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

4

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33 **Competing Financial Interests**

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

35

1 **Abstract**

2 Lead (Pb) interferes with various bodily functions. Although high blood Pb (Pb-B) levels
3 in residents from Kabwe, Zambia have been reported, the accumulation pattern of other
4 metals remains unknown. The study was designed to determine the Pb-B, blood cadmium
5 (Cd-B), and zinc (Zn-B) values of 504 representative samples from Kabwe, as well as the
6 potential associated adverse health effects. The Pb-B level ranged from 0.79 to 154.75
7 $\mu\text{g/dL}$ and generally increased in areas near the mine. A significant elevation of Cd-B
8 was observed in two areas (0.37 ± 0.26 and $0.32 \pm 0.30 \mu\text{g/L}$) where the two highest mean
9 Pb-B levels were recorded. By contrast, the Zn-B values did not differ greatly with respect
10 to area. Some blood biochemical parameters relating to hepatic and renal functions were
11 out of the normal range in approximately 20 to 50% of studied adult participants. The δ -
12 aminolevulinic acid dehydratase (δ -ALAD) activity was significantly inhibited in the two
13 areas contaminated by Pb and Cd. A significant negative relationship was observed
14 between metal levels and clinical parameters, e.g., between Pb-B and δ -ALAD for all the
15 age categories and between Cd-B and the estimated glomerular filtration rate for all the
16 age categories except 0 to 4 years. The elevated Cd-B in areas near the mine relative to
17 the other areas suggested the potential adverse health effects of Cd and/or the interaction
18 of Pb and Cd. A significant association of metal levels with clinical parameters also
19 indicated the effects of metal exposure on hematopoietic, hepatic, and renal systems.

20

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

22

23 **1. Introduction**

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

36 Lead is known to interfere with a number of bodily functions including nervous,
37 hematopoietic, hepatic, and renal systems (Lockitch 1993). Exposure to Pb causes
38 hematotoxicity through the restriction of hemoglobin synthesis by inhibiting key enzymes,
39 such as δ-aminolevulinic acid dehydratase (δ-ALAD), and shortening the life span of
40 circulating erythrocytes (Gonick 2011). Anemia, which is caused by these processes, is
41 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

61 not all age group, but children ages 1-5 years old (CDC 2012). The Pb-B level of
62 approximately 15% of the people exceeded 45 $\mu\text{g/dL}$, which is the threshold in children
63 ages 1-5 years old required for chelation therapy (CDC 2002). With the wide spread of
64 Pb poisoning across age groups in Kabwe, the accompanying adverse effects of Pb are
65 anticipated; however, no clinical observation has been conducted. Given these factors,
66 the current study aimed to assess the possible health effect of Pb poisoning in Kabwe.
67 Furthermore, we also targeted blood cadmium (Cd) and Zn levels, as high concentrations
68 of Cd in rats (N=20) (Nakayama et al. 2013), free range chickens (N=10) (Nakata et al.
69 2016) and children (N=190) (Yabe et al. 2018) as well as that of Zn in soil (N=101)
70 (Nakayama et al. 2011) have also been recorded in Kabwe. Cadmium exhibits
71 nephrotoxic activity, with a reduction in the estimated glomerular filtration rate (eGFR)
72 and albumin (Alb) loss in urine (ATSDR 2012). An observational study reported a
73 significant relationship between low-level environmental Cd exposure and renal
74 dysfunction (Ferraro et al. 2010). Additionally, according to both observational and
75 experimental studies, various adverse health effects can occur due to the co-exposure to
76 Pb and Cd (Hambach et al. 2013; Ni et al. 2014; Pan et al. 2018; Nakayama et al. 2019).
77 In contrast to Pb- and Cd-related diseases, disease relating to Zn excess is not common.
78 Rather, a protective effect of Zn on Pb and Cd intoxication has been suggested by
79 experimental studies using laboratory rats (Saxena et al. 1989; Soussi et al. 2018).

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

88 **2. Methods**

89

90 ***2.1. Sample collection and plasma preparation***

91 The study was approved by the University of Zambia Research Ethics
92 Committee (UNZAREC; REF. No. 012-04-16) and the Ministry of Health through the
93 Zambia National Health Research Ethics Board as well as the Kabwe District Medical
94 Office. The sampling was done in Kabwe, which is located approximately 130 km north
95 of Lusaka, the capital city of Zambia. Kabwe has a population of approximately 230,000
96 residents and an area of 1,547 km². The study was designed to select 1,000 households
97 from across Kabwe using two-stage random selection. After informed and written consent
98 was obtained from household heads, the data and samples were collected. Further details
99 of sample selection and collection have been described in recent papers (Hiwatari et al.
100 2019; Yabe et al. 2020). Heparinized blood was dispensed and immediately centrifuged
101 for 10 min at 1,500×g, after which the plasma was stored at –20°C. Analysis was
102 performed at the KAbwe Mine Pollution Amelioration Initiative (KAMPAI) project
103 monitoring laboratory in the Department of Biomedical Sciences, School of Veterinary
104 Medicine, the University of Zambia. The remaining whole blood samples were
105 transported to Japan in cooler boxes after obtaining a material transfer agreement (MTA)
106 from the Ministry of Health, Zambia, through the National Health Research Ethics

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

113

114 ***2.2. Data collection and physical measurement***

115 We asked the participants their age and measured their height and body weight
116 at the same time as blood collection. Data were recorded using Survey Solutions (version
117 5.22.20, the latest version at the time of our survey), developed by the World Bank. Body
118 mass index (BMI) was calculated using the following formula:

119

$$120 \text{ BMI} = \text{body weight (kg)}/\text{height (m)}^2$$

121

122 The BMI z-score for participants with the age between 5 to 17 years old was also
123 determined with reference to sex- and age-specific mean BMI values and distributions
124 using the charts and tables provided by WHO (2007) because the z-score gives a relative
125 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 (HNO₃) 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), lactate 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/1.73m² is
163 considered normal (Levey et al. 2009).

164

165
$$eGFR_{MDRD} = 175 \times (Cre^{-1.154}) \times (age(y)^{-0.203}) \times 1.212 \times 0.742 \text{ (for females)}$$

166
$$eGFR_{MDRD} = 175 \times (Cre^{-1.154}) \times (age(y)^{-0.203}) \times 1.212 \text{ (for males)}$$

167

168 The following formula was used to calculate $eGFR_{MDRD}$ for children and
169 adolescents aged below 18 years old (Schwartz et al. 2009). The value below 75
170 mL/min/1.73m² is considered normal (National Institute of Diabetes and Digestive and
171 Kidney Diseases).

172

173
$$eGFR_{MDRD} = 0.413 \times \text{height (cm)} / Cre$$

174

175 In cases where the T-bil level was below the limit of detection (LOD) of 0.2
176 mg/dL, the data were adjusted to the LOD value divided by the square root of 2 (0.14
177 mg/dL) for statistical analysis (Hornung and Reed, 1990). Similarly, the AST, ALT, and
178 GGT levels lower than the LOD of 10 international unit (IU)/L were adjusted to 7.07
179 IU/L. Furthermore, BUN values below the detection limit of 5 mg/dL were adjusted to
180 3.54 mg/dL. These changes were made to minimize the effect on statistical analysis where
181 the exact distribution of values below the detection limit was unknown.

182

183 2.6. δ -ALAD activity assay

184 Enzymatic activity and the ratio between non-activated and *in vitro*-activated
185 enzymes were measured as described in previous studies (Espín et al. 2015;
186 Scheuhammer 1987) with slight modifications. Three aliquots of 20 μ L of whole blood
187 of each participant were separated to measure non-activated δ -ALAD activity, reactivated
188 δ -ALAD activity, and the activity of the matrix. For the non-activated enzyme activity
189 assay, 80 μ L of 0.1% Triton X-100 (Sigma–Aldrich, MO, USA) was added to the blood
190 as a lysate. Then, 100 μ L of 0.5-M phosphate-buffered saline (PBS) (pH 6.8), 50 μ L of
191 60-mM 5-aminolevulinic acid (ALA) hydrochloride (Sigma–Aldrich) solution in PBS,
192 and 50 μ L of distilled water (DW) were added. For the reactivated activity assay, 0.1%
193 Triton X-100, 0.5-M PBS, and 60-mM ALA hydrochloride were added to the sample with
194 the same volume of each solution used in the non-activated enzyme activity assay. Then,
195 25 μ L of 0.8-mM Zn acetate (Himedia Laboratories Pvt. Ltd., Mumbai, India) and 25 μ L
196 of 1-M dithiothreitol (DTT) (Himedia Laboratories Pvt. Ltd.) were added. For the matrix
197 blank assay, 80 μ L of Triton X-100, 150 μ L of PBS, and 50 μ L of DW were added,
198 followed by 200 μ L of 0.4-M trichloroacetic acid (TCA) (Merck, Darmstadt,
199 Germany)/60-mM mercury chloride (HgCl_2) (Himedia Laboratories Pvt. Ltd.) as a stop
200 solution. After a 60-min incubation and termination of the reaction by HgCl_2 in both the
201 non-activated and reactivated activity assays, all samples were centrifuged at 10,000 g for

202 5 min. Supernatants were transferred to new tubes and mixed with 750 μ L of modified
203 Ehrlich's reagent, which consisted of dimethylaminobenzaldehyde (Nacalai tesque,
204 Kyoto, Japan) in acetic acid (glacial; 99.6%, Himedia Laboratories Pvt. Ltd.) and
205 perchloric acid (Merck KGaA, Darmstadt, Germany). After 10 min, the absorbance was
206 read at 555 nm against the appropriate blank using a UV spectrophotometer (Shimadzu
207 UV-2600, Shimadzu Inc., Kyoto, Japan). The activity was expressed as μ mol
208 porphobilinogen (PBG)/h/L red blood cells using the equation provided by Scheuhammer
209 (1987). Then, the ratio between the non-activated and the *in vitro* reactivated enzymes
210 was calculated.

211

212 **2.7. Statistical analysis**

213 IBM SPSS Statistics 26 (IBM Corporation, Armonk, NY, USA) was used to
214 evaluate significant differences in the data in all statistical analyses except principal
215 component analysis (PCA), which was carried out using JMP Pro version 14 (SAS
216 Institute, NC, USA). The data were log-transformed and fitted a normal distribution. The
217 Tukey–Kramer test was used to compare age, height, body weight, BMI, blood metal
218 levels, blood biochemical parameters, δ -ALAD enzyme activity, and the δ -ALAD
219 activity ratio among areas in Kabwe as well as groups categorized by age or Pb-B.
220 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

225 **3. Results**

226

227 *3.1. General outcomes of the randomly selected subjects*

228 In the random selection, subjects were drawn from people who were tested at the
229 following eight health centers: Kasanda, Makululu, Chowa, Natuseko, Bwacha, Mpima
230 prison, Kang'omba, and Hamududu (Supplementary Figure S1). The age of the 504
231 selected Kabwe residents ranged from 0 to 96 years (Supplementary Table S2). The mean
232 and median ages of all tested people were 28.1 and 27 years, respectively, and their mean
233 and median heights were 144.9 and 156.0 cm, respectively. Their body weight ranged
234 from 7 to 154 kg, with mean and median values of 47.8 and 53.0 kg, respectively. For
235 adults 18 years or older, the mean and median BMI values were 24.0 and 22.6,
236 respectively, whereas the mean and median BMI values for all age groups were 21.0 and
237 20.4, respectively.

238 Significant area differences in age, height, body weight, and BMI for all age
239 groups were recorded. The age, height, and body weight of people in Makululu were
240 significantly higher than those in Natuseko and Mpima prison. Similarly, the age, height
241 and body weight of people in Kasanda were significantly higher than those in Natuseko.
242 Significantly higher BMI values were recorded in Makululu and Bwacha compared to
243 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 $\mu\text{g/dL}$, 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 $\mu\text{g/dL}$ 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

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

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

270 ***3.3. Blood biochemical parameters***

271 The values of the blood biochemical parameters in 8 areas of Kabwe are
272 presented in Supplementary Table S5. The T-bil value recorded in Natuseko was
273 significantly higher than that recorded in Makululu and Kang'omba. Kasanda also
274 showed a significantly higher level of T-bil compared with Makululu. Hanududu had
275 significantly higher AST value than Makululu and Kang'omba. The LDH level in Chowa
276 was significantly higher than those in Makululu, Mpima prison, Kang'omba and
277 Hamududu. Bwacha recorded the highest mean value of GGT with statistical significant
278 difference compared to Kang'omba. A significantly lower Alb value was measured in
279 Makululu compared to those in Kasanda and Chowa. The significant increase of Cre was
280 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 adult 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 $\mu\text{g/dL}$ and above had significantly
324 lower δ -ALAD activity ratios than other two categories with Pb-B below 20.0 $\mu\text{g/dL}$.
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

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

356 **4. Discussion**

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

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

375 that children had greater Pb-B than adults (Yabe et al., 2020) although such a trend was
376 not clearly shown in general. Chowa and Natuseko followed Kasanda and Makululu, with
377 mean values close to 10 µg/dL. It should also be emphasized that some residents had Pb-
378 B values above the reference level of 5 µg/dL, even in rural areas away from the mine,
379 such as Mpima prison, Kang'omba, and Hamududu. Considering the possibility that the
380 reference level may be revised to 3.48 µg/dL based on the 2011–2014 National Health
381 and Nutrition Examination Survey (NHANES) (Caldwell et al. 2017), observed mean Pb-
382 B levels below 5 µg/dL in Kang'omba and Hamududu should also be carefully considered.
383 Compared with the results concerning other African countries, the Pb-B levels recorded
384 in the current study were higher, although elevated Pb-B values have been reported in
385 northwestern Nigeria where more than 400 children died because of Pb poisoning caused
386 by artisanal mining activities; the values ranged from 36.5 to 445 µg/dL in 86 children <
387 5 years of age (Dooyema et al. 2012; Pure Earth 2014). In a cross-sectional study in
388 Kinshasa, Democratic Republic of Congo, where leaded gasoline was still used at the
389 time, Pb-B levels in 275 representative residents ranged from 2.9 to 49.3 µg/dL, with a
390 median value of 9.9 µg/dL (Tuakuila et al. 2013). In Kibera slum, Nairobi, Kenya, a mean
391 Pb-B level of 6.0 µg/dL with a range of 3.3 to 24.7 µg/dL was recorded in a cross-
392 sectional study targeting 387 children aged 6 to 59 months (Olewe et al. 2009). Mean Pb-
393 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

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

437 Among the hepatic function parameters, most of the adult participants were
438 within the normal range of T-bil, AST and ALT despite some significant area differences
439 for T-bil and AST. The reason for the unexpected high LDH levels in Chowa and Bwacha
440 as well as GGT in Bwacha is unclear; however, the small sample size of Chowa and
441 Bwacha may have played a role. Although approximately 25 to 60% of the tested adult
442 participants showed the values of LDH, GGT and ALP above normal ranges, the
443 frequencies of abnormal values for these parameters were not Pb-B level-dependent.
444 Analysis of T-pro, Alb, UA, Cre and eGFR_{MDRD} for renal function screening showed
445 significant area differences. Interestingly, most of the observed area differences did not
446 reflect the ranking of the metal levels. The low Alb value in Makululu, as well as that of
447 Cre in Makululu and Hamududu, could be attributed to poverty and malnutrition in these
448 areas (Hiwatari et al. 2019). The eGFR_{MDRD} value was lowest in Kasanda, indicating the
449 inhibition of glomerular filtration function by Pb and Cd exposure. The activity of δ -
450 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).

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

489 Although it is known that Pb and Cd exhibit dose-dependent renal toxicity (ATSDR 2012;
490 2017; Bernard 2008), the reason for the significant relationship between Zn-B and UA is
491 unclear. The inverse correlation between eGFR_{MDRD} and Cd-B for the adult participants
492 in the current study is in agreement with earlier reports (Buser et al. 2016; Hwangbo et
493 al. 2011). High r^2 value was recorded between Pb-B and the δ -ALAD activity ratio, which
494 is widely considered a sensitive indicator of early Pb exposure (Fontanellas et al. 2002).
495 While a significant negative association was also observed between Cd-B and the δ -
496 ALAD activity ratio, the PCA and Pearson product-moment correlation analysis results
497 indicated that the reduction in the δ -ALAD activity ratio was caused mainly by increased
498 Pb-B rather than increased Cd-B. In addition, the confounding relationship between Pb-
499 B and Cd-B affected the association between Cd-B and the δ -ALAD activity ratio. In fact,
500 an inverse effect of Pb exposure on δ -ALAD has been widely reported (Fontanellas et al.
501 2002; Mani et al. 2018), contrary to that observed for Cd. The relationship between BMI
502 and age based on PCA agrees with a finding reported in Lusaka, the capital city of Zambia
503 (Rudatsikira et al. 2012). The association of Zn-B with BMI can also be explained by the
504 nutrition status, similar to that mentioned above for blood protein.

505

506 **5. Conclusion**

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

520

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542

543

544 **Figure captions**

545

546 **Figure 1. Comparison of blood biochemical parameters in plasma of adult female**
547 **(A) and male (B) with normal range (NR) by Pb-B range (0 – 4.9, 5 – 19.9, 20 – 44.9,**
548 **45 ≤ µg/dL).**

549

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

552

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

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

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

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

3 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
4 significant difference among age categories.

5

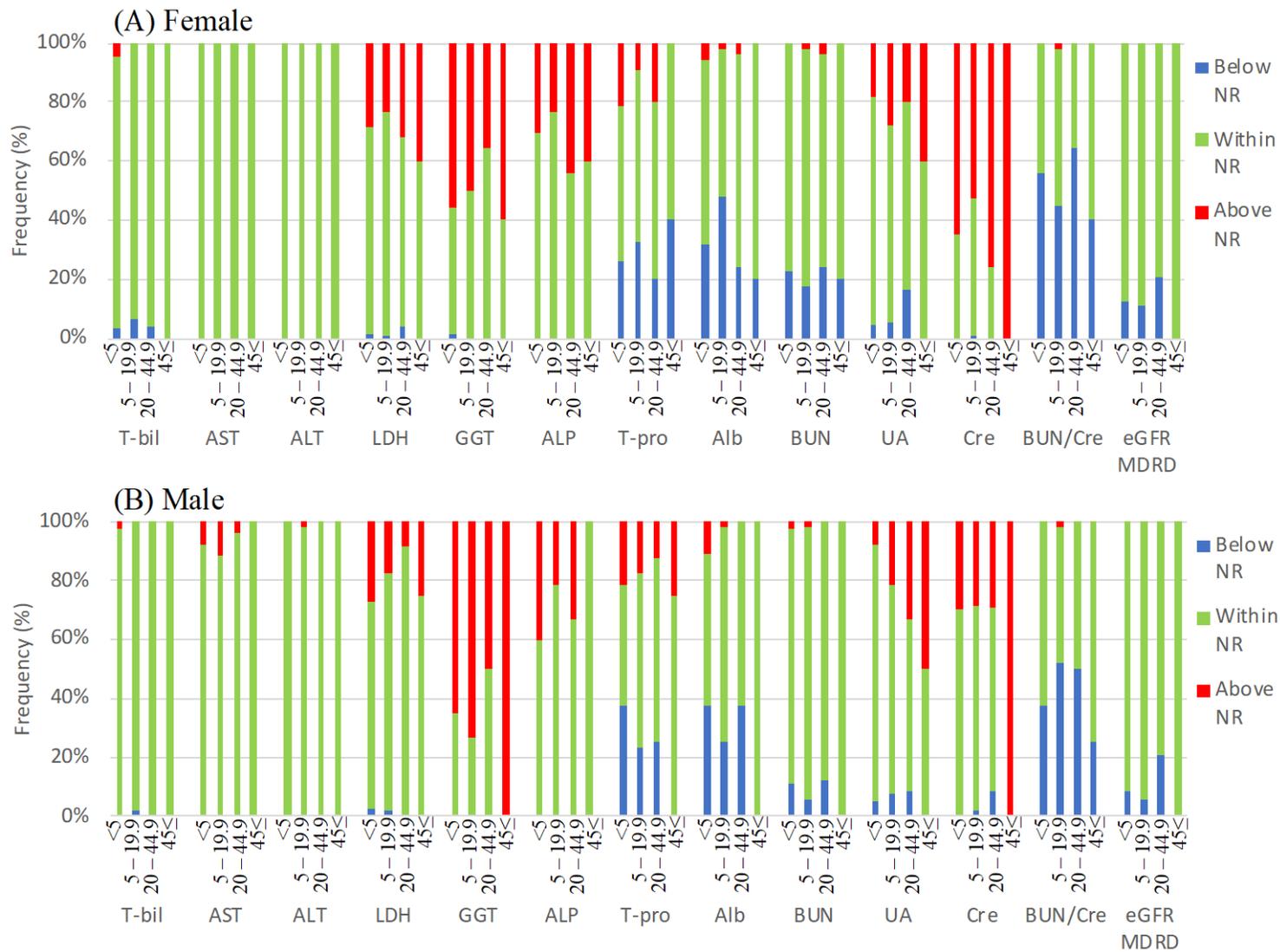
6 **Table 2. The δ -ALAD activity ratio in whole blood in Kabwe residents by groups categorized by age and Pb-B range (0 – 4.9, 5 – 19.9, 20 – 44.9, 45≤**
 7 **$\mu\text{g/dL}$).**

| 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 | |
| | 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 |
| 0 - 4 | 45≤ | 0.351 | 0.354 | 0.356 | 0.358 | 0.360 | 0.356 | 0.006 | 0.004 | b |
| | 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 |
| 5 - 17 | 45≤ | 0.303 | 0.401 | 0.431 | 0.445 | 0.463 | 0.411 | 0.058 | 0.024 | c |
| | 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 |
| 18 - | 45≤ | 0.308 | 0.333 | 0.393 | 0.484 | 0.545 | 0.412 | 0.093 | 0.031 | c |

8 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
 9 difference among Pb-B range categories.

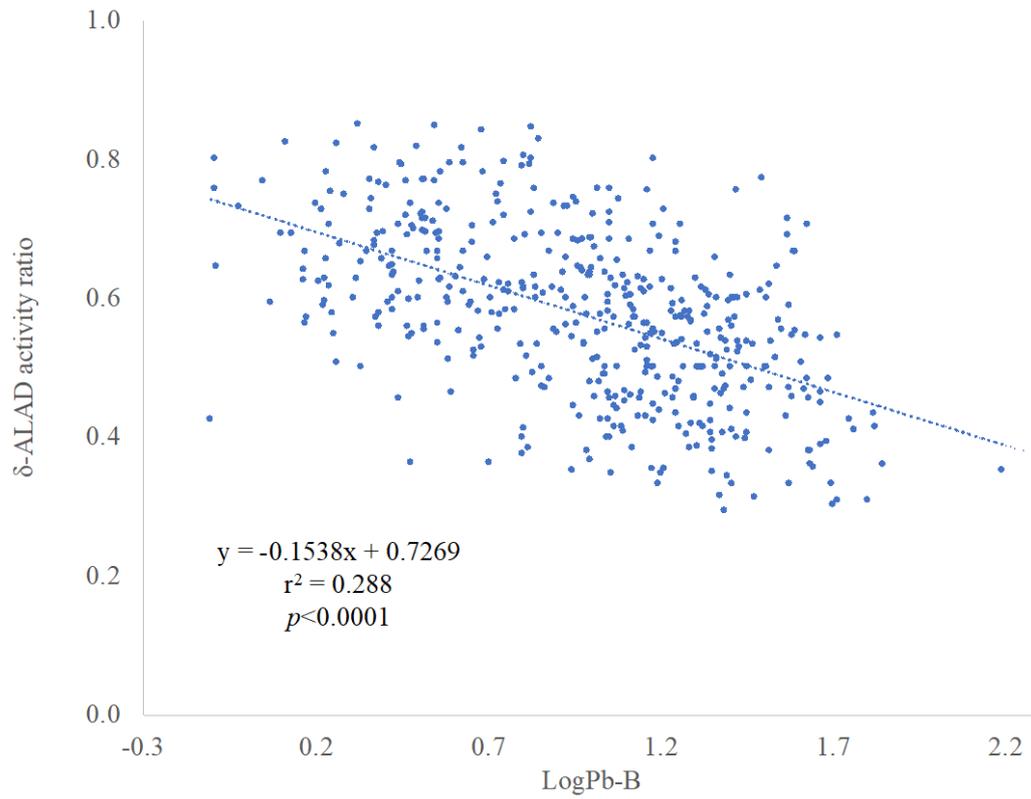
Table 3. Correlation coefficients (r^2) between blood metal levels, biochemical parameters and δ -ALAD activity ratio by age.

| 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 - | |
| | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | p | r^2 | 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 |



12

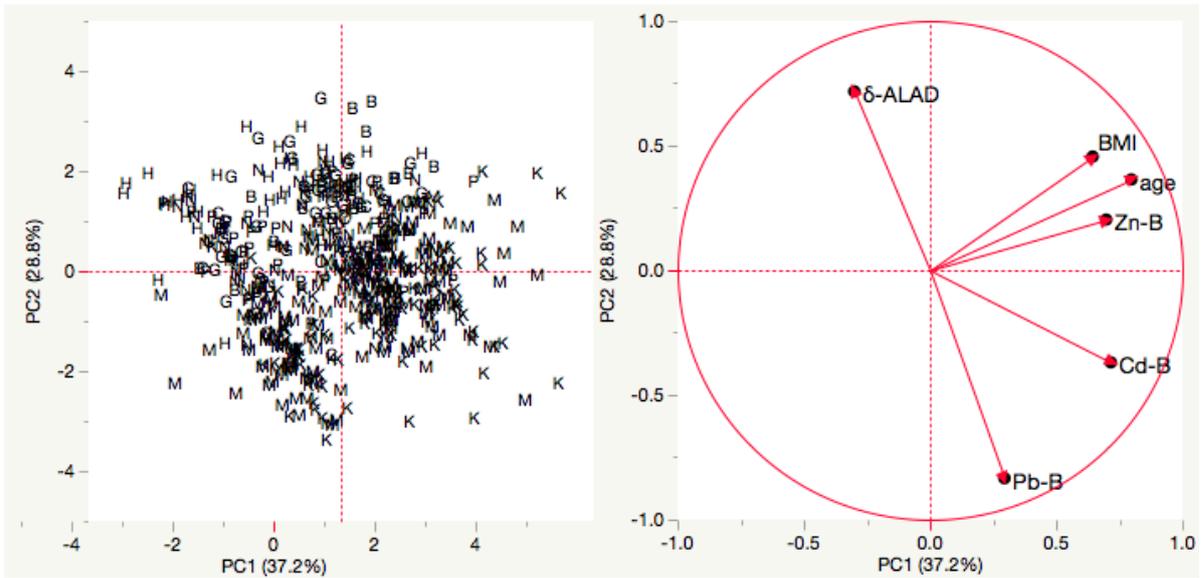
13 **Figure 1. Comparison of blood biochemical parameters in plasma of adult female (A) and male (B) with normal range (NR) by Pb-B range (0 – 4.9, 5 –**
 14 **19.9, 20 – 44.9, $45 \leq$ $\mu\text{g/dL}$).**



15

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

18



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

23