4.69 ± 0.42		1.46 ± 2.37		1.46 ± 2.37	
Sweet-potato leaves	29	71	24.69 ± 7.57		4.68 ± 0.45		1.29 ± 2.27		1.28 ± 2.27	
Chinese cabbage	14	34	25.07 ± 7.12		4.76 ± 0.44		1.58 ± 2.47		1.57 ± 2.47	
Cabbage	3	7	20.89 ± 5.53		4.54 ± 0.28		0.002 ± 0.0003		BDL ± 0.001	
Cassava leaves	11	27	29.02 ± 3.67		4.91 ± 0.44		1.39 ± 2.38		1.39 ± 2.37	
Pumpkin leaves	21	51	23.13 ± 6.73		4.62 ± 0.48		1.40 ± 2.28		1.39 ± 2.28	
Cowpea leaves	7	17	24.65 ± 6.31		4.72 ± 0.35		0.80 ± 2.12		0.80 ± 2.12	
White radish	3	7	16.59 ± 1.91		4.16 ± 0.12		0.002 ± 0.0001		0.002 ± 0.004	
Okra	2	5	23.29		4.53		0.002 ± 0.001		0.001 ± 0.002	

BDL: Below detection limit

Table 12: Concentration (g/L) of elements (Ca, K, Zn and Cu) in milk from healthy lactating mothers as influenced by fruits consumed frequently

Characteristic	Respondents		Ca		K		Zn		Cu	
	N	%	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Fruit consumption				0.468		0.333		0.444		0.444
Mango	38	93	23.93 ± 6.38		4.67 ± 0.42		1.12 ± 2.16		1.12 ± 2.16	
Pawpaw	6	15	30.39 ± 8.15		5.01 ± 0.52		2.02 ± 2.79		2.02 ± 2.79	
Banana	30	73	24.30 ± 5.53		4.64 ± 0.38		0.88 ± 1.96		0.88 ± 1.96	
Pears	3	7	23.54 ± 6.95		4.91 ± 0.12		0.88 ± 1.96		0.88 ± 1.96	
Pineapple	16	39	23.60 ± 7.54		4.63 ± 0.55		1.61 ± 2.47		1.61 ± 2.47	
Jackfruit	3	7	21.83 ± 4.99		4.53 ± 0.22		3.59 ± 3.11		3.59 ± 3.11	
Water melon	3	7	25.81 ± 3.27		4.56 ± 0.14		1.79 ± 3.09		1.79 ± 3.09	
Plums	1	2	24.41		5.29		5.13		5.13	
Passion fruit	1	2	15.37		3.89		0.002		BDL	
Orange	20	49	25.36 ± 8.39		4.71 ± 0.42		1.26 ± 2.24		1.25 ± 2.24	
Avocado	2	5	33.23 ± 14.07		5.07 ± 0.76		2.21 ± 3.21		2.21 ± 3.12	

BDL: Below detection limit

4. 6. 2 Fruit consumption

Table 12 presents mean ± standard deviation concentration (g/L) of macro (Ca and K) and micro (Zn and Cu) elements in milk from healthy lactating mothers in Morogoro Municipality as influenced by fruits consumed frequently. There were no statistical significant differences observed between mean Ca, K, Zn and Cu concentrations in human milk and fruit consumption ($p > 0.05$). As toxic elements Pb and Cd concentrations were below detection limits. Fruits and vegetables offer the most rapid and lowest cost source of providing vitamins, fiber and minerals to the majority of people in developing nations. Nutritional metals such as Ca, K, Cu and Zn do occur naturally in fruits and vegetables. Minor/trace elements, such as, Zn and Cu are needed for good health but they can be toxic when their concentrations exceed limits of safe exposure (Reilly, 1991; Skurikhin, 1993).

4. 6. 3 Fish consumption

Relationships between maternal nutrition and milk metal contents have been studied thoroughly. Consumption of fish seemed to influence Pb concentrations as revealed in Gundeker and Zödl's (2002) study that fish consumption was significantly related to milk Pb levels. Table 13 gives the mean ± standard deviation of Ca, K, Zn and Cu concentration in human milk in relation to frequency of fish consumption. Nevertheless, no statistical significant differences were found between mean Ca, K and Zn concentrations in human milk and fish consumption ($p > 0.05$). As toxic elements Pb and Cd concentrations were below detection limits.

Table 13: Concentration (g/L) of elements (Ca, K, Zn and Cu) in milk from healthy lactating mothers as influenced by fish consumption

Characteristic	Respondents		Ca		K		Zn		Cu	
	N	%	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Fish consumption				0.839		0.383		0.454		0.667
<3 times per week	20	49	24.15 ± 6.97		4.82 ± 0.48		0.34 ± 0.23		BDL ± 0.17	
3 times per week	9	22	24.10 ± 5.78		4.63 ± 0.34		0.24 ± 0.16		BDL ± 0.13	
3> times per week	12	29	25.68 ± 8.26		4.58 ± 0.45		0.33 ± 0.19		BDL ± 0.09	

BDL: Below detection limit

Table 14: Concentration (g/L) of elements (Ca, K, Zn and Cu) in milk from healthy lactating mothers as influenced by cereal consumption

Characteristic	Respondents		Ca		K		Zn		Cu	
	N	%	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Maize consumption				-		-		-		-
3> times per week	41	100	24.62 ± 6.96		4.69 ± 0.44		1.28 ± 2.25		1.28 ± 2.25	
Rice consumption				0.584		0.756		0.521		0.568
3 times per week	1	2	28.45		4.83		0.18		BDL	
3> times per week	40	98	24.51 ± 7.03		4.68 ± 0.44		0.32 ± 0.21		BDL ± 0.152	
Wheat consumption				0.601		0.050		0.308		0.094
<3 times per week	7	17	27.01 ± 9.51		5.06 ± 0.33		0.42 ± 0.22		BDL ± 0.057	
3 times per week	25	61	23.90 ± 6.55		4.64 ± 0.36		0.29 ± 0.20		BDL ± 0.092	
3> times per week	9	22	24.98 ± 6.95		4.49 ± 0.62		27 ± 0.18		0.077 ± 0.269	
Millet consumption				0.971		0.568		0.888		0.737
<3 times per week	4	10	25.76 ± 5.27		4.41 ± 0.01		0.33 ± 0.27		BDL ± 0.126	
3 times per week	4	10	24.80 ± 6.92		4.87 ± 0.69		0.27 ± 0.24		BDL ± 0.034	
3> times per week	33	80	24.52 ± 7.25		4.69 ± 0.44		0.32 ± 0.19		BDL ± 0.163	

- : Statistics could not be computed. BDL: Below detection limit

4. 6. 4 Cereal consumption

The study by Silva *et al.* (2005) has confirmed the importance of vegetables and cereals as significant sources for the dietary intake of toxic metals. Table 14 above provides the mean ± standard deviation of Ca, K, Zn and Cu concentration in human milk from healthy lactating mothers through cereal consumption. Statistics could not compute the significance in mean Ca, K, Zn and Cu concentrations in human milk and maize consumption because all respondents ate maize more than 3 times a week. There was no statistical significant difference found between mean Ca, K and Zn concentrations in human milk and rice consumption ($p > 0.05$). A weak statistical significant difference was observed between mean K concentration in human milk and wheat consumption ($p = 0.050$). Even so, statistical significant difference was not observed between mean Ca, Zn and Cu concentrations in human milk and wheat consumption ($p > 0.05$). No statistical significant difference was observed between mean Ca, K, Zn concentrations in human milk and millet consumption ($p > 0.05$). As toxic elements Pb and Cd concentrations were below detection limits.

4. 6. 5 Passive smoking

In a study by Gundeker and Zödl's (2002), smokers showed significantly higher Pb contents in breast milk than non-smokers; but Frkovic′ *et al.* (1997) found that breast milk of non-smokers had higher Pb contents compared with that of smokers. A study done by al-Saleh (1995) and Symanski and Hertz-Picciotto (1995) yielded a contrasting result: current smokers showed significantly increased Pb levels. Similar results were obtained by an average increase of approximately 15% in cord blood Pb levels was estimated by Rhainds and Levallois (1997) for every 10 cigarettes smoked per day when mothers smoked and consumed alcohol at the same time.

Cadmium levels in human milk have also been reported to be correlated strongly with exposure to cigarette smoke both when the mother smoked and when the father smoked and the mother did not smoke (Dabeka *et al.*, 1986). Table 15 below shows the mean ± standard deviation of Ca, K and Zn concentration in human milk for passive smoking mothers. Cu, Pb and Cd concentratios were all below detection limit. None of the respondents was actively smoking. No statistical significant difference was observed between mean Ca, K and Zn concentrations in human milk and passive smoking ($p > 0.05$).

4. 6. 6 Dental filling

Some authors reported positive correlations between the number of amalgam fillings and Hg concentrations in body fluids; but Pb and Cd have not been reported. Oskarsson (1996) and Jones (1999) stressed that the amount of Hg released from such fillings is minimal and that the uptake of food-related organic Hg is 6 times higher; moreover, food-related Hg is significantly more toxic, however, Drexler and Schaller (1998) concluded that the additional exposure of breast-fed infants from maternal amalgam fillings is of minor importance compared with maternal fish consumption. The mean ± standard deviation of Ca, K, Zn and Cu concentration in human milk with regard to dental filling is presented in Table 15. Cu, Pb and Cd concentrations were below detection limit. No significant differences were observed between mean Ca and K concentrations in human milk with either dental filling or dental filling frequency ($p > 0.05$) but a statistical significant difference was observed between mean Zn and Cu concentrations in human milk with dental filling and dental filling frequency ($p < 0.05$).

Table 15: Concentration (g/L) of elements (Ca, K, Zn and Cu) in milk from healthy lactating mothers as influenced by non-dietary factors

Characteristic	Respondents		Ca		K		Zn		Cu	
	N	%	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Passive smoking				0.326		0.645		0.676		0.908
Yes	4	10	21.36 ± 8.34		4.59 ± 0.51		0.27 ± 0.24		BDL ± 0.089	
No	37	90	25.04 ± 6.81		4.70 ± 0.43		0.32 ± 0.20		BDL ± 0.157	
Dental filling frequency				0.260		0.666		0.573		0.00000004
Once	1	2	16.79		4.49		0.19		0.678	
Never	40	98	24.85 ± 6.93		4.69 ± 0.44		0.32 ± 0.21		BDL ± 0.103	

BDL: Below detection limit

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Specification of mineral content in human milk, cow milk and infant formula is of prime importance in order to understand the bioavailability of elements for the newborn and to probably get better information about their metabolic pathways. The knowledge gained will be of significance for infant formula production as well as. There is an extensive variation in concentration of mineral content from the different types of milk as assessed by this study; this may be because of factors such as geographical location, dietary habits, contents of minerals in the soil and foodstuffs; and food processing procedures which differ considerably among and within sites.

Further more, each mammal's milk is naturally designed to meet the nutritional as well as growth and developmental requirements of its descendant which are very different from one species to another. The study revealed that the daily intake of Ca, K, Zn and Cu through human milk was inadequate; although this finding seems to contradict the preceding statement, the disparity could be influenced by nutritional and income factors. Daily Ca intake through fresh cow milk is highly elevated; however, cow milk is a very poor source of K, Zn and Cu as micro/trace elements. The daily intake of selected elements through tinned baby milk was significantly high in K, Zn and Cu; yet very low in Ca. Baby foods were lacking Zn and Cu but daily Ca intake matches with the WHO/FAO (1993) recommended level which is 21g/L/day for infants from birth up six months and 27g/L/day from seven months to one year. Daily Potassium intake observed was very high. Pb and Cd were all below the provisional tolerable daily intake (PTDI) levels of 3.0×10^{-7} and 1.0×10^{-6} g/kg$_{bw}$/day respectively (Kg$_{bw}$ means kilogram body weight).

The socio-demographic information in addition to dietary and non-dietary factors thought to influence presence and concentration of elements (Ca, K, Zn, Cu, Pb and Cd) in human milk did not prove so except for K concentration which showed a relationship between human milk and maternal age and a weak association between human milk and wheat consumption; and dental filling and dental filling frequency which showed that there is an association between Zn and Cu concentration in human milk with these factors. From a toxicological point of view the present study show that human milk, fresh cow milk and infant formula preparations are all safe and do not pose any likely harm to infants with respect to presence and daily intake of toxic elements, conversely, Zn and Cu as micro elements should be added in tinned baby foods through fortification to ensure that their biological functions in infant growth and development is not jeopardized.

Therefore, this study concludes that infants in Morogoro Municipality are not endangered by heavy metals (Pb and Cd) whether they are breastfed, fed with fresh cow milk or infant formula (tinned baby milk and tinned baby foods) but the intake of macro (Ca and K) and trace/micro (Zn and Cu) is below the recommended levels for human milk; cow milk provides a higher Ca and K intake but a low intake for Zn and Cu. Tinned baby milk indicates a high K and Cu intake but low intakes of Ca and Zn whereas tinned baby foods showed Ca intake is slightly above WHO/FAO recommended daily intake. K intake is very high while Zn and Cu were missing.

Potassium (K) concentration showed a relationship between human milk and maternal age and a weak association between human milk and wheat consumption; and dental filling and dental filling frequency also proved an association between Zn and Cu concentration in human milk with these factors.

5.2 Recommendations

Since the study shows that human milk does not supply enough macro and essential mineral elements to the new born due to poor nutrient intake before conception and during gestational period, there is an urgent need for maternal nutritional education through Reproductive and Child Health clinics and other different health stakeholders to impart nutritional knowledge and skills to general public but more specifically to women of child bearing age.

There is also a need for further research to better understanding the relationship between diet and macro and trace element supplies, which will evaluate the availability of these elements from diets typical of our environmental set up. The research should include an assessment of the feasibility of adopting realistic and culturally-accepted food preparation practices, such as fermentation, germination and soaking; and including locally available and inexpensive foods.

Again, a good monitoring system through Tanzania Food and Drug Authority (TFDA) and Tanzania Bureau of Standards (TBS) is needed to monitor fortification to ensure that neither deflation nor inflation expose infants food products at risk.

Since this study has not exhausted all important aspects of concentration and daily intake of mineral elements by infants through milk as well as dietary and non-dietary factors influencing nutritional composition of human milk; it is clear that, a lot more needs to be done (need for further research).

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APPENDICES

Appendix 1: AN INFORMED CONSENT DOCUMENT

Explanation given to mothers about the research/study

1. **Purpose of the research/study:** The research is going to determine the concentration and to estimate the daily intake of macro-, micro- and toxic elements by infants through milk (human, cows, tinned) and tinned baby foods. This research is done in collaboration with Department of Food Science and Technology (Sokoine University of Agriculture) and RCH Clinic at Mafiga Health Centre - Morogoro.
2. **Research procedure:** Procedure will involve collection of breast milk. The collection will be done only once when the lactating mothers attend to the RCH Clinic with their infants. If you agree to participate in this research you need to sign an informed consent statement form.
3. **Risk and discomfort:** Hardly any risk and discomfort is expected.
4. **Benefit(s) of the research:** Results obtained is expected to generate information about intake of toxic elements by infants and to help in developing a rationale for reducing their intake as well as their effects.
5. **Compensation:** You will not be given any compensation for participating in the research/study.
6. **Privacy and confidentiality:** The obtained information is confidential and will not be accessed by any other person except the research team. The information will be kept until when the objectives of the study are realized.

Statement of consent to participate in the study

I have read the above information/the above information has been read to me. I have had the opportunity to discuss this research/study with the researcher and I have had my questions answered by her in a language I understand. I am willing to take part in this research/study.

Consent:

If you agree to participate in this study please sign below;

....	....	....
Name of the mother	**Signature/thumb print**	**Date**
....	....	....
Name of the researcher	**Signature**	**Date**

Appendix 2: A QUESTIONNAIRE FOR LACTATING MOTHERS

Personal Information:

Name: ....Age: ....

Occupation: .... Area of residence: ....

Nutritional Information:

Weight: .... Height: .... Body mass index (BMI): ....

Three (3) types of green vegetables mostly consumed: ...., ... and ....

Three (3) types of fruits mostly consumed: ...., ... and ....

Fish consumption: YES / NO **(Tick the response)**

If yes, how many times per week: < 3 times a week

3 times a week

> Times a week

Cereal consumption: **(Tick the response)**

TYPE OF CEREAL	**YES**	**NO**	**LESS THAN THREE (3) TIMES A WEEK**	**THREE (3) TIMES A WEEK**	**MORE THAN THREE (3) TIMES A WEEK**
Maize					
Rice					
Wheat					
Millet					

Pregnancy History: (Tick the response)

Delivered a single full term infant (39 ± 2 weeks): YES / NO

Baby's birth weight: ....

Infant feeding information:

Breast feeding: YES / NO **(Tick the response)**

If yes, how many times a day:
- < 3 times
- 3 - 6 times
- 7 - 12 Times

Cows' milk: YES / NO **(Tick the response)**

If yes, how many litres per day:
- ≤ ½ litre
- 1 litre
- > 1 litre

Tinned milk (for infants less than 7 months): YES / NO **(Tick the response)**

If yes, which type:
- SMA
- NAN 1
- S – 26
- Lactogen. **(Tick the response)**

Tinned food (from 6 months onwards): YES / NO **(Tick the response)**

If yes, which type:
- Cerelac 1
- Cerelac 2
- Cerelac 3
- Heinz Farley's Rusk (banana) – stage 1
- Heinz Farley's Rusk (original) - for all ages **(Tick the response)**

Other Information: (Tick the response)

Has been sick for the past 2 weeks: YES / NO

In supplement(s): YES / NO

If yes, specify the kind of supplement(s):

Vitamins: ....

Minerals: ....

Smoking habits:

You personally: YES / NO

Your husband/partner: YES/ NO

Dental fillings: YES / NO

If yes, how many times: ....

THANK YOU FOR YOUR COOPERATION

Appendix 3: (SWAHILI VERSION OF QUESTIONNAIRE FOR LACTATING MOTHERS)

DODOSO KWA KINA MAMA WANYONYESHAO

Taarifa binafsi:

Jina: .... Umri: ....

Kazi: .... Mahali unapoishi: ....

Taarifa za lishe:

Uzito: .... Urefu: .... Fahirisi: ....

Mboga za majani za aina tatu (3) unazokula mara kwa mara: ...., ... na ....

Matunda ya aina tatu unayokula mara kwa mara: ...., ... na ....

Ulaji wa samaki: NDIYO / HAPANA **(Weka V kwenye jibu)**

Kama ndiyo, mara ngapi kwa wiki: Pungufu ya mara 3

Mara 3

Zaidi ya mara 3

Ulaji wa nafaka: **(Weka V kwenye jibu)**

AINA YA NAFAKA	NDIYO	HAPANA	PUNGUFU YA MARA TATU (3) KWA WIKI	MARA TATU (3) KWA WIKI	ZAIDI YA MARA TATU (3) KWA WIKI
Mahindi					
Mchele					
Ngano					
Mtama					

Historia ya ujauzito: (Weka V kwenye jibu)

Kujifungua mtoto aliyetimia (wiki 39 ± 2): NDIYO / HAPANA

Uzito wa mtoto ulipojifungua: ....

Taarifa za lishe za mtoto:

Maziwa ya mama: NDIYO / HAPANA **(Weka V kwenye jibu)**

Kama ndiyo, mara ngapi kwa siku: pungufu ya mara 3

Mara 3 - 6

Mara 7 - 12

Maziwa ya ng'ombe: NDIYO / HAPANA **(Weka V kwenye jibu)**

Kama ndiyo, lita ngapi kwa siku: ½ lita au pungufu yake

lita 1

zaidi ya lita 1

Maziwa ya kopo (kwa watoto chini ya miezi 7): NDIYO / HAPANA **(Weka V kwenye jibu)**

Kama ndiyo, aina gani: SMA

NAN 1

S – 26

Lactogen. **(Weka V kwenye jibu)**

Vyakula vya kopo (miezi 6 na kuendelea): NDIYO / HAPANA **(Weka V kwenye jibu)**

Kama ndiyo, aina gani: Cerelac 1

Cerelac 2

Cerelac 3

Heinz Farley's Rusk (banana) – stage 1

Heinz Farley's Rusk (original) - for all ages **(Weka V kwenye jibu)**

Taarifa nyinginezo: (Weka V kwenye jibu)

Kuwa mgonjwa kwa kipindi cha wiki 2 zilizopita: NDIYO / HAPANA

Utumiaji wa virutubisho: NDIYO / HAPANA

Kama ndiyo, vitaje: Vitamini: ....

Madini chumvi: ....

Uvutaji Sigara:

Wewe binafsi: NDIYO / HAPANA

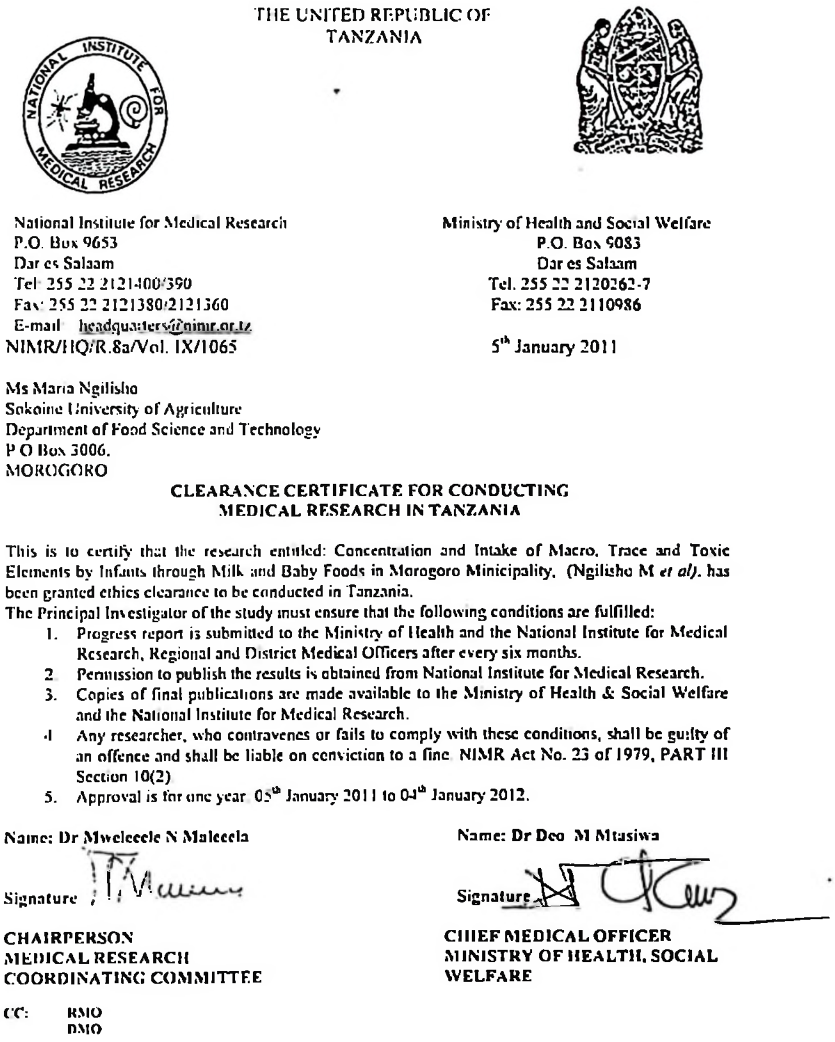
Mwenzi/mume: NDIYO/ HAPANA

Kujaza/kuziba/kuchomelea jino lililotoboka: NDIYO / HAPANA

Kama ndiyo, mara ngapi: ....

ASANTE KWA USHIRIKIANO WAKO

Appendix 4: CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH



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NIMR/HQ/R.8a/Vol. IX/1065

5th January 2011

Ms Maria Ngilisho
Sokoine University of Agriculture
Department of Food Science and Technology
P O Box 3006.
MOROGORO

**CLEARANCE CERTIFICATE FOR CONDUCTING
MEDICAL RESEARCH IN TANZANIA**

This is to certify that the research entitled: Concentration and Intake of Macro, Trace and Toxic Elements by Infants through Milk and Baby Foods in Morogoro Minicipality. (Ngilisho M *et al)*. has been granted ethics clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2)
5. Approval is for one year 05th January 2011 to 04th January 2012.

Name: Dr Mweleeele N Maleeela

Signature

**CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE**

Name: Dr Deo M Mtasiwa

Signature

**CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL
WELFARE**

CC: RMO
DMO