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#### **Abstract**

 Childhood lead (Pb) poisoning has devastating effects on neurodevelopment and can cause overt clinical signs including convulsions and coma. Health effects including hypertension and various reproductive problems have been reported in adults. Historical Pb mining in Zambia's Kabwe town left a legacy of environmental pollution and childhood Pb poisoning. However, the previous knowledge on Pb poisoning in Kabwe is limited to the close neighbourhood of the mine and exposure patterns among household members are not known. The current study aimed at establishing the extent of Pb poisoning and exposure differences among family members in Kabwe as well as determining populations at risk and identify children eligible for chelation therapy. Blood samples 36 were collected in July and August 2017 from 1,190 household members and Pb was measured using 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 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 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  $\mu$ g/dL, the threshold required for chelation therapy. A few children (total of 5) exceeded the 100 µg/dL whereas none of the parents exceeded the 100 µg/dL 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 46 children at highest risk. Exposure levels in parents were equally alarming. For prompt diagnosis and 47 treatment, a portable point-of-care devise such as a LeadCare II analyser would be preferable in 48 Kabwe. 

- **KEY WORDS**: Childhood lead poisoning; LeadCare II analyser; Pb exposure differences, Kabwe
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## **1. Introduction**

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

79 Clinical presentations of Pb poisoning vary widely depending upon the age, the amount and the 80 duration of exposure, with some individuals seeming well at a blood lead levels (BLLs) that in others 81 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 83 appropriate diagnosis and chelation therapy, which has been recommended to be initiated at levels > 84  $\frac{45 \text{ µg}}{\text{dL}}$  (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, 86 convulsions, coma and death (CDC 2002). Therefore, measurement of BLLs plays a pivotal role in 87 the diagnosis and management of patients (Lowry, 2010), as described in Pb poisoned children in 88 Nigeria (Thurtle et al., 2014). Traditionally, BLLs have been measured using atomic absorption 89 spectrophotometer (AAS), inductively coupled plasma mass spectrometry (ICP-MS), etc. Although 90 highly sensitive to Pb measurement, these equipment are laboratory-based and require trained 91 laboratory technologists. Moreover, they are expensive and would be time-consuming to ship 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*

107 Kabwe town, with a population of about 230, 000 inhabitants and area size of 1, 547 km², is the 108 fourth largest town in Zambia. It is the provincial capital of Zambia's Central Province and is located

- 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
- 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).



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- Fig. 1.

 Figure showing men scavenging for scrape metals at the Kabwe Pb-Zn mine tailings (left) and 116 houses located within 500 m to the tailings (right).

118 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).



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- Fig. 2.
- Map of Kabwe showing distribution of Pb (mg/kg) in township soils around the Pb-Zn mining
- complex (Bose-O'Reilly et al., 2018).

#### *2.2 Sample collection*

131 The 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 Medical Office. The study targeted households from areas diverse in the levels of Pb contamination based on the sample design in a 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 137 Central Statistical Office (CSO), which conducts official census in Zambia and has divided Kabwe 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 selected in the second stage.



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.

147 To conduct blood sampling, up to four household members (father, mother, and two children) 148 were invited to local health centres. Younger non-school-going children up to 3 years old and older 149 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.

 Thirteen health centres with catchments areas covering the 40 SEAs were included. These included Kasanda, Chowa, Makululu, Katondo, Railway, Pollen, Mahatma Ghandi, Bwacha, Ngungu, Natuseko, Mpima Prison, Kang'omba and Hamududu with distances between the mine and the health centres ranging from 1.5 - 30 km (Fig. 3). After informed and written consent were obtained from household heads, blood samples were collected as described earlier by Yabe et al. (2015). For each of the four family members included in the study, data on the age and sex were recorded. Sample collection and questionnaire administration were done by certified local nurses. In accordance with ethical requirements, confidentiality was upheld in the study.

159 To avoid sample contamination, all sample collection supplies were kept in plastic ziploc storage bags before sample collection. Moreover, the blood collection site on the arm was thoroughly cleaned and wiped with alcohol swabs before needle pricking to minimize contamination from dust. For infants, blood was collected by fingerstick after cleaning the finger with an alcohol swab. A new sterile lancet was used for each infant to penetrate a fingertip. The first drop of blood was wiped off with a clean and dry swab and 50 μL blood sample was collected with a pre-supplied LeadCare II capillary tube and transferred into the LeadCare II reagent vial. After collection, blood samples were 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.

#### *2.3 Blood Pb analysis*

171 Lead metal analysis in whole blood samples was done on-site immediately after blood sample 172 collection using a point-of-care blood Pb testing analyser, LeadCare© II (Magellan Diagnostics, 173 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  (Magellan Industries Inc., 2013). The analyser has been evaluated by several researchers including (Stanton and Fritsch, 2007; Sobin et al., 2011; Neria et al., 2014). Briefly, individual heparinized venous blood samples were drawn using the manufacturer-supplied LeadCare II capillary tubes (approximately 50 µL) and dispensed into labeled vials containing LeadCare II treatment reagent (250 µL of 0.1 % of HCl). These were thoroughly mixed by tipping the bottle ten times to enhance red blood cell lysis, which released the bound Pb. About 50 µL of the blood/reagent mixture was then transferred to a sensor using the provided transfer dropper and analyzed for blood Pb concentration. Single analyses were performed with results reflected within 3 minutes in µg/dL on the analyzer's screen. For quality assurance, the instrument was calibrated using a probe before each new lot of test supplies (every 48 tests). Standard controls, one high and one low blood-based controls supplied by the manufacturer were analyzed to assess accuracy, these fell within the manufacturer-specified acceptability limits of 6.9 - 13.7 μg/dL for the low control and 21.8 - 32.6  $\mu$ g/dL for the high control. Since limits of quantitation were 3.3 to 65  $\mu$ g/dL as the LeadCare II 188 Analyzer can only detect BLL above 3.3  $\mu$ g/dL. The precise values of BLLs below the 3.3  $\mu$ g/dL detection limit could not be determined. These BLLs below instrument detection limit were therefore treated as 1.65 μg/dL, the mean of 0 and 3.3 as suggested in other environmental studies (Wood et al., 2011; Ogden, 2010).

192 For samples above 65 µg/dL, a 3 times dilution was done using 0.1 % HCl. Briefly, 50 µL of 193 collected blood was added into 100 µL of 0.1 % HCl. Then 50 µL 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.

200 All data were combined into a single electronic database and checked for accuracy and outliers. Statistical analysis was performed using JMP version 10 (SAS Institute, USA). The data are 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, father and mother) as well as area difference. Different letters indicated significant difference. Principal component analysis (PCA) was used to evaluate the relatedness between BLL with age, wind direction and distance from the mine. The data of BLLs (µg/dL) were log-transformed before PCA analysis to stabilize variances.

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#### 209 **3. Results**

210 *3.1 Subjects and BLL*

211 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 217 recorded BLL ranged from 1.65 to 162 µg/dL (Table 1).

- 218
- 219 **Table 1**.
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221 BLL (µg/dL) exposure characteristics among household members in Kabwe, Zambia

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



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225 As shown in Table 2, of the 1, 190 participants, 30 % had BLL below 5 µg/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 µg/dL, the threshold required for chelation therapy. A few children (total of 5) exceeded the 100  $\mu$ g/dL whereas none of the parents exceeded the 100  $\mu$ g/dL value.

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- 231 **Table 2**.
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233 BLL (µg/dL) exposure characteristics among household members in Kabwe, Zambia

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#### *3.3 Pb exposure patterns among household members*

237 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 242 different (data not shown). A positive correlation was seen in the BLL of mothers and their infants 243 (data not shown).

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## *3.4 Relationship between BLL and age*

247 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.



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.

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#### *3.5 Pb exposure differences among townships*

260 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.

263 **Table 3**.

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

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 Residents in Kasanda Township, with mean BLL of 45.7 µg/dL accumulated higher BLL than 269 residents in the other 12 locations. Makululu Township had second highest mean BLL (29.3  $\mu$ 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 µg/dL.

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

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

 

## **4. Discussion**

293 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., 295 2015). Moreover, the LeadCare II analyser is less invasive and suitable for infants as it requires a 296 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  age was a significant risk factor given that BLL were highest in children, with peak levels recorded at the age of two, in agreement with similar trends in earlier studies (Yabe et al., 2015; Koller et al., 2004). This trend could be attributed to the hand-to-mouth or object-to-mouth (pica) behavior of children as they explore their environment after their onset of independent ambulation. In addition to increased exposure, children absorb a greater proportion of ingested Pb from the gastrointestinal tract than adults (Wani et al., 2015). Acute Pb poisoning exceeding 100 µg/dL can be fatal as seen in the Pb poisoning disaster in Nigeria, where more than 400 children died leaving numerous others with 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  $\geq 45 \mu g/dL$  as clinical symptoms such as abdominal pain, encephalopathy, convulsions, coma and death have 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. Moreover, the children were followed up for further assessment including neurodevelopmental

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

339 Similarly, increased Pb exposure in men from some Kabwe townships was recorded in the current study, with median BLLs of 8.60 µg/dL and maximum levels of 88.2 µg/dL. This is also the first time that Pb exposure is being investigated in men in Kabwe and the sources of exposure could 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 including reduced sperm quality (Wu et al., 2012; Apostoli et al., 1998), the findings of the current study highlight the reproductive health risks that men in Kabwe could be exposed to through chronic Pb exposure. Moreover, Pb exposure has an interactive relationship with socioeconomic factors. While socioeconomic conditions have been established as important predictors of exposure to Pb (Elias et al., 2007; Sargent et al., 1995), health effects of Pb exposure can be the sources of economic 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 current study, where residents from Kasanda Mine Township had the highest BLL followed by Makululu and Chowa Townships. BLLs in Railway, Natuseko, Katondo, Pollen, Mahatma Ghandi, Bwacha, Ngungu, Mpima Prison, Kang'omba were similar, with residents from Hamududu recording the lowest. These results reveal that severity to Pb poisoning risks among residents of Kabwe was different depending on area of residence. These differences could be attributed to distance from the mine and direction, with distance from the mine exerting the majority influence as 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 wind direction is from east to west in Kabwe, more Pb contaminated dusts emanating from the mine tailings are likely to settle in Kasanda and Makululu than the other townships. Of interest was Natuseko Township, which is located in similar direction with similar distance from the mine as Bwacha and Ngungu Townships but recorded slightly higher BLLs than these two townships. Although not established, this could be attributed to transportation and piling of contaminated soils and stones from the mine in Natuseko Township many years ago (verbal communication from community members).

#### **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  townships closer to the mine and lying on the western side of the mine were the most vulnerable. To avert overt Pb toxicity, children with BLL exceeding 45 µg/dL would require chelation therapy. These children were referred to the office of the District Medical Director. Regular BLL monitoring using a portable analyser such as the LeadCare II should be considered for prompt diagnosis and initiation of treatment to avoid the irreversible Pb-induced neurological dysfunction in children. A thorough clinical evaluation of Pb poisoning among the affected children, including neurodevelopmental and cognitive impairments, would reveal the true extend of Pb poisoning in Kabwe. Measuring blood Pb in pregnant women and breast milk will be significant to clarify the exposure pathway from mother to child and recommend appropriate medical management and advice for the mother. Socio-economic factors contributing to Pb exposure and socio-economic impacts of Pb exposure also need to be thoroughly investigated to fully understand the Pb exposure-effect cycle. Moreover, urgent environmental remediation is required to reduce Pb exposure in Kabwe.

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- **Conflict of interest**
- The authors declare no conflicts of interest



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