Title

Clinical biochemical parameters associated with the exposure to multiple environmental metals in residents from Kabwe, Zambia

Author names

Hokuto Nakata[1, a], Shouta M.M. Nakayama[1, a], John Yabe[2], Kaampwe Muzandu[2], Haruya Toyomaki[1], Yared Beyene Yohannes[1, 3], Andrew Kataba[1, 2], Golden Zyambo[2], Yoshinori Ikenaka[1, 4], Kennedy Choongo[2, 5], Mayumi Ishizuka [1, *)]

Authors' affiliation

1) Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku, Sapporo 060-0818, Japan

2) The University of Zambia, School of Veterinary Medicine, P.O. Box 32379, Lusaka, Zambia

3) Department of Chemistry, College of Natural and Computational Science, University of Gondar, Ethiopia

4) Water Research Group, School of Environmental Sciences and Development, North-West University, South Africa

5) Fiji National University, College of Agriculture, Fisheries & Forestry, School of Animal and Veterinary Sciences, Koronivia Campus, Suva, Fiji

(a) Both authors equally contributed to this study.

(*) Corresponding author

Mayumi Ishizuka

Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku, Sapporo 060-0818, Japan

Tel: +81-11-706-6949

Fax: +81-11-706-5105

E-mail: ishizum@vetmed.hokudai.ac.jp

Competing Financial Interests

The authors declare they have no actual or potential competing financial interests.

Abstract

Lead (Pb) interferes with various bodily functions. Although high blood Pb (Pb-B) levels in residents from Kabwe, Zambia have been reported, the accumulation pattern of other metals remains unknown. The study was designed to determine the Pb-B, blood cadmium (Cd-B), and zinc (Zn-B) values of 504 representative samples from Kabwe, as well as the potential associated adverse health effects. The Pb-B level ranged from 0.79 to 154.75 μg/dL and generally increased in areas near the mine. A significant elevation of Cd-B was observed in two areas (0.37 ± 0.26 and 0.32 ± 0.30 μg/L) where the two highest mean Pb-B levels were recorded. By contrast, the Zn-B values did not differ greatly with respect to area. Some blood biochemical parameters relating to hepatic and renal functions were out of the normal range in approximately 20 to 50% of studied adult participants. The δ-aminolevulinic acid dehydratase (δ-ALAD) activity was significantly inhibited in the two areas contaminated by Pb and Cd. A significant negative relationship was observed between metal levels and clinical parameters, e.g., between Pb-B and δ-ALAD for all the age categories and between Cd-B and the estimated glomerular filtration rate for all the age categories except 0 to 4 years. The elevated Cd-B in areas near the mine relative to the other areas suggested the potential adverse health effects of Cd and/or the interaction of Pb and Cd. A significant association of metal levels with clinical parameters also indicated the effects of metal exposure on hematopoietic, hepatic, and renal systems.

21 Keywords: Liver function; Kidney function; ALAD activity; lead; cadmium; mining site

22

1. Introduction

Lead (Pb) poisoning has been recognized as a major public health risk. According to the World Health Organization (WHO), Pb poisoning accounts for 0.6% of the global disease burden, which is highest in developing countries (WHO 2009). Lead is a persistent toxic substance that impacts human health through inhalation and ingestion pathways. Human exposure to Pb generally occurs via various sources, such as leaded gasoline, Pb-based paints, Pb-containing water pipes, battery recycling, and industrial processes including smelting and mining. The blood lead level (Pb-B) is used as the main bioindicator to monitor the current exposure level. The Center for Disease Control and Prevention (CDC) defined the blood reference value as 5 μg/dL in their new guidelines for assessing children's Pb-B (CDC 2012). Chronic environmental Pb poisoning with approximately 40–60 μg/dL of Pb-B has been widely reported (Bede-Ojimadu et al. 2018; Li et al. 2014; Tuakuila et al. 2013), whereas acute poisoning is relatively uncommon.

Lead is known to interfere with a number of bodily functions including nervous, hematopoietic, hepatic, and renal systems (Lockitch 1993). Exposure to Pb causes hematotoxicity through the restriction of hemoglobin synthesis by inhibiting key enzymes, such as δ-aminolevulinic acid dehydratase (δ-ALAD), and shortening the life span of circulating erythrocytes (Gonick 2011). Anemia, which is caused by these processes, is one of the most well-known toxicities of Pb. Renal toxicity occurs at Pb-B > 60 μg/dL

42 (Wang et al. 2002); however, even at lower levels, toxic effects appear (Harari et al. 2018).
43 Chronic nephropathy causes functional and morphological changes, resulting in renal
44 breakdown and hypertension (Rastogi 2008). Lead intoxication also causes hepatic injury,
45 namely, hepatic hyperplasia, and high serum levels of hepatic enzymes, such as alkaline
46 phosphatase (ALP) (Mudipalli 2007).

47 Kabwe is the fourth largest town and the administrative capital of Zambia's
48 central province, with a long history of Pb and zinc (Zn) mining activity, which operated
49 for nearly a century until 1994. Despite the end of the mining operation, some activities
50 have continued, such as the smelting of the mineral ores that were left in the mine dump
51 or transported from outside the town. Artisanal mining at the closed mine tailing dams
52 and the use of Pb-contaminated soil to make bricks are other activities commonly seen at
53 the site. Earlier observational studies reported serious Pb contamination in soil (Nakata
54 et al. 2016; Nakayama et al. 2011), wild rats (Nakayama et al. 2013), goats (Nakata et al.
55 2016), chickens (Nakata et al. 2016), dogs (Toyomaki et al. 2020), wild lizards (Doya et
56 al. 2020) and even humans (Yabe et al. 2015; 2018; 2020). Moreover, a recent cross-
57 sectional study with the sample size of 1190 using a LeadCare® II instrument, which
58 enables on-the-spot testing of Pb-B, revealed that Pb contamination has spread to wide
59 areas of Kabwe (Yabe et al. 2020). Among the tested people of all ages, 70% had higher
60 Pb-B than the reference level of 5 μg/dL although the reference level was determined for

not all age group, but children ages 1-5 years old (CDC 2012). The Pb-B level of approximately 15% of the people exceeded 45 μg/dL, which is the threshold in children ages 1-5 years old required for chelation therapy (CDC 2002). With the wide spread of Pb poisoning across age groups in Kabwe, the accompanying adverse effects of Pb are anticipated; however, no clinical observation has been conducted. Given these factors, the current study aimed to assess the possible health effect of Pb poisoning in Kabwe. Furthermore, we also targeted blood cadmium (Cd) and Zn levels, as high concentrations of Cd in rats (N=20) (Nakayama et al. 2013), free range chickens (N=10) (Nakata et al. 2016) and children (N=190) (Yabe et al. 2018) as well as that of Zn in soil (N=101) (Nakayama et al. 2011) have also been recorded in Kabwe. Cadmium exhibits nephrotoxic activity, with a reduction in the estimated glomerular filtration rate (eGFR) and albumin (Alb) loss in urine (ATSDR 2012). An observational study reported a significant relationship between low-level environmental Cd exposure and renal dysfunction (Ferraro et al. 2010). Additionally, according to both observational and experimental studies, various adverse health effects can occur due to the co-exposure to Pb and Cd (Hambach et al. 2013; Ni et al. 2014; Pan et al. 2018; Nakayama et al. 2019). In contrast to Pb- and Cd-related diseases, disease relating to Zn excess is not common. Rather, a protective effect of Zn on Pb and Cd intoxication has been suggested by experimental studies using laboratory rats (Saxena et al. 1989; Soussi et al. 2018).

In this study, we targeted the inhibition of δ-ALAD as a marker of hematotoxicity as well as various hepatic and renal function parameters. Studies focusing on the exact impact of metal poisoning on human health are limited. To the best of our knowledge, this is the first study that reports the widespread association of exposure to multiple metals with clinical screening parameters for humans on the African continent where serious Pb poisoning cases exist (Yabe et al. 2010). The present study was designed to clarify the exact health impact in Kabwe and to facilitate the implementation of possible countermeasures that can help us to overcome this type of pollution.

2. Methods

2.1. Sample collection and plasma preparation

The study was approved by the University of Zambia Research Ethics Committee (UNZAREC; REF. No. 012-04-16) and the Ministry of Health through the Zambia National Health Research Ethics Board as well as the Kabwe District Medical Office. The sampling was done in Kabwe, which is located approximately 130 km north of Lusaka, the capital city of Zambia. Kabwe has a population of approximately 230,000 residents and an area of 1,547 km^2. The study was designed to select 1,000 households from across Kabwe using two-stage random selection. After informed and written consent was obtained from household heads, the data and samples were collected. Further details of sample selection and collection have been described in recent papers (Hiwatari et al. 2019; Yabe et al. 2020). Heparinized blood was dispensed and immediately centrifuged for 10 min at 1,500×g, after which the plasma was stored at –20℃. Analysis was performed at the KAbwe Mine Pollution Amelioration Initiative (KAMPAI) project monitoring laboratory in the Department of Biomedical Sciences, School of Veterinary Medicine, the University of Zambia. The remaining whole blood samples were transported to Japan in cooler boxes after obtaining a material transfer agreement (MTA) from the Ministry of Health, Zambia, through the National Health Research Ethics

Committee (No. E03618) and analyzed in the Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Japan. After sample collection, we randomly ranked the 40 standard enumeration areas (SEAs) and selected them in sequential order until the total sample size of the selected SEAs exceeded 500. Consequently, 504 samples from 20 SEAs were chosen for laboratory analysis in the current study. Supplementary Figure S1 shows the locations of the selected SEAs.

2.2. Data collection and physical measurement

We asked the participants their age and measured their height and body weight at the same time as blood collection. Data were recorded using Survey Solutions (version 5.22.20, the latest version at the time of our survey), developed by the World Bank. Body mass index (BMI) was calculated using the following formula:

BMI = body weight (kg)/height (m)^2

The BMI z-score for participants with the age between 5 to 17 years old was also determined with reference to sex- and age-specific mean BMI values and distributions using the charts and tables provided by WHO (2007) because the z-score gives a relative measure of adiposity adjusted for sex and age.

126

127 ***2.3. Whole blood digestion and metal extraction***

128 Blood digestion and metal extraction were performed as described previously
129 (Nakata et al. 2016) with minor modifications. All laboratory materials and instruments
130 used in the metal extraction were washed in 2% nitric acid (HNO_3) and rinsed at least
131 twice with distilled water. Two hundred microliters of whole blood were placed in pre-
132 washed digestion vessels, followed by acid digestion using 5 mL of twofold diluted
133 ultrapure nitric acid (Cica reagent, specific gravity of 1.38, 60%; Kanto Chemical Corp.,
134 Tokyo, Japan) and 1 mL of ultrapure hydrogen peroxide (Cica reagent, 30%; Kanto
135 Chemical Corp.). The digestion and metal extraction were conducted using a microwave
136 digestion system (Speed Wave MWS-2; Berghof, Eningen, Germany) following the
137 manufacturer's instruction. After cooling, extracted solutions were transferred into 15-
138 mL plastic tubes and diluted to a final volume of 10 mL with double distilled and
139 deionized water (Milli-Q; Millipore, Bedford, MA).

140

141 ***2.4. Metal analysis***

142 The concentrations of metals (Pb, Cd, and Zn) were determined using
143 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (7700 series; Agilent
144 Technologies, Tokyo, Japan). Detailed operating conditions are shown in Supplementary

145 Table S1. Analytical quality control was performed using the certified reference material,
146 Seronorm™ Trace Elements Whole Blood L-2 (Sero, Billingstad, Norway). Replicate
147 analysis of these reference materials showed good accuracy (relative standard deviation
148 (RSD) was less than 3%) and recoveries (95–105%). The instrument detection limit was
149 0.001 μg/L.
150
151 ***2.5. Blood biochemical analysis***
152 A conventional blood biochemical analyzer (Spotchem EZ SP-4430, Arkray Inc.,
153 Kyoto, Japan) was used to analyze the levels of plasma total bilirubin (T-bil), aspartate
154 aminotransferase (AST), alanine aminotransferase (ALT), lactase dehydrogenase (LDH),
155 γ-glutamyltranspeptidase (GGT), ALP, total protein (T-pro), Alb, blood urea nitrogen
156 (BUN), urea acid (UA), and creatinine (Cre) in all participants. The analyses were done
157 following the manufacturer's instructions. Normal ranges of the parameters for
158 participants with the age of 18 years and above were presented in the manufacturer's
159 manual. The BUN/Cre ratio was determined because it is widely used to assess renal
160 function. The eGFR Modification of Diet in Renal Disease ($eGFR_{MDRD}$) value was also
161 calculated for participants older than or equal to 18 years for renal function screening
162 using the following formulas (Levey et al. 2006). A value < 60 mL/min/1.73m^2 is
163 considered normal (Levey et al. 2009).

$eGFR_{MDRD}$ = 175 × (Cre^–1.154) × (age (y)^ –0.203) × 1.212 × 0.742 (for females)

$eGFR_{MDRD}$ = 175 × (Cre^–1.154) × (age (y)^ –0.203) × 1.212 (for males)

The following formula was used to calculate $eGFR_{MDRD}$ for children and adolescents aged below 18 years old (Schwartz et al. 2009). The value below 75 mL/min/1.73m^2 is considered normal (National Institute of Diabetes and Digestive and Kidney Diseases).

$eGFR_{MDRD}$ = 0.413 × height (cm) / Cre

In cases where the T-bil level was below the limit of detection (LOD) of 0.2 mg/dL, the data were adjusted to the LOD value divided by the square root of 2 (0.14 mg/dL) for statistical analysis (Hornung and Reed, 1990). Similarly, the AST, ALT, and GGT levels lower than the LOD of 10 international unit (IU)/L were adjusted to 7.07 IU/L. Furthermore, BUN values below the detection limit of 5 mg/dL were adjusted to 3.54 mg/dL. These changes were made to minimize the effect on statistical analysis where the exact distribution of values below the detection limit was unknown.

2.6. δ-ALAD activity assay

Enzymatic activity and the ratio between non-activated and *in vitro*-activated enzymes were measured as described in previous studies (Espín et al. 2015; Scheuhammer 1987) with slight modifications. Three aliquots of 20 µL of whole blood of each participant were separated to measure non-activated δ-ALAD activity, reactivated δ-ALAD activity, and the activity of the matrix. For the non-activated enzyme activity assay, 80 µL of 0.1% Triton X-100 (Sigma–Aldrich, MO, USA) was added to the blood as a lysate. Then, 100 µL of 0.5-M phosphate-buffered saline (PBS) (pH 6.8), 50 µL of 60-mM 5-aminolevulinic acid (ALA) hydrochloride (Sigma–Aldrich) solution in PBS, and 50 µL of distilled water (DW) were added. For the reactivated activity assay, 0.1% Triton X-100, 0.5-M PBS, and 60-mM ALA hydrochloride were added to the sample with the same volume of each solution used in the non-activated enzyme activity assay. Then, 25 µL of 0.8-mM Zn acetate (Himedia Laboratories Pvt. Ltd., Mumbai, India) and 25 µL of 1-M dithiothreitol (DTT) (Himedia Laboratories Pvt. Ltd.) were added. For the matrix blank assay, 80 µL of Triton X-100, 150 µL of PBS, and 50 µL of DW were added, followed by 200 µL of 0.4-M trichloroacetic acid (TCA) (Merck, Darmstadt, Germany)/60-mM mercury chloride ($HgCl_2$) (Himedia Laboratories Pvt. Ltd.) as a stop solution. After a 60-min incubation and termination of the reaction by $HgCl_2$ in both the non-activated and reactivated activity assays, all samples were centrifuged at 10,000 g for

5 min. Supernatants were transferred to new tubes and mixed with 750 µL of modified Ehrlich's reagent, which consisted of dimethylaminobenzaldehyde (Nacalai tesque, Kyoto, Japan) in acetic acid (glacial; 99.6%, Himedia Laboratories Pvt. Ltd.) and perchloric acid (Merck KGaA, Darmstadt, Germany). After 10 min, the absorbance was read at 555 nm against the appropriate blank using a UV spectrophotometer (Shimadzu UV-2600, Shimadzu Inc., Kyoto, Japan). The activity was expressed as µmol porphobilinogen (PBG)/h/L red blood cells using the equation provided by Scheuhammer (1987). Then, the ratio between the non-activated and the *in vitro* reactivated enzymes was calculated.

2.7. Statistical analysis

IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY, USA) was used to evaluate significant differences in the data in all statistical analyses except principal component analysis (PCA), which was carried out using JMP Pro version 14 (SAS Institute, NC, USA). The data were log-transformed and fitted a normal distribution. The Tukey–Kramer test was used to compare age, height, body weight, BMI, blood metal levels, blood biochemical parameters, δ-ALAD enzyme activity, and the δ-ALAD activity ratio among areas in Kabwe as well as groups categorized by age or Pb-B. Pearson's product–moment correlation (r) was used to analyze the relationship between

221 blood metal levels, blood biochemical parameters, and the δ-ALAD activity ratio. PCA
222 was performed with blood metal levels, age, BMI, and the δ-ALAD activity ratio. All
223 statistical analyses were performed at the significance level of 0.05 ($p < 0.05$).
224

3. Results

3.1. General outcomes of the randomly selected subjects

In the random selection, subjects were drawn from people who were tested at the following eight health centers: Kasanda, Makululu, Chowa, Natuseko, Bwacha, Mpima prison, Kang'omba, and Hamududu (Supplementary Figure S1). The age of the 504 selected Kabwe residents ranged from 0 to 96 years (Supplementary Table S2). The mean and median ages of all tested people were 28.1 and 27 years, respectively, and their mean and median heights were 144.9 and 156.0 cm, respectively. Their body weight ranged from 7 to 154 kg, with mean and median values of 47.8 and 53.0 kg, respectively. For adults 18 years or older, the mean and median BMI values were 24.0 and 22.6, respectively, whereas the mean and median BMI values for all age groups were 21.0 and 20.4, respectively.

Significant area differences in age, height, body weight, and BMI for all age groups were recorded. The age, height, and body weight of people in Makululu were significantly higher than those in Natuseko and Mpima prison. Similarly, the age, height and body weight of people in Kasanda were significantly higher than those in Natuseko. Significantly higher BMI values were recorded in Makululu and Bwacha compared to those recorded in Mpima prison for all age groups.

244 The distribution patterns of BMI z-score for children and adolescents with the
245 age between 5 to 17 years old were summarized in Supplementary Table S3 and S4.
246 Generally, approximately 75% of both girl and boy showed the value of z-score between
247 -1 to 1, which are the values considered normal. The 16% of girls and 18% of boys had
248 the z-score below -2, indicating thinness, whereas 9% of girls and 4% of boys recorded
249 the z-score above 2 which means obesity. There was no clear area trend and relationship
250 with Pb-B.
251
252 ***3.2. Metal levels in blood***
253 Levels of Pb-B, Cd-B, and Zn-B in the whole blood samples are shown in Table
254 1. The minimum, maximum, mean, and median Pb-B levels of all subjects were 0.79,
255 154.75, 14.62, and 10.75 μg/dL, respectively. Values of Pb-B and Cd-B for all age groups
256 in Kasanda were significantly higher than those in other areas except for Cd-B in a
257 comparison between Kasanda and Makululu. The highest Pb-B level was measured in
258 Kasanda. All people tested in Kasanda had Pb-B levels higher than the 5 μg/dL reference
259 level. In Kang'omba and Hamududu, which are far from the mine site, the mean Pb-B
260 values for all age groups were lower than the reference level although the values for
261 children and adolescents in Kang'omba with the age below 18 were slightly greater than
262 the reference level. The Zn-B level showed less variation among areas, and significant

differences for all age groups were observed only between Kasanda and Natuseko and between Kasanda and Kang'omba.

In comparison among age groups, Pb-B for the group with the age between 0 to 4 in Kasanda was remarkably greater than that for the other age groups. In general, the group with age between 5 to 17 years had significantly elevated Pb-B than adult female and male groups. Contrary to Pb-B, Cd-B and Zn-B showed higher values in participants with the age of 18 and above compared to children and adolescent groups.

3.3. Blood biochemical parameters

The values of the blood biochemical parameters in 8 areas of Kabwe are presented in Supplementary Table S5. The T-bil value recorded in Natuseko was significantly higher than that recorded in Makululu and Kang'omba. Kasanda also showed a significantly higher level of T-bil compared with Makululu. Hanududu had significantly higher AST value than Makululu and Kang'omba. The LDH level in Chowa was significantly higher than those in Makululu, Mpima prison, Kang'omba and Hamududu. Bwacha recorded the highest mean value of GGT with statistical significant difference compared to Kang'omba. A significantly lower Alb value was measured in Makululu compared to those in Kasanda and Chowa. The significant increase of Cre was observed in Chowa compared to Makululu, Mpima prison and Hamududu. The

281 $eGFR_{MDRD}$ value in Kasanda was significantly lower than those in Makululu and
282 Hamududu. ALT, BUN and BUN/Cre did not show any significant area difference.
283 The distribution pattern of the parameters by age and Pb-B range is presented in
284 Supplementary Table S6. Moreover, the comparison of parameters with normal levels for
285 the participants with the age of 18 and above is summarized in Figure 1. Most of the
286 participants had T-bil values in the normal range or below, whereas only 1.5% of adult
287 female and 0.9% adult male participants had a value higher than the normal range. Adult
288 male participants recorded significantly greater T-bil level compared to other categories
289 although there was no significant difference of T-bil level by Pb-B range in adult male
290 group. Similarly, two major hepatic parameters, AST and ALT, were within the normal
291 range for most of the participants. In addition, those two parameters in adult male group
292 was significantly higher than those in other three categories as same as T-bil. However,
293 the LDH, GGT, and ALP levels exceeded the normal values in approximately 20 to 70%
294 of the adult participants. The highest values of LDH, GGT, and ALP were 1176, 390, and
295 1660 IU/L, respectively, which are approximately 5 to 10 times higher than the upper
296 limit of the normal range. For the LDH and ALP, adult female and male groups recorded
297 significantly lower values compared with other two age categories while GGT in these
298 two adult groups were significantly higher than that in two younger age categories. The
299 T-pro and Alb levels were lower than the normal range in approximately 30% of the adult

300 participants. The significantly higher T-pro and Alb levels were shown in adult female
301 and male groups than other two groups except the relationship of Alb value between the
302 group with age of 5 to 17 years and the adult female group. There was no Pb-B effect on
303 T-pro and Alb. Approximately 20% of the tested adult female and male participants had
304 values higher than the normal range for UA, whereas Cre levels of approximately 60% of
305 female and 30% male participants exceeded the normal range. The BUN/Cre ratio was
306 lower than the normal range for almost half of the adults. By contrast, BUN and
307 $eGFR_{MDRD}$ were mostly in the normal range. The significant effect of Pb-B on these
308 parameters relating to renal function were rarely recorded. Generally, male aduly group
309 showed significantly greater values of BUN, UA, Cre, BUN/Cre and $eGFR_{MDRD}$.
310

311 ***3.4. δ-ALAD activity***

312 The δ-ALAD activities recorded in the matrix blank and non-activated and
313 reactivated assays are shown in Supplementary Table S7 together with the activity ratio.
314 Significantly lower ratios were recorded in Kasanda and Makululu compared with those
315 recorded in the six other areas. The maximum ratio of 0.85 occurred in Kang'omba and
316 Hamududu, whereas the two minimum ratios, 0.29 and 0.30, occurred in Makululu and
317 Kasanda, respectively. The values of the non-activated and reactivated assays in
318 Hamududu were significantly the highest across the areas followed by those in

319 Kang'omba, while no significant area difference was found for the value of the matrix
320 blank assay.

321 Additionally, the comparison of the δ-ALAD activity ratios by age and Pb-B was
322 summarized in Table 2. In comparison of different Pb-B categories in the group with the
323 age from 0 to 4 years, two categories with Pb-B of 20.0 μg/dL and above had significantly
324 lower δ-ALAD activity ratios than other two categories with Pb-B below 20.0 μg/dL.
325 Similar trend was observed in the age groups of 5 to 17 years as well as 18 years and
326 above. There was no significant difference among different age groups.

327

328 ***3.5. Association among blood metal levels and other factors***

329 The association between Pb-B and the δ-ALAD activity ratio is shown in Figure
330 2. Within the group of all Kabwe residents who were randomly selected in the current
331 study, the Pb-B level and δ-ALAD activity ratio were negatively correlated, with an r^2
332 value of 0.288 ($p < 0.0001$). Correlation coefficients between blood metal levels and
333 blood biochemical parameters, as well as the δ-ALAD activity ratio, are shown in Table
334 3. The Pb-B and Cd-B were positively correlated with statistical significance for all the
335 age categories. A significant positive correlation between Pb-B and UA was recorded for
336 all the age categories except the category of 0 to 4 years, whereas there was a significant
337 negative correlation between the Pb-B level and T-bil, as well as the δ-ALAD activity

ratio, for the category of all age. The Pb-B and δ -ALAD activity ratio showed a strong negative correlation with a statistical significance for all the age categories as Figure 2 drew the statistical negative significance for the category of all age. The Cd-B level showed significant positive or negative correlations with most of the parameters other than T-bil, AST and $eGFR_{MDRD}$. However, those significant association with Cd-B did not appear for all the categorized age groups while UA and δ -ALAD activity ratio were positively and negatively correlated with Cd-B for the age categories of 5 to 17 years as well as 18 years and above, respectively. Similarly, Zn-B value was significantly correlated with most of the parameters for the category of all age whereas no significant association was observed for the age categories of 0 to 4 years as well as 5 to 17 years. PCA was performed on log-transformed data to evaluate the relationship between blood metal levels, age, BMI, and the δ-ALAD activity ratio for the category of all age (Figure 3). The results showed that the first principal component (PC1) accounted for 37.2% of the variation, and the second principal component (PC2) accounted for 28.8%. The Zn-B level was the factor that most positively contributed to PC1 followed by the Cd-B level, age, and BMI. PC2 had a strongly positive relationship with the δ-ALAD activity ratio and a negative relationship with Pb-B. The Pb-B level and δ-ALAD activity ratio had the strongest negative relationship.

4. Discussion

Although environmental metal pollution remains a significant risk factor for human health all over the world, many serious pollution cases associated with industrial activities have been recently reported especially in Africa where a rapid economic growth is being achieved (Dooyema et al. 2012; Olewe et al. 2009; Tuakuila et al. 2013; Yabe et al. 2010). However, exact effect on human health due to metal exposure remains unknown despite the big effort to evaluate metal accumulation status in the past studies. Assessment of adverse health effect would be a key to quantify the impact of pollution and to move toward resolution. In this sense, it is vitally important that our cross-sectional study of clinical screening relating to the exposure to multiple metals indicated the effect on hematopoietic, hepatic and renal functions.

Dispersibility of areas in Kabwe was maintained after the random selection of SEAs to choose 504 individuals in terms of the direction and distance from the mine, which are key factors that determine the Pb contamination level (Nakayama et al. 2011; Yabe et al. 2020). The selected subjects were considered representative of the Kabwe region. Kasanda and Makululu are seriously contaminated by Pb. This result agrees with previous reports of Pb contamination in children's blood (Yabe et al. 2015), feces, and urine (Yabe et al. 2018). Similarly, the result of higher Pb-B of younger generation in some areas including Kasanda, Natuseko and Bwacha supported the previous findings

that children had greater Pb-B than adults (Yabe et al., 2020) although such a trend was not clearly shown in general. Chowa and Natuseko followed Kasanda and Makululu, with mean values close to 10 µg/dL. It should also be emphasized that some residents had Pb-B values above the reference level of 5 µg/dL, even in rural areas away from the mine, such as Mpima prison, Kang'omba, and Hamududu. Considering the possibility that the reference level may be revised to 3.48 µg/dL based on the 2011–2014 National Health and Nutrition Examination Survey (NHANES) (Caldwell et al. 2017), observed mean Pb-B levels below 5 µg/dL in Kang'omba and Hamududu should also be carefully considered. Compared with the results concerning other African countries, the Pb-B levels recorded in the current study were higher, although elevated Pb-B values have been reported in northwestern Nigeria where more than 400 children died because of Pb poisoning caused by artisanal mining activities; the values ranged from 36.5 to 445 µg/dL in 86 children < 5 years of age (Dooyema et al. 2012; Pure Earth 2014). In a cross-sectional study in Kinshasa, Democratic Republic of Congo, where leaded gasoline was still used at the time, Pb-B levels in 275 representative residents ranged from 2.9 to 49.3 µg/dL, with a median value of 9.9 µg/dL (Tuakuila et al. 2013). In Kibera slum, Nairobi, Kenya, a mean Pb-B level of 6.0 µg/dL with a range of 3.3 to 24.7 µg/dL was recorded in a cross-sectional study targeting 387 children aged 6 to 59 months (Olewe et al. 2009). Mean Pb-B values of 5.85 (N=618) and 5.66 (N=1546) µg/dL at birth and the age of 13 years,

394 respectively, were reported in a cohort study conducted in the Johannesburg–Soweto
395 metropolitan area (Naicker et al. 2010). The elevated Pb-B level in Kabwe relative to that
396 in other countries has sounded the alarm due to the serious effects on human health.
397 Elevated fecal and urine Cd levels in Kasanda and Makululu residents have been
398 reported (Yabe et al. 2018), and supportive results were obtained in our blood analysis
399 study. A significantly higher Cd-B level was detected in Kasanda than in other areas
400 except Makululu. A similar significantly high level of Cd-B was also recorded in
401 Makululu compared with those recorded in the other areas except Mpima prison. In
402 comparison of Cd-B by age, it was found that adults accumulated greater level of Cd in
403 their blood than children and adolescents on the contrary to Pb accumulation pattern.
404 Although the exact Cd-B threshold that would cause adverse health effects is unclear, the
405 mean values for all residents in Kasanda and Makululu were markedly higher than those
406 reported for children in Koprivnica (Croatia), i.e., 0.17 μg/L (N=46), Prague (Czech
407 Republic), i.e., 0.13 μg/L (N=8), Wroclaw (Poland), i.e., 0.15 μg/L (N=27), Ban
408 (Slovakia), i.e., 0.14 μg/L (N=57), Landskrona (Sweden), i.e., 0.11 μg/L (N=41), Camilo
409 Ponce Enríquez (Ecuador), i.e., 0.26 μg/L (N = 69), and Fez (Morocco), i.e., 0.21 μg/L
410 (N=39) (Hrubá et al. 2012). By contrast, higher Cd-B values have been reported in Asian
411 countries where the dietary intake of rice and vegetables could be the major sources of
412 Cd in the general population. Mean Cd-B levels of 1.57 (N=955) and 1.49 (N=954) μg/L

413 have been reported in South Korean men and women, respectively (Hwangbo et al. 2011),
414 compared with 1.05 μg/L in 289 pregnant Taiwanese women (Lin et al. 2011). However,
415 in addition to the well-known fact that Cd is a nephrotoxin at high exposure levels (Järup
416 et al. 1995), Cd-related nephrotoxicity at relatively low exposure levels has also been
417 revealed (Åkesson et al. 2005; Ferrano et al. 2010; Thomas et al. 2008). Thus, the
418 potential health effect of Cd should be carefully observed in Kabwe. In contrast to Pb-B
419 and Cd-B, variation in Zn-B was small among areas, whereas some significant differences
420 that cannot be clearly explained were detected. Similarly, no elevation of Zn-B was
421 previously reported for goats and chickens in Kabwe (Nakata et al. 2016) or for Zn levels
422 in rat organs (Nakayama et al. 2013), although soil Zn concentrations increased around
423 the Kabwe mine area (Nakayama et al. 2011).

424 Analyzed blood biochemical parameters for adult participants were compared
425 with normal ranges which were presented in manual supplied by the manufacturer of the
426 instrument. However, the values for children and adolescents could not compare with the
427 manual's normal range because the physiological normal values of biochemical
428 parameters usually differ by age (Adeli et al., 2015). Besides, although the reference
429 values for the younger generation which were derived from developed countries are often
430 used for the African population, the normal values differ by race especially for the
431 younger generation (Dosoo et al. 2014; Quintó et al. 2006). Unfortunately, there is no

reference values specifically for Zambian children. These are common challenge and limitation of this kind of study. Thus, we compare the results of blood biochemical parameters for children and adolescents by Pb-B to evaluate the effect of Pb exposure. Additionally, Pearson product-moment correlation analysis was applied to assess the correlation between blood metal levels and blood biochemical parameters.

Among the hepatic function parameters, most of the adult participants were within the normal range of T-bil, AST and ALT despite some significant area differences for T-bil and AST. The reason for the unexpected high LDH levels in Chowa and Bwacha as well as GGT in Bwacha is unclear; however, the small sample size of Chowa and Bwacha may have played a role. Although approximately 25 to 60% of the tested adult participants showed the values of LDH, GGT and ALP above normal ranges, the frequencies of abnormal values for these parameters were not Pb-B level-dependent. Analysis of T-pro, Alb, UA, Cre and $eGFR_{MDRD}$ for renal function screening showed significant area differences. Interestingly, most of the observed area differences did not reflect the ranking of the metal levels. The low Alb value in Makululu, as well as that of Cre in Makululu and Hamududu, could be attributed to poverty and malnutrition in these areas (Hiwatari et al. 2019). The $eGFR_{MDRD}$ value was lowest in Kasanda, indicating the inhibition of glomerular filtration function by Pb and Cd exposure. The activity of δ-ALAD, an enzyme involved in heme biosynthesis that catalyzes the condensation of two

451 molecules of δ-aminolevulinic acid (δ-ALA) to form porphobilinogen (PBG) (Sakai and
452 Morita 1996), was also significantly inhibited in Kasanda and Makululu. Inhibition of δ-
453 ALAD leads to hemoglobin oxidation, which directly causes red blood cell (RBC)
454 hemolysis. The δ-ALAD assay results are indicative of both Pb-induced hematological
455 disorder and oxidative stress (Ahamed et al. 2006; Gurer-Orhan et al. 2004), suggesting
456 that δ-ALAD inhibition may imply other health effects in Kasanda and Makululu in
457 addition to hematotoxicity.

458 Some significant area differences were detected in the residents' characteristics,
459 such as age, height, body weight, and BMI. Thus, the statistical relationship among blood
460 metal levels and other factors was evaluated as one group without distinguishing between
461 areas. It should be emphasized that this is the first report about clinical outcomes affected
462 by metal exposure in Africa. Most of the blood biochemical parameters had a statistically
463 significant relationship with Cd-B and Zn-B for the category of all age. These significant
464 association would be understood by the fact that adult participants generally recorded
465 greater Cd-B, Zn-B and some biochemical parameters such as T-bil, ALT, GGT, T-pro,
466 Alb, UA, Cre and $eGFR_{MDRD}$ than the age groups of 0 to 4 years as well as 5 to 17 years.
467 Conversely, adult participants had lower parameters including LDH, ALP and BUN/Cre.
468 These higher or lower biochemical parameters in adults compared to children and
469 adolescents are well-studied and known fact as physiologically normal (Adeli et al. 2015).

It should be also noted that some biochemical parameters did not significantly correlate with Cd-B and Zn-B at comparison within each age category. Taken together, the change of parameters which significantly correlated with Cd-B and Zn-B only for the category of all age, but not for each age category, should not be considered as the effect of Cd or Zn. No statistical correlation with blood metal levels was observed for LDH, GGT, and ALP, whereas approximately 25 to 60% of tested subjects had elevated values compared with the normal range. An increase in LDH, GGT, and ALP normally suggests disorder of the liver or biliary system. However, the two major hepatic enzymes, AST and ALT, were within the normal range in most of the subjects. It should be also remembered that the increases of LDH, GGT and ALP were not Pb-B level-dependent. Taken together, our results suggested that biliary system disease is a common health issue in Kabwe due to reasons other than metal exposure. T-pro and Alb had significant positive correlations with Zn-B for the adult participants and were below normal levels in approximately 30% of the studied population. This finding suggests that people with insufficient Zn have lower amounts of blood protein. This is reasonable, as malnutrition is usually linked to a shortage of both blood protein and Zn. Another possible reason for a lower amount of blood protein is an increase in kidney Alb excretion due to renal dysfunction, which was suspected due to the elevated UA and Cre levels. In the statistical comparison, UA had a significant and positive correlation with Pb-B, Cd-B, and Zn-B for the adult participants.

Although it is known that Pb and Cd exhibit dose-dependent renal toxicity (ATSDR 2012; 2017; Bernard 2008), the reason for the significant relationship between Zn-B and UA is unclear. The inverse correlation between $eGFR_{MDRD}$ and Cd-B for the adult participants in the current study is in agreement with earlier reports (Buser et al. 2016; Hwangbo et al. 2011). High r^2 value was recorded between Pb-B and the δ-ALAD activity ratio, which is widely considered a sensitive indicator of early Pb exposure (Fontanellas et al. 2002). While a significant negative association was also observed between Cd-B and the δ-ALAD activity ratio, the PCA and Pearson product-moment correlation analysis results indicated that the reduction in the δ-ALAD activity ratio was caused mainly by increased Pb-B rather than increased Cd-B. In addition, the confounding relationship between Pb-B and Cd-B affected the association between Cd-B and the δ-ALAD activity ratio. In fact, an inverse effect of Pb exposure on δ-ALAD has been widely reported (Fontanellas et al. 2002; Mani et al. 2018), contrary to that observed for Cd. The relationship between BMI and age based on PCA agrees with a finding reported in Lusaka, the capital city of Zambia (Rudatsikira et al. 2012). The association of Zn-B with BMI can also be explained by the nutrition status, similar to that mentioned above for blood protein.

5. Conclusion

The current study provides new insights into the accumulation status of Pb, Cd, and Zn in Kabwe. It is noteworthy that Cd-B and Zn-B are reported for the first time, as it is important to consider interactions between metals. In addition to the elevated Pb-B levels in a wide area of Kabwe, an increase in Cd-B was also recorded in Kasanda and Makululu where high Pb-B levels were measured, whereas Zn-B had lower variation among areas. This is also the first clinical evaluation for hemato-, hepato-, and renal toxicity screening related to the exposure to multiple metals on the African continent. Significant correlations were found between blood metal levels and clinical parameters especially for adult participants, indicating potential adverse health effects due to metal exposure in Kabwe. Based on the observed scientific evidences in our study, immediate treatment of affected people and remediation of polluted environments are strongly recommended in addition to further studies to reveal exposure pathways and other toxic effects, such as neurodevelopmental disorders.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan awarded to M. Ishizuka (No. 16H0177906, 18KK0287), Y. Ikenaka (No. 17K2003807, 18H0413208), S.M.M. Nakayama (No. 16K16197, 17KK0009), and H. Nakata (No. 19K2047209) and

the foundation of JSPS Bilateral Open Partnership Joint Research Projects (JPJSBP120209902), and the Environment Research and Technology Development Fund (SII-1/3-2, 4RF-1802/18949907) of the Environmental Restoration and Conservation Agency of Japan. We also acknowledge financial support from The Soroptimist Japan Foundation, The Nakajima Foundation, The Sumitomo foundation, The Nihon Seimei Foundation, The Japan Prize Foundation, and The Akiyama Life Science Foundation. This research was also supported by JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development; No. JPMJSA1501) and Program for supporting introduction of the new sharing system (JPMXS0420100619). We are highly indebted to the communities that participated in this study. We are also grateful to the health centers in Kabwe, the Kabwe District Health Office, Kabwe Municipal Council, and the Ministry of Health, Zambia, for facilitating our work. We also want to thank all of the laboratory technicians and nurses at the health centers for their technical support. Our appreciation is extended to Mr. Hisao Odagiri, Ms. Michiko Nyanga, and Mr. Susumu Makino for their coordination, Ms. Sayako Yamamoto for her assistance, as well as Mr. Takahiro Ichise and Ms. Nagisa Hirano for their technical support.

Figure captions

Figure 1. Comparison of blood biochemical parameters in plasma of adult female (A) and male (B) with normal range (NR) by Pb-B range (0 – 4.9, 5 – 19.9, 20 – 44.9, 45≤ μg/dL).

Figure 2. Relationship between logPb-B and δ-ALAD activity ratio of the 504 representative Kabwe residents.

Figure 3. Principal component analysis on log-transformed data of the 504 representative Kabwe residents showing the relationship among blood metal levels, age, BMI and δ-ALAD activity ratio. K = Kasanda, M = Makululu, C = Chowa, N = Natuseko, B = Bwacha, P = Mpima Prison, G = Kang'omba, H = Hamududu.

559 **References**

560 Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL, Blais D. 2015.
561 Biochemical marker reference values across pediatric, adult, and geriatric ages:
562 establishment of robust pediatric and adult reference intervals on the basis of the Canadian
563 Health Measures Survey. *Clinical chemistry*, 61(8), 1049-1062. PMID: 26044506,
564 https://doi.org/10.1373/clinchem.2015.240515.

565

566 Agency for Toxic Substances and Disease Registry (ATSDR). 2012. Toxicological
567 profile for cadmium. U.S. Department of Health and Human Services, Public Health
568 Service, Atlanta, GA. Available: https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf
569 [accessed 27 January 2020].

570

571 Agency for Toxic Substances and Disease Registry (ATSDR). 2017. Lead Toxicity. What
572 are possible health effects from lead exposure? Available:
573 https://www.atsdr.cdc.gov/csem/lead/docs/csem-lead_toxicity_508.pdf [accessed 27
574 January 2020].

575

576 Ahamed M, Verma S, Kumar A, Siddiqui MK. 2006. Delta-aminolevulinic acid
577 dehydratase inhibition and oxidative stress in relation to blood lead among urban

578 adolescents. *Hum Exp Toxicol*, *25*(9), 547-553, PMID: 17017008,
579 https://doi.org/10.1191/0960327106het657oa.
580
581 Åkesson A, Lundh T, Vahter M, Bjellerup P, Lidfeldt J, Nerbrand C, Samsioe G,
582 Strömberg U, Skerfving S. 2005. Tubular and glomerular kidney effects in Swedish
583 women with low environmental cadmium exposure. *Environ Health Perspect*, *113*(11),
584 1627-1631. PMID: 16263522, https://doi.org/10.1289/ehp.8033.
585
586 Bede-Ojimadu O, Amadi CN, Orisakwe OE. 2018. Blood lead levels in women of child-
587 bearing age in Sub-Saharan Africa: a systematic review. *Frontiers in public health*, 6,
588 367. PMID: 30619808, https://doi.org/10.3389/fpubh.2018.00367
589
590 Bernard A. 2008. Cadmium & its adverse effects on human health. Indian J Med Res,
591 128(4), 557.
592
593 Buser MC, Ingber SZ, Raines N, Fowler DA, Scinicariello F. 2016. Urinary and blood
594 cadmium and lead and kidney function: NHANES 2007–2012. *Int J Hyg Envir*
595 *Heal*, *219*(3), 261-267. https://doi.org/10.1016/j.ijheh.2016.01.005.
596

597 Caldwell KL, Cheng PY, Jarrett JM, Makhmudov A, Vance K, Ward CD, Jones RL,
598 Mortensen ME. 2017. Measurement challenges at low blood lead
599 levels. *Pediatrics*, *140*(2), e20170272. PMID: 28771411,
600 https://doi.org/10.1542/peds.2017-0272.
601
602 Centers for Disease Control and Prevention (CDC). 2002. Managing elevated blood lead
603 levels among young children: recommendations from the Advisory Committee on
604 Childhood Lead Poisoning Prevention. Available: https://stacks.cdc.gov/view/cdc/26980
605 [accessed 27 January 2020].
606
607 Centers for Disease Control and Prevention (CDC). 2012. Low Level Lead Exposure
608 Harms Children: A Renewed Call for Primary Prevention. Available:
609 https://www.cdc.gov/nceh/lead/acclpp/Final_Document_030712.pdf
610 [accessed 27 January 2020].
611
612 Dooyema CA, Neri A, Lo YC, Durant J, Dargan PI, Swarthout T, Biya O, Gidado SO,
613 Haladu S, Sani-Gwarzo N, Nguku PM, Akpan H, Idris S, Bashir AM, Brown MJ. 2012.
614 Outbreak of fatal childhood lead poisoning related to artisanal gold mining in

615 northwestern Nigeria, 2010. *Environ Health Perspect*, *120*(4), 601-607.
616 https://doi.org/10.1289/ehp.1103965.
617
618 Dosoo DK, Asante KP, Kayan K, Adu-Gyasi D, Osei-Kwakye K, Mahama E, Danso S,
619 Amenga-Etego S, Bilson P, Koram KA, Owusu-Agyei S. 2014. Biochemical and
620 hematologic parameters for children in the middle belt of Ghana. *The American journal*
621 *of tropical medicine and hygiene*, 90(4), 767-773. PMID: 24591437,
622 https://doi.org/10.4269/ajtmh.13-0098.
623
624 Doya R, Nakayama SM, Nakata H, Toyomaki H, Yabe J, Muzandu K, Yohannes YB,
625 Kataba A, Zyambo G, Ogawa T, Uchida Y. Ikenaka Y, Ishizuka M. 2020. Land Use in
626 Habitat Affects Metal Concentrations in Wild Lizards Around a Former Lead Mining Site.
627 *Environmental Science & Technology*. PMID: 32490671,
628 https://doi.org/10.1021/acs.est.0c00150.
629
630 Espín S, Martínez-López E, Jiménez P, María-Mojica P, García-Fernández AJ. 2015.
631 Delta-aminolevulinic acid dehydratase (δALAD) activity in four free-living bird species
632 exposed to different levels of lead under natural conditions. Environ Res, 137, 185-198.
633 PMID: 25569843, https://doi.org/10.1016/j.envres.2014.12.017.

634

635 Ferraro PM, Costanzi S, Naticchia A, Sturniolo A, Gambaro G. 2010. Low level exposure
636 to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-
637 2006. *BMC Public Health*, *10*(1), 304. PMID: 20525263, https://doi.org/10.1186/1471-
638 2458-10-304.

639

640 Fontanellas A, Navarro S, Morán-Jiménez MJ, Sánchez-Fructuoso AI, Vegh I, Barrientos
641 A, de Salamanca RE. 2002. Erythrocyte aminolevulinate dehydratase activity as a lead
642 marker in patients with chronic renal failure. *Am J Kidney Dis*, *40*(1), 43-50. PMID:
643 12087560, https://doi.org/10.1053/ajkd.2002.33912.

644

645 Gonick HC. 2011. Lead-binding proteins: a review. *J Toxicol*, *2011*. PMID: 21941540,
646 https://doi.org/10.1155/2011/686050.

647

648 Gurer-Orhan H, Sabır HU, Özgüneş H. 2004. Correlation between clinical indicators of
649 lead poisoning and oxidative stress parameters in controls and lead-exposed
650 workers. *Toxicology*, *195*(2-3), 147-154. PMID: 14751670,
651 https://doi.org/10.1016/j.tox.2003.09.009.

652

Hambach R, Lison D, D'haese PC, Weyler J, De Graef E, De Schryver A, Lamberts LV, van Sprundel M. 2013. Co-exposure to lead increases the renal response to low levels of cadmium in metallurgy workers. *Toxicol Lett*, *222*(2), 233-238. PMID: 23806787, https://doi.org/10.1016/j.toxlet.2013.06.218.

Harari F, Sallsten G, Christensson A, Petkovic M, Hedblad B, Forsgard N, Melander O, Nilsson PM, Borné Y, Engström G, Barregard L. 2018. Blood lead levels and decreased kidney function in a population-based cohort. *Am J Kidney Dis*, 72(3), 381-389. PMID: 29699886, https://doi.org/10.1053/j.ajkd.2018.02.358.

Hiwatari M, Yamada D, Hangoma P, Narita D, Mphuka C, Chitah B, Yabe J, Nakayama SM, Nakata H, Choongo K, Ishizuka M. 2019. Assessing the population-wide exposure to lead pollution in Kabwe, Zambia: blood lead level estimation based on survey data. *Discussion Paper, Series A*, *338*, 1-28.

Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of nondetectable values. Applied occupational and environmental hygiene, 5(1), 46-51. https://doi.org/10.1080/1047322X.1990.10389587

672 Hrubá F, Strömberg U, Černá M, Chen C, Harari F, Harari R, Horvat M, Koppová K, Kos
673 A, Krsková A, Krsnik M, Laamech J, Li Y, Löfmark L, Lundh T, Lundström N, Lyoussi
674 B, Mazej D, Osredkar J, Pawlas K, Pawlas N, Prokopowicz A, Rentschler G, Spěváčková
675 V, Spiric Z, Tratnik J, Skerfving S, Bergdahl IA. 2012. Blood cadmium, mercury, and
676 lead in children: an international comparison of cities in six European countries, and
677 China, Ecuador, and Morocco. *Environ Int, 41*, 29-34. PMID: 22257910,
678 https://doi.org/10.1016/j.envint.2011.12.001.
679
680 Hwangbo Y, Weaver VM, Tellez-Plaza M, Guallar E, Lee BK, Navas-Acien A. 2011.
681 Blood cadmium and estimated glomerular filtration rate in Korean adults. *Environ Health*
682 *Perspect, 119*(12), 1800-1805. PMID: 21835726, https://doi.org/10.1289/ehp.1003054.
683
684 Järup L, Persson B, Elinder CG. 1995. Decreased glomerular filtration rate in solderers
685 exposed to cadmium. *Occup Environ Med, 52*(12), 818-822. PMID: 8563845,
686 https://doi.org/10.1136/oem.52.12.818.
687
688 Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Lente
689 FV. 2006. Using standardized serum creatinine values in the modification of diet in renal
690 disease study equation for estimating glomerular filtration rate. Annals of internal

691 medicine, 145(4), 247-254. PMID: 16908915, https://doi.org/10.7326/0003-4819-145-4-
692 200608150-00004.
693
694 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW,
695 Eggers P, van Lente F, Greene T, Coresh J. 2009. A new equation to estimate glomerular
696 filtration rate. *Ann Intern Med*, *150*(9), 604-612. PMID: 19414839,
697 https://doi.org/10.7326/0003-4819-150-9-200905050-00006.
698
699 Li MM, Cao J, Xu J, Cai SZ, Shen XM, Yan CH. 2014. The national trend of blood lead
700 levels among Chinese children aged 0–18 years old, 1990–2012. *Environment*
701 *international*, 71, 109-117. PMID: 25000556,
702 https://doi.org/10.1016/j.envint.2014.06.005
703
704 Lin CM, Doyle P, Wang D, Hwang YH, Chen PC. 2011. Does prenatal cadmium
705 exposure affect fetal and child growth?. *Occup Environ Med*, *68*(9), 641-646. PMID:
706 21186202, https://doi.org/10.1136/oem.2010.059758.
707
708 Lockitch G. 1993. Perspectives on lead toxicity. Clin Biochem, 26(5), 371-381. PMID:
709 8299207, https://doi.org/10.1016/0009-9120(93)90113-k.

Mani MS, Kunnathully V, Rao C, Kabekkodu SP, Joshi MB, D'Souza HS. 2018. Modifying effects of δ-Aminolevulinate dehydratase polymorphism on blood lead levels and ALAD activity. *Toxicol Lett*, *295*, 351-356. PMID: 30025905, https://doi.org/10.1016/j.toxlet.2018.07.014.

Mudipalli A. 2007. Lead hepatotoxicity & potential health effects. *Indian J Med Res*, 126(6), 518. PMID: 18219078.

Naicker N, Norris SA, Mathee A, von Schirnding YE, Richter L. 2010. Prenatal and adolescent blood lead levels in South Africa: child, maternal and household risk factors in the Birth to Twenty cohort. *Environ Res*, *110*(4), 355-362. 20226441, https://doi.org/10.1016/j.envres.2010.02.006.

Nakata H, Nakayama SM, Yabe J, Liazambi A, Mizukawa H, Darwish WS, Ikenaka Y, Ishizuka M. 2016. Reliability of stable Pb isotopes to identify Pb sources and verifying biological fractionation of Pb isotopes in goats and chickens. *Environ Pollut*, *208*, 395-403. PMID: 26549754, https://doi.org/10.1016/j.envpol.2015.10.006.

729 Nakayama SM, Ikenaka Y, Hamada K, Muzandu K, Choongo K, Teraoka H, Mizuno H,
730 Ishizuka M. 2011. Metal and metalloid contamination in roadside soil and wild rats
731 around a Pb–Zn mine in Kabwe, Zambia. *Environ Pollut*, *159*(1), 175-181. PMID:
732 20971538, https://doi.org/10.1016/j.envpol.2010.09.007.
733
734 Nakayama SM, Ikenaka Y, Hamada K, Muzandu K, Choongo K, Yabe J, Umemura T,
735 Ishizuka M. 2013. Accumulation and biological effects of metals in wild rats in mining
736 areas of Zambia. *Environ Monit Assess*, *185*(6), 4907-4918. PMID: 23054276,
737 https://doi.org/10.1007/s10661-012-2912-6.
738
739 Nakayama SM, Nakata H, Ikenaka Y, Yabe J, Oroszlany B, Yohannes YB, Bortey-Sam
740 N, Muzandu K, Choongo K, Kuritani T, Nakagawa M. Ishizuka M. 2019. One year
741 exposure to Cd-and Pb-contaminated soil causes metal accumulation and alteration of
742 global DNA methylation in rats. *Environ Pollut*, *252*, 1267-1276. PMID: 31252124,
743 https://doi.org/10.1016/j.envpol.2019.05.038.
744
745 Ni W, Huang Y, Wang X, Zhang J, Wu K. 2014. Associations of neonatal lead, cadmium,
746 chromium and nickel co-exposure with DNA oxidative damage in an electronic waste

747 recycling town. *Sci Total Environ, 472*, 354-362. PMID: 24295751,
748 https://doi.org/10.1016/j.scitotenv.2013.11.032.
749
750 National Institute of Diabetes and Digestive and Kidney Disease. Glomerular Filtration
751 Rate (GFR) Calculators, for children (Conventional Units). Available:
752 https://www.niddk.nih.gov/health-information/professionals/clinical-tools-patient-
753 management/kidney-disease/laboratory-evaluation/glomerular-filtration-rate-
754 calculators/children-conventional-units
755 [accessed 15 June 2020]
756
757 Olewe TM, Mwanthi MA, Wang'ombe JK, Griffiths JK. 2009. Blood lead levels and
758 potental environmental exposures among children under five years in Kibera slums,
759 Nairobi. *East Afr J Public Health*, *6*(1). PMID: 20000055,
760 https://doi.org/10.4314/eajph.v6i1.45734.
761
762 Pan S, Lin L, Zeng F, Zhang J, Dong G, Yang B, Jung Y, Chen S, Zhang G, Yu Z, Sheng
763 G, Ma H. 2018. Effects of lead, cadmium, arsenic, and mercury co-exposure on children's
764 intelligence quotient in an industrialized area of southern China. *Environ Pollut*, *235*, 47-
765 54. PMID: 29274537, https://doi.org/10.1016/j.envpol.2017.12.044.

766

767 Pure Earth. 2014. Project Completion Report: Nigeria Lead Poisoning Crisis. Available:

768 https://www.pureearth.org/wp-content/uploads/2014/10/Zamfara_Nigeria_PCR-April-

769 2011.pdf

770 [accessed 27 January 2020].

771

772 Quintó L, Aponte JJ, Sacarlal J, Espasa M, Aide P, Mandomando I, Guinovart C, Macete

773 E, Navia MM, Thompson R, Menéndez C, Alonso PL. 2006. Haematological and

774 biochemical indices in young African children: in search of reference intervals. *Tropical*

775 *Medicine & International Health*, *11*(11), 1741-1748. PMID: 17054755,

776 https://doi.org/10.1111/j.1365-3156.2006.01764.x.

777

778 Rastogi SK. 2008. Renal effects of environmental and occupational lead exposure. *Indian*

779 *J Occup Environ Med*, 12(3), 103. PMID: 20040966, https://doi.org/10.4103/0019-

780 5278.44689.

781

782 Rudatsikira E, Muula AS, Mulenga D, Siziya S. 2012. Prevalence and correlates of

783 obesity among Lusaka residents, Zambia: a population-based survey. *Int Arch Med*, *5*(1),

784 14. PMID: 22551418, https://doi.org/10.1186/1755-7682-5-14.

Sakai T, Morita Y. 1996. δ-Aminolevulinic acid in plasma or whole blood as a sensitive indicator of lead effects, and its relation to the other home-related parameters. *Int Arch Occup Environ Health*, *68*(2), 126-132. PMID: 8720283, https://doi.org/10.1007/bf00381245.

Saxena DK, Murthy RC, Singh C, Chandra SV. 1989. Zinc protects testicular injury induced by concurrent exposure to cadmium and lead in rats. *Res Commun Chem Pathol Pharmacol*, *64*(2), 317-329. PMID: 2740621.

Scheuhammer AM. 1987. Erythrocyte δ-aminolevulinic acid dehydratase in birds. I. The effects of lead and other metals in vitro. Toxicology, 45(2), 155-163. PMID: 3603581, https://doi.org/10.1016/0300-483X(87)90101-6.

Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. 2009. New equations to estimate GFR in children with CKD. Journal of the American Society of Nephrology, 20(3), 629-637. PMID: 19158356, https://doi.org/10.1681/ASN.2008030287.

804 Soussi A, Gargouri M, El Feki A. 2018. Effects of co-exposure to lead and zinc on redox
805 status, kidney variables, and histopathology in adult albino rats. *Toxicol Ind Health*, *34*(7),
806 469-480. PMID: 29702030, https://doi.org/10.1177/0748233718770293.
807
808 Thomas LD, Hodgson S, Nieuwenhuijsen M, Jarup L. 2008. Early kidney damage in a
809 population exposed to cadmium and other heavy metals. *Environ Health Perspect*, *117*(2),
810 181-184. PMID: 19270785, https://doi.org/10.1289/ehp.11641.
811
812 Toyomaki H, Yabe J, Nakayama SM, Yohannes YB, Muzandu K, Liazambi A, Ikenaka
813 Y, Kuritani T, Nakagawa M, Ishizuka M. 2020. Factors associated with lead (Pb)
814 exposure on dogs around a Pb mining area, Kabwe, Zambia. *Chemosphere*, 125884.
815 https://doi.org/10.1016/j.chemosphere.2020.125884.
816
817 Tuakuila J, Lison D, Mbuyi F, Haufroid V, Hoet P. 2013. Elevated blood lead levels and
818 sources of exposure in the population of Kinshasa, the capital of the Democratic Republic
819 of Congo. *J Expo Sci Env Epid*, 23(1), 81-87. PMID: 22617721,
820 https://doi.org/10.1038/jes.2012.49.
821

822 Wang VS, Lee MT, Chiou JY, Guu CF, Wu CC, Wu TN, Lai JS. 2002. Relationship
823 between blood lead levels and renal function in lead battery workers. *Int Arch Occup*
824 *Environ Health*, 75(8), 569-575. PMID: 12373319, https://doi.org/10.1007/s00420-002-
825 0362-0.
826
827 World Health Organization (WHO). 2007. Growth reference 5-19 years old, BMI-for-age
828 (5-19 years). Available: https://www.who.int/growthref/who2007_bmi_for_age/en/
829 [accessed 26 May 2020].
830
831 World Health Organization (WHO). 2009. Global health risks: mortality and burden of
832 disease attributable to selected major risks. Available:
833 http://whqlibdoc.who.int/publications/2009/9789241563871_eng.pdf [accessed 24 May
834 2020].
835
836 Yabe J, Ishizuka M, Umemura T. 2010. Current levels of heavy metal pollution in Africa.
837 *J Vet Med Sci*, 72(10), 1257-1263. PMID: 20519853, https://doi.org/10.1292/jvms.10-
838 0058.
839

Yabe J, Nakayama SM, 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. PMID: 25303652, https://doi.org/10.1016/j.chemosphere.2014.09.028.

Yabe J, Nakayama SM, Ikenaka Y, Yohannes YB, Bortey-Sam N, Kabalo AN, 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. PMID: 29554507, https://doi.org/10.1016/j.chemosphere.2018.03.079.

Yabe J, Nakayama SM, Nakata H, Toyomaki H, Yohannes YB, Muzandu K, Kataba A, Zyambo G, Hiwatari M, Narita D, Yamada D, Hangoma P, Munyinda NS, Mufune T, Ikenaka Y, Choongo K, Ishizuka M. 2020. Current trends of blood lead levels, distribution patterns and exposure variations among household members in Kabwe, Zambia. *Chemosphere*, *243*, 125412. PMID: 31995873, https://doi.org/10.1016/j.chemosphere.2019.125412.

Table 1. Blood Pb, Cd and Zn levels in whole blood among the 504 representative Kabwe residents from 8 areas by age (Mean±SD, minimum – maximum).

Area	Kasanda		Makululu		Chowa		Natuseko		Bwacha		Mpima prison		Kangomba		Hamududu		All area	
Pb-B (μg/dL)																		
all age	31.74 ± 19.05	a	16.38 ± 9.67	b	8.10 ± 7.72	c	8.20 ± 7.07	c	6.28 ± 6.71	cd	5.31 ± 3.85	cd	3.99 ± 3.34	de	2.99 ± 2.01	e	14.62 ± 14.40	
	(9.90 - 154.75)		(3.29 - 65.9)		(1.47 - 33.44)		(1.30 - 35.58)		(1.26 - 27.89)		(1.67 - 21.58)		(0.95 - 17.06)		(0.79 - 12.61)		(0.79 - 154.75)	
0 - 4	85.61 ± 48.04		24.55 ± 13.25		8.46		11.68 ± 7.52		23.70		5.60 ± 3.56		6.76 ± 5.37		3.67 ± 1.64		16.84 ± 26.35	AB
	(43.55 - 154.75)		(6.06 - 43.43)		(6.82, 10.09)		(3.94 - 31.32)		(19.50, 27.89)		(2.30 - 13.35)		(2.50 - 14.55)		(1.73 - 7.36)		(1.73 - 154.75)	
5 - 17	31.97 ± 12.16		25.17 ± 11.54		14.42 ± 13.56		6.74 ± 3.82		6.44 ± 1.69		5.68 ± 3.30		5.47 ± 4.76		3.10 ± 1.57		19.63 ± 14.64	A
	(12.31 - 66.89)		(9.74 - 65.94)		(3.24 - 33.44)		(2.78 - 15.35)		(5.23 - 8.90)		(2.69 - 15.11)		(1.78 - 17.06)		(1.62 - 5.77)		(1.62 - 66.89)	
18 -, female	25.47 ± 12.91		12.93 ± 6.10		3.62 ± 1.41		6.95 ± 8.16		3.28 ± 1.36		4.72 ± 4.90		3.00 ± 1.52		2.58 ± 2.77		11.63 ± 10.32	B
	(9.90 - 57.87)		(4.53 - 49.87)		(1.47 - 4.92)		(1.30 - 35.58)		(1.66 - 6.31)		(1.67 - 21.58)		(1.11 - 6.34)		(0.79 - 12.61)		(0.79 - 57.87)	
18 -, male	31.31 ± 12.84		13.00 ± 6.00		7.20 ± 7.15		6.37 ± 3.77		4.49 ± 4.67		5.53 ± 2.99		2.80 ± 1.15		3.04 ± 1.31		13.23 ± 12.07	B
	(10.41 - 63.63)		(3.29 - 26.80)		(5.62 - 8.90)		(2.43 - 11.39)		(1.26 - 9.85)		(3.32 - 10.64)		(0.95 - 4.80)		(1.18 - 6.36)		(0.95 - 63.63)	
Cd-B (μg/L)																		
all age	0.37 ± 0.26	a	0.32 ± 0.30	ab	0.16 ± 0.08	cde	0.17 ± 0.11	cde	0.12 ± 0.06	de	0.26 ± 0.26	bc	0.19 ± 0.11	cd	0.13 ± 0.14	e	0.27 ± 0.26	
	(0.12 - 1.38)		(0.04 - 2.27)		(0.06 - 0.33)		(0.05 - 0.52)		(0.05 - 0.26)		(0.04 - 1.30)		(0.06 - 0.56)		(0.02 - 0.99)		(0.02 - 2.27)	
0 - 4	0.30 ± 0.16		0.16 ± 0.15		0.09		0.11 ± 0.05		0.06		0.15 ± 0.13		0.15 ± 0.01		0.06 ± 0.03		0.13 ± 0.11	A
	(0.14 - 0.52)		(0.04 - 0.38)		(0.08, 0.10)		(0.05 - 0.25)		(0.05, 0.07)		(0.04 - 0.43)		(0.13 - 0.16)		(0.02 - 0.11)		(0.02 - 0.52)	
5 - 17	0.27 ± 0.17		0.21 ± 0.10		0.13 ± 0.09		0.14 ± 0.08		0.08 ± 0.01		0.19 ± 0.13		0.14 ± 0.03		0.07 ± 0.03		0.20 ± 0.13	B
	(0.12 - 1.04)		(0.05 - 0.52)		(0.06 - 0.26)		(0.06 - 0.31)		(0.07 - 0.10)		(0.05 - 0.40)		(0.10 - 0.19)		(0.05 - 0.12)		(0.05 - 1.04)	
18 -, female	0.38 ± 0.17		0.39 ± 0.36		0.21 ± 0.10		0.21 ± 0.13		0.16 ± 0.07		0.24 ± 0.16		0.19 ± 0.06		0.11 ± 0.07		0.31 ± 0.28	C
	(0.14 - 0.72)		(0.08 - 2.27)		(0.08 - 0.33)		(0.08 - 0.52)		(0.07 - 0.26)		(0.08 - 0.52)		(0.09 - 0.30)		(0.03 - 0.25)		(0.03 - 2.27)	

18 -, male	0.51 ± 0.39		0.34 ± 0.30		0.16 ± 0.05		0.23 ± 0.17		0.11 ± 0.07		0.70 ± 0.49		0.23 ± 0.19		0.21 ± 0.22		0.34 ± 0.32		C
	(0.18 - 1.38)		(0.07 - 1.46)		(0.11 - 0.21)		(0.13 - 0.48)		(0.05 - 0.19)		(0.19 - 1.30)		(0.06 - 0.56)		(0.05 - 0.99)		(0.05 - 1.46)		
Zn-B (mg/L)																				
all age	5.92 ± 1.37	a	5.52 ± 1.25	ab	5.14 ± 1.05	ab	5.01 ± 1.11	b	5.49 ± 1.13	ab	5.80 ± 1.46	ab	5.04 ± 1.09	b	5.37 ± 1.38	ab	5.51 ± 1.30		
	(3.45 - 10.20)		(2.30 - 10.06)		(3.53 - 7.02)		(2.95 - 7.53)		(3.72 - 7.36)		(2.99 - 9.27)		(3.05 - 6.85)		(2.78 - 8.53)		(2.30 - 10.20)		
0 - 4	5.31 ± 1.18		3.84 ± 1.15		4.55		4.49 ± 0.82		4.16		4.50 ± 0.84		4.26 ± 0.73		3.99 ± 1.58		4.36 ± 1.06		A
	(4.17 - 6.84)		(2.30 - 5.14)		(4.29, 4.81)		(2.95 - 5.96)		(3.72, 4.60)		(2.99 - 5.93)		(3.46 - 4.91)		(2.78 - 7.75)		(2.30 - 7.75)		
5 - 17	4.95 ± 0.95		4.95 ± 0.95		4.48 ± 0.90		4.51 ± 0.85		5.27 ± 1.20		5.04 ± 0.98		4.19 ± 0.94		4.94 ± 1.24		4.71 ± 1.02		A
	(3.45 - 7.34)		(3.45 - 7.34)		(3.53 - 5.69)		(3.40 - 5.88)		(4.27 - 6.70)		(3.40 - 6.79)		(3.05 - 6.19)		(3.40 - 6.93)		(3.03 - 9.70)		
18 -, female	6.22 ± 1.13		5.67 ± 1.00		5.59 ± 1.16		5.49 ± 1.04		5.78 ± 0.94		6.78 ± 1.27		5.54 ± 1.00		5.33 ± 0.94		5.79 ± 1.09		B
	(4.61 - 8.84)		(3.48 - 8.82)		(4.36 - 6.82)		(3.05 - 7.53)		(4.45 - 7.36)		(4.93 - 9.27)		(3.93 - 6.83)		(3.16 - 7.09)		(3.05 - 9.27)		
18 -, male	6.97 ± 1.32		6.44 ± 1.02		5.52 ± 1.06		5.71 ± 1.70		5.69 ± 1.68		7.17 ± 0.81		5.48 ± 0.84		6.36 ± 1.03		6.40 ± 1.17		C
	(4.94 - 10.20)		(4.64 - 10.06)		(4.57 - 7.02)		(4.00 - 7.24)		(3.92 - 7.26)		(6.25 - 8.06)		(3.66 - 6.85)		(4.61 - 8.53)		(3.66 - 10.20)		

Note: Different small letters (a, b, c, d and e) between columns indicate a significant difference among areas. Different capital letters (A, B and C) indicate a significant difference among age categories.

Table 2. The δ-ALAD activity ratio in whole blood in Kabwe residents by groups categorized by age and Pb-B range (0 – 4.9, 5 – 19.9, 20 – 44.9, 45≤ μg/dL).

age group	Pb-B range	min	25% quartile	median	75% quartile	max	mean	SD	SE		
all age	all Pb-B	0.294	0.490	0.581	0.667	0.850	0.577	0.122	0.006		
0 - 4	all Pb-B	0.333	0.532	0.629	0.686	0.773	0.596	0.126	0.022	A	
	<5	0.463	0.633	0.646	0.679	0.742	0.643	0.072	0.020		a
	5 - 19.9	0.483	0.565	0.619	0.706	0.758	0.636	0.092	0.028		a
	20.0 - 44.9	0.333	0.388	0.486	0.542	0.773	0.499	0.158	0.065		b
	45≤	0.351	0.354	0.356	0.358	0.360	0.356	0.006	0.004		b
5 - 17	all Pb-B	0.303	0.462	0.560	0.634	0.850	0.558	0.125	0.011	A	
	<5	0.553	0.613	0.678	0.759	0.850	0.686	0.090	0.019		a
	5 - 19.9	0.355	0.532	0.595	0.669	0.800	0.590	0.108	0.016		b
	20.0 - 44.9	0.313	0.411	0.500	0.558	0.714	0.496	0.099	0.013		c
	45≤	0.303	0.401	0.431	0.445	0.463	0.411	0.058	0.024		c
18 -	all Pb-B	0.294	0.500	0.587	0.667	0.849	0.583	0.120	0.007	A	
	<5	0.364	0.594	0.667	0.727	0.849	0.659	0.097	0.010		a
	5 - 19.9	0.333	0.469	0.564	0.633	0.847	0.560	0.115	0.009		b
	20.0 - 44.9	0.294	0.481	0.533	0.600	0.755	0.526	0.091	0.014		b
	45≤	0.308	0.333	0.393	0.484	0.545	0.412	0.093	0.031		c

Note: Only one capital letter (A) indicate that there was no significant difference among age groups. Different small letters (a, b and c) indicate a significant difference among Pb-B range categories.

Table 3. Correlation coefficients (r^2) between blood metal levels, biochemical parameters and δ-ALAD activity ratio by age.

Age category	Pb-B								Cd-B								Zn-B							
	all age		0 - 4		5 - 17		18 -		all age		0 - 4		5 - 17		18 -		all age		0 - 4		5 - 17		18 -	
	r2	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*	*r2*	*p*
Pb-B	-	-	-	-	-	-	-	-	0.46	**<.0001**	0.48	**<.01**	0.56	**<.0001**	0.56	**<.0001**	0.03	0.47	0.27	0.15	0.03	0.74	0.19	**<.001**
Cd-B	0.46	**<.0001**	0.48	**<.01**	0.56	**<.0001**	0.56	**<.0001**	-	-	-	-	-	-	-	-	0.32	**<.0001**	0.35	0.07	0.12	0.19	0.16	**<.005**
Zn-B	0.03	0.47	0.27	0.15	0.03	0.74	0.19	**<.001**	0.32	**<.0001**	0.35	0.07	0.12	0.19	0.16	**<.005**	-	-	-	-	-	-	-	-
T-bil	-0.12	**<.05**	0.08	0.68	-0.22	**<.05**	-0.04	0.44	0.06	0.22	0.11	0.57	-0.07	0.43	-0.04	0.46	0.23	**<.0001**	0.09	0.63	-0.04	0.65	0.19	**<.001**
AST	0.04	0.35	0.28	0.14	-0.02	0.84	0.01	0.80	0.00	0.98	0.21	0.29	0.01	0.94	0.07	0.23	-0.02	0.74	0.14	0.46	-0.13	0.16	0.15	**<.01**
ALT	-0.05	0.27	0.30	0.11	0.00	0.97	-0.02	0.69	0.14	**<.005**	0.13	0.49	0.08	0.39	0.06	0.25	0.20	**<.0001**	0.08	0.67	-0.06	0.53	0.11	**<.05**
LDH	0.09	0.05	0.41	**<.05**	0.01	0.91	-0.03	0.58	-0.12	**<.01**	0.21	0.28	-0.06	0.52	0.00	0.93	-0.23	**<.0001**	0.24	0.20	-0.15	0.09	-0.03	0.65
GGT	-0.08	0.09	0.31	0.11	-0.03	0.71	-0.01	0.87	0.14	**<.005**	0.20	0.30	-0.03	0.74	0.00	0.93	0.30	**<.0001**	0.11	0.58	0.04	0.68	0.11	0.05
ALP	0.16	**<.001**	-0.05	0.79	-0.04	0.66	0.00	0.93	-0.24	**<.0001**	-0.10	0.61	0.06	0.53	0.05	0.37	-0.44	**<.0001**	0.09	0.66	-0.04	0.63	0.02	0.79
T-pro	-0.06	0.23	-0.20	0.29	-0.11	0.20	0.07	0.22	0.15	**<.005**	0.13	0.49	-0.10	0.28	0.08	0.14	0.20	**<.0001**	-0.05	0.78	-0.05	0.58	0.11	**<.05**
Alb	-0.07	0.12	-0.09	0.66	-0.15	0.10	0.00	0.94	0.10	**<.05**	0.24	0.21	-0.05	0.56	0.00	0.94	0.22	**<.0001**	0.00	0.98	-0.03	0.74	0.21	**<.0005**
BUN	0.00	0.95	-0.08	0.67	-0.03	0.70	0.08	0.14	0.12	**<.05**	0.14	0.45	-0.10	0.27	0.08	0.14	0.18	**<.0001**	0.02	0.92	0.14	0.13	0.07	0.19
UA	0.11	**<.05**	0.22	0.25	0.24	**<.01**	0.19	**<.005**	0.29	**<.0001**	0.23	0.22	0.18	**<.05**	0.18	**<.005**	0.35	**<.0001**	0.12	0.55	0.10	0.28	0.23	**<.0001**
Cre	-0.07	0.12	0.13	0.51	-0.13	0.15	0.08	0.15	0.28	**<.0001**	0.37	**<.05**	-0.01	0.88	0.05	0.34	0.44	**<.0001**	0.15	0.44	0.12	0.20	0.17	**<.01**
BUN/Cre	0.07	0.15	-0.14	0.46	0.11	0.22	0.02	0.79	-0.12	**<.01**	-0.16	0.40	-0.06	0.54	0.03	0.60	-0.20	**<.0001**	-0.15	0.45	0.03	0.70	-0.04	0.52
$eGFR_{MDRD}$	-0.10	**<.05**	-0.17	0.37	0.14	0.12	-0.10	0.07	0.03	0.53	-0.27	0.15	0.04	0.63	-0.13	**<.05**	0.13	**<.005**	-0.02	0.90	-0.08	0.35	-0.09	0.10
δ-ALAD activity ratio	-0.53	**<.0001**	-0.63	**<.0005**	-0.61	**<.0001**	-0.48	**<.0001**	-0.25	**<.0001**	-0.25	0.20	-0.33	**<.0005**	-0.26	**<.0001**	-0.02	0.60	-0.04	0.82	0.02	0.86	-0.11	0.05

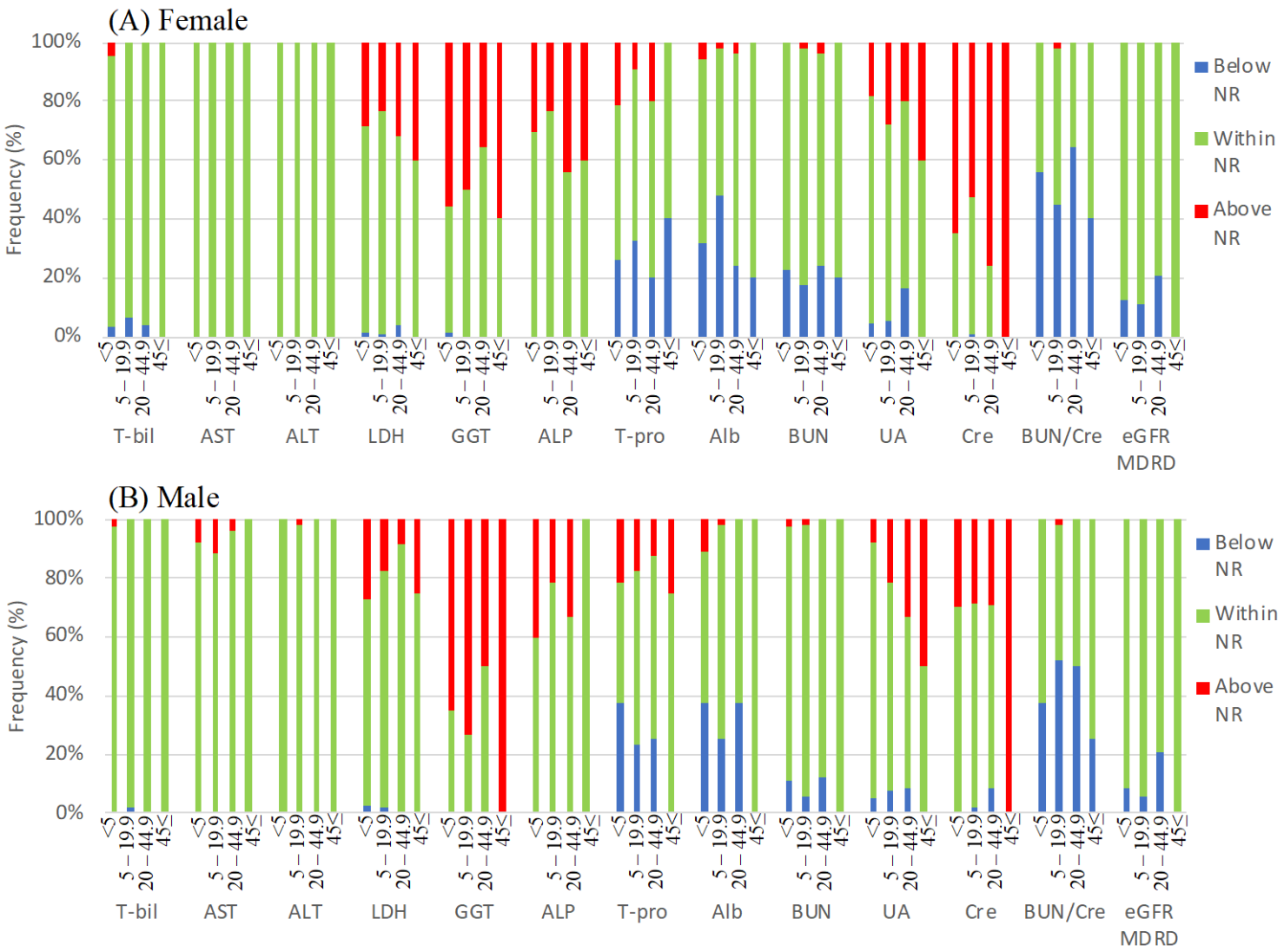

Figure 1. Comparison of blood biochemical parameters in plasma of adult female (A) and male (B) with normal range (NR) by Pb-B range (0 – 4.9, 5 – 19.9, 20 – 44.9, 45≤ μg/dL).

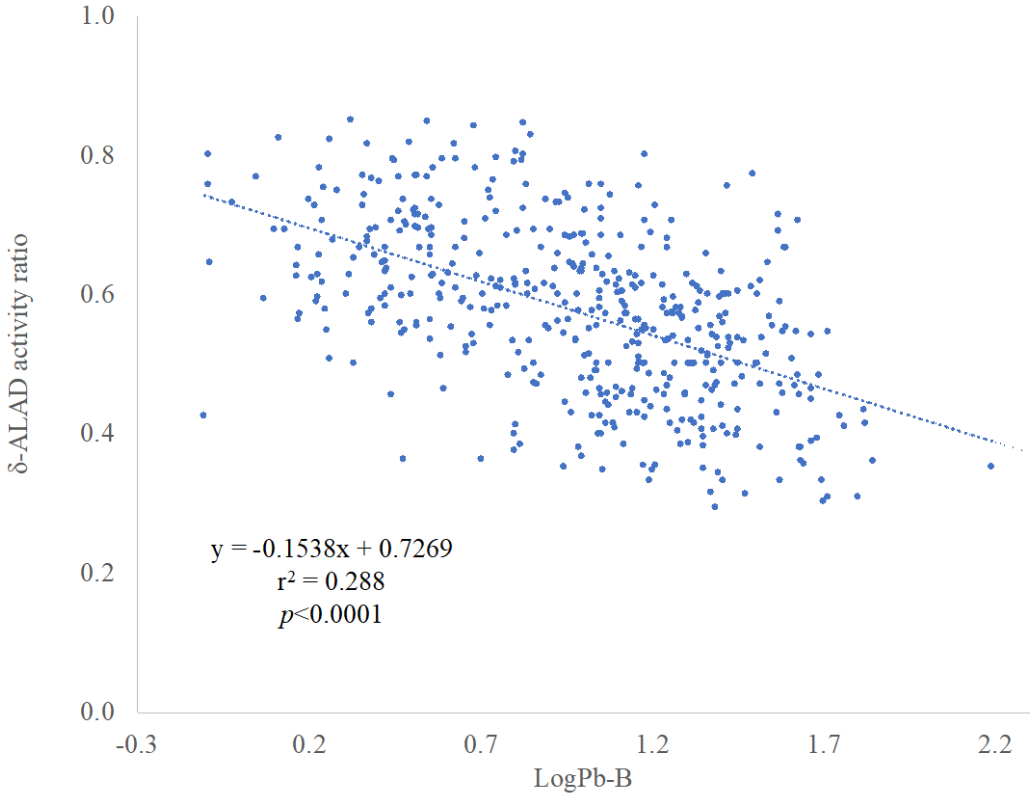

Figure 2. Relationship between logPb-B and δ-ALAD activity ratio of the 504 representative Kabwe residents.

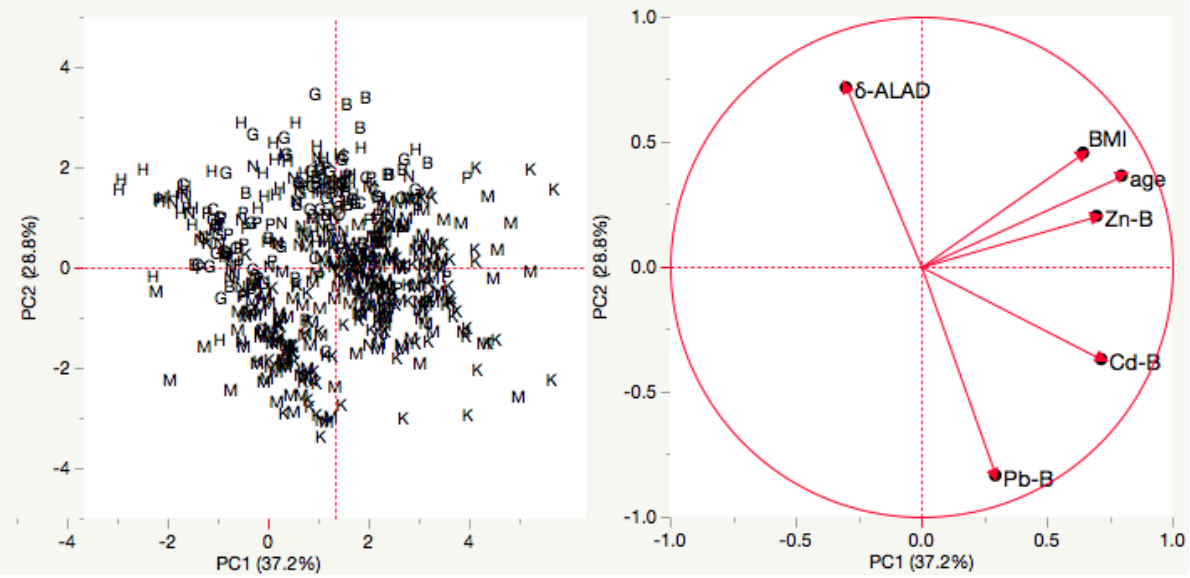

Figure 3. Principal component analysis on log-transformed data of the 504 representative Kabwe residents showing the relationship among blood metal levels, age, BMI and δ-ALAD activity ratio. K = Kasanda, M = Makululu, C = Chowa, N = Natuseko, B = Bwacha, P = Mpima Prison, G = Kang'omba, H = Hamududu.