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Author(s)	Toyomaki, Haruya; Yabe, John; Nakayama, Shouta M. M.; Yohannes, Yared B.; Muzandu, Kaampwe; Mufune, Tiza; Nakata, Hokuto; Ikenaka, Yoshinori; Kuritani, Takeshi; Nakagawa, Mitsuhiro; Choongo, Kennedy; Ishizuka, Mayumi
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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

5	Haruya Toyomaki ¹ , John Yabe ^{2, 8} , Shouta M.M. Nakayama ^{1*} , Yared B. Yohannes ^{1,3} ,
6	Kaampwe Muzandu ^{1,2} , Tiza Mufune ⁴ , Hokuto Nakata ¹ , Yoshinori Ikenaka ^{1,5,9,10} ,
7	Takeshi Kuritani ⁶ , Mitsuhiro Nakagawa ⁶ , Kennedy Choongo ^{2, 7} and Mayumi Ishizuka ¹
8	(*Corresponding author)
9	
10	1) Laboratory of Toxicology, Department of Environmental Veterinary Sciences,
11	Faculty of Veterinary Medicine, Hokkaido University, Japan
12	2) The University of Zambia, School of Veterinary Medicine, Zambia
13	3) Department of Chemistry, College of Natural and Computational Science, University
14	of Gondar, Ethiopia
15	4) Ministry of Health, District Health Office, Kabwe, Zambia
16	5) Water Research Group, School of Environmental Sciences and Development, North-

- 17 West University, South Africa
- 18 6) Department of Earth and Planetary Sciences, Graduate School of Science, Hokkaido
- 19 University, Sapporo, Japan
- 20 7) Fiji National University, College of Agriculture, Fisheries & Forestry, School of
- 21 Animal and Veterinary Sciences, Koronivia Campus, Suva, Fiji
- 22 8) Department of Pathobiology, School of Veterinary Medicine, University of Namibia,
- 23 Windhoek, Namibia

24	9) Translational Research Unit, Veterinary Teaching Hospital, Faculty of Veterinary
25	Medicine, Hokkaido University, Sapporo, 060-0818, Japan
26	10) One Health Research Center, Hokkaido University, Japan
27	
28	
29	
30	
31	*Address Correspondence to
32	Shouta M.M. Nakayama
33	Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Faculty
34	of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-
35	0818, Japan.
36	Tel: +81-11-706-5105, Fax: +81-11-706-5105.
37	E-mail: shouta-nakayama@vetmed.hokudai.ac.jp
38	or shoutanakayama0219@gmail.com
39	

41 Abstract

42 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 43 44 breastmilk. The present study was undertaken to determine the Pb exposure and the 45 contribution of lactation as one of the exposure pathways to infants in a Pb mining area, 46 Kabwe, Zambia. Blood, breastmilk and infants' feces were collected from 418 pairs of 47 infants and mothers. The Pb concentrations, isotope ratios in the samples, and 48 biochemistry in mothers' plasma were analyzed. The overall mean of blood lead levels 49 (BLLs) in infants and mothers were 18.0 and 11.3 µg/dL, respectively. High Pb 50 concentration in breastmilk (range: 0.4-51.9, mean: 5.3 µg/L) above the WHO 51 acceptable level between 2 and 5 μ g/L were found and could be one of the sources of Pb 52 exposure in infants. The Pb isotope ratios in infants' feces were the most similar to Pb 53 ratios in the soil samples. The results suggest that infants are also exposed to Pb from 54 the environment. Pb exposure in infants through breastfeeding and soil ingestion could potentially exceed daily intake of Pb which causes neurodevelopmental toxicity. In 55 56 contrast to the high BLLs in mothers, the plasma biochemical profiles of most analyzed 57 parameters were interestingly within, or close to, the standard reference values. Our data 58 suggest that environmental remediation is urgently needed to reduce the Pb exposure in 59 infants and mothers from the environment in Kabwe in parallel with chelation therapy.

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

63 **1. Introduction**

64 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 65 reported in both developed and developing countries (Ajumobi et al., 2014; Haefliger et 66 67 al., 2009; Ruckart et al., 2019). Lead poisoning causes various symptoms, including 68 anemia, nephropathy, and death (Meyer et al., 2008). Children, especially infants are 69 more vulnerable to Pb, compared to adults. To measure exposure to Pb, blood lead level 70 (BLL) has been widely used. In the blood, Pb has a short half-life of 30-40 days 71 (Barbosa et al., 2005). Even at low levels, Pb exposure can cause pediatric 72 neurodevelopmental impairments, such as a reduction in intelligence quotient (IQ) 73 (Canfield et al., 2003). Due to this, the blood Pb reference value for Pb exposure has 74 been set to 5 µg/dL (CDC, 2019, 2012). A BLL above 45 µg/dL is considered the level 75 where treatment is required (CDC, 2012, 2002; Needleman, 2004), and a BLL above 76 100 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 77 78 European Food Safety Authority (EFSA) observed that Pb dietary intake of 0.5 µg/kg 79 body weight (bw)/day would be associated with developmental neurotoxicity in young 80 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).

89 Lead exposure primarily occurs via ingestion and inhalation, and can be traced 90 to numerous sources, including battery recycling, gasoline, paint, as well as mining 91 (Calabrese and Stanek, 1995; Meyer et al., 2008; Schoning et al., 1996; Yabe et al., 92 2010). Identifying the source of Pb exposure is important to prevent exposure. One such 93 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: ²⁰⁸Pb (52%), 94 ²⁰⁷Pb (23%), ²⁰⁶Pb (24%), and ²⁰⁴Pb (1%) (Komárek et al., 2008). The compositions of 95 96 these isotopes are not affected to a measurable extent by physicochemical fractionation 97 processes (Bollhöfer and Rosman, 2001; Veysseyre et al., 2001).

98 The Zambian town of Kabwe accommodates a Pb-zinc (Zn) mining area which, 99 up until its closure in 1994, was operated without adequate pollution laws to regulate 100 mining emissions. Elevated BLLs and Pb concentrations in the feces and urine of 101 children near the mine have been reported, all of which exceeded the 5 µg/dL blood Pb 102 reference value (Yabe et al., 2018, 2015). Yabe et al. (2015) found that BLLs in 103 children between the ages of one and two years old were higher compared with those in 104 children between the ages of four and seven years old in Kabwe, as has been observed 105 in many other studies. It is necessary to reduce and prevent Pb exposure in children. 106 This is especially important in infants, who are more vulnerable to Pb poisoning. 107 Moreover, a more recent study has revealed a high Pb exposure also in mothers, where 108 approximately 5% of mothers were found to have a BLL above 45 µg/dL which 109 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 110

111 for infants in Kabwe. However, the precise sources and routes of Pb exposure in infants 112 have not yet been determined. Furthermore, no clinical studies of Pb poisoning have 113 been done in Kabwe, despite high BLLs being reported in the local people. Some 114 previous studies have reported that metallothionein concentrations, which is a cysteine-115 rich protein that binds and detoxifies toxic metals, increase as metal concentrations in 116 the blood increase (Bizoń and Milnerowicz, 2014; Kowalska et al., 2015). 117 Metallothionein may therefore play an important role in reducing Pb toxicity in the 118 people of Kabwe.

119 The current study aimed to determine the Pb exposure and the contribution of 120 lactation as one of the exposure pathways to infants in a lead mining area, Kabwe, 121 Zambia. Pb concentrations in mothers' breastmilk, infants' feces, and both infants' and 122 mothers' blood, were analyzed. Daily intake of Pb in infants through breastfeeding and 123 soil ingestion was calculated to estimate the burden of routes of Pb exposure. The Pb 124 isotope ratios in samples were analyzed in a limited number of samples to determine the 125 source of Pb exposure. Furthermore, a plasma biochemical analysis including 126 metallothionein concentrations was conducted in the mothers to evaluate the health 127 impact of Pb exposure in this population.

128

129 **2. Materials and Methods**

130 **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.



144

145 Fig. 1. Map of the sampling sites in Kabwe, Zambia (image modified from Google146 Earth).

148 **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.

153 A sensitization campaign about the research activities was conducted by 154 community health workers before sampling in their catchment areas around the health 155 centers. Mothers and guardians were encouraged to participate in the study, and were 156 asked to take their breastfed infants under the age of 1 year and 6 months to the selected 157 health centers for sample collection. Only infants with mothers/guardians that willingly 158 agreed to participate and signed the informed consent were included in the present study. 159 After informed and written consent were obtained from the mothers/guardians, blood 160 samples were collected as described by Yabe et al. (2015). The mothers/guardians were 161 also interviewed to obtain necessary personal details about themselves and their infants, 162 such as age and sex. Sample collection and questionnaire administration were 163 undertaken by certified local nurses. In accordance with ethical requirements, 164 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.

177 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 178 179 transfer agreement (MTA) for human samples from the Zambia National Health 180 Research Ethics Committee (approval No. E00417) was obtained before transportation. 181 Similarly, the phytosanitary certificate from plant quarantine and phytosanitary service, 182 Zambia Ministry of Agriculture, and import permission by plant protection station, 183 Japanese Ministry of Agriculture, Forestry and Fisheries (approval No. 28-313) was 184 also granted for soil samples. The human samples were transported in temperature-185 controlled boxes with ice packs, and the soil samples in temperature-controlled boxes, 186 for further analysis at Hokkaido University, Japan.

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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.

196 2.4 Calculation of Daily Intake of Pb in Infants through Breastfeeding and Soil 197 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.

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205 Daily intake of Pb = (Pb \text{ concentrations in breastmilk} \times Amount of daily breastmilk})
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206 *intake*) + (*Pb concentrations in soil* × *amount of daily soil ingestion*)

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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).

210

211 **2.5 Stable Pb Isotope Analysis**

212 Only 26 sample sets with high Pb concentrations of infants' and mothers' blood, 213 breastmilk, and infants' feces from Kasanda and Makululu, were chosen and analyzed 214 during Pb isotope analysis as well as 32 soil samples. As mentioned above, these soil 215 samples were collected at the preliminary survey in 2016. The sampling area between 216 blood and soil are same, but not all the locations were exactly from the same household. 217 The sample dissolution procedure was similar to previously described methods 218 (Kuritani and Nakamura, 2002; Nakayama et al., 2019). The detailed methods of the 219 high precision isotope analysis were described in the supplementary materials. We have

220 measured duplicates of breastmilk samples and the results of Pb isotope ratios had the 221 similar trends. As sample amounts decrease, the uncertainty of ion beam intensity measurements for the minor isotope ²⁰⁴Pb tends to increase, and the accuracy and 222 precision of the isotopic ratios involving ²⁰⁴Pb decrease (²⁰⁴Pb error; Hamelin et al., 223 224 1985). Therefore, the isotopic composition of Pb is commonly expressed as ratios of ²⁰⁸Pb, ²⁰⁷Pb, and ²⁰⁶Pb. However, since normalization to ²⁰⁴Pb yields the largest 225 variability between reservoirs (Komárek et al., 2008), we included ²⁰⁴Pb data with 226 correction (Kuritani et al., 2003, 2002). This was done in order to observe the detail 227 variability of isotope ratio among the breastmilk, blood, feces and soil samples in this 228 229 study.

230

231 **2.6 Plasma Biochemical Analysis and Metallothionein ELISA**

232 A conventional blood biochemical analyzer (FUJI DRICHEM 7000V; FUJIFILM corporation, Tokyo, Japan) was used to analyze the concentrations of 233 234 (ALT), alkaline alanine aminotransferase phosphatase (ALP), aspartate 235 aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactase dehydrogenase 236 (LDH), total bilirubin (T-Bil), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), and urea acid (UA) in mothers' plasma samples. 237 238 Metallothionein in mothers' plasma samples was measured by ELISA using an antibody 239 against iso-Metallothionein I and II (Metallothionein ELISA kit; Frontier Institute Co., 240 Ltd., Hokkaido, Japan). The standard reference ranges for each parameter in humans 241 were provided by the kit manufacturers.

243 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

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3.1 Characteristics of the Infants and Mothers

254 A total of 418 pairs of infants and mothers participated in this study. Of these, 255 333 participants came from four sites near the mine, and 85 came from Bwacha, which 256 is located about 6 km from the mine (Table 1; Fig. 1). None of the infants and mothers 257 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 258 259 significantly shorter and infants from Katondo were significantly taller than infants 260 from other areas (p < 0.05). Body weight of the infants from Chowa was significantly 261 lower than that of the infants from other areas (p < 0.05).

262 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 263 264 (p < 0.01) and in Makululu alone (p < 0.01), Supplementary Table S3). In Katondo, the 265 height of boys tended to be greater than that of girls, but no significant difference was 266 recorded (p = 0.09). The weight of boys was significantly higher than that of girls when 267 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 (p268 269 < 0.045) alone.

Area (N)	Sex infants, boy:girl	of	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		$\begin{array}{l} 7.1 \pm 3.8 \\ (417, 0.1- \\ 16.8) \end{array}$	$\begin{array}{c} 64.2 \pm 7.7 \\ (361, 45.0 - \\ 95.0) \end{array}$	$7.3 \pm 1.7 \\ (411, 1.9 - 12.1)$	$\begin{array}{c} 26.1 \pm 6.5 \\ (412, 16.3 - \\ 46.1) \end{array}$	18.0 ± 18.1 ^A (406, 0.8–93.4)	11.3 ± 9.2 ^в (417, 1.5–82.6)	5.3 ± 7.0 [°] (407, 0.4–51.9)	39.2 ± 217.7 ^D (212, 0.08– 3002.7)
Near the mine (333)	171:162		$\begin{array}{l} 7.2 \pm 4.0 \\ (332, 0.1 - \\ 16.8) \end{array}$	$\begin{array}{c} 64.5\pm 8.0\\ (280, 45.0-\\ 95.0)\end{array}$	$\begin{array}{c} 7.2 \pm 1.7 \\ (329, 1.9 - \\ 12.1) \end{array}$	$\begin{array}{c} 26.1 \pm 6.6 \\ (329, 16.3 - \\ 46.1) \end{array}$	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)	$49.2 \pm 248.0 **, D$ (162, 0.09– 3002.7)
Kasanda (82)	43:39		7.3 ± 3.8 ^a (82, 0.6–16.7)	$\begin{array}{c} 64.5\pm8.4~^{\rm a} \\ (74,49.095.0) \end{array}$	7.6 ± 1.5 ^a (79, 4.0–12.1)	$27.1 \pm 6.7 \\ (82, 17.4 - 45.4)$	$24.8 \pm 20.9^{a, A}$ (80, 3.7–93.4)	$15.8 \pm 10.6^{a, B}$ (82, 2.3–82.6)	9.2 ± 8.9 ^{a, C} (79, 1.9–50.4)	$\begin{array}{l} 38.8\pm88.9 \ ^{ab,D} \\ (28,0.6451.0) \end{array}$
Makululu (102)	44:58		7.7 ± 3.7^{a} (102, 1.5– 16.7)	$\begin{array}{l} 63.0 \pm 7.0 \\ (102, 45.0 - \\ 85.0) \end{array}$	$\begin{array}{ll} 7.4 \pm 1.4 \\ ^{a} \\ (101, 4.1 - \\ 11.9) \end{array}$	$\begin{array}{c} 26.2 \pm 7.0 \\ (102, 16.6- \\ 45.9) \end{array}$	$\begin{array}{l} 30.8 \pm 19.4 \ {}^{b,A} \\ (102,5.6{-}82.7) \end{array}$	$\begin{array}{c} 17.6 \pm 10.1 \ ^{a,B} \\ (102,3.367.3) \end{array}$	7.1 ± 7.1 ^{ab, C} (100, 1.1–40.2)	$\begin{array}{l} 82.4\pm 362.7 \ ^{\rm a,\ D} \\ (69,\ 1.13002.7) \end{array}$
Chowa (58)	35:23		$5.3 \pm 3.9^{\text{ b}} \\ (58, 0.1 15.2)$	$56.2 \pm 7.1 \ ^{\text{b}} \\ (13, 49.067.0)$	5.6 ± 1.4 ^b (58, 3.5–8.7)	$\begin{array}{c} 26.9 \pm 6.3 \\ (58, 17.6- \\ 41.8) \end{array}$	15.7 ± 15.5 °, A (49, 3.2–62.3)	$11.2 \pm 6.1^{\text{b, A}}$ (58, 3.0–36.2)	5.6 ± 6.5 ^{b, B} (57, 1.4–42.1)	$\begin{array}{l} 33.7 \pm 139.4 \ ^{\rm bc, \ C} \\ (31, \ 0.09 - 780.4) \end{array}$
Katondo (91)	49:42		$\begin{array}{c} 7.7 \pm 4.3 \ ^{a} \\ (90, 0.9 16.8) \end{array}$	$\begin{array}{c} 67.5 \pm 7.6 \ ^{\rm c} \\ (91, 48.0 {-} 94.0) \end{array}$	7.8 ± 1.7 ª (91, 1.9–11.1)	$\begin{array}{c} 24.6 \pm 5.9 \\ (87, 16.3 - \\ 46.1) \end{array}$	$\begin{array}{l} 10.9 \pm 9.9 \ ^{c, A} \\ (90, 1.6 {-} 51.0) \end{array}$	6.3 ± 3.5 °, ^B (91, 1.9–21.6)	2.3 ± 5.5 °, ^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)	$26.2 \pm \overline{5.9} \\ (83, 16.6 - 43.3)$	$5.2 \pm 4.1^{\text{d, A}}$ (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)	$6.9 \pm 27.6^{\text{ d, C}}$ (50, 0.08–184.0)

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).

Note: ** indicates a significant difference (p < 0.01) between sites near the mine and Bwacha. Various small letters indicate a significant

273 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 $1048 \pm 1470 \text{ mg/kg}$ (dry weight) and ranged from 346 to 6327 mg/kg.

279 The overall mean values of BLLs in infants and mothers were 18.0 ± 18.1 and 280 $11.3 \pm 9.2 \ \mu g/dL$, respectively (Table 1 and Fig. 2a). We found 76.8% of infants 281 (312/406) and 73.6% of mothers (307/417) had BLLs above the reference value for Pb 282 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 μ g/dL, the recommended threshold BLL for 283 284 chelation therapy (Meyer, 2008). No infants or mothers had BLLs above the lethal level 285 for Pb exposure (100 μ g/dL), however, the highest BLL in an infant in the present study 286 was 93.4 µg/dL. The overall mean of Pb concentrations in breastmilk and infants' feces 287 were $5.3 \pm 7.0 \,\mu\text{g/L}$ and $39.2 \pm 217.7 \,\text{mg/kg}$ (dry weight), respectively (Fig. 2b and 2c). 288 Overall, 30.0% of breastmilk samples (122/407) had Pb concentrations of more than 5 289 μ g/L, which is above the accepted level for breastfeeding (WHO 1989).

290 There were significant differences in Pb concentrations among sample types in 291 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 292 293 differences in Pb concentrations between infants' and mothers' blood. Pb concentrations 294 in infants' blood were 1.8 ± 1.5 and 60.2 ± 67.6 times higher than those in the mothers' 295 blood and Pb concentrations in breastmilk, respectively (Supplementary Table S4). On 296 the other hand, Pb concentrations in infants' blood were $5.3 \pm 7.4\%$ of the Pb 297 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).

309 Supplementary Tables S6, S7, S8, and S9 show Fe, Cu, Zn, and Ag 310 concentrations in the samples, respectively. Fe concentrations in infants' blood and Fe, 311 Cu, Zn, and Ag concentrations in mothers' blood from sites near the mine were 312 significantly lower than the concentrations in samples from Bwacha. On the other hand, 313 Fe concentrations in breastmilk from sites near the mine were significantly higher than concentrations 314 the in samples from Bwacha.







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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 μ g/dL, respectively. The red dotted line in Fig 2b indicates the 5 μ g/L level.

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326 **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 333 334 (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 335 breastmilk (p < 0.05, $\rho = -0.13$). In the samples from Bwacha, the same trend was 336 337 found as in the samples from all other sites, except for the relationship between Pb 338 concentrations in breastmilk and other samples, and the BLLs in mothers and Pb 339 concentrations in infants' feces (Supplementary Table S11). Supplementary Tables S12, 340 13, and 14 show the relationships among Pb and other metals in the samples from all 341 areas, from sites near the mine, and Bwacha, respectively. In the samples from all areas, 342 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 343 344 negative correlations between Pb and Fe concentrations in infants' blood as well as in 345 mothers' blood.

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	Age	of	Height	Weight	Age	of	BLLs	in	BLLs	in	Pb	in	Pb in	infants'
	infants				mothers		infants		mothers		breastmilk		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														

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

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

348 **3.4 Daily Intake of Pb in Infants through Breastfeeding and Soil Ingestion**

349 Table 3 shows the results of daily intake of Pb in infants through breastfeeding 350 and soil ingestion. The results ranged from 0.3 to 40.5 µg/day through breastfeeding and 351 from 10.4 to 189.8 µg/day through soil ingestion depending on Pb concentrations in the 352 samples. Daily intake of Pb through combined breastfeeding and soil ingestion ranged 353 from 10.7 to 230.3 µg/day. The reference value of daily intake of Pb which would be 354 associated with developmental neurotoxicity in infants in the present study was 3.7 355 μ g/day (the mean bw: 7.3 kg) and ranged from 1.0 μ g/day (the minimum bw: 1.9 kg) to 356 6.1 μ g/day (the maximum bw: 12.1) based on the EFSA's reference value (0.5 μ g/kg 357 bw/day) (EFSA, 2010). The minimum of calculated daily intake of Pb even only 358 through soil ingestion exceeded the maximum of the limit.

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361	Table 3. Sum of poss	sible daily intake	of Pb (μ g/day) th	rough breastfee	ding and soil	ingestion.
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			Daily intake	e of Pb through soil inges	tion (30 mg/day)	362
			Maximum Pb (6326.7 mg/kg)	Mean Pb (1047.6 mg/kg)	Minimum Pb (34 mg/kg)	^{45.7} 363
			189.8 μg/day	31.4 µg/day	10.4 µg/day	364
Daily intake of Pb through breastmilk	Maximum Pb	40.5 µg/day	230.3	71.9	50.8	365
(0.78 L/day)	(51.9 µg/L)					366
	Mean Pb (5.3 μg/L)	4.1 μg/day	193.9	35.5	14.5	367
	Minimum	0.3 µg/day	190.1	31.8	10.7	368
	Pb (0.4 μg//L)					369
						370

371 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).

374 **3.5 Pb Isotope Ratio Analysis**

Table 4 shows the mean values of the ²⁰⁸Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, ²⁰⁸Pb/²⁰⁴Pb, and 375 ²⁰⁷Pb/²⁰⁴Pb ratios in the samples. The ²⁰⁸Pb/²⁰⁶Pb Pb isotope ratios in mothers' blood 376 377 (2.127 ± 0.006) were significantly different from those in infants' blood and feces and the soil $(2.129 \pm 0.006, 2.130 \pm 0.004, \text{ and } 2.131 \pm 0.002, \text{ respectively, } p < 0.05)$. On 378 the other hand, there was no significant difference in ²⁰⁷Pb/²⁰⁶Pb ratios between infants' 379 and mothers' blood. Both 208Pb/204Pb, and 207Pb/204Pb ratios in mothers' blood were 380 significantly different from those in other samples (p < 0.05), except for those in 381 breastmilk. Pb isotope ratios in soil samples were similar to those reported for Kabwe 382 383 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).

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

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391 Table 4. Mean \pm SD values of the Pb isotope ratios in different samples from Kasanda

392 an	d N	/laku	lulu.
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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)	$2.129\pm0.006~^{\text{ac}}$	$0.8724\pm0.003~^{\text{ac}}$	$38.271 \pm 0.063 ^{acd}$	15.691 ± 0.012 $^{\mathrm{a}}$	$17.997 \pm 0.062 \ ^{ab}$
Mothers' blood (26)	$2.127\pm0.006~^{\rm b}$	0.8726 ± 0.002 $^{\text{a}}$	$38.223 \pm 0.080 \ ^{bc}$	15.680 ± 0.015 $^{\text{b}}$	17.970 ± 0.059 $^{\mathrm{a}}$
Breastmilk (26)	$2.129 \pm 0.001 \ ^{bc}$	0.8729 ± 0.0005 $^{\text{b}}$	38.271 ± 0.046 $^{\rm c}$	15.691 ± 0.010 b	17.972 ± 0.018 $^{\mathrm{a}}$
Infants' feces (26)	$2.130\pm0.004~^{\mathrm{ac}}$	0.8725 ± 0.002 $^{\text{c}}$	$38.349 \pm 0.067 \ ^{d}$	15.709 ± 0.012 $^{\mathrm{a}}$	$18.005 \pm 0.048 \ ^{b}$
Soil (32)	$2.131\pm0.002~^a$	$0.8724 \pm 0.001 \ ^{d}$	$38.407 \pm 0.036 \ ^{e}$	15.723 ± 0.005 $^{\rm c}$	18.022 ± 0.027 $^{\rm c}$
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

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

394 Note: The number of significant digits in each ratio value were adjusted with those of395 the reference (Kamona et al., 1999)





Fig. 3. Pb isotope ratios of individual sample (3a: ²⁰⁸Pb/²⁰⁶Pb and ²⁰⁷Pb/²⁰⁶Pb and 3b:
²⁰⁸Pb/²⁰⁴Pb and ²⁰⁷Pb/²⁰⁴Pb) 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.

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406 **3.6 Plasma Biochemical Analysis**

407 Supplementary Table S15 shows the mean values of the plasma biochemical 408 analysis on mothers' plasma. The mean values of all parameters in mothers were within 409 the reference range or slightly higher, except for ALP. The ALT and ALP 410 concentrations in the mothers from sites near the mine were significantly higher than 411 those in the mothers from Bwacha (p < 0.05). On the other hand, LDH (p < 0.05), Alb 412 (p = 0.049), and metallothionein (p < 0.001) concentrations in the mothers from Bwacha 413 were significantly higher than those in mothers from sites near the mine. T-Bil 414 concentrations in the mothers from Bwacha were higher than those in the mothers from 415 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 420 increased (p = 0.08, $\rho = 0.13$). The Alb (p < 0.05, $\rho = -0.14$) and metallothionein 421 concentrations (p < 0.001, $\rho = -0.53$) significantly decreased as the Pb concentrations 422 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.

430 **4. Discussion**

431 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 432 433 Pb concentration in breastmilk was 5.3 µg/L, which was marginally above the 434 acceptable level of 2 to 5 μ g/L for breastfeeding (WHO, 1989), and 30.0% of breastmilk 435 samples contained Pb levels above the acceptable level. Compared with previous 436 studies that reported elevated Pb concentrations in breastmilk ranged from 8.8 to 35.4 437 µg/L (Isaac et al., 2012; Turan et al., 2001), Pb concentrations in the breastmilk of 438 mothers from sites near the mine were comparable or even lower. On the other hand, the 439 mean values of Pb concentrations in breastmilk of mothers from Katondo and Bwacha 440 $(2.3 \mu g/L)$ were within the acceptable level, which agrees with the results obtained in 441 unpolluted areas in other reports (Ettinger et al., 2014; Klein et al., 2017), although the 442 highest individual Pb breastmilk concentration in this study was found in Katondo (51.9 443 μ g/L). Pb in breastmilk may be one of the sources of Pb exposure in infants. Pb 444 concentrations in breastmilk in this study were 5.6% of Pb concentrations in maternal 445 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 446 al., 2010; Koyashiki, et al., 2010; Gulson et al., 1998). In an evaluation of 447 448 breastmilk/mothers' blood relationships, Gulson et al. (1998) suggested that a ratio of 449 Pb concentration in breast milk to the concentration of Pb in maternal blood greater than 450 15% should be treated with caution, higher values than this arising probably from 451 sampling and/or analytical contamination.

452 High overall mean Pb concentration in infants' feces (39.2 mg/kg) was recorded453 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.

464 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 465 466 Makululu, which is further from the mine than Kasanda, were the highest among the 467 area. Following Makululu, those from Kasanda and Chowa were the second and the 468 third highest, respectively. Yabe et al. (2015) reported a similar trend for BLLs in 469 children under seven years old, but the highest BLL mean was found in Kasanda, 470 among the three sites. Since most areas in Makululu are dusty and unpaved compared to 471 Kasanda, residents could easily come in to contact with polluted soils or dusts in the 472 area. Pb exposure in infants and mothers from Kasanda and Makululu, which are 473 located on the western side of the mine and in the direction of prevailing winds, could 474 be higher than that in Chowa, which lies in the opposite direction. Pb concentrations in 475 breastmilk and infants' feces showed similar trends to BLLs in infants and mothers. 476 These trends agreed with those determined in earlier studies by Tembo et al. (2006) and 477 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).

484 In the present study, Fe, Cu, Zn and Ag were also analyzed. In contrast to Pb, 485 the concentrations of Fe, Cu, Zn and Ag in the blood of infants and mothers tended to 486 be lower in areas surrounding the mine area than in Bwacha. Given that Pb poisoning is 487 known to cause anemia, the negative correlation between Pb and Fe concentrations 488 suggested that the higher Pb concentrations negatively affected Fe metabolism, 489 especially absorption (Hegazy et al., 2010; Chen et al., 2019). Positive correlations were 490 found between Pb and Cu, Zn, as well as Ag. These positive co-exposures could be 491 attributed to the presence of Pb, Zn and Ag in the galena in Kabwe as confirmed in 492 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.

500 Significant positive correlations between BLLs in infants and age of the infants 501 were found. The hand-to-mouth or object-to-mouth (pica) behavior of children, and 502 high absorbance of ingested Pb from the gastrointestinal tract are well known factors 503 attributed to high Pb exposure in children (Wani et al., 2015). However, younger infants 504 who are not ambulatory could display less hand-to-mouth behaviors, as they are under 505 the care of their mothers or guardians although inhalation may be an important pathway 506 for very young children in windy environments (Gulson et al., 2009). Given that BLLs 507 in infants increases from birth to around two years of age in Kabwe, it is important to 508 pay more attention to activities of infants during this period. Moreover, cleaning floors 509 in the house where infants spend most of the time and the kitchen where food could be 510 contaminated by house dust would be important to reduce Pb exposure in infants. On 511 the other hand, only BLLs in mothers from Bwacha significantly decreased as the age of 512 mothers increased. Adults in Bwacha, which is farthest from the mine, could be less 513 exposed to Pb, thus, Pb in the blood of adults may mainly occur from redistribution of 514 endogenous bone-derived Pb (Gulson et al. 1998; Manton et al. 2003). From this point 515 of view, adults even from sites far from the mine in Kabwe could be chronically 516 exposed to Pb via endogenous exposure.

517 The Pb isotope ratios in infants' samples, especially feces, were almost identical 518 to those in the soil samples. The soil samples exhibited Pb isotope ratios similar to those 519 in Kabwe galena (Kamona et al., 1999). Furthermore, a Pb exposure study on rats 520 exposed to lead in the Kabwe soil revealed that the Pb isotope ratios in these biological 521 samples were also similar to those in Kabwe galena (Kamona et al., 1999; Nakayama et 522 al., 2019). These results suggest that contaminated soil or dust from the mine could be 523 one of the important sources of Pb exposure in infants of Kabwe as well as breastmilk. 524 Understanding which infant behaviors and activities are related to their Pb exposure is 525 required to determine the routes and to minimize the exposure. Pb isotope ratios in

526 infants' blood were similar to those in mothers' blood as infants could be exposed to Pb 527 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 528 529 infants' feces and the soil samples. Pb isotope ratios in chicken, goats, and vegetables in 530 Kabwe reported by Nakata et al. (2016) were similar to the results in the present study, 531 suggesting that consuming contaminated food could be an important route of Pb exposure in mothers. In the present study, both ²⁰⁶Pb and ²⁰⁴Pb ratios were used to 532 533 compare the differences of Pb isotope ratios among sample types. Both results were similar, but ²⁰⁴Pb-based ratios displayed clear differences among sample types. 534 Therefore, ²⁰⁴Pb ratios could be more useful than ²⁰⁶Pb ratios to elucidate the source of 535 536 Pb exposure as recommended by Gulson (2008).

537 Daily intake of Pb in infants through breastfeeding and soil ingestion was 538 calculated to estimate the burden of the possible routes of Pb exposure. Given that Pb 539 exposure can cause pediatric neurodevelopmental impairments even at low level 540 (Canfield et al., 2003), the reference value 0.5 µg/kg bw/day was used (US EPA, 2011). 541 From the results obtained in the present study, daily intake of Pb even through soil 542 ingestion alone highly exceeded the reference value. Although the bioavailability of Pb 543 in galena is known to be minimal (Rasmussen et al., 2011), Pb exposure through soil 544 ingestion could be the important source due to the high concentrations. Moreover, the 545 results of daily intake of Pb through breastfeeding using the maximum Pb 546 concentrations were larger than the reference value. These results suggest that both Pb 547 though breastfeeding and soil ingestion are important sources of Pb exposure in infants. 548 In the present study, the daily intake of Pb from the environment was only calculated 549 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.

554 In contrast to the high BLLs in mothers, the plasma biochemical profiles of most 555 analyzed parameters were interestingly within, or close to, the standard reference values, 556 except in the case of ALP. These results indicate that Pb exposure in Kabwe mothers 557 did not significantly impact their health, as was observed during sampling, where all 558 sampled mothers appeared healthy. More specifically, the ALT and ALP values in the 559 mothers from sites near the mine were significantly higher than those in the mothers 560 from Bwacha although these values were not significantly correlated with BLLs in 561 mothers. Therefore, these results could not only be attributed to Pb exposure, but also 562 other factors. On the other hand, LDH and AST, which are indicators of liver function, 563 significantly increased as BLLs increased. These results suggest that Pb exposure in 564 mothers may have caused some mild liver damage. High Pb exposure is known to cause 565 kidney damage in conjunction with an increase in BUN and a decrease in Alb. However, 566 both biomarkers significantly decreased as BLLs increased in mothers. During a 567 previous study on Pb poisoning in refugee children in the United States, the CDC 568 reported chronic and acute malnutrition as risk factors for Pb poisoning (CDC, 2005). 569 Further studies in Kabwe should therefore focus on the relationship between Pb 570 exposure and nutrition status. In our study, metallothionein concentrations significantly 571 decreased as BLLs increased. A previous study by Mustonen et al. (2014) found 572 constant metallothionein expression in earthworms from a contaminated site, and therefore suggested that the inducibility of the metallothionein response could be lost in 573

574 earthworms with a history of metal exposure. It is probable that local people in the sites 575 near the mine may be chronically exposed to metals, including Pb, over a long period of 576 time compared to people in sites far from the mine, such as Bwacha. Therefore, 577 metallothionein expression in people residing near the mine was lower than that in 578 people residing far from the mine. Further studies should focus on both metallothionein 579 concentrations and gene expression. In the present study, it is difficult to exclude the 580 possibility of other factors or diseases since a detailed questionnaire survey or medical 581 check-ups were not performed.

582 The findings in the present study suggest that one of the important sources of Pb 583 exposure in infants could be Pb from the environment, especially from soils. Daily 584 intake of Pb in infants through soil ingestion could be enough to exceed the EFSA's 585 reference value of daily intake of Pb. It is important to minimize Pb exposure in 586 infants from soil and dust.. Thus, remediation of the environment in Kabwe is 587 urgently needed to reduce Pb exposure. High BLLs in mothers could also be one of the 588 important sources of Pb exposure in infants via breastfeeding. Also, high BLLs in 589 mothers may cause their fetus to be exposed to Pb during pregnancy. In the current 590 situation, chelation therapy for Pb poisoning is prioritized more in children than in 591 adults, as children are more vulnerable to Pb. However, in utero exposure to 592 environmental lead may be adversely associated with neurodevelopment at two years of 593 age (Lin et al., 2013). Pilsner et al. (2009) reported that the epigenome of the 594 developing fetus can be influenced by the maternal cumulative lead burden, which may 595 influence long-term epigenetic programming and disease susceptibility throughout a 596 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 597

necessary that mothers with high BLLs are treated with chelation therapy, as well astheir children.

601 **5. Conclusions**

602 High Pb concentrations in breastmilk, which were above the WHO acceptable 603 level for breastfeeding, could be one of the important sources of Pb exposure in infants. 604 The results of the isotope ratio analysis suggest that Pb from the environment, such as 605 contaminated soil is one of the important sources of Pb exposure in infants. Moreover, 606 Pb exposure in infants through breastfeeding and soil ingestion potentially exceeded the 607 EFSA's reference value of daily intake of Pb. Therefore, environmental remediation, in 608 parallel with chelation therapy, is urgently needed to reduce the Pb exposure in infants 609 and mothers in Kabwe. Moreover, mothers with high BLLs in Kabwe should be treated 610 with chelation therapy to reduce the Pb exposure of their infants via breastfeeding.

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635

636 **Conflict of interest**

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The authors declare no conflict of interest.

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