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

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Abstract

Lead (Pb) poses a serious public health concern. 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 Pb concentration in breastmilk (range: 0.4–51.9, mean: 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. The Pb isotope ratios in infants' feces were the most similar to Pb ratios in the soil samples. The results suggest that infants are also exposed to Pb from the environment. Pb exposure in infants through breastfeeding and soil ingestion could potentially exceed daily intake of Pb which causes neurodevelopmental toxicity. In contrast to the high BLLs in mothers, the plasma biochemical profiles of most analyzed parameters were interestingly within, or close to, the standard reference values. Our data suggest that environmental remediation is urgently needed to reduce the Pb exposure in infants and mothers from the environment in Kabwe in parallel with chelation therapy.

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

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 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) (Canfield et al., 2003). 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, 2012, 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). The European Food Safety Authority (EFSA) observed that Pb dietary intake of 0.5 μg/kg body weight (bw)/day would be associated with developmental neurotoxicity in young children (EFSA, 2010).

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 μg/L. Mothers with confirmed BLLs above 40 μg/dL should pump and discard their breastmilk (CDC, 2010).

Lead exposure primarily occurs via ingestion and inhalation, and can be traced to numerous sources, including battery recycling, gasoline, paint, as well as 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 exposure. One such method for identifying sources is the use of Pb isotopic tracing (Komarek et al., 2008; Gulson, 2008). 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; Veysseyre et al., 2001).

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 μg/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, as has been observed in many other studies. 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 μg/dL which indicated that treatment was required (Yabe et al., 2020, Nakata et al., 2021). 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.

The current study aimed 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. Daily intake of Pb in infants through breastfeeding and soil ingestion was calculated to estimate the burden of routes of Pb exposure. The Pb isotope ratios in samples were analyzed in a limited number of samples to determine the source of Pb exposure. Furthermore, a plasma 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, metallic residues 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 rainy season from January to March of 2017 (Fig. 1). Kasanda, Makululu, Chowa, Katondo, and Bwacha health centers were located about 0.9, 2.6, 1.4, 4.5, and 6.1 km from the mine, respectively. Kasanda and Makululu are located on the western side of the mine and in the direction of prevailing winds.

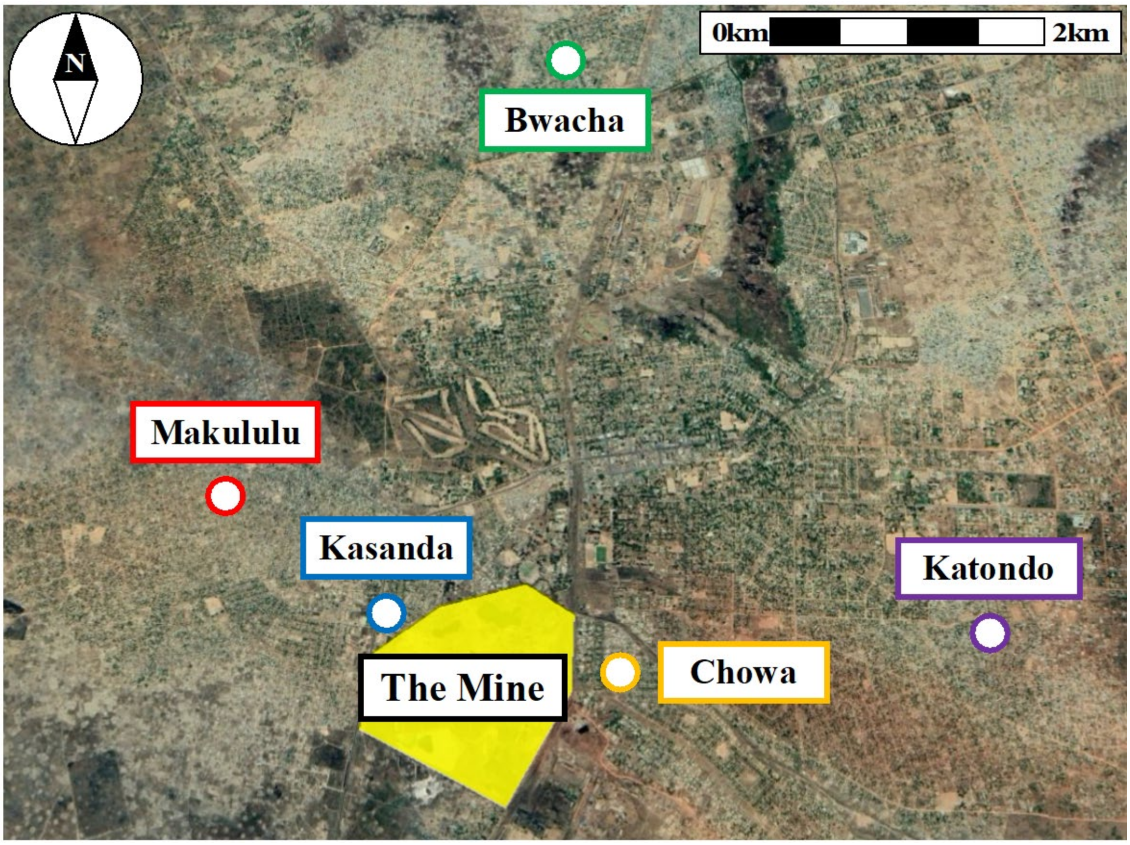


Fig. 1. Map of the sampling sites in Kabwe, Zambia (image modified from Google Earth).

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. Breastmilk samples from mothers were collected in clean sample cups by gentle compression of the breast and transferred to 2 mL sample tubes for storage and transportation. To avoid contamination, the hands as well as venipuncture and breast sites were cleaned and sanitized with an ethanol swab before the sample collection. 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 used diaper in the morning of the following day. Household soil samples were collected in June 2016 from Kasanda (n = 12) and Makululu (n = 20) as a reference of environmental samples for Pb stable isotope analysis.

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 Hokkaido University, Japan.

2.3 Pb and Metal Concentration Analysis

Pb and other metals (iron (Fe), copper (Cu), Zn, and silver (Ag) were extracted from the samples. Thawed fecal and bulk soil samples were weighed on heat-resistant tissue drying plates and dried for 48 h in a tissue drying oven at 60 °C, whereas whole blood and breastmilk samples were only thawed. Each blood and breastmilk sample (1mL) and 50 mg of each dried fecal and soil sample were analyzed. Detailed method was described in the supplementary materials.

2.4 Calculation of Daily Intake of Pb in Infants through Breastfeeding and Soil Ingestion

Daily intake of Pb in infants through breastfeeding and soil ingestion was calculated. The calculation was conducted using the maximum, mean, and minimum Pb concentrations in breastmilk and soil samples in the present study using the formulas below. The amounts of daily breastmilk intake and soil ingestion in infants were set as 0.78 L/day and 30 mg/day reported by Costa et al. (2010) and United States Environmental Protection Agency (US EPA, 2011), respectively.

Daily intake of Pb = (Pb concentrations in breastmilk × Amount of daily breastmilk intake) + (Pb concentrations in soil × amount of daily soil ingestion)

In the present study, 0.5 μg/kg bw/day was used as the reference value as the limit of daily intake of Pb for infants (EFSA, 2010).

2.5 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 as well as 32 soil samples. As mentioned above, these soil samples were collected at the preliminary survey in 2016. The sampling area between blood and soil are same, but not all the locations were exactly from the same household. The sample dissolution procedure was similar to previously described methods (Kuritani and Nakamura, 2002; Nakayama et al., 2019). The detailed methods of the high precision isotope analysis were described in the supplementary materials. We have measured duplicates of breastmilk samples and the results of Pb isotope ratios had the similar trends. 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, since normalization to ^{204}Pb yields the largest variability between reservoirs (Komárek et al., 2008), we included ^{204}Pb data with correction (Kuritani et al., 2003, 2002). This was done in order to observe the detail variability of isotope ratio among the breastmilk, blood, feces and soil samples in this study.

2.6 Plasma 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.

2.7 Statistical Analysis

All data from the experiments and questionnaires were combined into a single electronic database and checked for accuracy and outliers. 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 about 6 km from the mine (Table 1; Fig. 1). 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 ($p < 0.05$). Body weight of the infants from Chowa was significantly lower than that of the infants from other areas ($p < 0.05$).

The overall 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 ($p < 0.01$, Supplementary Table S3). 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 higher than that of girls when considering all infants ($p < 0.01$), and for 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.

Table 1. General characteristics of infants and mothers, as well as Pb concentrations in blood, breastmilk and fecal samples in Kabwe, Zambia; mean ± 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, μg/dL	BLLs in mothers, μg/dL	Pb in breastmilk, μg/L	Pb in infants' feces, mg/kg dry weight
Overall (418)	221:197	7.1 ± 3.8 (417, 0.1–16.8)	64.2 ± 7.7 (361, 45.0–95.0)	7.3 ± 1.7 (411, 1.9–12.1)	26.1 ± 6.5 (412, 16.3–46.1)	18.0 ± 18.1 ^{A} (406, 0.8–93.4)	11.3 ± 9.2 ^{B} (417, 1.5–82.6)	5.3 ± 7.0 ^{C} (407, 0.4–51.9)	39.2 ± 217.7 ^{D} (212, 0.08–3002.7)
Near the mine (333)	171:162	7.2 ± 4.0 (332, 0.1–16.8)	64.5 ± 8.0 (280, 45.0–95.0)	7.2 ± 1.7 (329, 1.9–12.1)	26.1 ± 6.6 (329, 16.3–46.1)	21.4 ± 18.9 $^{**, A}$ (321, 1.6–93.4)	13.0 ± 9.5 $^{**, B}$ (333, 1.9–82.6)	6.1 ± 7.5 $^{**, C}$ (324, 0.4–51.9)	49.2 ± 248.0 $^{**, D}$ (162, 0.09–3002.7)
Kasanda (82)	43:39	7.3 ± 3.8 ^{a} (82, 0.6–16.7)	64.5 ± 8.4 ^{a} (74, 49.0–95.0)	7.6 ± 1.5 ^{a} (79, 4.0–12.1)	27.1 ± 6.7 (82, 17.4–45.4)	24.8 ± 20.9 $^{a, A}$ (80, 3.7–93.4)	15.8 ± 10.6 $^{a, B}$ (82, 2.3–82.6)	9.2 ± 8.9 $^{a, C}$ (79, 1.9–50.4)	38.8 ± 88.9 $^{ab, D}$ (28, 0.6–451.0)
Makululu (102)	44:58	7.7 ± 3.7 ^{a} (102, 1.5–16.7)	63.0 ± 7.0 ^{a} (102, 45.0–85.0)	7.4 ± 1.4 ^{a} (101, 4.1–11.9)	26.2 ± 7.0 (102, 16.6–45.9)	30.8 ± 19.4 $^{b, A}$ (102, 5.6–82.7)	17.6 ± 10.1 $^{a, B}$ (102, 3.3–67.3)	7.1 ± 7.1 $^{ab, C}$ (100, 1.1–40.2)	82.4 ± 362.7 $^{a, D}$ (69, 1.1–3002.7)
Chowa (58)	35:23	5.3 ± 3.9 ^{b} (58, 0.1–15.2)	56.2 ± 7.1 ^{b} (13, 49.0–67.0)	5.6 ± 1.4 ^{b} (58, 3.5–8.7)	26.9 ± 6.3 (58, 17.6–41.8)	15.7 ± 15.5 $^{c, A}$ (49, 3.2–62.3)	11.2 ± 6.1 $^{b, A}$ (58, 3.0–36.2)	5.6 ± 6.5 $^{b, B}$ (57, 1.4–42.1)	33.7 ± 139.4 $^{bc, C}$ (31, 0.09–780.4)
Katondo (91)	49:42	7.7 ± 4.3 ^{a} (90, 0.9–16.8)	67.5 ± 7.6 ^{c} (91, 48.0–94.0)	7.8 ± 1.7 ^{a} (91, 1.9–11.1)	24.6 ± 5.9 (87, 16.3–46.1)	10.9 ± 9.9 $^{c, A}$ (90, 1.6–51.0)	6.3 ± 3.5 $^{c, B}$ (91, 1.9–21.6)	2.3 ± 5.5 $^{c, C}$ (88, 0.4–51.9)	4.4 ± 5.1 $^{c, D}$ (34, 0.1–19.4)
Bwacha (85)	50:35	6.9 ± 3.2 ^{ab} (85, 1.6–13.5)	63.0 ± 6.6 ^{a} (81, 51.0–86.0)	7.6 ± 1.6 ^{a} (82, 4.6–11.4)	26.2 ± 5.9 (83, 16.6–43.3)	5.2 ± 4.1 $^{d, A}$ (85, 0.8–22.0)	4.7 ± 3.4 $^{d, A}$ (84, 1.5–23.1)	2.3 ± 2.9 $^{c, B}$ (83, 0.5–17.9)	6.9 ± 27.6 $^{d, C}$ (50, 0.08–184.0)

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

3.2 Pb and Other Metals Concentrations in Blood, Breastmilk, Infants' Feces, and Soil.

The overall mean of Pb concentrations in the soil samples (n = 32) was 1048 ± 1470 mg/kg (dry weight) and ranged from 346 to 6327 mg/kg.

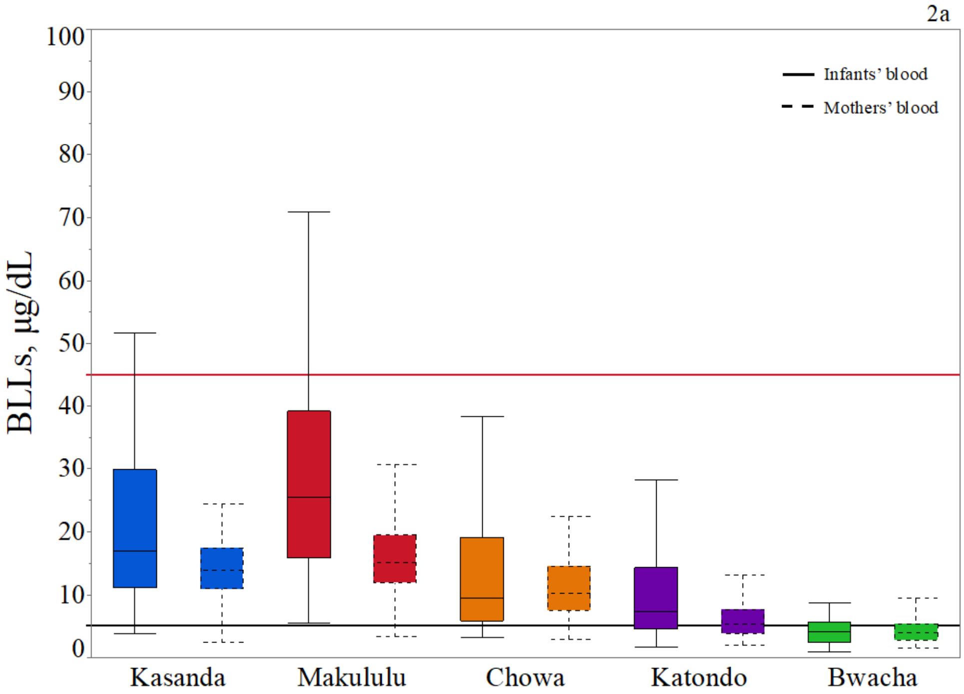
The overall mean values of BLLs in infants and mothers were 18.0 ± 18.1 and 11.3 ± 9.2 μg/dL, respectively (Table 1 and Fig. 2a). We found 76.8% of infants (312/406) and 73.6% of mothers (307/417) had BLLs above the reference value for Pb exposure (5 μg/dL; CDC, 2019, 2012). Moreover, BLLs in 8.9% of infants (36/406) and 1.2% of mothers (5/417) were above 45 μg/dL, the recommended threshold BLL for chelation therapy (Meyer, 2008). No infants or mothers had BLLs above the lethal level for Pb exposure (100 μg/dL), however, the highest BLL in an infant in the present study was 93.4 μg/dL. The overall mean of Pb concentrations in breastmilk and infants' feces were 5.3 ± 7.0 μg/L and 39.2 ± 217.7 mg/kg (dry weight), respectively (Fig. 2b and 2c). Overall, 30.0% of breastmilk samples (122/407) had Pb concentrations of more than 5 μg/L, which is above the accepted level for breastfeeding (WHO 1989).

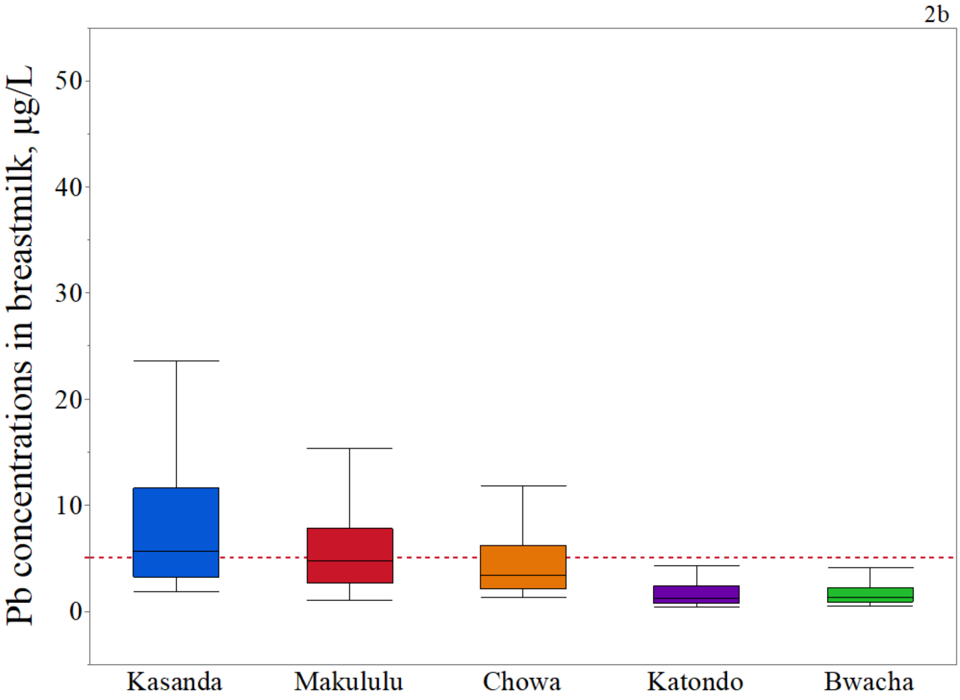
There were significant differences in Pb concentrations among sample types in the samples from all sites: infants' feces > infants' blood > mothers' blood > breastmilk ($p < 0.05$). Among the samples from Chowa and Bwacha, there were no significant differences in Pb concentrations between infants' and 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 (Supplementary Table S4). On the other hand, Pb concentrations in infants' blood were 5.3 ± 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 each sample type from Makululu were the 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 higher in Bwacha ($p = 0.06$, Supplementary Table S5). 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, although the association was not statistically significant ($p = 0.08$).

Supplementary Tables S6, S7, S8, and S9 show Fe, Cu, Zn, and Ag concentrations in the samples, respectively. Fe concentrations in infants' blood and Fe, Cu, Zn, and Ag concentrations in mothers' blood from sites near the mine were significantly lower than the concentrations in samples from Bwacha. On the other hand, Fe concentrations in breastmilk from sites near the mine were significantly higher than the concentrations in samples from Bwacha.


2a
Infants' blood
Mothers' blood
BLLs, µg/dL
100
90
80
70
60
50
40
30
20
10
0
Kasanda
Makululu
Chowa
Katondo
Bwacha


2b
Pb concentrations in breastmilk, µg/L
50
40
30
20
10
0
Kasanda
Makululu
Chowa
Katondo
Bwacha

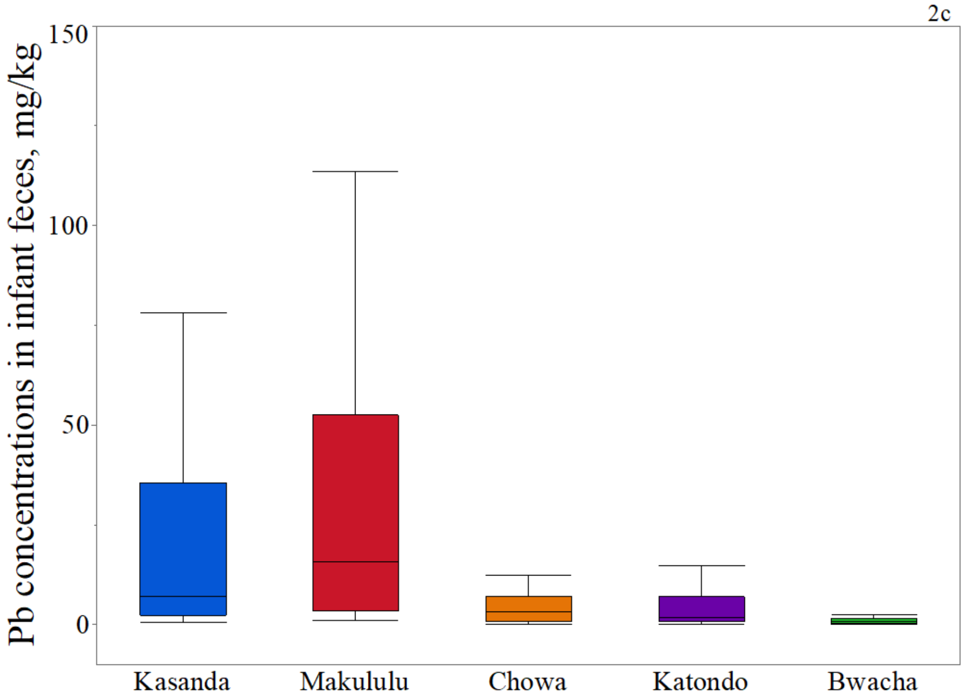


Fig. 2. BLLs in infants and mothers (2a), Pb concentrations in breastmilk (2b), and infants' feces (2c) among areas. The blue, red, orange, violet, and green colors indicate samples from Kasanda, Makululu, Chowa, Katondo, and Bwacha, respectively. Solid and dot box plots in Fig. 2a indicate BLLs in infants and mothers, respectively. Red and black lines in Fig. 2a indicate 45 and 5 µg/dL, respectively. The red dotted line in Fig 2b indicates the 5 µg/L level.

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 2, $p < 0.001$). There were significant positive correlations of Pb concentrations among all sample types ($p < 0.001$, Supplementary Fig. S2). BLLs in infants and Pb concentrations in infants' feces had a significant positive correlation with the age (Supplementary Fig. S3), height, and weight of infants ($p < 0.001$).

In all samples from sites near the mine, the same trend was found (Supplementary Table S10). 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 (Supplementary Table S11). Supplementary Tables S12, 13, and 14 show the relationships among Pb and other metals in the samples from all areas, from sites near the mine, and Bwacha, respectively. In the samples from all areas, Pb concentrations in infants' blood significantly increased as Cu ($p < 0.001$, $\rho = 0.34$) and Ag ($p < 0.001$, $\rho = 0.28$) concentrations in infants' blood. There were significant negative correlations between Pb and Fe concentrations in infants' blood as well as in mothers' blood.

Table 2. 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.

3.4 Daily Intake of Pb in Infants through Breastfeeding and Soil Ingestion

Table 3 shows the results of daily intake of Pb in infants through breastfeeding and soil ingestion. The results ranged from 0.3 to 40.5 µg/day through breastfeeding and from 10.4 to 189.8 µg/day through soil ingestion depending on Pb concentrations in the samples. Daily intake of Pb through combined breastfeeding and soil ingestion ranged from 10.7 to 230.3 µg/day. The reference value of daily intake of Pb which would be associated with developmental neurotoxicity in infants in the present study was 3.7 µg/day (the mean bw: 7.3 kg) and ranged from 1.0 µg/day (the minimum bw: 1.9 kg) to 6.1 µg/day (the maximum bw: 12.1) based on the EFSA's reference value (0.5 µg/kg bw/day) (EFSA, 2010). The minimum of calculated daily intake of Pb even only through soil ingestion exceeded the maximum of the limit.

Table 3. Sum of possible daily intake of Pb (µg/day) through breastfeeding and soil ingestion.

			Daily intake of Pb through soil ingestion (30 mg/day)		
			Maximum Pb (6326.7 mg/kg) 189.8 µg/day	Mean Pb (1047.6 mg/kg) 31.4 µg/day	Minimum Pb (345.7 mg/kg) 10.4 µg/day
Daily intake of Pb through breastmilk (0.78 L/day)	Maximum Pb (51.9 µg/L)	40.5 µg/day	230.3	71.9	50.8
	Mean Pb (5.3 µg/L)	4.1 µg/day	193.9	35.5	14.5
	Minimum Pb (0.4 µg//L)	0.3 µg/day	190.1	31.8	10.7

Note: The reference value of daily intake Pb in the present study was 3.7 µg (the mean bw: 7.3 kg) ranged from 1.0 µg (the minimum bw: 1.9 kg) to 6.1 µg (the maximum bw: 12.1) based on the EFSA's reference value (0.5 µg/kg bw/day) (EFSA, 2010).

3.5 Pb Isotope Ratio Analysis

Table 4 shows the mean values of the $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$, $^{208}Pb/^{204}Pb$, and $^{207}Pb/^{204}Pb$ ratios in the samples. The $^{208}Pb/^{206}Pb$ Pb isotope ratios in mothers' blood (2.127 ± 0.006) were significantly different from those in infants' blood and feces and the soil (2.129 ± 0.006, 2.130 ± 0.004, and 2.131 ± 0.002, respectively, $p < 0.05$). On the other hand, there was no significant difference in $^{207}Pb/^{206}Pb$ ratios between infants' and mothers' blood. Both $^{208}Pb/^{204}Pb$, and $^{207}Pb/^{204}Pb$ 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).

Pb isotope ratios in infants' and mothers' blood, and infants' feces were closer to those reported for Kabwe galena (Kamona et al., 1999) as the reciprocal of Pb concentrations decreased (Supplementary Fig. S4).

The $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ ratios (Fig. 3b) clearly show trends of samples compared to $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ ratios (Fig. 3a and Supplementary Fig. S5).

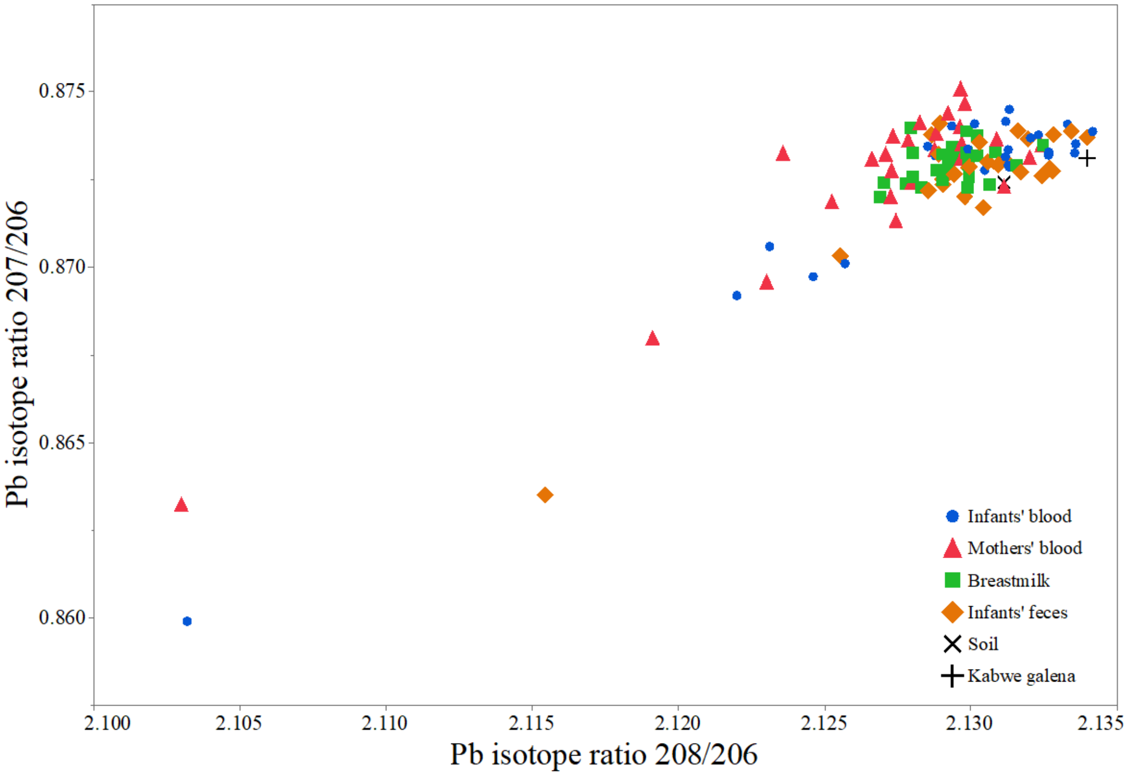
Table 4. Mean ± SD values of the Pb isotope ratios in different samples from Kasanda and Makululu.

Samples (N)	$^{208}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$
Infants' blood (26)	2.129 ± 0.006^{ac}	0.8724 ± 0.003^{ac}	38.271 ± 0.063^{acd}	15.691 ± 0.012^{a}	17.997 ± 0.062^{ab}
Mothers' blood (26)	2.127 ± 0.006^{b}	0.8726 ± 0.002^{a}	38.223 ± 0.080^{bc}	15.680 ± 0.015^{b}	17.970 ± 0.059^{a}
Breastmilk (26)	2.129 ± 0.001^{bc}	0.8729 ± 0.0005^{b}	38.271 ± 0.046^{c}	15.691 ± 0.010^{b}	17.972 ± 0.018^{a}
Infants' feces (26)	2.130 ± 0.004^{ac}	0.8725 ± 0.002^{c}	38.349 ± 0.067^{d}	15.709 ± 0.012^{a}	18.005 ± 0.048^{b}
Soil (32)	2.131 ± 0.002^{a}	0.8724 ± 0.001^{d}	38.407 ± 0.036^{e}	15.723 ± 0.005^{c}	18.022 ± 0.027^{c}
Kabwe galena (Kamona et al., 1999)	2.134 ± 0.0009	0.8731 ± 0.0003	38.410 ± 0.033	15.713 ± 0.010	17.997 ± 0.007

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

Note: The number of significant digits in each ratio value were adjusted with those of the reference (Kamona et al., 1999)

3a


Pb isotope ratio 207/206
0.875
0.870
0.865
0.860
Pb isotope ratio 208/206
2.100
2.105
2.110
2.115
2.120
2.125
2.130
2.135
● Infants' blood
▲ Mothers' blood
■ Breastmilk
◆ Infants' feces
× Soil
+ Kabwe galena

3b

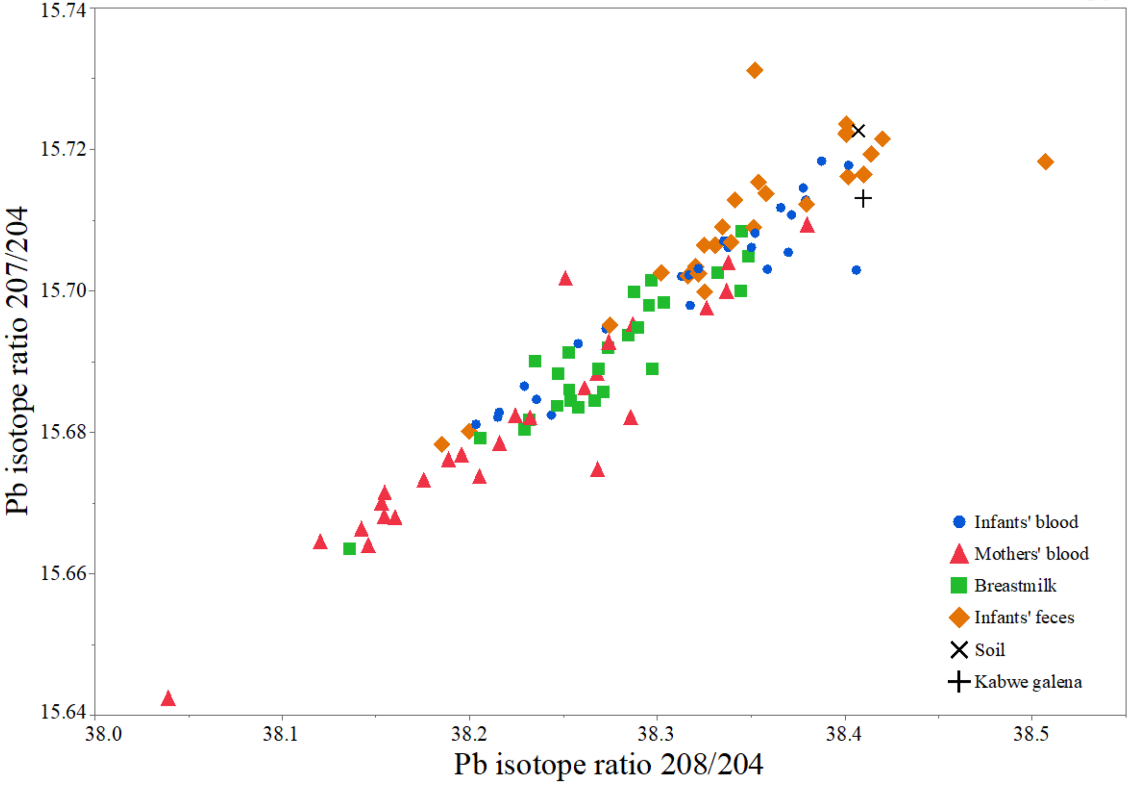

Pb isotope ratio 207/204
15.74
15.72
15.70
15.68
15.66
15.64
Pb isotope ratio 208/204
38.0
38.1
38.2
38.3
38.4
38.5
● Infants' blood
▲ Mothers' blood
■ Breastmilk
◆ Infants' feces
× Soil
+ Kabwe galena

Fig. 3. Pb isotope ratios of individual sample (3a: $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ and 3b: $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$) in different samples from Kasanda and Makululu. The blue circle, red triangle, green square, orange rhombus, and black cross markers indicate infants' blood, mothers' blood, breastmilk, infants' fecal, and soil samples, respectively. The reference value of Kabwe galena was obtained from a report by Kamona et al. (1999) and is indicated by a plus sign.

3.6 Plasma Biochemical Analysis

Supplementary Table S15 shows the mean values of the plasma biochemical analysis on mothers' plasma. The mean values of all parameters in mothers 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, there was a significant positive correlation between the Pb and AST concentrations (Supplementary Table S16, $p < 0.05$, $\rho = 0.16$), and Fe and metallothionein concentrations (Supplementary Table S17, $p < 0.05$, $\rho = 0.17$). 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 ($p < 0.001$, $\rho = -0.53$) significantly decreased as the Pb concentrations increased.

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. Moreover, the metallothionein concentrations significantly associated with decreased Cu concentrations ($p < 0.05$, $\rho = -0.21$). In the mothers from Bwacha, there were no significant correlations between Pb and plasma 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 marginally 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 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; Gulson et al., 1998). In an evaluation of breastmilk/mothers' blood relationships, Gulson et al. (1998) suggested that a ratio of Pb concentration in breast milk to the concentration of Pb in maternal blood greater than 15% should be treated with caution, higher values than this arising probably from sampling and/or analytical contamination.

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 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 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. Toyomaki et al. (2020) reported that Pb concentrations in dog blood decreased with the distance from the mine in Kabwe, and that the exposure to Pb in dogs remarkably decreased about 5 km away from the mine. These findings suggested that location of the townships in the relation to the wind direction and distance from the mine influence the extent of the exposure to Pb in infants and mothers in Kabwe, as has been shown in other studies (Soto-Jiménez and Flegal, 2011; Yun et al., 2018).

In the present study, Fe, Cu, Zn and Ag were also analyzed. In contrast to Pb, the concentrations of Fe, Cu, Zn and Ag in the blood of infants and mothers tended to be lower in areas surrounding the mine area than in Bwacha. Given that Pb poisoning is known to cause anemia, the negative correlation between Pb and Fe concentrations suggested that the higher Pb concentrations negatively affected Fe metabolism, especially absorption (Hegazy et al.,2010; Chen et al., 2019). Positive correlations were found between Pb and Cu, Zn, as well as Ag. These positive co-exposures could be attributed to the presence of Pb, Zn and Ag in the galena in Kabwe as confirmed in previous studies (Nakayama et al., 2011).

Blood lead levels 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). Moreover, Pb concentrations in infants' feces of boys were significantly higher than those of girls in Chowa. This difference could be attributed to differences in breastmilk consumptions as reported by Costa et al. (2010) were boys consumed breastmilk 0.05 kg/d more than girls. These results suggest that boys are more exposed to Pb via ingestion than girls.

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 although inhalation may be an important pathway for very young children in windy environments (Gulson et al., 2009). 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. Moreover, cleaning floors in the house where infants spend most of the time and the kitchen where food could be contaminated by house dust would be important to reduce Pb exposure in infants. On the other hand, only BLLs in mothers from Bwacha significantly decreased as the age of mothers increased. Adults in Bwacha, which is farthest 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 (Gulson et al. 1998; Manton et al. 2003). 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 Pb isotope ratios in infants' samples, especially feces, were almost identical to those in the soil samples. The 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 Kabwe 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 contaminated soil or dust from the mine could be one of the important sources of Pb exposure in infants of Kabwe as well as breastmilk. Understanding which infant behaviors and activities are related to their Pb exposure is required to determine the routes and to minimize the exposure. Pb isotope ratios in infants' blood were similar to those in mothers' blood as infants could be exposed to Pb through breastfeeding and through the placenta before birth. On the other hand, Pb isotope ratios in mothers' samples, especially blood were different from those in infants' feces and the soil samples. Pb isotope ratios in chicken, goats, and vegetables in Kabwe reported by Nakata et al. (2016) were similar to the results in the present study, suggesting that consuming contaminated food could be an important route of Pb exposure in mothers. In the present study, both ^{206}Pb and ^{204}Pb ratios were used to compare the differences of Pb isotope ratios among sample types. Both results were similar, but ^{204}Pb-based 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 as recommended by Gulson (2008).

Daily intake of Pb in infants through breastfeeding and soil ingestion was calculated to estimate the burden of the possible routes of Pb exposure. Given that Pb exposure can cause pediatric neurodevelopmental impairments even at low level (Canfield et al., 2003), the reference value 0.5 μg/kg bw/day was used (US EPA, 2011). From the results obtained in the present study, daily intake of Pb even through soil ingestion alone highly exceeded the reference value. Although the bioavailability of Pb in galena is known to be minimal (Rasmussen et al., 2011), Pb exposure through soil ingestion could be the important source due to the high concentrations. Moreover, the results of daily intake of Pb through breastfeeding using the maximum Pb concentrations were larger than the reference value. These results suggest that both Pb though breastfeeding and soil ingestion are important sources of Pb exposure in infants. In the present study, the daily intake of Pb from the environment was only calculated from soil ingestion. However, US EPA (2011) estimated the same amount (30 mg/day) of dust ingestion in parallel with soil ingestion. This implies that the actual daily intake in the field could be underestimated in the present study. Thus, future studies need to evaluate the detail of Pb exposure in infants as well as mothers in Kabwe including other routes, such as dust ingestion.

In contrast to the high BLLs in mothers, the plasma 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 in Kabwe should therefore focus on the relationship between Pb exposure and nutrition status. In our 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 probable 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 was lower than that in people residing far from the mine. Further studies should focus on both metallothionein concentrations and gene expression. 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 one of the important sources of Pb exposure in infants could be Pb from the environment, especially from soils. Daily intake of Pb in infants through soil ingestion could be enough to exceed the EFSA's reference value of daily intake of Pb. It is important to minimize Pb exposure in infants from soil and dust.. Thus, remediation of the environment in Kabwe is urgently needed to reduce Pb exposure. High BLLs in mothers could also be one of the important sources of Pb exposure in infants via breastfeeding. Also, 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. Conclusions

High Pb concentrations in breastmilk, which were above the WHO acceptable level for breastfeeding, could be one of the important sources of Pb exposure in infants. The results of the isotope ratio analysis suggest that Pb from the environment, such as contaminated soil is one of the important sources of Pb exposure in infants. Moreover, Pb exposure in infants through breastfeeding and soil ingestion potentially exceeded the EFSA's reference value of daily intake of Pb. 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.

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Conflict of interest

The authors declare no conflict of interest.

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