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to contribute to the settlement of Pb pollution
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ザンビア・カブウェ鉱床地域の鉛汚染被害解決に向けた
動物及びヒトの鉛中毒に関するフィールド研究

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Contents

ABBREVIATIONS	3
PREFACE	6
CHAPTER 1	
Factors associated with lead (Pb) exposure on dogs around a Pb mining area, Kabwe, Zambia	
Abstract	18
1. Introduction.....	20
2. Materials and Methods.....	24
3. Results.....	29
4. Discussion	35
5. Conclusion	45
Figures and Tables	46
References.....	59

CHAPTER 2

Lead concentrations and isotope ratios in blood, breastmilk, and feces in humans:

Contribution of both lactation and soil/dust exposure to infants in a lead mining area, Kabwe, Zambia

Abstract	69
1. Introduction.....	71
2. Materials and Methods.....	74
3. Results.....	80
4. Discussion	86
5. Conclusion	94
Figures and Tables	95
References.....	110
SUMMARY	119
ACKNOWLEDGEMENTS	123

ABBREVIATIONS

ATSDR: Agency for Toxic Substances and Disease Registry

Alb: Albumin

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

As: Arsenic

AST: Aspartate aminotransferase

BLL: Blood Lead Level

BUN: Blood Urea Nitrogen

Cd: Cadmium

CDC: Centers for Disease Control and Prevention

Cl: Chlorine

Co: Cobalt

Cr: Chromium

Cre: Creatinine

Cu: Copper

dL: Deciliter

Fe: Iron

Fig: Figure

Ge: Germanium

GGT: Gamma glutamyl transpeptidase

GPS: Global Positioning System

Hg: Mercury

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

IQ: Intelligence Quotient

IU: International Unit

JICA: Japanese International Cooperation Agency

kg: Kilogram

km: Kilometer

L: Liter

LDH: Lactase dehydrogenase

MC-ICP-MS: Multiple Collector-Inductively coupled plasma mass spectrometry

mg: Milligram

min: Minute

mL: Milliliter

MTA: Material Transfer Agreement

Ni: Nickel

NIST: National Institute of Standards and Technology

O: Oxygen

Pb: Lead

PFA: Perfluoroalkoxy alkanes

S: Sulfur

SD: Standard Deviation

Tl: Thallium

TP: Total protein

T-bil: Total bilirubin

U: Unit

UA: Urea Acid

UNZAREC: University of Zambia Research Ethics Committee

W: Watt

WHO: World Health Organization

Zn: Zinc

µg: Microgram

µl: Microliter

PREFACE

Environmental Pollution

Environmental pollution is currently one of the most serious problems which humans as well as animals are facing. There are various pollutants such as metals and chemical compounds which originated from both naturally and anthropogenically. Pollutants spread over the border of countries by wind and water as well as human related dispersal activities. Moreover, pollutants released into environments are generally difficult to be removed, and it takes time to remediate as before contamination occurs. Therefore, the prevention of environmental pollution is necessary and important, such as laws related to emission regulation. The settlement of current environmental pollution is an urgent issue to protect humans and animals in parallel with the prevention of further pollution.

Heavy Metal Pollution in Africa

A heavy metal is a natural element that is usually presents in the environment. While some of heavy metals are essential elements for humans and animals, they are concerned as one of the environmental pollutants with their toxicity. Heavy metal pollution emanates from both natural and anthropogenic activities. However, anthropogenic activities, such as industries and mining, often bring them in large amount than naturally occurring amount of heavy metals into environment. In African countries, rapid population growth and high urbanization rates have resulted in a recent expansion of cities in the absence of proper planning (JICA, 2002; Yabe et al., 2010). Moreover, resources for environmental management in most African countries are limited, as most developmental programs are focused on economic growth and industrialization (Snoussi and Awosika, 1998). As a result,

industrial expansion and increased extraction of natural resources have resulted in widespread heavy metal pollution (Abou-Arab, 2001; Heyden and New, 2004).

Lead Poisoning

Lead is one of the earliest metals used by human and still used in manufacturing industries nowadays. On the other hand, Pb toxicity is also known from Ancient Roman era and causes various non-specific symptoms, and death in the worst case. Currently, Pb concentration in whole blood is used as a main biomarker of Pb exposure and has been broadly used in epidemiological studies. In 2003, a new observation was revealed that blood lead levels (BLLs), even those below 10 µg/dL, are inversely associated with children's IQ scores (Canfield et al., 2003) and Centers for Disease Control and Prevention (CDC) of USA now uses a blood Pb reference value of 5 µg/dL (CDC, 2012). A BLL above 45 µg/dL is considered the level where treatment is required (CDC, 2002; Needleman, 2004), and a BLL above 100 µg/dL is considered a fatal level in children, which causes serious clinical symptoms such as encephalopathy, even in adults (Meyer et al., 2008; NAS, 1972). Children are more susceptible than adults to the effects of lead exposure because a proportionately greater amount of the lead they ingest is absorbed, more circulating lead enters their brain, and their developing nervous system is more vulnerable to lead's toxic effects (Lidsky and Schneider, 2003; Meyer et al., 2008). Therefore, children, especially infants should be protected from lead exposure for their health and the future.

Pb poses a serious public health concern in both developed and developing countries, that accounted for 0.6% of the Global Burden of Disease (WHO, 2010). In Africa, there were two large incidents of lead poisoning with children deaths in Dakar, Senegal (Haeffliger et al., 2009) and Zamfara, Nigeria (Ajumobi et al., 2014). In Flint, Michigan, USA, Pb contamination of the water supply resulted in state and federal state-of-emergency

declarations in 2016 (Ruckart et al., 2019). The Agency for Toxic Substances and Disease Registry (ATSDR) listed Pb as the second substance in the Substance Priority List in 2017, which was compiled to determine the most significant potential threat to human health (ATSDR, 2017). Lead exposure primarily occurs via ingestion and inhalation, and can be traced to numerous sources, including gasoline, battery recycling, paint, and mining (Calabrese and Stanek, 1995; Meyer et al., 2008; Schoning et al., 1996; Yabe et al., 2010).

As in humans, Pb poisoning in animals including wildlife, livestock and pet has also been reported (Bates, 2018; Ishii et al., 2017; Nakata et al., 2015). Lead poisoning in animals cause harmful effects in their gastrointestinal and central nervous systems as it does in humans. The concentration of Pb in the whole blood can also be used to confirm exposure of animals. Moreover, Pb concentrations in tissues are also analyzed in animal studies, whereas Pb concentrations in tissues are difficult to analyze in human studies due to ethical impediments. The sources of Pb exposure of animals are similar to those for humans (Bates, 2018): therefore, the risk of Pb poisoning in animals must also be considered.

To reduce Pb exposure and prevent further exposure, identifying and eliminating the source of Pb exposure is important. Pb has four stable isotopes: ^{208}Pb , ^{207}Pb , ^{206}Pb , and ^{204}Pb . The compositions of these isotopes are not affected to a measurable extent by physicochemical fractionation processes (Bollhöfer and Rosman, 2001; Veyseyre et al., 2001). Therefore, Pb isotope ratios are currently used to determine the source of Pb (Binkowski et al., 2016; Cao et al., 2014).

Lead poisoning in Kabwe

Kabwe is the capital city of the Zambian central province. The lead-zinc mining in Kabwe was operational for over 90 years with no pollution laws regulating emissions from the mine. The mine has left the city with poisons and hazardous concentrations of Pb in biota,

soil, and water. High Pb and other metal contamination in soils around the mine have been reported (Nakayama et al., 2011; Tembo et al., 2006). Moreover, high Pb exposure in wild rats, cattle, chicken, and goats were found (Nakata et al., 2016; Nakayama et al., 2011; Yabe et al., 2013, 2011). Elevated blood Pb concentrations in children near the mine, for whom all samples exceeded the 5 µg/dL blood Pb reference value (CDC, 2012), were also found (Yabe et al., 2015). Pb exposure in Kabwe, Zambia is a serious public concern. However, the scale of Pb pollution in Kabwe have not been determined yet because the previous studies mainly focused on Pb exposure on animals and humans near the mine. Moreover, the sources and routes of Pb exposure on animals and humans have not been determined yet. To control Pb exposure in Kabwe, understanding these kinds of information is important. While high elevated BLLs in children more than a fatal level have been reported, no clinical studies of Pb poisoning have been implemented in Kabwe. Compared to the acute Pb poisoning outbreaks with death cases in Africa (Ajumobi et al., 2014; Haeffliger et al., 2009), local people in Kabwe could be chronically exposed to Pb. There could be some differences of the toxicity between acute and chronic Pb exposure. Moreover, local people in Kabwe might have some biological defense mechanism to reduce Pb toxicity such as metal binding proteins. Thus, revealing the mechanism could be useful and applicable for Pb pollution not only in Kabwe, but also in other areas. Therefore, further field studies are necessary to understand and solve Pb exposure in humans and animals in Kabwe.

The Objectives of My PhD Thesis

The aim of this study is to contribute to the settlement of Pb pollution in a lead mining area, Kabwe, Zambia. Firstly, this thesis was conducted to understand the current scale of Pb exposure on animals and humans in Kabwe, Zambia and related factors such as distance from the mine and the differences of sites. Moreover, the determination of the sources and routes

of Pb exposure on humans and animals using Pb isotope analysis were carried out to control the exposure. The effects of Pb exposure on humans and animals were evaluated to determine the health impact.

Structure of this thesis

This thesis consists of two chapters.

Chapter1: Factors associated with lead (Pb) exposure on dogs around a Pb mining area, Kabwe, Zambia

Pets share similar risk factors, including the areas of habitation and living conditions with their owners. Dogs have been reported as a useful sentinel for Pb exposure in humans. They can be used a feasible, low-cost alternative to a large-scale survey of humans (Kucera, 1988). Dogs are commonly kept as a guardian in Kabwe. In this study, I focused on the current circumstance of Pb exposure, the potential source of Pb exposure, and the health impact of Pb exposure in dogs.

This content was announced in the following journals.

- Toyomaki et al., 2020. Factors associated with lead (Pb) exposure on dogs around a Pb mining area, Kabwe, Zambia. Chemosphere 125884.
<https://doi.org/10.1016/j.chemosphere.2020.125884>

Chapter2: Lead concentrations and isotope ratios in blood, breastmilk, and feces in humans: Contribution of both lactation and soil/dust exposure to infants in a lead mining area, Kabwe, Zambia

Children, especially infants are more vulnerable to Pb. Therefore, it is necessary to prevent Pb exposure in infants. Breastmilk is important for infants, to ensure normal development and to prevent infectious diseases. However, Pb can be transferred from the maternal blood to breastmilk. Breastfeeding from mothers with high BLLs could be a potential source of Pb exposure in infants. Based on the foregoing, blood, breastmilk and infants' feces were collected from infants and mothers in Kabwe, Zambia to determine the Pb exposure and the contribution of lactation as one of the exposure pathways to infants. Moreover, the health impacts of Pb exposure on mothers were evaluated.

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CHAPTER 1

Factors associated with lead (Pb) exposure on dogs around a Pb mining area, Kabwe, Zambia

Abstract

Lead (Pb) poisoning is a serious public health concern and dogs have been useful as a sentinel animal for Pb exposure of humans. In the present study, the blood Pb concentrations, isotope ratios ($^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$), and biochemistry of 120 domestically owned dogs living around a Pb mining area, in Kabwe, Zambia were analyzed to determine factors associated with Pb exposure. The overall mean value of Pb in dog blood in the present study was 271.6 $\mu\text{g/L}$. The Pb concentrations in the dogs from sites near the mine were significantly higher than those in the dogs from a site 4 km from the mine ($352.9 \pm 205.1 \mu\text{g/L}$ versus $28.0 \pm 13.9 \mu\text{g/L}$). The blood Pb concentrations significantly decreased with both increasing age of the dogs and distance from the mine. The Pb isotope ratios in the blood of the dogs that resided near the mine showed values similar to those reported at the galena mine in Kabwe, which is considered to be the source of Pb exposure. In contrast to the high metal exposure that was determined in these dogs, the mean values of most analyzed parameters in the blood biochemical analysis were surprisingly within or close to the standard reference values. Moreover, none of the dogs showed overt signs of Pb poisoning or other clinical symptoms. The results of analysis of Pb exposure of the dogs obtained in the present study, which are similar to the previously reported results in human in this location, suggest that dogs could be useful as a sentinel animal for Pb exposure of humans in Kabwe.

Keywords: Dog, Sentinel animal, Lead poisoning, Lead stable isotope, Metal, Zambia

Highlight

- Lead (Pb) levels in blood of 120 dogs around a Pb mining area, Kabwe were measured.
- The overall mean of Pb in dog blood in the present study was 271.6 µg/L.
- Pb levels significantly decreased with increasing age and distance from the mine.
- Pb isotope ratios in blood showed values close to those reported for Kabwe galena.
- Dogs could be useful as a sentinel animal of Pb exposure on human in Kabwe.

1. Introduction

Lead (Pb) poisoning is a serious public health concern, that accounted for 0.6% of the Global Burden of Disease (WHO, 2010) and 540,000 deaths worldwide (IHME, 2017). The Agency for Toxic Substances and Disease Registry (ATSDR) listed Pb as the second substance in the Substance Priority List in 2017, which was compiled to determine the most significant potential threat to human health (ATSDR, 2017). Pb exposure primarily occurs via ingestion and inhalation (Calabrese and Stanek, 1995; Schoning et al., 1996). Numerous sources of Pb exposure are known, including gasoline, smelters, battery recycling, paint, and mining (Meyer et al., 2008; Yabe et al., 2010). Eliminating the source of Pb exposure is necessary to reduce and prevent further exposure. Pb has four stable isotopes: ^{208}Pb , ^{207}Pb , ^{206}Pb , and ^{204}Pb . The compositions of these isotopes are not affected to a measurable extent by physicochemical fractionation processes (Bollhöfer and Rosman, 2001; Veyseyre et al., 2001). The $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios are well known to be useful in determining the source of Pb exposure (Binkowski et al., 2016; Cao et al., 2014).

The Pb concentration in whole blood is the main biomarker that is used to monitor the exposure; it has been widely used in epidemiological studies. Symptoms of Pb poisoning are varied, including anemia, vomiting, nephropathy, encephalopathy,

and, in the worst case, death (Meyer et al., 2008). At low-level concentrations, Pb exposure can cause neurodevelopmental impairment such as a reduction of the intelligence quotient (IQ) in children. Therefore, the Centers for Disease Control and Prevention (CDC) has set 5 µg/dL of Pb in the whole blood as the blood Pb reference value for Pb exposure (CDC, 2019, 2012).

As in humans, Pb poisoning in companion animals such as dogs and cats has also been reported (Bates, 2018). Lead poisoning in companion animals affects their gastrointestinal and central nervous systems as it does in humans. The concentration of Pb in the whole blood can also be used to confirm exposure of companion animals and a toxic concentration is generally considered to be > 400 µg/L (Bates, 2018). The sources of Pb exposure of animals are similar to those for humans: therefore, the risk of Pb poisoning in animals must also be considered. Companion animals, which share similar risk factors, including the areas of habitation and living conditions with their owners, have been useful as sentinels for toxic substances and infectious diseases (Rabinowitz et al., 2006; Sévère et al., 2015). Thomas et al. (1976) reported an association between high blood Pb concentrations in dogs and children from the same family. Moreover, the socioeconomic characteristics of the dog-owning family were reported to be reliably associated with abnormally high blood Pb concentrations in dogs (Thomas et al., 1975).

Dogs have been reported as a useful indicator of Pb exposure for humans. They can be used as a feasible, low-cost alternative to a large-scale survey of humans (Kucera, 1988).

The lead-zinc (Zn) mine in Kabwe, Zambia was operational for over 90 years without adequate pollution laws regulating emissions from the mine, and it was closed in 1994. These mining operations have left the city with hazardous concentrations of Pb in the biota and soil. The high accumulation of Pb and other metals in various environmental and biota samples has been reported in Kabwe (Nakayama et al., 2011; Yabe et al., 2013, 2011). High blood Pb concentrations in children near the mine, for whom all samples exceeded the 5 µg/dL blood Pb reference value (CDC, 2019, 2012), were also found (Yabe et al., 2015). Further studies are necessary to determine the scale and impact of Pb poisoning in Kabwe; however, large-scale surveys of humans possess some difficulties such as cost and use of human resources. In Kabwe, guard dogs are commonly owned and could be exposed to a similar amount of Pb as humans. Pb residues in dogs can serve as a potential indicator for predicting the extent of Pb contamination in the environment and Pb exposure of their owners. However, Pb exposure of dogs in Kabwe has not yet been reported.

Therefore, the present study was undertaken to assess the trends of Pb and other metals in the blood of domestic dogs residing in areas around the mine for use as

sentinel animals. The factors associated with their exposure to Pb and other metals in Kabwe, Zambia are also discussed. The Pb isotope ratios of $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ in the blood of dogs were analyzed to determine the source of Pb exposure. Moreover, a blood biochemical analysis was conducted to evaluate the health impact of Pb exposure of dogs.

2. Materials and Methods

2.1 Sampling Sites

Kabwe is the provincial capital of Zambia's Central Province and is located at about 28°26'E and 14°27'S . It is the fourth largest town in Zambia, with a population of about 230, 000 inhabitants and an area of 1, 547 km². Kabwe has a long history of open-pit Pb-Zn mining that lasted from 1902 to 1994. Despite the closure of this mine, metal scraps from the abandoned tailings and wastes stored in the mine have continued to serve as sources of metal pollution, especially dusts that emanate from the mine dumps. Moreover, the high Pb exposure of some households within 500 m of the tailings and the residential areas close to the mine could result from these sources of metal pollution. The present study was conducted in three sites near the mine (Kasanda, Mutwe Wansofu, and Chowa) and one site far from the mine (Lukanga) in June and July of 2016 (Fig. 1).

2.2 Sampling

We conducted a sensitization campaign in which dog owners from the selected townships were requested to take their dogs to designated locations within their townships for free rabies vaccination programs, which acted as part of the recruitment

strategy for this study. The rabies vaccinations were performed by government personnel from the Kabwe District Veterinary Office. Only dogs with owners that willingly agreed to participate in the present study were used in the sample collection. The dog owners were also interviewed to obtain necessary information about their dogs such as age, sex, and breed.

Blood sample of up to 10 mL were collected from the cephalic veins of each dog into heparinized blood collection tubes for laboratory analysis. To avoid sample contamination, all sample collection supplies were stored in plastic Ziploc storage bags before sampling. The venipuncture site was carefully cleaned and sanitized with an ethanol swab to avoid contamination before sample collection. The blood samples were transported to the laboratory and the plasma samples were separated after they were centrifuged. The processed samples were kept at -20°C at the University of Zambia, School of Veterinary Medicine, and transported to Japan in temperature-controlled boxes with ice packs. The samples were analyzed in the Toxicology Laboratory at the Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan (Approval Number: Vet-17010).

2.3 Pb and Metal Concentration Analysis

The extraction of Pb and other metals (cadmium (Cd), nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), cobalt (Co), and arsenic (As)) from the whole blood samples was performed. In brief, 1 mL of each blood sample was placed in prewashed digestion vessels, followed by acid digestion using 5 mL of two-fold diluted ultrapure nitric acid (Cica reagent, Specific gravity of 1.38, 60%; Kanto Chemical Corp.) and 1 mL of ultrapure hydrogen peroxide (Cica reagent, 30%; Kanto Chemical Corp.). Microwave digestion was performed using a Speedwave MWS-2 (Berghof) according to the instruction of the manufacturer, as has been described previously (Yabe et al., 2015). The concentrations of Pb and other metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, 7700 series; Agilent Technologies, Tokyo, Japan). Analytical quality control was conducted using DORM-3 (fish protein; National Research Council of Canada, Ottawa, Canada) and DOLT-4 (dogfish liver; National Research Council of Canada) certified reference materials. Replicate analyses of these reference materials showed good recovery rates (95–105%) with an instrument detection limit of 0.001 µg/L.

2.4 Pb Stable Isotope Analysis

The sample dissolution procedure was similar to a previously described method (Kuritani and Nakamura, 2002; Nakayama et al., 2019). The extracted solutions of blood were transferred into Teflon tubes after the Pb concentrations were analyzed. $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios were determined using a multiple collector (MC)-ICP-MS (Neptune Plus, Thermo Finnigan, California, USA) in static mode with Faraday cup configuration. The other general parameters are provided in Table 1.

2.5 Blood biochemical analysis

A conventional blood chemical analyzer (COBAS Ready; Roche Diagnostic Systems, Basel, Switzerland, and Spotchem panels I and II; Arkray, Kyoto, Japan) was used to analyze the concentrations of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), total bilirubin (T-Bil), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), and urea acid (UA) in the plasma. The standard reference ranges for each parameter of the Spotchem Series in dogs were provided by Arkray, Kyoto, Japan.

2.6 Statistical analysis

All data from the experiments and questionnaire were combined into a single electronic database. All statistical analyses were performed at a significance level of $p < 0.05$ using R version 3.4.3 and JMP 13.1.0 (SAS Institute, USA). Mean values were indicated in addition to standard deviation (SD) values. The collinearity between factors was analyzed using Spearman's rank correlation test. A Steel–Dwass multiple comparison test was used to compare the differences between the factors among differing areas.

3. Results

3.1 Characteristics of Dogs in the Present Study

A total of 120 domestic dogs were sampled in the present study, with 90 coming from the three sites near the mine and 30 from Lukanga which is located farther from the mine (Table 2). All of the dogs were crossbreeds with a mean age of 32.0 ± 24.3 months. There was a significant difference in age between the dogs from Kasanda and Chowa ($p < 0.01$). The mean distance between the mine and the location of the dogs was 2.58 ± 1.83 km. There was a significant difference in the distances from the mine between the dogs from sites near the mine (Kasanda, Mutwe Wansofu, and Chowa) and from Lukanga ($p < 0.01$). None of the dogs had overt signs of lead poisoning or other clinical symptoms.

3.2 Pb and Other Metals Concentrations in Blood of Dogs

The overall mean of Pb concentrations in dog blood in the present study was $271.6 \mu\text{g/L}$ (Table 1). Of the 120 dogs sampled, 24% (29/120) were above the toxic level for companion animals, which is $400 \mu\text{g/L}$ (Fig. 2). The mean concentration of Pb in the blood of the dogs from Kasanda ($525.3 \mu\text{g/L}$) was above the toxic level. On the other hand, the mean concentration of Pb in the blood of the dogs from Lukanga was

28.0 µg/L, and all dogs from Lukanga showed concentrations below the toxic level. The mean of all metals in the dogs residing at the three sites near the mine were significantly higher than those in the dogs from Lukanga ($p < 0.01$). Most of the other metal (Cd, Ni, Cr, Co, and As) concentrations in the dogs from Kasanda were significantly higher than those concentrations determined in other areas, except for Pb, Cu, and Zn. In contrast, all metal concentrations in the dogs from Lukanga were significantly lower than those from all other sites, except for Co.

There was no significant difference in the metal concentrations in blood between male and female dogs in all locations as well as in the dogs from sites near the mine. However, the Pb ($p = 0.015$), Cr ($p = 0.015$), and Cu ($p < 0.01$) concentrations in male dogs were significantly higher than those in female dogs from Chowa. The Cd concentrations in male dogs from Mutwe Wansofu ($p = 0.059$), Chowa ($p = 0.09$), and Lukanga ($p = 0.06$) were higher than the concentrations in female dogs, but not significantly. In contrast, the Pb concentrations in female dogs were significantly higher than those in male dogs from Kasanda ($p = 0.047$). The Ni concentrations in female dogs were also higher than those in male dogs from Lukanga ($p = 0.094$).

In all dogs, there were significant positive correlations among all metals (Table 3, $p < 0.05$). Most metal concentrations ($p < 0.05$) significantly decreased with

increasing age, except for Cu ($p = 0.14$, Spearman's $\rho = -0.13$) and Cd ($p = 0.07$, $\rho = -0.17$). All of the metals concentrations in the dogs significantly decreased with increasing distance between the mine and the location of the dogs ($p < 0.05$). Fig. 3 and 4 respectively show the relationships between the Pb concentrations and the age, and the Pb concentrations and distance from the mine.

In the dogs from sites near the mine, there were also significant positive correlations among all of metals (Table 4, $p < 0.05$). The concentrations of all metals in the dogs significantly decreased with increasing age and distance from the mine ($p < 0.05$). There was a significantly positive correlation between the age of the dog and distance from the mine ($p < 0.05$, $\rho = 0.21$).

In the dogs from Lukanga, there were significant positive correlations between Pb and Zn ($p < 0.01$, $\rho = 0.50$), as well as between Ni and Cr ($p < 0.01$, $\rho = 0.60$) (Table 5). The As concentrations were found to be nearly significantly correlated with Co ($p = 0.09$, $\rho = 0.31$) and Zn ($p = 0.09$, $\rho = -0.31$) concentrations. The Cd concentrations were almost significantly correlated with Cr ($p = 0.07$, $\rho = 0.33$) and Co ($p = 0.07$, $\rho = 0.34$) concentrations. The Pb concentrations decreased with the age of the dog, but not significantly ($p = 0.052$, $\rho = -0.36$). The concentrations of Pb ($p < 0.05$, $\rho = -0.42$) and Cu ($p < 0.05$, $\rho = -0.42$) were significantly correlated with the distance from the mine.

The Ni concentrations increased with the distance, but not significantly ($p = 0.059$, $\rho = 0.35$).

3.3 Pb Isotope Ratio Analysis

The mean values of the $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in the blood of all dogs were 2.129 ± 0.006 and 0.8727 ± 0.003 , respectively (Table 6). Both Pb isotope ratios in the blood of the dogs from sites near the mine ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.131 ± 0.003 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8734 ± 0.0014) were significantly different from those of the dogs from Lukanga ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.122 ± 0.007 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8708 ± 0.0047 , $p < 0.05$). The SD values of the isotope ratios of the dogs from sites near the mine were smaller than those of the dogs from Lukanga, indicating small individual differences in the isotope ratios of the dogs from sites near the mine. Both ratios in the blood of the dogs from sites near the mine (Fig. 5, 6, and 7), especially from Kasanda ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.133 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8735) showed values similar to those reported for Kabwe galena ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.134 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8731, Kamona et al., 1999).

3.4 Blood Biochemical analysis

Table 7 shows the mean values of the blood biochemical analysis. The means values of all parameters in the dogs in the present study were within the reference range or slightly higher. The LDH concentrations in the dogs from Lukanga (279.9 IU/L) was significantly higher than that in the dogs from sites near the mine (192.0 IU/L, $p < 0.01$). The GGT ($p = 0.08$) and Alb ($p = 0.052$) concentrations in the dogs from Lukanga were higher than those in the dogs from sites near the mine, although these differences were not significant. There was no significant difference in the blood biochemistry parameters between male and female dogs.

In all dogs in the present study, there was a significant positive correlation between Pb and ALP concentrations (Table 8, $p = 0.047$, $\rho = 0.18$). The LDH ($p = 0.08$, $\rho = -0.16$), BUN ($p = 0.08$, $\rho = -0.16$), and Cre ($p = 0.07$, $\rho = -0.17$) concentrations decreased as the Pb concentrations increased, but not significantly.

In the dogs from sites near the mine, the Pb concentrations were significantly associated with increased ALT ($p < 0.01$, $\rho = 0.29$), ALP ($p < 0.01$, $\rho = 0.29$), and AST ($p = 0.014$, $\rho = 0.29$) concentrations. On the other hand, a significant negative correlation between Pb and BUN concentrations ($p < 0.05$, $\rho = -0.26$) was observed.

The Cre ($p = 0.07$, $\rho = -0.20$) and UA ($p = 0.099$, $\rho = -0.20$) concentrations almost significantly decreased as Pb concentrations increased.

In the dogs from Lukanga, the ALP concentrations almost significantly increased with Pb concentrations ($p = 0.06$, $\rho = 0.35$), as was found in other groups.

There was also a significantly negative correlation between Pb and TP ($p < 0.05$, $\rho = -0.39$) in the dogs from this area, while BUN ($p = 0.06$, $\rho = -0.36$) and Cre ($p = 0.09$, $\rho = -0.32$) appeared to decrease with Pb concentrations increased, but not significantly.

4. Discussion

In the present study, high blood Pb concentrations (271.6 $\mu\text{g/L}$) have been recorded in dogs in townships around the mine in Kabwe, wherein cases of Pb poisoning in animals and humans have been reported (Nakayama et al., 2011; Yabe et al., 2015, 2013, 2011). Of all dogs that were surveyed, 24% (29/120) had Pb concentrations in their blood that are above the toxic level (400 $\mu\text{g/L}$) for Pb exposure in companion animals (Bates, 2018). The Pb concentrations in the dogs from sites near the mine that were determined in a present study (352.9 $\mu\text{g/L}$) were similar to or higher than the blood Pb concentrations in polluted sites in other countries where the concentrations ranged from 28.3 to 262 $\mu\text{g/L}$ (Balagangatharathilagar et al., 2006; Brownie et al., 2009; Thomas et al., 1975). In the present study, elevated Pb concentrations in the blood were found in the dogs from Kasanda, with the highest concentration being 1233.5 $\mu\text{g/L}$. Several studies have reported severe clinical cases of Pb poisoning due to elevated blood Pb concentrations in dogs, such as those found in Kasanda in the present study (Hamir et al., 1985; King, 2016; Langlois et al., 2017). These findings suggest that Pb exposure of the dogs residing in sites near the mine in Kabwe, Zambia, is a serious health risk. On the other hand, the dogs from Lukanga had lower mean blood Pb concentrations (28.0 $\mu\text{g/L}$), which agree with the results obtained

for dogs from unpolluted areas in previous reports (Balagangatharathilagar et al., 2006; Brownie et al., 2009; Thomas et al., 1975).

The present study found different trends of Pb and other metals concentrations in the blood of dogs among the different observed areas in Kabwe. The mean blood Pb concentrations in the dogs from Kasanda (525.3 $\mu\text{g/L}$), was higher than that in the dogs from Mutwe Wansofu (392.3 $\mu\text{g/L}$), whereas the locations of the dogs from Mutwe Wansofu (0.85 km from the mine) were closer to the mine than those of the dogs from Kasanda (1.09 km from the mine). Kasanda is located on the western side of the mine, which is in the direction of the prevailing winds. The accumulation of metal in the dogs from Chowa (2.00 km from the mine) were lower than those in the dogs from the other two sites that were located near the mine. A similar trend was also found in a previous study of the children in these arears, in which the mean blood Pb concentration in the children in Kasanda (822 $\mu\text{g/L}$) was higher than that in Chowa (390 $\mu\text{g/L}$, Yabe et al., 2015). In the present study, the Pb concentrations in dog blood significantly decreased with increasing distance from the mine, as did the concentrations of other metals. These trends agree with those determined in studies by Tembo et al. (2006) and Nakayama et al. (2011), who reported similar trends in the soil in Kabwe. Therefore, these trends suggest that the location of the townships in the relation to the wind direction and

distance from the mine are key factors that influence the severity of the exposure to Pb and other metals in dogs in Kabwe. The obtained results also suggest that the exposure to Pb and other metals in dogs remarkably decreased about 5 km away from the mine. Therefore, severe metal exposure in Kabwe may only occur in areas near the mine.

The significant positive correlations between Pb and other metals in the blood of the dogs analyzed in the current study agreed with the findings of previous studies that reported high concentrations of Pb and other metals in both soil and animal samples in Kabwe (Nakata et al., 2016; Nakayama et al., 2011; Tembo et al., 2006; Yabe et al., 2013, 2011). Moreover, Kamona and Friedrich (2007) reported that the ore in Kabwe contained sphalerite (ZnS) with Cd, galena (PbS), bismutite [$\text{Cu}_2(\text{Fe,Zn})\text{GeS}_4$], and mimetite [$\text{Pb}_5(\text{AsO}_4)_3\text{Cl}$]. These findings suggest that in addition to Pb, other toxic metal contaminants including As and Cd, may spread into the environment and pose a health risk to humans and animals. However, the toxicity of other metals in dogs is unknown, although many studies concerning Pb poisoning in dogs have been reported. Therefore, the health risks associated with the exposure of dogs in Kabwe to other metals should be further investigated.

The mean Pb concentrations in the blood of dogs from Kasanda and Chowa in the present study were 525.3 and 246.5 $\mu\text{g/L}$, respectively. The concentrations were

lower than the findings of a previous study examining children in the same township which reported the mean blood Pb concentrations in children from Kasanda and Chowa as 822 and 390 $\mu\text{g/L}$, respectively (Yabe et al., 2015). This result agrees with the findings of Thomas et al. (1976) who reported higher blood Pb concentrations in children than those in dogs from the same household environment. Therefore, children may be more vulnerable to Pb exposure than dogs are. Thomas et al. (1976) also reported that an abnormally high blood Pb concentration in a family dog increase by six-fold the probability that at least one child in that family would also have an abnormally high blood Pb concentration. Therefore, children from households with dogs that have high blood Pb concentrations should be examined for Pb exposure.

Significant negative correlations between the blood Pb concentrations and age of the dogs were found, indicating that the concentrations of blood Pb reduced as the dog age. Langlois et al. (2017) reported a similar trend in the Pb poisoning outbreak in Flint, Michigan, wherein the blood Pb concentrations in young dogs (≤ 2 years of age) were higher than those in older dogs (≥ 6 years of age). This trend was similar to that found in humans, as significant negative correlations were observed between the blood Pb concentrations and ages of children in Kabwe (Yabe et al., 2015). The hand-to-mouth or object-to-mouth (pica) behavior of children are known to be factors related to high Pb

exposure. In addition, children absorb a greater proportion of ingested Pb from the gastrointestinal tract than adults do (Wani et al., 2015). Therefore, similar behaviors and high absorbances from the gastrointestinal tracts of young dogs may also be attributed to high Pb exposure of young dogs compared with older dogs. Pb poisoning during a vulnerable period of development can lead to negative neurodevelopmental impacts such as a low IQ and cognitive impairments in humans (ATSDR, 2007; Lanphear et al., 2005). Mielke and Zahran (2012) found a strong association between air Pb concentrations and the latent aggravated assault rate at the scale of city. This finding provides insight into latent behavioral effects resulting from environmental Pb exposure of children that were subjected to lead dust during their most sensitive developmental years. Pb exposure of dogs might also results in negative developmental and behavioral impacts such as increased aggression. On the other hand, Schoning et al. (1996) reported that the lung dust concentrations of substances including silicate and other metals increased linearly with age in dogs. High Pb accumulation in the organs of adult dogs might occurred even with low blood Pb concentrations, especially when dogs are chronically exposed to Pb because Pb is deposited in bone and has a long half-life.

In the present study, blood Pb concentrations were significantly higher in male dogs than in female dogs from Chowa, but this trend was reversed in dogs from

Kasanda. It was also previously determined that the blood Pb concentrations in boys were significantly higher than those in girls in Makululu Township, which is located on the western side of the mine (Yabe et al., 2015). Yabe et al. (2015) suggested that the different behaviors between boys and girls could be one of the factors contributing to this difference, as boys are more likely than girls to wander farther from home and play near the mine dumps. Considering studies other locations, Koh and Bidge (1986) reported blood Pb concentrations in male dogs that are significantly higher than those in female dogs at four different locations in Australia. On the other hand, several studies did not report any such association between sex and Pb exposure or accumulation (Balagangatharathilagar et al., 2006; Esposito et al., 2019; Serpe et al., 2012; Tomza-Marciniak et al., 2012). The differences in the behaviors of dogs due to differing sex may influence their exposure to Pb, especially in mature male dogs that are likely to travel across wide areas to find female dogs for reproduction, while female dogs do not move around so freely as they need to care for their offspring. However, the sex of dogs might not be strongly associated with exposure to Pb and other metals in the present study.

The $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios in dog blood were analyzed in the present study to determine the source of Pb exposure of the dogs. There were significant

differences in the Pb isotope ratios in the dogs between the sites near the mine ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.131 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8734) and Lukanga ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.122 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8708). Moreover, the isotope ratios in the dogs from Kasanda ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.133 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8735) were significantly different from those in the dogs from Mutwe Wansofu ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.130 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8747) and Chowa ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.130 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8729). With increasing blood Pb concentrations, the variance of the Pb isotope ratios decreased and approached those of the Kabwe galena ($^{208}\text{Pb}/^{206}\text{Pb}$: 2.134 and $^{207}\text{Pb}/^{206}\text{Pb}$: 0.8731), which is considered to be the exposure source (Kamona et al., 1999). Similar trends were found in a previous study of goats and chicken located in Kabwe (Nakata et al., 2016). Moreover, a Pb exposure study on rats that used soils from Kabwe revealed that the Pb isotope ratios in the biological samples of high exposure groups were more similar to those in Kabwe galena (Kamona et al., 1999), than to those of the control and low exposure groups (Nakayama et al., 2019). These results suggest that the source of Pb exposure of dogs in Kabwe could be the galena from the mine. Understanding the route of Pb exposure in dogs is required to minimize their exposure. Future studies should include environmental and biological samples that relate to the various routes of exposure, such as lung and feces sample, for further clarification.

The blood biochemical profiles of the plasma of the dogs in Kabwe were analyzed in the present study. In contrast to the high metal exposure of these dogs, the mean values of most analyzed parameters were surprisingly within or close to the standard reference values. This indicates that Pb exposure in the dogs in Kabwe did not significantly impact their health, as was observed during sampling, in which all sampled dogs appeared healthy. Only the LDH values in the dogs from Lukanga were significantly higher than those of the dogs from sites near the mine; therefore this could not be attributed to Pb exposure related to distance from the mine. However, the ALP concentrations in all dogs as well as the dogs from sites near the mine significantly increased as the blood Pb concentrations increased. There were significant positive correlations between ALT and Pb concentrations, as well as AST and Pb concentrations in the dogs from sites near the mine. These relationships were also almost significant when the dogs from all locations were considered. These results suggest that Pb exposure in dogs may have caused some mild liver damage. Of course, it is difficult to exclude the possibility of other factors or diseases since a detailed questionnaire survey or medical check-up was not performed in the present study. On the other hand, the BUN and Cre concentrations which are indicators of kidney functions, were not elevated and negatively related to blood Pb concentrations in the dogs. High Pb

exposure is known to cause kidney damage in conjunction with an increase the selected biomarkers. However, King (2016) reported that two dogs with elevated blood Pb concentrations had glucosuria and proteinuria in their urinalysis that was consistent with damage to the proximal renal tubules, whereas the BUN and Cre concentrations in the dogs were within the reference range. There is therefore a possibility that Pb exposure could damage and disrupt kidney functions without increasing the BUN and Cre concentrations. The reasoning behind these antithetical results could not be established and would require further studies of dogs using both blood biochemical analysis and urinalysis.

The findings of Pb exposure of dogs obtained in the present study, which were similar to the results of previous studies of humans in Kabwe, suggest that dogs could be a useful sentinel animal for Pb exposure. Although similar trends were seen in humans, a direct relationship between the Pb exposure in dogs and their owners was not determined in the present study. Berny et al. (1995) reported the likelihood of finding one person with a blood Pb concentrations of greater than above 10 was significantly increased when there was one pet with a high blood Pb concentration in the same household. Further studies should focus on the relationship between Pb exposure of dogs and their owners. The present study revealed large individual differences in Pb

exposures observed in sites near the mine. A similar trend was seen in the blood Pb concentrations in children in Kabwe (Yabe et al., 2015). Determining the factors that influence the differing exposures to Pb would be helpful in reducing the Pb exposures observed in this area. Because most dogs in Kabwe roam freely, the difference in exposure levels could be attributed to their different activities. As opposed to humans monitoring, the monitoring of animals has become popular with the development of new monitoring techniques such as GPS. Therefore, future studies may focus on the relationship between Pb exposure of dogs and their behaviors by using a GPS monitoring system.

5. Conclusion

The present study is the first report to reveal high exposures of dogs in Kabwe to Pb and other metals. The locations of the dogs and their ages were related to their Pb and metal exposures. The trends of the exposures of dogs were shown to be largely similar to those previously reported for humans, although some differences between dogs and humans were found. These results suggest that dogs could be useful as sentinel animals for Pb exposure of human residing in Kabwe. Moreover, different trends of exposure among individual dogs were found, and the trends were previously found in humans. The factors contributing to the individual differences of Pb exposure must be investigated to reduce Pb exposure. The source of Pb exposure of dogs was determined to result from the galena in mine in Kabwe. The findings of this study suggest that environmental remediation is urgently needed to reduce Pb exposure in Kabwe.

Figures and Tables

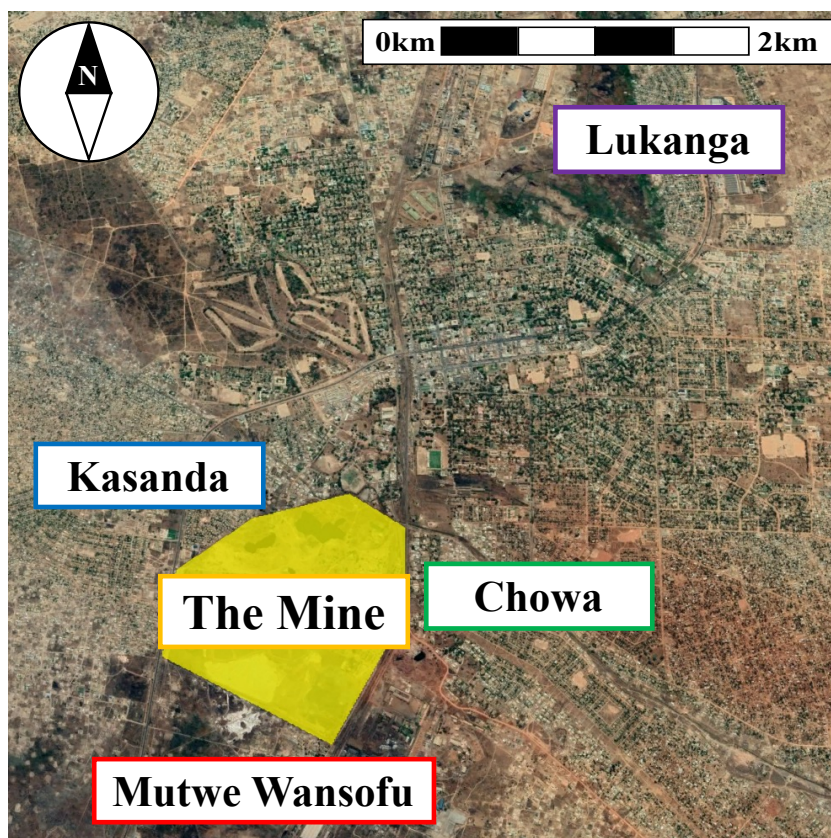


Fig. 1. Map of the sampling areas in Kabwe, Zambia (modified from Google Earth).

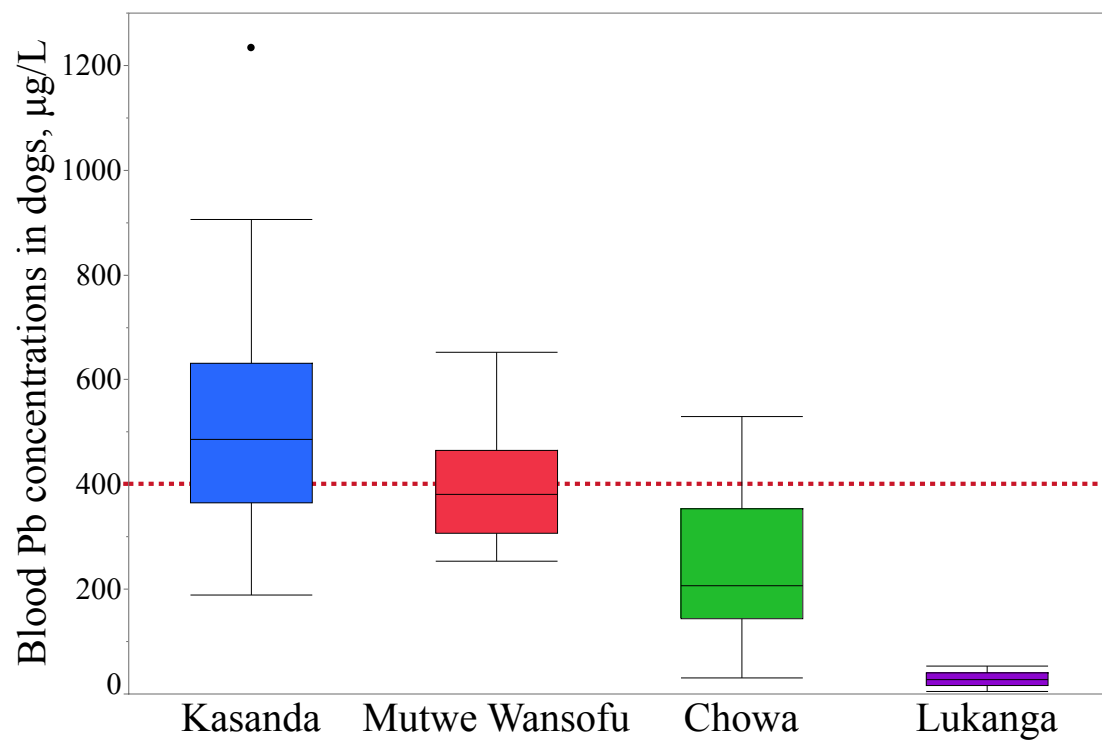


Fig. 2. Blood Pb concentrations in the dogs from Kasanda (blue, $n = 27$), Mutwe Wansofu (red, $n = 14$), Chowa (green, $n = 49$), and Lukanga (violet, $n = 30$). Red dash line shows the toxic level of Pb in whole blood for dogs (Bates, 2018).

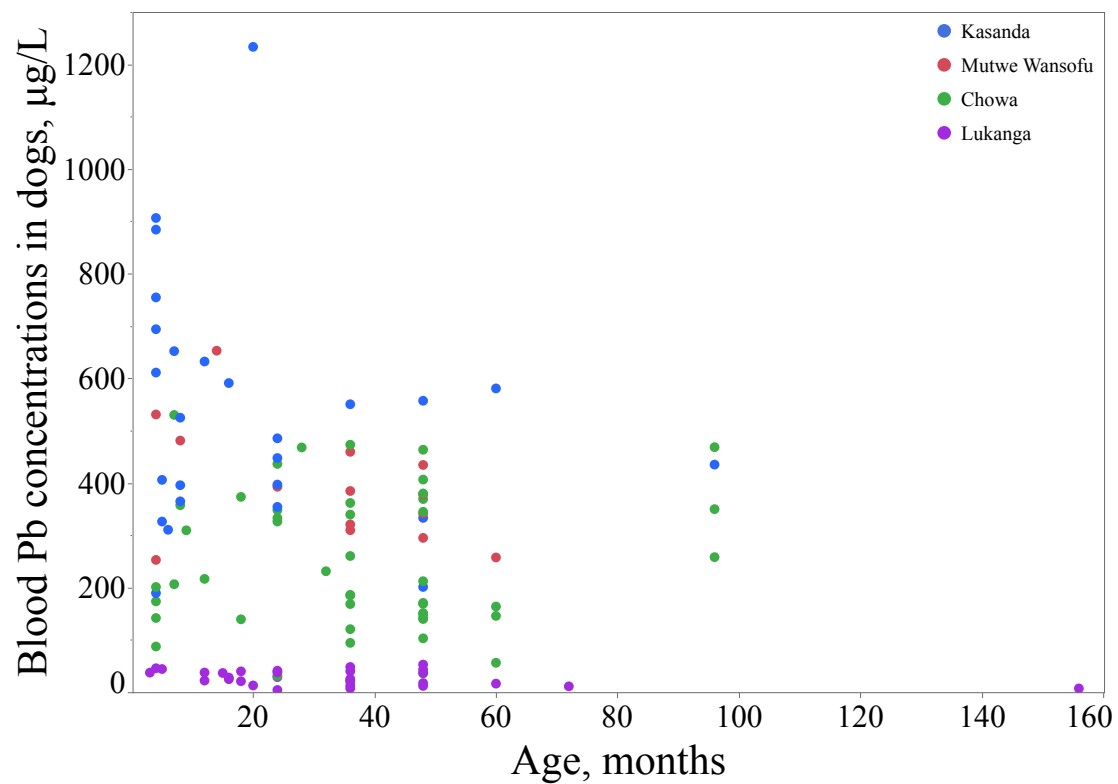


Fig. 3. Relationship between the blood Pb concentrations in the dogs and their age (n = 119). The blue, red, green, and violet circles indicate samples from Kasanda, Mutwe Wansofu, Chowa, and Lukanga, respectively.

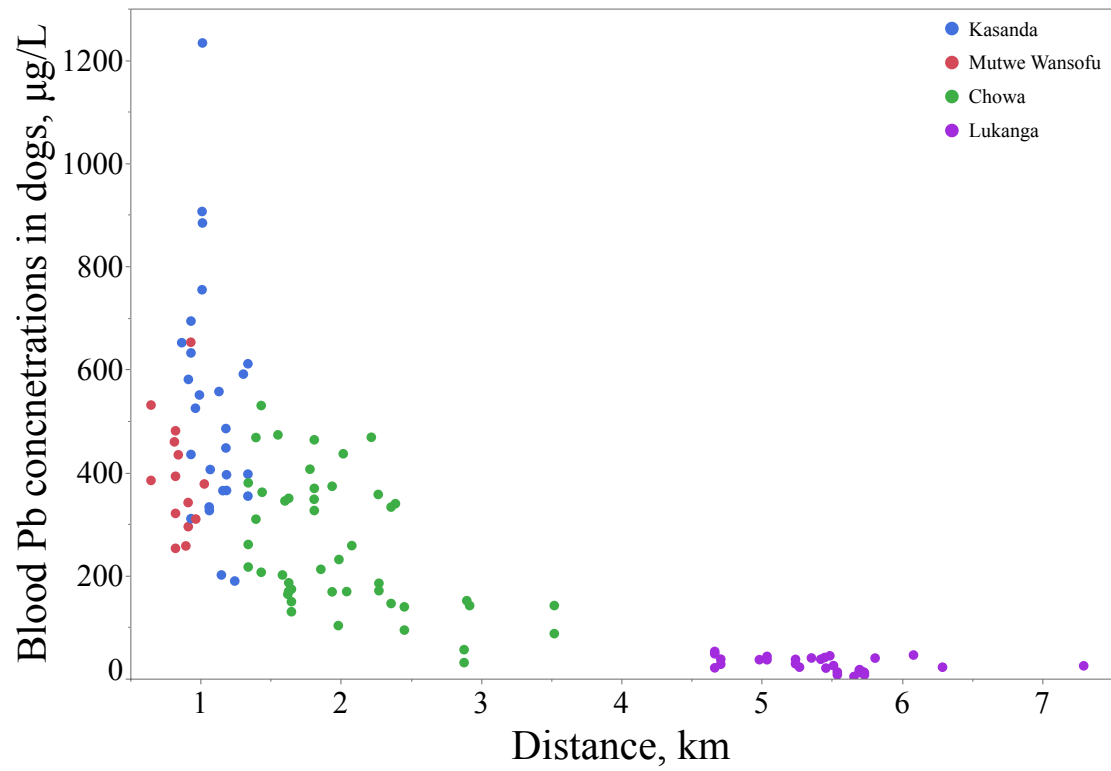


Fig. 4. Relationship between the blood Pb concentrations in the dogs and the distance between the mine and the location of the dogs (km, n = 117). The blue, red, green, and violet circles indicate samples from Kasanda, Mutwe Wansofu, Chowa, and Lukanga, respectively.

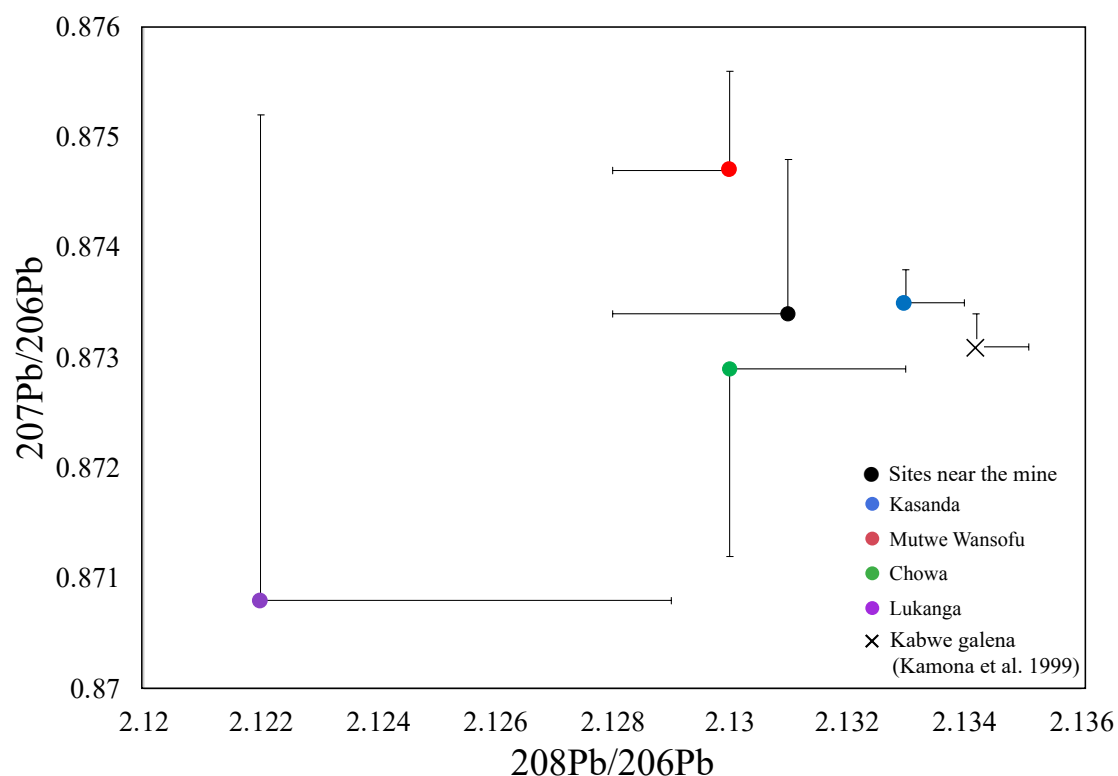


Fig. 5. Pb isotope ratios ($^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$) in the blood of the dogs residing in different areas ($n = 119$). The mean values are shown with error bars indicating the SD.

The blue, red, green, and violet circles indicate samples from Kasanda, Mutwe Wansofu, Chowa, and Lukanga, respectively. The black circle indicates the mean values of samples from three sites near the mine (Kasanda, Mutwe Wansofu, and Chowa). The reference value of Kabwe galena was obtained from a report by Kamona et al. (1999) and is indicated by a cross mark.

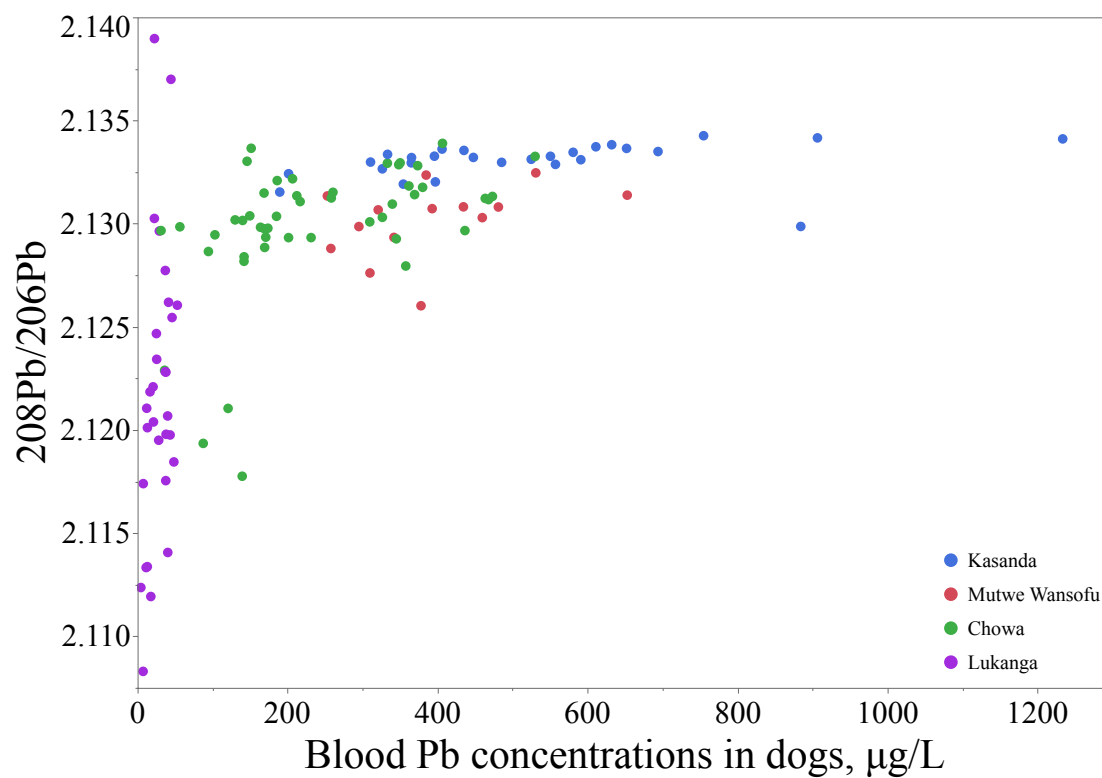


Fig. 6. Relationship between blood Pb concentrations in the dogs and $^{208}\text{Pb}/^{206}\text{Pb}$ isotope ratios (n = 119). Blue, red, green, and violet circles indicate samples from Kasanda, Mutwe Wansofu, Chowa, and Lukanga, respectively.

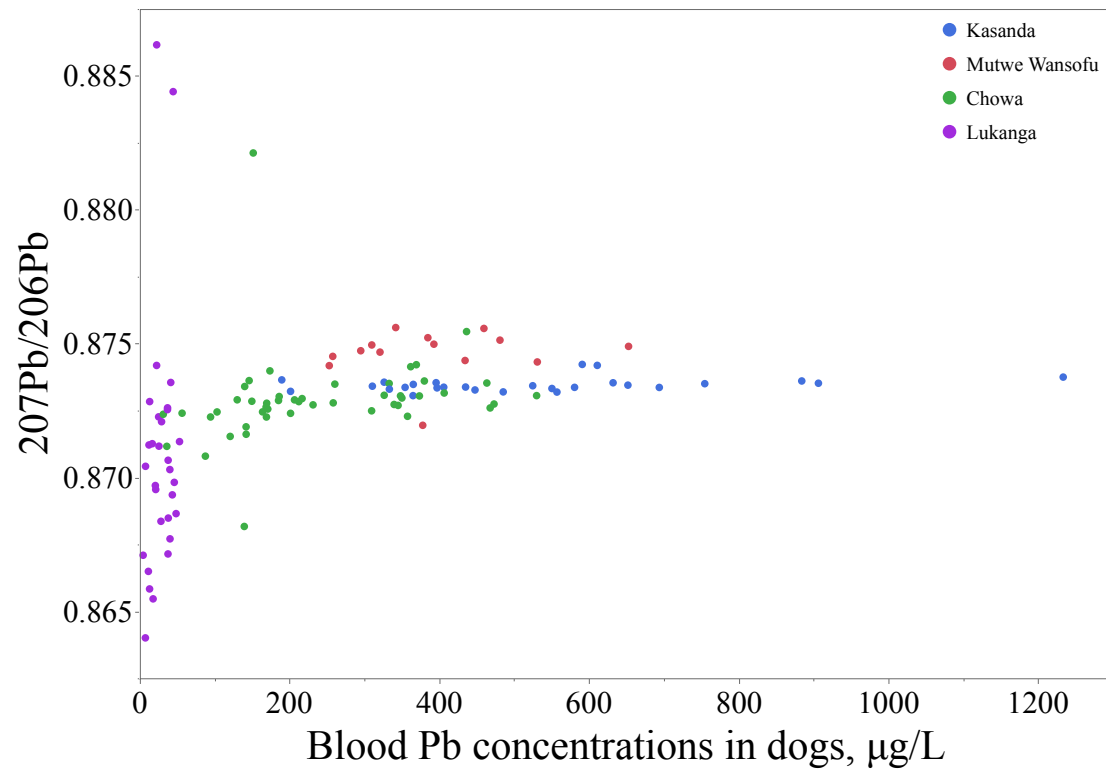


Fig. 7. Relationship between blood Pb concentrations in the dogs and $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios (n =119). Blue, red, green, and violet circles indicate samples from Kasanda, Mutwe Wansofu, Chowa, and Lukanga, respectively.

Table 1. MC-ICP-MS operating conditions for Pb stable isotope analysis.

Detector	Mass
L3	²⁰² Hg
L2	²⁰³ Tl
L1	²⁰⁴ Pb
Center or axial	²⁰⁵ Tl
H1	²⁰⁶ Pb
H2	²⁰⁷ Pb
H3	²⁰⁸ Pb
Frontend parameters	
Resolution	Low
Cool gas (L/min)	16
Auxiliary gas	0.7
Typical sample gas flow (L/min)	1.1-1.2
PFA nebulizer uptake rates (μL/min)	~200
Typical forward Power (W)	1200
Note: L = low mass side, H = high mass side	

Table 2. Characteristics of dogs and metal concentrations in dog blood ($\mu\text{g/L}$) in the present study in Kabwe, Zambia; mean $\pm$ SD values (minimum-maximum).

Area	Sex, Male:Female	Age, months	Distance between the mine and the location of dogs, km	Pb	Cd	Ni	Cr	Cu	Zn	Co	As
Overall (120)	67:52	32.0 $\pm$ 24.3 (3-156)	2.58 $\pm$ 1.83 (0.65-7.30)	271.6 $\pm$ 226.9 (4.3-1233.5)	1.5 $\pm$ 1.6 (0.01-6.1)	86.9 $\pm$ 261.8 (2.5-2054.8)	67.2 $\pm$ 75.4 (2.0-316.6)	1108.8 $\pm$ 634.5 (396.3-3438.0)	6537.1 $\pm$ 3509.9 (1976.5-18394.0)	10.5 $\pm$ 21.6 (0.2-173.7)	5.2 $\pm$ 4.5 (0.6-18.6)
Near the mine (90)	49:40	31.4 $\pm$ 22.9 (4-96)	1.53 $\pm$ 0.65** (0.65-3.52)	352.9 $\pm$ 205.1** (31.2-1233.5)	2.0 $\pm$ 1.6** (0.2-6.1)	114.6 $\pm$ 298.1** (11.0-2054.8)	88.8 $\pm$ 75.9** (11.6-316.6)	1284.3 $\pm$ 643.6** (419.5-3438.0)	7494.6 $\pm$ 3555.8** (2076.4-18394.0)	12.8 $\pm$ 24.6** (0.2-173.7)	6.4 $\pm$ 4.6** (0.6-18.6)
Kasanda (27)	15:12	20.7 $\pm$ 22.4 ^a (4-96)	1.09 $\pm$ 0.14 ^b (0.87-1.34)	525.3 $\pm$ 230.8 ^a (189.5-1235.5)	3.4 $\pm$ 1.0 ^a (1.7-6.1)	213.3 $\pm$ 370.0 ^a (90.6-2054.8)	175.5 $\pm$ 49.9 ^a (100.7-316.6)	1802.9 $\pm$ 530.8 ^a (1043.9-3438.0)	10675.6 $\pm$ 3252.9 ^a (5055.5-18394.0)	16.6 $\pm$ 7.9 ^a (5.4-44.0)	11.5 $\pm$ 2.3 ^a (6.9-18.6)
Mutwe Wansofu (14)	5:9	32.1 $\pm$ 18.4 ^{a, b} (4-60)	0.85 $\pm$ 0.11 ^a (0.65-1.03)	392.3 $\pm$ 112.0 ^a (253.0-652.8)	2.9 $\pm$ 0.4 ^b (2.2-3.4)	199.5 $\pm$ 521.4 ^b (37.9-2007.6)	74.3 $\pm$ 19.6 ^b (54.0-127.2)	1571.1 $\pm$ 146.4 ^a (1246.8-1780.0)	9158.9 $\pm$ 1109.7 ^a (7394.9-11245.7)	8.4 $\pm$ 5.1 ^b (4.3-24.2)	9.2 $\pm$ 1.9 ^b (7.3-12.6)
Chowa (49)	29:19	37.2 $\pm$ 22.6 ^b (4-96)	2.00 $\pm$ 0.55 ^c (1.34-3.52)	246.5 $\pm$ 130.6 ^b (31.2-530.0)	0.9 $\pm$ 1.3 ^c (0.2-5.0)	34.3 $\pm$ 34.1 ^c (11.0-160.4)	44.2 $\pm$ 53.7 ^c (11.6-294.4)	908.9 $\pm$ 539.2 ^b (419.5-2782.3)	5219.9 $\pm$ 2357.3 ^b (2076.4-12633.4)	12.0 $\pm$ 32.8 ^{b, c} (0.2-173.7)	2.7 $\pm$ 2.3 ^c (0.6-8.6)
Lukanga (30)	18:12	33.8 $\pm$ 28.6 ^{a, b} (3-156)	5.44 $\pm$ 0.55 ^d (4.67-7.30)	28.0 $\pm$ 13.9 ^c (4.3-53.0)	0.03 $\pm$ 0.01 ^d (0.01-0.08)	5.0 $\pm$ 1.4 ^d (2.5-8.6)	3.4 $\pm$ 1.1 ^d (2.0-6.8)	588.0 $\pm$ 79.8 ^c (396.3-742.6)	3696.4 $\pm$ 774.1 ^c (1976.5-5196.5)	3.5 $\pm$ 2.7 ^c (1.0-12.4)	1.5 $\pm$ 0.8 ^d (0.6-3.9)

Note: ** indicates a significant difference ($p < 0.01$) between sites near the mine and Lukanga. Different letters indicate a significant difference among areas ($p < 0.05$). In Chowa, the following data was not recorded: the age of one dog, the sex of one dog, and the locations of three dogs (home address).

Table 3. Correlation coefficients (R^2) among metal and factors in all dogs in the present study.

	Pb	Cd	Ni	Cr	Cu	Zn	Co	As	Age	Distance
Pb		0.86**	0.80**	0.83**	0.78**	0.79**	0.52**	0.69**	-0.20*	-0.82**
Cd			0.92**	0.90**	0.90**	0.86**	0.66**	0.80**	NS	-0.83**
Ni				0.95**	0.88**	0.81**	0.67**	0.80**	-0.19*	-0.79**
Cr					0.85**	0.81**	0.64**	0.77**	-0.18*	-0.77**
Cu						0.87**	0.60**	0.77**	NS	-0.76**
Zn							0.57**	0.72**	-0.20*	-0.73**
Co								0.70**	-0.27**	-0.58**
As									-0.29**	-0.75**
Age										NS
Distance										

Note: * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. NS means not significant

Table 4. Correlation coefficients (R^2) among metals and factors in the dogs from sites near the mine.

	Pb	Cd	Ni	Cr	Cu	Zn	Co	As	Age	Distance
Pb		0.72**	0.59**	0.64**	0.68**	0.70**	0.43**	0.62**	-0.30**	-0.59**
Cd			0.85**	0.78**	0.92**	0.89**	0.61**	0.79**	-0.29**	-0.63**
Ni				0.90**	0.86**	0.83**	0.65**	0.79**	-0.32**	-0.54**
Cr					0.81**	0.80**	0.61**	0.77**	-0.32**	-0.49**
Cu						0.92**	0.81**	0.76**	-0.27*	-0.61**
Zn							0.80**	0.75**	-0.32**	-0.59**
Co								0.68**	-0.42**	-0.48**
As									-0.36**	-0.70**
Age										0.21*
Distance										

Note: * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. NS means not significant.

Table 5. Correlation coefficients (R^2) among metals and factors in the dogs from Lukanga.

	Pb	Cd	Ni	Cr	Cu	Zn	Co	As	Age	Distance
Pb		NS	NS	NS	NS	0.50**	NS	NS	NS	-0.42*
Cd			NS	NS	NS	NS	NS	NS	NS	NS
Ni				0.60**	NS	NS	NS	NS	NS	NS
Cr					NS	NS	NS	NS	NS	NS
Cu						NS	NS	NS	NS	0.42*
Zn							NS	NS	NS	NS
Co								NS	NS	NS
As									NS	NS
Age										NS
Distance										

Note: * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. NS means not significant.

Table 6. Mean $\pm$ SD values of the Pb isotope ratios.

	$^{208}\text{Pb}/^{206}\text{Pb}$	$^{207}\text{Pb}/^{206}\text{Pb}$
Overall (119)	2.129 ± 0.006	0.8727 ± 0.003
Near the mine	$2.131 \pm 0.003^{**}$	$0.8734 \pm 0.0014^{**}$
Kasanda (27)	2.133 ± 0.001^a	0.8735 ± 0.0003^a
Mutwe Wansofu (14)	2.130 ± 0.002^b	0.8747 ± 0.0009^b
Chowa (48)	2.130 ± 0.003^b	0.8729 ± 0.0017^c
Lukanga (30)	2.122 ± 0.007^c	0.8708 ± 0.0047^d
Kabwe galena (Kamona <i>et al.</i> , 1999)	2.134 ± 0.0009	0.8731 ± 0.0003

Note: ** indicates a significant difference ($p < 0.01$) between sites near the mine and Lukanga. Different letters indicate a significant difference among areas.

Table 7. Mean $\pm$ SD values (minimum-maximum) of the blood biochemical analysis of the dogs.

Factors	Overall (115)	Near the mine (86)	Lukanga (29)	Reference range
ALT, IU/L	15.3 ± 23.9 (5-214)	13.5 ± 16.2 (5-103)	20.8 ± 38.5 (5-214)	0-113
ALP, IU/L	126.2 ± 129.9 (25-747)	126.7 ± 128.7 (25-747)	124.8 ± 135.7 (25-745)	0-132
AST, IU/L	16.6 ± 12.9 (5-106)	16.3 ± 13.3 (5-106)	17.8 ± 12.0 (5-73)	0-47
GGT, IU/L	19.6 ± 26.3 (5-170)	18.16 ± 26.8 (5-170)	23.8 ± 24.6 (5-105)	0-20
LDH, IU/L**	214.1 ± 132.5 (15-704)	192.0 ± 120.9 (15-704)	279.9 ± 145.2 (57-632)	0-201
T-Bil, mg/dL	0.4 ± 0.3 (0.1-2.3)	0.4 ± 0.4 (0.1-2.3)	0.4 ± 0.3 (0.1-1.2)	0-0.3
TP, g/dL	7.2 ± 1.4 (4.2-9.8)	7.2 ± 1.5 (4.3-9.8)	7.1 ± 1.3 (4.2-9.4)	4.7-7.3
Alb, g/dL	2.1 ± 0.4 (0.5-3.1)	2.1 ± 0.4 (0.5-2.9)	2.3 ± 0.4 (1.1-3.1)	1.8-3.1
BUN, mg/dL	12.7 ± 7.4 (3.0-53.0)	13.1 ± 7.7 (3.0-53.0)	12.6 ± 6.9 (3.0-34.0)	0-29
Cre, mg/dL	1.4 ± 0.4 (0.5-2.3)	1.3 ± 0.4 (0.5-2.3)	1.4 ± 0.4 (0.6-2.2)	0-1.6
UA, mg/dL	1.2 ± 0.3 (0.5-2.2)	1.2 ± 0.3 (0.5-2.2)	1.3 ± 0.2 (1.0-2.0)	0-1.0

Note: ** indicates $p < 0.01$ between sites near the mine and Lukanga.

Table 8. Correlation coefficients (R^2) between Pb and blood biochemical parameters in the dogs.

	Pb in all dogs	Pb in the dogs from sites near the mine	Pb in the dogs from Lukanga
ALT	NS	0.29**	NS
ALP	0.18*	0.29**	NS
AST	NS	0.29**	NS
GGT	NS	NS	NS
LDH	NS	NS	NS
T-Bil	NS	NS	NS
TP	NS	NS	-0.39*
Alb	NS	NS	NS
BUN	NS	-0.26*	NS
Cre	NS	NS	NS
UA	NS	NS	NS

Note: * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. NS means not significant.

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CHAPTER 2

Lead concentrations and isotope ratios in blood, breastmilk, and feces in humans: Contribution of both lactation and soil/dust exposure to infants in a lead mining area, Kabwe, Zambia

Abstract

Lead (Pb) poses a serious public health concern and children, especially infants are vulnerable to Pb. Breastmilk may be a possible source of Pb exposure in infants, as Pb can be transferred from the maternal blood to breastmilk. The present study was undertaken to determine the Pb exposure and the contribution of lactation as one of the exposure pathways to infants in a Pb mining area, Kabwe, Zambia. Blood, breastmilk and infants' feces were collected from 418 pairs of infants and mothers. The Pb concentrations, isotope ratios in the samples, and biochemistry in mothers' plasma were analyzed. The overall mean of blood lead levels (BLLs) in infants and mothers were 18.0 and 11.3 µg/dL, respectively. High overall mean Pb concentration in breastmilk (5.3 µg/L) above the WHO acceptable level between 2 and 5 µg/L were found and could be one of the sources of Pb exposure in infants. Mothers with high BLLs in Kabwe should be treated with chelation therapy to reduce the Pb exposure in infants via breastfeeding. The Pb isotope ratios in infants' feces were the most similar to Pb ratios in the soil samples. The results suggest that infants are exposed to Pb mainly from environment. In contrast to the high BLLs in mothers, the blood biochemical profiles of most analyzed parameters were interestingly within, or close to, the standard reference values. My data strongly suggested that environmental remediation is urgently needed to reduce the Pb exposure in infants and mothers from environment in Kabwe.

Keywords: Lead poisoning, Infant, Mother, Breastmilk, Lead stable isotope, Zambia

Highlight

- Sample: blood, breastmilk and infants' feces from 418 pairs of infants and mothers.
- The highest BLLs in infants and mothers were 93.4 and 82.6 $\mu\text{g/dL}$, respectively.
- High Pb concentrations in breastmilk above the acceptable level (WHO) were found.
- Pb isotope ratios in infants' feces were similar to those in Pb contaminated soil.
- Pb in breastmilk could not be the main source of Pb exposure in infants in Kabwe.

1. Introduction

Lead (Pb) poses a serious public health concern, accounting for 0.6% of the global burden of disease (WHO, 2010). Serious cases of Pb exposure have been reported in both developed and developing countries (Ajumobi et al., 2014; Haefliger et al., 2009; Ruckart et al., 2019). Lead poisoning causes various symptoms, including anemia, nephropathy, and in the worst cases, death (Meyer et al., 2008). Children, especially infants are more vulnerable to Pb, compared to adults. To measure exposure to Pb, blood lead level (BLL) has been widely used. In the blood, Pb has a short half-life of 30–40 days (Barbosa et al., 2005). Even at low levels, Pb exposure can cause pediatric neurodevelopmental impairments, such as a reduction in intelligence quotient (IQ). Due to this, the blood Pb reference value for Pb exposure has been set to 5 µg/dL (CDC, 2019, 2012). A BLL above 45 µg/dL is considered the level where treatment is required (CDC, 2002; Needleman, 2004), and a BLL above 100 µg/dL is considered a fatal level in children, which causes serious clinical symptoms such as encephalopathy, even in adults (Meyer et al., 2008; NAS, 1972).

Lead exposure primarily occurs via ingestion and inhalation, and can be traced to numerous sources, including gasoline, battery recycling, paint, and mining (Calabrese and Stanek, 1995; Meyer et al., 2008; Schoning et al., 1996; Yabe et al., 2010). Identifying the source of Pb exposure is important to prevent further exposure. Lead is present in the environment as four main isotopes: ^{208}Pb (52%), ^{207}Pb (23%), ^{206}Pb (24%), and ^{204}Pb (1%) (Komárek et al., 2008). The compositions of these isotopes are not affected to a measurable extent by physicochemical fractionation processes (Bollhöfer and Rosman, 2001; Veyseyre et al., 2001). Therefore, isotope ratios are used to determine the source of Pb (Binkowski et al., 2016; Cao et al., 2014). As sample amounts decrease, the uncertainty of ion beam intensity measurements for the minor isotope ^{204}Pb tends to increase, and the accuracy and precision of the isotopic ratios involving ^{204}Pb decrease (^{204}Pb error; Hamelin et al., 1985).

Therefore, the isotopic composition of Pb is commonly expressed as ratios of ^{208}Pb , ^{207}Pb , and ^{206}Pb . However, normalization to ^{204}Pb yields the largest variability between reservoirs (Komárek et al., 2008).

The Zambian town of Kabwe accommodates a Pb-zinc (Zn) mining area which, up until its closure in 1994, was operated without adequate pollution laws to regulate mining emissions. Elevated BLLs and Pb concentrations in the feces and urine of children near the mine have been reported, all of which exceeded the 5 $\mu\text{g}/\text{dL}$ blood Pb reference value (Yabe et al., 2018, 2015). Yabe et al. (2015) found that BLLs in children between the ages of one and two years old were higher compared with those in children between the ages of four and seven years old in Kabwe. Thus, it is necessary to reduce and prevent Pb exposure in children. This is especially important in infants, who are more vulnerable to Pb poisoning. Moreover, a more recent study has revealed a high Pb exposure also in mothers, where approximately 5% of mothers were found to have a BLL above 45 $\mu\text{g}/\text{dL}$ which indicated that treatment was required (Yabe et al., 2020). Breastmilk is vital for infants, to ensure normal development and to prevent infectious diseases. However, breastmilk may be a possible source of Pb exposure in infants, as Pb can be transferred from the maternal blood to breastmilk. As a result, BLLs in mothers should be monitored to prevent Pb exposure in infants via breastfeeding. Therefore, to minimize Pb exposure in infants through breastmilk, WHO (1989) has set the acceptable level of breastmilk Pb concentration to be between 2 and 5 $\mu\text{g}/\text{L}$. Mothers with confirmed BLLs above 40 $\mu\text{g}/\text{dL}$ should pump and discard their breastmilk (CDC, 2010). The breastfeeding practices of mothers with high BLLs are a possible source of Pb exposure for infants in Kabwe. However, the precise sources and routes of Pb exposure in infants have not yet been determined. Furthermore, no clinical studies of Pb poisoning have been done in Kabwe, despite high BLLs being reported in the local people. Some previous studies have reported that metallothionein concentrations, which is a

cysteine-rich protein that binds and detoxifies toxic metals, increase as metal concentrations in the blood increase (Bizoń and Milnerowicz, 2014; Kowalska et al., 2015). Metallothionein may therefore play an important role in reducing Pb toxicity in the people of Kabwe.

Thus, the present study was undertaken to determine the Pb exposure and the contribution of lactation as one of the exposure pathways to infants in a lead mining area, Kabwe, Zambia. Pb concentrations in mothers' breastmilk, infants' feces, and both infants' and mothers' blood, were analyzed. The Pb isotope ratios in samples were analyzed to determine the source of Pb exposure. Moreover, a blood biochemical analysis including metallothionein concentrations was conducted in the mothers to evaluate the health impact of Pb exposure in this population.

2. Materials and Methods

2.1 Sampling Sites

The town of Kabwe is located at about 28°26'E and 14°27'S, and is the provincial capital of Zambia's Central Province. It is the fourth largest town in Zambia, with a population of about 230,000 inhabitants and an area of 1547 km². In Kabwe, metal scraps from abandoned tailings and waste stored in the mine have continued to serve as potential sources of metal pollution even after the closure of the mine. Dust emanates from the mine dumps, and residents in townships close to the mine may be exposed to high levels of Pb in contaminated dust and soil.

The present study was conducted at health centers in four sites near the mine (Kasanda, Makululu, Chowa and Katondo) and one site far from the mine (Bwacha) in the period from January to March of 2017 (Fig. 1).

2.2 Sampling

This study was approved by the University of Zambia Research Ethics Committee (UNZAREC; REF. No. 012-04-16). Further approvals were granted by the Ministry of Health through the Zambia National Health Research Ethics Board and the Kabwe District Health Office.

A sensitization campaign about the research activities was conducted by community health workers before sampling in their catchment areas around the health centers. Mothers and guardians were encouraged to participate in the study, and were asked to take their breastfed infants under the age of 1 year and 6 months to the selected health centers for sample collection. Only infants with mothers/guardians that willingly agreed to participate and signed the informed consent were included in the present study. After informed and written consent were obtained from the mothers/guardians, blood samples were collected as

described by Yabe et al. (2015). The mothers/guardians were also interviewed to obtain necessary personal details about themselves and their infants, such as age and sex. Sample collection and questionnaire administration were undertaken by certified local nurses. In accordance with ethical requirements, confidentiality was upheld in the study.

Blood samples up to 2 mL and 5 mL were collected from the cephalic veins of each infant and mother, respectively, and were placed into heparinized blood collection tubes for laboratory analysis. The venipuncture site was carefully cleaned and sanitized with an ethanol swab to avoid contamination before blood sample collection. Breastmilk samples from mothers were collected in clean sample cups and transferred to 2 mL sample tubes for storage and transportation. To avoid sample contamination, all sample collection supplies were stored in plastic Ziploc® storage bags before sampling. Plasma samples were separated only from the mothers' blood after centrifugation. For infants' fecal samples, mothers/guardians were handed 30 mL stool containers equipped with scoops and were instructed to scoop feces into the container from a soiled diaper in the morning of the following day. Household soil samples were collected in June 2016 from Kasanda and Makululu as a reference of environmental samples for Pb stable isotope analysis, as described previously (Nakayama et al., 2011).

The processed samples were transported to the laboratory of The University of Zambia, School of Veterinary Medicine, Zambia, and stored at -20°C . The material transfer agreement (MTA) for human samples from the Zambia National Health Research Ethics Committee (approval No. E00417) was obtained before transportation. Similarly, the phytosanitary certificate from plant quarantine and phytosanitary service, Zambia Ministry of Agriculture, and import permission by plant protection station, Japanese Ministry of Agriculture, Forestry and Fisheries (approval No. 28–313) was also granted for soil samples. The human samples were transported in temperature-controlled boxes with ice packs, and the

soil samples in temperature-controlled boxes, for further analysis at the Toxicology Laboratory at the Faculty of Veterinary Medicine, Hokkaido University, Japan.

2.3 Pb Concentration Analysis

Pb was extracted from the samples. Thawed fecal samples were weighed on heat-resistant tissue drying plates and dried for 48 h in a tissue drying oven at 60 °C, whereas blood and breastmilk samples were only thawed. In brief, 1 mL of each blood and breastmilk sample and 50 mg of each dried fecal sample were placed separately in prewashed digestion vessels with 5 mL of two-fold diluted ultrapure nitric acid (Cica reagent, Specific gravity of 1.38, 60%; Kanto Chemical Corp., Tokyo, Japan) and 1 mL of ultrapure hydrogen peroxide (Cica reagent, 30%; Kanto Chemical Corp.). Microwave digestion was performed using a Speedwave MWS-2 (Berghof, Eningen, Germany) according to the instructions of the manufacturer, as previously described (Table 1; Nakata et al., 2016; Yabe et al., 2015). The concentrations of Pb were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, 7700 series; Agilent Technologies, Tokyo, Japan). Analytical quality control was conducted using Seronorm™ Trace Elements Whole Blood L-2 (Sero, Billingstad, Norway), SRM 1944 (New York/New Jersey Waterway Sediment), and DOLT-4 (dogfish liver; National Research Council of Canada) certified reference materials. Replicate analyses of these reference materials showed good recovery rates (Seronorm™ Trace Elements Whole Blood L-2 and DOLT-4: 95–105% and SRM 1944: 80–110%) with an instrument detection limit of 0.001 µg/L.

2.4 Stable Pb Isotope Analysis

Only 26 sample sets with high Pb concentrations of infants' and mothers' blood, breastmilk, and infants' feces from Kasanda and Makululu, were chosen and analyzed during

Pb isotope analysis. The sample dissolution procedure was similar to previously described methods (Kuritani and Nakamura, 2002; Nakayama et al., 2019). The extracted solutions of samples were transferred into Teflon tubes after the Pb concentrations were analyzed. The ratios of $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, and $^{206}\text{Pb}/^{204}\text{Pb}$ were determined using a multiple collector (MC)-ICP-MS (Neptune Plus, Thermo Finnigan, California, USA) in static mode with Faraday cup configuration (Nakayama et al., 2019). Other general parameters are provided in Table 2. Mass fractionation factors were corrected using Tl as an external standard. Additional external corrections were performed by applying a standard bracketing method using NIST981, and normalizing to $^{206}\text{Pb}/^{204}\text{Pb} = 16.9424$, $^{207}\text{Pb}/^{204}\text{Pb} = 15.5003$, and $^{208}\text{Pb}/^{204}\text{Pb} = 36.7266$ (Kuritani and Nakamura, 2003).

Fig. 2 shows the results of replicate analyses on NIST981 for variable sample sizes ($n = 5$ for each sample size). As the beam intensity of ^{208}Pb decreases from $\sim 5\text{V}$, the $^{206}\text{Pb}/^{204}\text{Pb}$ ratios tend to deviate significantly from the recommended ratio, and the analytical reproducibility also tends to worsen. We found that the variations of the isotopic ratios and the analytical errors (2σ) with the ^{208}Pb beam intensity can be approximated using power functions as follows:

For isotopic ratios:

$$^{206}\text{Pb}/^{204}\text{Pb} : 16.9424 - \{0.010785 \times (^{208}\text{Pb intensity, V})^{-1.1134}\}$$

$$^{207}\text{Pb}/^{204}\text{Pb} : 15.5003 - \{0.010801 \times (^{208}\text{Pb intensity, V})^{-1.1228}\}$$

$$^{208}\text{Pb}/^{204}\text{Pb} : 36.7266 - \{0.023533 \times (^{208}\text{Pb intensity, V})^{-1.2089}\}$$

For analytical errors:

$$^{206}\text{Pb}/^{204}\text{Pb} : 0.0102110 \times (^{208}\text{Pb intensity, V})^{-1.0765}$$

$$^{207}\text{Pb}/^{204}\text{Pb} : 0.0093576 \times (^{208}\text{Pb intensity, V}) - 1.0463$$

$$^{208}\text{Pb}/^{204}\text{Pb} : 0.0225340 \times (^{208}\text{Pb intensity, V}) - 1.0722$$

Therefore, for unknown samples with ^{208}Pb intensity < 5 V, additional corrections were performed on the externally-corrected isotopic ratios using the above equations (e.g., $^{206}\text{Pb}/^{204}\text{Pb} = (^{206}\text{Pb}/^{204}\text{Pb})$ externally corrected / $[16.9424 - \{0.010785 \times (^{208}\text{Pb intensity, V}) - 1.1134\}] \times 16.9424$), and the analytical errors were also estimated using the above equations.

2.5 Blood Biochemical Analysis and Metallothionein ELISA

A conventional blood biochemical analyzer (FUJI DRICHEM 7000V; FUJIFILM corporation, Tokyo, Japan) was used to analyze the concentrations of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactase dehydrogenase (LDH), total bilirubin (T-Bil), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), and urea acid (UA) in mothers' plasma samples. Metallothionein in mothers' plasma samples was measured by ELISA using an antibody against iso-Metallothionein I and II (Metallothionein ELISA kit; Frontier Institute Co., Ltd., Hokkaido, Japan). The standard reference ranges for each parameter in humans were provided by the kit manufacturers.

2.6 Statistical Analysis

All data from the experiments and questionnaires were combined into a single electronic database. All statistical analyses were performed at a significance level of $p < 0.05$ using JMP 13.1.0 (SAS Institute, USA). Mean values were indicated in addition to standard

deviation (SD) values. The collinearity between factors was analyzed using Spearman's rank correlation test. A Steel–Dwass multiple comparisons test was used to compare the differences between the factors among areas and samples.

3. Results

3.1 Characteristics of the Infants and Mothers

A total of 418 pairs of infants and mothers participated in this study. Of these, 333 participants came from four sites near the mine, and 85 came from Bwacha, which is located farther from the mine (Table 3). None of the infants and mothers had overt signs of Pb poisoning. Infants from Chowa were younger than those from other areas, except for Bwacha ($p < 0.05$). In regard to height, infants from Chowa were significantly shorter and infants from Katondo were significantly taller than infants from other areas (each $p < 0.05$). Body weight of the infants from Chowa was significantly smaller than that of the infants from other areas ($p < 0.05$).

The height of boys was greater than that of girls overall ($p < 0.01$), with boys found to be significantly taller in the four areas near the mine considered together ($p < 0.01$) and in Makululu alone ($p < 0.01$, Table 4). In Katondo, the height of boys tended to be greater than that of girls, but no significant difference was recorded ($p = 0.09$). The weight of boys was significantly larger than that of girls when considering all infants ($p < 0.01$), infants from near the mine ($p < 0.01$), and infants in Kasanda ($p < 0.05$), Makululu ($p < 0.01$), Katondo ($p < 0.01$), and Bwacha ($p < 0.045$) alone.

3.2 Pb Concentrations in Blood, Breastmilk, and Infants' Feces

The overall mean values of BLLs in infants and mothers were 18.0 ± 18.1 and 11.3 ± 9.2 $\mu\text{g/dL}$, respectively (Table 3). Fig. 3a shows the difference of BLLs in infants and mothers among sites. We found 76.8% of infants (312/406) and 73.6% of mothers (307/417) had BLLs above the reference value for Pb exposure (5 $\mu\text{g/dL}$; CDC, 2019, 2012). Moreover, BLLs in 8.9% of infants (36/406) and 1.2% of mothers (5/417) were above 45 $\mu\text{g/dL}$, the threshold BLL for chelation therapy (Meyer, 2008). No infants or mothers had BLLs above the lethal level for Pb exposure (100 $\mu\text{g/dL}$), however, the highest BLL in an infant in the present study was 93.4 $\mu\text{g/dL}$. The overall mean of Pb concentrations in breastmilk and infants' feces were 5.3 ± 7.0 $\mu\text{g/L}$ and 39.2 ± 217.7 mg/kg (dry weight), respectively. Fig. 3b and 3c shows the difference of Pb concentrations in breastmilk and infants' feces among sites, respectively. Overall, 30.0% of breastmilk samples (122/407) had Pb concentrations of more than 5 $\mu\text{g/L}$, which is above the accepted level for breastfeeding (WHO, 1989).

There were significant differences in Pb concentrations among sample types from all sites: infants' feces > infants' blood > mothers' blood > breastmilk ($p < 0.05$). Among the samples from Chowa and Bwacha, there were no significant differences in Pb concentrations between infants' and mothers' blood. Pb concentrations in infants'

blood were 1.8 ± 1.5 and 60.2 ± 67.6 times higher than those in the mothers' blood and Pb concentrations in breastmilk, respectively (Table 5). On the other hand, Pb concentrations in infants' blood were $5.3 \pm 7.4\%$ of the Pb concentrations in infants' feces.

Pb concentrations in infants' and mothers' blood, breastmilk, and infants' feces from sites near the mine (Kasanda, Makululu, Chowa, and Katondo) were significantly higher than the concentrations in samples from Bwacha. Among sites, the mean of Pb concentrations in samples from Makululu were highest, except in breastmilk. Pb concentrations in samples from Bwacha were significantly lower than those from other sites ($p < 0.05$), except in breastmilk.

BLLs in boys were significantly higher than those in girls ($p = 0.04$) in Makululu, and almost significantly higher in Bwacha ($p = 0.06$, Table 6). Pb concentrations in infants' feces of boys were significantly higher than those of girls in Chowa ($p < 0.01$). The same trend was found in all infants, without a significant association ($p = 0.08$).

3.3 Relationships among Samples and Factors

In the samples from all areas, significant positive correlations existed among infants in the factors of age, height, and weight (Table 7, $p < 0.001$). BLLs in infants and Pb concentrations in infants' feces had a significant positive correlation with the age, height, and weight of infants ($p < 0.001$). There were significant positive correlations of Pb concentrations among all sample types ($p < 0.001$, Fig. 4).

In all samples from sites near the mine, the same trend was found (Table 8). Moreover, the height of infants had a significant negative correlation with BLLs in mothers ($p < 0.01$, $\rho = -0.19$) and Pb concentrations in breastmilk ($p < 0.05$, $\rho = -0.13$).

In the samples from Bwacha, the same trend was found as in the samples from all other sites, except for the relationship between Pb concentrations in breastmilk and other samples, and the BLLs in mothers and Pb concentrations in infants' feces (Table 9).

3.4 Pb Isotope Ratio Analysis

Table 10 shows the mean values of the $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$, and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios in the samples. The $^{208}\text{Pb}/^{206}\text{Pb}$ Pb isotope ratios in mothers' blood (2.126 ± 0.006) were significantly different from those in infants' feces and the soil

(2.129 ± 0.005 and 2.131 ± 0.002 , respectively, $p < 0.05$). On the other hand, there was no significant difference in $^{207}\text{Pb}/^{206}\text{Pb}$ ratios among sample types. Both $^{208}\text{Pb}/^{204}\text{Pb}$, and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios in mothers' blood were significantly different from those in other samples ($p < 0.05$), except for those in breastmilk. Pb isotope ratios in soil samples were similar to those reported for Kabwe galena (Kamona et al., 1999). The $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios (Fig. 5b) clearly show differences among samples compared to $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ ratios (Fig. 5a).

3.5 Blood Biochemical Analysis

Table 11 shows the mean values of the blood biochemical analysis on mothers' plasma. The mean values of all parameters in mothers in the present study were within the reference range or slightly higher, except for ALP. The ALT and ALP concentrations in the mothers from sites near the mine were significantly higher than those in the mothers from Bwacha ($p < 0.05$). On the other hand, LDH ($p < 0.05$), Alb ($p = 0.049$), and metallothionein ($p < 0.001$) concentrations in the mothers from Bwacha were significantly higher than those in mothers from sites near the mine. T-Bil concentrations in the mothers from Bwacha were higher than those in the mothers from sites near the mine, although this difference was not significant.

In all mothers in the present study, there was a significant positive correlation between the Pb and AST concentrations (Table 12, $p < 0.05$, $\rho = 0.16$). The ALT concentrations displayed an almost significant increase as Pb concentrations increased ($p = 0.08$, $\rho = 0.13$). The Alb ($p < 0.05$, $\rho = -0.14$) and metallothionein concentrations significantly decreased as the Pb concentrations increased ($p < 0.001$, $\rho = -0.53$).

In the mothers from sites near the mine, the Pb concentrations were significantly associated with increased AST ($p < 0.05$, $\rho = 0.17$) and LDH ($p < 0.05$, $\rho = 0.19$) concentrations. On the other hand, the BUN ($p < 0.05$, $\rho = -0.26$) and metallothionein concentrations ($p < 0.001$, $\rho = -0.43$) significantly decreased as Pb concentrations increased.

In the mothers from Bwacha, there were no significant correlations between Pb and blood biochemical factors.

4. Discussion

To the authors' knowledge, the present study undertook the first ever analysis of Pb in breastmilk of mothers from the sites around the mine in Kabwe. The overall mean Pb concentration in breastmilk was 5.3 µg/L, which was above the acceptable level of 2 to 5 µg/L for breastfeeding (WHO, 1989), and 30.0% of breastmilk samples contained Pb levels above the acceptable level. Compared with previous studies that reported elevated Pb concentrations in breastmilk ranged from 8.8 to 35.4 µg/L (Isaac et al., 2012; Turan et al., 2001), Pb concentrations in the breastmilk of mothers from sites near the mine were comparable or even lower. On the other hand, the mean values of Pb concentrations in breastmilk of mothers from Katondo and Bwacha (2.3 µg/L) were within the acceptable level, which agrees with the results obtained in unpolluted areas in other reports (Ettinger et al., 2014; Klein et al., 2017), although the highest individual Pb breastmilk concentration in this study was also found in Katondo (51.9 µg/L). Pb in breastmilk may be one of the sources of Pb exposure in infants. Pb concentrations in breastmilk in this study were 5.6% of Pb concentrations in maternal blood. This result agreed with previous studies reporting breastmilk/mothers' blood ratios between 1% and 10% (Anastácio et al., 2004; Ettinger et al., 2005; Koyashiki et al., 2010; Koyashiki, et al., 2010).

High overall mean Pb concentration in infants' feces (39.2 mg/kg) was recorded in the present study. The highest Pb concentration in infants' feces was 3002.7 mg/kg. These results are in agreement with a previous study conducted in Kabwe (Yabe et al., 2018). Even in Bwacha, which is far from the mine, high Pb concentrations were found in infants' feces. This suggests that infants in Kabwe were highly exposed to Pb via ingestion.

Among sample types, Pb concentrations in infants' feces were significantly higher than in blood and breastmilk. There were significant positive correlations among samples. BLLs in infants significantly increased with BLLs in mothers ($p < 0.001$, $\rho = 0.68$), Pb concentrations in breastmilk ($p < 0.001$, $\rho = 0.43$), and infants' feces ($p < 0.001$, $\rho = 0.82$). These results suggest that mothers, as well as infants, are exposed to Pb from the environment, as they share the same living conditions. Pb concentrations in infants' feces may be a useful indicator of Pb exposure in infants, in addition to BLLs.

The present study found different trends of Pb exposure in infants and mothers among the studied areas in Kabwe. The mean BLL in infants and mothers from Makululu, which is further from the mine than Kasanda, were the highest among the area. Following Makululu, those from Kasanda and Chowa were the second and the third highest, respectively. Yabe et al. (2015) reported a similar trend for BLLs in

children under seven years old, but the highest BLL mean was found in Kasanda, among the three sites. Since most areas in Makululu are dusty and unpaved compared to Kasanda, residents could easily come in to contact with polluted soils or dusts in the area. Pb exposure in infants and mothers from Kasanda and Makululu, which are located on the western side of the mine and in the direction of prevailing winds, could be higher than that in Chowa, which lies in the opposite direction. Pb concentrations in breastmilk and infants' feces showed similar trends to BLLs in infants and mothers. These trends agreed with those determined in earlier studies by Tembo et al. (2006) and Nakayama et al. (2011), who reported similar trends in the soils in Kabwe. In chapter 1, there was a significant negative relationship between Pb concentrations in dog blood and the distance from the mine in Kabwe. Moreover, the result indicates that the exposure to Pb and other metals in dogs remarkably decreased about 5 km away from the mine. Therefore, these trends suggest that the location of the townships in the relation to the wind direction and distance from the mine are key factors that influence the severity of the exposure to Pb in infants and mothers in Kabwe.

In the present study, BLLs in boys were significantly higher than those in girls in Makululu. The same trend for children under seven years old in the same township was previously reported (Yabe et al., 2015). Yabe et al. (2015) suggested that the

difference in behavior between boys and girls could contribute to this difference, as boys are more likely than girls to wander farther from home and play near the mine dumps. Moreover, Pb concentrations in infants' feces of boys were significantly higher than those of girls in Chowa. These results suggest that boys are more exposed to Pb via ingestion. However, the sex of infants may not be strongly associated with exposure to Pb in this study.

Significant positive correlations between BLLs in infants and age of the infants were found. The hand-to-mouth or object-to-mouth (pica) behavior of children, and high absorbance of ingested Pb from the gastrointestinal tract are well known factors attributed to high Pb exposure in children (Wani et al., 2015). However, younger infants who are not ambulatory could display less hand-to-mouth behaviors, as they are under the care of their mothers or guardians. Given that BLLs in infants increases from birth to around two years of age in Kabwe, it is important to pay more attention to activities of infants during this period. On the other hand, only BLLs in mothers from Bwacha significantly decreased as the age of mothers increased. Adults in Bwacha, which is far from the mine, could be less exposed to Pb, thus, Pb in the blood of adults may mainly occur from redistribution of endogenous bone-derived Pb. From this point of view,

adults even from sites far from the mine in Kabwe could be chronically exposed to Pb via endogenous exposure.

The $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$, and $^{207}\text{Pb}/^{204}\text{Pb}$ isotope ratios in samples were analyzed in the present study to determine the source of Pb exposure in infants. The Pb isotope ratios in infants' feces were the most similar to Pb ratios in the soil samples among biological samples. These soil samples exhibited Pb isotope ratios similar to those in Kabwe galena (Kamona et al., 1999). Furthermore, a Pb exposure study on rats exposed to lead in the soil revealed that the Pb isotope ratios in these biological samples were also similar to those in Kabwe galena (Kamona et al., 1999; Nakayama et al., 2019). These results suggest that the main source of Pb exposure in infants of Kabwe could be contaminated soil from the mine. Understanding which infant behaviors and activities are related to ingestion of soils is required to minimize their exposure. In the present study, both ^{206}Pb and ^{204}Pb ratios were analyzed. Both results were similar, but ^{204}Pb ratios displayed clear differences among sample types. Therefore, ^{204}Pb ratios could be more useful than ^{206}Pb ratios to elucidate the source of Pb exposure.

In contrast to the high BLLs in mothers, the blood biochemical profiles of most analyzed parameters were interestingly within, or close to, the standard reference

values, except in the case of ALP. These results indicate that Pb exposure in Kabwe mothers did not significantly impact their health, as was observed during sampling, where all sampled mothers appeared healthy. More specifically, the ALT and ALP values in the mothers from sites near the mine were significantly higher than those in the mothers from Bwacha although these values were not significantly correlated with BLLs in mothers. Therefore, these results could not only be attributed to Pb exposure, but also other factors. On the other hand, LDH and AST, which are indicators of liver function, significantly increased as BLLs increased. These results suggest that Pb exposure in mothers may have caused some mild liver damage. High Pb exposure is known to cause kidney damage in conjunction with an increase in BUN and a decrease in Alb. However, both biomarkers significantly decreased as BLLs increased in mothers. During a previous study on Pb poisoning in refugee children in the United States, the CDC reported chronic and acute malnutrition as risk factors for Pb poisoning (CDC, 2005). Further studies should therefore focus on the relationship between Pb exposure and nutrition status. In my study, metallothionein concentrations significantly decreased as BLLs increased. A previous study by Mustonen et al. (2014) found constant metallothionein expression in earthworms from a contaminated site, and therefore suggested that the inducibility of the metallothionein response could be lost in

earthworms with a history of metal exposure. It is possible that local people in the sites near the mine may be chronically exposed to metals, including Pb, over a long period of time compared to people in sites far from the mine, such as Bwacha. Therefore, metallothionein expression in people residing near the mine is lower than that in people residing far from the mine. Further studies should focus on both metallothionein concentrations and gene expression. However, metallothionein in people in Kabwe may not play a role of biological defense mechanisms, which I expected that a metal binding protein in high abundance reduces Pb toxicity. In the present study, it is difficult to exclude the possibility of other factors or diseases since a detailed questionnaire survey or medical check-ups were not performed.

The findings in the present study suggest that the main source of Pb exposure in infants could be Pb from the environment, and especially from soils. Thus, remediation of the environment in Kabwe is urgently needed to reduce Pb exposure. It is important to keep away infants from contact with soil or dust. On the other hand, breastfeeding practices in mothers with high BLLs could also be a source of Pb exposure in infants. Moreover, high BLLs in mothers may cause their fetus to be exposed to Pb during pregnancy. In the current situation, chelation therapy for Pb poisoning is prioritized more in children than in adults, as children are more vulnerable to Pb. However, in

utero exposure to environmental lead may be adversely associated with neurodevelopment at two years of age (Lin et al., 2013). Pilsner et al. (2009) reported that the epigenome of the developing fetus can be influenced by the maternal cumulative lead burden, which may influence long-term epigenetic programming and disease susceptibility throughout a child's life. Reducing the Pb exposure in mothers is important to reduce Pb exposure in fetuses via the placenta, as well as Pb exposure in infants, via breastfeeding. Thus, it is necessary that mothers with high BLLs are treated with chelation therapy, as well as their children.

5. Conclusion

The present study is the first to reveal the relationship between Pb exposure in infants and mothers via breastfeeding in Kabwe. High Pb concentrations in breastmilk, which were above the WHO acceptable level for breastfeeding, could be one source of Pb exposure in infants. However, the results of the isotope ratio analysis suggest that the main source of Pb exposure in infants is Pb from environment, such as from soil. Therefore, environmental remediation, in parallel with chelation therapy, is urgently needed to reduce the Pb exposure in infants and mothers in Kabwe. Moreover, mothers with high BLLs in Kabwe should be treated with chelation therapy to reduce the Pb exposure of their infants via breastfeeding.

Figures and Tables

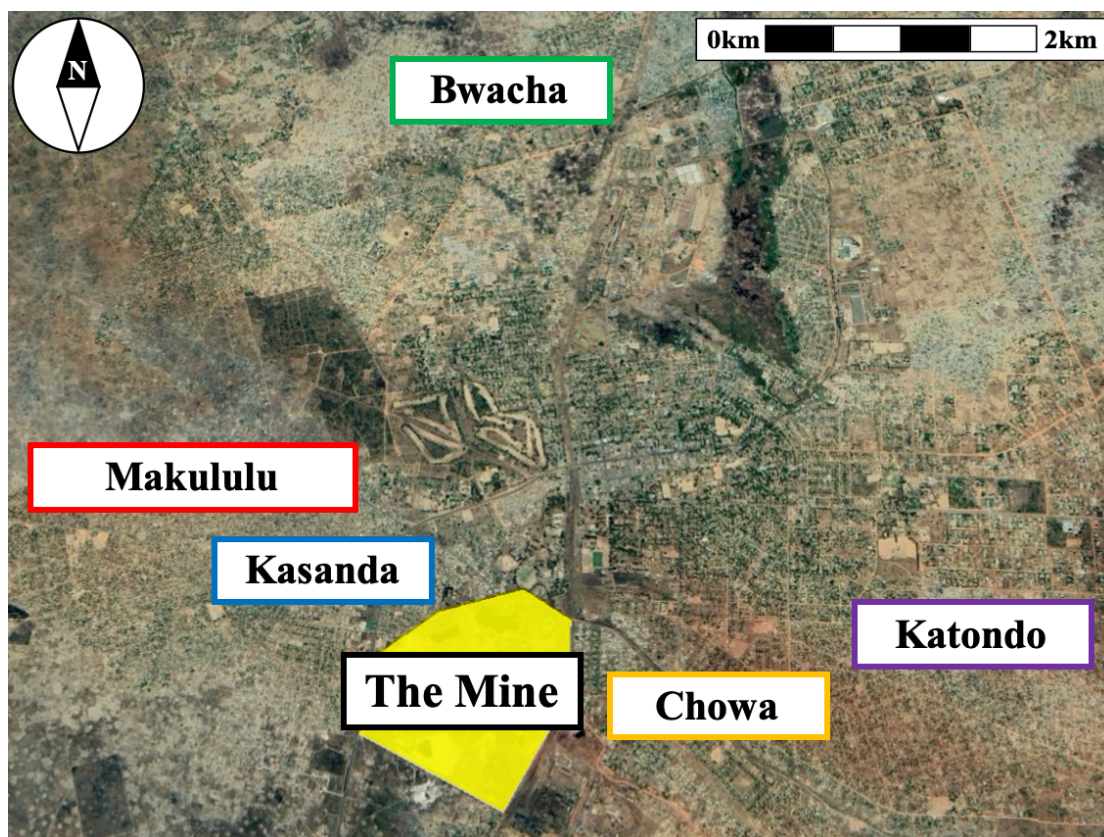


Fig. 1. Map of the sampling sites in Kabwe, Zambia (image modified from Google Earth).

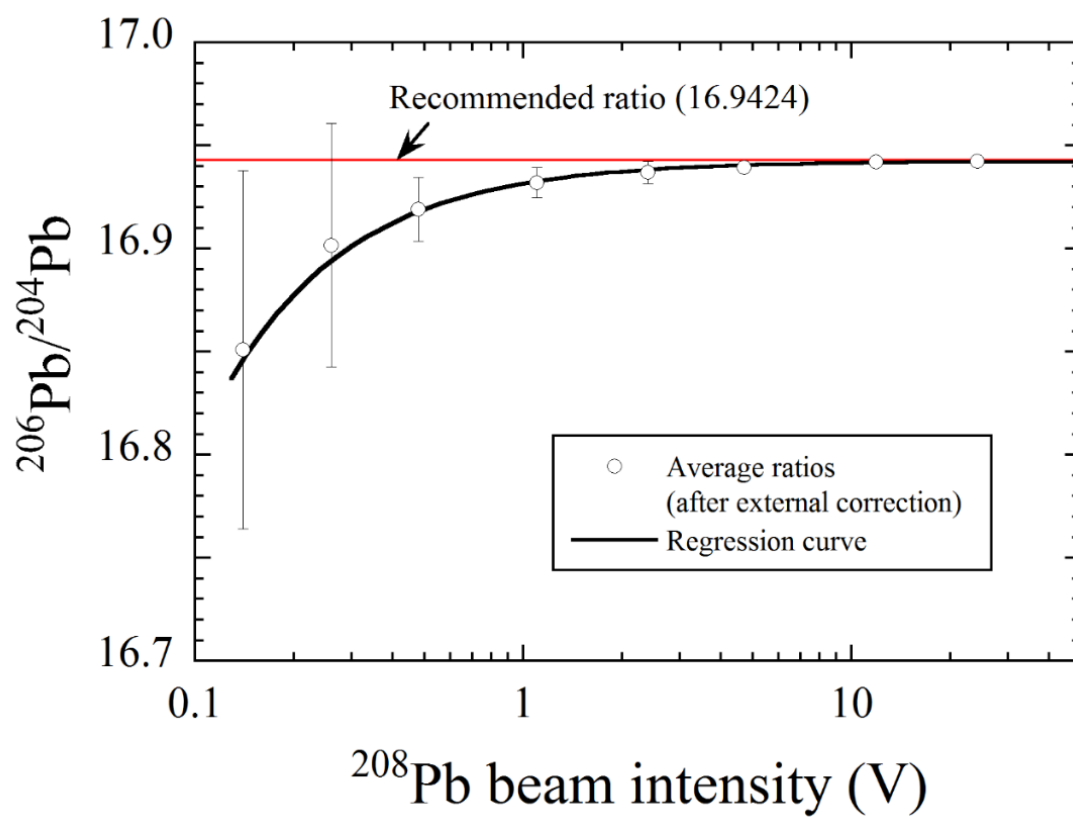
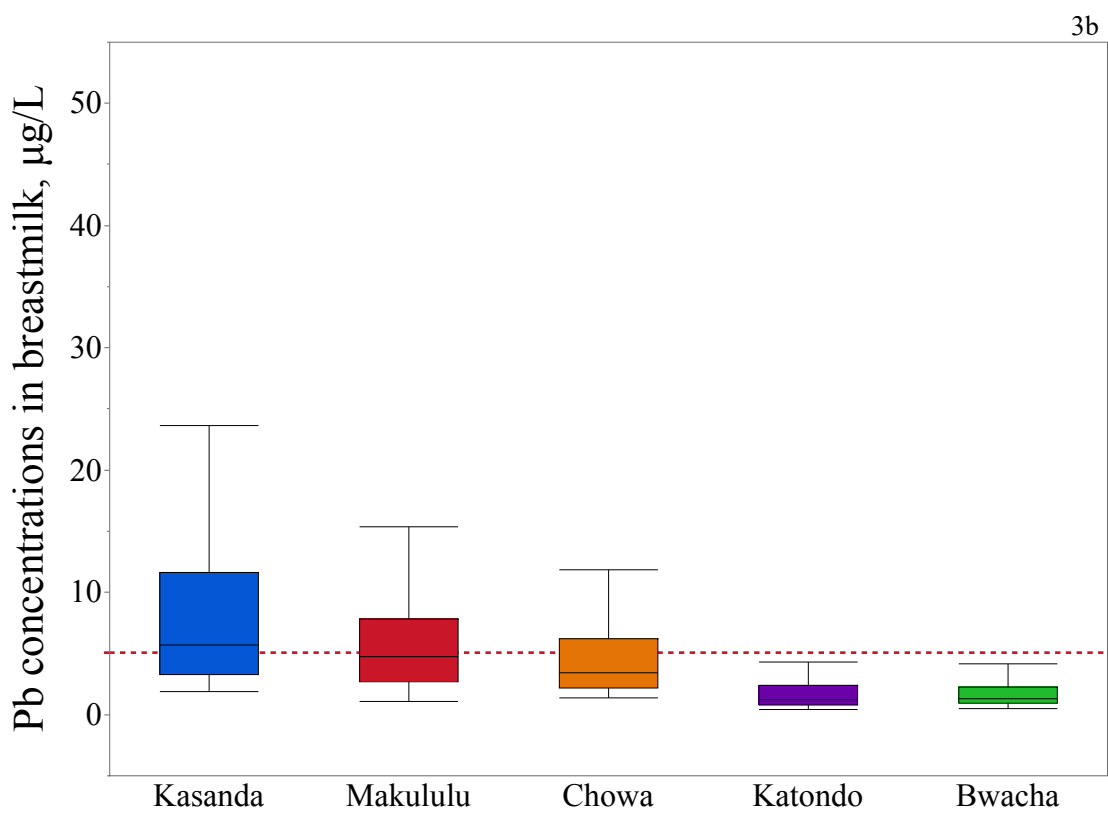
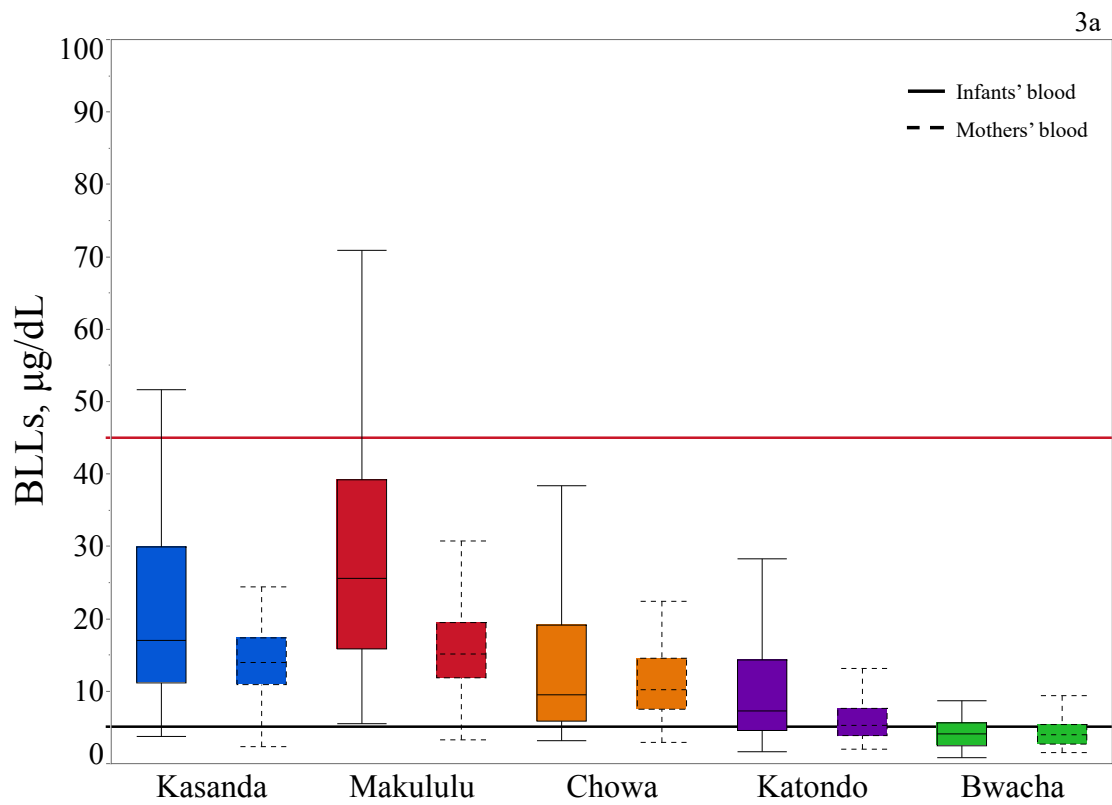


Fig. 2. The results of replicate analyses on NIST981 for variable sample sizes, showing the average $^{206}\text{Pb}/^{204}\text{Pb}$ ratios with error bars (2σ) as a function of the ^{208}Pb beam intensity (V).



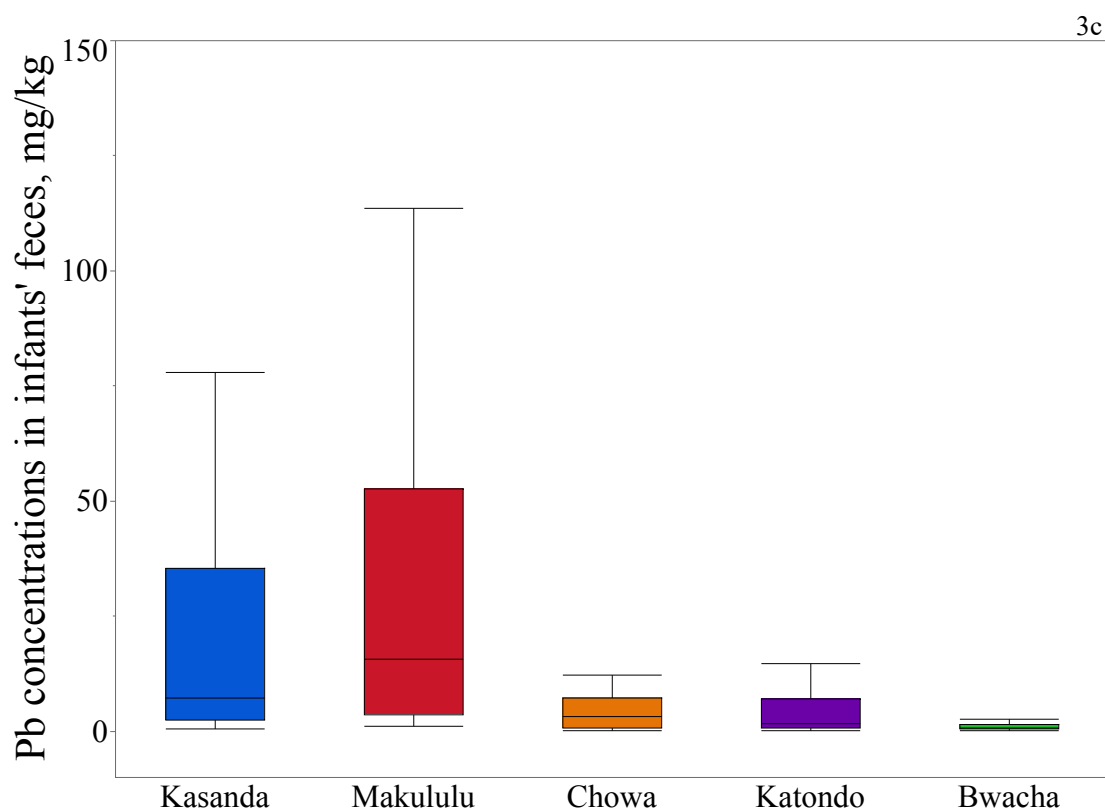
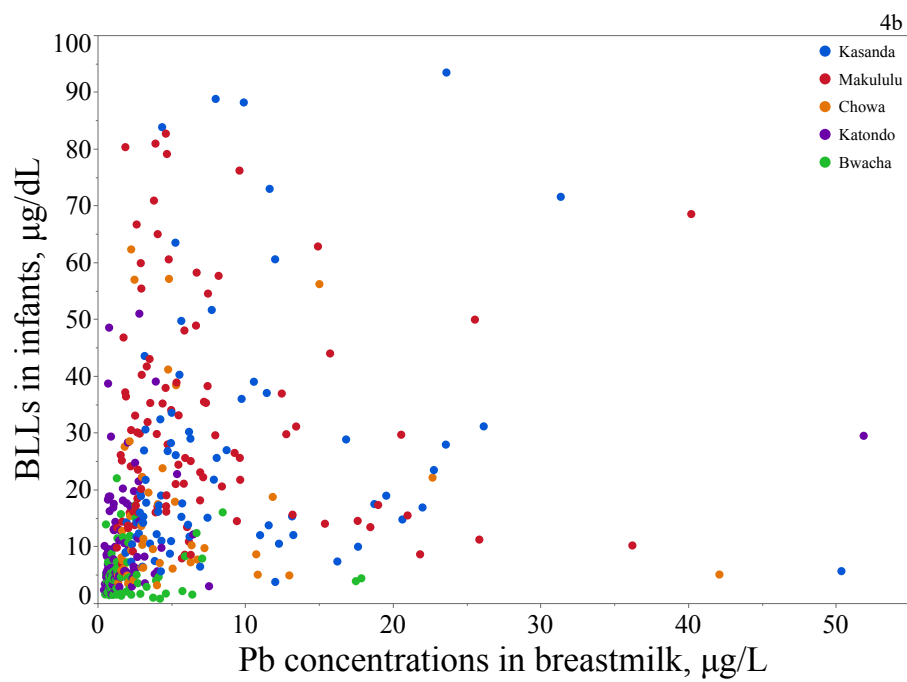
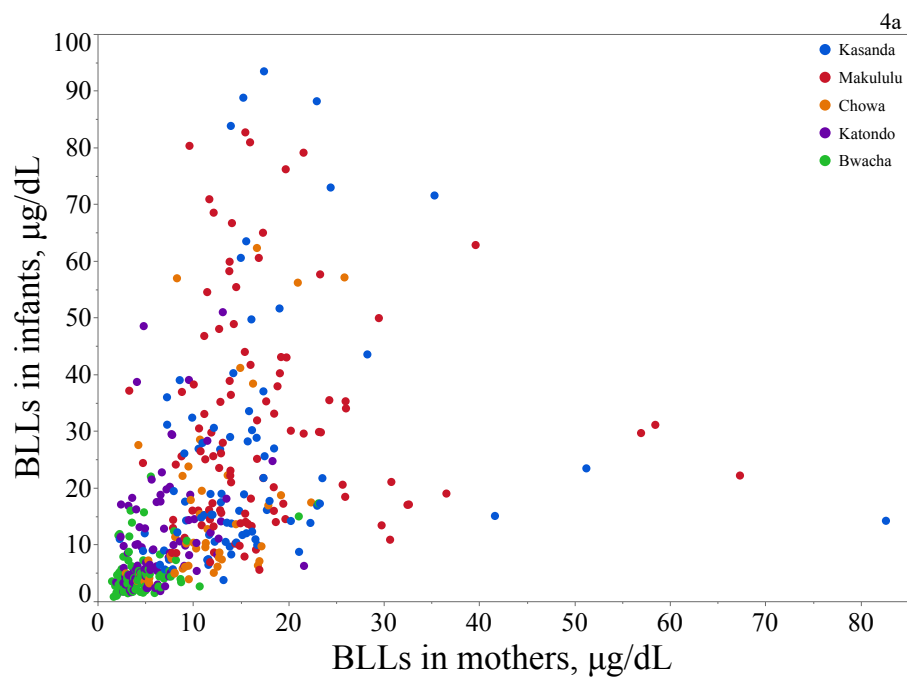


Fig. 3. BLLs (3a) in infants (n = 406) and mothers (n = 417), Pb concentrations in breastmilk (3b, n = 407), and infants' feces (3c, n = 212) among areas. The blue, red, orange, violet, and green colors indicate samples from Kasanda, Makululu, Chowa, Katondo, and Bwacha, respectively. Straight and dot box plots in Fig. 3a. indicate BLLs in infants and mothers, respectively. Red and black horizontal lines in Fig. 3a indicate 100 and 45 $\mu\text{g}/\text{dL}$, respectively. The red dotted line in Fig 3b indicates the 5 $\mu\text{g}/\text{L}$ level.



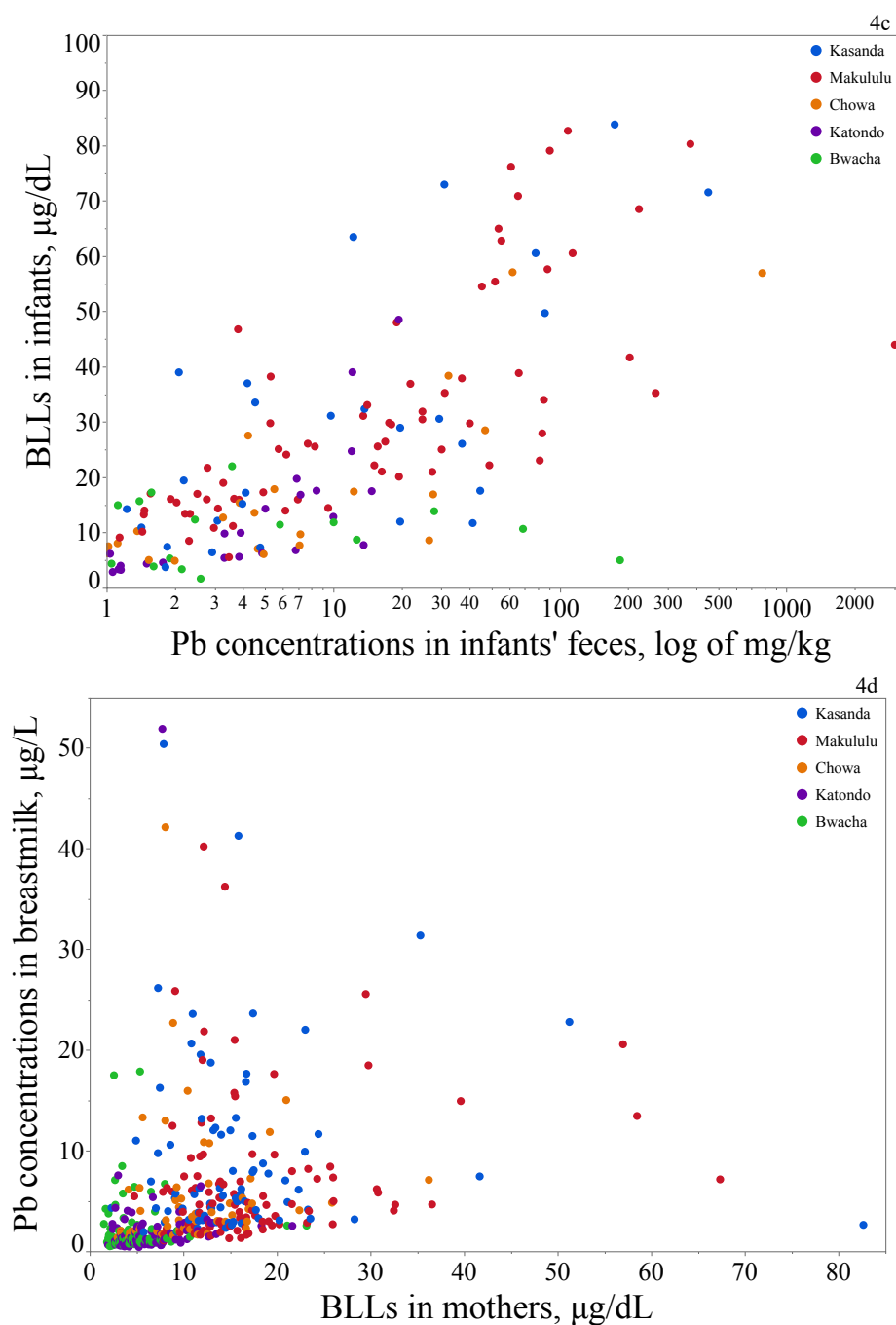


Fig. 4. Relationship between blood lead levels (BLLs) in infants and mothers (4a, $n = 405$), BLLs in infants and Pb concentrations in breastmilk (4b, $n = 395$), BLLs in infants and Pb concentrations in infants' feces (4c, $n = 210$), and BLLs in mothers and Pb concentrations in breastmilk (4d, $n = 406$). The blue, red, orange, violet, and green circles indicate samples from Kasanda, Makululu, Chowa, Katondo, and Bwacha, respectively.

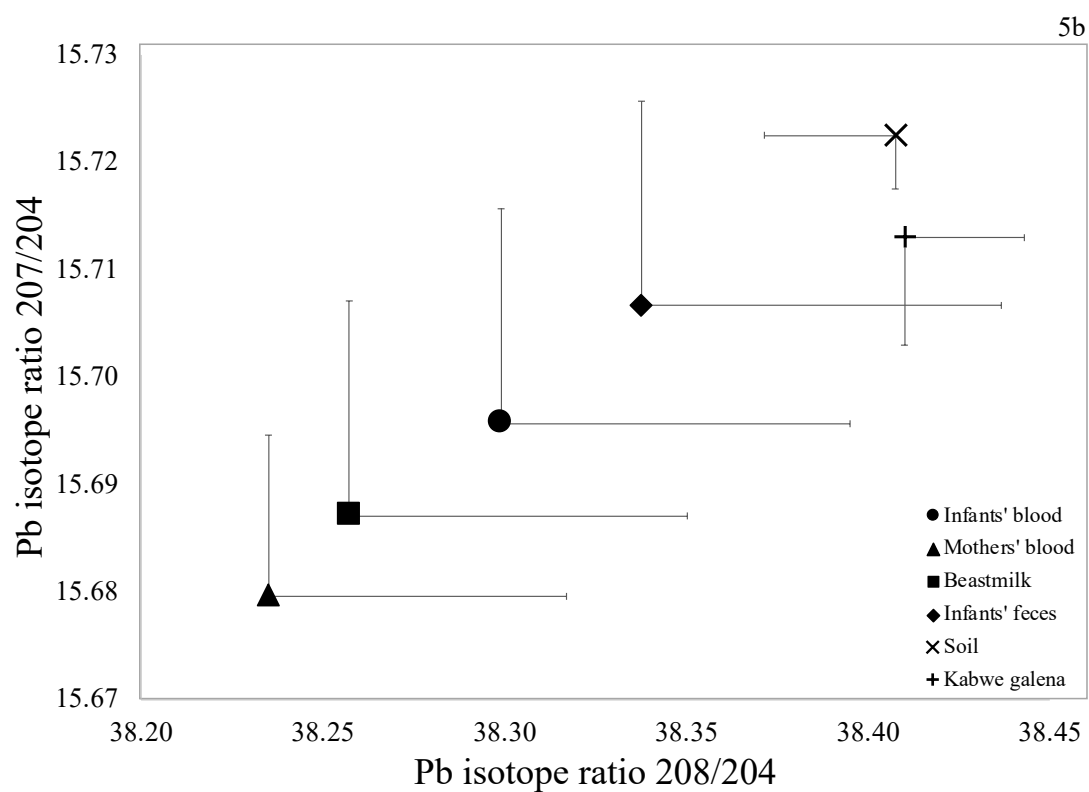
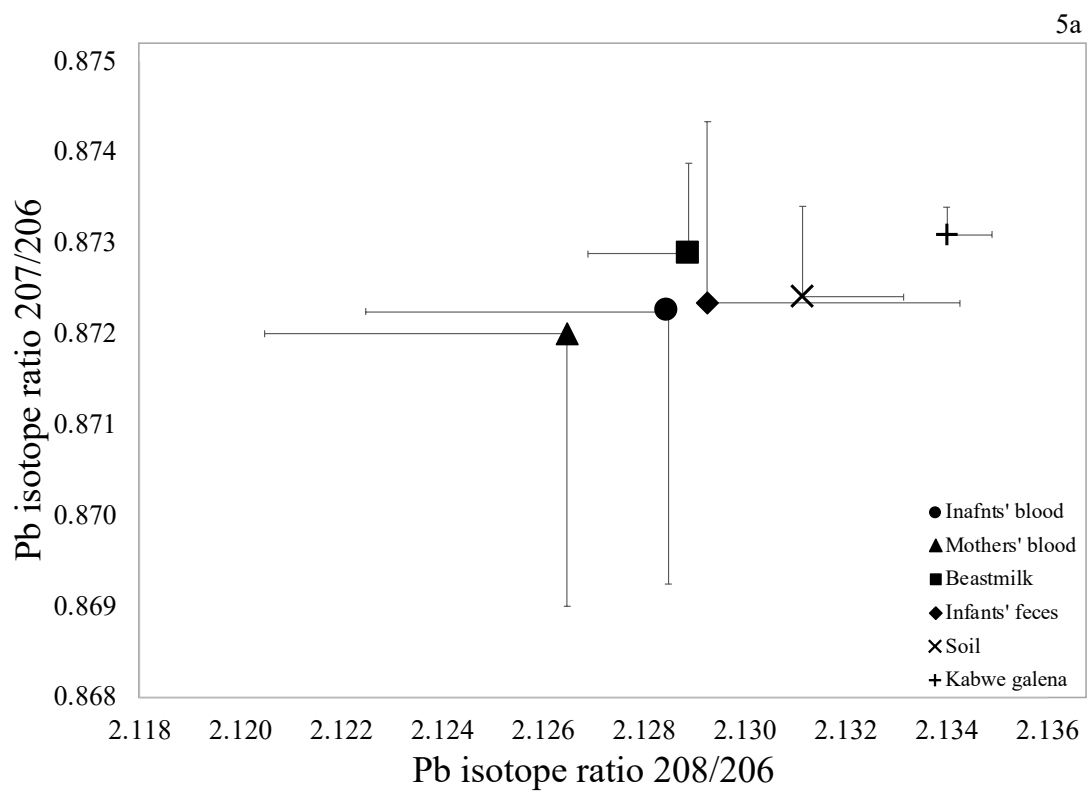


Fig. 5. Pb isotope ratios (5a: $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ and 5b: $^{208}\text{Pb}/^{204}\text{Pb}$, and $^{207}\text{Pb}/^{204}\text{Pb}$) in different samples from Kasanda and Makululu (28 sample sets). The mean values are shown with error bars indicating the SD. The triangle, square, circle, rhombus, and cross markers indicate infants' blood, mothers' blood, breastmilk, infants' fecal, and soil samples from Kasanda and Makululu, respectively. The reference value of Kabwe galena was obtained from a report by Kamona et al. (1999) and is indicated by a plus sign.

Table 1. Microwave operating conditions for blood, breastmilk, and infants' feces digestions.

Blood and breastmilk		Infants' feces	
Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
160	5	150	5
190	10	175	5
75	10	200	20
		180	10

Table 2. MC-ICP-MS operating conditions for Pb stable isotope analysis.

Detector	Mass
L3	²⁰² Hg
L2	²⁰³ Tl
L1	²⁰⁴ Pb
Center or axial	²⁰⁵ Tl
H1	²⁰⁶ Pb
H2	²⁰⁷ Pb
H3	²⁰⁸ Pb
Frontend parameters	
Resolution	Low
Cool gas (L/min)	16
Auxiliary gas	0.7
Typical sample gas flow (L/min)	1.1-1.2
PFA nebulizer uptake rates (μL/min)	~200
Typical forward power (W)	1200

Note: L = low mass side, H = high mass side

Table 3. General characteristics of infants and mothers, as well as Pb concentrations in blood, breastmilk and fecal samples in Kabwe, Zambia; mean $\pm$ SD values (sample size, minimum–maximum).

Area (N)	Sex of infants, boy:girl	Age of infants, months	Height, cm	Weight, kg	Age of mothers, years	BLLs in infants, $\mu\text{g/dL}$	BLLs in mothers, $\mu\text{g/dL}$	Pb in breastmilk, $\mu\text{g/L}$	Pb in infants' feces, mg/kg dry weight
Overall (418)	221:197	7.1 $\pm$ 3.8 (417, 0.1–16.8)	64.2 $\pm$ 7.7 (361, 45.0–95.0)	7.3 $\pm$ 1.7 (411, 1.9–12.1)	26.1 $\pm$ 6.5 (412, 16.3–46.1)	18.0 $\pm$ 18.1 ^A (406, 0.8–93.4)	11.3 $\pm$ 9.2 ^B (417, 1.5–82.6)	5.3 $\pm$ 7.0 ^C (407, 0.4–51.9)	39.2 $\pm$ 217.7 ^D (212, 0.08–3002.7)
Near the mine (333)	171:162	7.2 $\pm$ 4.0 (332, 0.1–16.8)	64.5 $\pm$ 8.0 (280, 45.0–95.0)	7.2 $\pm$ 1.7 (329, 1.9–12.1)	26.1 $\pm$ 6.6 (329, 16.3–46.1)	21.4 $\pm$ 18.9 ^{**A} (321, 1.6–93.4)	13.0 $\pm$ 9.5 ^{**B} (333, 1.9–82.6)	6.1 $\pm$ 7.5 ^{**C} (324, 0.4–51.9)	49.2 $\pm$ 248.0 ^{**D} (162, 0.09–3002.7)
Kasanda (82)	43:39	7.3 $\pm$ 3.8 ^a (82, 0.6–16.7)	64.5 $\pm$ 8.4 ^a (74, 49.0–95.0)	7.6 $\pm$ 1.5 ^a (79, 4.0–12.1)	27.1 $\pm$ 6.7 (82, 17.4–45.4)	24.8 $\pm$ 20.9 ^{aA} (80, 3.7–93.4)	15.8 $\pm$ 10.6 ^{aB} (82, 2.3–82.6)	9.2 $\pm$ 8.9 ^{aC} (79, 1.9–50.4)	38.8 $\pm$ 88.9 ^{abD} (28, 0.6–451.0)
Makululu (102)	44:58	7.7 $\pm$ 3.7 ^a (102, 1.5–16.7)	63.0 $\pm$ 7.0 ^a (102, 45.0–85.0)	7.4 $\pm$ 1.4 ^a (101, 4.1–11.9)	26.2 $\pm$ 7.0 (102, 16.6–45.9)	30.8 $\pm$ 19.4 ^{bA} (102, 5.6–82.7)	17.6 $\pm$ 10.1 ^{aB} (102, 3.3–67.3)	7.1 $\pm$ 7.1 ^{abC} (100, 1.1–40.2)	82.4 $\pm$ 362.7 ^{aD} (69, 1.1–3002.7)
Chowa (58)	35:23	5.3 $\pm$ 3.9 ^b (58, 0.1–15.2)	56.2 $\pm$ 7.1 ^b (13, 49.0–67.0)	5.6 $\pm$ 1.4 ^b (58, 3.5–8.7)	26.9 $\pm$ 6.3 (58, 17.6–41.8)	15.7 $\pm$ 15.5 ^{cA} (49, 3.2–62.3)	11.2 $\pm$ 6.1 ^{bA} (58, 3.0–36.2)	5.6 $\pm$ 6.5 ^{bB} (57, 1.4–42.1)	33.7 $\pm$ 139.4 ^{bcC} (31, 0.09–780.4)
Katondo (91)	49:42	7.7 $\pm$ 4.3 ^a (90, 0.9–16.8)	67.5 $\pm$ 7.6 ^c (91, 48.0–94.0)	7.8 $\pm$ 1.7 ^a (91, 1.9–11.1)	24.6 $\pm$ 5.9 (87, 16.3–46.1)	10.9 $\pm$ 9.9 ^{cA} (90, 1.6–51.0)	6.3 $\pm$ 3.5 ^{cB} (91, 1.9–21.6)	2.3 $\pm$ 5.5 ^{cC} (88, 0.4–51.9)	4.4 $\pm$ 5.1 ^{cD} (34, 0.1–19.4)
Bwacha (85)	50:35	6.9 $\pm$ 3.2 ^{ab} (85, 1.6–13.5)	63.0 $\pm$ 6.6 ^a (81, 51.0–86.0)	7.6 $\pm$ 1.6 ^a (82, 4.6–11.4)	26.2 $\pm$ 5.9 (83, 16.6–43.3)	5.2 $\pm$ 4.1 ^{dA} (85, 0.8–22.0)	4.7 $\pm$ 3.4 ^{dA} (84, 1.5–23.1)	2.3 $\pm$ 2.9 ^{cB} (83, 0.5–17.9)	6.9 $\pm$ 27.6 ^{dC} (50, 0.08–184.0)

Note: ** indicates a significant difference ($p < 0.01$) between sites near the mine and Bwacha. Different small letters indicate a significant difference among areas ($p < 0.05$). Different capital letters indicate a significant difference among infants' and mothers' blood, breastmilk, and infants' feces ($p < 0.05$).

Table 4. Differences in characteristics between boys and girls in the present study in Kabwe, Zambia; mean $\pm$ SD values (sample size, minimum–maximum).

Area (N)	Age of infants, months		Height, cm		Weight, kg		Age of mothers, years	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Overall (418)	7.3 $\pm$ 3.9 (221, 0.1–16.8)	7.0 $\pm$ 3.8 (196, 0.1–16.7)	65.6 $\pm$ 7.9** (186, 45.0–95.0)	62.3 $\pm$ 7.2 (175, 47.0–86.0)	7.6 $\pm$ 1.7 ** (216, 3.5–11.9)	7.0 $\pm$ 1.6 (195, 1.9–12.1)	26.2 $\pm$ 6.5 (218, 16.6–45.9)	26.1 $\pm$ 6.4 (194, 16.3–46.1)
Near the mine (333)	7.4 $\pm$ 4.1 (171, 0.1–16.8)	7.0 $\pm$ 3.9 (161, 0.1–16.7)	66.3 $\pm$ 8.5 ** (138, 45.0–95.0)	62.7 $\pm$ 7.1 (142, 47.0–84.0)	7.5 $\pm$ 1.7 ** (168, 3.5–11.9)	6.9 $\pm$ 1.6 (161, 1.9–12.1)	26.2 $\pm$ 6.6 (169, 16.8–45.9)	26.1 $\pm$ 6.6 (160, 16.3–46.1)
Kasanda (82)	7.4 $\pm$ 3.8 (43, 1.2–16.7)	7.2 $\pm$ 3.9 (39, 0.6–15.8)	65.9 $\pm$ 8.8 (38, 53.0–95.0)	62.9 $\pm$ 7.9 (36, 49.0–84.0)	7.9 $\pm$ 1.3 * (40, 5.0–10.0)	7.3 $\pm$ 1.7 (39, 4.0–12.1)	27.2 $\pm$ 6.8 (43, 17.4–41.0)	27.0 $\pm$ 6.8 (39, 18.1–45.4)
Makululu (102)	8.2 $\pm$ 3.9 (44, 1.5–15.0)	7.3 $\pm$ 3.5 (58, 1.5–16.7)	65.5 $\pm$ 8.0 ** (44, 45.0–85.0)	61.4 $\pm$ 5.4 (58, 47.0–71.0)	7.8 $\pm$ 1.5 ** (44, 4.4–11.9)	7.1 $\pm$ 1.2 (57, 4.1–9.0)	26.5 $\pm$ 7.5 (44, 17.1–45.9)	26.0 $\pm$ 6.7 (58, 16.6–45.2)
Chowa (58)	5.3 $\pm$ 3.8 (35, 0.1–15.2)	5.3 $\pm$ 4.1 (23, 0.1–14.5)	56.4 $\pm$ 8.4 (7, 49.0–67.0)	56.0 $\pm$ 6.0 (6, 50.0–66.0)	5.6 $\pm$ 1.2 (35, 3.5–8.1)	5.6 $\pm$ 1.6 (23, 3.6–8.7)	27.4 $\pm$ 6.4 (35, 18.0–39.8)	26.2 $\pm$ 6.2 (23, 17.6–41.8)
Katondo (91)	8.1 $\pm$ 4.3 (49, 1.4–16.8)	7.2 $\pm$ 4.1 (41, 0.9–14.7)	68.8 $\pm$ 7.8 (49, 52.0–94.0)	65.9 $\pm$ 7.3 (42, 48.0–77.0)	8.3 $\pm$ 1.6 ** (49, 5.1–11.1)	7.2 $\pm$ 1.7 (42, 1.9–9.9)	24.1 $\pm$ 5.4 (47, 16.8–43.1)	25.3 $\pm$ 6.5 (40, 16.3–46.1)
Bwacha (85)	7.0 $\pm$ 3.1 (50, 1.6–12.8)	6.8 $\pm$ 3.5 (35, 1.8–13.5)	63.4 $\pm$ 5.5 (48, 53.0–76.0)	62.4 $\pm$ 8.1 (33, 51.0–86.0)	7.9 $\pm$ 1.4 * (48, 4.6–11.4)	7.2 $\pm$ 1.7 (34, 4.6–10.8)	26.3 $\pm$ 6.3 (49, 16.6–43.3)	26.0 $\pm$ 5.5 (34, 17.0–38.9)

Note: * and ** indicate $p < 0.05$ and $p < 0.01$ between boys and girls, respectively.

Table 5. Pb concentration ratios among samples.

Area	Pb in infants' blood/mothers' blood (times higher)	Pb in infants' blood/breastmilk (times higher)	Pb in infants' blood/infants' feces (%)	Pb in breastmilk/mothers' blood (%)
Overall	1.8 $\pm$ 1.5 (405, 0.2–11.2)	60.2 $\pm$ 67.6 (395, 1.1–623.2)	5.3 $\pm$ 7.4 (210, 0.01–68.7)	5.6 $\pm$ 8.0 (406, 0.3–67.8)
Near the mine	1.9 $\pm$ 1.6 (321, 0.2–11.2)	65.8 $\pm$ 72.8 (312, 1.1–623.2)	4.6 $\pm$ 6.4 (160, 0.01–39.5)	5.4 $\pm$ 7.6 (324, 0.3–67.0)
Bwacha	1.3 $\pm$ 1.1 (84, 0.2–5.3)	38.8 $\pm$ 36.1 (83, 1.9–246.0)	7.7 $\pm$ 9.8 (50, 0.03–68.7)	6.5 $\pm$ 9.5 (82, 1.1–67.8)

Table 6. Differences in Pb concentrations in samples ($\mu\text{g/dL}$) between boys and girls in Kabwe, Zambia; mean $\pm$ SD values (sample size, minimum–maximum).

Area (N)	BLLs in infants, $\mu\text{g/dL}$		BLLs in mothers, $\mu\text{g/dL}$		Pb in breastmilk, $\mu\text{g/L}$		Pb in infant's feces, mg/kg dry weight	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Overall	19.1 $\pm$ 20.6	16.9 $\pm$ 14.9	11.1 $\pm$ 9.0	11.6 $\pm$ 9.4	5.6 $\pm$ 7.7	4.9 $\pm$ 6.2	23.0 $\pm$ 61.9	57.7 $\pm$ 311.5
(418)	(213, 1.0–93.4)	(193, 0.8–83.8)	(220, 2.0–82.6)	(197, 1.5–67.3)	(215, 0.4–51.9)	(192, 0.5–50.4)	(113, 0.08–451.0)	(99, 0.1–3002.7)
Near the mine	23.3 $\pm$ 21.9	19.5 $\pm$ 15.1	12.9 $\pm$ 9.3	13.1 $\pm$ 9.6	6.5 $\pm$ 8.3	5.5 $\pm$ 6.6	30.4 $\pm$ 71.1	68.4 $\pm$ 345.5
(333)	(163, 1.6–93.4)	(158, 1.7–83.8)	(171, 2.3–82.6)	(162, 1.9–67.3)	(167, 0.4–51.9)	(157, 0.5–50.4)	(82, 0.09–451.0)	(80, 0.1–3002.7)
Kasanda	27.7 $\pm$ 24.5	21.7 $\pm$ 16.1	17.1 $\pm$ 12.7	14.2 $\pm$ 7.4	9.5 $\pm$ 8.6	8.9 $\pm$ 9.3	53.2 $\pm$ 117.9	24.4 $\pm$ 45.3
(82)	(41, 5.6–93.4)	(39, 3.7–83.8)	(43, 2.3–82.6)	(39, 4.8–51.2)	(41, 1.9–41.3)	(38, 1.9–50.4)	(14, 0.6–451.0)	(14, 1.2–174.2)
Makululu	37.5 $\pm$ 23.8 *	25.8 $\pm$ 13.2	16.7 $\pm$ 7.5	18.3 $\pm$ 11.7	8.0 $\pm$ 8.0	6.4 $\pm$ 6.3	53.1 $\pm$ 78.3	105.0 $\pm$ 479.1
(102)	(44, 7.9–82.7)	(58, 5.6–70.9)	(44, 7.8–39.6)	(58, 3.3–67.3)	(44, 1.1–40.2)	(56, 1.3–36.2)	(30, 2.3–375.9)	(39, 1.1–3002.7)
Chowa	15.7 $\pm$ 14.6	15.7 $\pm$ 17.1	11.6 $\pm$ 6.3	10.6 $\pm$ 5.9	6.3 $\pm$ 8.0	4.5 $\pm$ 3.5	3.6 $\pm$ 6.5 *	70.3 $\pm$ 205.3
(58)	(30, 3.5–62.3)	(19, 3.2–57.1)	(35, 3.3–36.2)	(23, 3.0–25.9)	(34, 1.4–42.1)	(23, 1.3–13.3)	(17, 0.09–27.6)	(14, 0.1–780.4)
Katondo	11.1 $\pm$ 9.4	10.6 $\pm$ 10.6	6.6 $\pm$ 3.9	6.0 $\pm$ 3.0	2.8 $\pm$ 7.4	1.6 $\pm$ 1.3	4.6 $\pm$ 4.9	4.1 $\pm$ 5.5
(91)	(48, 1.6–39.0)	(42, 1.7–51.0)	(49, 2.4–21.6)	(42, 1.9–13.5)	(48, 0.4–51.9)	(40, 0.5–6.5)	(21, 0.1–14.8)	(13, 0.3–19.4)
Bwacha	5.5 $\pm$ 4.1	4.7 $\pm$ 4.2	4.8 $\pm$ 3.1	4.7 $\pm$ 3.8	2.5 $\pm$ 3.6	1.9 $\pm$ 1.7	3.5 $\pm$ 12.3	12.5 $\pm$ 42.1
(85)	(50, 1.0–22.0)	(35, 0.8–17.3)	(49, 2.0–21.1)	(35, 1.5–23.1)	(48, 0.6–17.9)	(35, 0.5–6.7)	(31, 0.08–68.7)	(19, 0.1–184.0)

Note: * and ** indicate $p < 0.05$ and $p < 0.01$ between boys and girls, respectively. BLL, blood lead level.

Table 7. Correlation coefficients (R^2) among factors and Pb concentrations in samples in all infants and mothers in the present study.

	Age of infants	Height	Weight	Age of mothers	BLLs in infants	BLLs in mothers	Pb in breastmilk	Pb in infants' feces
Age of infants		0.64***	0.59***	NS	0.46***	NS	NS	0.44***
Height			0.76***	NS	0.36***	NS	NS	0.27***
Weight				NS	0.32***	NS	NS	0.28***
Age of mothers					NS	NS	NS	NS
BLLs in infants						0.68***	0.52***	0.83***
BLLs in mothers							0.58***	0.57***
Pb in breastmilk								0.46***
Pb in infants' feces								

Note: *** indicates $p < 0.001$. BLL, blood lead level; NS, not significant.

Table 8. Correlation coefficients (R^2) among factors and Pb concentrations in samples in all infants and mothers from sites near the mine.

	Age of infants	Height	Weight	Age of mothers	BLLs in infants	BLLs in mothers	Pb in breastmilk	Pb in infant's feces
Age of infants		0.65***	0.61***	NS	0.51***	NS	NS	0.48***
Height			0.79***	NS	0.36***	-0.19**	-0.13*	0.25**
Weight				NS	0.42***	NS	NS	0.35***
Age of mothers					NS	NS	NS	NS
BLLs in infants						0.58***	0.43***	0.82***
BLLs in mothers							0.53***	0.48***
Pb in breastmilk								0.38***
Pb in infant's feces								

Note: *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. BLL, blood lead level; NS, not significant

Table 9. Correlation coefficients (R^2) among factors and Pb concentrations in samples in infants and mothers from Bwacha in the present study.

	Age of infants	Height	Weight	Age of mothers	BLLs in infants	BLLs in mothers	Pb in breastmilk	Pb in infant's feces
Age of infants		0.57***	0.47***	NS	0.65***	NS	NS	0.48***
Height			0.65***	NS	0.48***	NS	NS	0.36*
Weight				NS	0.42***	NS	NS	0.40**
Age of mothers					NS	-0.31**	NS	NS
BLLs in infants						0.29**	NS	0.68***
BLLs in mothers							NS	NS
Pb in breastmilk								NS
Pb in infant's feces								

Note: *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. BLL, blood lead level; NS, not significant

Table 10. Mean $\pm$ SD values of the Pb isotope ratios in different samples from Kasanda and Makululu.

Samples (N)	$^{208}\text{Pb}/^{206}\text{Pb}$	$^{207}\text{Pb}/^{206}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$
Infants' blood (26)	2.128 ± 0.006^{abc}	0.8723 ± 0.003	38.299 ± 0.096^{ac}	15.696 ± 0.020^a
Mothers' blood (26)	2.126 ± 0.006^a	0.8720 ± 0.003	38.235 ± 0.082^b	15.680 ± 0.015^b
Breastmilk (26)	2.129 ± 0.002^{ab}	0.8729 ± 0.001	38.257 ± 0.093^{ab}	15.687 ± 0.020^{ab}
Infants' feces (26)	2.129 ± 0.005^{bc}	0.8723 ± 0.002	38.338 ± 0.099^c	15.707 ± 0.019^c
Soil (32)	2.131 ± 0.002^c	0.8724 ± 0.001	38.408 ± 0.036^d	15.723 ± 0.005^d
Kabwe galena (Kamona et al., 1999)	2.134 ± 0.0009	0.8731 ± 0.0003	38.410 ± 0.033	15.713 ± 0.010

Note: Various small letters indicate a significant difference among areas ($p < 0.05$).

Table 11. Mean $\pm$ SD values (minimum-maximum) of the blood biochemical analysis of the mothers.

Parameter	Overall (194)	Near the mine (155)	Bwacha (39)	Reference range
ALT, U/L*	11.3 ± 4.4 (7–38)	11.5 ± 4.5 (7–38)	10.3 ± 3.6 (7–28)	4–44
ALP, U/L*	198.3 ± 54.1 (69–366)	202.1 ± 55.9 (69–366)	183.3 ± 43.9 (119–333)	104–138
AST, U/L	19.8 ± 6.0 (0.2–54)	20.0 ± 6.0 (0.2–54)	19.2 ± 5.7 (15–47)	8–38
GGT, U/L	20.9 ± 16.7 (2–139)	21.8 ± 17.9 (2–139)	17.3 ± 10.2 (4–61)	16–73
LDH, U/L*	124.3 ± 44.7 (43–301)	121.7 ± 45.6 (43–301)	134.3 ± 39.7 (51–229)	106–221
T-Bil, mg/dL	0.3 ± 0.5 (0.1–7.0)	0.3 ± 0.6 (0.1–7.0)	0.3 ± 0.2 (0.1–0.9)	0.1–1.2
TP, g/dL	7.7 ± 0.7 (0.9–10.6)	7.7 ± 0.8 (0.9–10.6)	7.7 ± 0.4 (6.7–8.6)	6.7–8.3
Alb, g/dL*	4.3 ± 0.3 (3.5–5.1)	4.3 ± 0.3 (3.5–5.1)	4.4 ± 0.3 (3.9–4.9)	3.8–5.0
BUN, mg/dL	9.8 ± 2.6 (4.0–18.6)	9.8 ± 2.7 (4.0–18.6)	9.7 ± 2.4 (5.9–15.4)	8–23
Cre, mg/dL	0.8 ± 1.0 (0.4–13.9)	0.8 ± 1.1 (0.4–13.9)	0.7 ± 0.1 (0.6–1.0)	0.46–0.79
UA, mg/dL	4.3 ± 0.9 (1.8–7.7)	4.3 ± 0.9 (1.8–6.5)	4.4 ± 1.0 (2.8–7.7)	3.0–5.5
Metallothionein, $\mu\text{g/L}^{***}$	28.0 ± 36.0 (0.6–374.6)	19.1 ± 15.4 (0.6–126.1)	61.7 ± 62.8 (6.7–374.6)	18.4–27.6

Note: * and *** indicate $p < 0.05$ and $p < 0.001$ between sites near the mine and Bwacha, respectively.

Table 12. Correlation coefficients (R^2) between Pb and blood biochemical parameters in mothers.

	BLLs in all mothers	BLLs in mothers from sites near the mine	BLLs in mothers from Bwacha
ALT	NS	NS	NS
ALP	NS	NS	NS
AST	0.16*	0.17*	NS
GGT	NS	NS	NS
LDH	NS	0.19*	NS
T-Bil	NS	NS	NS
TP	NS	NS	NS
Alb	-0.14*	NS	NS
BUN	NS	-0.26*	NS
Cre	NS	NS	NS
UA	NS	NS	NS
Metallothionein	-0.53***	-0.43***	NS

Note: * and *** indicate $p < 0.05$ and $p < 0.001$, respectively. BLL, blood lead level

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SUMMARY

The final goal of my thesis is to contribute to solving the Pb poisoning problem in a Pb mining area, Kabwe Zambia. The studies presented here focused on the scale of Pb exposure, and the sources and routes of Pb exposure in animals and humans. I elucidated the Pb exposure situation in domestic dogs, infants and mothers in the area, and the potential sources of Pb exposure in them. Moreover, the health impacts of Pb exposure in dogs and mothers were evaluated.

In chapter 1, I revealed high exposures of dogs in Kabwe to Pb and other metals as the first report. The locations of the dogs and their ages were significantly related to their Pb and metal exposures. The trends of the exposures of dogs were shown to be largely similar to those previously reported for humans. Dogs could be useful as sentinel animals for Pb exposure of human residing in Kabwe. Moreover, the obtained results also suggest that the exposure to Pb and other metals in dogs remarkably decreased about 5 km away from the mine. The scale of Pb exposure in dogs could be a part of Kabwe, especially near the mine, not a whole area. The source of Pb exposure of dogs was determined to result from the galena in mine in Kabwe. It is necessary to reduce Pb exposure from environment, such as soils. Moreover, different trends of exposure among individual dogs were found. The factors contributing to the individual differences of Pb exposure must be investigated to reduce Pb exposure.

On the other hand, the mean values of most blood biochemical parameters were surprisingly within or close to the standard reference values, in contrast to the high metal exposure of these dogs. This indicates that Pb exposure in the dogs in Kabwe did not significantly impact their health, as was observed during sampling, in which all sampled dogs

appeared healthy. These results suggest that the adverse effects of chronic Pb exposure on dogs might be different from those of acute exposure. Moreover, these results support a hypothesis that dogs in Kabwe could have some biological defense mechanism for Pb exposure. Further studies are necessary to elucidate the health impacts of chronic Pb exposure and the mechanism how dogs mitigate Pb toxicity.

In chapter 2, the study is the first report to reveal the relationship between Pb exposure in infants and mothers via breastfeeding in Kabwe. High Pb concentrations in breastmilk, which were above the WHO acceptable level for breastfeeding, were found and could be one of the sources of Pb exposure in infants. On the other hand, Pb concentrations in infants' and mothers' blood, breastmilk, and infants' feces from Bwacha, which is a site far from the mine, were remarkably lower than those from sites near the mine. These results suggest that the high Pb exposure in humans could occur only in a part of Kabwe, especially near the mine as well as Pb exposure in dogs. The results of the isotope ratio analysis suggest that the main source of Pb exposure in infants is Pb from environment, such as from soil. Therefore, environmental remediation is urgently needed to reduce the Pb exposure in both infants and mothers in Kabwe.

On the other hand, the blood biochemical profiles of most analyzed parameters in mothers were interestingly within, or close to, the standard reference values. These results indicate that Pb exposure in mothers did not significantly impact their health. Moreover, these results support the hypothesis that the health impacts of chronic Pb exposure could be different from those of acute Pb exposure. However, a detailed questionnaire survey or medical check-ups were not performed in the present study. Further study should reveal the health impacts of chronic Pb exposure using a detailed questionnaire survey or medical check-ups. The metallothionein, which was considered to play a role for a biological defense

for Pb exposure, were not positively related with high Pb exposure. To determine the mechanism, a detailed research is necessary in parallel with the assessment of chronic Pb exposure in local people.

High BLLs in mothers were found in the study, suggest the possibility of Pb exposure in fetus via placenta as well as Pb exposure in infants via breastfeeding. Thus, mothers in Kabwe should be treated with chelation therapy to reduce the Pb exposure in their fetus and infants.

My research revealed high Pb exposure in dogs and humans in a Pb mining area, Kabwe, Zambia and could grasp the scale of Pb exposure briefly. Moreover, one of the sources of Pb exposure in dogs and humans were determined to be of environmental origin, such as soils. However, the health impacts of Pb exposure in dogs and humans were not clearly determined using blood biochemical test. The results strongly suggest the difference of health impacts between acute and chronic Pb exposure, and the possibility that animals and humans in Kabwe have some biological defense mechanism for Pb exposure. Further study should focus on the detailed survey on the health impact and the biological defense mechanism to mitigate the Pb toxicity.

Reducing Pb exposure in dogs and humans in Kabwe, Zambia are necessary because Pb has a toxicity even at low level, such as impairment of IQ. The results of my studies suggest that the main source of Pb exposure in both dogs and humans is contaminated soil from the mine. Therefore, environmental remediation is urgently necessary to reduce the Pb exposure. However, it takes long time to remediate the contaminated area in Kabwe. In parallel with environmental remediation, local people should be treated with chelation therapy. Children who are vulnerable Pb must be treated as priority. High BLLs in mothers

could be a potential source of Pb exposure in their fetus and infants. Thus, chelation therapy for mothers is highly recommended as well as children.

In conclusion, my researches clarified the current circumstances of Pb poisoning in dogs and humans. My works provides useful data to help reducing Pb exposure in them and preventing further exposure. I hope that my researches contribute to solve Pb pollution in Kabwe, Zambia as soon as possible, and local people live without the threat of Pb poisoning. Moreover, it would really appreciate if my thesis could contribute to solving Pb poisoning and environmental pollution in the world also.

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