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Lead and cadmium excretion in feces and urine of children from polluted townships near a lead-zinc mine in Kabwe, Zambia

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1 **Abstract**

2 Lead (Pb) and cadmium (Cd) are toxic metals that exist ubiquitously in the environment.
3 Children in polluted areas are particularly vulnerable to metal exposure, where clinical
4 signs and symptoms could be nonspecific. Absorbed metals are excreted primarily in
5 urine and reflect exposure from all sources. We analyzed Pb and Cd concentrations in
6 blood, feces and urine of children from polluted townships near a lead-zinc mine in
7 Kabwe, Zambia, to determine concurrent childhood exposure to the metals. Moreover,
8 the study determined the Pb and Cd relationships among urine, feces and blood as well
9 as assessed the potential of urine and fecal analysis for biomonitoring of Pb and Cd
10 exposure in children. Fecal Pb (up to 2252 mg/kg, dry weight) and urine Pb (up to 2914
11 µg/L) were extremely high. Concentrations of Cd in blood (Cd-B) of up to 7.7 µg/L,
12 fecal (up to 4.49 mg/kg, dry weight) and urine (up to 18.1 µg/L) samples were elevated.
13 metal levels were higher in younger children (0 - 3 years old) than older children (4 - 7).
14 Positive correlations were recorded for Pb and Cd among blood, urine and fecal samples
15 whereas negative correlations were recorded with age. These findings indicate children
16 are exposed to both metals at their current home environment. Moreover, urine and
17 feces could be useful for biomonitoring of metals due to their strong relationships with
18 blood levels. There is need to conduct a clinical evaluation of the affected children to
19 fully appreciate the health impact of these metal exposure.

20

21 **Keywords:** Lead; Cadmium; Excretion; Children's health; Kabwe; Zambia

22 **1. Introduction**

23 Lead (Pb) and cadmium (Cd) are ubiquitous environmental toxicants as a result of
24 contamination from a variety of sources including natural and anthropogenic causes.
25 Children in polluted environments are particularly vulnerable to Pb exposure because of
26 their inclination to ingest soil through pica and to assimilate a relatively greater amount
27 of ingested Pb than adults (Calabrese et al., 1997., Manton et al., 2000 and Caravanos et
28 al., 2013). The detrimental effects of low blood lead levels (BLLs) are usually
29 subclinical and may include neurodevelopmental impairment such as decreased IQ in
30 children (CDC 2002; Canfield et al. 2003). It has been observed that high BLLs in
31 children can cause abdominal pain, encephalopathy, convulsions, coma and death
32 (Needleman 2004). Recently, more than 400 children died of Pb poisoning due to
33 artisanal mining activities in Nigeria, where long-term neurological impairment
34 including blindness and deafness were also recorded (Pure Earth, 2014, Dooyema et al.,
35 2012 and Lo et al., 2012).

36 Similarly, Cd toxicity results in a wide range of biochemical and physiological
37 dysfunctions in humans (Ercal et al., 2001). One of the most severe forms of chronic Cd
38 toxicity is *itai itai* disease (a Japanese term meaning “ouch-ouch”), which is
39 characterized by nephrotoxicity, osteoporosis and cardiovascular diseases (Kido et al.,
40 1990 and Uno 2005). Cadmium has also been classified as a group I carcinogen as
41 chronic inhalation exposure can produce lung cancer in humans (IARC, 1993).
42 Although the toxic effects of Cd are mainly seen in adults (Kido et al., 1990 and
43 Umemura, 2000), exposure to children in even low amounts has associated with
44 neurodevelopmental defects (Ciesielski et al., 2012). Moreover, exposure may have

45 long-term consequences since Cd is a cumulative toxin and has a very long half time in
46 the body.

47 Major sources of Pb and Cd pollution in many African countries include mining,
48 industrial activities, municipal wastes and agricultural activities (Yabe et al., 2010). In
49 Zambia, the closed Pb-Zn mine that operated from 1902 to 1994 in Kabwe town has
50 contributed to extensive metal pollution in the surrounding residential areas, especially
51 with Pb and Cd that was produced as by-product. Despite closure of the mine, dust
52 emanating from the mine dumps has continued to serve as a source of metal pollution.
53 In earlier studies, extensive Pb and Cd contamination of township soils in the vicinity of
54 the mine were reported and pose a serious health risk to children in these townships
55 (Water Management Consultants Ltd, 2006, Nakayama et al., 2011). Recently, clear
56 evidence of Pb poisoning was reported in children from townships around the mine in
57 Kabwe (Yabe et al., 2015). Using stable Pb isotope analysis, Nakata et al. (2016)
58 revealed that soil was likely the main source of Pb exposure in Kabwe.

59 Clinical presentations of metal poisoning vary widely depending upon the age at
60 exposure, the amount of exposure and the duration of exposure. Since chronic Pb
61 poisoning in children is asymptomatic and may result in a delay in the appropriate
62 diagnosis, measurement of concentrations in biological samples plays a pivotal role in
63 the diagnosis and management of patients (Lowry, 2010). Currently, Pb concentration
64 in whole blood (Pb-B) is the main biomarker used to monitor exposure and has been
65 widely used in epidemiological studies (CDC, 2012). However, independent of the
66 mode of exposure, absorbed metals such as Pb and Cd are excreted primarily in urine
67 and the biliary-fecal route (Gwiazda et al., 2005; Swaran and Vidhu 2010). Therefore,

68 Pb and Cd biomonitoring using fecal and urine samples could be useful as they are easy
69 to collect and are non-invasive. Moreover, whereas blood Cd (Cd-B) is the most
70 common marker of recent exposure, urinary Cd (Cd-U) may reflect the kidney burden
71 and is associated with renal health effects (Akerstrom et al., 2013). Evaluating
72 relationships of Pb and Cd among blood, urine and fecal compartments may be useful
73 for understanding exposure patterns. Therefore, the current study measured Pb and Cd
74 concentrations in blood, feces and urine of children with known BLLs (Yabe et al.,
75 2015), from contaminated townships in the vicinity of a Pb-Zn mine in Kabwe, Zambia
76 to determine concurrent childhood exposure. Moreover, the study analysed Pb and Cd
77 relationships in matched feces, urine and blood as well as assessed the potential of urine
78 and fecal analysis for biomonitoring of Pb and Cd exposure in children

79

80 **2. Materials and methods**

81 *2.1 Sampling sites*

82 Kabwe town, the fourth largest town and the provincial capital of Zambia's Central
83 Province, is located at about 28°26'E and 14°27'S. Kabwe has a long history of open-pit
84 Pb-Zn mining. The mine operated almost continuously from 1902 to 1994 without
85 addressing the potential risks of metal pollution. Cadmium was obtained as a by-product
86 of processing zinc-containing ores. As shown in the survey by Water Management
87 Consultants Ltd. (2006), soils in townships in the vicinity of the closed mine and homes
88 downwind from the mine dumps were highly polluted with Pb exceeding acceptable
89 levels for residential areas (Fig. 1). In the current study, fecal and urine samples were

100

101

102 2.2 *Sample collection*

103 The study was approved by the University of Zambia Research Ethics Committee
104 (UNZAREC) and the Ministry of Health, Zambia. Before sampling commenced, an
105 awareness campaign about the research activities was conducted by community health
106 workers in each township to encourage parents/guardians to take their children under
107 the age of 7 to the selected health centres for sample collection. After informed and
108 written consent was obtained from the children's parents or guardians, paired fecal and
109 urine (morning spot-urine) samples were collected in clean metal-free specimen
110 containers at Chowa, Kasanda and Makululu clinics. Blood samples were collected as
111 described earlier by Yabe et al. (2015). For each child, data on the age, sex, residential
112 area, medical history and past or current metal chelation therapy were recorded. Sample
113 collection and questionnaire administration were done by laboratory technicians and
114 nurses, respectively. In addition to selecting children under the age of 7 years, other
115 inclusion criteria included children that were residing in communities in the vicinity of
116 the Pb-Zn mine. The children must have been born or resided in the selected
117 communities for at least 1 year. Only the children whose parents responded to the
118 awareness campaign and signed the informed consent were selected. Efforts were made
119 to collect urine samples in 50 ml urine containers in the morning of sample collection at
120 the health centres. To avoid sample contamination, all sample collection supplies were
121 kept in plastic ziploc storage bags before sample collection. For fecal samples,
122 parents/guardians were handed 50 ml stool containers equipped with scoops and

123 instructed to let their children deposit their stool on a clean plastic/paper in the morning
124 of the following day. Only the top surface was scooped into a stool container and
125 returned to the health centre the same day to avoid sample storage at home. For infants,
126 fecal samples were scooped from a soiled diaper. After submission, samples were then
127 transferred into 15 ml falcon tubes for storage and transportation. The samples were
128 immediately stored at -20 °C after sampling and then transported in cooler boxes on dry
129 ice to the laboratory of the Kabwe District Health Offices where they were again stored
130 at - 20 °C. After obtaining the material transfer clearance from the Zambia National
131 Health Research Ethics Committee (NHREC), the samples were transported to Japan in
132 cooler boxes on dry ice and analyzed for metal concentrations in the Laboratory of
133 Toxicology, Graduate School of Veterinary Medicine, Hokkaido University.

134 2.3 *Sample preparation and metal extraction*

135 All laboratory materials and instruments used in metal extraction were washed in 2 %
136 nitric acid (HNO₃) and oven dried. The metals were extracted in fecal and urine samples
137 using microwave digestion system (Speedwave MWS-2; Berghof) according to the
138 manufacture's instruction and published reports (Fukui et al., 2004; Yabe et al., 2011).
139 Thawed fecal samples were weighed onto heat-resistant tissue drying plates and dried
140 for 24 h in a tissue drying oven at 60 °C while urine samples were just thawed. Briefly,
141 1 mL of each urine sample and 50 mg of oven-dried fecal sample were separately
142 placed in prewashed microwave digestion flasks. To these samples, 5 mL of 60 % nitric
143 acid (Kanto Chemical) and 1 mL of 30 % hydrogen peroxide (Kanto Chemical) were
144 added. After digestion in the microwave for 52 minutes and temperatures of up to

145 190 °C, the digested samples were each transferred into 15 ml plastic tubes. The volume
146 was then made up to 10 mL with bi-distilled and de-ionized water (Milli-Q).

147

148 2.4 *Metal analysis*

149 Blood samples for Cd measurements were prepared and analysed as described earlier
150 (Yabe et al., 2015). Fecal and urine metals (Pb and Cd) concentrations were analyzed by
151 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; 7700 series, Agilent
152 technologies, Tokyo, Japan). The precision and accuracy of the applied analytical
153 method was evaluated by analyzing the recovery rate using digested urine samples and
154 spiking Pb and Cd standard solutions. Using this method, good recoveries of 95 % for
155 both Pb and Cd were obtained. Certified Reference Materials, DORM-3 (Fish protein,
156 National Research Council of Canada, Ottawa, Canada) and DOLT-4 (Dogfish liver,
157 National Research Council of Canada, Ottawa, Canada) were used to evaluate
158 recoveries. Replicate analysis of these reference materials also showed good accuracy
159 (relative standard deviation, RSD, $\leq 3\%$) and recovery rates ranged from (95-105%).
160 Using the Certified Reference Materials, the detection limits of Cd and Pb were 0.0005
161 $\mu\text{g/L}$ and 0.0022 $\mu\text{g/L}$, respectively (Ogbomida et al., 2018). The instrument detection
162 limit (IDL) was 0.001 $\mu\text{g/L}$. Replicate urine samples were used at 4 different spike
163 concentrations of 0.01 ppb, 0.1 ppb, 1 ppb and 10 ppb. These yielded detection limits of
164 0.006 $\mu\text{g/L}$ (Cd) and 0.043 $\mu\text{g/L}$ (Pb) as well as recovery rates of 85.5 - 99.7% (Cd) and
165 104.8 - 107.7% (Pb). To compensate for variations in urine dilution, measured urine-Pb
166 (Pb-U) and urine-Cd (Cd-U) concentrations were adjusted for specific gravity (SG).
167 Urinary SG was measured by a hand refractometer (ATAGO, PAL-095, Tokyo, Japan).

168 Obtained mean SG for Kasanda (1.012) and Makululu (1.021) were used to adjust
169 urinary metal concentrations as illustrated in other studies (Suwazono et al., 2005;
170 Nermell et al., 2008). For example, SG adjusted Pb-U_{SG} was calculated using the
171 obtained mean value of 1.012 and the following formula: $Pb-U_{SG} = Pb-U \times (1.012 -$
172 $1)/(SG - 1.000)$ where Pb-U_{SG} is the adjusted value for SG and Pb-U is the measured
173 concentration. The same was done for Pb-U in Makululu as well as Cd-U in Makululu
174 and Kasanda.

175 2.5 *Statistical analysis*

176 Specific gravity-adjusted concentrations of Pb and Cd in urine are presented as Pb-
177 U_{SG} and Cd-U_{SG}, respectively. The data of blood Cd (Cd-B), fecal Pb (Pb-F), Pb-U_{SG},
178 fecal-Cd (Cd-F) and Cd-U_{SG} were log-transformed to stabilize variances. Statistical
179 analysis was performed using JMP version 10 (SAS Institute, USA). The data are
180 presented as mean, geometric mean (GM), median and minimum-maximum values in
181 mg/kg (feces) and µg/L (urine). Student's *t* test was used to analyze area differences of
182 metal accumulation. Pearson's correlation was used to analyse associations between Pb
183 and Cd in matched blood, feces and urine. Multiple logistic regression analyses on log-
184 transformed data were used to estimate the influence of area, sex and age on Pb and Cd
185 excretions in feces and urine. Samples from Chowa were not included in the
186 comparisons due to small number of sampled children less than 7 years old compared
187 with Kasanda and Makululu. A *p* value of less than 0.05 was considered to indicate
188 statistical significance. Data of blood Pb (Pb-B) from the already published results
189 (Yabe et al., 2015) were used (with permission from the journal) for correlations with
190 matched fecal and urine samples.

191 **2. Results**

192 *3.1 Sample sizes and characteristics*

193 A total of 190 fecal samples were collected from children, up to 7 years old, at
194 Chowa (n = 8 samples), Kasanda (n = 88) and Makululu (n = 94) health centres. The
195 children were classified as male/female and younger (8 months – 3 years)/older (4 – 7
196 years) as shown in Table 1. The data on mean age (4.2 years), median (4 years) and
197 ranges (8 months – 7 years) are not shown.

198 Table 1.

199 Sample sizes and sample characteristics of children from Chowa, Kasanda and
200 Makululu townships near the Pb-Zn in Kabwe, Zambia

	Chowa	Kasanda	Makululu	Totals
Sample size	8	88	94	190
Males	4	40	39	83
Females	4	48	55	107
Median age	5.9	3.6	4.6	4.2
Younger children (8 months – 3 years)	1	42	29	72
Older children (4 – 7 years)	7	46	65	118

201

202

203

204 3.2 *Fecal lead (Pb-F) and Urine lead (Pb-U) levels*

205 As shown in Table 2, concentrations of Pb in fecal samples (mg/kg, dry weight) were
 206 high in all the sampled children. Similarly, a total of 190 urine samples were collected
 207 at Chowa ($n = 8$ samples), Kasanda ($n = 88$) and Makululu ($n = 94$) health centres. The
 208 concentrations of Pb in urine (Pb-U_{SG}) were extremely high, with concentration up to
 209 2914 µg/L recorded in Kasanda Township (Table 2). Only five (about 2.6 %) of the
 210 total sampled children had a history of metal chelation therapy.

211

212 Table 2.

213 Pb-F (mg/kg, dry weight) and Pb-U_{SG} (µg/L, adjusted for SG) concentrations of
 214 children from Chowa, Kasanda and Makululu townships near the Pb-Zn mine in Kabwe,
 215 Zambia

Sample	<i>n</i>	Mean	GM	Median	Minimum	Maximum	IQR
Pb-F (mg/kg)							
Chowa	8	11.6	9.32	10.3	3.03	92.7	19.9 – 5.17
Kasanda	88	90.6	35.3	31.9	3.45	1259	71.2 – 15.4
Makululu	94	67.8	20.3	15.0	2.27	2252	53.3 – 7.99
Pb-U_{SG} (µg/L)							
Chowa	8	13.4	12.1	13.5	4.62	19.9	17.8 – 8.88
Kasanda	88	207	67.8	59.6	1.84	2914	117.8 – 31.2
Makululu	94	81.3	35.1	29.7	2.57	1113	56.4 – 18.6

216 Pb-F = fecal Pb; Pb-U = urinary Pb; *n* = number of samples; IQR = Interquartile Range

217

218 3.3 *Fecal cadmium (Cd-F), Urine cadmium (Cd-U) and Blood (Cd-B) levels*

219 As shown in Table 3, concentrations of Cd in fecal samples (mg/kg, dry weight) were
 220 elevated in all the sampled children with a maximum concentration of 4.49 mg/kg. The

221 concentrations of Cd in urine (Cd-U_{SG}) were elevated, especially for Kasanda with a
 222 mean (GM) of 0.46 µg/L. Similarly, concentrations of Cd in blood were higher in
 223 Kasanda, where Cd-B concentrations of up to 7.70 µg/L were recorded.

224

225 Table 3.

226 Cd-F (mg/kg) and Cd-U_{SG} (µg/L, adjusted for SG) concentrations of children from
 227 Chowa, Kasanda and Makululu townships in vicinity of the Pb-Zn mine in Kabwe,
 228 Zambia

229

Sample	<i>n</i>	Mean	GM	Median	Minimum	Maximum	IQR
Cd-F (mg/kg)							
Chowa	8	0.18	0.15	0.16	0.07	0.43	0.23 – 0.09
Kasanda	88	0.54	0.31	0.28	0.04	4.49	0.57 – 0.15
Makululu	94	0.26	0.18	0.17	0.04	1.58	0.29 – 0.10
Cd-U_{SG} (µg/L)							
Chowa	8	0.43	0.19	0.13	0.06	1.67	0.93 – 0.09
Kasanda	88	1.47	0.46	0.38	0.02	18.1	0.79 – 0.19
Makululu	94	0.71	0.35	0.30	0.03	7.66	0.61 – 0.17
Cd-B (µg/L)							
Chowa	8	0.69	0.66	0.67	0.46	1.06	0.80 – 0.51
Kasanda	88	1.10	0.84	0.72	0.24	7.70	1.31 – 0.40
Makululu	94	0.52	0.44	0.49	0.08	1.56	0.68 – 0.32

230 Cd-F = fecal Cd; Cd-U_{SG} = urinary Cd adjusted for SG; Cd-B = blood CD; *n* = number
 231 of samples; IQR = Interquartile Range

232

233 3.4 Measured Pb-U and Cd-U vs Biomonitoring Equivalent (BE) values

234 As shown in Table 4, the measured Cd-U_{SG} and Cd-B concentrations were compared
 235 with the current BE values that are consistent with established exposure guideline

236 values to evaluate if measured values in the current study were of low, medium, or high
 237 priority for risk assessment follow-up of (Hays et al., 2008). The measured Cd-U_{SG} and
 238 Cd-B were below the BE values.

239

240 Table 4.

241 Comparison between Cd-U and Cd-B concentrations ($\mu\text{g/L}$) measured in urine and
 242 blood samples of children from Chowa, Kasanda and Makululu townships in vicinity of
 243 the Pb-Zn mine in Kabwe, Zambia with Biomonitoring Equivalents (BE) values

Data set	Chowa	Kasanda	Makululu	USEPA (BE value)	ATSDR (BE value)
Cd-U ($\mu\text{g/L}$)	0.19 $\mu\text{g/L}$	0.46 $\mu\text{g/L}$	0.35 $\mu\text{g/L}$	1.5 $\mu\text{g/L}$	1.2 $\mu\text{g/L}$
Cd-B ($\mu\text{g/L}$)	0.66 $\mu\text{g/L}$	1.10 $\mu\text{g/L}$	0.44 $\mu\text{g/L}$	1.7 $\mu\text{g/L}$	1.4 $\mu\text{g/L}$

244 USEPA and ATSDR Biomonitoring Equivalents (BE) values of blood Cd and
 245 creatinine-adjusted urinary Cd (Hays et al., 2008).

246

247 3.5 Site, age and sex differences

248 Multiple logistic regression analyses were performed on log-transformed data to
 249 estimate the influence of independent variables (age as continuous variable, sex
 250 represented as 0 for girls and 1 for boys, location (area) represented as 0 for Makululu
 251 and 1 for Kasanda) on Pb-F. Similar analyses were done on Cd-F, Pb-U_{SG} and Cd-U_{SG}
 252 (Table 5). Fecal Pb and Cd as well as urinary Pb concentrations in children from
 253 Kasanda were higher than those from Makululu ($p < 0.05$). Children from Kasanda and
 254 Makululu had similar concentrations of urinary Cd ($p > 0.05$). Similarly, there was no

255 difference in the concentration of Pb-F and Cd-F between boys and girls. However, girls
256 excreted more urinary Pb and Cd than boys ($p < 0.05$), with the difference in Cd-U
257 being substantial considering an estimated increase of 1.26 $\mu\text{g/L}$ against median
258 concentrations of 0.40 $\mu\text{g/L}$ (female) and 0.31 $\mu\text{g/L}$ (male children). Fecal Pb levels in
259 younger children aged between 8 months to 3 years old were slightly higher than levels
260 in children aged 4 - 7 years ($p = 0.05$) but not Cd-F. There were urinary Pb and Cd
261 concentration differences between the younger (8 months to 3 years) and older (4 to 7
262 years) children ($p < 0.05$).

263

264 Table 5.

265 Log-transformed fecal (Pb and Cd) and urine (Pb and Cd) concentration differences
266 (site, age and sex) multiple logistic regression analyses in children from Kasanda and
267 Makululu townships in Kabwe, Zambia.

268

Pb-F (mg/kg)						
Parameter	Estimate	nDF	SS	F Ratio	<i>p</i> value (Prob>F)	
Intercept	1.59	1.00	0.00	0.00	1	
Area{Makululu-Kasanda	-0.10	1.00	1.75	5.89	0.016	
Age	-0.042	1.00	1.14	3.85	0.051	
Sex{M-F}	-0.055	1.00	0.53	1.79	0.183	
Cd-F (mg/kg)						
Parameter	Estimate	nDF	SS	F Ratio	<i>p</i> value (Prob>F)	
Intercept	-0.61	1.00	0.00	0.00	1.00	
Area{Makululu-Kasanda	-0.123	1.00	2.47	16.09	0.0001	
Age	-0.005	1.00	0.01	0.09	0.77	
Sex{M-F}	-0.02	1.00	0.11	0.69	0.41	
Pb-U (µg/L)						
Parameter	Estimate	nDF	SS	F Ratio	<i>p</i> value (Prob>F)	
Intercept	2.05	1.00	0.00	0.00	1.00	
Area{Makululu-Kasanda	-0.10	1.00	1.76	6.53	0.01	
Age	-0.09	1.00	4.90	18.21	0.00003	
Sex{M-F}	-0.13	1.00	2.74	10.18	0.00168	
Cd-U (µg/L)						
Parameter	Estimate	nDF	SS	F Ratio	<i>p</i> value (Prob>F)	
Intercept	-0.22	1.00	0.00	0.00	1.00	
Area{Makululu-Kasanda	-0.043	1.00	0.32	1.16	0.28	
Age	-0.045	1.00	1.28	4.63	0.03	
Sex{M-F}	-0.10	1.00	1.89	6.84	0.01	

269

270 Kasanda ($n = 88$) and Makululu ($n = 94$) townships in the vicinity of the Pb-Zn mining
271 area in Kabwe; Age – children between 8 months – 3 years ($n = 71$) years old vs
272 children between 4 – 7 ($n = 111$) years; Sex – M ($n = 79$) vs F ($n = 103$); *P* values in
273 bold indicate significant ($p < 0.05$); nDF - number if degrees of freedom for a term; SS -
274 Sequential Sum of Squares.

275

276 3.6 *Pb and Cd correlations*

277 Using Pearson correlation analysis, strong positive correlations were observed between
278 Pb and Cd in feces ($r = 0.81$; $p < 0.0001$) and urine ($r = 0.84$; $p < 0.0001$) of children
279 from Kasanda and Makululu. Lead concentrations also showed positive correlations
280 among blood, feces and urine of children from the polluted townships. Cadmium
281 concentrations showed similar positive associations (Table 6).

282 Table 6.

283 Correlations among Pb concentrations in blood (Pb-B), feces (Pb-F) and urine (Pb-U) as
284 well as Cd in blood (Cd-B), feces (Cd-F) and urine (Cd-U) of children from polluted
285 townships in Kabwe, Zambia

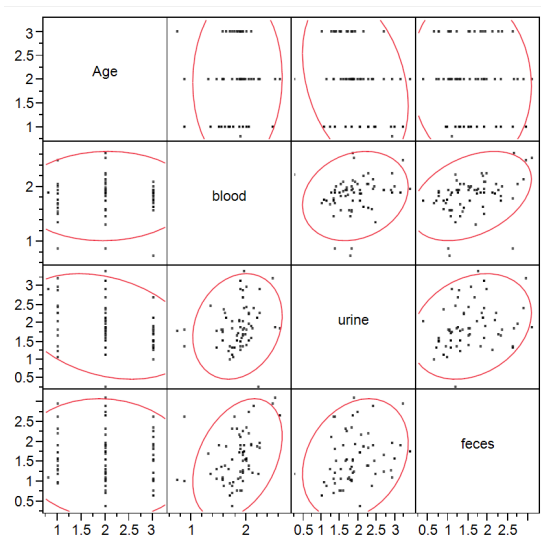
Township	<i>r</i> value	<i>p</i> value
Pb (Kasanda and Makululu)		
Pb-B: Pb-U	0.27	=.0005
Pb-B: Pb-F	0.36	<.0001
Pb-U: Pb-F	0.33	<.0001
Cd (Kasanda and Makululu)		
Cd-B: Cd-U	0.26	=.0005
Cd-B: Cd-F	0.37	<.0001
Cd-U: Cd-F	0.21	=.007

286 *r* = Pearson's correlation coefficient; *n* = 182

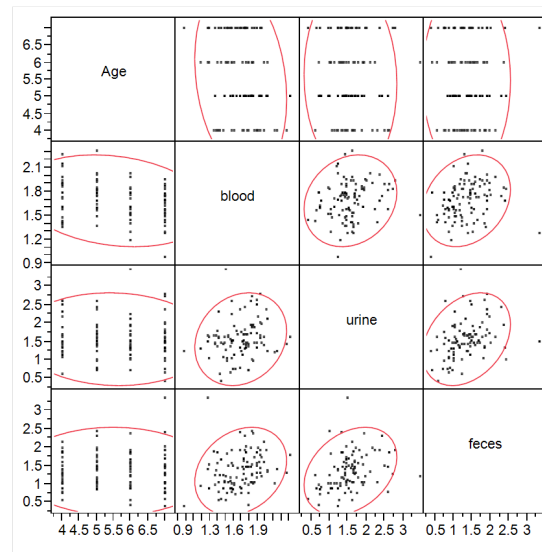
287

288 When presented by age groups of 8 months – 3 years and 4 – 7 years, Pb concentrations
289 had strong positive associations ($p < 0.05$) among blood, feces and urine (Fig. 2).
290 Correlations of Pb in blood, feces and urine with age were negative but not significant
291 ($p > 0.05$).

Pb in younger children (both sites and sexes)



Pb in older children (both sites and sexes)



292

293 Fig. 2.

294 Positive correlations among Pb concentrations in blood, feces and urine of younger
 295 children (8 months – 3 years, ($n = 71$)) and older children (4 – 7 years, ($n = 111$)) from
 296 Kasanda and Makululu townships of Kabwe, Zambia. Strong positive correlations were
 297 also observed between fecal Pb and Cd ($p = 0.81, <.0001$) as well as between urinary Pb
 298 and Cd ($p = 0.83, <.0001$).

299

300 **4. Discussion**

301 The current study has demonstrated Pb and Cd excretion in urine and feces of children
 302 from polluted townships in Zambia’s Kabwe mining town. This highlighted concurrent
 303 toxic metal exposure, especially in children from Kasanda Township, which is closest to
 304 the mine. The study targeted children under the age of 7 years from Kasanda, Makululu
 305 and Chowa townships following preliminary studies where extremely elevated Pb-B

306 levels were revealed in the same children by Yabe et al. (2015). All of the sampled
307 children in the current study showed alarming Pb exposure with geometric means up to
308 35.3 mg/kg (Pb-F) and 67.8 µg/L (Pb-U). The health risk due to Cd was evident as Cd
309 levels up to 4.49 mg/kg (Cd-F), 18.1 µg/L (Cd-U) and 7.70 µg/L (Cd-B) indicated
310 increased exposure. Elevated childhood exposure to Pb could be hazardous as the
311 developing nervous system is sensitive to its neurotoxic effects (Lidsky and Schneider
312 2003; Bellinger, 2004). Although Cd toxicity in children is not clear, low-level exposure
313 in children has been implicated with adverse neurodevelopmental outcomes with
314 increasing evidence of learning disabilities and need for special education (Jiang et al.,
315 1990; Ciesielski et al., 2012). Therefore, the findings in the current study are worrisome
316 as simultaneous exposure to Pb and Cd could have detrimental effects on the
317 neurodevelopment of the exposed children given that neurodevelopmental toxicity is
318 dependent on co-exposure to multiple neurotoxicants (Bellinger, 2008).

319 Biomonitoring methods using fecal and urine metal concentrations may provide
320 alternatives to blood analysis in children from polluted environments (Dos Santos et al.,
321 2018). As such, measurement of fecal and urine metals have been used to estimate the
322 overall magnitude of metal intake and elimination (Iwao, 1977, Kjellstrom et al., 1978
323 and Moon et al., 1999). According to Gwiazda et al. (2005), fecal Pb content reflects an
324 integrated measure of Pb exposure from all sources, including dietary. Although most of
325 the metals in feces represent the unabsorbed fraction of ingested metals, their presence
326 in feces may also reflect their endogenous biliary excretion into feces (Hammond et al.,
327 1980; Gregus and Klaassen, 1986; Gwiazda et al., 2005). Mean (GM) concentrations of
328 Pb-F of 9.32 mg/kg (Makululu) and 35.3 mg/kg (Kasanda) in the current study were

329 extremely high and showed that children from the polluted townships in Kabwe are
330 exposed to high levels of Pb. Similarly, Cd-F concentrations of up to 4.49 mg/kg in the
331 current study could raise health concerns in the children from the polluted townships.
332 Since the living space are important sources of environmental exposure for young
333 children (Hornberg and Pauli, 2007), findings of the current study indicate that the
334 current home environment of the children in Kabwe could be the source of metal
335 exposure. This is because young children spend most of their time at home and ingested
336 metals are expected to be eliminated in the feces probably within 24-48 hours after
337 exposure (Smith, 2013).

338 Although fecal metal measurements may be convenient than urinalysis due to
339 difficulties in collecting urine samples in infants, urinary metal biomonitoring is
340 preferred because absorbed Pb and Cd are excreted primarily in urine (Heitland and
341 Koster, 2006). In contrast to blood, urine is equally easy to collect and non-invasive
342 (Zhang et al., 2016). In the current study, recorded mean (GM) Pb-U of 12.1 µg/L
343 (Makululu) and 67.8 µg/L (Kasanda) with levels up to 2914 µg/L were extremely higher
344 than Pb-U_{SG} of 4.08 µg/L recorded in children between 4 - 10 years from a general
345 population in Korea (Moon et al., 2003). Moreover, Pb-U levels in the current study
346 markedly exceeded concentrations of 0.9 µg/L (adjusted for creatinine) recorded in
347 children in USA (Shao et al., 2017). When compared with records in children from the
348 US National Health and Nutrition Examination Survey (NHANES) of 2013-2014, Pb-U
349 levels in the current study extremely exceeded the 0.22 µg/L in US (CDC - Fourth
350 National Report on Human Exposure to Environmental Chemicals, 2017). These

351 findings reveal high Pb exposure among children in Kabwe, Zambia, and could have
352 serious health implications.

353 Mean (GM) urinary Cd-U_{SG} of 0.19 µg/L (Chowa), 0.35 µg/L (Kasanda) and 0.46
354 µg/L (Makululu) in the current study were lower than the biomonitoring equivalent
355 values of Hays et al. (2008) for urine Cd according to USEPA (1.5 µg/L) and ATSDR
356 (1.2 µg/L). The current findings were however, similar to median Cd-U_{SG}
357 concentrations of 0.23 µg/L (girls) and 0.22 µg/L (boys) in a cross-sectional study
358 among school children in Belgium (Wang et al., 2017). Although Cd-U concentrations
359 in the current study exceeded the mean (GM) urine level of 0.185 µg/L (adjusted for
360 creatinine) set by ATSDR (2012) in unexposed children, they were of low priority for
361 risk assessment follow-up according to the current health-based exposure guidelines
362 (Hays et al., 2008). However, this should be interpreted with caution given that 23
363 percent of the 171 sampled children had urinary Cd concentrations exceeding the urine
364 Cd BE values and could be at risk of nephrotoxicity. The means (GM) in the current
365 study also extremely exceeded US children's Cd-U records of 0.057 µg/L in 2009-2010
366 NHANES (CDC - Fourth National Report on Human Exposure to Environmental
367 Chemicals, 2017). Moreover, 7 percent of the 181 sampled children in the current study
368 had Cd-B concentrations exceeding the BE values for Cd in blood.

369 The difference in Pb-U between the two age groups appeared minimal as the
370 concentration of Pb-U marginally increased by 1.23 µg/L (estimate log value = -0.09) in
371 younger children in relation to the higher median of 66.7 µg/L (younger children) and
372 31.2 µg/L (older children). On the other hand, the Cd-U difference between the two age
373 groups was wide considering an increase estimate of about 1.11 µg/L (estimate log

374 value = -0.045) in relation to the lower median values of 0.38 µg/L (younger children)
375 and 0.31 µg/L (older children). Given that urinary Cd, which reflects body burden
376 increases with age (Hays et al., 2008; Jarup and Akesson, 2009), the higher Cd-U in
377 younger children in the current study could be attributed to behavioural differences as
378 younger children are more exposed to metals due to increased hand-mouth activities.
379 Moreover, the age difference between the two age groups was minimal for the older
380 group to have accumulated more Cd.

381 Since findings in the current study do not imply that urinary Cd reduces with age,
382 regular biomonitoring of the exposed children up to adulthood in Kabwe need to be
383 conducted, particularly pregnant women as Cd from the placenta may impair fetal
384 development including neurodevelopmental impairment (Ciesielski et al., 2012; Kippler
385 et al., 2012; Kippler et al., 2010, Llanos and Ronco, 2009, Salpietro et al., 2002; Zhang
386 et al., 2004). The finding of higher excretion levels of Pb and Cd in the urine of girls
387 compared with boys from both Kasanda and Makululu townships was interesting. More
388 studies need to be conducted to establish gender differences in metal accumulation and
389 excretion in children as the high absorption rate following oral exposures in women is
390 associated with iron deficiency (ATSDR, 2008), which might not be the case in children.

391 Concurrent exposure to Pb and Cd can result in metal interactions, which may be
392 characterized by alterations in both tissue metal concentrations and toxicity (Mahaffey
393 et al., 1981). In the current study, strong positive correlations were seen between Pb and
394 Cd in both feces and urine of the sampled children, thus indicating concurrent exposure
395 to Pb and Cd. This was not surprising as soils from the selected townships are highly
396 polluted with Pb and Cd (Nakayama et al., 2011). Data on correlations between Pb and

397 Cd levels in feces and urine of children from polluted areas in rare. In a study among
398 adults in the general population in Japan, no close correlations between Pb-U and Cd-U
399 were detected (Fukui et al., 2004). Joint toxicity can result in various effects including
400 greater than additive (synergism and potentiation), additive (no interaction) and less
401 than additive (antagonism and inhibition). However, since additivity is the default
402 assumption for evaluating health effects of multiple chemicals, evaluation of the
403 simultaneous effects of Pb and Cd in Kabwe is needed as it is now known that children
404 from the polluted townships are exposed to both Pb and Cd in their current environment.
405 The current study also revealed positive associations of Pb and Cd concentrations
406 among blood, urine and feces. These findings indicate that either urine or feces could be
407 useful for biomonitoring of Pb and Cd in polluted environments.

408 **5. Conclusions**

409 Childhood Pb and Cd co-exposure in Kabwe poses serious implications on the health of
410 the exposed children and should be given attention. A thorough clinical evaluation of Pb
411 and Cd exposure among children in townships surrounding the Pb-Zn mine in Kabwe is
412 long over-due as it has never been done despite alarming metal exposure. Regular fecal
413 and urine biomonitoring should be considered for prompt remedial measures to avoid
414 irreversible Pb-induced neurological dysfunction. Urgent interventions are required to
415 reduce Pb and Cd exposure in the affected townships. This can be done through
416 community-based programs to educate the affected communities about the health effects
417 of Pb and Cd, sources of the metals and practical ways of reducing exposure in their
418 homes and communities.

419

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437

438

439 **Conflict of interest**

440 The authors declare no conflicts of interest.

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