LEAD CONTAMINATION AND HUMAN HEALTH RISK ASSESSMENT THROUGH CONSUMPTION OF COW MILK IN KABWE, ZAMBIA

By

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Declaration

I hereby declare that this thesis is my original work and that it has not been previously submitted to any other institution for the award of any degree, diploma or certificate.

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Dedication

I dedicate this thesis to the almighty God who enabled me to accomplish my goal. I also dedicate this mammoth work to my beloved wife Audrey Chirwa Zyambo and my children, brothers and sisters for their delightful cooperation and unwavering patience even when I denied them my presence on several auspicious and memorable family occasions to enable me complete this work. I also dedicate this work to my father James Zyambo and my mother Lila M. Zyambo as well as to my in-laws, Mr and Mrs Abrazio Chirwa for their kind support and encouragement during my research work.

Finally, I also dedicate this report to my late Grandfather Mr. Benson Chaponda Zgambo and his wife Nomi M. Zgambo for illuminating my academic path with success early enough in my childhood years when they conferred me with the title “teacher”.

Certificate of approval

'This thesis of Golden Zyambo has been approved in fulfilling of the requirements for the Degree of Master of Science Degree in Toxicology by the University of Zambia'.

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Abstract

Lead (Pb) contamination in the vicinity of lead mines and smelters affects both humans and animals. Chronic exposure to Pb via dietary intake of animal products such as milk from contaminated areas poses a health risk to consumers. Therefore, the aim of the current study was to evaluate the human health risk impact of Pb exposure through consumption of cow milk as well as to assess the seasonal variations of Pb concentrations in cow milk and blood of lactating cows in Kabwe, which has a long history of lead-zinc mine in Zambia. The cow milk and blood samples were collected from traditional smallholder, emerging smallholder and medium-sized dairy farms located in Kang'omba, Mukobeko, Mpima, Munga, and Kafulamase of Kabwe during the wet and dry seasons. The Pb metal concentrations were determined using Graphite Atomic Absorption Spectrophotometer (GFAAS) after samples were acid digested in a microwave-optimized system. Cow milk and blood lead levels (BLLs) showed seasonal significant differences at $p = 0.05$ using Dunn's Multiple Comparison Test (DMCT, $p < 0.05$). The mean Pb metal level obtained in cow milk during the wet season ranged from 0.98 (± 0.30) to 2.32 (± 1.87) µg/kg while in the dry season the mean concentrations varied from 0.50 (± 0.24) to 4.24 (± 2.24) µg/kg. Similarly, the mean blood Pb concentrations ranged from 3.84 (± 3.22) to 21.8 (± 15.9) µg/kg in the wet season while in the dry season the mean ranged from 0.55 (± 0.24) to 23.8 (± 18.6) µg/kg. Of all the cow's blood samples analysed, 27% and 60% in Kang'omba and Munga respectively, exceeded the baseline value of 20 µg/kg. A higher concentration percentage of 37% in Kang'omba was recorded in the dry season while in Munga it remained unchanged at 60% in the wet season. Notably, the factors that influenced different Pb concentration patterns were the season, distance, and location of the farms from the Pb–Zn mine. The overall mean Pb concentration, chronic daily intake (CDIs), target hazard quotients (THQs), and incremental lifetime cancer risk (ILCR) results obtained were all below the maximum permissible limits of 20 g/kg, 3 and 12.5 g/kg-BW/day (for children and adults), 1 and 1.0×10^{-4} to 1.0×10^{-6}, recommended by FAO/WHO, the joint FAO/WHO, FDA and USEPA respectively. In conclusion, although the study showed that Pb was present in all Kabwe studied regions, the health risk effects of Pb exposure associated with the consumption of milk in both adults and children were insignificant.

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ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
BLLs	Blood lead levels
BW	Average body weight of an individual (kg)
CAC	Codex Alimentarius Commission
Cd	Cadmium
CDI	Chronic Daily Intake
Cm	Metal concentration in mg/kg
CR	Carcinogenic risk
CRM	Certified Reference material
CSF	Oral carcinogenic slope factor
Cu	Copper
DDW	Double distilled water
DMCT	Dunn's Multiple Comparison Test
EFSA	European Food Safety Authority
FAO	Food Agricultural Organization (of the United Nations)
GF AAS	Graphite Atomic absorption spectrophotometer
GPS	Global position system
H_2O_2	Hydrogen peroxide
HI	Hazard index
HNO_3	Nitric acid
ILCR	Incremental life carcinogenic risk
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
MCCs	Milk collection centres
Pb	Lead
RfD	Oral Reference Dose
SD	Standard deviation
THQ	Target hazardous quotients

UNZABREC	University of Zambia Biomedical Science Ethics Committee
USEPA	United States Environmental Protection Agency
UWE	University of the West of England
WHO	World Health Organization (of the United Nations)
Zn	Zinc

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of the study

Rapid industrialization, urbanization, and various other anthropological activities are the main causes of wide dispersion of heavy metals in the environment (Islam et al., 2018). Environmental contamination can also occur from natural or geological sources which include metal–bearing rocks and volcanic eruptions (Ali et al., 2019). In recent years, the pollution of heavy metals has become a serious problem in the vicinity of mining sites and this has attracted increasing attention (Derakhshesh and Rahimi, 2012). Their accumulation and persistence in the human food chain is recognized as a public health hazard worldwide (Yabe, et al., 2013). Heavy metal pollutants have become a threat to animal life due to their toxic effects and consequently have led to a strain on the livelihood and health of people (Kasozi et al., 2018).

Among the heavy metals, lead (Pb) is one of the most abundant environmental pollutants that is often implicated in human and animal poisoning (Derakhshesh and Rahimi, 2012). Lead poisoning with varying degrees of toxicities has been reported in animals around polluted sites (Swarup et al., 2005). The potential source of Pb exposure in cattle is often the forage produced on agricultural surface or grazing on pastures contaminated by emissions from smelters (Zadnik, 2010). The emissions of toxic heavy metals such as Pb can also contaminate agricultural soils, food crops, surface and ground water, and these pose health risks to the population through different pathways (Tong et al., 2000). Exposure to Pb is a global public health issue that recently has been recognized as a potential problem in many developing countries. According to World Health Organization (WHO, 2010), some countries recognize that they have a childhood lead-poisoning problem in relation to certain exposure sources, but have not yet implemented assessment and exposure prevention programs.

Milk is considered to be an important component of human diet due to its positive influence that it has on human health (Muhib et al., 2016). Typically, milk is known to be a good source of proteins, fats, vitamins supplements and major minerals, and because of its unique nutritional composition, milk is considered a nearly complete food (Muhib et al., 2016). In many traditions, milk is a basic food in the human diet, both in its unprocessed form and as various dairy products (Licata et al., 2004). However, despite milk being part of the human diet, reports of contamination with Pb from various sources are well documented (Swarup et al., 2005; Pilarczyk et al., 2013; Bischoff et al., 2014). Mostly, animals that are reared in the vicinity of Pb-Zn smelting units excrete high levels of Pb in the milk (Swarup et al., 2005). Incidentally, children are the most vulnerable to Pb exposure due to their high milk consumption rates (Ismail et al., 2017). Thus, Pb residues in milk are of particular concern (Derakhshesh and Rahimi, 2012). Since milk and dairy products form a large part of human diet, especially in early childhood, consumption of heavy metal contaminated milk and its products therefore, could lead to high proportionate intake of trace and toxic elements (Suturović et al., 2014). Data indicate that children who are exposed to toxic metals from an early age, progressively accumulate the metals in different organs, developing a high possibility of having metal toxicosis when they become older (Gonzalez et al., 2017). According to (WHO, 2010), Pb exposure in children is associated with neurobehavioral disorders at blood Pb levels (BLLs) of 5 µg/dl and even lower; this is because children's nervous system is still in developmental stages (UWE, 2013).

Although, consumption of Pb contaminated milk is unlikely to cause clinical Pb poisoning in people, subclinical exposure has been associated with intellectual and cognitive deficits in children and has also been associated with spontaneous abortion and stillbirth in women (Sharpe & Livesey, 2006). In adult men, chronic (long-term) exposure to Pb has been found to reduce their fertility (Wani et al., 2015). Because milk is largely consumed by children who are more susceptible to toxic metal exposure risks (Gonzalez et al., 2017), it's now under strict regulatory attention. In this present study, the human risk assessment via milk consumption pathway is of particular interest. Therefore, the current study aims were to: (i) quantify the Pb concentration in cow milk; (ii) determine the total BLLs in lactating animals; (iii) determine variations of Pb concentrations in the blood and milk of cows across different seasons; (iv) evaluate the human health risk exposure to Pb associated with the consumption of cow milk produced in the vicinity of the Pb-Zn mining town of Kabwe, Zambia.

1.2 Problem statement

Lead is a pervasive environmental pollutant with potential public health hazard as a contaminant of food of animal origin (Swarup et al., 2005). According to literature, the natural exposure of lactating cows to the environmental toxicants, influences trace mineral composition of milk and significantly affects the milk quality and nutritional values (Patra et al., 2008). Of particular concerns are the Pb residues in milk because it is largely consumed by infants and children (Derakhshesh and Rhimi, 2012). Since milk consumers are at a great health risk from Pb exposure in milk especially infants and children, regular monitoring and evaluation of cow milk is necessary.

Numerous studies that have been conducted in Kabwe focused on Pb levels in soil (Tembo et al., 2006; Nakayama et al., 2011; Ikenaka et al., 2010). The existing data reveal that Pb pollution in Kabwe was extensive (Tembo et al., 2006; Ikenaka et al., 2012), approximately covering the radius of 20 kilometres from the point source (Tembo et al., 2006). In extreme cases, the Pb levels exceeding the threshold of 1,000 mg/kg in the soil are reported to have been detected (Tembo et al., 2006). Recently, high levels of Pb and other heavy metals such as Cadmium (Cd), Copper (Cu) and Zinc (Zn) have been detected in livestock particularly in free-range chickens (Yabe et al., 2013) and in cattle (Yabe et al., 2011). Interestingly, the concentrations of Pb obtained in the edible tissues exceeded the benchmark levels of 500 µg/kg wet weight in chicken and cattle liver, offal and kidneys for human consumption (Yabe et al., 2011). However, the resultant health risk to human consumers in the aforementioned studies, were not established. Additionally, cow milk, which presumably has been consumed for an immeasurable time could probably be one of the sources of childhood Pb poisoning to Kabwe residents. Similarly, Pb content in cow milk and its potential health risk to human consumers have not been determined.

For this reason, it is therefore, necessary to evaluate cow milk produced in Kabwe for food safety purposes with regard to human risk exposure to Pb, assess the prominence of Pb status in the farms proximal to the old Pb-Zinc mine where milk is produced for the establishment of critical data which may be vital for regulation and management of metal pollution in the region.

1.3 Justification

Lead contamination in Kabwe is extensive, covering a radius of approximately 20 kilometres (Tembo et al., 2006). Lead can enter the food chain through plant uptake from a contaminated environment (Nachiyunde et al., 2013). Animals accumulate heavy metals such as Pb in their tissues and ultimately get excreted in milk (Younus et al., 2016).

Elevated BLLs in cattle reared in Kabwe have been detected ranging from 90.6 ± 67.6 mg/kg dry weight (Ikenaka et al., 2012). In addition, a study by Yabe et al. (2011) indicated high Pb concentrations in cattle offal including kidney and liver, suggesting that cattle reared near the Pb-Zn mine in Kabwe were highly exposed to Pb from the polluted environment.

Despite alarming levels of Pb concentrations found in edible tissues of cattle reared in the region, cow milk has not been investigated, and health risk assessment on ingestion of cow milk has not been evaluated. Therefore, the current study will be of great importance for toxicological and environmental purposes to both humans and cattle in Kabwe region.

1.4 Significance of the study

Milk is an important source of essential nutrients in the diet, especially for infants and children. However, heavy metal contamination of the environmental can result in excretion of toxic metals in exposed cows including Pb in milk. This can pose a public health hazard, particularly in infants who have higher consumption rates of milk compared to adults (Chandrakar et al., 2018).

In Kabwe town of Zambia, high levels of Pb and other metals have been reported in the liver and kidneys from cattle reared in the vicinity of the lead-zinc mine (Yabe et al., 2011). However, concentrations of Pb in milk from cattle in Kabwe and potential health risks to human consumers have not been determined. Since Pb contamination in milk from cows reared in Kabwe has not been determined, there is high likelihood that children may have been exposed to Pb in cow milk from an early age. Therefore, evaluation of the cow milk in Kabwe is necessary.

1.5 Research questions

1. How elevated are the levels of Pb in the milk and blood of the cows reared near the Pb-Zn mine in Kabwe?
2. Does the exposure of Pb through consumption of cow milk in people in Kabwe exceed the public health safety benchmarks?

1.6 The research study objectives

1.6.1 The general study objective

To evaluate Pb levels in cow milk, blood, and the health risk impact of Pb on humans through consumption of cow milk in Kabwe, Zambia.

1.6.2 Specific objectives

(i) To determine the Pb levels in cow milk and blood in the vicinity of the Pb-Zn mine in Kabwe

(ii) To determine the seasonal variations of Pb concentrations in cow milk and blood in the sampled animals.

(iii) To evaluate the health risks associated with the consumption of milk produced in Kabwe region.

1.7 Scope of the study

The research is a field-based study that was conducted in peri-urban areas of Kabwe. The samples were obtained from traditional smallholder, emerging smallholder, and medium-sized dairy farmers who reared local breed, mixed-breed or dairy animals for milk production on free-range practices.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

Natural and human activities, such as industrial, agricultural and domestic wastes greatly contribute to environmental pollution, which causes adverse effects to human and animal health. Environmental pollutants have consequently continued to be a world concern and one of the great challenges faced by the global society. Pollution is identified as the largest environmental cause of disease and premature death in the world today (Mathee et al., 2018). In dairy cattle, Pb exposure is associated with economic losses due to mortality and treatment costs, but with production animals, there is also a risk to the human food chain (Bischoff et al., 2014).

Due to human activity, levels of Pb are reported to have increased in the environment by more than a thousand fold over the last three centuries (Wang et al., 2019). Particularly in Africa, metal pollution has recently been reported to have reached unprecedented levels in the past decade. This phenomenon has now become a major health risk to the continent causing human exposure to toxic metals. In this regard, soil was pinpointed to be the key medium of exposure to pollution (Mathee et al., 2018).

Lead is a naturally occurring heavy metal present in the earth's crust (Wang et al., 2019). It is one of the global environmental pollutants that is mainly prevalent in industrial regions and animals that are in proximity to such areas are easily exposed to Pb pollution. Lead is considered as one of the most hazardous and cumulative environmental pollutants that affect all biological systems through exposure to air, water and food sources (Assi et al., 2016). Because of its cumulative and toxic nature in the human food chain, it is recognized as a public health hazard (Yabe et al., 2013).

Continuous transfer of Pb occurs between air, water, and soil by natural chemical and physical processes such as weathering, runoff and precipitation, dry deposition of dust and stream/river flow. Soil and sediments appear to be important sinks for Pb according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2007). Atmospheric deposition generally is the main source of Pb found in the soils not impacted by other non-air sources such as dust from deteriorating leaded paints (ATSDR, 2007). Although Pb is a natural environmental contaminant, its use in the past especially in water pipes, paint and petrol increased its presence as reported by the European Food Safety Authority (EFSA) (EFSA, 2012). Industrial exposure significantly, accounts for a common route of exposure for adults while ingestion is the most common route of exposure in children (Masindi & Muedi, 2018). Due to its toxic nature, childhood poisoning has now become a public health concern worldwide (Yabe et al., 2015).

Lead is a toxic and possible carcinogenic element (Miclean et al., 2019). As one of the heavy metals, Pb accumulates in tissues of dairy animals and ultimately gets excreted in milk because of its non-biodegradable and persistent in nature (Abah et al., 2017). Milk is a basic food in the human diet, both in its natural form and other different dairy products (Licata et al., 2004). Thus, consumption of Pb contaminated milk is reported to be the most dangerous aspect (Pilarczyk et al., 2013) since it is consumed regularly (Wiley, 2017). According to Abah (2017), consumption of milk from cattle reared in polluted sites leads to chronic (prolonged) exposure to toxic metals which results in human health risks.

Lead is as a systemic neurotoxic metal that has been linked to visual deterioration, central and peripheral nervous system disorders, renal dysfunction and hypertensive cardiovascular disease according to the University of West England (UWE, 2013). Children are more vulnerable to Pb exposure mainly because they are more at risk of ingesting environmental Pb through normal mouthing behaviours, and their absorption rate in the gastrointestinal tract is higher than in adults (UWE, 2013). Additionally, their developing nervous system is thought to be immature such that it is far more vulnerable to the toxic effects of Pb than the mature brain. Unfortunately, epidemiological studies indicate that exposure to Pb during the early stages of children's development is linked to a drop in intelligence. Further, studies suggest that the intelligence quotient (IQ) is reduced by at least 1-3 for each 10 µg/dl (microgram per decilitre) elevation of blood Pb concentration (UWE, 2013).

Cow milk consumption was recently introduced in Zambia as one of the routine school feeding programs among many children from grade 1-9 (Tesliuc et al., 2013). As in other traditions reported elsewhere (Gonzalez et al., 2017), milk in Zambia is one of the common dietary food supplements that is routinely consumed by many families with urban dwellers consuming four times higher than the rural households (Neven et al., 2017). Urbanization, rising income levels and other related lifestyle changes contribute to the increasing demand on milk in Zambia. On the other hand, milk supplies in Zambia were observed to be on the upsurge trends (Neven et al., 2017). Because young children, infants, pregnant women, elderly, and immunecompromised people who are the primary groups at risk for milk safety problems, regular monitoring, and evaluation of toxic heavy metals such as Pb in cow milk is necessary (Girma et al., 2014).

2.2 Lead contamination in the environment: sources and exposure pathways

Lead in the environment can result from multiple sources (UWE, 2013). Some of the most important sources of metal contamination are the natural environment, chemical and metallurgical industries (Akoto et al., 2017). For example, metal concentrations have been found to be proportionately high close to towns, signifying their characteristic urban/industrial origins (Akoto et al., 2017). The sink for Pb is the soil and sediments from which plants and animals may bio-concentrate Pb deposited in the environment (ATSDR, 2007). Thus, the metal present in the soil-plant system can easily be transferred to the food chain and ultimately constitute a risk factor for humans, animals, plants and the entire modern ecosystem (Chandrakar et al., 2018).

Anthropogenic factors such as mining and smelting, battery manufacturing, recycling of waste batteries, burning coal, and use of leaded petrol, leaded paints and Pb piping have primarily contributed to the high levels of Pb in the environment (Wang et al., 2019). Due to adverse effects associated with high levels of Pb, excessive occupational exposure is now strictly controlled (Assi et al., 2016). However, food is still the major source of human exposure to Pb (EFSA, 2012) accounting for over 90 % compared to other exposure pathways such as inhalation and dermal contact (Loutfy et al., 2006).

2.3 Lead contamination in milk

Lead and other trace elements have been detected in cow milk. Such contaminants, are not natural part of the milk, but they are excreted into milk from the animal body (Tunegova et al., 2016).

Lead levels, resulting from the natural exposure of lactating cows to the environmental toxicants, influences trace mineral composition of milk and significantly affects the milk quality and nutritional values (Patra et al., 2008). Apart from seasonal variations in different weather conditions, herbage growth has been identified to have a significant effect on the bioavailability of metals from which the grazers were exposed (Roggeman et al., 2013). Most frequently, the prevalence of metal contaminants in milk above the maximum residual limits (MRLs) have been reported to be common in developing countries mainly due to the unhygienic conditions of the processing technologies, contaminated feed and contaminated water for the animals as well as unawareness of personnel involved in dairy business (Chandrakar et al., 2018). Comparatively, data shows that raw milk produced in Europe and North America has been found to have low content of macro and micro minerals while other regions such as Brazil, Croatia, Egypt, Mexico, Nigeria, Palestine, Romania, Serbia, and Turkey, the values exceed bench mark levels (Zwierzchowski & Ametaj, 2018).

Metal concentration variations in milk are influenced by its excretion from the mammary gland such as breed of the animal, season of the year, feeding and factors related to animal handling by humans (Ikirić et al., 2003). Since milk is an important food received from animal origin, transfer of toxic elements to human food chain is a concrete danger (Chandrakar et al., 2018), therefore, it is necessary to obtain it from healthy animals that are free from environmental contamination (Soares et al., 2010). Thus, with increasing environmental pollution, implementation of priority safety regulatory measures in addressing safety milk concerns for public health is essential (Girma et al., 2014).

2.4 Lead toxicity in cattle

Lead exposure is a common cause of heavy metal toxicity in cattle and may cause economic loss in beef and dairy herds. Lead poisoning particularly in animals can be found from numerous sources that may be linked to the contamination of feed, soil from industrial pollution and agricultural practices (Assi et al., 2016). Toxicity can occur in cattle after ingestion of toxic amount of lead from a variety of sources such as auto batteries, discarded crankcase oil, paint, solder, greases, oil well pipes, asphalt, and roofing material (Galey et al., 1990). Because of differences in the dose of Pb consumed, Pb poisoning in cattle may be acute or subacute (Checkley et al., 2002).

The absorption, distribution, storage, and elimination of Pb in animals depend on several factors, which include the chemical form of Pb, species, age and physiologic state of the animal, nutrition, and rate of ingestion (Aslani et al., 2012). Once absorbed from the gastrointestinal tract, Pb is initially distributed to soft tissues, kidneys, and liver, by the blood (Aslani et al., 2012). The absorbed Pb is then finally excreted in the bile, urine as well as mobilized in milk in lactating animals (Aslani et al., 2012). Galey (1990) asserts that Pb has a relatively short half-life of 1-2 months in blood and soft tissues. As a result of the short half-life, there is rapid elimination of Pb via the kidneys and lactation and redistribution of Pb to the bone (Galey et al., 1990).

Chronic Pb poisoning in cattle is normally caused by ingestion of low amounts of lead-contaminated fodder over extended periods, and general signs that often result in loss of appetite and weight, reproduction abnormalities, anaemia, osteoporosis, and immunosuppression characterize its consequence (Zadnik, 2010). Chronically Pb affected cattle may charge around, press their heads against a wall, and later they develop ataxia. Mostly, their symptoms relate to neurotoxicity (Zadnik, 2010). Pb exposure in dairy cattle is associated with economic losses due to mortality and treatment costs, but with production animals, there is also a risk to the human food chain (Bischoff et al., 2014).

2.5 Toxic effects of lead exposure on humans

The effects of Pb exposure are a health concern for humans, particularly during early childhood (WHO, 2010). Although, cow milk is considered as the major source of nutrition recommended for children (Gonzalez et al., 2017), evidence of Pb excretion in milk in the vicinity of highly polluted areas increases the health risks due to natural exposure to the lactating cows (Swarup et al., 2005; Patra et al., 2008). The toxic effects of Pb exposure extend from acute, clinically obvious, symptomatic poisoning at elevated levels of exposure down to subclinical (but still severe) effects at lower levels (WHO, 2010).

Lead accumulates in the body, primarily in the skeleton. At extreme exposure, Pb attacks the central nervous system to cause coma, convulsions and even death. At lower levels of exposure that cause no obvious symptoms, and that previously were considered safe, Pb is now known to cause multiple injuries across various body systems (WHO, 2010).

An epidemiological study by Popovic et al. (2005) on the impact of occupational exposure levels of Pb in women indicated that the blood Pb concentrations remain elevated in women long after the cessation of occupational exposure. Further, the study particularly indicated that the endogenous exposure relation found for postmenopausal exposed females was consistent with the data on their counterpart male smelter workers, whereas the relationship established for premenopausal females was significantly lower. Literature reveals that endogenous exposure levels of Pb was more evident during periods or conditions associated with increased bone resorption; for example pregnancy and lactation periods as well as during menopausal transitions (Nie et al. 2009). Because of such differences related to endogenous exposure factors, sex is considered to be an important aspect that plays a significant role in metabolism of Pb, therefore, data from males on Pb effects may not be appropriate for use on females (Popovic et al., 2005).

2.5.1 Health effects of lead on men

Lead toxicity in men result in reduced performance and damage to the sperm producing organs (Younus et al., 2016) causing decreased sperm count, including other alterations that occur in the volume of sperm when BLLs exceed 40 µg/dL (Wani et al., 2015). Also, Pb exposure in adults causes anaemia, hypertension, renal damage and immunotoxicity (WHO, 2010).

2.5.2 Health effects of lead on women.

Lead is reported to be an important paediatric environmental health problem in both developed and undeveloped countries despite remarkable successes that have been scored in combating the key sources and exposure pathways in recent years (Liu et al., 2014). Once Pb is absorbed into the body, it is stored for a long time in mineralizing tissues such as teeth and bones (ATSDR, 2017). And the stored Pb however, may be released into the blood stream again particularly in times of calcium deficit which is often experienced during pregnancy, lactation, osteoporosis (WHO, 2010; ATSDR, 2017).

In pregnant women, Pb is reported to have the ability to cross the placental and blood-brain barrier and induce neurotoxicity to the developing foetus (Liu et al., 2014). The nature, magnitude and persistence of the adverse effects on human health resulting from low-level exposure to environmental Pb, particularly in early childhood causes cognitive development deficits (Tong et al., 2000). Documented evidence reveals that, the health impact of lead in pregnant women causes numerous cases of premature births and small/underdeveloped infants (Blacksmith, 2007). On comparison terms, literature indicates that there is an increased threat of hypertension risks in postmenopausal women than in premenopausal women associated with high BLLs (ATSDR, 2007). On the other hand, BLLs above 3 µg/dL in girls are associated with delayed attainment of menarche (onset of menstruation) and pubertal development (breast and pubic hair) (Schoeters et al., 2008).

2.5.3 Health effects of lead on children

Children have a greater potential for adverse effects of Pb exposure compared to adults because their intake per unity body weight is higher and they are subject to more ingestion of dust (WHO, 2010). Moreover, children have high gastrointestinal absorption rate up to 50% of ingested Pb compared with 10% in adults (WHO, 2010).

Studies indicate that Pb toxicity has devastating effects on neurodevelopment in children related to mental retardation and lowering of IQ. Further, toxic effects of Pb in children ultimately result in poor school performance, lower tertiary education attainment, behavioural disorders and poor lifetime earnings (Lidsky and Schneider, 2003). Interestingly, adverse effects of exposure to Pb in children occur even at BLLs <5 µg /dL (WHO, 2010) that once were thought to be a "safe level", which is now known to be associated with decreased IQ in children, cause behavioural difficulties and learning problems (WHO, 2010). Unfortunately, childhood neurological effects resulting from Pb exposure, relates to attention and deficit hyperactivity disorder (ADHD), which persist into adulthood (ATSDR, 2017).

2.6 Factors influencing lead toxicity

Intake of milk has been identified as one of the many ways by which Pb released into the environment can become available for human exposure (Swarup et al., 2005; Bischoff et al., 2014). The most important metal exposure routes for grazers is their food (Roggeman et al., 2013). Metal toxicity depends on several factors such as the route of exposure, age and sex of the exposed person, and level of consumption, state of the metal, duration of exposure, frequency of intake, absorption rate mechanism or extraction efficiency (Tunegova et al., 2016; Chandrakar et al., 2018). According to the European Food Safety Authority (EFSA, 2012), chronic toxicity of Pb metal due to its long half-life, is of the most concern when considering the potential risk to humans. As a highly persistent metal in the environment (Wani, et al., 2015) Pb toxicity induced by excessive levels in the environment is well known (Tunegova et al., 2016).

2.7 Biomonitoring of lead exposure

The use of biomarkers is widespread in biological monitoring of susceptible populations (Mañay et al., 2011) because they suggest the occurrence of toxicological events much earlier than the emergence of those effects that can be evaluated (Alimonti & Mattei, 2008). Several biomarkers exist for monitoring exposure to Pb (ATSDR, 2007) and have been reviewed by several authors (Sakai, 2000; Barbosa et al., 2005; Alimonti & Mattei, 2008). Biomonitoring for exposure to Pb reflects an individual's current body burden, which is a function of recent and/or past exposure (Barbosa et al., 2005). Apart from Pb concentration in whole blood, other biological markers of exposure used in environmental studies include urine, faeces, hair and other tissues etc. (Alimonti & Mattei, 2008). Accordingly, the appropriate choice and measurement of biomarkers of Pb exposure is important for health care management purposes (Barbosa et al., 2005).

2.7.1 Biomarkers of exposure

Biomarker of exposure, used in biomonitoring studies, may be an exogenous compound or its metabolite such as a metal or a metal compound inside the body, an interactive product between the compound (or metabolite) and an endogenous component, or another event related to the exposure (Alimonti & Mattei, 2008). Biomarkers of exposure may be used to identify exposed individuals or groups, quantify their exposure, assess their health risks, or to assist in diagnosis of diseases(Alimonti & Mattei, 2008).

2.7.2 Biomarkers of effect

Biomarkers of effect are referred to as reversible biochemical and functional alterations than can be measured in a target tissue of the organism. Typically, a biomarker of effect by definition may be an endogenous component, or a measure of the functional capacity, or some other marker of the state or balance of the body or organ system, as affected by the exposure of the agent. For example, the urinary excretion of proteins with a small molecular weight, e.g., albumin, may be used as a biomarker indicative of early kidney damage (Alimonti & Mattei, 2008).

2.7.3 Lead residues in milk

Several studies have shown that cow milk is a good biological matrix that could be used in non-invasive procedure as a biomarker matrix for evaluating Pb exposure risks to both animal and human health (Koyashiki, et al., 2010). On the other hand, residual concentrations of metals in milk could be an important "direct indicator" of the hygienic status of the milk as well as an "indirect indicator" of the degree of the environmental pollution in which the milk is produced (Licata et al., 2004).

2.7.4 Blood lead

Blood Pb is regarded as the most reliable index of exposure to Pb (Sakai, 2000) because it is reflective of the recent metal exposure (Mañay et al., 2011). Lead levels in the blood of exposed cattle provide a reliable indicator of the lead-contaminated environment. In normal ruminants, the whole BLLs are usually below the range of 50-250 µg/kg, and for the poisoned animals the Pb levels are usually above 350.0 µg/kg and deaths begin at 1000 µg/kg according to Zadnik (2010). Although blood or tissue samples are analysed for Pb diagnosis of animals in clinical cases, routine screening for Pb is not done in asymptomatic animals for evidence of exposure (Checkley et al., 2002). However, asymptomatic animals may accumulate sufficient Pb in their tissues and thus unsuitable for human consumption. Therefore, routine screening in cattle reared in contaminated environments should be considered (Checkley et al., 2002).

2.8 Milk production levels in Zambia

The total milk production in Zambia is estimated to be over 253 million litres per year (Mumba et al., 2013). For example, milk production from 15 districts was estimated at 57,5521,087 litres per annum out of which 3,633,272 litres go into the formal sector through established milk collection centres (MCCs). And Kabwe was one of the milk-producing districts in the country with an estimated production of 1,610,700 litres per year (Mumba et al., 2013).

2.9 Milk consumption: Implications for human health and nutritional value

Worldwide, milk consumption in recent years has increased, especially in developing countries and it is now considered to be a significant dietary supplement for a high proportion of the global population (Handford et al., 2016). The variations in milk production and consumption levels between urban and rural areas that have been reported from different regions were attributed to historical differences and cultural preferences (Handford et al., 2016).

Consumption of raw milk is characteristic of many populations. Typically, as a representation of popular customs, raw milk is used to make special and traditional products (Barreto et al., 2019). Cow milk is considered to be one of the foods of the greatest nutritional value that is recommended by the Food and Agriculture Organization (FAO) of the United Nations and the United Nations Educational, Scientific and Cultural Organization (UNESCO) (Gonzalez et al., 2017). In its natural form, milk has high food value comprising of a wide variety of nutrients that are essential for growth and maintenance of the human body and also, many communities depend on milk for their source of protein supplement (Gonzalez et al., 2017).

Routinely, cow milk is now consumed by many children across the world, and it has recently been promoted as one of the common components of the school feeding programs (Wiley, 2017). For example, ‘milk for schools’ program was launched in Zambia in 2011 with multiple objectives among school going children from grade 1-9. Apart from increasing school attendance and nutritional value, the program was also designed to provide a new market share to the local milk producers for their products (Tesliuc et al., 2013).

Intake of milk has been identified as one of the modes by which Pb released into the environment can become available for human exposure (Swarup et al., 2005; Bischoff et al., 2014). Since intake of heavy metals such as Pb through milk consumption is a food safety problem, it is therefore necessary to estimate the potential risk to the health of the population

(Lara-Severino et al., 2019). Barreto et al. (2019) asserts that milk per capita consumption levels have increased almost two folds since early 1960s. Unfortunately, there is no recommended tolerable intake level as there is no evidence of thresholds for a number of critical health effects. Data suggest that, with the capita milk consumption range of 30 to 150 kg/person/year, milk could be an important vector in terms of consumer exposure to contaminants (Barreto et al., 2019). Mumba et al. (2013) reported that the average per capita consumption of milk in sub-Saharan Africa was about 36 litres with Kenya being the highest at about 100 litres. Further, the report indicated that the per capita milk consumption in Zambia averaged between 16.5 to 19.4 litres, against the 200 litres recommended by WHO (Kawambwa et al., 2014). Because milk and milk based products were widely consumed in human diet especially in early childhood, they could contribute a large fraction of the intake of trace and toxic elements (Suturović et al., 2014).

2.10 Legal framework for human health safety on lead exposure

Lead is one of the heavy metal pollution that is now emerging as a matter of concern at local, regional and global scales (Chandrakar et al., 2018), therefore, a number of regulatory agencies have established permissible limits to control its levels in milk (Ismail et al., 2017; Chandrakar et al., 2018). To ensure the safety of milk for human consumption, codex food standard, which is a FAO/WHO Codex Alimentarius Commission set 20 μg/kg as the maximum residual limit (MRL) for Pb (FAO/WHO , 2011) while the maximum permissible limit for milk established by EFSA of the European Union Commission is 100.0 μg/kg (EFSA, 2012; Chandrakar et al., 2018). The U.S. on the other hand, the U.S. Food and Drug Administration (FDA) recommends interim reference levels of 3 and 12.5 μg/kg for children and adults (women of childbearing age) (Flannery et al., 2020), respectively, which correspond to the blood lead level (BLL) of 0.5 μg/kg for a general population. In developing countries where monitoring and remediation measures are relatively poor or completely absent, toxic metal levels in milk could pose a serious threat to human health (Ismail et al., 2017).

2.11 Health risk assessment

Food consumption has been identified as the major exposure pathway of heavy metals in humans (EFSA, 2012). Since the intake of heavy metals through food consumption is considered to be a food safety problem that severely impacts the health of consumers, the information about food intake and heavy metal concentrations in food products are required to estimate the potential health risk of the population (Lara-severino et al., 2019). The health risk assessment of each heavy metal such as Pb or metalloid is usually based on quantification of the risk level and is expressed in terms of a carcinogenic or non-carcinogenic health risk (Bortey-Sam et al., 2015; Muhib et al., 2016; Ismail et al., 2017; Kasozi et al., 2018).

2.12 Knowledge gap

Lead contamination in Kabwe has so far been reported mostly in soil and recently in biological samples involving humans and animals. Despite high levels of Pb previously established in different sample matrices, dietary Pb exposure and its associated risk impact have not been evaluated. As a matter of public safety, particularly for children and adults, the current study was conducted seasonally to investigate the Pb concentrations in cow milk; assess the human health risk of Pb exposure associated with the intake of cow milk from free-range cattle reared around the Pb-Zn mine in Kabwe.

CHAPTER 3

3.0 RESEARCH METHODOLOGY

3.1 Study design

The study was a cross sectional in design that was conducted between 2018 and 2021. A convenient sampling technique was used to increase the likelihood of selecting healthy cows from the traditional smallholder, emerging smallholder and medium-sized farms composed of mixed breed and dairy animals. Appendix 5 was used as a criterion for selecting healthy cows for sampling. With particular interest to monitor seasonal variations of Pb concentrations in milk and blood samples, a follow-up design study from the selected subjects was carried out during the wet and dry seasons.

3.2 Study site

The study was conducted around the mining area in Kabwe district in Central Province and in Chongwe district (reference site), which is a non-mining area of Lusaka province. Kabwe is situated at approximately 14°27'S and 28°26'E. The study sites included a cluster of farms situated around Kabwe town from five zones namely, Kang'omba, Munga, Mukobeko, Mpima, Kafulamase and, in Chongwe (Kanakantapa farm block) situated approximately 131 kilometres away from the pollution source in Kabwe. Sampling locations were accurately marked using a global positioning system (GPS).

3.3 Cow milk and blood samples

3.3.1 Sample **size determination**

The sample size of the population was determined according to the formula described by Fosgate, (2009) as presented in equation 1 as follows:

$$n = \frac{Z^2\, P(1-P)}{d^2} \qquad \textbf{Equation (1)}$$

Where,

n = required sample size,

Z = 1.96 (confidence level at 95%), the standard distribution corresponding to a significant value of α = 0.05,

p = prevalence rate of the Pb pollution set at 50%,

d = level of precision at 5% (standard value of 0.05).

Sample size, n = $(1.96)^2$ x 0.5 x (1-0.5)/ $(0.05)^2$

= (3.84 x 0.5 x 0.5 x)/ 0.25

= 384

Therefore, the required sample size was 384.

Although n = 384 was the required estimated sample size, only 233 (60.6%) milk and 246 (64.1%) blood samples were collected from Kabwe including the reference site in Chongwe both in wet (February/March) and dry (October) seasons. The number of targeted samples was lower than the actual number of samples collected because of the lower number of the lactating animal populations reared on free-range practices in the selected regions.

3.3.2 Sampling strategy and sample distribution

The study targeted five (5) farm areas within a radius of 25 kilometres from the Pb-Zn mine in each sampling zone for equal distribution of samples. Farms having a size greater than or equal to five (5) lactating cows were registered for sampling. If the farm had more than five but less than ten (10) lactating cows (5≥10), all the available lactating cows were considered as study subjects as long as they meet the inclusion or exclusion criteria (appendix 5). However, in a case where lactating cows exceeded 10, the extra number of subjects was calculated based on the 10 per cent fraction of the total herd to cow ratio, following a random selection criteria. To cater for the non-responsive, loss of animals due to theft or death and diminished lactation, a 10 % proportion of the lactating population was considered. Figure 3.1 below shows the sampling areas.

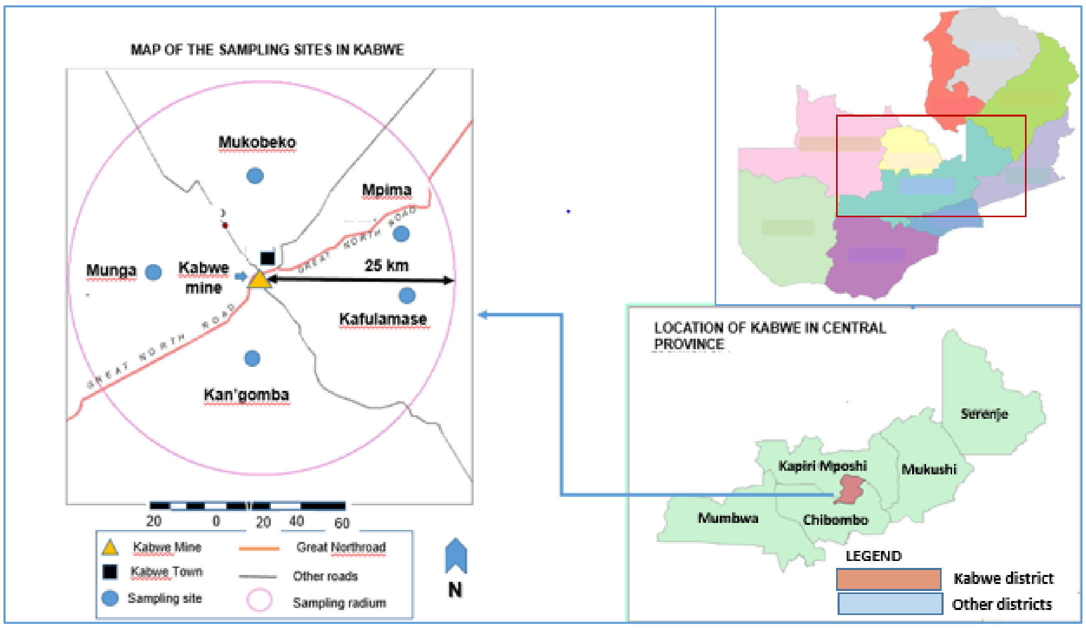


Figure 3.1: Map showing the sampling areas in Kabwe
(Modified from https://en.wikipedia.org/wiki/Central_Province, _Zambia)

3.4. Sampling frame

3.4.1 Inclusion criteria

The study included free-range cows that were healthy and not on medication with an estimated period of not less than at least 7 months of lactation with high likelihood of repeated sampling in wet and dry seasons.

3.4.2 Exclusion criteria

The study excluded cows that had colostrum milk (at least not less than 6 weeks of lactation), animals on medication, and those that were reared for less than 30 days on the respective farms for uniformity purposes since the half-life of Pb concentration in the animal blood is 30 days. In addition, cows from independent units (commercial farms) were excluded from the study. Both inclusion and exclusion criteria were based on appendix 5.

3.5 Sample collection

3.5.1 Physical examination of cows

Prior to sample collection, all lactating cows were screened for any clinical conditions by the local veterinarian and the selection was done with the help of the farmer as described by Bischoff et al. (2014) following the procedure outlined in appendix 5. The selected cows were then isolated and restrained in a cattle crush pen to ensure safety procedures were observed during sampling.

3.5.2 Collection of milk and blood samples

Milk sampling was performed during routine morning milking (Gonzalez et al., 2017) from healthy cows according to the methods described by (Belete et al., 2014). Briefly, the udder of each cow was washed before manually expressing about 10 ml of milk directly into the sterile 15 ml polypropylene tubes (SuperClear^{R} Labcon, USA). Also, approximately 6 ml of blood were collected via venepuncture of the jugular or tail vein from the milk sampled cows using syringes and heparinized tubes containing (5000 unit/5ml of heparin) by an authorized veterinarian as per the method of (Patra et al., 2008). After collection, the samples (milk and blood) were instantly homogenized by inverting the tubes several times and temporarily stored in separate cooler boxes with ice packs before transportation for storage at -20^{0}C (Defy, South Africa) until analysis, at the University of Zambia.

3.6 Instrumentation

3.6.1 Graphite furnace atomic absorption spectrophotometer instrumentation conditions

The experimental approach for the metal determination were performed using graphite furnace atomic absorption spectrophotometer (GFAAS), ((Z-2010; Hitachi High-Technologies) equipped with graphite furnace according to a technique described by Yabe et al., 2013.

Table 3.1: Graphite furnace atomic absorption spectrophotometer (GFAAS) operating conditions for the analysis of milk and blood

Analytical GFAAS operating conditions							
Parameter		**Furnace temperature Program**					
Lamp current	7.5 V	Stage		Temperature (Degrees)		Time (Sec)	
Wavelength	283.3 nm			Start	End	Ramp	Hold
Slit	1.3 nm	1	Dry	80		40	
Cuvette	Pyro Tube HR	2	Ash	600	600	20	
Carrier gas (Ar)	30 mL/min	3	Atomize	2400	2400		5
Injection volume	20 µL	4	Clean	2700	2700		4
PMT Voltage	298 V						
Time constant	0.1 s						
Single Mode	BKG correction						
Calc. Mode	Peak height						

Graphite furnace atomic absorption spectrophotometer (GFAAS) has good detection limits for a majority of elements, with a small sample size of 20 µl for analysis and minimum requirements for sample preparation. And in many applications, GFAAS has demonstrated acceptable level of high precision and accuracy (Khalid et al., 2016). Because of its proficiency, GFAAS has been used for metals measurements at low concentration levels after reducing the interference problems by various techniques (Sherman and Muehlhoff, 2007). According to the manufacturer's guide, the use of the Pyro Tube HR cuvette in GFAAS analysis offers great advantage compared to other types of cuvettes for similar analytical work due to its ultrahigh density graphite coating that provides optimum sample measurement. To enhance the analysis of

Pb concentrations which are generally low in milk (Chen et al., 2008), the furnace temperature program was optimized to provide matrix decomposition without loss of analyse as described in the manufacture's guide. The furnace program is given in Table 3.1.

3.6.2 Microwave conditions

The microwave closed-vessel system (Berghof MSW-3+, Eningen, Germany) offers high reliability coupled with ease of operation and extremely low operating costs as described in the manufacturer's manual. According to Ataro et al. (2008), the advantage of this procedure is that it consumes minimum amounts of reagents and the digestion time is short. It also gives clarity of the digest's absence of the undigested milk sample, simplicity and low heating temperatures (Belete et al., 2014).

The Berghof MSW-3+ closed-vessel microwave system was used to digest the milk and blood samples as per modified method described by Toyomaki et al. (2020). The optimized microwave digestion system under automated temperature and pressure-control conditions for milk and blood were set for 31 minutes according to the manufacturer's program as shown in Table 3.2 below.

set according to the manufacturer's program as shown in Table 3.2 below.

Table 3.2: Microwave operating conditions for milk and blood

Digestion operating conditions			
Step	Ramp (min)	Time (min)	Temperature (^{0}C)
1	5	5	160
2	1	10	190
3	1	10	75

3.7 Quality control and methodology validation

3.7.1 Quality control

Quality control was performed using a method blank in every 10 samples prepared alongside the samples. Blank replicates were measured directly and their intensities were used to correct background sample reading intensities whenever they were significant according to Ataro et al. (2008) and Salazar-Flores et al. (2019). Further, constant memory stability of the GFAAS equipment during analysis was checked by replicate measurement of Pb standard concentration in every 10 samples measured.

3.7.2 Calibration curve

The standard solution of Pb metal was prepared at six different concentrations as described by Muhib et al. (2016) with slight modification. Calibration curves were constructed from a stock solution of 1000 mg/L of Pb by diluting the intermediate standard solution with double distilled water. The standard calibration curve of Pb was established using the linear regression analysis of the standard solutions against absorbance values. Each standard solution was measured in duplicate.

3.7.3 Limit of detection and limits of quantification

The limits of detection (LOD) and limits of quantification (LOQ) was calculated from the calibration curve according to the following mathematical equations 2 and 3 previously used by de Oliveira et al. (2017):

$$\mathrm{LOD} = \frac{3\mathrm{SD}}{\mathrm{m}}$$ **Equation (2)**

$$\mathrm{LOQ} = \frac{10\mathrm{SD}}{\mathrm{m}}$$ **Equation (3)**

Where,

SD = the standard deviation of 10 consecutive measurement of blank solution

m = slope of analytical curve.

3.7.4 Method validation and accuracy

Method validation and accuracy was performed using DOLT-5 (dogfish liver, National Research Council of Canada) certified reference material.

3.8 Sample preparation, digestion and metal determination

3.8.1 Reagents

The ultrapure double distilled water from a Milli-Q-Element system (Millipore, Milford, MA, USA) was used throughout to prepare of all solutions as described in the method by Yabe et al. (2013). The stock solutions of metals (1000 mg/L) was obtained by dissolving appropriate metal salts (Merck, Darmstadt, Germany) as per the method of Ataro et al. (2008). Concentrated metal nitric acid (69 %), HNO_3 and hydrogen peroxide (30 %), H_2O_2 were metal-free grade. Before use, metal-free polythene laboratory ware was soaked overnight in 2 % (v/v) dilute nitric acid, pre-washed and thoroughly rinsed with ultrapure double distilled water from a MiliQ-Element System.

3.8.2 Milk and blood sample digestion

The digested milk and blood sample preparation was carried out as per the methods described by (Muhib et al., 2016; Yabe et al. 2013) with slight modifications. The frozen milk samples were removed from -20^{0}C freezer and let to stand for thawing overnight at 4 ^{0}C. After the attainment of constant room temperature and homogenization with a vortex, 1 ml of the milk sample were transferred to the digestion vessel (Berghof DAP-60K, Eningen, Germany) and acid digested in a mixture of 5 ml of 30 % HNO_3 and 1 ml of 30 % H_2O_2. Similarly, blood samples were equilibrated to room conditions, homogenized by inverting the tubes several times followed by the measurement and transfer of 0.3 ml of sample to the digestion vessels to which subsequently, HNO_3/H_2O_2 (5:1) mixture was added. The mineralization program was performed in a closed optimized microwave digestion system (Berghof MWS-3+, Eningen, Germany) as indicated Table 3.2. After mineralization and cooling of vessels for about 20 minutes, the contents were quantitatively transferred to the polypropylene tubes (SuperClear^R Labcon, USA) and the volume was adjusted to 10 ml with milli-Q double distilled water. To ensure homogeneity, the contents were thoroughly mixed by inverting the tube ten times. Similarly, blank samples were also prepared alongside the milk and blood samples maintaining uniform digestion parameters.

3.8.3 Lead metal determination in milk and blood

Pb metal concentration in the digested milk and blood digested samples were measured directly by a GFAAS equipped with a Zeeman graphite furnace according to the manufacturer's analysis manual. Graphite flameless atomic absorption spectrophotometer method required the use of a matrix modifier of 0.5% ammonium hydrogen sulphate ($NH_4H_2PO_4$) (Nakagyo-Ku Kyoto, Japan) concomitantly with the samples. The auto sampler programmed to inject 20 µl of sample per time performed this automatically. Sample solutions were nebulized followed by the introduction of double distilled and de-ionized water for at least 1 minute, to rinse the sampling system to avoid contamination of other solutions. Measurement of Pb concentrations in samples was determined in duplicate.

3.9. Human health risk assessment

3.9.1 Risk assessment

The risk assessment process involves standard steps prescribed by the United States Environmental Protection Agency (USEPA), which has previously been used by other authors (Muhib et al., 2016; Liang et al., 2017; Miclean et al., 2019). In the current study, the risk assessment encompassed:

3.9.2. Hazard identification

The hazard identification is the first step in the risk assessment process that defines the hazard and nature of harm to the exposed populations (USEPA, 2010). In this study, Pb was identified essentially as a potential hazard (contaminant) that affects the residents of Kabwe in close proximity to the Pb-Zn mine (Blacksmith, 2007).

3.9.3. Exposure assessment

In the current study, the exposure assessment was based on chronic daily intake (CDI). The CDI is a value related to the metal concentration in milk that is associated with its daily consumption and the bodyweight of the consumer (Pb) in milk (Miclean et al., 2019); in this case, CDI values were determined according to the equation (1) (Muhib et al., 2016; Miclean et al., 2019) shown below in equation 4.

$$\text{CDI} = \frac{\text{Cm x D (daily intake of milk)}}{\text{BW}}$$ **Equation (4)**

Where,

CDI = estimated chronic daily intake of Pb (µg/kg -1 BW day-1)

Cm = mean concentration of Pb in milk (µg/kg);

D = average consumption intake of milk per person (µg/day);

BW = average body weight (kg) of an individual (children or adults).

3.9.4. Dose-response assessment

The dose-response assessment is a quantitative relationship that indicates a degree of contaminant toxicity to exposed species (USEPA, 2010). In the current study, the oral reference dose value of 4.00 µg/kg/day for Pb was used (Miclean et al., 2019).

3.9.5. Risk characterization

The risk characterization is the final stage of the health risk assessment in which all the information gathered in the previous three stages of evaluating the risk associated with ingestion of food is incorporated (USEPA, 2010). In the current study, risk characterization process involved the analysis of carcinogenic and non-carcinogenic risk assessment.

3.9.5.1. Incremental lifetime cancer risk

The Incremental Lifetime Cancer Risk (ILCR), which represents the probability of developing cancer during a 70-year lifetime continuous exposure, is as a measure of adverse health effect of metal exposure (Zhang et al., 2019). The potential cancerous effects due to Pb exposure in adults and children through consumption (ingestion) of milk was evaluated by using ILCR equation 5 (USEPA, 2010) as follows;

$$ILCR = CDI \times CSF \qquad \textbf{Equation (5)}$$

Where,

ILCR = incremental life carcinogenic risk

CDI = chronic daily intake averaged (µg/kg/day)

CSF = cancer slope factor (µg/kg/day); The CSF for Pb is 8.5 µg /kg/day, a known value that estimates the upper-bound probability of an individual developing cancer as a result of a lifetime of exposure through an ingestion route (Miclean et al., 2019).

3.9.5.2. Non-carcinogenic risk effects

Non-carcinogenic risks were evaluated by comparing an exposure level (dose) over a specified period (e.g., lifetime), with a reference dose derived for a similar exposure period based on target hazard quotients (Liang et al., 2017). The non-carcinogenic risk is characterized in terms of the target hazard quotient (THQ), which has been recognized as a useful parameter for the evaluation of risks associated with the consumption of metal contaminated food. Thus, the potential non-cancer risks for exposure to Pb via consumption of cow milk is assessed by comparison of CDI from the oral exposure route with the chronic dose (RfD) to find the THQ value (USEPA, 2010), which in this study it was calculated using equation (6) as follows:

$$THQ = \frac{CDI}{RfD} \times 10^{-3} \qquad \text{Equation (6)}$$

Where,

CDI = chronic daily intake average (µg/kg/day)

RfD = oral reference dose (µg/kg/day) which is 3.5×10^{-3} for Pb

THQ>1 assumes that there may be a concern for potential non-carcinogenic cancer risks. In a case where THQ<1, it means that the hazard is unlikely to cause adverse health effects for the exposed populations (Liang et al., 2017).The exponential 10^{-3} is the conversion factor.

3.10 Data analysis

Descriptive statistical analysis of the data was performed using GraphPad Prism software (Prism 7 for Windows; Version 5.02, GraphPad Software, Inc., CA, USA). The data obtained were presented as sample number (n); mean value ± standard deviation (SD); coefficient to of variation; range (Min-Max); median of Pb levels in milk and blood; percentiles of 25% and 75% measured at 95% confidence level (CL). Prior to analyses, data were examined for normality of distribution by the Kolmogorov-Smirnov normality test. The test performed showed the departure of data from normality. In order to ascertain if there were significant variations at $p < 0.05$ for Pb in each sampled site in relation to milk and blood, data were subjected to Kruskal-Wallis Test followed by Dunn's Multiple Comparison Test (DMCT) to differentiate the means. Correlation relationships of Pb concentration in milk and blood in different selected sites in each season were determined using Pearson's correlation analysis.

CHAPTER 4

4.0 RESULTS

4.1 Quality assurance data

In the current study, the GFAAS method for Pb analysis included measurement of analytical parameters such as linear range, coefficient of correlation, LOD and LOQ**.** The LOD value was initially calculated by multiplying 3 times standard deviation (SD) for 10 replications of the blank while the LOQ was calculated by multiplying 10 times SD of the slope/intercept. Replicate analysis of DOLT-5 certified reference material used to validate the method gave good accuracy and recovery rate of 93.1-119.8% at 0.999 coefficient of determination (Figure 4. 1 and Table 4.1) below show the methodology performance results.

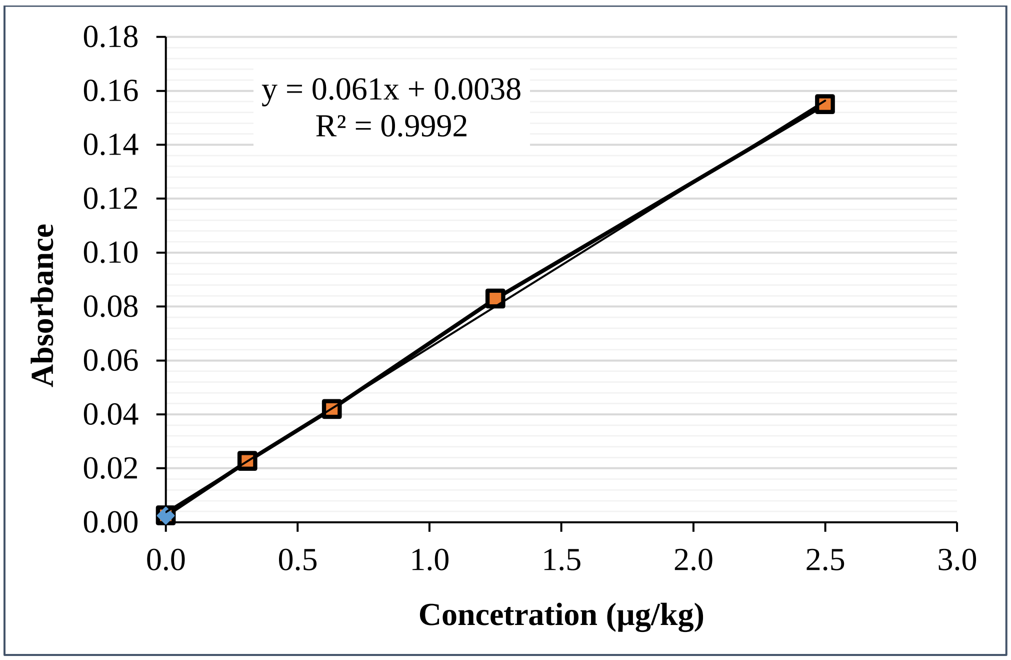


Figure 4.1: Calibration curve of lead on DOLT-5 analysis

Table 4.1: Analytical performances of lead

Parameter	Value
Certified value (µg/kg)	0.005
Measured Value (µg/kg)	0.006
Standard Error (SE)	0.002
Standard Deviation (SD)	0.007
Limit of detection (LOD = µg/kg)	0.026
Limit of quantitation (LOQ = µg/kg)	0.079
Slope (Sensitivity)	0.061
Intercept	0.004
Limit of decision (µg/kg)	0.013
Linearity (working range, µg/kg)	0.020
Mean recovery rate (X ± SD, n= 8), %	111.0
Correlation coefficient	0.999

4.2 Samples of milk and blood collected from each site per season

Two sample types from each lactating cow, blood and milk, were collected for investigation. Table 4.3 below shows the number of the samples collected from Kabwe and Chongwe, the control site for analysis.

Table 4.2: The number of the blood and milk samples collected from Kabwe and Chongwe in each season

S/N	Sample type	Kabwe study group		Chongwe reference group		Total
		Wet season	Dry season	Wet season	Dry season	
1	Milk	101	114	9	9	233
2	Blood	113	115	9	9	246
Total	2	214	229	18	18	**479**

4.2.1 Milk lead levels

Table 4.3 presents descriptive statistics of the cow milk data for Kang'omba, Kafulamase, Mpima, Mukobeko, Munga and Chongwe. The highest mean Pb concentration detected in milk was from Munga area in the dry season followed by Kang'omba in the same season. However, the lowest mean Pb concentrations measured in milk in the dry season were found in Mukobeko followed by Mpima region.

Table 4.3: Summary of the descriptive statistics of the cow milk Pb levels showing number (n), mean lead concentration (µg/kg-wt. /wt.), range (min-max), standard deviation (SD ±), standard error, (S.E), percentiles, Median, lower and upper bound 95% confidence level (Cl)

Region	**Kang'omba**		**Kafulamase**		**Mpima**		**Mukobeko**		**Munga**		**Chongwe**	
Season	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Number (n)	22	34	16	19	28	26	29	30	5	5	9	9
Minimum	0.50	0.70	0.59	0.06	0.20	0.41	0.10	0.09	0.78	1.76	0.30	0.40
25% Percentile	0.90	1.36	0.78	0.59	0.62	0.61	0.59	0.30	0.81	2.36	0.35	0.50
Median	1.45	1.85	0.89	1.01	1.13	0.81	0.69	0.50	1.08	4.05	0.50	0.60
75% Percentile	3.04	3.66	1.16	1.25	1.74	0.99	1.89	0.66	3.54	6.23	0.64	0.69
Maximum	6.90	9.66	1.80	10.8	2.76	1.70	2.77	1.15	3.70	7.70	0.89	0.81
Mean	2.32	2.93	0.98	1.72	1.20	0.84	1.11	0.50	1.96	4.24	0.52	0.60
Std. Deviation	1.87	2.43	0.30	2.58	0.67	0.32	0.74	0.24	1.46	2.24	0.19	0.13
Std. Error	0.40	0.42	0.08	0.59	0.13	0.06	0.14	0.04	0.65	1.00	0.06	0.04
Lower 95% CI	1.49	2.08	0.81	0.47	0.94	0.71	0.83	0.41	0.15	1.46	0.37	0.49
Upper 95% CI	3.15	3.78	1.14	2.96	1.46	0.97	1.39	0.59	3.76	7.02	0.66	0.70

On the contrary, Kang'omba in the wet season had highest Pb levels in milk compared to Munga followed by Kafulamase (Table 4.3 and Figure 4.2). Further, Mpima and Mukobeko regions had higher mean Pb concentrations in the wet season than in the dry season, a pattern that was different from the other study sites as illustrated in Figure 4.2 below.

The general trend of the mean Pb concentrations in milk during the wet season followed the decreasing order of Kang'omba>Munga>Mpima>Mukobeko>Kafulamase>Chongwe while in the dry season the trend followed the order of Munga>Kang'omba>Kafulamase>Mpima>Chongwe> Mukobeko as illustrated in Figure 4.3.

The mean Pb concentrations measured in milk samples in the dry season were higher than the samples in the wet season, especially in farms closer to the mine.

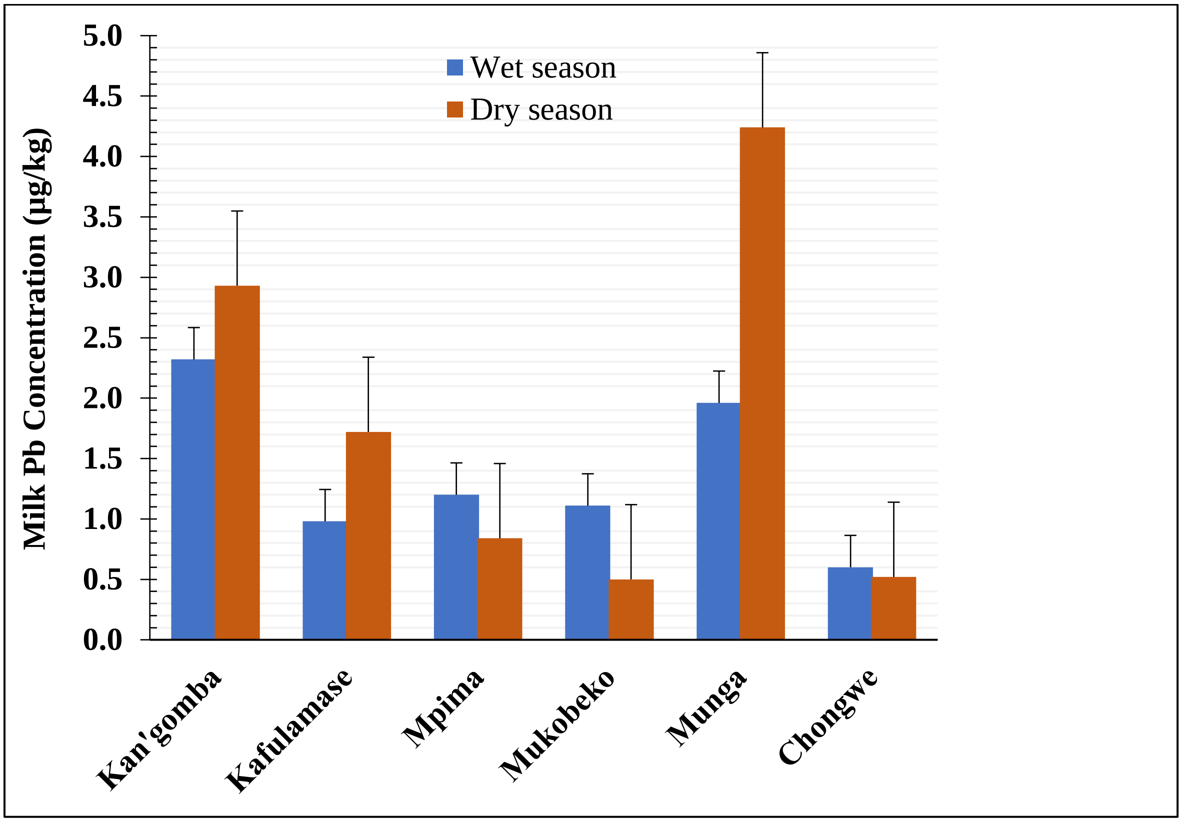


Figure 4.2: Mean Pb concentration in cow milk (µg/kg-wt. /wt.) per region and per season.

Based on the results of the current study, Pb was present in all sampled sites including Chongwe (reference site). The study revealed that Chongwe, the reference site, had the least Pb results and correspondingly, the interval Pb concentration was remarkably minimal (Table 4.3 and Figure 4.4).

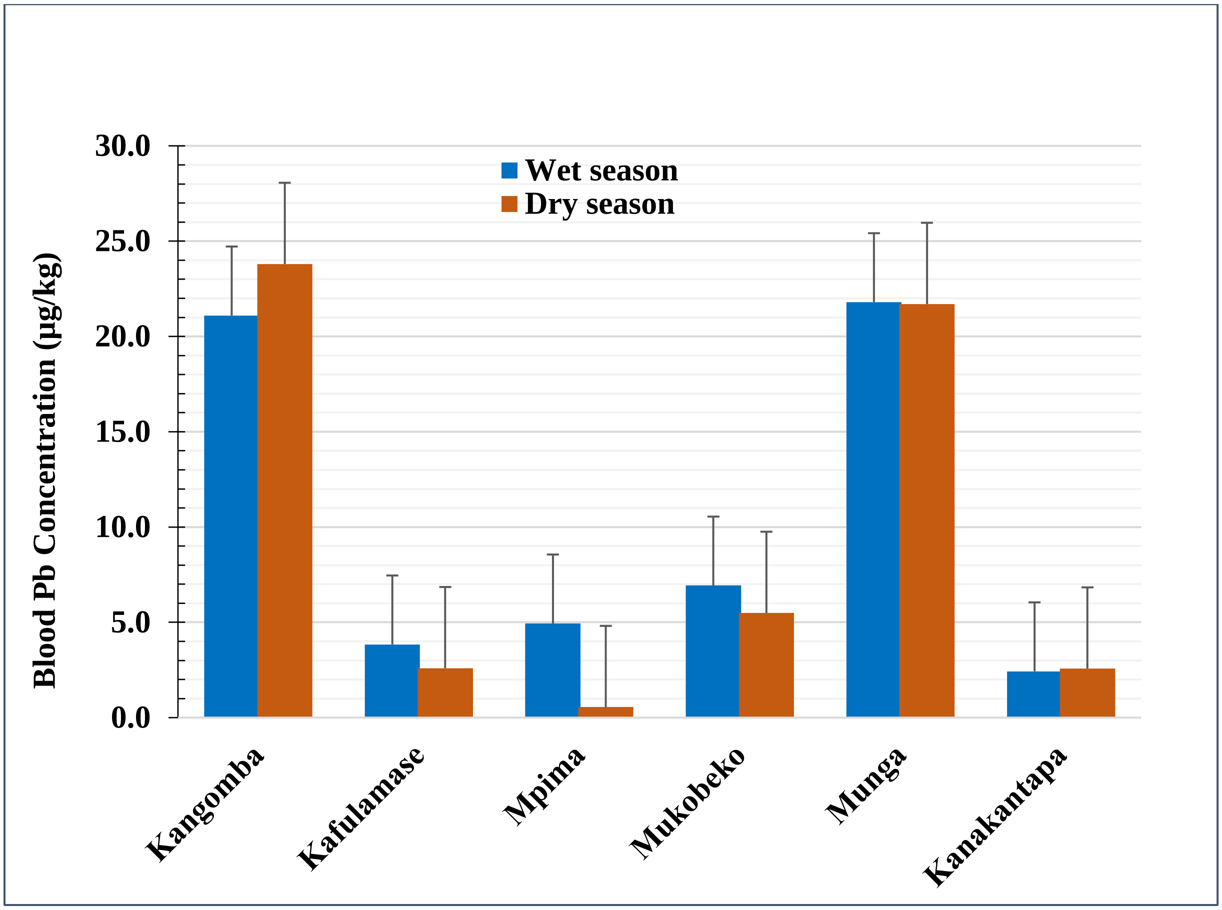
4.2.2 Blood lead levels

Mean BLLs from all sampled sites are presented in Table 4.4**.** Data for Pb in cow blood are presented as mean Pb concentration (µg/kg-wt. /wt.), range (Min-Max, standard deviation (SD ±), standard error, (S.E), percentiles, median, lower and upper bound 95 % confidence level (Cl). The results in Table 4.4 indicate that the highest mean concentration of Pb was recorded in Kang'omba, in the dry season followed by Munga in the wet season while the least was found in Mpima in the dry season. Interestingly, it was observed that the BLLs in Chongwe (reference site) were slightly higher than the Pb levels found in Kafulamase and Mpima regions in the dry season. Data descriptive summary of the Pb in cow blood is presented in Table 4.4 below.

Table 4.4: Summary of the descriptive statistics of the cow blood Pb levels showing number (n), mean lead concentration (µg/kg-wt. /wt.), range (min-max), standard deviation (SD ±), standard error, (S.E), percentiles, median, lower and upper bound 95 confidence level (Cl).

Region	**Kang'omba**		**Kafulamase**		**Mpima**		**Mukobeko**		**Munga**		**Chongwe**	
Season	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Number (n)	34	35	16	18	28	25	28	30	5	5	9	9
Minimum	1.88	6.51	1.20	0.05	1.00	0.14	2.19	0.62	5.89	17.0	0.47	1.00
25% Percentile	7.26	13.2	1.83	0.29	2.47	0.35	4.05	3.90	6.62	17.6	1.61	1.96
Median	12.3	18.4	3.23	1.48	3.90	0.54	6.04	4.34	21.1	21.1	2.41	2.41
75% Percentile	23.6	26.9	4.09	4.24	6.91	0.72	8.49	5.90	37.2	26.1	3.38	3.35
Maximum	93.7	78.1	14.8	9.76	17.8	1.15	25.6	31.3	43.3	27.4	3.91	3.64
Mean	21.1	23.8	3.84	2.59	4.94	0.55	6.93	5.49	21.8	21.7	2.44	2.57
Std. Deviation	21.6	18.6	3.22	2.89	3.54	0.24	4.49	5.10	15.9	4.38	1.11	0.88
Std. Error	3.70	3.15	0.80	0.68	0.67	0.05	0.85	0.93	7.11	1.96	0.37	0.29
Lower 95% CI	13.5	17.4	2.13	1.15	3.57	0.45	5.19	3.59	2.01	16.3	1.59	1.89
Upper 95% CI	28.6	30.2	5.56	4.03	6.32	0.65	8.669	7.39	41.5	27.1	3.29	3.25

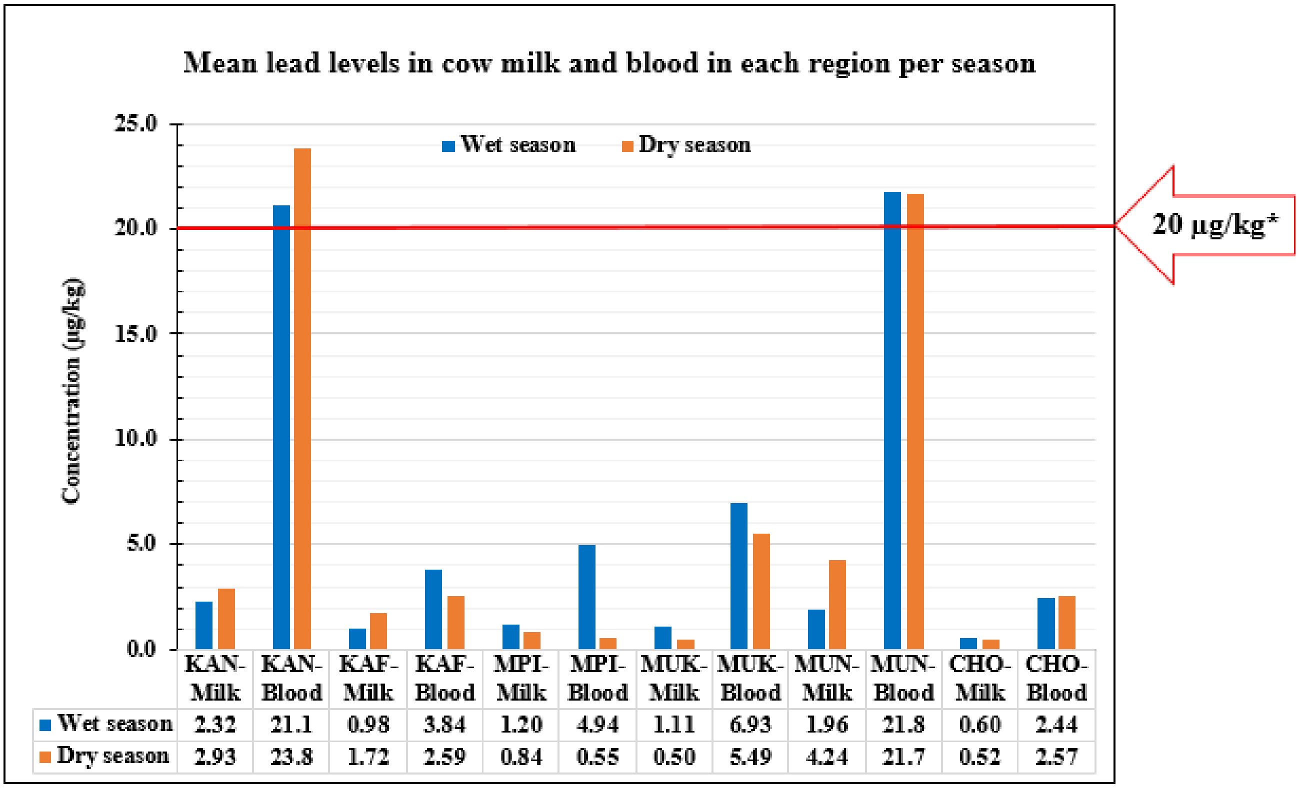
Further analysis of the mean Pb content in blood and milk samples as shown in Figure 4.3, Graphical data (Figure 4.3) show a similar Pb concentrations pattern in Mpima, and Mukobeko in which case, the Pb content in each sample type was higher in the wet season than in the dry season. On the contrary, the mean BLLs in Kang'omba were observed to be higher in the dry season than in the wet season. Strangely, the mean BLLs in Mpima during the dry season were found to be lower than the mean Pb concentrations measured in the blood samples from the reference site in Chongwe during the sesame season (Figure 4.3). However, Pb concentrations measured from Chongwe (reference site) indicate that Pb content in milk was slightly higher in the wet season than in the dry season. The mean Pb concentration changes found in both blood and milk in the present study are presented in Figures 4.3 (a) and (b) below.



(a) (b)

Figure 4.3: Comparison of the mean Pb concentrations (µg/kg-wt. /wt.) (a) in cow blood and (b) in milk per region per season.

A statistical summary of the comparisons of the mean Pb concentrations in cow blood and milk Pb in each region and per season are shown in Figure 4.4. The graphical information also shows that the mean Pb concentrations in Kang'omba and Munga exceeded the baseline level of 20 µg/kg (Figure 4.4).



- 20 µg/kg* is base level of Pb concentration in cow blood

Figure 4.4: Statistical summary of the comparisons of the mean Pb concentrations in cow blood and milk Pb in each region and per season

In the present study, all the blood Pb samples analysed from Mpima and Kafulamase were below 20 µg/kg both during the wet and dry seasons. The blood Pb in 9 (27%), 13 (37%) samples in Kang'omba and 5 (60%) samples in Munga were found to exceed 20 µg/kg. In Mukobeko, only 1 blood sample in each season exceeded the benchmark level of 20 µg/kg, translating into 0.04 and 0.03 % in wet and dry season, respectively. Figure 4.5 below shows the percentage distribution of the blood Pb samples above the minimum baseline of 20 µg/kg in each site per season.

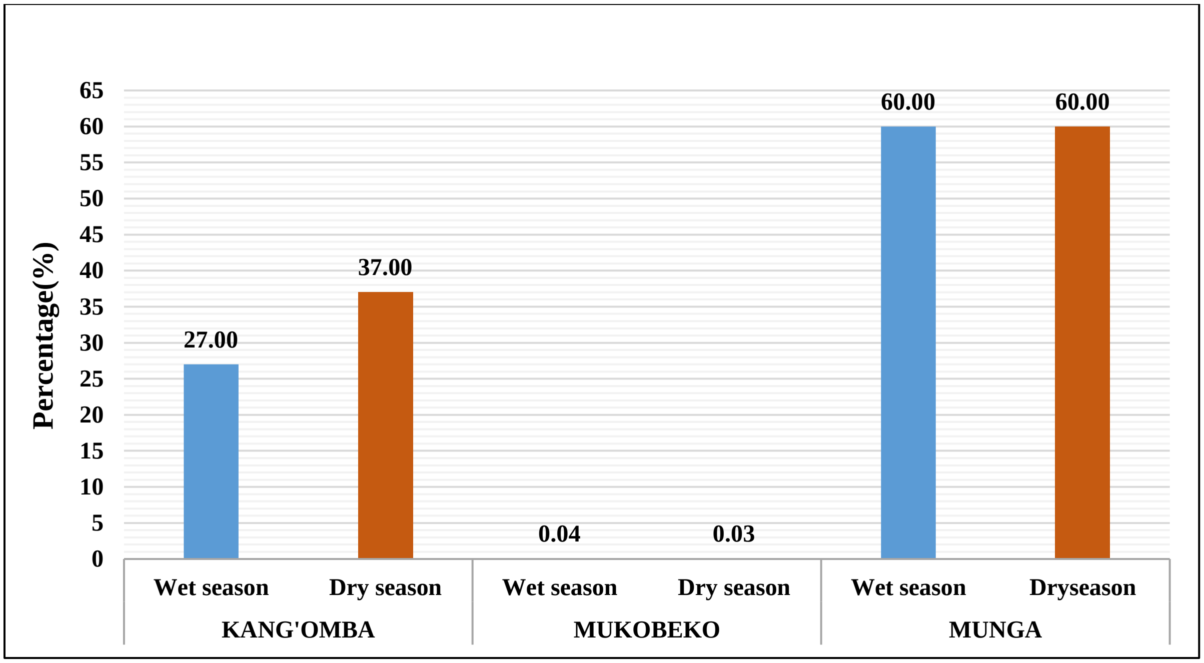


Figure 4.5: Percentages of blood Pb samples above the baseline level of 20 µg/kg in each season per region.

Figure 4.5 above indicate that cows in the two regions that recorded the highest BLLs in Kang'omba seemed to have more cows exposed to high Pb concentrations in the dry season compared to the wet season, while in Munga the concentrations were also found to be high but uniform across seasons.

4.2.3 Cluster comparison analysis of Pb concentrations in cow blood µg/kg per site in each season

A comparison of lead concentrations across different sites indicates that most of the animals were within the range of 0-20 µg/kg although a few cases particularly in Kang'omba ranged from 20- 97.3 µg/kg in the wet and dry seasons (Figure 4.6) and (Figure 4.7). Similarly, Munga and Mukobeko regions recorded BLLs above the baseline of 20.2 to 78.1 µg/kg in the dry season. Cluster comparison analyses of the Pb concentrations are given in Figure 4.6 for the wet season and Figure 4.7 for the dry season.

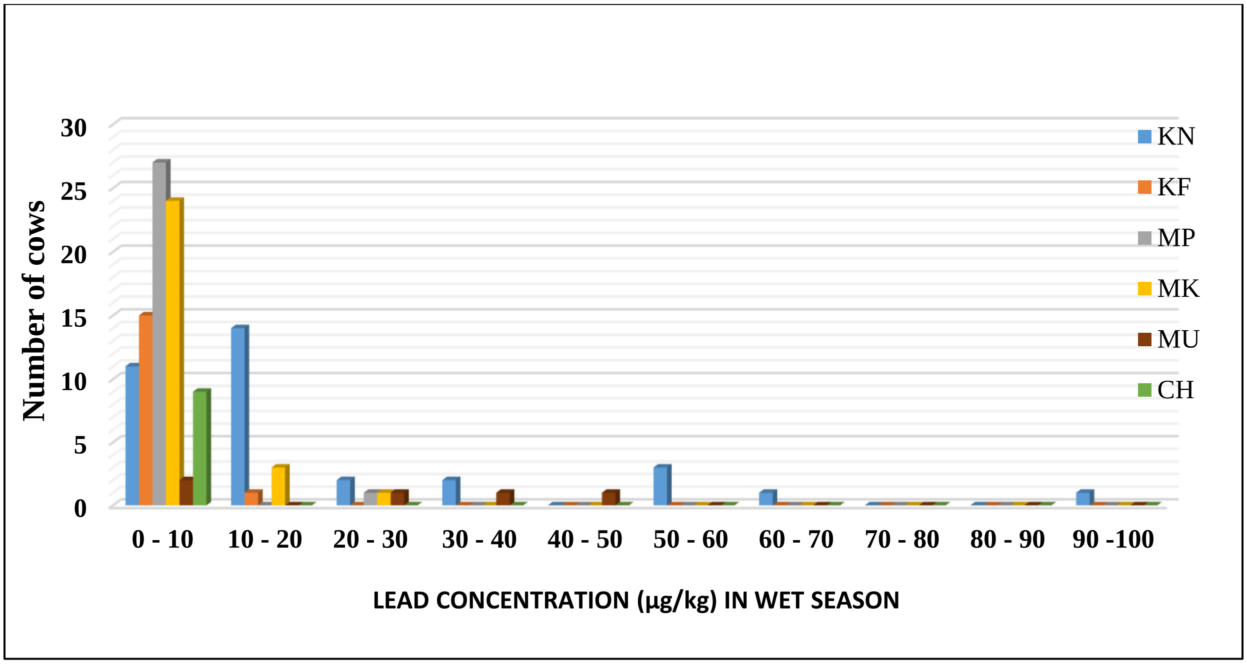


Figure 4.6: Clustered blood Pb concentration (µg/kg) comparisons per site (KN; Kang'omba, KF; Kafulamase, MP; Mpima, MK; Mukobeko, MU; Munga, CH; Chongwe) in wet season

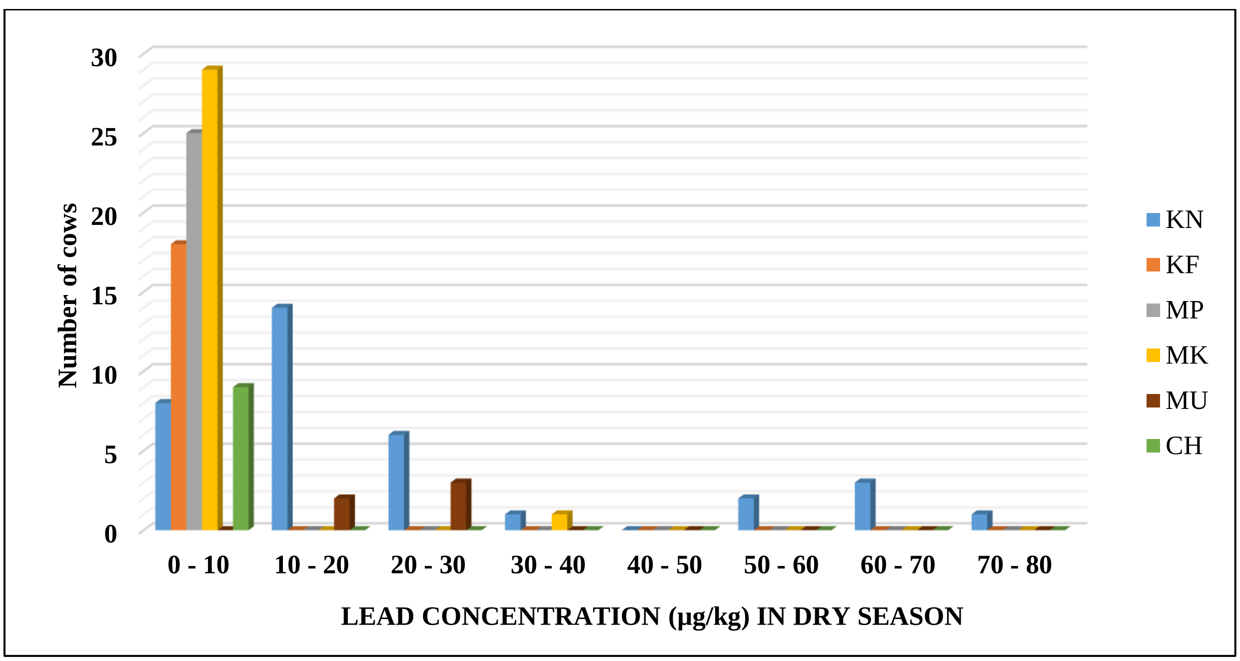


Figure 4.7: Clustered blood Pb concentration (µg/kg) comparisons per site (KN; Kang'omba, KF; Kafulamase, MP; Mpima, MK: Mukobeko, MU; Munga, CH; Chongwe) in dry season

4.3 Seasonal variations of Pb concentrations (µg/kg) in milk and Blood

The descriptive results of the seasonal variation of Pb concentration are presented in the whisker boxplots shown as Figure 4.8. The results indicate that the variability of Pb concentrations in both milk and blood were observed to be higher in the wet season than in the dry season (Figure 4.8). However, the interquartile range for both milk and blood in the dry season was observed to be higher during the dry compared to the wet season. The outlook of the Pb concentrations in the milk and blood is presented in Figure 4.8 below with significant differences per season ($p < 0.05$, DMCT).

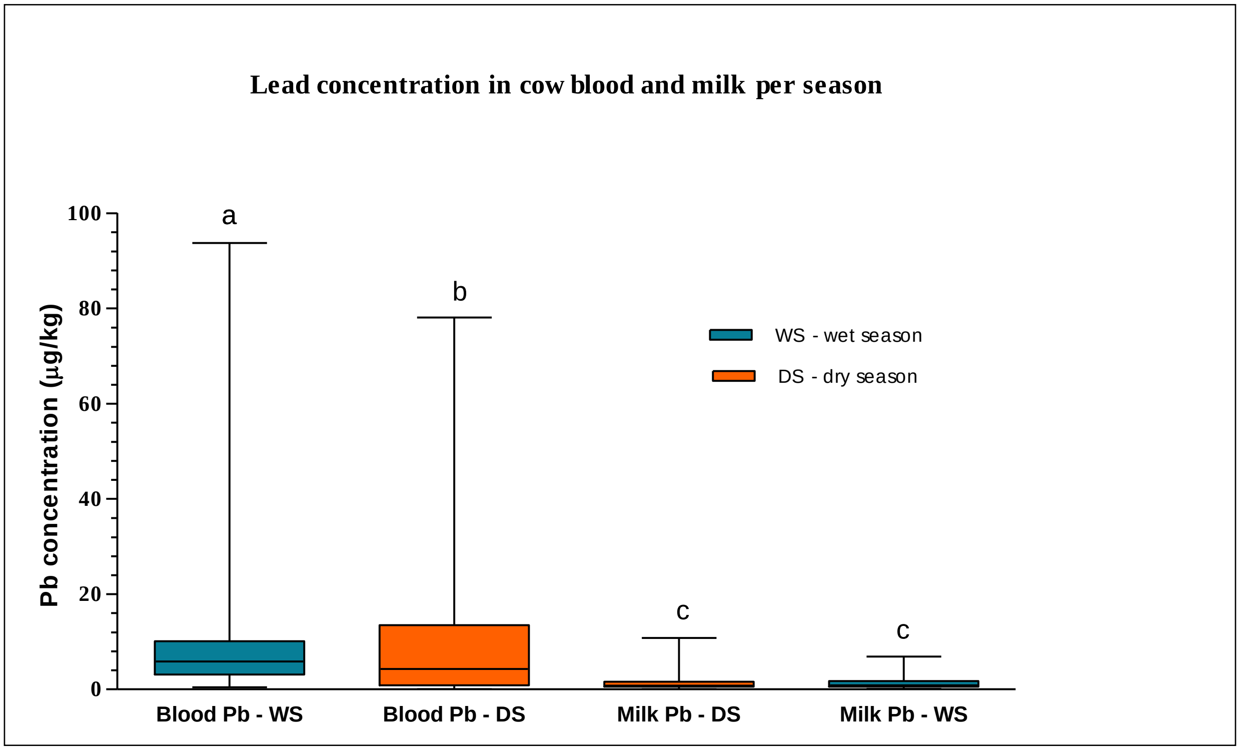


Figure 4.8: Seasonal variation of Pb concentrations (µg/kg) in cow milk and blood; paired letters 'a, b and c' represent significantly different ($p < 0.05$, DMCT).

A summary of results of box and whisker plot results illustrated above in Figure 4.8 are descriptively presented in Table 4.5 below.

Table 4.5: Descriptive statistics of the seasonal Pb concentration (µg/kg) variations in blood and milk per season

	Blood Pb Wet season	**Milk Pb Wet season**	**Blood Pb Dry season**	**Milk Pb Dry season**
Minimum	0.47	0.10	0.05	0.06
25% Percentile	3.11	0.60	0.86	0.59
Median	5.83	0.89	4.32	0.81
75% Percentile	10.1	1.78	13.5	1.59
Maximum	93.7	6.90	78.1	10.8
Mean	10.3	1.36	9.75	1.79
Std. Deviation	14.3	1.16	14.2	2.01
Std. Error	1.31	0.11	1.29	0.18
Lower 95% CI of mean	7.76	1.13	7.20	1.23
Upper 95% CI of mean	12.9	1.57	12.3	1.95

According to the results in Table 4.5, the results show that the 75% percentile of the blood samples recorded in the wet season had 10.1µg/kg of Pb concentration while in the dry season it was 13.5 µg/kg. However, the concentration of Pb in milk in the wet season was higher than in the dry season. Notably, 75% percentile of the milk samples had 1.78 µg/kg in the wet season compared to the dry season in which the results showed a slight decrease to about 1.59 µg/kg. The results of correlations in terms of r squared (RSQ) and r squared p-values (RSQ p-values) are presented in Table 4.6.

Table 4.6: Spearman RSQ correlation and RSQ p-values (in brackets) between milk and blood per season

	Milk Pb Wet season	**Blood Pb Wet season**	**Milk Pb Dry season**	**Blood Pb Dry season**
Milk Pb - Wet season	1.00			
Blood Pb - Wet season	0.07	1.00		
Milk Pb - Dry season	0.12	0.35 (9.01×10^{-5})	1.00	
Blood Pb - Dry season	0.057	0.55 (5.31×10^{-11})	0.50 (6.50×10^{-09})	1.00

- In brackets are p-values indicating significance difference (P<0.05, DMCT)

The results in Table 4. 6 show that there was a positive association ($p < 0.05$, DMCT) between Pb in blood and milk in the dry season while in the wet season, the correlation was not significant ($p > 0.05$, DMCT).

4.4 Human health risk assessment of Pb through consumption of cow milk from Kabwe

The human health risk assessment based on the determination of the CDI, THQ, and ILCR of Pb exposure through consumption of cow milk are presented in Tables 4**.**7**,** 4.8 and 4.9 are below.

4.3.1 Chronic daily intake of metal assessment

Table 4.7 shows the summary of the calculated CDI levels in both children and adults obtained for each season based on the average concentrations found in cow milk. In comparison with provisional tolerable daily intake set by FAO/WHO (JEFCA, 2010), the CDI values (Table 4.7) in children ranged from 5.10×10^{-7}- 4.98×10^{-6} while in adults, results ranged from 1.75×10^{-7}- 1.49×10^{-6} were below the permissible limits.

Table 4.7: Chronic daily intake (CDI) (µg/kg/day) of lead through consumption of milk in children and adults

	Wet season CDI CDI (µg/kg/day)		**Dry season CDI (µg/kg/day)**	
Region	**Children**	**Adult**	**Children**	**Adult**
Kang'omba	3.77×10^{-6}	6.66×10^{-7}	4.98×10^{-6}	8.79×10^{-7}
Kafulamase	8.33×10^{-7}	3.43×10^{-7}	1.46×10^{-6}	6.02×10^{-7}
Mpima	1.02×10^{-6}	4.20×10^{-7}	1.02×10^{-6}	4.20×10^{-7}
Mukobeko	9.44×10^{-7}	3.89×10^{-7}	4.25×10^{-7}	1.75×10^{-7}
Munga	1.67×10^{-6}	6.86×10^{-7}	3.60×10^{-6}	1.48×10^{-6}
Average	$\mathbf{8.24 \times 10^{-6}}$	$\mathbf{2.50 \times 10^{-6}}$	$\mathbf{1.15 \times 10^{-5}}$	$\mathbf{3.56 \times 10^{-6}}$

- 3 and 12.5 µg/kg-BW/day is recommended CDI for children and adults (Flannery et al., 2020)

The results in Table 4.7 show that the average CDI value in children both in the wet season and dry indicate higher values than in adults ($8.24 \times 10^{-6} > 2.50 \times 10^{-6}$ and $1.15 \times 10^{-5} > 3.56 \times 10^{-6}$) for the same period, respectively. The general trend showed that the results in the dry season had a high chronic exposure effect in both the children and adults. Further, results indicate that chronic exposure risk of Pb to children compared to adults in both the wet and dry seasons was three times higher than in adults (Figure 4.9).

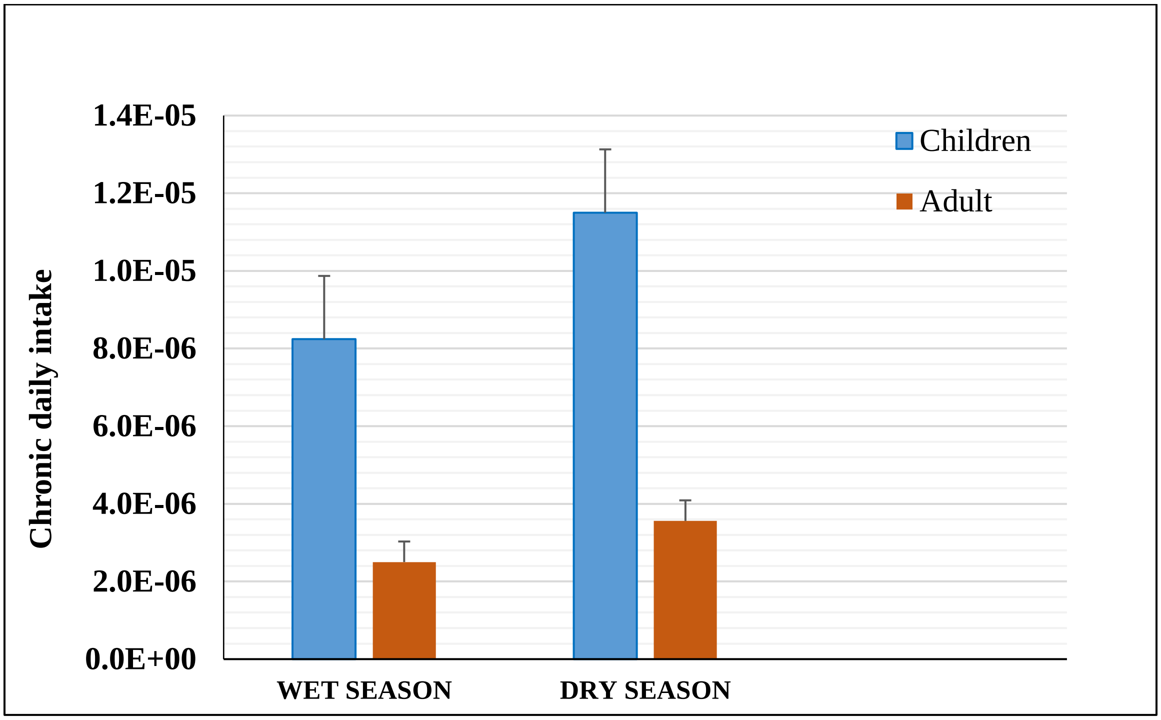


Figure 4.9: Analysis of the chronic daily intake (CDI) for children and adults per season

4.3.2 Non-cancer risk assessment

The calculated potential adverse effects results based on the THQ parameter from Pb in cow milk are presented in Table 4. 8. The average THQ values in children from wet to dry season were higher than in adults ranging from 2.36 x 10^{-3} to 3.28 x 10^{-3} while in adults values were seen to vary from 7.15 x 10^{-4} to 1.02 x 10^{-3} respectively.

Table 4.8: Target hazard quotient (THQ) of Pb through consumption of cow milk in children and adults

	Wet season THQ		**Dry season THQ**	
Region	Children	Adult	Children	Adult
Kang'omba	1.08×10^{-3}	1.90×10^{-4}	1.42×10^{-3}	2.51×10^{-4}
Kafulamase	2.38×10^{-4}	9.80×10^{-5}	4.18×10^{-4}	1.72×10^{-4}
Mpima	2.91×10^{-4}	1.20×10^{-4}	2.91×10^{-4}	1.20×10^{-4}
Mukobeko	2.70×10^{-4}	1.11×10^{-4}	1.21×10^{-4}	5.00×10^{-5}
Munga	4.76×10^{-4}	1.96×10^{-4}	1.03×10^{-3}	4.24×10^{-4}
Average	**2.36** $\times 10^{-3}$	**7.15** $\times 10^{-4}$	**3.28** $\times 10^{-3}$	**1.02** $\times 10^{-3}$

- THQ < 1 means that the sampled milk was safe for consumption (USEPA, 2010)

Further, the results in Table 4.8 show that 1.42 x 10^{-3} was the highest THQ value calculated in children for the milk samples collected during the dry season in Kang'omba, whereas, the highest value of 4.24 x 10^{-5} in adults was associated with the Munga region in the same period. All the values were < 1 indicating that the hazard exposure risk of Pb to humans via milk consumption was negligible.

Moreover, Figure 4.10 demonstrates that the average THQ was higher in children compared to adults. The dry season indicates higher average THQs in both children and adults.

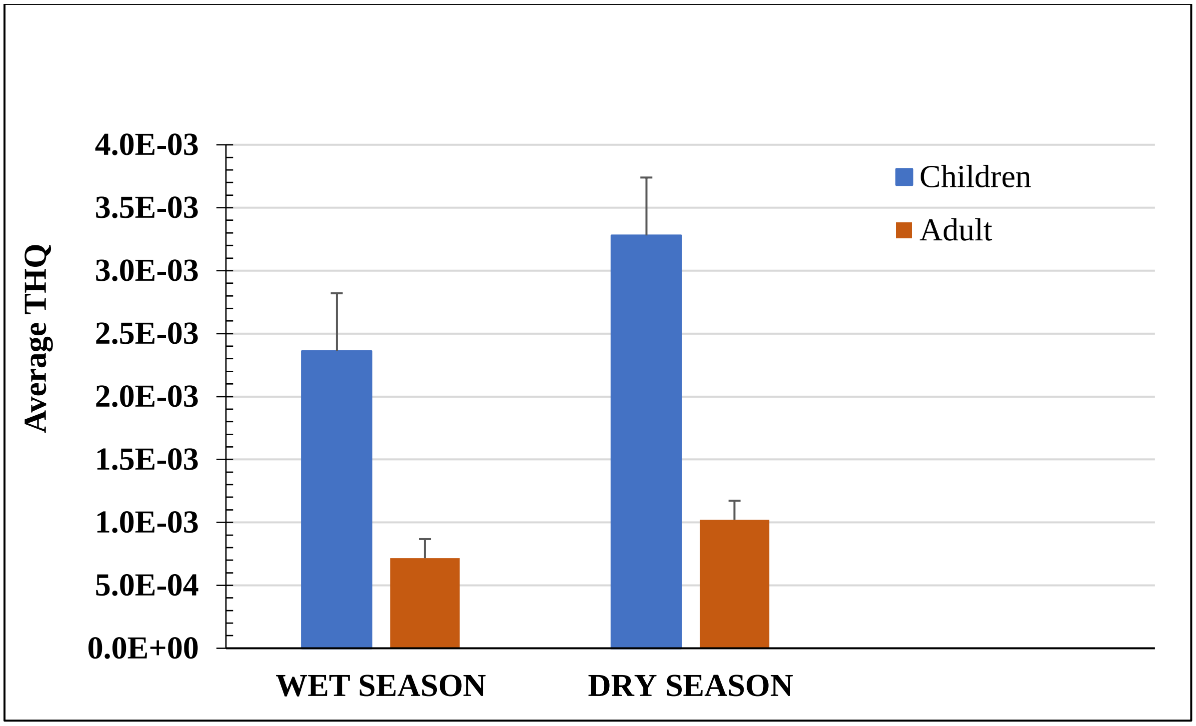


Figure 4.10: Average target hazard quotient (THQ) of Pb through consumption of cow milk in children and adults

4.3.3 Cancer risk assessment

Table 4.9 shows the cancer risk assessment results based on the ILCR calculations. The average ILCR values in children in both the wet season and dry ranged from 7.01 x 10^{-8} to 9.76 × 10^{-8} while in adults ranged from 2.13 x 10^{-8} to 3.03 x 10^{-8}.

Table 4.9: Incremental lifetime cancer risk (ILCR) of lead through consumption of cow milk in children and adults

	Wets season ILCR		**Dry season ILCR**	
Region	Children	Adult	Children	Adult
Kang'omba	3.21×10^{-8}	5.66×10^{-9}	4.23×10^{-8}	7.47×10^{-9}
Kafulamase	7.08×10^{-9}	2.92×10^{-9}	1.24×10^{-8}	5.12×10^{-9}
Mpima	8.67×10^{-9}	3.57×10^{-9}	8.67×10^{-9}	3.57×10^{-9}
Mukobeko	8.02×10^{-9}	3.30×10^{-9}	3.61×10^{-9}	1.49×10^{-9}
Munga	1.42×10^{-8}	5.83×10^{-9}	3.06×10^{-8}	1.26×10^{-8}
Average	**7.01** $\times 10^{-8}$	**2.13** $\times 10^{-8}$	**9.76** $\times 10^{-8}$	**3.03** $\times 10^{-8}$

- 10^{-6} to 10^{-4} is the ILCR acceptable (safe) reference range (USEPA, 2010)

The graphical information below in Figure 4.11 clearly illustrates that both children and adults were appreciably more prone to increased cancer risk effects in the dry season than in the wet season.

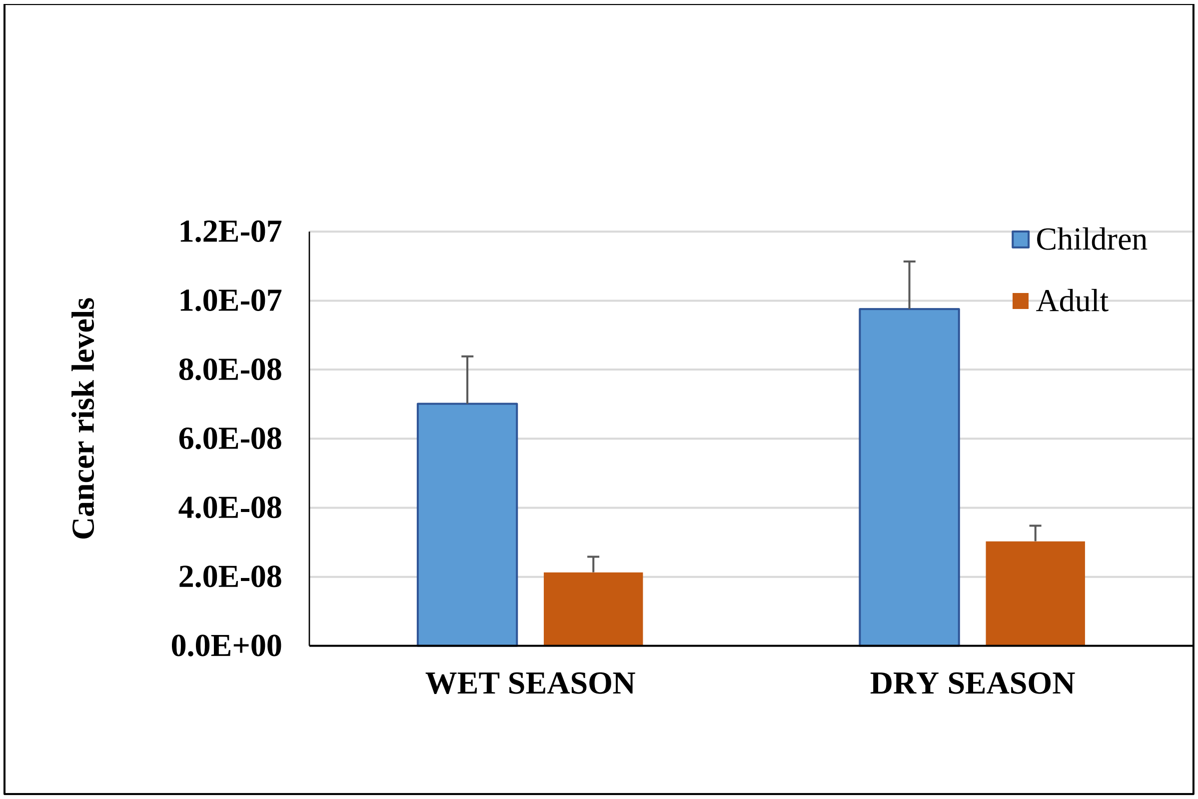


Figure 4.11: Average potential incremental life cancer risk (ILCR) due to consumption of cow milk in children and adults

As observed in Figures 4.9, 4.10 and 4.11 for the chronic daily intake, target hazard risk and the ILCR assessments respectively, results showed a similar trend with regard to the exposure risk level being higher in children compared to adults.

CHAPTER 5

5. DISCUSSION

5.1. Lead concentrations in cow milk and blood

5.1.1 Lead concentrations in cow milk per region in each season

The study analysed mean concentrations of Pb in milk from the studied areas in Kabwe and the reference site in Chongwe. The mean milk Pb concentrations in the wet season ranged from 0.98 ± 0.30 to 2.32 ± 1.87 µg/kg while in the dry season the mean concentrations varied from 0.50 ± 0.24 to 4.24 ± 2.24 µg/kg. The presence of Pb in cow milk samples suggest that the farms in the studied areas around the Pb-Zn mine in Kabwe were contaminated with Pb. However, the mean concentrations detected were below the Codex Alimentarius permissible level of 20 µg/kg of Pb set for human consumption in cow milk by FAO/WHO (2011). Additionally, the mean Pb concentrations did not exceed the maximum permissible limit for milk established by the European Union Commission of 100 µg/kg (EFSA, 2012).

In contrast, authors in Romania (Miclean et al., 2019), India (Kumar, 2019), Iran (Sobhanardakani, 2018) and Kazakhstan (Sarsembayeva et al., 2020) reported higher Pb concentration levels of 24 ± 15 µg/kg , 32.83 ± 20.80 µg/kg, and 11.6 ± 10 µg/kg in cow milk, respectively. Additionally, higher Pb concentration levels than those found in the current study have been reported in previous studies on cow milk by Elsaim and Ali (2018) in Sudan (below detectable limit) but within the range that was found in China (3.6 ± 2.3 µg/kg) by Wang et al. (2019).

Generally, the mean Pb metal content in cow milk in the current study (Table 4.3) was observed to be in the decreasing order of Kang'omba>Munga>Mpima>Mukobeko>Kafulamase during the wet season while in the dry season the mean content of Pb in milk was found to be in the decreased order of Munga> Kang'omba> Kafulamase> Mpima> Mukobeko. The highest Pb concentration in milk near the mine was observed in the dry season in Munga while the lowest Pb amount was recorded in the dry season Mukobeko, a place located further away from the point source (Figure 4.2**)**. The low mean concentration in Mukobeko was attributed to low environmental pollution because of extended distance from the Pb-Zn mine. It was observed that during the wet season, animals usually did not roam far away from their resident farmlands for pasture and water. Most often however, animals in dry season walked long distances in search of grass and water, increasing the probability of their Pb exposure through inhalation of contaminated dust depending on the distance and location of the farm from the Pb-Zn mine.

5.1.2 Lead concentrations in cow blood per region in each season

Lead poisoning is reported to be common in farm ruminants (Waldner et al., 2002). Blood is the most frequently used sample for monitoring the Pb status in cattle because it is a good indicator of recent Pb exposure (Rumbeiha et al., 2001). High incidences of Pb ingestion in cattle are associated with their natural curiosity and their habits of licking and indiscriminate eating (Sharpe and Livesey, 2006). Recently, Pb poisoning is both a food safety issue and an important cause of economic loss in beef and dairy herds (Waldner et al., 2002). According to Yakub and Iqbal, 2010, the half-life of Pb is approximately 30 days in which blood Pb is expected to return to background levels in asymptomatic animals. Thus, animals that are reared in Pb polluted environment should be screened for lead poisoning before they are slaughtered for the market for food safety reasons.

Mean Pb concentrations in the current study decreased in the order of Munga > Kang'omba > Mukobeko > Mpima > Kafulamase in the wet season while in the dry season the order was Kang'omba > Munga > Mukobeko > Kafulamase > Mpima. Contaminated pasture close to the mine in the dry period and the particulate matter influenced by the south-easterly winds could have caused high Pb levels in Kang'omba. The observed Pb concentrations in Kafulamase could be attributed to the effluents from the mine site and discharge of the municipal contaminated waste causing the elevation of Pb concentrations in the stream and other surface water drinking points for cows. Also, the stream overflows during the rainy season could have caused increased pollution of the pasture in the area.

Most of the animals in the current study recorded BLLs ranging between 0-10 µg/kg. On the other hand, the Mukobeko region had the highest number with over 25 cows recording between 0-10 µg/kg blood concentrations. Generally, most of the regions had a few animals exceeding the range of 10-15 µg/kg blood lead concentrations both in the wet and dry seasons. According to the literature, the Pb concentration baseline levels range from 20-60 µg/kg (Roggeman et al., 2013). Of all the samples analysed, no blood Pb concentrations exceeded the toxicosis value of 350 µg/kg for cattle (Checkley et al., 2002). Data in (Figure 4.5) show that 27% and 37% of blood Pb samples in Kang'omba in the wet and dry seasons, respectively exceeded 20 µg/kg of concentration while in Munga 60% were found to be above this value in the same seasons. Mukobeko had the least percentages of cows recording 0.04 and 0.03 % (1 cow each season), above 20 µg/kg baseline level in both the wet and dry seasons.

Previously, the occurrence of Pb in cattle was detected in the peripheral blood from Kabwe and Lusaka although the levels established in Kabwe were remarkably greater than those found in Lusaka, the control area (Ikenaka et al., 2012). According to Ikenaka et al. (2012), the average Pb concentration in cattle blood from the Kabwe area was 90600 ± 67600 µg/kg weight, which is much higher than the mean average value found in the current study.

Despite being a non-mining area, Pb was detected in Chongwe (reference site) ranging from 0.47-3.91 ± 1.11 µg/kg. Further, the source of the Pb found in Chongwe was unknown. These results obtained in Chongwe in the present study are in line with the results found by Yabe et al. (2012), in which they established the presence of Pb measuring 61.0 µg/kg in cattle offal in agricultural areas of Lusaka province. The presence of Pb in the food chain resulting from environmental pollution (Elatrash and Atoweir, 2014) is an important food safety issue (de Oliveira, 2017), thus investigation of the source of Pb in Chongwe is necessary.

5.1.3 Lead concentration levels in cow milk and blood

Numerous studies have documented the analysis of Pb in both cow milk and blood. Several authors have found that Pb in blood is higher than in milk (Patra et al., 2008). Aslani et al. (2012) found high Pb levels in the blood than in milk samples varying from 250-590 and 60-290 µg/kg in a herd of Holsten cattle (n = 9) that was affected by Pb poisoning associated with residuals of battery recycling. Also, values of 80 ± 40 µg/kg and 360 ± 40 µg/kg of blood and milk Pb respectively, were found earlier in a study by Oskarsson et al. (1992) in Sweden after animals were accidentally exposed to Pb. Moreover, Tahir et al. (2017) reported Pb concentration variations in blood ranging from 1382 to 2979 µg/kg and 300–800 µg/kg in milk samples that were collected quarterly from the cows for a period of one year. In the present investigation of Pb concentrations in blood and milk, the results were consistent with the literature (Aslani et al., 2012; Tahir et al., 2017).

Similarly, in human milk, the median Pb concentration in Mexico was found to be 300.0 µg/kg, varying from 1000.0 to 8000.0 µg/kg while the median of Pb in blood was 27,000 µg/kg varying from 10000 to 55000 µg/kg (Koyashiki et al., 2010). Recently, Toyomaki et al. (2021), found a different scenario in lactating mothers in Kabwe, as the mean Pb concentration in blood (113 µg/kg) was lower than the concentration in breastmilk (5.3 µg/kg). Further, Toyomaki et al., 2021 reported that breastmilk could be one of the sources of Pb exposure in infants in Kabwe because the levels established in milk were higher than the WHO recommended range of 20-50 µg/kg. Compared to the Pb concentrations in human milk which were found to exceed the permissible levels (Toyomaki et al., 2021), Pb concentrations in the present study were below the WHO safe limits destined for human consumption in cow milk. Although the levels of Pb in the present study could be low, chronic exposure to low concentrations of Pb may cause adverse health effects in children.

Previously in animal studies, Yabe et al. (2011) reported that cattle reared on the farms in the vicinity of the Pb-Zn mine in Kabwe accumulated high levels of toxic metals such as Pb and Cd. The findings by Yabe et al. (2011) were consistent with the earlier report by Tembo et al. (2006) that revealed elevated concentrations of Pb and Cd in soil samples from the farms near the Pb-Zn mine. Moreover, Yabe et al. (2011) established using the cluster analysis method that the Pb contamination in animal tissues was undoubtedly from the point source, the Pb-Zn mine.

Descriptive data for blood Pb analysis for the current study show that although the Kafulamse region was remote on the eastern side of the Pb-Zn mine, the mean blood Pb concentration measured in the dry season was higher than the mean Pb level found in Mpima, despite it being closer to the pollution source. The concentrations variations could be attributed to the source of drinking water for the animals in the dry season in Mpima from the boreholes in contrast to dam and the shallow water wells in Munga and Kang'omba. Additionally, Munga and Kang'omba are located in the western and southern parts of the mine, respectively, which are mostly affected by the contaminated dust emanating from the mine. Munga and Kang'omba are subject to high environmental Pb pollution due to the proximity to the mine compared to other studied regions in Kabwe. As for the Kafulamase region, animals largely depended on the water downstream that also flows from the Pb-Zn mine drainage upstream.

According to the EPA (1989), surface water bodies are normally subject to contamination from many sources such as pesticide runoff, storm water, waste water discharges, acid mine drainage, etc. Differential hydrology induces variable heavy metal speciation and mobility in Pb-Zn mine tailings (Kovács et al., 2006). Moreover, according to the Kabwe Municipal Council Report, of 2021, it is stated that Kabwe is situated east of the main watershed; thus, the influence of hydrological factors in the distribution of Pb from the tailings ponds in the neighbouring communities cannot be doubted. The larger eastern part of the surface water in Kabwe drains towards the east while the smaller western part flows towards the Lukanga swamps (Kabwe Municipal Council, 2021). Therefore, the present study has demonstrated that Kafulamase, which is a region situated far away from the point source of pollution compared to the other sampled sites has a high potential impact of Pb contaminant on livestock mostly during the dry season because it sits along with the drainage system.

Tembo et al. (2006) previously reported that both distance and direction from the source of pollution are significant factors affecting Pb accumulation with regard to the dust dispersal in the environment. In the subsequent studies, other authors confirmed a similar concentration Pb pattern. For example, Yabe et al. (2020) in a study on human BLLs indicated that Pb exposure differences in Kabwe were attributed to distance and direction from the mine, with younger children at the highest risk.

Similarly, a study by Toyomaki et al. (2020), demonstrated that Pb concentrations in the dogs significantly decreased with increasing distance between the mine and the location of the dogs. Also, Doya et al. (2020) confirmed that Pb concentrations in lizards (Trachylepis Wahlberg) living in bare fields were higher than expected based on distance from the contaminant source. Recently, Kataba et al. (2021) analysed Pb in the crown incisors of Pb-exposed wild rats (Rattus rattus) from residential sites within varying distances from an abandoned lead-zinc mine.

Accordingly, the Pb-Teeth accumulation data in wild rats were found to decrease with increased distance away from the Pb–Zn mine Kataba et al. (2021). Mainly, the prevailing winds in Kabwe are easterly, southeasterly, and northeasterly (Tembo et al., 2006). In the current study, the farms that are in proximity to the mine namely, Kang'omba and Munga areas showed high mean levels of Pb with 23.8 ± 18.6 and 21.8 ± 15.9 µg/kg in the blood, respectively. Interestingly, the Pb concentration patterns in the milk and blood were observed to be consistent with those previously found by other authors, suggesting that distance and the direction of the wind were significant factors in influencing Pb pollution levels from the point source to the nearby farms. In addition, animals grazing in pastures bordering highways have higher background Pb concentrations resulting from the accumulated Pb in the environment where leaded gasoline was previously used (Checkley et al., 2002).

Studies have shown that elevated temperatures, solar radiation, humidity, and anthropogenic pollution seasonally result in increased acidification (low pH) in air, water and soil. Because of increased bioavailability and mobility of Pb due to environmental acidification, Pb exposure in humans, animals, and plants may significantly be high (Levin et al., 2020). Besides heavy metals such as Pb residues, increases during the summer season due to water evaporation leaves behind relatively higher concentrations (Aslam et al., 2011). The high mean Pb levels detected in milk and blood from exposed cows in the dry season could probably be due to the high intake of high Pb concentrations in water due to seasonal factors mentioned above.

Animals grazing in the contaminated pasture have elevated BLLs and consequentially, excrete high levels of Pb in milk (Ogundiran et al., 2012). In general, the presence of Pb in all the sampled sites is indicative of the extent of exposure of Pb contaminant in the food-producing animal

5.2 Seasonal Pb concentration variations in cow milk and blood in each season

The study revealed that Pb concentrations in milk were significantly lower than in the blood. Further, it was observed that the mean Pb concentrations in the milk were appreciably higher in the dry season compared to the results obtained in the wet season. Similarly, although the Pb concentrations in the cow blood showed higher variability in the wet season (0.47-93.7

μg/kg) than in the dry season (0.05-78.1 μg/kg), the interquartile range of the Pb concentrations were higher in the dry season than in the wet season. A further scrutiny of data showed that the 75 per cent percentile of the blood samples recorded 10.1μg/kg of Pb concentration in the wet season while in the dry season it was 13.5 μg/kg. Hence, this phenomenon indicates that more cows were exposed to Pb in the dry season compared to the wet season. Thus, the results in present study confirm that the concentration variations of Pb in milk and blood were affected by seasonal changes. It is also known that blood Pb reflects recent exposure with a half-life of approximately 30 days (Yakub and Iqbal, 2010) while Pb in milk is influenced by several factors including the physiology of the mammary gland (Kumar, 2019) and lactation stage (Oskarsson et al., 1992). According to the studies by Oskarsson et al. (1992), Pb levels in milk decreased rapidly after delivery, a clear confirmation that Pb levels in colostrum are higher than in the subsequent milk.

5.3 Correlation between blood and milk in each season

A correlation between milk and blood Pb content was observed (Table 4.6). A closer look at the data shows that there were significant differences ($P<0.05$, DMCT) in the Pb levels in blood and milk of the lactating cows. Most evident, the significant differences were seen between the milk Pb in dry season and blood Pb in dry season; milk Pb in dry season and blood Pb in wet season; blood Pb in dry season and blood Pb in wet season. Although the association was positive, the study results showed a relatively weak dependence of milk Pb concentration level on blood Pb concentration. However, data showed that the higher the blood Pb concentration the higher the Pb level excreted in the milk. On the other hand, there was no significant difference observed for the Pb in cow milk in the wet and dry seasons. Since the results were non-significantly different, it indicates that the change of the dry season to wet season or vice versa had no effect on the Pb concentration in milk despite the Pb in blood sample concentrations being significantly different.

5.4 Human health risk assessment of lead exposure through milk consumption

Data on Pb concentrations in lactating cows are important for evaluating the extent of the metal pollutant on cattle, as well as the potential risk posed to humans from consumption of the milk from Pb exposed animals.

5.4.1 Chronic daily intake of metal

Chronic daily intake of Pb in cow milk represents the lifetime average daily dose of exposure to a chemical (Kasozi et al., 2018) in this case Pb. Chronic Pb poisoning arises from prolonged exposure to lower doses of Pb metal in the environment (Sharpe and Livesey, 2006). Ingestion accounts for a larger percentage (> 90 %) of the exposure effects compared to inhalation and dermal contact exposure pathways (Loutfy et al., 2006). Due to the scarcity of updated data on the consumption rates of milk in Zambia, the calculated results in the present study were based on the average daily intake values of 21 and 17 g/day/person in children and adults, respectively. Accordingly, the consumption intake limits and risk levels were calculated on the assumption of body weights of 10 kg for children and 70 kg for adults (Haakonde et al., 2021; Zambia, Milk Consumption 1992–2007–Knoema.Com, n.d). The average chronic exposure levels obtained in the present study indicate that children had higher exposure levels than adults. Because children are at a greater risk of chronic Pb exposure and poisoning than adults due to their higher CDI levels in relation to their lower body weight (Abedi et al., 2020; Sharpe & Livesey, 2006), milk should be evaluated regularly for food safety reasons. On the other hand, it was observed CDI trends regionally showed a decreasing order of Kang'omba > Munga > Kafulamase > Mpima > Chongwe > Mukobeko in the dry season.

According to the published information (JEFCA, 2010), there is no established tolerable Pb intake, for which Pb cannot cause adverse effects. Despite this alarming revelation, the U.S. Food and Drug Administration (FDA) recommends interim reference levels of 3 and 12.5 µg/day for children and adults (women of childbearing age), respectively which correspond to the BLL of 0.5 µg/dL for a general population (Flannery et al., 2020). Exposure levels in the present study did not exceed the interim reference benchmarks recommended by FDA (Flannery et al., 2020). On the contrary, Salah et al. (2013) and Meshref et al. (2014) reported higher CDI values

of 158.5 and 1.7 x 10^{-4} µg/kg/day respectively, compared to the levels found in the present study. Moreover, much higher values several folds higher than the findings of the current study ranging from 0.069-0.946 in both children and adult milk consumers were reported by Ismail et al. (2017). However, Muhib et al. (2016) and Norouzirad et al. (2018) reported CDI values of 5.4 x 10^{-6}, and 3.4 x 10^{-5} similar to the results of the current study.
Although the chronic Pb exposure levels found were lower than is recommended by the FDA in cow milk, Pb contamination in milk could probably pose a potential health risk due to other complimentary dietary and non-dietary factors to the consumers.

5.4.2 Non-cancer risk assessment

Non-cancer risk assessment, expressed as THQ, is one of the methods that has previously been used by numerous authors to estimate lifetime exposure to Pb metal through the milk diet (Kasozi et al., 2018; Zhang et al., 2019; Abedi et al., 2020; Haakonde et al., 2021; Salazar-Flores et al., 2019) using equation 2. THQ < 1 is an indicative safe limit to non-carcinogenic risk effects (USEPA, 2010). The THQ values of Pb in all sampled sites per season varied from 1.08 x 10^{-3} to 4.76 x 10^{-3} in children and 5.00 x 10^{-5} to 1.11 x 10^{-4} in adults in the wet season. On the other hand, the values ranged from 1.21 x 10^{-4} to 1.42 x 10^{-3} and 5.00 x 10^{-5} to 4.24 x 10^{-4} in the dry season for children and adults respectively. The findings in the present study were lower than the results reported in Iran by Abedi et al. (2020) ranging from 0.009 to 0.032, and those found in Mexico by Gonzalez et al. (2017) between 0.039 to 0.059. Similarly, higher THQ values than the values obtained in the current study were reported in Europe ranging from 7.0 x 10^{-3} to 4.9 x 10^{-2} (Miclean et al., 2019). Although these values from the reported regions were slightly higher than findings in the current study, they were also found within the safe limit of THQ>1. However, in Uganda, Kasozi et al. (2018) recorded far much higher values in children and adults ranging from 6.2648 and 2.116 respectively.

Although the THQ for children and adults were less than 1 in the current study, it was observed that the THQs in children in the dry season were higher than in the wet season compared to adults, implying a higher impact of non-carcinogenic effect in children than in adults. Therefore, investigated Pb contamination in cow milk and its health risk impact on humans through consumption of milk in Kabwe suggest that the non-cancer risk effects of Pb exposure in both in both children and adult populations were negligible.

5.4.3 Carcinogenic risk assessment

The ILCRs caused by the ingestion of Pb in cow milk obtained in the present study for both children and adults on average ranged from 7.01×10^{-8} to 9.76×10^{-8} and 2.13×10^{-8} to 3.03×10^{-8}, respectively. Comparatively, the ILCR results obtained in the study were far lower than the results reported by Kasozi et al. (2018) in Uganda as 8.37×10^{-4}, and 2.83×10^{-4} in children and adults, respectively. In addition, Abedi et al. (2020) in Iran reported higher values of ILCR ranging from 1.89×10^{-3} to 2.45×10^{-3} and 2.96×10^{-4} to 3.85×10^{-4}, respectively compared to the results in the current study. Incremental lifetime cancer values are deemed significant if they exceed the USEPA acceptable risk range of 1.0×10^{-6} to 1.0×10^{-4} (1 in 1,000,000 to 1 in 10,000) (Proshad et al., 2019).

The ILCR results in the present study were far lower than the reference range given by the international standards, implying that carcinogenic risk effects in the Kabwe residents were insignificant. It is worth noting that milk produced in Kabwe is delivered to the milking collection centre in Mpima area where it is mixed with other milk from other farms from distant places in the district. The present study revealed that milk samples measured from individual cows and between farms showed high Pb variability. However, any possible contaminants in the milk from individual cows are not likely to increase the concentration in the final volume appreciably (Bertha, 2007). Thus, milk from the surrounding farms in Kabwe could even be safer to consume after it has been mixed with other milk supplies from other farms in non-lead exposed areas after processing.

Although the findings presented in this study indicate that consumption of cow milk did not constitute a health hazard to consumers in the residents of Kabwe, the results should be considered with caution because the study was subject to a few limitations. These limitations included a sampling study that was conveniently restricted to cattle reared on free-range practices, excluding the animals confined in the commercial farms with different Pb exposure factors. In addition, the study encountered a challenge with non-commercial farmers who only reared small herds of mixed-breed cattle. For example, a small number of the animal population in the Munga region incidentally determined the sample size, which ultimately had a bearing effect on the statistical data analysis. Further, the study did not take into account the similarities in milk yield, age and lactation stage of the sampled subjects (Pilarczyk et al., 2013). Moreover, a shorter lactation period among traditional cow breeds compared to dairy cows (Neven et al. 2006), as well as the outbreak of foot and mouth disease during the sampling period, made the follow-up sampling study design a great challenge.

CHAPTER 6

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study results indicate that Pb metal was present in cow milk and blood samples from all studied sites. The Pb levels in the samples analysed showed different concentration patterns according to season, distance, and location of the farms from the Pb–Zn mine within the perimeter distance of 25 kilometres. Further, the findings evidently showed that the regions that were located along the wind flow direction particularly, on the southern part of the mine in Kang'omba area and Munga on the west exhibited high Pb concentration levels. As such, about 37% of samples from cows in Kang'omba and 60% in Munga exceeded the baseline level of 20 µg/kg of Pb concentration in cows. Moreover, the change of season from dry to wet season induced significant differences in milk and BLLs, with blood samples generally showing remarkably higher Pb content compared to milk samples in all the studied regions.

Although Pb was detected in all selected regions in our present study in Kabwe, the concentrations measured in cow milk were below the safety standard threshold of 20 µg/kg set by Codex Alimentarius Commission for human consumption. Further, due to low Pb concentrations found in cow milk, the health risk assessment of Pb based on the CDI, THQ, and ILCR parameters indicated that adverse health effects of Pb exposure in milk were not likely to be significant. However, although the detected Pb concentrations in cow milk were remarkably low, sites influenced by discharge from the mine through either wind or drainage water, had high levels of Pb compared to the sites that were not affected by such factors. Therefore, the prolonged intake of the milk may probably cause adverse health effects to humans, especially children whose exposure levels were found to be appreciably higher than in adults in the study.

6.2 Recommendations

The average consumption rates of milk used in tandem with the body weights of the children and adults in the current study were literature-based values, therefore, a field broad-based questionnaire survey is recommended to estimate accurate average values of intake rates of cow milk in the studied regions. Although the established Pb levels in cow milk from Kabwe were remarkably low to constitute a health hazard to consumers, regular monitoring and evaluation are necessary because Pb even in low-dose exposure can cause adverse health effects to humans.

Considering that Pb is an accumulative and persistent metal and that its threshold at which it cannot cause health complications has not been identified especially for children's health, risk assessment from time to time is recommended. Furthermore, evaluation of a wide range of other heavy metals of toxicological interest in cow milk in in future studies e.g., Cd, is necessary for the determination of the combined toxic metal effects in the residents of Kabwe.

Finally, for food safety reasons, the presence of Pb in a non-mining area of Chongwe, which in the present study was mapped as a reference site, should further be investigated since its source in the cow milk and blood samples analysed was unknown. Since Pb has a wide natural availability and use, it would be prudent to further study factors that affect its solubility and bioavailability in both animals and humans.

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Appendices

Appendix 1: Geographical sampling locations

S/N	Region	Sampling Area	Sample ID	Latitude	Longitude
1	Kabwe	Kang'omba	B	14° 29' 25.73"S	28° 23' 23.93"E
3	Kabwe	Kang'omba	D	14° 29' 45.20"S	28° 23' 21.37"E
2	Kabwe	Kang'omba	F	14° 27' 55.48"S	28° 24' 27.83"E
4	Kabwe	Kang'omba	G	14° 28' 44.40"S	28° 24' 54.99"E
5	Kabwe	Kang'omba	H	14° 28' 39.22"S	28° 24' 05.72"E
6	Kabwe	Mpima	I	14° 22' 09.73"S	28° 34' 04.15"E
7	Kabwe	Mpima	J	14° 22' 26.40"S	28° 33' 32.40"E
8	Kabwe	Mpima	K	14° 22' 48.22"S	28° 33' 45.93"E
9	Kabwe	Mpima	L	14° 22' 58.08"S	28° 30' 25.85"E
10	Kabwe	Mpima	M	14° 33' 35.96"S	28° 30' 21.85"E
11	Kabwe	Kafulamase	N	14° 33' 35.96"S	28° 30' 36.50"E
12	Kabwe	Kafulamase	O	14° 33' 35.10"S	28° 31' 21.86"E
13	Kabwe	Kafulamase	P	14° 33' 39.60"S	28° 30' 08.96"E
14	Kabwe	Mukobeko	Q	14° 23' 36.20"S	28° 24' 45.47"E
15	Kabwe	Mukobeko	R	14° 23' 27.92"S	28° 24' 56.63"E
16	Kabwe	Mukobeko	S	14° 23' 17.88"S	28° 24' 56.63"E
17	Kabwe	Mukobeko	T	14° 23' 37.46"S	28° 24' 51.41"E
18	Kabwe	Munga	U	14° 26' 57.16"S	28° 22' 21.14"E
19	Kabwe	Munga	V	14° 26' 57.30"S	28° 22' 20.42"E
20	Kabwe	Munga	X	14° 27' 33.66"S	28° 20' 58.23"E
21	Kabwe	Munga	Y	14° 23' 36.85"S	28° 24' 18.18"E
22	Chongwe	Kanakantapa	W	15° 14' 33.01"S	28° 37' 43.01"E
23	Chongwe	Kanakantapa	Z	15° 14' 38.62"S	28° 37' 41.44"E
24	Chongwe	Kanakantapa	ZZ	15° 15' 40.88"S	28° 37' 06.72"E

Appendix 2: Participant Information Sheet

THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
DEPARTMENT OF BIOMEDICAL SCIENCES

Informed Consent Form for: ____

Name of Principle Investigator: Golden Zyambo

Name of the Organization: University of Zambia

Name of Sponsor: KAMAPA/JICA Project and UNZA

This letter is an invitation to consider participating in a study that I am conducting as part of my Master's degree in Toxicology at the University of Zambia under the supervision of Dr. John Yabe. I would like to provide you with more information about this study and your involvement would entail if you decide to take part.

Cow milk is a very important source of food value because it contains essential minerals and vitamins that are required by the body for the growth, development, and long-term health of infants and children. It is also recommended for the aged and the sick for the revitalization of their health conditions. The nutrition of the milk, however, is altered by the presence of the heavy metal pollutants in the food chain of animals that graze freely on open fields near the contaminated mining areas and smelters. Among the heavy metals, Pb is the most frequently evaluated heavy metal due to its high persistence in the environment and its highly toxic effect on both humans and animals. It is important to acknowledge that the diagnosis of lead in non-symptomatic poisoned animals is often omitted. This is because the diagnostic procedures involved are costly and the equipment used in the determination of the heavy metal concentrations is sophisticated. In addition, for animals that may show signs of being unwell due to undiagnosed lead-related conditions, farmers are unable to correctly give the right treatment. Additionally, lack of knowledge about the Pb levels in production animals is a major source of concern because milk contaminated with Pb for example may have an adverse effect on the consumers. Although lead studies have become an important phenomenon in Kabwe, the amount of lead in cow milk has not been evaluated; also, the human health risks associated with the consumption of the milk produced in the region have not been determined.

The purpose of this study, therefore, is to evaluate the levels of Pb seasonally in lactating cows reared by smallholder farmers located in the surrounding areas of Kabwe town. The primary aim of the study is to assess the quality of milk for human consumption for health risks associated with lead contamination.

The focus of the study will be in five regions of Kabwe namely, Kang'omba, Kafulamse, Mpima, Mukobeko, and Munga while Chongwe district, will be considered as a reference sampling area.

Numerous studies indicate that Pb levels in lactating cows can easily be monitored using milk while the total Pb burden in animals can be evaluated using blood as a biomarker. Lead can also be released in urine and stool in animals that may be exposed to it and because such samples can easily be collected, lead pollution can easily be quantified and monitored.

To meet the objectives of the study, the following samples from your farm would be required to be collected:

1. 10 ml of milk
2. 6 ml of blood
3. 10 ml of urine
4. 50 g stool
5. 50 g Soil
6. 50 ml of water

In addition, for mapping and easy identification location purposes, the Global Position System (GPS) numbers will be recorded for your farm. The sampling cycles in the selected farms will be conducted in October, February, and June. Your participation in this study is voluntary and you can terminate your involvement at any time.

Your participation in the study will help bridge up the knowledge gap in Pb pollution studies especially in human health assessment, which is key to this research. All the information you will provide is considered completely confidential. Your name will not appear in any thesis or report resulting from this study, however, with your permission anonymous quotations may be used. According to research ethics, the data collected in this study will be kept for 2 years in a lockable cabinet or on a password-protected computer, and then confidentially destroyed or deleted. Only researchers associated with this project will have access to the data with personal access details.

In this study, you will not be given any incentive due to its academic nature. However, you will be given a final report with recommendations as a form of gratitude for your time and involvement. Before the collection of data and samples, you will be asked to sign a consent form confirming that you understand the information presented in this information sheet.

If you have any questions regarding this study or would like to have additional information to assist you in deciding on participation, please call me on +260 97 887872. Alternatively, you can email me on goldzgambo@gmail.com or you can also contact my supervisor, Dr. John Yabe on +260973258703 or email him on mjyabe@yahoo.co.uk.

I would like to assure you that this study has been reviewed and received ethics clearance through the Directorate of Research and Graduate Studies (DRGS) at the University of Zambia. If you have any reservations or concerns resulting from your participation in this study, please contact the office of the DRGS on +260 – 211-290258/293937.

Golden Zyambo (Principal Investigator)
Master's Candidate
Department of Biomedical Sciences
School of Veterinary Medicine
University of Zambia
Cell N. +260 0977887278/WhatsApp line: +260977887278
E-mail: goldzgambo@gmail.com

Appendix 3: Certificate of Informed Owner Consent

Lead contamination and human health risk assessment through consumption of cow milk in Kabwe, Zambia

==

1. Purpose of the project: The current study was designed to assess the human health risks associated with the consumption of cow milk as one of the high potential sources of exposure to lead (Pb). Smallholder farmers living around the peri-urban areas of Kabwe town will be the key focus of the study. Farmers selected to participate in this study will be asked to provide lactating cows for seasonal sampling during October – November, February – March, and June - July months. The cow milk will be analysed only for Pb levels. Blood, urine, and stool will also be collected alongside the milk samples for Pb concentration comparison purposes. Additional to these samples for Pb) analysis, soil and water samples will be collected from each farm. The residual concentration of Pb that will be determined in cow milk will serve as an important direct indicator of the hygienic status of the milk, as well as an indirect indicator of the extent of environmental Pb pollution in which the milk is produced. This is because animals that graze freely on open fields are considered as bio-indicators of environmental pollution.

2. Eligibility for participation: The cow must have calved down after two weeks or more and just a possibility of an extended lactation period of 8 months for the planned sampling cycles to be achieved.

3. Expected duration of participation: It is expected that 15 minutes will be required to physically examine the cows by the veterinary doctor/veterinary assistants and to provide the details of each selected animal as well as the details of the farm owner. An additional 1 hour 15 minutes will be required to collect samples from at least 10 animals.

4. Description of Procedure: Approximately 10 ml of cow milk (2 teaspoons) will manually be expressed directly into the sterile 15 ml tube and about 6 ml of blood will be obtained from a jugular/tail vein using a needle and heparinized vacutainers from each randomly selected animal.

5. Possible discomforts and risks: The collection of blood from a jugular or tail vein can Pb to transient pain, swelling, bruising, or in rare cases, infection.

6. Possible benefits of study: There are no direct benefits to participating cows or owners except that the study will provide the lead diagnosis in few representatives sampled animals which often not is done by farmers even the farm (s) may be located in a highly contaminated area due to high costs of metal analysis.

7. Confidentiality records/data: Research records will be kept confidential to the extent possible. No disclosure of information shall be made that would identify you, your animals, or farm when reporting or publishing the data arising from this study unless you provide written authorization to do so.

8. Voluntary nature of participation: Your participation in this study is voluntary. Even after you sign this consent document, you may decide to stop further participation in the study at any time without any penalty. Data and specimen that may have been collected after the termination of your participation will remain in the study.

9. Documentation of the consent: One copy of this document will be kept together with research records of this study, also you be given a copy to keep.

10. Contact: To obtain further information regarding this study contact:

Golden Zyambo

Phone: +260 97 7887278/ +260 96 8652075

Physical Address: Plot M/D3 Handsworth Court Lusaka Zambia

Print Name of Owner/agent: ____

Signature of Owner/agent: __________________________**Date:** ____________________

Appendix 4: Informed Owner Consent

Lead contamination and human health risk assessment through consumption of cow milk in Kabwe, Zambia

==

I,____(name),of____________________

____(address)

____(City/place)

, hereby consent to the participation of the following cows in the study cited above. I certify that I am the legal owner (or agent of the owner) and I am responsible for the under-listed animals. I have accurately read (or it has accurately been read for me), received a copy, and understood the informed Owner Consent Form. I have had the opportunity to ask questions about the study and any question I have asked has been answered to my satisfaction.

Animal Details

S/N	**Name of animal**	**Breed**	**Age cow**	**Age of calf**

Name of Owner/Agent taking consent: ____

Signature of Owner/Agent taking consent: ____

Date: __________________

Appendix 5: Statement by the researcher/ person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure the participant understands.

I confirm that the participant was allowed to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this information Consent Form has been provided to the participant.

Name of Researcher (Person taking consent):____

Signature of Researcher/ person taking consent: ____

Date: __________________

Appendix 6: Physical examination of cows included in the study – visual assessment and record

Part A: Preliminary information

No	Description	Remark/Record
1	Introduce the appraisal team	
2	Take precautionary biosafety measures	
3	Identify the respondent (s)	
4	Confirm eligibility (lactating cows/free-range)	
5	Provide purpose of study/information	
6	Identify livestock breeds kept	
7	Identify husbandry systems	
8	Drinking water locations (using GPS)	
9	Grazing sites	

Part B: Physical Examination of cows – Veterinarian Key

No.	Parameter -	Yes	No
1	Have no injury or lame		
2	Have low or lowered ears		
3	Have foam around their nose or mouth		
4	Grind their teeth frequently		
5	Have abscesses on their body or in their mouths		
6	Weak hind limbs		
7	Have signs of weakness		
8	Isolated or rejected by the rest of the herd		
9	Are the eyes bright, clean and alert		
10	Breathing laboured, loud, wheezy, sneezy, whistling, or squeaky.		
11	General body condition		
12	Hair coat condition		

13	Normal joints in their legs and shoulders		
14	Have perineum (no diarrhoea or blood in stool)		
15	Feces (normal, watery, melena, constipated, mucus/fibrin or other)		
16	Have no symptoms of mastitis (udders not hot, swollen, tough, or painful etc.)		

N.B: Animals selected for sampling were constrained in a crush pen for physical examination

Other findings: ____

____

____

Name of the Veterinarian______________________________Signature____________________

Table S 1 adapted from:

1. Jerry Bertoldo, 2015. https://docplayer.net/storage/63/50084955/50084955.pdf

Terrs and Reynolds, 2020. https://veteriankey.com/ruminant-history-physical-

UNIVERSITY OF ZAMBIA
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IRB00001131 of IORG0000774

20th October 2021

Your REF. No. 1903-2021

Mr. Golden Zyambo,
University of Zambia,
Department of Biomedical Sciences,
Lusaka.

Dear Mr. Zyambo,

RE: HUMAN HEALTH RISK ASSESSMENT OF LEAD EXPOSURE THROUGH CONSUMPTION OF COW'S MILK IN KABWE, ZAMBIA (REF. NO. 1903-2021)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 7th October, 2021. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

APPROVAL NUMBER : REF. 1903-2021

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 19th October 2021**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 18th October 2022**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.

- **NHRA**: You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No. +260977925304 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You are also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,

Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Chalala Office Lot No. 18961/M, Off Kasama Road, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@nhra.org.zm | www.nhra.org.zm

Ref No: NHRA000004/15/11/2021 **Date: 15th November, 2021**

The Principal Investigator,
Golden Zyambo,
University of Zambia,
Lusaka, Zambia.

Dear Golden Zyambo,

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for authority to conduct research titled **"HUMAN HEALTH RISK ASSESSMENT OF LEAD EXPOSURE THROUGH CONSUMPTION OF COW'S MILK IN KABWE, ZAMBIA."**

I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **APPROVED** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

Prof. Godfrey Biemba
Director/CEO
National Health Research Authority

International Journal of
Environmental Research and Public Health

MDPI

Article

Human Health Risk Assessment from Lead Exposure through Consumption of Raw Cow Milk from Free-Range Cattle Reared in the Vicinity of a Lead–Zinc Mine in Kabwe

Golden Zyambo [1,2], John Yabe [1,3,*], Kaampwe Muzandu [1], Ethel M'kandawire [1], Kennedy Choongo [1], Andrew Kataba [1,2], Kenneth Chawinga [4], Allan Liazambi [4], Shouta M. M Nakayama [1,2], Hokuto Nakata [2] and Mayumi Ishizuka [2,*]

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check for updates

Citation: Zyambo, G.; Yabe, J.; Muzandu, K.; M'kandawire, E.; Choongo, K.; Kataba, A.; Chawinga, K.; Liazambi, A.; Nakayama, S.M.M.; Nakata, H.; et al. Human Health Risk Assessment from Lead Exposure through Consumption of Raw Cow Milk from Free-Range Cattle Reared in the Vicinity of a Lead–Zinc Mine in Kabwe. *Int. J. Environ. Res. Public Health* **2022**, *19*, 4757. https://doi.org/10.3390/ijerph19084757

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Abstract: Lead (Pb) contamination in the environment affects both humans and animals. Chronic exposure to Pb via dietary intake of animal products such as milk from contaminated areas poses a health risk to consumers; therefore, the present study investigated Pb contamination in cow milk and its health risk impact on humans through consumption of milk from cattle reared in the proximity of a Pb–Zn mine in Kabwe, Zambia. Fresh milk samples were collected from cows from Kang'omba (KN), Kafulamse (KF), Mpima (MP), Mukobeko (MK), and Munga (MN) farming areas. Pb determination was performed using Graphite Flame Absorption Atomic Spectrophotometry (GFAAS). Cow milk Pb levels showed different concentration patterns according to season, distance, and location of the farms from the Pb–Zn mine. The overall mean Pb levels were ranged 0.60–2.22 μg/kg and 0.50–4.24 μg/kg in the wet and dry seasons, respectively. The mean Pb concentration, chronic daily intake (CDIs), target hazard quotients (THQs), and incremental lifetime cancer risk (ILCR) results obtained were all within the permissible limits of 20 μg/kg, 3 and 12.5 μg/kg-BW/day, <1 and 10^{-4} to10^{-6}, respectively. In conclusion, although Pb was detected in milk from cows reared in Kabwe, the health risk effects of Pb exposure associated with the consumption of milk in both adults and children were negligible.

Keywords: human health risk; milk; lead; food safety; ingestion

1. Introduction

Lead (Pb) is a toxic and possible carcinogenic element [1] that results from multiple sources in the environment [2]. Because of its widespread use, Pb has caused extensive environmental contamination and health problems in many parts of the world [3]. Irrespective of the source, human exposure to Pb is one of the most serious health problems facing populations, especially children [4]. In animals, grazing on contaminated pastures is one of the major sources of Pb content in animal tissues [5]. In exposed animals, milk contamination with Pb is caused by the excretion of the metal into milk [5–9]. Numerous authors have reported milk contamination with Pb [6,8,10–15].

Primarily, the food chain is subject to Pb contamination through plant uptake from a contaminated environment [16], where animals accumulate Pb in their tissues and excrete it in milk [17]. Because cow milk is considered a good biological matrix, it can thus be used to evaluate the Pb exposure risks in both animals and humans [18]. On the other hand, metal residues in milk could be an important "direct indicator" or an "indirect indicator" of the hygienic status of the milk and the degree of environmental pollution [11]. Apart from environmental contamination and seasonal variations, climatic conditions, lactation period of cows, health conditions, and animal annual feed composition have been identified to have a significant effect on the variability of the mineral content in raw cow milk [11].

Milk is regarded as nature's single most complete food [19]. It is the most valuable and regularly consumed food that is best known for its good composition of major minerals, proteins, fats, and vitamins [20]. As a basic food in the human diet, it is consumed both in its unprocessed form and as various dairy products [11]. Since milk is largely consumed by infants and children, Pb residues in milk are of particular concern [21], especially in children where even low-level exposure in early childhood is known to cause cognitive development deficits [22]. In adults, Pb poisoning causes multiple health effects, including anemia, hypertension, renal damage, immunotoxicity, etc. [3]. Chronic exposure to Pb is also associated with spontaneous abortions and stillbirth in women [13], as well as infertility in men [23].

High levels of environmental Pb are attributed to anthropogenic activities such as mining and smelting, battery manufacturing, recycling of waste batteries, and burning coal [24]. In Kabwe, the capital of Zambia's Central Province, extensive Pb pollution in the nearby townships caused by emissions from a Pb–Zn mine and smelters has been reported [25,26]. As a result, high Pb concentrations have been detected in animals, including livestock and dogs [26–32]. In addition, high levels of Pb concentrations in humans, especially children, have been reported [33]. Despite these numerous reports about Pb contamination in Kabwe, dietary Pb exposure and its associated risks have not been evaluated. To address this knowledge gap, the current study was conducted to: (1) quantify the Pb concentrations in milk from cattle reared around the Pb–Zn mine in Kabwe; (2) investigate the seasonal variations of Pb concentrations in cow milk; (3) assess the health risk impact of exposure to Pb through cow milk consumption by the local residents of Kabwe.

2. Methods and Materials

2.1. *Location of the Study Area and Study Design*

The current study was conducted using a convenient sampling technique. The farms located in the proximities of the mining area in Kabwe town in Central Province were selected as study sites; Chongwe town in Lusaka Province Zambia was included as a reference site (a non-mining area). The geographical location of Kabwe is 14°27′ S and 28°26′ E, about 150 km north of Lusaka, the capital city of Zambia; Chongwe is located at 14°27′ S and 28°26′ E, 45 km east of Lusaka.

The focus of the study was on five regions of Kabwe mainly with a target selection of farms composed of traditional smallholder milk producers, emerging dairy farms of small and medium-size, namely Kang'omba (KN), Kafulamse (KF), Mpima (MP), Mukobeko (MK), and Munga (MN) (Figure 1). Chongwe (CN) town in the Kanakantapa area, as it is a non-mining area, was considered a reference zone for comparative purposes with the samples from Kabwe. The sampling locations were marked using the Global Positioning System (GPS) (Table S1). The sampling zones were chosen based on the high dependence on traditional or mix-breed cattle reared using free-range practices by farmers for both beef and milk production. According to Mumba et al. [34], Kabwe produced about 1,610,700 L of milk per year. Farms having a herd size greater than or equal to five lactating cows were registered for sampling. If the farm had more than five but less than ten lactating cows ($5 \geq 10$), all the available lactating cows were considered as study subjects, as long as they met the selection criteria (Table S2); however, in a case where lactating cows exceed 10, the extra number of subjects was calculated based on the 10 percent fraction of the total herd to cow ratio, following random selection criteria.

Figure 1. GPS map (modified from Google My Maps) showing sampling locations around the Pb-Zn mine in five regions of Kabwe (Kang'omba n = 56; Mpima, n = 54; Kafulamase, n = 35; Mukobeko, n = 59; Munga, n = 10).

2.2. Milk Sampling

Cow milk sampling was conducted between 2018 and 2021 during the wet and dry seasons. Generally, seasons are divided into two distinctive halves, a dry half from May to October and a wet half from November to April. The coldest month is July, with temperatures in the range of 3.6–12.0 °C, while the hottest month is October, with temperatures averaging 27.7–36.5 °C. On average, annual rainfall ranges from 700 mm in the extreme southwest to 1400 mm in the north and is 1001 mm on average [35]. To evaluate seasonal variations of Pb in the milk of the study participants, samples were collected in a follow-up design study in February/March (wet season) and October (dry season). Samples were collected during morning routine milking [36] from healthy cows according to the standard methods [37,38]. Before sample collection, the udder of each cow was washed using distilled water and about 10 mL was expressed manually directly into sterile 15 mL polypropylene tubes (SuperClear® Labcon, Petaluma, CA, USA). After collection, the samples were then homogenized by inverting the tubes ten times and temporarily stored in a cooler box with ice packs before transportation for storage at −20 °C at the University of Zambia, School of Veterinary Medicine, Lusaka, Zambia until analysis.

2.3. Sample Preparation and Microwave Acid Digestion

For the preparation of all solutions, double distilled water from a Milli-Q-Element system (18 MΩ-cm, Millipore®, Milford, MA, USA) was used. Metal-free polypropylene vials (Kanto Chemical Co., Inc., Tokyo, Japan) were pre-cleaned with 2% diluted HNO_3 (Kanto Chemical Co., Inc., Tokyo, Japan) for 24 h and rinsed thoroughly with Milli-Q double distilled water before use. The milk samples were thawed at room temperature and homogenized by vortex. Approximately 1 g of milk was accurately weighed in a pre-cleaned Berghof digestion vessel (DAP-60K, Enigen, German), followed by the metal extraction in a closed microwave digestion system (Berghof, Speed Wave®ENTRY, Eningen, Germany) as described by Toyomaki et al. [27]. Briefly, metal extraction was conducted using a closed, optimized microwave digestion system under automated temperature and pressure-control conditions for 31 min (Table S3) after the addition of 5 mL of 30% nitric acid (69% HNO_3 pure, HI-AR™, Kanto Chemical Co., Inc., Tokyo, Japan), and 1 mL of 30% hydrogen peroxide (Kanto Chemical Co., Inc., Tokyo, Japan). The digested samples were then cooled for 20 min and transferred into 15 mL sterile polypropylene tubes. To ensure homogeneity, the contents were thoroughly mixed by inverting the tube for ten min. Similarly, blank samples were also prepared alongside the milk samples to maintain the uniformity of digestion parameters.

2.4. Lead Determination in Milk

Lead analysis in milk was performed using Zeeman-corrected graphite furnace absorption spectrophotometry (GFAAS), (Hitachi-Zeeman 2010 model, High Technologies Corporation, Tokyo, Japan) equipped with a graphite furnace according to a technique described by Yabe et al. [26]. In this graphite method of Pb analysis, a matrix modifier of 0.5% ammonium hydrogen sulfate ($NH_4H_2PO_4$) (Nakagyo-Ku, Kyoto, Japan) was used to minimize the matrix effect according to the manufacturer's guide. Concomitantly, the auto-programmed sampler per time injected 20 µL of sample and the modifier for analysis. The operation conditions of the GFAAS are as given in Table S4.

2.5. Quality Assurance

The Pb analysis by the GFAAS method included linear range, coefficient of correlation limits of detection (LOD), and limits of quantification (LOQ) analytical parameters. The LOD value was calculated by multiplying 3 times standard deviation (SD) for 10 replications of the blank, while the LOQ was calculated by multiplying 10 times SD of the slope/intercept (Table S4). Replication of blank samples analysis was measured directly, and their measurements were subtracted from the sample intensities [39]. Method validation and accuracy were performed according to Yabe et al. [26] using DOLT-5 (Dogfish liver, National Research Council of Canada, Ottawa, ON, Canada). Replicate analysis of DOLT-5 reference material indicated the accuracy and recovery rate of 93.1–119.8% (relative standard deviation, RSD ≤ 5%). The confidence in the integrity of data was increased by duplicate measurement of samples in µg/kg (ppb) anchored on a multipoint calibration curve prepared from 1000 mg/L of Pb (Himea, New Deli, India) stock solution.

2.6. Data Analysis

Descriptive statistical analysis of the data was performed using GraphPad Prism software (Prism 7 for Windows; Version 5.02, GraphPad Software, Inc., San Diego, CA, USA). The explorative parameters are presented as n—sample number; m—mean value; (SD±)—standard deviation; Min-Max—range of Pb levels in milk. Prior to analyses, data were examined for normality of distribution by Kolmogorov–Smirnov normality test. The test performed showed the departure of data from normality. To stabilize the variances, we transformed the data by a base 10 logarithm. Statistical comparison analysis was performed to determine the differences in the mean concentrations of Pb in the milk samples collected in the wet and dry seasons and among different sampled regions using analysis of variance (ANOVA) and Tukey's multiple comparison test, respectively. All significant differences were set at $p < 0.05$.

2.7. Probabilistic Health Risk Assessment of Lead in Milk

To evaluate the Pb exposure through ingestion of contaminated cow milk in the local residents of Kabwe, the risk assessment was performed based on chronic daily intake (CDIs), target hazard quotients (THQs), and incremental lifetime cancer risk (ILCR) parameters according to the guidelines prescribed by the United States Environmental Protection Agency (USEPA) [40].

2.7.1. Chronic Daily Intake

The CDI is a value related to the metal concentration in milk, the daily consumption of milk, and the body weight of the consumer, which influences tolerance to a contaminant [1]; in this case, Pb. The CDI values were determined according to Equation (1) [41].

$$CDI = (C_m \times D)/BW \quad (1)$$

where CDI is the estimated chronic daily intake of Pb (μg/kg $^{-1}$ BW day^{-1}); C_m, mean Pb concentration (μg/kg); D, daily milk intake (μg/day); and BW is the average body weight (kg). Owing to the scarcity of updated data on the consumption rates of milk in Zambia, the average daily intake of milk in children and adults is 21 and 17 g/day/person for body weights of 10 kg and 70 kg, respectively [41,42], were used for the calculations in the present study.

2.7.2. Risk Characterization

The risk characterization analysis using carcinogenic and non-carcinogenic risk assessment via ingestion was considered an important tool for identifying the health risk effects in humans and providing risk evidence for decision-making.

Non-Carcinogenic Risk

Non-carcinogenic risk is evaluated by comparing an exposure level (dose) over a specified period, such as a lifetime, with a reference dose derived for a similar exposure period [43].

The non-carcinogenic risk is characterized in terms of THQ, which has been recognized as a useful parameter for the evaluation of risks associated with the consumption of metal-contaminated food. Thus, the potential non-carcinogenic risks for the exposure to Pb via consumption of cow milk are assessed by comparison of CDI from the oral exposure route with the chronic dose (RfD) to find the THQ value described by the USEPA [40] as follows:

$$THQ = (CDI/RfD) \times 10^{-3} \quad (2)$$

where THQ is unitless, CDI is the chronic daily intake average (mg/kg/day); and RfD is mg/kg/day. The oral reference dose for Pb is 3.5×10^{-3}. THQ > 1 assumes that there may be a concern for potential non-carcinogenic risks. In a case where THQ < 1, it means that the hazard is unlikely to cause adverse health effects for the exposed populations [43].

Carcinogenic Risk

Carcinogenic risk refers to the incremental probability of an individual developing any kind of cancer in a lifetime because of exposure to carcinogens [43]. In the current study, carcinogenic risks were calculated as the incremental probability of an individual developing cancer over a lifetime as a result of exposure to Pb according to the linear equation [36,43,44] as follows:

$$\text{Carcinogenic risk} = CDI \times CSF \quad (3)$$

where CDI is the daily chronic intake; CSF, cancer slope factor (mg/kg/day)$^{-1}$. According to the USEPA [40], the acceptable safe risk ranges from 10^{-6} to 10^{-4}.

3. Results and Discussion

3.1. *Lead Concentrations in Cow Milk*

The overall summarized concentrations of Pb analyzed in cow milk samples from the studied regions are presented in Table 1. The mean Pb levels detected in cow milk varied in the range of 0.60–2.32 μg/kg in the wet season and 0.50–4.24 μg/kg in the dry season. The lowest mean value recorded was in Mukobeko, which surprisingly showed a slightly lower mean value than the level that was recorded in Chongwe, the reference site. According to the results, Munga had the highest mean Pb concentration. Table 1 below shows the descriptive results obtained in the study.

Table 1. Summary of mean lead concentrations (µg/kg-wt./wt.).

Season	Region	No. of Farms	Samples (n)	Mean (m)	Standard Deviation (SD±)	Min-Max (µg/kg)
Wet season	Kan'gomba	5	22	2.32	1.87	0.05–6.90
	Kafulamase	3	16	0.98	0.30	0.59–1.80
	Mpima	5	28	1.20	0.67	0.20–2.76
	Mukobeko	5	29	1.11	0.74	0.10–2.77
	Munga	3	5	1.96	1.46	0.78–3.70
	Chongwe	3	9	0.60	0.19	0.89–4.66
	Average			**1.36**	**0.88**	**0.05–6.90**
Dry season	Kan'gomba	4	34	2.93	2.43	0.70–9.66
	Kafulamase	3	19	1.72	2.58	0.06–10.8
	Mpima	5	26	0.84	0.32	0.41–1.70
	Mukobeko	5	30	0.50	0.24	0.09–1.15
	Munga	3	5	4.24	2.24	1.76–7.70
	Chongwe	3	9	0.51	0.13	0.40–0.81
	Average			**1.79**	**1.32**	**0.06–10.8**

The variation of mean Pb concentration results in cow milk obtained showed significant differences among the sampled regions when compared with Tukey's multiple comparison test set at 0.05 ($p < 0.05$). Data in Figure 2 show the graphical variations of mean Pb concentrations obtained in each sampled region.

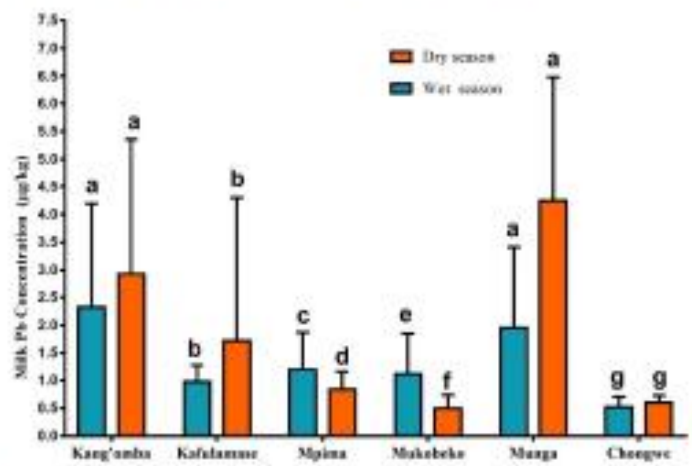

Figure 2. The variation of mean Pb concentration results in cow milk (µg/kg-wt./wt.) per region and per season. The lower-case letters a–g represent significant differences among the sampled regions using Tukey's multiple comparison test set at 0.05 ($p < 0.05$).

The general trend of the mean Pb concentrations in milk during the wet season followed the decreasing order of Kang'omba > Munga > Mpima > Mukobeko > Kafulamase > Chongwe, while in the dry season, the trend followed the order of Munga > Kang'omba > Kafulamase > Mpima > Chongwe > Mukobeko as illustrated in Figure 3. The mean Pb concentrations measured in milk samples in the dry season were higher than the samples in the wet season, especially in farms closer to the mine.

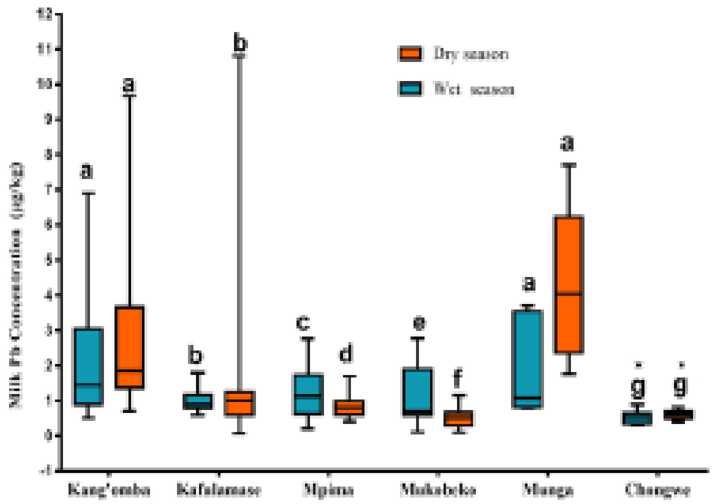

Figure 3. A summary of Pb concentrations (µg/kg) in cow milk analyzed from different sites per season in the mining area (Kang'omba, n = 56; Mpima, n = 54; Kafulamase, n = 35; Mukobeko, n = 59; Munga = 10) and in a non-mining area of Chongwe (reference site), n = 18. Data are presented in box and whisker plots: lines marked across the boxes indicate the medians; box limits represent 25th and 75th percentiles; ends of the whisker show minimum and maximum values of Pb concentrations measured. The lower-case letters a–g represent significant differences among the sampled regions using Tukey's multiple comparison test ($p < 0.05$). Asterisk (*), means reference site.

Based on the results of the current study, Pb was present in all sampled sites, including Chongwe (reference site). The source of Pb in cattle from the sampled farms around Chongwe town, which has no history of Pb mining, is unknown; however, the current study indicates that Chongwe, the reference site, had the least Pb results, and correspondingly, the interval Pb concentration was remarkably minimal (Figure 3). The milk Pb concentrations in the mining areas of Kabwe showed higher variability compared to the non-mining in Chongwe (Figure 3).

Generally, the findings indicate that the spatial distribution pattern of the Pb contamination in the cow milk samples investigated corresponded with the prevailing wind direction and the distance to the point source of the Pb pollution. For example, Munga on the west and Kang'omba on the southwest were subject to high Pb pollution because of their proximity and geographical location to the Pb–Zn mine. The most likely explanation for the elevated Pb concentrations in the two areas compared to other sites in the dry season could be due to the airborne Pb in dust and contaminated forage produced on agricultural surfaces or cattle grazing on pastures contaminated by emissions from smelters [5].

On the other hand, the least Pb concentration in the current study was observed in the dry season in Mukobeko (0.5 µg/kg), followed by Mpima (0.84 µg/kg), regions that were located on the northern and northwestern sides of Kabwe town. Interestingly, the highest Pb concentration (10.8 µg/kg) during the same season was recorded in Kafulamase, a sampling location that was further on the southeastern side of the Pb–Zn mine. Studies reveal that large amounts of tailings and wastewater produced in the mining process account for high metal contamination in the surrounding environment [43]. Mostly, near the smelters [45], there is an increased uptake of Pb through contaminated fodder, which subsequently results in enhanced excretion of Pb residues in milk [12]. Moreover, studies indicate that rainwater aids metal dispersion, causing widespread contamination and affecting agricultural fields and water bodies [2]. Thus, intake of Pb contaminated water emanating from the Pb–Zn mine carried downstream during the rainy season could be associated with the appreciably high Pb content in milk observed from the Kafulamse region, 15 km away from the mine. The overall results in the present investigation showed that Pb levels in cow milk were lower than most of the findings that have been reported previously from various countries (Table 2).

Table 2. Lead levels in cow milk from various countries in recent five years (2015–2021).

Year	Country	Source of Milk	N	Method Used	Mean (µg/kg)	Range	Reference
2021	Zambia	Farms near the mining area	233	GFAAS	2.22 ± 1.69	LOD-9.66	Present study
2020	Kazakhstan	Farms	120	AAS	11.6 ± 10.0	-	[46]
2020	Slovakia	Farms	40	EDA-AAS	10.0	-	[47]
2019	China	Wholesale markets and stores	208	ICP-MS	3.6 ± 2.30	-	[48]
2019	Romania	Small cattle farms	10	GFAAS	24. ± 15.0	0.010–0.048	[1]
2018	Iran	Dairies and markets	36	ICP-OES	32.83 ± 20.80	15.70–68.0	[49]
2018	Sudan	Farms	9	AAS	<LOD	-	[50]
2018	Iran	Dairy, industrial and traditional farms	118	GFAAS	47.0 ± 3.9	N.D-250	[51]
2018	Uganda	District	20	AAS	10.48 ± 1.82	6.62–14.34	[44]
2017	India	Farms	30	ICP-AES	124.0	0.016–0.356	[52]
2017	Pakistan	Farms	240	AAS	0.021	0.007–0.041	[53]
2017	Egypt	Dairy shops	18	AAS	93.4 ± 18.8	0.007–0.341	[54]
2017	Mexico	Sub-basin of industrial and urban region	40	ICP-OES	46.0± 28.	0.039–0.05	[36]
2016	Bangladesh	Branded, dairy farm and household farmers	27	FAAS	0.012 ± 0.001	-	[20]
2016	Romania	Rural area	19	ICP-MS	15.8 ± 5.45	0.01–0.48	[38]
2015	Egypt	Dairy shops and groceries	30	GFAAS	414.0 ± 34.2	0.09–0.52	[15]

Compared to the established values in the present study, other authors in Romania [1], India [55], Iran [49], and Kazakhstan [46] recently reported higher Pb concentrations of 24.0 ± 15.0 µg/kg, 209 ± 0.70 µg/kg, 32.83 ± 20.80 µg/kg, and 11.6 ± 10 µg/kg, respectively. Nonetheless, values obtained in the current study were remarkably higher than those reported by Elsaim and Ali [50] in Sudan but within the range that was found in China (3.60 ± 2.30 µg/kg) by Wang et al. [24]. Although Pb was detected in all Kabwe regions in our present study, the concentrations found in cow milk were below the 20.0 µg/kg safety standard threshold set by Codex Alimentarius Commission [56], and 100 µg/kg, the benchmark value established by the European Food Safety Authority of the European Union (EU) [57].

3.2. Seasonal Variations of Lead Concentration Trends in Milk

The general trends of Pb concentrations observed in each region are presented in Figure 4a–d below.

The study showed varied Pb concentration trends in each season, despite the animals generally sharing the same grazing pasture in each region. For example, in Kan'gomba and Munga, the paired results indicate that the mean Pb concentrations in the dry season were higher than in the wet season. Similarly, the results obtained in Kafulamase in the paired samples analyzed indicate remarkable high Pb content in the milk in the dry season compared to the results obtained in the wet season; however, the mean Pb concentration trends in Mukobeko and Mpima were similar but different from the pattern observed in Kang'omba, Kafulamse, and Munga. On the contrary, in Chongwe (reference area), samples analyzed showed an irregular Pb pattern, although the concentrations seemed to oscillate within the same magnitude in each season.

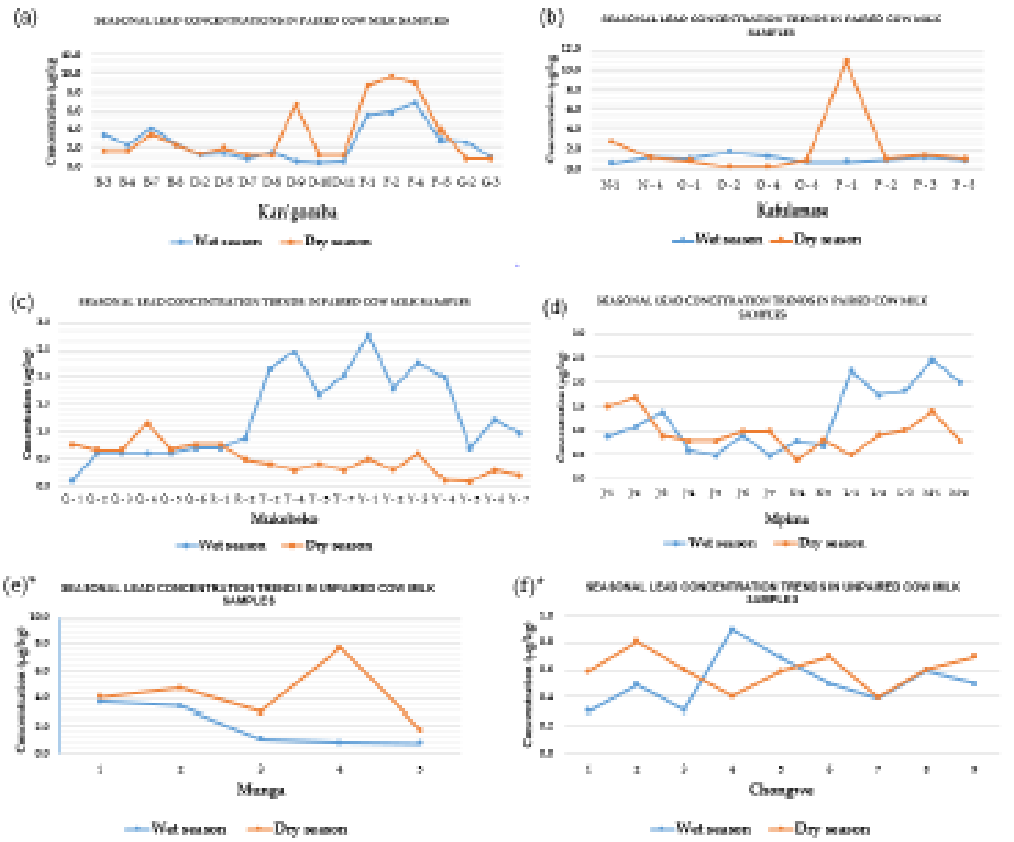

Figure 4. Pb concentration trends in paired cow milk samples collected in the wet and dry seasons in: (a) Kang'omba; (b) Kafulamase; (c) Mukobeko; (d) Mpima regions. Asterisk on (e)* and (f)* indicates unpaired milk samples, i.e., milk samples collected from different selected subjects in Munga and Chongwe regions during the wet and dry seasons. The letters on graph (a–d) represent identity numbers of paired samples from the same individual cows, while the numbers on graph (e)* and (f)* represent sample identity numbers for unpaired samples from different animals of the same herd.

The difference in seasons evoked considerable Pb concentration variations either between the region in the same sampling sites and/or among different regions. Factors such as the location and distance of the farms from the Pb–Zn mine could have also contributed to the observed Pb concentration fluctuations. The literature indicates that the degree of heavy metal contamination in cow milk is not constant but differs depending on the exposure routes, environmental condition, animal's nutrition, stage of lactation, and animal breed [58]. Since the present study focused on cattle with mixed breeds reared on free-range practices, variability of Pb residues in milk in different regions was expected.

3.3. Health Risk Assessment

Health risk is defined as the likelihood of harmful effects on human health as a result of environmental pollution [43]. Thus, exposure assessment is the process of measuring or estimating the intensity, frequency, and duration of human exposures to an environmental agent [59]. Exposure assessment results can be used to evaluate risks to human health [24].

Resulting from such an evaluation process in the current study, the summarized data are presented in Tables 3–5 below.

Table 3. Chronic daily intake (CDI) of lead through consumption of milk for the resident children and adults of Kabwe.

	Wet Season CDI (µg/kg/day)		Dray Season CDI (µg/kg/day)	
Region	**Children**	**Adult**	**Children**	**Adult**
Kan'gomba	3.77×10^{-6}	6.66×10^{-7}	4.98×10^{-6}	8.79×10^{-7}
Kafulamase	8.33×10^{-7}	3.43×10^{-7}	1.46×10^{-6}	6.02×10^{-7}
Mpima	1.02×10^{-6}	4.20×10^{-7}	1.02×10^{-6}	4.20×10^{-7}
Mukobeko	9.44×10^{-7}	3.89×10^{-7}	4.25×10^{-7}	1.75×10^{-7}
Munga	1.67×10^{-6}	6.86×10^{-7}	3.60×10^{-6}	1.48×10^{-6}
Chongwe *	5.10×10^{-7}	2.10×10^{-7}	4.34×10^{-7}	1.79×10^{-7}
Average	$\mathbf{8.75 \times 10^{-6}}$	$\mathbf{2.71 \times 10^{-6}}$	$\mathbf{1.19 \times 10^{-5}}$	$\mathbf{3.74 \times 10^{-6}}$

* indicate reference site.

Table 4. Target hazard quotient (THQ) of lead through consumption of cow milk (in children and adults) in the residents of Kabwe.

	Wet Season THQ		Dry Season THQ	
Region	**Children**	**Adult**	**Children**	**Adult**
Kan'gomba	1.08×10^{-3}	1.90×10^{-4}	1.42×10^{-3}	2.51×10^{-4}
Kafulamase	2.38×10^{-4}	9.80×10^{-5}	4.18×10^{-4}	1.72×10^{-4}
Mpima	2.91×10^{-4}	1.20×10^{-4}	2.91×10^{-4}	1.20×10^{-4}
Mukobeko	2.70×10^{-4}	1.11×10^{-4}	1.21×10^{-4}	5.00×10^{-5}
Munga	4.76×10^{-4}	1.96×10^{-4}	1.03×10^{-3}	4.24×10^{-4}
Chongwe *	1.46×10^{-4}	6.00×10^{-5}	1.24×10^{-4}	5.10×10^{-5}
Average	$\mathbf{2.50 \times 10^{-3}}$	$\mathbf{7.75 \times 10^{-4}}$	$\mathbf{3.41 \times 10^{-3}}$	$\mathbf{1.07 \times 10^{-3}}$

* indicate reference site.

Table 5. Incremental lifetime cancer risk (ILCR) of lead through consumption of cow milk (in children and adults) in the residents of Kabwe.

	Wet Season ILCR		Dry Season ILCR	
Region	**Children**	**Adult**	**Children**	**Adult**
Kan'gomba	3.21×10^{-8}	5.66×10^{-9}	4.23×10^{-8}	7.47×10^{-9}
Kafulamase	7.08×10^{-9}	2.92×10^{-9}	1.24×10^{-8}	5.12×10^{-9}
Mpima	8.67×10^{-9}	3.57×10^{-9}	8.67×10^{-9}	3.57×10^{-9}
Mukobeko	8.02×10^{-9}	3.30×10^{-9}	3.61×10^{-9}	1.49×10^{-9}
Munga	1.42×10^{-8}	5.83×10^{-9}	3.06×10^{-8}	1.26×10^{-8}
Chongwe	4.34×10^{-9}	1.79×10^{-9}	3.68×10^{-9}	1.52×10^{-9}
Average	$\mathbf{7.43 \times 10^{-8}}$	$\mathbf{2.31 \times 10^{-8}}$	$\mathbf{1.01 \times 10^{-7}}$	$\mathbf{3.18 \times 10^{-8}}$

3.3.1. Chronic Daily Intake of Lead Metal in Milk

Table 3 below shows the summary of the CDI values calculated based on the average concentrations found in cow milk analyzed from each sampled region. These were compared with provisional tolerable daily intake as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [60]. The results obtained in the present study showed comparatively high CDI for children than for adults; however, in both cases, the values were all within the permissible limits. The CDI results established in the present study ranged from 5.10×10^{-7} to 4.98×10^{-6} in children, while in adults, it ranged from 1.75×10^{-7} to 1.487×10^{-6}; however, the highest CDI value (4.98×10^{-6}) was recorded for children in the milk samples that were collected in the Kang'omba region during the dry season, whereas the lowest value (1.75×10^{-7}) in adults was obtained in samples that were collected from the Mukobeko region in the same period (Table 3).

The average CDI results in children both in the wet season and dry indicate higher values than in adults ($8.75 \times 10^{-6} > 2.71 \times 10^{-6}$ and $1.19 \times 10^{-5} > 3.74 \times 10^{-6}$) for the same period, respectively. In all cases, CDI values were below the oral reference dose of 3.57 µg/kg/day of Pb [21]. According to the present results, the observed CDI trends regionally in both children and adults were found in the decreasing order of Kang'omba > Munga > Kafulamase > Mpima > Chongwe > Mukobeko > in the dry season.

The CDI values established in the present study were lower than the values found in Africa by Salah et al. [61] and Meshref et al. [62] of 158.5 and 1.70×10^{-4} µg/kg/day, respectively. Similarly, other authors [38,63] in Europe reported a CDI range of 4.40×10^{-4} to 8.00×10^{-5}, which was several folds higher than what was found in the current study. On the contrary, CDI values of 5.40×10^{-6} and 3.40×10^{-5} that were similar to the current study were reported in Asia by Muhib et al. [20] and Norouzirad et al. [51], respectively. On the other hand, Ismail et al. [53] reported much higher values in the range of 0.069 to 0.946 in both children and adult milk consumers.

Food intake has been identified to be the major route of human exposure to Pb, although, for children, ingestion of soil and dust can also be an important contributor [56]. In other studies, it is reported to account for over 90% compared to other exposure pathways such as inhalation and dermal contact [64]. Consequently, in a general population, the most probable route of exposure that leads to elevated blood Pb levels is ingestion [3]. Children are at a greater risk of chronic Pb exposure and poisoning than adults due to their higher CDI coupled with lower body weight [13,65]. There is no established tolerable Pb intake for which Pb cannot cause adverse effects [60]; however, the U.S. Food and Drug Administration (FDA) recommends interim reference levels of 3 and 12.5 µg/day for children and adults (women of childbearing age), respectively. This corresponds to the blood lead level (BLL) of 0.5 µg/dL for a general population [66]. Analysis of samples in the present study, however, showed that the calculated values of Pb in cow milk were lower than the interim reference benchmarks recommended by FDA.

Although the low levels were found to be below the recommended risk level by the FDA in cow milk from Kabwe, potential risks may probably occur due to dietary and non-dietary contributing factors that may magnify the Pb exposure effects in the population; therefore, strict regular monitoring of Pb contamination of milk and milk products is recommended [61] for the food safety and quality aspects of the consumers.

3.3.2. Evaluation of Non-Cancer Risk Assessment for the Kabwe Population

The calculated potential adverse effects for the current study associated with non-carcinogen Pb in cow milk are presented in Table 4. The estimation of the maximum permissible risk on the human population was calculated based on THQ according to Kasozi et al. [44].

Target THQ is a method developed by the US environmental protection agency (EPA) for estimation of potential human health risk exposure to chemical pollutants associated with non-carcinogens [58], as expressed in Equation (2). The average THQ values in children and adults both in the wet season and dry were higher than in adults (7.75×10^{-4} to 2.5×10^{-3}) and 1.07×10^{-3} to 3.41×10^{-3}), respectively. As shown in Table 4, 1.42×10^{-3} was the highest THQ value calculated in children for the milk samples collected in the Kang'omba region in the dry season, whereas the highest value of 4.24×10^{-3} in adults was calculated in the analyzed samples from the Munga region in the same season. Considering the THQ value of less than one as non-hazardous to the consumers [36], and since all the THQ values obtained in the current study were consistent with this benchmark, consumption of cow milk in the sampled regions was considered safe from non-cancer risk effects due to Pb exposure. Our findings with a similar matrix agree with the results reported in Asia by Abedi et al. [65] in Iran (0.009 to 0.032) and those found in South America by Gonzalez et al. [36] in Mexico (0.039 to 0.059).

Similarly, results in the present study were consistent with the findings reported in Asia (1.2×10^{-3} to 9.8×10^{-3}) [20,51]; however, in Europe, the THQ values reported ranged from

1.3×10^{-2} to 7.6×10^{-2} [38,63,67]. Although these values from the reported regions were also lower than the safe limit of THQ > 1, they were slightly higher than the findings in the current study. In Uganda, Kasozi et al. [44] also found far much higher values in children and adults, ranging from 6.2648 to 2.116, respectively, compared to our results.

3.3.3. Evaluation of Carcinogenic Risk Assessment for the Kabwe Population

The cancer risk assessment was evaluated as ILCR, and the results are indicated in Table 5.

The ILCRs caused by the ingestion of Pb in cow milk obtained in the present study for both children and adults on average ranged from 7.43×10^{-8} to 1.01×10^{-7} and 2.31×10^{-8} to 3.18×10^{-8}, respectively. Our ILCR results obtained in the study indicate that they were far lower than the results reported by Kasozi et al. [44] in Uganda as 0.847×10^{-4} and 0.283×10^{-4} in children and adults, respectively. Further, Abedi et al. [65] in Iran reported higher values of ILCR than findings in the current study in children and adults, ranging from 1.89×10^{-3} to 2.45×10^{-3} and 2.96×10^{-4} to 3.85×10^{-4}, respectively.

For safety reasons, incremental lifetime cancer risks ranging from 1.0×10^{-6} to 1.0×10^{-4} are acceptable [68]. In the current study, both in the wet and dry seasons, the ILCR results were negligible and far lower than the reference range given. The ILCR values, therefore, suggested that cow milk from Kabwe did not pose carcinogenic risks to human consumers; however, long-term exposure to metals such as Pb and their complete absorption through the digestive tract is considered to be the worst-case scenario [1] because of their cumulative deleterious effect on human health [69]; therefore, regular assessment of their dietary exposure levels and impact to humans is essential.

Although the current study results indicated that consumption of cow milk in smallholder farming communities did not constitute a health hazard to consumers according to the Codex Alimentarius Commission set standard [56], the study had limitations. Limitations included a small sample size in the Munga region due to a small animal population, which could have some effect on the statistical analysis of data. Moreover, a shorter lactation period among traditional cow breeds compared to dairy cows, as well as the outbreak of foot and mouth disease during the sampling period, made the follow-up sampling study design a great challenge.

4. Conclusions

The intake of cow milk from free-range cattle in the studied regions indicated that the adverse health effects were not likely to be significant, based on the CDI, THQ, and ILCR parameters measured due to low Pb exposure in milk. The current study also revealed that the change of season did not cause a significant difference in mean Pb concentrations in Kabwe; however, since Pb is accumulative and can cause adverse health effects to humans even in low-dose exposure, regular assessment is necessary for quantifying the risks. Considering also the important influence of the aggregate potential risk factors due to dietary Pb exposure, a broad-based questionnaire survey is recommended to determine the consumption levels of cow milk in the studied regions. Furthermore, evaluation of a characteristic wide range of toxic heavy metals should also be considered for the determination of accurate combined toxic metal exposure effects that may be associated with the consumption of cow milk, including other dietary sources such as vegetable crops grown in the affected areas.

Further, given the high variability of results in certain sampled sites, even in places far away from the Kabwe Pb-Zn mine, further seasonal evaluation of Pb concentrations in animal drinking water from various sources such as ponds/dams, wells, streams, and rivers, including soil and fodder from the individual farms, is strongly recommended.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph19084757/s1, Table S1: Sampling locations in the study area of Kabwe and Chongwe (reference site), Table S2: Microwave operating conditions for milk digestion, Table S3: Summary of Graphite furnace atomic absorption spectrophotometer (GFAAS), Table S4: Analytical performances of lead.

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