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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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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 assessed 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

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

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

Clinical presentations of metal poisoning vary widely depending upon the age at exposure, the amount of exposure and the duration of exposure. Since chronic Pb poisoning in children is asymptomatic and may result in a delay in the appropriate diagnosis, measurement of concentrations in biological samples plays a pivotal role in the diagnosis and management of patients (Lowry, 2010). Currently, Pb concentration in whole blood (Pb-B) is the main biomarker used to monitor exposure and has been widely used in epidemiological studies (CDC, 2012). However, independent of the mode of exposure, absorbed metals such as Pb and Cd are excreted primarily in urine 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).

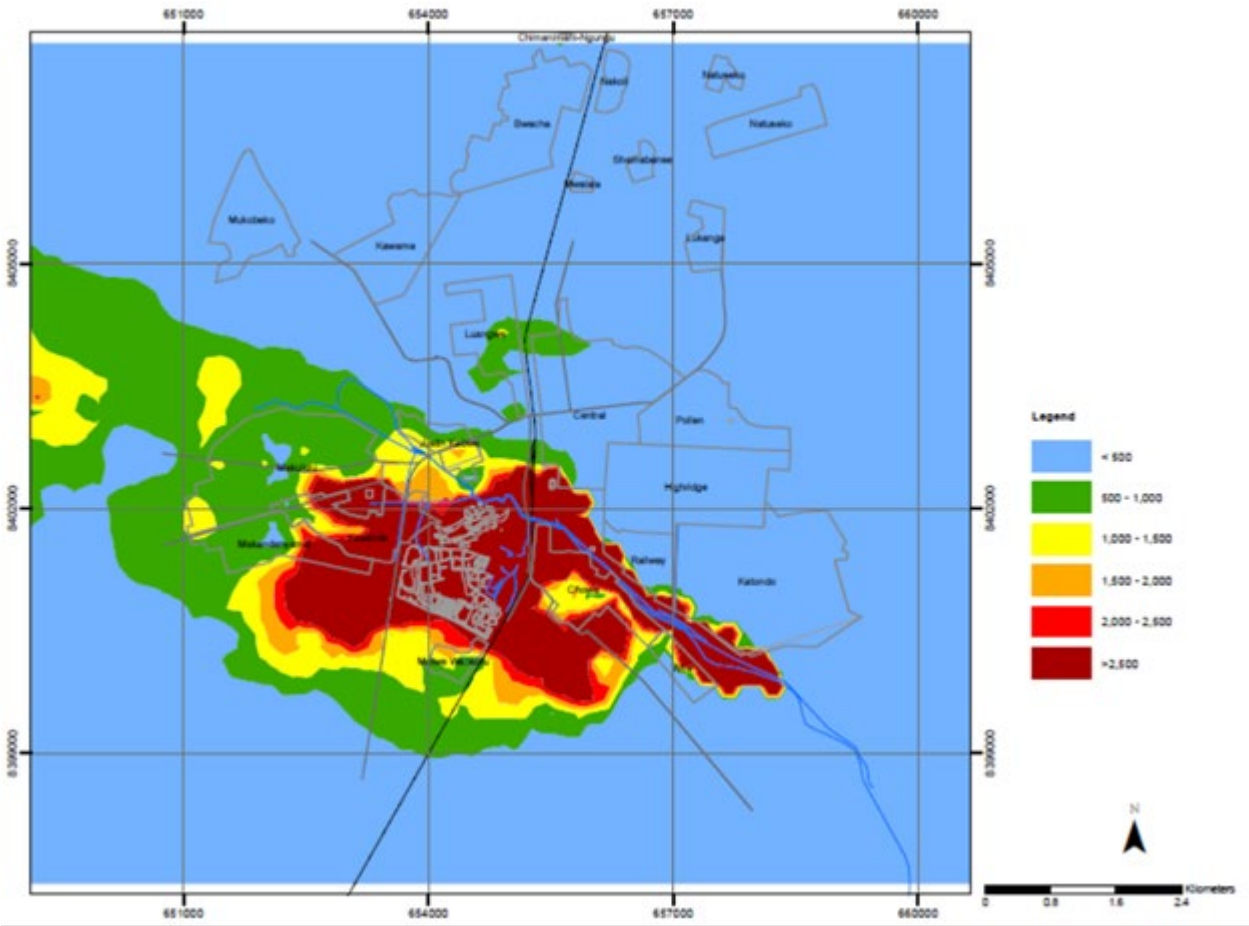


Fig. 1.
Map of Kabwe showing distribution of Pb (mg/kg) in township soils around the Pb-Zn mining complex (Water Management Consultants Ltd, 2006).

2.2 Sample collection

The study was approved by the University of Zambia Research Ethics Committee (UNZAREC) and the Ministry of Health, Zambia. Before sampling commenced, an awareness campaign about the research activities was conducted by community health workers in each township to encourage parents/guardians to take their children under the age of 7 to the selected health centres for sample collection. After informed and written consent was obtained from the children's parents or guardians, paired fecal and urine (morning spot-urine) samples were collected in clean metal-free specimen containers at Chowa, Kasanda and Makululu clinics. Blood samples were collected as described earlier by Yabe et al. (2015). For each child, data on the age, sex, residential area, medical history and past or current metal chelation therapy were recorded. Sample collection and questionnaire administration were done by laboratory technicians and nurses, respectively. In addition to selecting children under the age of 7 years, other inclusion criteria included children that were residing in communities in the vicinity of the Pb-Zn mine. The children must have been born or resided in the selected communities for at least 1 year. Only the children whose parents responded to the awareness campaign and signed the informed consent were selected. Efforts were made to collect urine samples in 50 ml urine containers in the morning of sample collection at the health centres. To avoid sample contamination, all sample collection supplies were kept in plastic ziploc storage bags before sample collection. For fecal samples, 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 (HNO₃) 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, $\leq 3\%$) and recovery rates ranged from (95-105%). Using the Certified Reference Materials, the detection limits of Cd and Pb were 0.0005 $\mu\text{g/L}$ and 0.0022 $\mu\text{g/L}$, respectively (Ogbomida et al., 2018). The instrument detection limit (IDL) was 0.001 $\mu\text{g/L}$. 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 $\mu\text{g/L}$ (Cd) and 0.043 $\mu\text{g/L}$ (Pb) as well as recovery rates of 85.5 - 99.7% (Cd) and 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).

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

2.5 Statistical analysis

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

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	<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

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 Equivalent (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 $\mu\text{g/L}$ 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.

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

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 of 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).

Table 6.

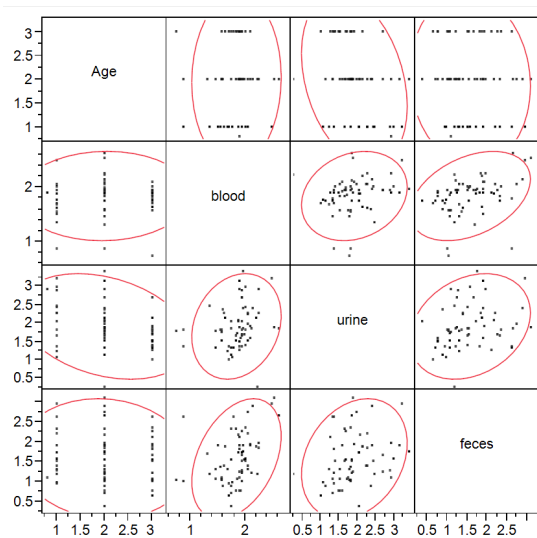
Correlations among Pb concentrations in blood (Pb-B), feces (Pb-F) and urine (Pb-U) as well as Cd in blood (Cd-B), feces (Cd-F) and urine (Cd-U) of children from polluted townships in Kabwe, Zambia

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)		<.0001
Cd-B: Cd-U	0.26	=.0005
Cd-B: Cd-F	0.37	<.0001
Cd-U: Cd-F	0.21	=.007

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

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

Pb in younger children (both sites and sexes)



Pb in older children (both sites and sexes)

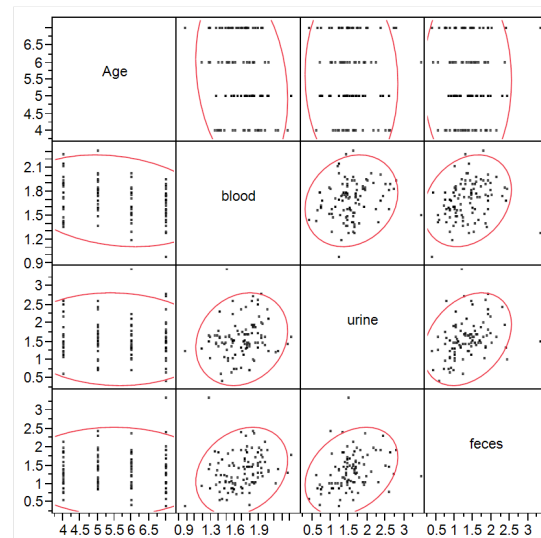


Fig. 2.

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

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

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

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

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

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

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

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