LEVELS OF HEAVY METALS IN CATTLE AND HUMAN MILK COLLECTED AT AGBOGBLOSHE, AN E-WASTE DUMPSITE.

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DECLARATION

I, Eunice Matilda Mends hereby declare that, with the exception of cited literature, this dissertation is the result of my own original research and this has not been presented elsewhere either in part or in whole for purposes of the award of another Degree.

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DEDICATION

This work is dedicated to my family and friends for their support and prayers.
ACKNOWLEDGEMENT

I give thanks and glory to God for how far He has brought me. I wish to thank Dr John Arko-Mensah and Prof. Julius Fobil for their advice and immense support during the course of this study. Further appreciation goes to Prince of the Ecological Laboratory at the Department of Geography and Resource Development. This study was funded by the West Africa-Michigan Collaborative Health Alliance for Reshaping Training, Education and Research in Global Environmental and Occupational Health (WEST AFRICA-MICHIGAN CHARTER II).
ABSTRACT

Background: Milk is the best source of infant nutrition containing the ideal balance of carbohydrates, proteins and fats for developing infants and young children providing a range of benefits for growth, development and immunity. Through e-waste recycling activities, significant amounts of toxic substances such as heavy metals are released into the atmosphere, which could contaminate human and cow milk. The issue of toxic chemicals in milk (human and cow milk) raises important issues for public health as it could be deleterious for infant health as main consumers of milk and also due to their high vulnerability and susceptibility which may result in debilitating health conditions.

Objectives: The aim of this study was to determine the concentration of heavy metals (lead, mercury, arsenic and nickel) in cattle and human milk and to compare these levels with the internationally accepted limits (standards).

Methods: The study was conducted at the e-waste recycling site at Agbogbloshie. Milk was obtained from both cattle and humans, and analysed for heavy metals concentration using Atomic Absorption Spectrophotometry.

Results: The overall mean concentration of heavy metals in breast milk obtained were as follows: lead (100.08±133.67 µg/l), arsenic (38.96±20.83 µg/l), mercury (135.39±63.71 µg/l) and nickel (66.18±83.35 µg/l). All metals detected in human samples were above the permissible limits. Only lead and mercury were detected in cow milk analysed and their mean concentrations obtained were 11.58 ± 5.22µg/l and 124.38 ± 25.35µg/l respectively. Mercury concentration was above the permissible limit.

Conclusion: Mercury levels were high in both human and cow milk samples. Arsenic and nickel levels were undetected in the cow milk samples.
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LIST OF ACRONYMS

AAS  Atomic Absorption Spectrophotometer
Ar   Arsenic
BLL  Blood lead levels
Cd   Cadmium
CNS  Central Nervous System
DNA  Deoxyribonucleic acid
DRC  Dynamic reaction cell
EBF  Exclusive breastfeeding
EEE  Electronic and Electrical Equipments
FAO  Food and Agriculture Organization
HBCD Hexabromocyclododecane
Hg   Mercury
IQ   Intelligent Quotient
JEFCA Joint FAO/WHO Expert Committee on Food Additives
LIMS laboratory Information management system
Ni   Nickel
Pb   lead
PBDEs Polybrominated diphenyl ethers
PCB  Polychlorinated biphenyl
RF   Radio frequency
RNA  Ribonucleic acid
SIDS Sudden Infant Death Syndrome
TDS  Total Dissolved Solids
USBC United States Breastfeeding Committee
DEFINITION OF TERMS

Exclusive breastfeeding
Is defined as feeding an infant no other food or drink, not even water, except breast milk (including milk expressed or from a wet nurse) for 6 months of life, but allows the infant to receive ORS, drops and syrups; vitamins, minerals and medicines (WHO).

Electronic and Electrical Equipment (EEE)
Any equipment that is dependent on electric currents or electromagnetic fields to work properly.

Polybrominated diphenyl ethers
Polybrominated diphenyl ethers (PBDEs) are flame-retardant chemicals added to plastics and foam products to make them difficult to burn.

Polychlorinated biphenyl
A class of toxic aromatic compounds, often formed as waste in industrial processes, whose molecules contain two benzene rings in which hydrogen atoms are replaced by chlorine atoms often used in plasticizers, surface coatings, inks, adhesives, flame-retardants, pesticide extenders, paints, and microencapsulation of dyes for carbonless duplicating paper.

Sudden Infants Death Syndrome (SIDS)
This is the unexplained death, usually during sleep, of a seemingly healthy baby less than a year old and is sometimes referred to as crib death because the infants often die in their crib
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Milk and milk products form an essential part of the daily human diet, especially for infants, school age children and old people (Belete, Hussen, & Rao, 2014). Infants and children are most especially vulnerable due to their immature body systems, physiology and large surface area to volume ratio for uptake of contaminants compared to adults which increases their organ susceptibility to the effects of toxic contaminants (Cooper et al., 2000; Tchounwou, Yedjou, Patlolla, & Sutton, 2012; Malhat, Hagag, Saber, & Fayz, 2012).

Human milk is the best natural source of nutrition for infants as it provides the optimal balance of fats, carbohydrates, proteins, minerals and vitamins necessary for their right development (Hafez & Amal, 2008). Milk also contains potent immune factors that help infants fight infections (Hafez & Amal, 2008). Human milk unfortunately could be contaminated with toxic environmental pollutants that are released from e-waste recycling activities particularly the open burning procedure (Akormedi, 2012).

The level of health risk faced by breastfeeding infants and children in exposure to heavy metal residues in human milk is dependent on the mother's dietary patterns, the nature and levels of chemical residues in her milk (Hafez & Amal, 2008).

Milk has the ability to store xenobiotic substances which present as threats to public health. For example, metal residues can bioaccumulate in milk and adversely affect the health of consumers especially infants and children as they are the largest consumers (Solis et al., 2009; Pilarczyk et al., 2013).

Infant formula, an alternative for non-breastfed infants has also been found to be contaminated with toxic metals and may contain them in excess levels that pose serious
health threats to infants (Hafez & Amal, 2008; Rezaei et al., 2014). Cow milk is considered the standard source for milk products and infant formula throughout the world (Kodrik et al., 2011).

Unlike breastmilk, formula does not provide the optimal immune protection for infants because it does not contain protective substances and also because infant formula and many industrial baby dairy foods can contain the same harmful chemical residues found in breastmilk, but often in higher concentrations (Cattaneo, 2013).

Attention has been focused on the quality of baby formula and other milk products implying that the environmental conditions lactating cattle are exposed to can impact the quality of milk. Milk from lactating cows can be contaminated when cattle are exposed to high amounts of heavy metals in the environment through air, water and ingestion of polluted feeds (Sayed et al., 2011).

Dumping of electronic waste (e-waste) especially in developing countries has been on the increase in the past decade. Ghana currently receives high volumes of e-waste which is either recycled to retrieve re-usable parts or dumped (Grant et al., 2013). In this regard, Agbogbloshie, the largest e-waste recycling and dumpsite in Ghana and one of the most notable in Sub Saharan Africa is a final destination for bulk of these e-waste in the country (Akormedi, Asampong, & Fobil, 2013; Nukpezah, Okine, & Ofori, 2014). Several studies have reported large-scale environmental pollution from e-waste processing activities. Crude methods used in recycling e-waste releases toxic pollutants such as persistent organic pollutants and heavy metals like lead, mercury and arsenic into the atmosphere (Oteng-Ababio, 2012; Amankwaa, 2014). The effect of these toxic substances released is not limited to workers at the e-waste dumpsite but extends to
the markets, the Agbogbloshie slums and schools located in the vicinity (Akormedi, 2012).

Exposure of cattle and humans to hazardous components of e-waste most likely occurs through dust ingestion, inhalation, oral intake and dermal contact absorption (Grant et al., 2013; Lundgren, 2012).

Heavy metal toxicity has a significantly negative impact on both cattle and humans as studies have shown that heavy metals are known to cause neurotoxicity, nephrotoxicity, fetotoxicity and teratogenicity in humans. They may also cause disturbance in the blood and cardiovascular system, changes in the detoxification pathways (colon, kidney, liver, and skin) as well as disturbances in gastrointestinal, reproductive and nervous systems (Iftikhar, Arif, Siddiqui, & Khattak, 2014; Aal, Awad, & Kamal, 2012; Abdulkhaliq, A, Swaileh, K.M, Hussein, R.M, Matani, 2012). Consequently it could lead to changes in mental and neurological functions, alteration in neurotransmitter production and utilization, intellectual and behavioural deficits, hyperactivity, neurodisorders, decreased intelligence quotient, endocrine disorders commonly observed among infants and young children directly exposed to heavy metals contamination (Lanphear, 2005, Patrick, 2006; Jusko et al., 2007; Bischoff et al., 2014). Due to increasing environmental pollution, milk could easily be contaminated with toxic chemicals and is therefore necessary to assess and monitor the levels of heavy metals in milk as they can influence human health considerably.

1.2 Problem statement

Heavy metal contamination is of great concern globally due to the adverse nature of their ill health effects (Patra et al., 2008). E-waste recycling activities at the Agbogbloshie scrap metal site in Accra releases huge quantities of pollutants that could
affect the quality of life of humans and livestock found in range of site (Nukpezah, Okine, & Ofori, 2014; Akormedi, Asampong, & Fobil, 2013).

Most developing countries like Ghana lack the requisite infrastructure for the appropriate handling and recycling of the hazardous materials present in e-waste resulting in massive environmental pollution (Akormedi, 2012). The open-air burning process during recycling at the Agbogbloshie dumpsite releases significant amounts of heavy metals into the atmosphere (Amoyaw-Osei & Agyekum, 2011). These potentially toxic substances accumulate in biological systems and can cause adverse effects in exposed humans (Pilarczyk et al., 2013). Unfortunately, breast milk is one of the various ways by which a mother’s environmental toxic chemical burden is eliminated (Yurdakök, 2015).

The ability of heavy metals to also bioaccumulate in ruminants results in metal residues being deposited in milk and body which is of great concern in humans especially infants and children as large consumers of milk (Patra et al., 2008; Pilarczyk et al., 2013).

Children rely solely on milk as their main meal during their early formative years i.e. the first 1000 days of life, which spans from conception until two years. The first 1000 days are critical for the right development and as such, any impairment during these times could lead to irreversible lifetime changes. Exposure to contaminated milk and dairy products predisposes infants to an early heavy metal contamination which could be deleterious for their health as they have an increased uptake of heavy metals due mainly to organ susceptibility, large surface and being involuntarily exposed (Cooper et al., 2000; Solis et al., 2009; Malhat, Hagag, Saber, & Fayz, 2012).
Contamination of milk with heavy metals is considered to be a great public health concern due to their toxic effects on humans especially infants and young children and in spite of the essential benefits of consuming milk, the contamination of milk is deleterious and demands urgent attention (Jigam et al. 2011). It is therefore of utmost importance to assess and compare the levels of heavy metals (mercury, arsenic, lead and nickel) in milk samples from humans and cattle exposed to heavy metal contamination.

1.3 Objectives

1.3.1 General Objective

The general objective of this study is to assess the levels of heavy metals in human breastmilk and milk from cattle raised near Agbogbloshie, an e-waste recycling site.

1.3.2 Specific Objectives

The study specifically seeks to

- To determine the levels of selected heavy metals (As, Hg, Ni and Pb) in cow milk.
- To determine the levels of selected heavy metals (As, Hg, Ni and Pb) in human milk.
- To compare the levels of heavy metals analysed (As, Hg, Ni and Pb) in human and cow milk.

1.4 Conceptual Framework

Figure 1 below shows the conceptual framework for the route of exposure of humans and cattle to heavy metal contamination which eventually could impact the health of infants and young children.
Infants and young children are exposed to contaminated milk from both human and animal (cow) sources after consumption. E-waste recycling activities at Agbogbloshie releases huge amounts of toxic substances such as heavy metals into the atmosphere which can directly enter lactating mothers or cows via different routes and ultimately
be passed on to infants. Processing indices such as manufacturing and packaging processes as well as improper storage can also add on to the contamination of cow’s milk. Diets prepared with already contaminated raw cow milk such as wagaashie and brukina and contaminated meat and fish could be sources of contamination that could be passed on from mothers to infants. All these factors eventually affect infant health negatively.

1.5 Justification

Many studies have shown increased levels of heavy metals in both human and cattle milk (Landrigan et al., 2002; Aal et al., 2012; Abdollahi et al., 2014; Iftikhar et al., 2014) but there is still paucity of data on contamination in Ghana. Milk and milk products are consumed mainly by infants and young children serving as the major source of proteins, fats and minerals (FAO, 2013).

Human exposure to heavy metals through milk and dairy products may have influences on food safety (Kodrik et al., 2011) as contaminated milk may have a direct negative impact on the health of infants and children due to the incipient development of their gastrointestinal tract which allows for easy absorption of the harmful trace metals (Malhat et al., 2012). Exposure to heavy metals could lead to neurodisorders, intellectual and behavioural deficits, decreased intelligence quotient and endocrine disorders (Jusko et al., 2007; WHO, 2008; Bischoff et al., 2014).

The vulnerability and susceptibility of infants and young children call for more attention to be focused on heavy metal contamination in milk and dairy products. There is therefore the need to conduct studies on heavy metal concentrations and other toxic substances in milk obtained from cattle reared near polluted sites, as well as in
breastmilk of women who live, stay, work or ply these polluted sites. A typical polluted site for such studies is the Agbogbloshie e-waste dumpsite in Ghana.

Very few researches in Ghana have documented the impact contaminated milk has on the health of infants and young children and there is currently no data in Ghana on contamination of milk and dairy products by toxic substances. Therefore, findings of this study will be useful in policy formulation, public health education and strategic measures to reduce or eliminate (if possible) the contamination of milk obtained from these sites.

1.6 Research Questions

- Is there a difference in the heavy metal concentrations in cow milk from cows reared near e-waste recycling activities and those reared on normal pastures?
- Is there a difference in heavy metal concentrations in human milk from women living close to an e-waste recycling site and those who live in far ranges?
CHAPTER TWO
2.0 LITERATURE REVIEW

The heavy metal content of milk and dairy products is particularly influenced by environmental conditions and the possible contamination steps during the manufacturing process (Aal et al., 2012).

2.1 E-waste recycling

E-waste recycling basically refers to the sequence of activities including the collection and manual dismantling of electronic and electrical equipment (EEE), repair and recovery of valuable parts for sale (Amoyaw-Osei & Agyekum, 2011). In Ghana, e-waste is predominantly recycled by the informal sector where crude methods including crushing and burning are employed for separation and recovery of reusable components such as steel, aluminium, copper, gold, silver and other rare earth metals (Akormedi, Asampong, & Fobil, 2013; Heacock et al., 2015).

At the Agbogbloshie dumpsite, the main recycling processes are manual dismantling and open-air burning. Some e-waste workers known as scavengers move through homes collecting electronic waste and other scrap metals such as refrigerators, faulty televisions and computers and some other household equipment to sell to e-waste workers for processing (Akormedi et al., 2013).

Dismantling is then done to remove vital components that though do not function, contain valuable materials like copper, aluminium and steel (Oteng-Ababio, 2012; Nukpezah, Okine, & Ofori, 2014). Manual dismantling of the gadgets is done using basic tools such as hammers, screwdrivers and cutters with no protective clothing (Oteng-Ababio, 2012; Akormedi et al., 2013).
The final recycling activity is to recover valuable components through open-air burning (Oteng-Ababio, 2012). Materials to be burnt are received by those engaged in burning from dismantlers through assistants, commonly referred to as “boys” (Oteng-Ababio, 2012; Akormedi et al., 2013; Amankwaa, 2014).

In most developing countries like Ghana, the handling and disposal of discarded EEE is unregulated and pose a health and environmental hazard to workers who work without basic personal protective equipment and those in close environs (Nukpezah, Okine, & Ofori, 2014; Heacock et al., 2015). Crude recycling of EEE raises issues of safety because e-waste contains hazardous substances such as polychlorinated biphenyl (PCB), polybrominated diphenyl ethers (PBDEs), flame retardants and heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (Ar) (Nukpezah et al., 2014; Heacock et al., 2015).

Food safety regarding contaminants such as heavy metals is of great concern for consumers due to their damaging impacts on human health with more attention gained when such contaminants are found in basic food items such as milk, commonly consumed by the most vulnerable age groups (infants, young children and the aged) (Ismail, 2015).

2.2 Milk and Milk products
Milk and milk products are fundamental diets of infants and young children, and contain adequate nutrients and promotes growth and development. The WHO promotes exclusive breastfeeding of infants during the first six months of life to attain optimum growth, development and health and thereafter should continually be given nutritionally adequate and safe complementary foods to meet their growing nutritional requirements while continuing breastfeeding up to two years or more (Allen, 2012; WHO; Essential
Nutritional Action, 2013). Milk is an excellent source of protein, calcium, riboflavin, and phosphorus, potassium, vitamins (A, B3, B-12 and D) (Belete et al., 2014). Breastmilk, a unique nutritional source of milk for infants and young children provides a range of important health benefits: more resistance to disease and infection early in life, reduces the likelihood of children contracting diseases later in life (juvenile diabetes, heart diseases and cancer), strengthens the immune system as antibodies are transferred to infants during breastfeeding, reduces diarrheal diseases and reduce the probability of Sudden Infant Death Syndrome (SIDS) (United States Breastfeeding Committee (USBC), 2002).

2.3 Exposure of Milk to Heavy metal Contamination

Exposure routes of milk and milk products to contaminants are mainly through human and cattle exposures. Milk tends to attract heavy metals and other contaminants due to its high-fat and protein content (Matt et al., 2011). The exposure routes for milk contamination for both humans and cattle involve ingestion, inhalation and skin absorption (Ogabiela et al., 2011; Örün et al., 2011; Bischoff et al., 2014).

2.3.1 Ingestion

Contaminated food when ingested could eventually enter into the bloodstream and contaminate milk during lactation. For humans, a mother’s diet could pose a threat to breastmilk contamination with heavy metals (Mead, 2012). Diets prepared with already contaminated raw cow milk such as “wagaashie” and “brukina” and contaminated meat and fish when consumed in large quantities over a period of time could lead to heavy metal toxicity in breastmilk which could be passed on to infants during breastfeeding (Tchounwou at al., 2012).
Cattle are exposed to metal contamination from ingestion of contaminated forage and water (Ogabiela et al., 2011; Ogundiran & Ogundele, 2012; Oyebadejo et al., 2014). Heavy metals are transferred into milk when cattle are fed with metal-contaminated fodder or drink water originating from industrial effluents or sewage waste (Ismail et al., 2015). Heavy metals have the ability to accumulate along the food chain and remain persistently present in plants and could be passed along the food chain (Jiwan & Kalamdhad, 2011; Jaishankar et al., 2014). Continuous consumption of contaminated forage and water could eventually lead to heavy metal toxicity in cow milk.

2.3.2 Inhalation

Inhalation is the major route of entry for most chemicals in the vaporized, gaseous, mists or particulates forms (UNL Environmental Health and Safety, 2002). Inhaled, chemicals are either exhaled or accumulated in the respiratory tract. Accumulation of toxic substances leads to damage through direct contact with tissues or through the diffusion into the blood through the lung-blood interface. Substances absorbed into the blood are circulated and distributed to organs and tissues that have an affinity for that specific chemical (UNL Environmental Health and Safety, 2002).

Cattle reared at the Agbogbloshie e-waste dumpsite could have their milk contaminated possibly through the inhalation of smoke released during the burning of electronic gadgets which release heavy metals into the atmosphere. Breast milk could be contaminated with heavy metals when lactating mothers continuously inhale the smoke that arise from the burning of the electronic waste.

Factors that could affect the inhalation of toxic substances include the concentration of such metals in the air, its solubility in the blood and tissues, respiration rate, duration
of exposure, condition of respiratory tract and the size of the toxic particle (Tchounwou et al., 2012).

2.3.3 Skin absorption

Many chemicals may cross the skin barrier and be absorbed into the blood stream and easily contaminate breastmilk. Once absorbed, they may cause systemic damage to internal organs. Factors that affect dermal absorption of toxic substances include the condition of the skin, chemical constitution of the substance and exposure time (UNL Environmental Health and Safety, 2002).

2.4 Children’s health and the environment

Children’s unique and specific susceptibilities to certain environmental risks such as heavy metal contamination has gained increased attention among the scientific community and the general public due to its deleterious effect (Tchounwou et al., 2012). Children should not be considered as little adults because relative to their size, they breathe more air, consume more food and drink more water than adults and thus may have a relatively higher exposure to contaminants per body weight (Pronczuk-Garbino, 2011).

In vast dissimilarity to adults, infants and young children are particularly vulnerable and delicate because their bodies and physiological systems are still undergoing extensive growth and development. They are also typically more often involuntarily exposed to environmental chemicals (Cooper et al., 2000; Lanphear, 2005). There are specific periods of vulnerability, from conception through infancy and childhood when children may be particularly sensitive to the deleterious effects of environmental contaminants (Commission for Environmental Cooperation, 2006).
2.5 Factors influencing children’s exposure and susceptibility to environmental contaminants

2.5.1 Exposure to Contaminants

The cognitive abilities of infants and young children are insufficiently developed to evade environmental exposures on their own accord (Cooper et al., 2000; Commission for Environmental Cooperation, 2002). As large consumers of milk and dairy products, infants and young children could in a greater extent be involuntarily exposed to contaminants from milk (breastmilk and cow milk) through consumption (Cooper et al., 2000).

2.5.2 Uptake of Contaminants

Children usually have a greater relative uptake of contaminants by all routes (ingestion, inhalation and dermal absorption) as they are smaller and have a large surface area to volume ratio that is about three times that for adults (Cooper et al., 2000). Young children are known to breathe more rapidly, with new-borns taking 60 breaths per minute as compared to 12 breaths for adults and therefore they take in a larger volume of air per minute of breathing time consequently exposing them to greater quantities of air pollutants (Commission for Environmental Cooperation, 2002).

2.5.3 Specific organ susceptibility

Specific systems and body structures are more susceptible to the effects from exposure to heavy metals as they undergo continuous differentiation before maturity (Cooper et al., 2000; Tchounwou et al., 2012). According to the Cooperation for Environmental Cooperation of North America (2002), the brain, nervous system and lungs are specifically sensitive throughout childhood as they undergo extensive growth after birth. Other organ systems in infants and young children are also immature leaving
them particularly ill-equipped to handle heavy metal contamination (Cooper et al., 2000).

The Toxicology and Exposure guidelines set by the University of Nebraska (2003) has stated that the permeability of the digestive tract and the large surface area of the developing lungs enhances easy absorption of chemicals in an infant’s body. In the initial stages of life, an infant’s immune, excretory and de-toxifying organs (physiological mechanisms) are also incapable of protecting it from the invasion of chemicals because they are underdeveloped in its earliest stages of life (Cooper et al., 2000).

Generally, children are characteristically more susceptible to the biological effects of contaminants because of their immature systems and physiology (WHO, 2003; Lanphear, 2005).

### 2.6 Heavy metals and their known effects

Heavy metals are widely recognized as cumulative noxious substances due to its minimal elimination rates from the body (Alissa & Ferns, 2011). Heavy metals cannot be metabolized, thus persist in the body exerting their toxic effects resulting in severe health hazards in humans, depending on their levels of contamination (Ibrahim et al., 2005).

Potential effects from heavy metal contaminants vary depending on the type and nature of the metal, timing of exposure, frequency and duration of exposure and exposure dose (Tchounwou et al., 2012). Their effects is also dependent on many intrinsic factors in the exposed child (Bose-O’Reilly et al., 2010).
2.6.1 Lead

Lead is a naturally occurring stable, ubiquitous heavy metal released in small amounts into the environment by natural processes (Juberg, 2000). Lead accumulating in the environment is non-biodegradable and maintains its toxicity over time (Flora, Gupta, & Tiwari, 2012).

Physiological uptake rates of lead in children are higher than those in adults as they can absorb considerably more (30-40%) of ingested lead (Juberg, 2000). Young children undergo rapid development, have underdeveloped systems, and consequently are more susceptible than adults to the effects of lead (Cooper et al., 2000). Some studies indicate that 36–80% of all blood Pb in breast-fed infants originates from mother’s milk during the first three months of their lives and also through infant formula in recent times (Mead, 2012; Abdollahi, Tadayon, & Amirkavei, 2014). Infants and young children may absorb up to 50% of dietary Pb, compared with only 10% for adults (Lanphear, 2005; Gundacker et al., 2015).

Lead impairs several organ systems, but the main concern is Central Nervous System damage, particularly in young infants who are exposed to lead during the critical stages of brain development (Patrick, 2006; Flora et al., 2012). The developing young human brain undergoes rapid growth, development and differentiation, and lead can interfere with these delicate processes consequently leading to its damage (Lanphear, 2005). Lead exposure in children at minimal levels can be associated with intellectual and cognitive deficits characterized by reduction in Intelligence Quotient (IQ), short attention span, reduced short-term memory reading and learning disabilities (Jusko et al., 2007). It could also result in hyperactivity and behavioural problems resulting in aggressive behaviour, renal impairment, hypertension and impaired growth and visual and motor functions cataracts (Patrick, 2006; Bischoff et al., 2014).
At high levels, lead exposure in children could lead to adverse health effects including anaemia, brain, liver, kidney, nerve and stomach damage, convulsions, coma, insanity and eventually death (Levin et al., 2008). The manifestations of the effects of lead poisoning are non-specific, and majority of children may be asymptomatic and diagnosed only by routine screening of blood lead levels (BLL) (WHO, Children's Health and Environment, 2008).

Many studies have been conducted around the world on lead contamination of both breast milk and cow milk and the impact it has on child health. Studies in China, USA, Turkey, Hungary, Congo and Egypt have reported high levels of lead contamination in breast milk (Koyashiki et al., 2010; Orun et al., 2011; Masiala et al., 2015). On the other hand other studies conducted in Mexico, Brazil and Greece reported low levels of lead contamination in breast milk below the WHO limit for lead in breast milk (5µg/l) (Leotsinidis et al., 2005; Koyashiki et al., 2010; Ettinger et al., 2016). Studies on lead contamination of cow milk in Iran, Egypt, Croatia, Poland and Nigeria all reported low levels of Pb in raw cow milk (below the WHO permissible limit of 25µg/l) (Pavlovic et al., 2004; Hafez & Amal, 2008; Pilarczyk et al., 2013; Nejatolahi, Mehrjo, Sheykhi, & Bineshpor, 2014).

### 2.6.2 Mercury

Mercury is a persistent element occurring both anthropogenically and naturally (Bose-O’Reilly et al., 2010). There are three main forms of mercury; elemental, inorganic and organic which vary with respect to their toxicokinetics in relation to their absorption, distribution, and accumulation and its related health outcomes in the human body (Bose-O’Reilly, 2010). The Commission for Environmental Cooperation (2002) states that methyl mercury, an organic form of mercury is the most toxic form of mercury with ingestion of contaminated food (milk for infants and young children) as the main
route of exposure. The organic-bound methylmercury from food is very well absorbed from the gastrointestinal tract and can be excreted into breastmilk (Bose-O’Reilly et al., 2010).

Infants and young children are especially vulnerable to mercury exposures with special attention in the development of the central nervous system. Damage of the nervous system caused by mercury is likely to be permanent (Bose-O’Reilly, 2010). Exposure to mercury could result in brain damage, mental retardation altering normal thinking and learning, poor coordination inability to speak, blindness, seizures, nervous and digestive system problems, kidney damage and could also affect the immunologic system (Besser, 2009; Abdollahi et al., 2014).

Acrodynia characterized by pain in the extremities, pinkish discoloration and desquamation hypertension, sweating, insomnia, irritability and apathy in children, nephrotoxicity, neurotoxicity are all effects of exposure of mercury (WHO; Children's health and Environment, 2008).

Worldwide, there is limited information on mercury levels in human and cow milk (Najarnezhad & Akbarabadi, 2013). Few studies conducted in Germany, Sweden, Austria and Faroe Islands reported high levels of mercury in breastmilk above the WHO 1.4-1.7µg/l limit (Najarnezhad & Akbarabadi, 2013; Grandjean et al., 1995; Gundacker et al., 2002; Bose-O’Reilly et al., 2010). In Iran low levels of mercury were reported in raw cow milk (Arianejad et al., 2015).

2.6.3 Arsenic

Arsenic (As) is ubiquitous in the environment both in organic and inorganic forms (Hojsak et al., 2015). Inorganic arsenic is highly toxic and is classified as a human carcinogen based on sufficient epidemiological evidence for carcinogenicity in humans.
In greatly exposed children, inorganic arsenic exposure was reported to be related to the development of cancers and lung disease later in life (Hojsak et al., 2015). Epidemiological studies in recent time show that children may be susceptible to inorganic arsenic which could be associated with impaired cognitive function in school-aged children, increased infant mortality and morbidity mainly due to impaired immune function (Bjerselius et al., 2013).

Acute arsenic poisoning may result in the following symptoms; vomiting, abdominal pain and diarrhoea followed by numbness and tingling of the extremities, muscle cramping and death, in extreme cases (WHO, 2010). Long term effects of exposure results in skin pigmentation, gastrointestinal symptoms, conjunctivitis, diabetes, renal system effects, neuropathy, enlarged liver, bone marrow depression, destruction of erythrocytes and could eventually lead to cancer (WHO; Exposure to Arsenic, 2010).

High levels of arsenic in breastmilk have been documented in India and Bangladesh (Watanabe et al., 2003; Samanta et al., 2007) in contrast to low levels reported by other studies (Sternowsky, Moser, & Szadkowsky, 2002; Gurbay et al., 2016). Low levels of arsenic has been reported in raw cow milk from Iran whiles other studies in certain regions of Japan, China and Argentina reported no detectable arsenic levels in cow milk (Qin & Wang, 2009; Sigrist, Beldoméncio, & Repetti, 2010).

### 2.6.4 Nickel

In humans, nickel can also be eliminated through sweat and milk. The most common harmful health effect of nickel in humans is an allergic skin reaction in those who are sensitive to nickel as it causes skin irritation (Das, Das, & Dhundasi, 2008). Nickel toxicity could lead to pneumonia and brain damage and could eventually lead to cancer. Long term nickel exposure could lead to damage of the heart, lungs and nasal cavity.
and adversely affects the kidneys, blood, liver and immune systems (Ogabiela, Udiba, et al., 2011). Very few studies have been conducted on nickel contamination of breast milk. A study by Gurbay et al., (2012) in Turkey reported high levels above the permissible limit of 16µg/l in contrast to a study in Japan that recorded low nickel levels. A study on cow milk from Egypt and Japan reported low nickel levels in cow milk (Enb & Donia, 2009) whiles a study in Poland reported high nickel levels from two different regions (Dobrzański et al, 2005).

The toxicity of metals is dependent on a number of factors such as the ingestion rate and route, bioavailability, gender, age, excretion rate and the chemical state, retention and excretion percentage and the frequency of exposure to these heavy metals (Enb & Donia, 2009; Aal et al., 2012).

2.7 Heavy metal contamination of milk in Ghana
There is currently no data in Ghana on heavy metal contamination in milk and dairy products. Very few researches have been conducted on heavy metal contamination of human and cow milk in Ghana. A study conducted by Bentum, Essumang, & Coast, (2010) in Odumase-Atua in the Eastern region reported low levels of lead and arsenic in breastmilk of women in the Manya Krobo District. Another study conducted in Accra and Tema among healthy lactating women who were engaged in commercial activities for at least five years also reported low mean concentrations of lead in breast milk (Koka, Koranteng-Addo, Bentum, Koka, & Kamoah, 2013). Other biological samples such as urine, blood and hair samples have been documented to also be contaminated with heavy metals (Asante & Ntow, 2009; Armah, Quansah, & Luginaah, 2014). Another study in Ghana on breast milk analysed the PCB, PBDE and HBCD levels in human milk (Asante et al., 2011).
Analysis of raw cow milk in Ghana have been centred mostly on the microbiological safety of the milk. Amponsah, (2014) however in her study analysed the levels of heavy metals (lead, arsenic, cadmium and mercury) present in tin milk produced in Ghana. Results from the study showed contamination of tin milk with levels above the permissible limits.
CHAPTER THREE

3.0 METHODOLOGY

3.1 Overview
The general approach and specific analytical techniques that were used in achieving the stated objectives for this study are described including information on measures that were taken to ensure collection of samples and how they were analysed and interpreted. In addition, a description and highlights of ethical issues associated with the study are provided.

3.2 Study design
This research is a cross-sectional and analytic study involving collection of both human and cattle milk samples and analysing them for heavy metals using laboratory-based techniques.

3.3 Study Area
Human milk samples were collected from lactating mothers residing close to the Agbogbloshie e-waste dump site and lactating cows reared near the same site. The Agbogbloshie e-waste recycling site located in the city of Accra is known to be one of the largest e-waste dumpsites in Sub-Saharan Africa, processing millions of tons of e-waste yearly thus releasing huge amounts of toxic substances into the environment. The Agbogbloshie suburb also serves residential, industrial and commercial purposes including a large food market. The settlement is situated on the left bank of the Odaw River and in the upper regions of the Korle Lagoon in Accra. The dumping ground at the Agbogbloshie e-waste site also serves as grazing grounds for livestock especially cattle.
3.4 Variables of interest

The independent variable is the heavy metals present in the environment due to the e-waste recycling activities at Agbogbloshie and the dependent variable is the concentration of heavy metals in human and cow milk.
3.5 Sampling Technique and sample size

Milk samples were collected from lactating cows reared at Agbogbloshie and mothers who lived and work around the Agbogbloshie e-waste dumpsite. At the time of this study, 4 cows and 25 women were lactating, and they were all recruited for this study.

3.5.1 Inclusion criteria

Lactating cows reared only at the Agbogbloshie e-waste dumpsite were considered for the study. Lactating mothers who lived around the Agbogbloshie e-waste dumpsite who were willing to participate in the study were included in the study.

3.5.2 Exclusion criteria

Cows and mothers with any of the following conditions were excluded:

(i) Cows reared outside Agbogbloshie and lactating mothers who did not work or live in Agbogbloshie and its close environs.

(ii) Mothers who did not give their consent for the provision of breast milk.

3.6 Sample Collection

Ten (10) to 20ml of milk samples were collected from both cows and mothers using standard procedures from both cattle and humans.

3.6.1 Collection of Cow milk

- Hand milking was used in extracting milk from the cow.

- To ensure clean hands and prevent contamination, hands were washed thoroughly.

- The cows were securely tied with a rope to prevent escape during milking.

- Loose manure, dirt or bedding particles from the udder and teats were brushed off and cleaned thoroughly.
• Milking was done using the forefinger and thumb. The teats were squeezed gently and squirts of milk was collected directly into sterile sample tubes.

• The sample containers were opened immediately before the sample was extracted and not before. Precautions were taken so as not to touch the inside of the sample tubes or let the teat end touch the tubes and the tubes were held at an angle to keep loose dirt or hair from falling into it as streams of milk were being collected into the tubes.

• Using a waterproof marking pen, the sample tubes (sterile) were labelled clearly with the date and cow ID.

• The sample tubes were then placed immediately on ice and transported to the laboratory for analysis.

3.6.2 Collection of Breast milk

• Lactating mothers were asked to express a little amount of their breastmilk into sterile tubes after thorough cleaning of the breast with alcohol.

• Breast milk was collected from women in their homes and it was ensured that their hands were clean before expressing.

• The thumb was placed on top of the nipple and the rest of the fingers were placed under the breast for support.

• The nipple was then pressed gently to release squirts of milk which was directed into the sterile tubes.

• Using a waterproof marking pen, the sample tubes (sterile) were labelled clearly with the date and ID.

• The sample tubes were then placed immediately on ice and transported to the laboratory for analysis.
3.7 Laboratory Procedures

After samples had been collected from cattle and humans, heavy metal concentrations were analysed using the PerkinElmer PinAAcle 900T Atomic Absorption Spectrophotometer (Shelton, CT, USA) (AAS) at the Ecological Laboratory (ECOLAB), University of Ghana, Legon.

3.7.1 Sample Preparation

Samples analysed by the AAS were made into a clear solution. The milk samples were brought into a clear solution for analysis by the AAS.

- For this reason, the samples were ashed and digested with chemicals (HNO₃) to break the organic matrix of milk (García & Báez, 2012).

- About 3g of milk was weighed into a porcelain crucible and transferred to a Thermo scientific graphite furnace (Thermolyne) for ashing. Ashing lasted for 2 hours at 600°C.

- Fifty (50) ml of deionised water and 10ml of concentrated nitric acid (70% HNO₃) was added to ash. The solution was gently stirred after which it was filtered to remove undissolved particles.

- The solution was then transferred into a 100ml volumetric flask and made up to the mark using deionised water.

3.7.2 Analytical Method

The atomic absorption spectrophotometer (PerkinElmer PinAAcle 900T, Shelton, CT, USA) was used in measuring the levels of heavy metals (arsenic, lead, nickel and mercury). Measurements with AAS was done using the following procedures;
• The capillary tube of the AAS drew a volume of sample to be analysed into the nebulizer. The nebulizer turned the liquid sample into a fine mist and with the help of acetylene gas and compressed air, the sample moved upwards into the burner slot.

• The nebulized sample was then atomized using the flame or graphite furnace atomizers to break compounds in milk into free atoms.

• A hallow cathode tube was then used as a source of light to excite free atoms present in the sample.

• The atoms absorbed ultraviolet light and transitioned to higher electronic energy levels.

• The monochromator then selected specific wavelengths of the specific heavy metal to be measured and directed it to a detector.

• The detector then converted the light signal into an electrical signal proportional to the light intensity.

• The signal was then displayed for readout on the computer.

• Results of concentrations of metals were generated using a laboratory information system (LIMS) i.e. WINLAB AA Furnace software.

### 3.8 Quality Control

#### 3.8.1 Accuracy

To ensure the validity and accuracy of results from the AAS, blank samples were measured before any reading with samples was done. Also, appropriate Standard Reference Materials of known concentration for each metal were measured before the actual measurements of samples.
3.8.2 Precision
Concentrations of heavy metals were measured in triplicates and the means of each heavy metal recorded with corresponding standard deviation values.

3.8.3 Detection limit
The detection limit is the concentration of the element that corresponds to an absorbance value equal to the blank signal plus three times the noise level. Thus,

\[ A_{LOD} = b + 3s \]

where \( A_{LOD} \) is the absorbance that corresponds to the limit of detection, ‘b’ is the intercept of the calibration curve, and ‘s’ is the computed standard error.

The limit of detection, \( C_{LOD} \), can be estimated using

\[ C_{LOD} = \frac{(A_{LOD} - b)}{m} \]

where \( A_{LOD} \) is the absorbance that corresponds to the limit of detection, \( b \) is the intercept of the calibration curve and \( m \) is the computed least-squares slope of the calibration curve. Final equation yields;

\[ C_{LOD} = \frac{3s}{m} \]

The detection limits of these metals are as follows; Arsenic = 0.05 µg/l, Mercury = 0.6 µg/l, Lead = 0.05 µg/l and Nickel = 0.07 µg/l.

3.9 Data Processing
Data collected was entered into Microsoft Excel and exported to STATA (version13 College station, Texas, USA) for statistical analysis. Means, standard deviations, p-values as well as percentages were used to describe obtained data. Graphical representation of data was done where appropriate using tables.
The Kruskal-Wallis test was used to determine the statistical difference between metals in breast milk and the Mann Whitney test was used in comparing the levels of heavy metals in human and cow milk.

3.10 Ethical Consideration Issues

The proposal was submitted to the Ghana Health Service Ethical Review Board for approval before embarking on the study. Permission and informed consent was also sought from the chiefs of the study area, cattle owners and lactating mothers to collect milk samples for analysis. The lactating mothers were given a detailed description of the study and its importance for them to gain understanding into the study before they consented to give their breast milk.

3.10.1 Potential Risks and Benefits

The procedure did not cause any risk to participants and participants bore no cost. The results of the study will however provide evidence for breastmilk contamination from exposure to e-waste crude recycling activities at Agbogbloshie.

3.10.2 Right to refuse

Participation in the study was voluntary. Participants had the right to choose not to participate and were at liberty to withdraw from the study at any time. Full participation was however encouraged.

3.10.3 Anonymity and confidentiality

Participation was strictly confidential. Personal Information of participants was not taken. Privacy was ensured during breast milk extraction.

3.10.4 Consent

Consent was sought from mothers before their participation in the study. At the initial meeting, the study was read and explained to prospective participant. The written consent form which contained study procedure was read by the participant and/or by a
translator to all persons who showed interest in partaking in the study and any questions raised by the participant was addressed. Participants were required to sign a statement of declaration, indicating they have understood the purpose, procedures, risks and benefits of the study and their free will to participate.

The procedure was explained to participants who could not read in a language (Daagbani) they understood. A witness was required to sign the consent form on behalf of the said participant as confirmation of the latter’s willingness to participate.

3.10.5 Privacy during extraction
Privacy of mothers was ensured during breastmilk extraction as the procedure was done in their homes. Mothers were given sterile tubes and taught how to express the milk on their own. There was no need for spouses of women to be present. A few husbands were however present during the extraction.

3.10.6 Data storage/ Security and Usage
All research specimen, data and records were protected against inappropriate use or disclosure in order to protect the confidentiality of subject data. Electronic data files have been stored in password protected folder with access limited to only the Principal Investigator and Supervisors.

3.10.7 Discard of study samples and/or specimens
Specimens of breast milk were destroyed as well as the identifiers on their storage containers after heavy metal analysis.

3.10.8 Conflict of Interest
There was no conflict of interest pertaining to the study.
CHAPTER FOUR

4.0 RESULTS

4.1 Study participants.
Breastmilk was collected from women living in a slum located at Agbogbloshie. The slum which is situated close to the e-waste recycling site at Agbogbloshie is home to many of the e-waste recyclers and their families. Most houses from which study participants were recruited were single rooms with very poor ventilation. Women living in these slums are constantly exposed to contaminants from e-waste especially from the inhalation of smoke that arises from the burning of EEEs. A total of 25 lactating mothers were recruited for the study. Study participants have been residents of Agbogbloshie for more than a year.

The cow milk obtained for this study was collected from cows reared in the same yard as the e-waste recycling site. These cows are reared in the open and confined to a specific area by means of a wooden fence. The cows are exposed to the harsh weather conditions as the cow pen has no roofing or shed. Burning of EEEs occurs few metres away from the cow pens and cows continually inhale the smoke that arise from open-burning. The cows from which milk was obtained have been kept at Agbogbloshie for close to 3 years.

4.2 Mean concentrations of heavy metals in individual breast milk samples
A total of twenty-five (25) breast milk samples were analysed for the presence of lead, arsenic, mercury and nickel. Lead was detected in all 25 (100%) breast milk samples, arsenic 8 (32%), mercury 15 (60%) and nickel 24 (96%). Lead was detected in all 4 cow milk samples (100%) whiles 2 (50%) samples had detectable levels of mercury. Arsenic and Nickel were however not detected in any of the samples.
Table 1 below shows the mean concentrations of all the heavy metals analysed in human and cow milk. The WHO permissible limits for heavy metal residues for both cow and human milk are also represented in the table below.

**Table 1: Mean Concentrations (µg/l) of heavy metals in human and cow milk from Agbogbloshie.**

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Arsenic ± SD</th>
<th>Lead ± SD</th>
<th>Mercury ± SD</th>
<th>Nickel ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow milk</td>
<td>Nd</td>
<td>11.58 ± 5.22</td>
<td>124.38 ± 25.35</td>
<td>nd</td>
</tr>
<tr>
<td>WHO/FAO Rec</td>
<td>10</td>
<td>20-25</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Human Milk</td>
<td>38.96 ± 20.83</td>
<td>100.08 ± 26.73</td>
<td>135.39 ± 63.71</td>
<td>65.63 ± 81.64</td>
</tr>
<tr>
<td>WHO Rec</td>
<td>0.2-0.6</td>
<td>2-5</td>
<td>1.4-1.7</td>
<td>11-16</td>
</tr>
</tbody>
</table>

nd- not detected (below detection limit) WHO Rec =WHO permissible limits of heavy metal residue in milk 1= 283.3nm (wavelength for Pb measurements) ,2=193.70nm (wavelength of As measurement), 3=253.65nm (wavelength of Hg measurement), 4=232.00nm wavelength of Ni measurements)

4.3 Comparison of mean concentrations of heavy metals in cow and human milk from Agbogbloshie

Comparison of mean concentrations of heavy metals in milk (Table 2) showed a statistical significant difference (p-value< 0.05) in the levels of metals obtained in human milk but not in cow milk (p-value>0.05).

**Table 2: Comparison of mean concentrations of heavy metals in cow and human milk from Agbogbloshie**

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Mean concentrations of heavy metals in human and cattle milk in µg/l</th>
<th>Kruskal wallis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>As</td>
<td>Hg</td>
</tr>
<tr>
<td>Cow Milk</td>
<td>11.58</td>
<td>nd</td>
<td>124.38</td>
</tr>
<tr>
<td>Human Milk</td>
<td>100.08</td>
<td>38.96</td>
<td>135.39</td>
</tr>
</tbody>
</table>

* = P-value <0.05 (statistically significant)
4.4 Comparison of heavy metals (lead and mercury) in human and cow milk

The comparison of concentrations of lead and mercury are summarised in Table 3 below. Concentrations of lead and mercury were higher in human milk compared to cow milk. The differences in lead concentration in cow and human milk are statistically significant (p-value= 0.002).

Table 3: Comparison of lead and mercury concentrations (µg/l) in cow and human milk from Agbogbloshie.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Cow milk Median</th>
<th>Human milk Median</th>
<th>z-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>10.89</td>
<td>48.15</td>
<td>-0.310</td>
<td>0.002*</td>
</tr>
<tr>
<td>Mercury</td>
<td>124.38</td>
<td>132.83</td>
<td>-0.140</td>
<td>0.888</td>
</tr>
</tbody>
</table>

p-values were calculated using Mann Whitney test  *statistically significant (p-value<0.05).
CHAPTER FIVE

5.0 DISCUSSION

The aim of this study was to assess whether milk obtained from lactating mothers who are resident at Agbogbloshie, an e-waste recycling and dumpsite or cattle reared around the dumpsite are contaminated with heavy metals (i.e. lead, mercury, arsenic and nickel). This is because e-waste is known to contain several toxic chemicals including heavy metals, which could be released into the environment. Breast milk is a unique option for infant nutrition and though it has considerable protective mechanisms against being contaminated by heavy metals, monitoring environmental contaminants in milk gives useful information on the extent of environmental pollution (Gürbay et al., 2012).

A mother’s lifetime exposure to heavy metals is the greatest determinant of the levels in her breast milk. Heavy metals are known to be excreted through breast milk, and therefore an important route of elimination for the mother’s environmental toxic chemical burden (Yurdakök, 2015). Unfortunately, contaminated breast milk is directly ingested by the breastfeeding infant which could pose serious health threats (Grant et al., 2013; Yurdakök, 2015).

Overall, lead was detected in each milk sample from both mothers and cows. Levels ranged from 15.76 µg/l to 548.10 µg/l in mothers, compared to 6.60 µg/l to 17.92 µg/l in cow milk.

Twenty-four (24) (96%) of the 25 breast milk samples had detectable levels of nickel, with concentrations ranging from as low as 3.61 µg/l to 408.00 µg/l. In contrast to breast milk, nickel was undetected in all 4 cow milk samples. Mercury was detected in 15 (60%) of the 25 breast milk sampled, concentration ranging from 45.64 µg/l to 258.80 µg/l, whereas 2 (50%) of the 4 cow milk analysed had detectable levels. Finally, arsenic
was detected in only 8 (32%) of the 25 breast milk sampled, while no detectable levels were found in cow milk.

Generally, all chemicals enter breast milk by passive transfer from plasma, and their concentration in breast milk is dependent on their solubility and lipophilicity (Landrigan et al., 2002). The mammary glands of cows are known to be the most physiologically active part and clearly indicates a cow’s exposure to heavy metals through the levels detected in their milk (Gimba & Turoti, 2006).

According to Gürbay et al., (2012) and Örün et al., (2011), there is currently no safe level of lead exposure. However, the World Health Organization (WHO) has reported 2–5 ng/g (5 µg/l) (García-esquinas et al., 2011; Yurdakök, 2015) to be the acceptable lead levels in breast milk and 20 µg/l for cow milk (Najarnezhad & Akbarabadi, 2013; Pilarczyk et al., 2013). Compared to the acceptable levels set by WHO, lead concentrations detected in all breast milk samples were far above the permissible limit in contrast to lead levels detected in cow milk that were below the permissible limit for lead residue in cow milk. Low levels of lead in cow milk found in this study is comparable to studies conducted in Nigeria and Iran which detected low levels of lead (0.63, 5.50, 7.10 µg/l and 12.9 µg/l), all below the permissible limits (Jigam et al., 2011; Ogabiela, Udiba, et al., 2011; Najarnezhad & Akbarabadi, 2013).

Reports on concentrations of lead in breast milk varies in wide ranges around the world. Mean lead level of 100.08 µg/l measured in this study is comparable with results obtained from studies conducted in countries such as China, Hungary, Turkey, Congo and Egypt that also reported high lead levels of 250, 213, 196.6 and 101 µg/l respectively (Gurbay et al., 2012; Orun et al., 2011; Masiala et al., 2015). The difference in the breast milk lead levels (in this study and other studies) may be dependent on
factors such as the difference in sampling time (morning or night) and method (pump or manual), the time of lactation (colostrum or mature milk), maternal factors (maternal lead burden, parity), as well as environmental factors (place of residence/work and exposure level/duration) (Örün et al., 2011).

Breast milk lead levels are influenced both by existing environmental exposures and by the redistribution of previously accumulated maternal lead due to bone resorption associated with pregnancy and lactation (Ettinger, et al., 2016). Accumulated lead in maternal bones could be mobilized along with calcium in order to meet the increased calcium needs during lactation (Yurdakök, 2015; Ettinger et al., 2016). Lead is assumed to be transported from maternal plasma to mammary gland and secreted into milk during lactation (Pilarczyk et al. 2013).

Contrary to findings concluding that breast milk levels of mercury are usually lower than levels of breast milk lead (García-esquinas et al., 2011; Yurdakök, 2015), higher levels of mercury (mean = 135.39µg/l) were detected in this study as compared to lead (mean = 100.08µg/l). Mean concentration of mercury detected in breast milk samples were all higher than the 1.7 µg/l limit set by WHO (Yurdakök, 2015) as the maximum mercury residue in breast milk. Secretion of mercury into breast milk is affected by a number of maternal constitutional factors such as maternal age and lactation stage (Dorea, 2004).

Two major forms of mercury (inorganic and organic) can enter breast milk (Dorea, 2004) with the most hazardous, the organic form (methylmercury) entering at low rates because it is attached to red blood cells. However, even the small amounts of organic mercury in breast milk may be readily absorbed by the infant (Yurdakök, 2015).
Infants and young children have a particularly high vulnerability to mercury poisoning due to efficient gastrointestinal absorption, predisposition to accumulate rather than excrete mercury from brain cells, and undeveloped detoxification mechanisms (Kampalath & Jay, 2015). High mercury concentrations in breast milk can lead to neurodevelopmental issues, blindness, seizures, speech impairment and could also affect the immunologic system (Besser, 2009; Abdollahi et al., 2014).

Two longitudinal studies conducted on children’s exposure to methylmercury via breastmilk documented no adverse effect (Grandjean et al., 1993; Jensen et al., 2005). The earlier study conducted on a cohort of 583 infants in Denmark found that babies exposed to methylmercury via breast milk had higher developmental scores than formula fed babies. The study concluded that the effects of methylmercury from breastmilk are outweighed by the advantages of breastfeeding (Grandjean et al., 1995). Another cohort study on infants who were followed for 7 years found that exposure to methylmercury in breastmilk was not associated with any deficit in neurophysiological performance at age 7 after a detailed neurobehavioral examination of the cohort (Jensen et al., 2005).

Worldwide, there is limited information on mercury levels in cow milk (Najarnezhad & Akbarabadi, 2013). Mean mercury levels (135.39 µg/l) detected in cow milk was higher than the permissible limit set (10 µg/l) (Najarnezhad & Akbarabadi, 2013) for mercury residue in cow milk. Extremely high mercury levels found could be partly attributed to the release of toxic mercury into the atmosphere by the crude e-waste recycling activities at the Agbogbloshie dumpsite where these cattle are reared.

In this study, mean arsenic levels present in breastmilk (38.96µg/l) was found to be consistent with other studies which also reported maximum breast milk arsenic
concentrations. For example, Watanabe et al., (2003) reported breast milk arsenic concentration of 38 μg/l from Bangladesh, and Samanta et al., (2007) reported arsenic levels up to 49 μg/l from West Bengal in India which a known high arsenic zones (Bhattacharya, Chatterjee, & Sarkar, 2012). In contrast, lower levels of 0.15, 0.29, 0.55 and 0.26 μg/l have also been reported by other studies (Sternowsky, Moser, & Szadkowsky, 2002; Gurbay et al., 2011). The reasons for obtaining high arsenic values of arsenic in breastmilk from other studies were reported as due to exposure to high arsenic levels through contaminated drinking water and dietary intake of mothers (Gürbay et al., 2012). In this study however, high values of arsenic in breast milk could be attributed to exposure to high arsenic levels from environmental contamination from the crude activities of e-waste recycling in Agbogbloshie.

There is however proof of limited transfer of arsenic into the mammary gland that serves as protection for infants against arsenic exposure (Gurbay et al., 2012) and this mechanism could account for the non-detection of arsenic in most of the breast milk samples. The excretion mechanism of arsenic through breast milk and the factors that affect its excretion rate are however unclear and not well-known (Gürbay et al., 2012).

In general, the mean arsenic levels detected in breast milk samples in this study were far above the WHO permissible limits for arsenic residues in breast milk (0.6μg/l) (WHO, 1989; Parr et al., 1991). Exposure of infants and young children is an important risk for their vulnerable bodies as it could lead to impaired cognitive functions, neuropathy, gastrointestinal disorders and in extreme cases death (WHO, 2010; Bjerselius et al., 2013).

Arsenic was not detected in all cow samples analysed which is consistent with a study conducted by Sigrist, Beldoménico, & Repetti (2010) in which arsenic was not undetectable in all 12 cow milk samples although cows were exposed to water
contaminated with arsenic. Arsenic was also not detected in fresh cow milk collected from certain regions in China and Japan (Qin et al., 2009). The levels of arsenic were below the arsenic detection limit (0.05µg/l) of the AAS machine used; a higher machine with greater precision such as the ICP-MS could have been able to detect the minute concentrations of arsenic that could be present in the cow milk samples.

Almost all detected levels of nickel were above WHO permissible limit level of 16 µg/l (Iftikhar et al., 2014). Reported levels of nickel in breast milk in other studies were in the range of 0.79–20 ng/ml and 3 and 50 ng/ml (Gurbay et al., 2012). The maximum nickel concentration detected in this study were above these reported levels and could be attributed to mothers living in Agbogbloshie being exposed to high levels of environmental pollution from the smoke from the burning of EEE. Nickel is an essential metal needed in minute quantities in the body and serves as a cofactor in the absorption of iron from the intestine. High levels in breast milk could lead to toxicity in breast feeding infants which could lead to allergic skin reactions, brain damage and immune dysfunctions (Das et al., 2008; Ogabiela, et al., 2011).

Contrary to expectation, nickel levels were undetectable in cow samples, i.e. below the detection limit of nickel for the spectrophotometer (0.07µg/l), which was also inconsistent with findings from other studies conducted in various countries which recorded the presence of nickel in fresh cow milk samples. Ogabiela, et al., (2011) reported nickel levels of 3.013 and 2.097mg/l in Kano and Zaria all in Nigeria, whilst 53.54-68.84µg/l was reported by Dobrzański et al., (2005) in Poland. A study conducted in four farms in the Borena zone in Ethiopia had nickel levels also below the detection limit of the machine used (Belete et al., 2014). Nickel is generally accepted as an essential trace element for animals and therefore traces of the metal should be found in biological samples of humans.
There is limited data on the actual nickel content of milk and the reported values are open to substantial variation due to significant differences in geographical regions and characteristics of the area as well as the extent of environmental contamination in the various study sites (Amaro et al., 1998).

In general, the heavy metal content of milk may vary and is influenced by several factors such as the lactation period, climate, season, breed of animal, type of feeding, level of exposure to environmental pollutants and the environmental contamination in study sites (Sejian et al., 2013; Amaro et al., 1998).

The Kruskal Wallis non-parametric test was performed on the mean concentrations of the various heavy metals in both human and cow milk. The test showed statistical significant differences in the levels of heavy metals detected in the breast milk samples with $\chi^2(3) = 17.945$ and p-value of 0.005 with highest and lowest concentration being mercury and arsenic respectively. However in cow milk, there was no statistical difference in the levels of heavy metals detected ($\chi^2(1) = 3.429$ and p-value of 0.0641).

A comparison of mean concentrations of heavy metals in human and cow milk showed differences in mean lead levels which was statistically significant (p-value of 0.002 <0.05). The differences in mercury in human and cow milk was not statistically significant (p-value of 0.888>0.05).

### 5.1 LIMITATIONS OF THE STUDY

The study was limited by small sample size as during the period of this study, 25 lactating mothers from the study site were available to be recruited. Also, four cows reared at the Agbogbloshie e-waste dumpsite were lactating at the time of the study. These limitations however do not affect the validity of the results obtained from the analysis of heavy metals. However, a sample size of 4 cows may not be representative of the whole cow population in the study area (Agbogbloshie).
CHAPTER SIX

6.1 CONCLUSION
The results of this study showed that breast milk and cow milk collected around Agbogbloshie were contaminated with lead, mercury, arsenic and nickel at varying degrees with most levels higher than recommended WHO acceptable limits. This could be as a result of exposure of women to environmental contamination due to the crude e-waste recycling activities at Agbogbloshie. Although the levels of heavy metals in breast milk depend on several factors and fluctuate with time, exposure of infants to these kinds of heavy metals pose a health risk for their vulnerable bodies. Despite the possibility of harm from environmental contaminants in breast milk, breastfeeding is still recommended as the best infant feeding method.

Lead and mercury levels were detected in cow milk samples. Results revealed that all detected lead levels were below the recommended limits (permissible) for lead residue in cow milk. Mercury levels were however above the recommended set limits. Nickel and arsenic levels were below detection limits of the machine.

6.2 RECOMMENDATIONS
1. Electronic waste recycling activities at the Agbogbloshie e-waste dumpsite releases toxic pollutants into the atmosphere which, could eventually be secreted into breast milk and cow milk when mothers and cows are exposed. Activities at the recycling site must be regulated by stakeholders such as the Environmental Protection Agency and the Ministry of Environment, Science and Technology to minimize release of toxic substances into the environment.

2. The recent Hazardous and Electronic Waste Control and Management Bill passed by Parliament of Ghana to regulate the usage and disposal of electronic
waste need to be fully implemented to prevent further environmental contamination.

3. Large numbers of infants and young children suffer from heavy metal contamination in milk. Breastmilk of lactating mothers are contaminated due to the foods consumed and the environment to which they are exposed. It is therefore necessary for massive education and awareness to be created especially among mothers to prevent contamination of their breast milk during the antenatal and post-natal visits to the health facilities. Community Health nurses who deal directly with mothers and babies should also give expert advice in that regard.

4. Cattle rearing in Ghana should be regulated by appropriate institutions such as the Veterinary Services Directorate under the Ministry of Food and Agriculture to ensure that standard operating procedures are employed and the environment is conducive for cattle rearing.

5. Further studies are required using a larger sample size and negative controls from non-exposed communities.

6. A more sensitive equipment is required for quality control checks on the results.
REFERENCES


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APPENDICES

APPENDIX I

INFORMED CONSENT FORM

**Project Title**: Levels of heavy metals in cattle and human milk around an e-waste dumpsite.

**Institution affiliation Introduction**

My name is Eunice Matilda Mends, a student of the Department of Biological, Environmental and Occupational Health Sciences of the School of Public Health, College of Health Sciences, University of Ghana, Legon.

Address: P.O Box CT 2267, Cantonments, Accra.

Contacts: 0208088164 / 0262165034

Email address: eunicemends@gmail.com

**Background**

I am conducting a study on the levels of heavy metals present in cattle and human milk from cattle and humans around Agbogbloshie, an e-waste dumpsite. The purpose of this study is to determine the concentrations of selected heavy metals in milk and how it could eventually impact on infant health. This study would be conducted from January to July 2016 and study participants include lactating women around Agbogbloshie exposed to e-waste recycling activities.

**Procedure**

Study participants will be asked to express 10-20ml of breastmilk for heavy metal analysis. Your participation in this study will be appreciated. This is purely an academic research which forms part of my work for the award of a Master’s degree.
Risks and Benefits

This procedure will not cause any discomfort to you the participant. The results of the study will provide evidence for breastmilk contamination from exposure to e-waste crude recycling activities at Agbogbloshie.

There are no costs involved to you the participant.

Discomfort during Extraction

There might be slight discomfort during extraction of breastmilk. This will however not pose any harm to the mother or the baby.

Privacy

Privacy will be ensured during breast milk extraction. A makeshift structure that will be fully covered will be used for extraction for mothers who work at Agbogbloshie but do not live close by. The extraction will be done preferably in the homes of participants. To ensure confidentiality, participants will be identified by ID numbers without their names.

Compensation/ Payment

There are no incentives/ payment for participating in this study.

Right to refuse

Participation in this study is voluntary and you can choose not to partake. You are at liberty to withdraw from the study at any time. However, I will encourage your full participation since your participation is important.

Anonymity and confidentiality

I would like to assure you that your participation will be confidential.
If you have questions you may contact

The Principal Investigator,
Eunice Matilda Mends
Department of Biological, Environmental and Occupational Health Sciences
Contact: 0262165034/ 0208088164
Email address: eunicemends@gmail.com

**Voluntary Consent**

I ……………………………………………, declare that I have read the above information, or it has been read to me. The purpose, procedures risks and benefits of the study have been thoroughly explained to me and I have understood. I have also had the opportunity to ask questions about it and any question I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study at any time.

I hereby agree to partake in the study and extract my breastmilk for analysis

Signature of participant…………………………Date ……../……../………

**If the respondent cannot read the form themselves a witness must sign here:**

I was present while the benefits risks and procedures were read to the respondent. All questions were answered and the volunteer has agreed to take part in the research

Signature of witness……………………………….Date……./………./………
APPENDIX II

ETHICAL APPROVAL

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted

My Ref. GHS/RED/ERC/Admin/App/16/
Year Ref. No.

Eunice Matilda Mens
University of Ghana
School of Public Health
Legon, Accra

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

<table>
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<tr>
<th>GHS-ERC Number</th>
<th>GHS-ERC 60/12/15</th>
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<tr>
<td>Project Title</td>
<td>“Levels of Heavy Metals in Cattle and Human Milk around Agbogbloshie, An E-Waste Dumpsite”</td>
</tr>
<tr>
<td>Approval Date</td>
<td>12th April, 2016</td>
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<tr>
<td>Expiry Date</td>
<td>11th April, 2017</td>
</tr>
<tr>
<td>GHS-ERC Decision</td>
<td>Approved</td>
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This approval requires the following from the Principal Investigator:

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED: DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra