

Title	Current trends of blood lead levels, distribution patterns and exposure variations among household members in Kabwe, Zambia
Author(s)	Yabe, John; Nakayama, Shouta M. M.; Nakata, Hokuto; Toyomaki, Haruya; Yohannes, Yared B.; Muzandu, Kaampwe; Kataba, Andrew; Zyambo, Golden; Hiwatari, Masato; Narita, Daiju; Yamada, Daichi; Hangoma, Peter; Munyinda, Nosiku Sipilanyambe; Mufune, Tiza; Ikenaka, Yoshinori; Choongo, Kennedy; Ishizuka, Mayumi
Citation	Chemosphere, 243, UNSP 125412 https://doi.org/10.1016/j.chemosphere.2019.125412
Issue Date	2020-03-01
Doc URL	http://hdl.handle.net/2115/84250
Rights	©2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Туре	article (author version)
File Information	Chemosphere243_UNSP125412.pdf



1	Current Trends of Blood Lead Levels, Distribution Patterns and Exposure Variations among
2	Household Members in Kabwe, Zambia

4	John Yabe ^{1a} , Shouta MM Nakayama ^{2a} , Hokuto Nakata ² , Haruya Toyomaki ² , Yared B Yohannes ² ,
5	Kaampwe Muzandu ¹ , Andrew Kataba ^{1,2} , Golden Zyambo ¹ , Masato Hiwatari ³ , Daiju Narita ⁴ , Daichi
6	Yamada ⁴ , Peter Hangoma ⁵ , Nosiku Sipilanyambe Munyinda ⁵ , Tiza Mufune ⁶ , Yoshinori Ikenaka ² ,
7	Kennedy Choongo ¹ , Mayumi Ishizuka ^{2*}
8	
9	1) The University of Zambia, School of Veterinary Medicine, P.O. Box 32379. Lusaka, Zambia
10	2) Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo
11	060-0818, Japan
12	3) Faculty of Economics & Business, Hokkaido University, Kita 9, Nishi 7, Kita-ku, Sapporo
13	060-0809, Japan
14	4) Graduate School of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo
15	153-8902, Japan
16	5) The University of Zambia, School of Public Health, P.O. Box 32379. Lusaka, Zambia
17	6) Ministry of Health, District Health Office, P.O. Box 80735. Kabwe, Zambia
18	
19	^a) The authors contributed equally to this study.
20	*Corresponding author
21	Mayumi Ishizuka
22	E-mail: <u>ishizum@vetmed.hokudai.ac.jp</u>
23	Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo
24	060-0818, Japan
25	Tel: +81-11-706-6949, Fax: +81-11-706-5105

27 Abstract

28 Childhood lead (Pb) poisoning has devastating effects on neurodevelopment and can cause overt 29 clinical signs including convulsions and coma. Health effects including hypertension and various 30 reproductive problems have been reported in adults. Historical Pb mining in Zambia's Kabwe town 31 left a legacy of environmental pollution and childhood Pb poisoning. However, the previous 32 knowledge on Pb poisoning in Kabwe is limited to the close neighbourhood of the mine and 33 exposure patterns among household members are not known. The current study aimed at establishing 34 the extent of Pb poisoning and exposure differences among family members in Kabwe as well as 35 determining populations at risk and identify children eligible for chelation therapy. Blood samples were collected in July and August 2017 from 1,190 household members and Pb was measured using 36 37 a portable LeadCare[®] II analyser. Participants included 291 younger children (3 months to 3 years old), 271 older children (4 - 9 years old), 412 mothers and 216 fathers from 13 townships with 38 39 diverse levels of Pb contamination. The Blood Lead Levels (BLL) ranged from 1.65 to 162 µg/dL, with residents from Kasanda Township (mean BLL of 45.7 µg/dL) recording the highest BLL while 40 41 Hamududu residents recorded the lowest (mean BLL of 3.3 µg/dL). Of the total number of children 42 sampled (n = 562), 23 % exceeded the 45 μ g/dL, the threshold required for chelation therapy. A few 43 children (total of 5) exceeded the 100 μ g/dL whereas none of the parents exceeded the 100 μ g/dL 44 value. Children had higher BLL than parents, with peak BLL recorded at the age of 2 years old. Lead exposure differences in Kabwe were attributed to distance and direction from the mine, with younger 45 46 children at highest risk. Exposure levels in parents were equally alarming. For prompt diagnosis and treatment, a portable point-of-care devise such as a LeadCare II analyser would be preferable in 47 48 Kabwe. 49

- 50 **KEY WORDS**: Childhood lead poisoning; LeadCare II analyser; Pb exposure differences, Kabwe
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- -
- 58
- 59

61 **1. Introduction**

62 Lead (Pb) poisoning accounts for about 0.6 % of the global burden of disease (WHO 2010), 63 posing a serious public health concern worldwide. While acute toxicity is related to occupational 64 exposure and is quite uncommon, low level chronic toxicity due to environmental pollution is much 65 more common (ATSDR, 2017). Lead poisoning has devastating effects on neurodevelopment such as mental retardation and lowering of intelligence quotient (IO) in children, which may further result in 66 poor school performance, lower tertiary education attainment, behavioural disorders and poor 67 68 lifetime earnings (WHO, 2018; Dapul and Laraque, 2014; Miranda et al., 2007; Canfield et al., 2003; 69 Lidsky and Schneider, 2003;). If not treated, Pb poisoning is characterized by persistent vomiting, 70 anaemia, encephalopathy, lethargy, delirium, convulsions, coma and death (WHO, 2018; Flora et al., 71 2012; Pearce, 2007). The Institute for Health Metrics and Evaluation (IHME, 2017) estimated that in 72 2016 Pb exposure accounted for 540,000 deaths worldwide. In chronically exposed adults, 73 significant health effects including renal dysfunction, hypertension and various reproductive 74 problems have been shown even at low Pb exposures (Kumar 2018; Wani et al., 2015). Cases of 75 reduced fertility following chronic exposure have been reported in males (Benoff et al., 2003; 76 Telisman et al., 2000; Benoff et al. 2000) as well as miscarriages in pregnant women (Wani et al., 77 2015). Moreover, childhood Pb exposure poses significant economic losses in affected countries, 78 especially in low- and middle-income countries (Attina and Trasande, 2013).

Clinical presentations of Pb poisoning vary widely depending upon the age, the amount and the duration of exposure, with some individuals seeming well at a blood lead levels (BLLs) that in others results in overt clinical signs (Bellinger 2004). Given that detrimental effects of chronic Pb exposure are usually subclinical (Yabe et al., 2015; Yabe et al., 2018), it may result in a delay in the appropriate diagnosis and chelation therapy, which has been recommended to be initiated at levels \geq 45 µg/dL (CDC 2002; Needleman 2004). Early diagnosis and chelation therapy are crucial as it has

85 been reported that high BLLs exceeding 100 µg/dL in children can cause encephalopathy, convulsions, coma and death (CDC 2002). Therefore, measurement of BLLs plays a pivotal role in 86 the diagnosis and management of patients (Lowry, 2010), as described in Pb poisoned children in 87 88 Nigeria (Thurtle et al., 2014). Traditionally, BLLs have been measured using atomic absorption spectrophotometer (AAS), inductively coupled plasma mass spectrometry (ICP-MS), etc. Although 89 90 highly sensitive to Pb measurement, these equipment are laboratory-based and require trained laboratory technologists. Moreover, they are expensive and would be time-consuming to ship 91 92 samples to appropriate laboratories. 93 In a set-up like Kabwe town in Zambia, where historical Pb mining has resulted in alarming Pb 94 poisoning, especially in children from townships in the vicinity of the closed mine and its tailing 95 wastes (Yabe et al., 2018; Bose-O'Reilly et al., 2018; Yabe et al., 2015), prompt diagnosis and 96 immediate chelation therapy would be required. Therefore, a portable point-of-care devise such as a 97 LeadCare II analyser, which can be used on-site in remote medical facilities like Kabwe would be 98 appropriate and preferable. Given that BLL results are read within 3 minutes, Pb poisoning would be 99 diagnosed and chelation therapy initiated promptly. Therefore, the current study investigated trends 100 of BLL using a LeadCare II Analyser in Kabwe to identify children that required medical 101 management to minimize the toxic effects of Pb. In addition, factors influencing Pb exposure in 102 Kabwe were analysed and exposure patterns among household members including fathers, mothers 103 and children were evaluated.

104

105 **2.** Materials and methods

106 2.1 Sampling sites

Kabwe town, with a population of about 230, 000 inhabitants and area size of 1, 547 km², is the
fourth largest town in Zambia. It is the provincial capital of Zambia's Central Province and is located

- 109 at about 28°26'E and 14°27'S. Kabwe has a long history of open-pit Pb-Zn mining, from 1902 to
- 110 1994. As observed by the Blacksmith Institute (2013), despite closure of the mine, scavenging of
- 111 metal scraps from the abandoned tailings and wastes stored on the mine has continued to serve as a
- source of metal pollution, especially dusts emanating from the mine dumps (Fig. 1).



- 113
- 114 Fig. 1.

Figure showing men scavenging for scrape metals at the Kabwe Pb-Zn mine tailings (left) and houses located within 500 m to the tailings (right).

117

Moreover, some households were within 500 m of the tailings. As shown in Fig. 2, soils in townships in the vicinity of the mine and homes downwind from the tailings were highly polluted with Pb exceeding acceptable levels for residential areas (Bose-O'Reilly et al., 2018). In the current study, blood samples were collected from family members including fathers, mothers and children at health centres around the town of Kabwe, in July and August of 2017. More details about the study site and descriptions of townships that are within the vicinity of the mine can be obtained from the previous study (Yabe et al., 2015).



- 127 Map of Kabwe showing distribution of Pb (mg/kg) in township soils around the Pb-Zn mining
- 128 complex (Bose-O'Reilly et al., 2018).

¹²⁶ Fig. 2.

130 2.2 Sample collection

131 The study was approved by the University of Zambia Research Ethics Committee (UNZAREC; 132 REF. No. 012-04-16). Further approvals were granted by the Ministry of Health through the Zambia 133 National Health Research Ethics Board and the Kabwe District Medical Office. The study targeted 134 households from areas diverse in the levels of Pb contamination based on the sample design in a 135 parallel socioeconomic survey under the KAMPAI project (Hiwatari et al., 2018). 1,000 target households were randomly chosen in two steps. In the first step, following the sampling frame of 136 Central Statistical Office (CSO), which conducts official census in Zambia and has divided Kabwe 137 138 town into 384 Standard Enumeration Areas (SEAs). Forty SEAs falling within the catchment area of 139 health facilities were randomly selected (Fig. 3) while 25 households from each SEA were randomly 140 selected in the second stage.



142 Fig. 3.

Map of Kabwe showing the 40 selected SEAs (numbers 1 - 40 in white circles) widely distributed across the whole Kabwe town and the 13 health centres (yellow blocks) that were included in the study.

146

To conduct blood sampling, up to four household members (father, mother, and two children) were invited to local health centres. Younger non-school-going children up to 3 years old and older school-aged children older than 4 years were selected in the study. The age criterion was according to Yabe et al. (2015) who found significant differences BLL in children of the two age groups. 151 Thirteen health centres with catchments areas covering the 40 SEAs were included. These included 152 Kasanda, Chowa, Makululu, Katondo, Railway, Pollen, Mahatma Ghandi, Bwacha, Ngungu, 153 Natuseko, Mpima Prison, Kang'omba and Hamududu with distances between the mine and the 154 health centres ranging from 1.5 - 30 km (Fig. 3). After informed and written consent were obtained 155 from household heads, blood samples were collected as described earlier by Yabe et al. (2015). For 156 each of the four family members included in the study, data on the age and sex were recorded. 157 Sample collection and questionnaire administration were done by certified local nurses. In 158 accordance with ethical requirements, confidentiality was upheld in the study.

159 To avoid sample contamination, all sample collection supplies were kept in plastic ziploc 160 storage bags before sample collection. Moreover, the blood collection site on the arm was thoroughly 161 cleaned and wiped with alcohol swabs before needle pricking to minimize contamination from dust. 162 For infants, blood was collected by fingerstick after cleaning the finger with an alcohol swab. A new 163 sterile lancet was used for each infant to penetrate a fingertip. The first drop of blood was wiped off 164 with a clean and dry swab and 50 µL blood sample was collected with a pre-supplied LeadCare II 165 capillary tube and transferred into the LeadCare II reagent vial. After collection, blood samples were 166 immediately analysed for Pb using a LeadCare© II analyser. The remaining samples were 167 immediately stored at -20 °C at the health centres before being transported in cooler boxes on dry ice 168 to the laboratory of the Kabwe District Health Offices where they were again stored at - 20 °C.

169

170 2.3 Blood Pb analysis

Lead metal analysis in whole blood samples was done on-site immediately after blood sample
collection using a point-of-care blood Pb testing analyser, LeadCare© II (Magellan Diagnostics,
USA) according to the manufacturer's instructions. The analyser uses an electrochemical technique
called Anodic Stripping Voltammetry (ASV) to determine the amount of Pb in a blood sample

175 (Magellan Industries Inc., 2013). The analyser has been evaluated by several researchers including 176 (Stanton and Fritsch, 2007; Sobin et al., 2011; Neria et al., 2014). Briefly, individual heparinized 177 venous blood samples were drawn using the manufacturer-supplied LeadCare II capillary tubes 178 (approximately 50 µL) and dispensed into labeled vials containing LeadCare II treatment reagent 179 (250 µL of 0.1 % of HCl). These were thoroughly mixed by tipping the bottle ten times to enhance 180 red blood cell lysis, which released the bound Pb. About 50 µL of the blood/reagent mixture was 181 then transferred to a sensor using the provided transfer dropper and analyzed for blood Pb 182 concentration. Single analyses were performed with results reflected within 3 minutes in µg/dL on 183 the analyzer's screen. For quality assurance, the instrument was calibrated using a probe before each 184 new lot of test supplies (every 48 tests). Standard controls, one high and one low blood-based 185 controls supplied by the manufacturer were analyzed to assess accuracy, these fell within the 186 manufacturer-specified acceptability limits of 6.9 - 13.7 µg/dL for the low control and 21.8 - 32.6 187 $\mu g/dL$ for the high control. Since limits of quantitation were 3.3 to 65 $\mu g/dL$ as the LeadCare II Analyzer can only detect BLL above 3.3 µg/dL. The precise values of BLLs below the 3.3 µg/dL 188 189 detection limit could not be determined. These BLLs below instrument detection limit were therefore 190 treated as 1.65 µg/dL, the mean of 0 and 3.3 as suggested in other environmental studies (Wood et al., 191 2011; Ogden, 2010).

For samples above 65 μ g/dL, a 3 times dilution was done using 0.1 % HCl. Briefly, 50 μ L of collected blood was added into 100 μ L of 0.1 % HCl. Then 50 μ L of diluted blood was pipetted into the LeadCare II reagent. This was mixed thoroughly and analyzed in the same way as for undiluted blood. The blood specimens and blood/reagent mixtures were maintained at room temperature throughout the analytical process.

197

200 All data were combined into a single electronic database and checked for accuracy and outliers. 201 Statistical analysis was performed using JMP version 10 (SAS Institute, USA). The data are 202 presented as mean, geometric mean (GM), median and minimum-maximum values in µg/dL. Tukey Kramer test was used to analyze BLL differences among family members (younger child, older child, 203 204 father and mother) as well as area difference. Different letters indicated significant difference. 205 Principal component analysis (PCA) was used to evaluate the relatedness between BLL with age, 206 wind direction and distance from the mine. The data of BLLs (μ g/dL) were log-transformed before 207 PCA analysis to stabilize variances.

208

- 209 **3. Results**
- 210 3.1 Subjects and BLL

The current study focused on blood samples that were collected from a total number of 1,190 household members including 291 younger children (3 months to 3 years old) with an average age of 1.9 years; 271 older children (4 - 9 years old) with an average age of 6.5 years; 412 mothers with an average age of 39 years and 216 fathers with an average age of 46 years. Participants were drawn from 13 health centres servicing Kasanda, Chowa, Makululu, Katondo, Railway, Pollen, Mahatma Ghandi, Bwacha, Ngungu, Natuseko, Mpima Prison, Kang'omba and Hamududu townships. The recorded BLL ranged from 1.65 to 162 µg/dL (Table 1).

- 218
- 219 **Table 1**.
- 220

221 BLL (µg/dL) exposure characteristics among household members in Kabwe, Zambia

Catagory	All	Younger child	Older child	Mother	Father
Calegory	<i>n</i> = 1190	<i>n</i> = 291	<i>n</i> = 271	<i>n</i> = 412	<i>n</i> = 216
Mean	20.8	29.9	24.3	14.8	15.7
Geo. Mean	11.1	17.0	14.2	8.2	8.1

Standard Error	0.62	1.59	1.32	0.74	1.20
Median	13.0	22.0	17.3	10.8	8.6
Standard Deviation	21.4	27.1	21.7	15.0	17.7
Minimum	1.65	1.65	1.65	1.65	1.65
Maximum	162	162	103	86.7	88.2

223

224	32	Critical BLL value	es amono	, household	memhers
<i></i>	5.4		s unions	ς ποαδεποια	members

As shown in Table 2, of the 1, 190 participants, 30 % had BLL below 5 μ g/dL, which is the level of concern. These comprised 57 younger children, 59 older children, 151 mothers and 85 fathers. Of the total number of children sampled (n = 562), a total of 130 (23 %) exceeded the 45 μ g/dL, the threshold required for chelation therapy. A few children (total of 5) exceeded the 100 μ g/dL whereas none of the parents exceeded the 100 μ g/dL value.

- 230
- 231 **Table 2**.
- 232

233 BLL (µg/dL) exposure characteristics among household members in Kabwe, Zambia

234

Category	All	Young child	Child	Mother	Father	
BLL ranges	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	
BLL < 5 μ g/dL	352 (30)	57 (20)	59 (22)	151 (37)	85 (39)	
BLL 5 - 44 μg/dL	666 (56)	154 (53)	162 (60)	239 (58)	111 (51)	
BLL 45-99 μg/dL	167 (14)	76 (26)	49(18)	22 (5.3)	20 (9.3)	
BLL > 100 μg/dL	5 (0.4)	4 (1.4)	1 (0.4)	0 (0.0)	0 (0.0)	

236 3.3 *Pb exposure patterns among household members*

Tukey test was performed to analyse age differences in BLL accumulation among family members. Children had significantly higher BLL than parents. However, there was no accumulation difference in BLL between younger children between the ages of 3 months to 3 years and older children aged 4 - 9 years. Moreover, BLL between fathers and mothers were not different. Similarly, there was no sex difference in blood Pb concentrations as the BLL between boys and girls were not different (data not shown). A positive correlation was seen in the BLL of mothers and their infants (data not shown).

- 244
- 245

246 3.4 Relationship between BLL and age

A combined dot plot and box-whisker plot was performed to evaluate the relationship between BLL and age (Fig. 4). In terms of the median BLL, a general trend indicated a high peak in children around the age of 2 years and lower BLL in older children, albeit with fluctuations. Very high BLLs are also more frequently observed among young children although BLL above 45 µg/dL is observed in any age group.



253

254 Fig. 4.

Figure of combined dot plot and box-whisker plot showing relationship between BLL and age, with peak BLL recorded at 2 years old.

- 257
- 258

259 3.5 *Pb exposure differences among townships*

In order to fully understand the Pb exposure patterns in Kabwe, differences in blood Pb accumulations in residents from the 13 townships were compared. Descriptive statistics of the BLL in residents enrolled at the 13 health centres are shown in Table 3. **Table 3**.

265 Area differences in BLL (μ g/dL) among Kabwe residents from 13 health centres

	Kasanda	Makululu	Chowa	Railway	Natuseko	Bwacha	Ngungu	Pollen	Mahatma	Mpima	Katondo	Kang'omba	Hamududu
									Ghandi	Prison			
Mean	45.7	29.3	16.5	11.4	8.58	6.78	5.38	4.70	4.51	5.41	6.51	8.48	3.31
St'd Error	1.64	1.01	1.02	1.97	0.98	1.10	0.59	0.98	0.63	0.59	1.09	1.01	0.41
Median	44.9	24.3	16.6	10.5	6.95	3.90	4.80	1.65	4.60	4.90	3.80	5.40	1.65
Standard	23.5	19.0	10.5	6.81	6.92	11.1	3.50	4.69	2.36	4.13	7.17	9.94	4.08
Deviation													
Minimum	1.65	1.65	1.65	3.30	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Maximum	162	119	48.3	26.2	34.3	94.8	14.2	16.8	9.00	23.3	38.7	63.5	35.6
Count	204	355	105	12	50	103	35	23	14	49	43	96	101

Residents in Kasanda Township, with mean BLL of 45.7 μ g/dL accumulated higher BLL than residents in the other 12 locations. Makululu Township had second highest mean BLL (29.3 μ g/dL) followed by Chowa and Railway townships. Similar but lower BLL were recorded in residents from Natuseko, Kang'omba, Ngungu, Mpima Prison, Katondo and Mahatma Ghandi followed by Bwacha and Pollen townships. Residents in Hamududu community had the lowest BLL, with a mean value of 3.3 μ g/dL.

274

275 *3.6 Factors contributing to Pb exposure patterns in Kabwe*

276 Principle component analysis (PCA) was performed on log-transformed data to evaluate the 277 relationships among BLL, age, direction and distance from the mine to the township health centres. 278 As shown in Fig. 5, the results of PCA accounted for 44.3% of the variation by the first principal 279 component (PC1) and 26.4% by the second principal component (PC2). Whereas PC1 was positively 280 determined by distance as well as a slight positive influence by age and direction, it was negatively 281 influenced by BLL. On the other hand, PC2 had a strongly positive relationship with age, but rarely 282 with distance and BLL. It was indicated that distance from the mine had a strong and bigger negative 283 relationship with BLL while direction and age had lower negative relationship with BLL.



Principal component analysis on log transformed data showing the influence of age, distance andwind direction on BLL among Kabwe residents.

4. Discussion

A portable LeadCare© II analyzer was used and proved to be an effective point of care blood Pb analyzer in Kabwe, where alarming childhood Pb poisoning was previously reported (Yabe et al., 2015). Moreover, the LeadCare II analyser is less invasive and suitable for infants as it requires a smaller finger stick blood sample. In an environment like Kabwe where non-specific clinical symptoms of cumulative Pb poisoning can easily be confused with other diseases like malaria, a rapid and appropriate diagnosis of Pb poisoning cannot be overemphasized. The current study analyzed Pb exposure patterns among family members in Kabwe, where household members shared similar risk factors such as area, direction and living conditions. The study revealed that not only children were at risk of the toxic effects of Pb in Kabwe town but women and men as well. Young 302 age was a significant risk factor given that BLL were highest in children, with peak levels recorded 303 at the age of two, in agreement with similar trends in earlier studies (Yabe et al., 2015; Koller et al., 304 2004). This trend could be attributed to the hand-to-mouth or object-to-mouth (pica) behavior of 305 children as they explore their environment after their onset of independent ambulation. In addition to 306 increased exposure, children absorb a greater proportion of ingested Pb from the gastrointestinal tract 307 than adults (Wani et al., 2015). Acute Pb poisoning exceeding 100 µg/dL can be fatal as seen in the 308 Pb poisoning disaster in Nigeria, where more than 400 children died leaving numerous others with 309 long-term neurological impairment (Dooyema et al., 2012; Lo et al., 2012). To minimize the 310 pernicious effects of Pb toxicity in children, chelation therapy is recommended at levels $\geq 45 \ \mu g/dL$ 311 as clinical symptoms such as abdominal pain, encephalopathy, convulsions, coma and death have 312 been observed in BLLs > 60 (CDC, 2002; Needleman, 2004). The current study revealed that of the 556 children, 29 % had BLL that exceeded 45 µg/dL and were recommended for chelation therapy. 313 314 Moreover, the children were followed up for further assessment including neurodevelopmental

315 impairment assessment (data not provided).

316 For the first time, the current study revealed high BLL in women in some areas in Kabwe, with 317 concentrations up to 86 µg/dL. These findings were similar to BLLs reported in women of child-bearing age in Sub-Saharan Africa where the overall weighted mean BLLs of 24.73 µg/dl was 318 319 recorded, with the highest mean of 99 µg/dl being recorded in women from Nigeria (Bede-Ojimadu 320 et al., 2018). Most of the mothers that participated in current the study (58 %) had BLL ranging 321 between 5 - 44 µg/dL, a few (5 %) were above 45 µg/dL with none exceeded 100 µg/dL. Exposure to 322 Pb in the women could be attributed to multiple sources including dust inhalation, ingestion via diet 323 or soil (pica), a habit that is common among pregnant women in Zambia, including Kabwe. Although 324 most studies are focused on childhood Pb exposure, the findings in the current study should be 325 considered carefully as increased BLLs in women of child-bearing age in Sub-Saharan Africa were associated with incidences of preeclampsia and hypertension (Bede-Ojimadu et al., 2018). Delayed 326

327 puberty due to Pb exposure has also been observed in girls (Schoeters et al., 2008). With a half-life 328 of many years to decades in adults, endogenous exposure to Pb due to increased bone resorption as 329 seen in women during pregnancy and lactation (Rothenberg et al., 2000; Tellez-Rojo et al., 2002; 330 Gulson et al., 2003; Manton et al., 2003) could also not be ruled out in the exposed mothers in 331 Kabwe. When pregnant, blood Pb accumulation in women could pose a threat to the developing fetus 332 given that maternal-fetal transfer is a major source of early life exposure to Pb (Chen et al., 2006; 333 Gardella, 2001; Li et al., 2000; Lin et al., 1998). Additional Pb exposure to the infant can occur via 334 breast milk as breastfeeding is a recognized source of postnatal Pb exposure (Counter et al., 2014). 335 These exposure pathways could explain the alarmingly high BLL in infants in the current study, even 336 before their ambulatory stage. This is critical as pediatric Pb poisoning during a vulnerable period of 337 development can lead to negative neurodevelopmental impacts such as low IQ and cognitive 338 impairments (ATSDR, 2007; Lanphear et al., 2005).

339 Similarly, increased Pb exposure in men from some Kabwe townships was recorded in the 340 current study, with median BLLs of 8.60 µg/dL and maximum levels of 88.2 µg/dL. This is also the 341 first time that Pb exposure is being investigated in men in Kabwe and the sources of exposure could 342 be similar to those of women, with the exception of pica, a practice common especially among 343 expectant mothers. Findings in the current study were similar to reports in Iran where mean BLL of 344 41.41 µg/dl were reported in male workers at a battery manufacturing plant (Sadeghniat haghighi et 345 al., 2013). Given that chronic low level Pb exposure has been associated with health complications 346 including reduced sperm quality (Wu et al., 2012; Apostoli et al., 1998), the findings of the current 347 study highlight the reproductive health risks that men in Kabwe could be exposed to through chronic 348 Pb exposure. Moreover, Pb exposure has an interactive relationship with socioeconomic factors. 349 While socioeconomic conditions have been established as important predictors of exposure to Pb 350 (Elias et al., 2007; Sargent et al., 1995), health effects of Pb exposure can be the sources of economic 351 losses that can impact families negatively (UMRSC and MNCEH, 2014; Attina and Trasande, 2013; Gould, 2009; Ogunseitan and Smith, 2007). While many studies may place emphasis only on health effects of Pb exposure, the impact of Pb exposure and poisoning in Kabwe could be broad and include healthcare, social, and behavioral costs.

355 Area differences in BLL exposure patterns among Kabwe residents were established in the 356 current study, where residents from Kasanda Mine Township had the highest BLL followed by 357 Makululu and Chowa Townships. BLLs in Railway, Natuseko, Katondo, Pollen, Mahatma Ghandi, 358 Bwacha, Ngungu, Mpima Prison, Kang'omba were similar, with residents from Hamududu 359 recording the lowest. These results reveal that severity to Pb poisoning risks among residents of 360 Kabwe was different depending on area of residence. These differences could be attributed to 361 distance from the mine and direction, with distance from the mine exerting the majority influence as 362 seen on PCA analysis. It was shown that townships closest to the mine and lying in the western direction of the mine were affected the most, especially Kasanda, followed by Makululu. Since the 363 364 wind direction is from east to west in Kabwe, more Pb contaminated dusts emanating from the mine 365 tailings are likely to settle in Kasanda and Makululu than the other townships. Of interest was 366 Natuseko Township, which is located in similar direction with similar distance from the mine as 367 Bwacha and Ngungu Townships but recorded slightly higher BLLs than these two townships. 368 Although not established, this could be attributed to transportation and piling of contaminated soils 369 and stones from the mine in Natuseko Township many years ago (verbal communication from 370 community members).

371

372 **5.** Conclusions

This is the first study that has revealed the true extent of Pb exposure in the whole Kabwe town, which poses a serious public hazard and should be given urgent attention. Exposure to Pb does not only affect children but their parents as well. Factors contributing to Pb exposure included age, distance and direction, with distance playing the major role. Therefore, younger children in 377 townships closer to the mine and lying on the western side of the mine were the most vulnerable. To 378 avert overt Pb toxicity, children with BLL exceeding 45 µg/dL would require chelation therapy. 379 These children were referred to the office of the District Medical Director. Regular BLL monitoring 380 using a portable analyser such as the LeadCare II should be considered for prompt diagnosis and 381 initiation of treatment to avoid the irreversible Pb-induced neurological dysfunction in children. A 382 thorough clinical evaluation of Pb poisoning among the affected children, including 383 neurodevelopmental and cognitive impairments, would reveal the true extend of Pb poisoning in 384 Kabwe. Measuring blood Pb in pregnant women and breast milk will be significant to clarify the 385 exposure pathway from mother to child and recommend appropriate medical management and advice 386 for the mother. Socio-economic factors contributing to Pb exposure and socio-economic impacts of 387 Pb exposure also need to be thoroughly investigated to fully understand the Pb exposure-effect cycle. 388 Moreover, urgent environmental remediation is required to reduce Pb exposure in Kabwe.

389 Acknowledgments

390 We are highly indebted to the families in Kabwe that participated in the study. We are also grateful 391 to the 13 health centers in Kabwe, the Kabwe District Health Office and the Ministry of Health, 392 Zambia, for facilitating the study. We also want to thank all the laboratory technicians and nurses at 393 the health centers for their technical support. This work was supported by Grants-in-Aid for 394 Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of 395 Japan awarded to M. Ishizuka (No. 16H0177906, 18K1984708) and Y. Ikenaka (17K2003807, 396 18H0413208), and S.M.M. Nakayama (No. 16K16197, 17KK0009), and the foundation of JSPS 397 Core to Core Program (AA Science Platforms), the Environment Research and Technology 398 Development Fund (SII-1/3-2, 4RF-1802/18949907) of the Environmental Restoration and 399 Conservation Agency of Japan. We also acknowledge financial support from The Soroptimist Japan 400 Foundation, The Nakajima Foundation, The Sumitomo foundation, The Nihon Seimei Foundation 401 and The Japan Prize Foundation. This research was also supported by JST/JICA, SATREPS (Science

- 402 and Technology Research Partnership for Sustainable Development; No. JPMJSA1501). We also
- 403 acknowledge the contribution of the Kabwe Municipal Council, especially Mr. Paul Mukuka, the
- 404 Director of Public Health, for facilitating the study.
- 405
- 406 **Conflict of interest**
- 407 The authors declare no conflicts of interest

409 References

- Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Toxicological profile for lead.
 Available: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf [accessed 16 December 2018].
- 413Agency for Toxic Substances and Disease Registry (ATSDR). 2017. Lead Toxicity. What are414possiblehealtheffectsfromleadexposure?Available:415https://www.atsdr.cdc.gov/csem/lead/docs/csem-lead_toxicity_508.pdf [accessed 21 January4162019].
- Apostoli P, Kiss P, Porru S, Bonde JP, Vanhoorne M., 1998. Male reproductive toxicity of lead in
 animals and humans. Occup. Environ. Med. 55, 364-374.
- Attina TM, Trasande L., 2013. Economic costs of childhood Pb exposure in low- and middle-income
 countries. Environ. Health Perspect. 121, 1097-1102.
- 421 Bede-Ojimadu O, Amadi CN, Orisakwe OE., 2018. Blood Lead Levels in Women of Child-Bearing
 422 Age in Sub-Saharan Africa: A Systematic Review. Front. Public Health. 6, 367.
- Benoff S, Centola GM, Millan C, Napolitano B, Marmar JL, Hurley IR., 2003. Increased seminal
 plasma lead levels adversely affect the fertility potential of sperm in IVF. Hum. Reprod. 18,
 374-383.
- 426 Benoff S, Jacob A, Hurley IR., 2000. Male infertility and environmental exposure to lead and 427 cadmium. Hum. Reprod. Update 6, 107-21.
- Blacksmith Institute (PureEarth). 2013. The world's worst 2013: the top ten toxic threats. Available:
 https://www.worstpolluted.org [accessed 29 October 2019].
- Bose-O'Reilly S, Yabe J, Makumba J, Schutzmeier P, Ericson B, Caravanos J., 2018. Lead
 intoxicated children in Kabwe, Zambia. Environ. Res. 168, 420-424.
- 432 Canfield RL, Henderson Jr CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP., 2003.
 433 Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter.
 434 N. Engl. J. Med. 348, 1517-1526.
- 435 Centers for Disease Control and Prevention (CDC). 2012. Low level lead exposure harms children: a
 436 renewed call for primary prevention. Report of the Advisory Committee on Childhood Lead
 437 Poisoning Prevention of the Centers for Disease Control and Prevention. Atlanta, Ga. [online].
 438 Available at URL: www.cdc.gov/nceh/lead/acclpp/final_document_030712.pdf [accessed 16]
- 439 December 2018].

- Centers for Disease Control and Prevention (CDC). 2002. Managing elevated blood lead levels
 among young children: recommendations from the Advisory Committee on Childhood Lead
 Poisoning Prevention. Available: <u>https://stacks.cdc.gov/view/cdc/26980</u> [accessed 29
 December 2018].
- Chen PC, Pan IJ, Wang JD., 2006. Parental exposure to lead and small for gestational age births. Am.
 J. Ind. Med. 49, 417-422.
- Counter SA, Buchanan LH, Ortega F, Chiriboga R, Correa R, Collaguaso MA., 2014. Lead levels in
 the breast milk of nursing Andean mothers living in a lead-contaminated environment. J.
 Toxicol. Environ. Health A. 77, 993-1003.
- 449 Dapul H, Laraque D., 2014. Lead Poisoning in Children. Adv. Pediatr., 61, 313-333.

Dooyema CA, Neri A, Lo YC, Durant J, Dargan PI, Swarthout T, Biya O, Gidado SO, Haladu S,
Sani-Gwarzo N, Nguku PM, Akpan H, Idris S, Bashir AM, Brown MJ., 2012. Outbreak of
fatal childhood lead poisoning related to artisanal gold mining in northwestern Nigeria, 2010.
Environ. Health Perspect. 120, 601-607.

- Elias SM, Hashim Z, Marjan ZM, Abdullah AS, Hashim JH., 2007. Relationship between blood Pb
 concentration and nutritional status among Malay primary school children in Kuala Lumpur,
 Malaysia. Asia. Pac. J. Public Health 19, 29-37.
- Flora G, Gupta D, Tiwari A., 2012. Toxicity of lead: a review with recent updates. Interdiscip.
 Toxicol. 5, 47-58.
- Gardella C., 2001. Lead exposure in pregnancy: a review of the literature and argument for routine
 prenatal screening. Obstet. Gynecol. Surv. 56, 231-238.
- Gould E., 2009. Childhood lead poisoning: conservative estimates of the social and economic
 benefits of lead hazard control. Environ. Health Perspect. 117, 1162-1167.
- Gulson BL, Mizon KJ, Korsch MJ, Palmer JM, Donnelly JB., 2003. Mobilization of lead from
 human bone tissue during pregnancy and lactation a summary of long-term research. Sci.
 Total Environ. 303, 79-104.
- 466 Hiwatari M, Yamada D, Hangoma P, Narita D, Mphuka C, Chitah B., 2018. Kabwe Household
 467 Socioeconomic Survey (KHSS) 2017 Report. Kabwe Mine Pollution Amelioration Initiative

- 468 (KAMPAI). pp 1-91 (ISBN978-4-909032-02-7), available at 469 <u>http://satreps-kampai.vetmed.hokudai.ac.jp/publications/</u>
- 470 Institute for Health Metrics and Evaluation (IHME). 2017. Global Burden of Disease (GBD)
 471 Compare. Seattle, WA: IHME, University of Washington. Available:
 472 https://vizhub.healthdata.org/gbd-compare/ [accessed 10 February 2019].
- 473
- Koller K, Brown T, Spurgeon A, Levy L., 2004. Recent developments in low-level lead exposure
 and intellectual impairment in children. Environ. Health Perspect. 112, 987-994.
- 476 Kumar S., 2018. Occupational and Environmental Exposure to Lead and Reproductive Health
 477 Impairment: An Overview. Indian J. Occup. Environ. Med. 22, 128-137.
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN,
 Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano
 J, Roberts R., 2005. Low-level environmental lead exposure and children's intellectual
 function: an international pooled analysis. Environ. Health Perspect. 113, 894-899.
- Li PJ, Sheng YZ, Wang QY, Gu LY, Wang YL., 2000. Transfer of lead via placenta and breast milk
 in human. Biomed. Environ. Sci. 13, 85-89.
- 484 Lidsky TL, Schneider JS., 2003. Lead neurotoxicity in children: basic mechanisms and clinical
 485 correlates. Brain 126, 5-19.
- 486 Lin S, Hwang SA, Marshall EG, Marion D., 1998. Does paternal occupational lead exposure increase
 487 the risks of low birth weight or prematurity? Am. J. Epidemiol. 148, 173-181.
- Lo YC, Dooyema CA, Neri A, Durant J, Jefferies T, Medina-Marino A, de Ravello L, Thoroughman
 D, Davis L, Dankoli RS, Samson MY, Ibrahim LM, Okechukwu O, Umar-Tsafe NT, Dama
 AH, Brown MJ., 2012. Childhood lead poisoning associated with gold ore processing: a
 village-level investigation-Zamfara State, Nigeria, October-November 2010. Environ. Health
 Perspect. 120, 1450-1455.
- Manton WI, Angle CR, Stanek KL, Kuntzelman D, Reese YR, Kuehnemann TJ., 2003. Release of
 lead from bone in pregnancy and lactation. Environ. Res. 92, 139-151.

- 495 Magellan Industries Inc. 2013. LeadCare II Blood Lead Analyzer User's Guide (v 1.09, Rev 04), 496 Industries Magellan Inc. North Billerica, Mass. USA, 497 http://www.leadcare2.com/Product-Support/Product-Literature-Downloads [accessed 20 498 February 2019].
- Miranda ML, Kim D, Galeano MA, Paul CJ, Hull AP, Morgan SP., 2007. The relationship between
 early childhood blood lead levels and performance on end-of-grade tests. Environ. Health
 Perspect. 115, 1242-1247.
- Nakayama SMM, Ikenaka Y, Hamada K, Muzandu K, Choongo K, Teraoka H, Mizuno N, Ishizuka
 M., 2011. Metal and metalloid contamination in roadside soil and wild rats around a Pb-Zn
 mine in Kabwe, Zambia. Environ. Pollut. 159, 175-181.
- 505 Needleman H., 2004. Lead poisoning. Annu. Rev. Med. 55, 209-222.
- Neria AJ, Royb J, Jarrettc J, Panc Y., Dooyemaa C, Caldwellc K, Umar-Tsafed NT, Olubiyoe R,
 Brownf MJ., 2014. Analysis of a novel field dilution method for testing samples that exceed
 the analytic range of point-of-care blood lead analyzers. Int. J. Environ. Health Res. 24,
 418-428.
- 510 Ogden TL., 2010. Handling results below the level of detection. Ann. Occup. Hyg. 54, 255-256.
- 511 Ogunseitan OA, Smith TR., 2007. The Cost of environmental lead (Pb) poisoning in Nigeria. Afr. J.
 512 Environ. Sci. Technol. 1, 27-36.
- 513 Pearce JM., 2007. Burton's line in lead poisoning. Eur. Neurol. 57, 118-119.
- Rothenberg SJ, Khan F, Manalo M, Jiang J, Cuellar R, Reyes S, Acosta S, Jauregui M, Diaz M,
 Sanchez M, Todd AC, Johnson C., 200. Maternal bone lead contribution to blood lead during
 and after pregnancy. Environ. Res. 82, 81-90.
- 517
- Sadeghniat haghighi K, Aminian O, Chavoshi F, Sadat BL, Soltani S, Rahmati NF., 2013.
 Relationship between blood lead level and male reproductive hormones in male lead exposed
 workers of a battery factory: A cross-sectional study. Iran J. Reprod. Med. 11, 673-676.
- 521

- Sargent JD, Brown MJ, Freeman JL, Bailey A, Goodman D, Freeman Jr DH., 1995. Childhood Pb
 Poisoning in Massachusetts Communities: it's association with sociodemographic and housing
 characteristics. Am. J. Public Health 85, 528-534.
- Schoeters G, Den Hond E, Dhooge W, van Larebeke N, Leijs M., 2008. Endocrine disruptors and
 abnormalities of pubertal development. Basic Clin. Pharmacol. Toxicol. 102, 168-75.
- Sobin C, Parisi N, Schaub T, de la Riva E., 2011. A Bland-Altman comparison of the lead Care®
 System and Inductively Coupled Plasma Mass Spectrometry for detecting low-level lead in
 child whole blood samples. J. Med. Toxicol. 7, 24-32.
- Stanton NV, Fritsch TBS., 2007. Evaluation of a second-generation portable blood lead analyzer in
 an occupational setting. Am. J. Ind. Med. 50, 1018-1024.

532 Téllez-Rojo MM, Hernández-Avila M, González-Cossío T, Romieu I, Aro A, Palazuelos E,
533 Schwartz J, Hu H., 2002. Impact of breast-feeding on the mobilization of lead from bone. Am.
534 J. Epidemiol. 155, 420-428.

- Telisman S, Cvitkovic P, Juraasovic J, Pizent A, Gavella M, Rocic B., 2000. Semen quality and
 reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc and copper
 in men. Environ. Health Perspect. 108, 45-53.
- University of Michigan Risk Science Center (UMRSC) and Michigan Network for Children's
 Environmental Health (MNCEH). 2014. Economic Impacts of Lead Exposure and
 Remediation in Michigan. Available at:
 http://www.mnceh.org/sites/www.mnceh.org/files/mnceh/press-releases/Lead_Cost_Report_
 MI 2014 smaller.pdf [accessed 11 April 2019].
- 543 Wani AL, Ara A, Usmani JAH., 2015. Lead toxicity: a review. Interdiscip. Toxicol. 8, 55-64.
- Wood MD, Beresford NA, Copplestone D., 2011. Limit of detection values in data analysis: Do they
 matter? Radioprotection 46, S85-S90.
- 546 World Health Organization. 2010. Childhood lead poisoning. WHO Press. Available:
 547 <u>http://www.who.int/ceh/publications/leadguidance.pdf</u> [accessed 12 February 2019].

- 548 World Health Organization. 2018. Lead poisoning and health. Available:
 549 <u>https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health</u> [accessed 12
 550 February 2019].
- Wu HM, Lin-Tan DT, Wang ML, Huang HY, Lee CL, Wang HS, Soong YK, Lin JL., 2012. Lead
 level in seminal plasma may affect semen quality for men without occupational exposure to
 lead. Reprod. Biol. Endocrinol. 10, 91.
- Yabe J, Ishizuka M, Umemura T., 2010. Current levels of heavy metal pollution in Africa. J. Vet.
 Med. Sci.72, 1257-1263.
- Yabe J, Nakayama SMM, Ikenaka Y, Yohannes YB, Bortey-Sam N, Oroszlany B, Muzandu K,
 Choongo K, Kabalo AN, Ntapisha J, Mweene A, Umemura T, Ishizuka M., 2015. Lead
 poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia.
 Chemosphere 119, 941-947.
- Yabe J, Nakayama SMM, Ikenaka Y, Yohannes YB, Bortey-Sam Kabalo A.N, Ntapisha J,
 Mizukawa H, Umemura T, Ishizuka M., 2018. Lead and cadmium excretion in feces and urine
 of children from polluted townships near a lead-zinc mine in Kabwe, Zambia. Chemosphere
 202, 48-55.