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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Abstract

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

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

1. Introduction

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

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

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

2. Materials and methods

2.1 Sampling sites

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

collected from children at health centers located in Chowa, Kasanda and Makukulu townships, in May-June of 2012. Matched samples were collected from the same children and townships where extremely high levels of Pb-B were reported by Yabe et al. (2015). More details about the study site and township description, which are in the vicinity of the mine can be obtained from the previous study (Yabe et al., 2015).

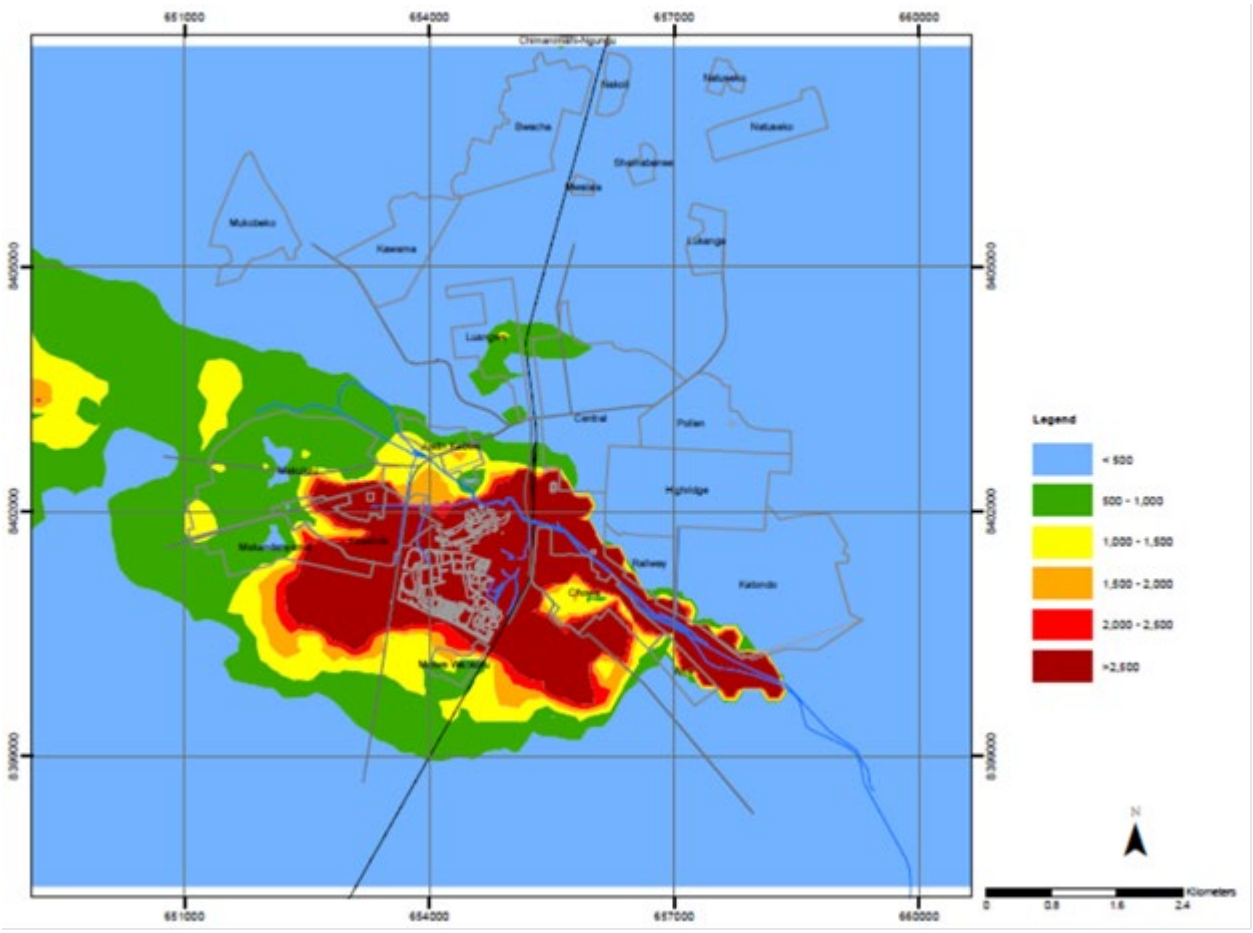

Fig. 1.

Map of Kabwe showing distribution of Pb (mg/kg) in township soils around the Pb-Zn mining complex (Water Management Consultants Ltd, 2006).

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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

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

2.3 Sample preparation and metal extraction

All laboratory materials and instruments used in metal extraction were washed in 2 % nitric acid (HNO_3) and oven dried. The metals were extracted in fecal and urine samples using microwave digestion system (Speedwave MWS-2; Berghof) according to the manufacture's instruction and published reports (Fukui et al., 2004; Yabe et al., 2011). Thawed fecal samples were weighed onto heat-resistant tissue drying plates and dried for 24 h in a tissue drying oven at 60 °C while urine samples were just thawed. Briefly, 1 mL of each urine sample and 50 mg of oven-dried fecal sample were separately placed in prewashed microwave digestion flasks. To these samples, 5 mL of 60 % nitric acid (Kanto Chemical) and 1 mL of 30 % hydrogen peroxide (Kanto Chemical) were added. After digestion in the microwave for 52 minutes and temperatures of up to

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

2.4 Metal analysis

Blood samples for Cd measurements were prepared and analysed as described earlier (Yabe et al., 2015). Fecal and urine metals (Pb and Cd) concentrations were analyzed by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, Japan). The precision and accuracy of the applied analytical method was evaluated by analyzing the recovery rate using digested urine samples and spiking Pb and Cd standard solutions. Using this method, good recoveries of 95 % for both Pb and Cd were obtained. Certified Reference Materials, DORM-3 (Fish protein, National Research Council of Canada, Ottawa, Canada) and DOLT-4 (Dogfish liver, National Research Council of Canada, Ottawa, Canada) were used to evaluate recoveries. Replicate analysis of these reference materials also showed good accuracy (relative standard deviation, RSD, ≤ 3%) and recovery rates ranged from (95-105%). Using the Certified Reference Materials, the detection limits of Cd and Pb were 0.0005 µg/L and 0.0022 µg/L, respectively (Ogbomida et al., 2018). The instrument detection limit (IDL) was 0.001 µg/L. Replicate urine samples were used at 4 different spike concentrations of 0.01 ppb, 0.1 ppb, 1 ppb and 10 ppb. These yielded detection limits of 0.006 µg/L (Cd) and 0.043 µg/L (Pb) as well as recovery rates of 85.5 - 99.7% (Cd) and 104.8 - 107.7% (Pb). To compensate for variations in urine dilution, measured urine-Pb (Pb-U) and urine-Cd (Cd-U) concentrations were adjusted for specific gravity (SG). 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 × (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.

2. Results

3.1 Sample sizes and characteristics

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

Table 1.

Sample sizes and sample characteristics of children from Chowa, Kasanda and 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

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

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

Table 2.

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

Sample	*n*	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

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

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

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

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

Table 3.

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

Sample	*n*	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

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

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

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

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

Table 4.

Comparison between Cd-U and Cd-B concentrations (µg/L) measured in urine and blood samples of children from Chowa, Kasanda and Makululu townships in vicinity of 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 (µg/L)	0.19 µg/L	0.46 µg/L	0.35 µg/L	1.5 µg/L	1.2 µg/L
Cd-B (µg/L)	0.66 µg/L	1.10 µg/L	0.44 µg/L	1.7 µg/L	1.4 µg/L

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

3.5 Site, age and sex differences

Multiple logistic regression analyses were performed on log-transformed data to estimate the influence of independent variables (age as continuous variable, sex represented as 0 for girls and 1 for boys, location (area) represented as 0 for Makululu and 1 for Kasanda) on Pb-F. Similar analyses were done on Cd-F, Pb-U_{SG} and Cd-U_{SG} (Table 5). Fecal Pb and Cd as well as urinary Pb concentrations in children from Kasanda were higher than those from Makululu ($p < 0.05$). Children from Kasanda and 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 µg/L against median
258 concentrations of 0.40 µg/L (female) and 0.31 µg/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	*p* 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	*p* 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	*p* 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	*p* 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**

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

3.6 Pb and Cd correlations

Using Pearson correlation analysis, strong positive correlations were observed between Pb and Cd in feces (r = 0.81; $p < 0.0001$) and urine (r = 0.84; $p < 0.0001$) of children from Kasanda and Makululu. Lead concentrations also showed positive correlations among blood, feces and urine of children from the polluted townships. Cadmium 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	*r* value	*p* 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)		<.0001
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 ($p > 0.05$).

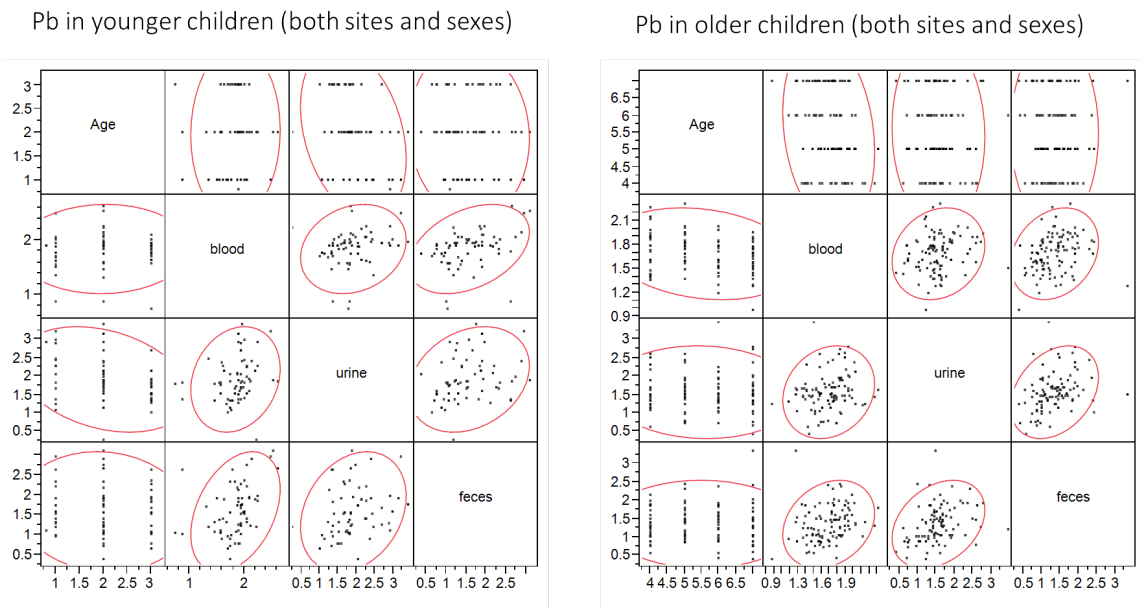

Fig. 2.

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

4. Discussion

The current study has demonstrated Pb and Cd excretion in urine and feces of children from polluted townships in Zambia's Kabwe mining town. This highlighted concurrent toxic metal exposure, especially in children from Kasanda Township, which is closest to the mine. The study targeted children under the age of 7 years from Kasanda, Makululu 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

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

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

The difference in Pb-U between the two age groups appeared minimal as the concentration of Pb-U marginally increased by 1.23 µg/L (estimate log value = -0.09) in younger children in relation to the higher median of 66.7 µg/L (younger children) and 31.2 µg/L (older children). On the other hand, the Cd-U difference between the two age 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

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

5. Conclusions

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

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Conflict of interest

The authors declare no conflicts of interest.

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