Tanzania Journal of Science 46(2): 190-204, 2020
ISSN 0856-1761, e-ISSN 2507-7961

Variations of Concentrations of Lead, Zinc, Iron, Copper and Cadmium in Urine of Primary School Pupils in Relation to Age, Sex and Academic Performance

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Received 1 February 2020, Revised 4 April 2020, Accepted 4 April 2020, Published June 2020
DOI: https://dx.doi.org/10.4314/tjs.v46i2.1

Abstract
This study was conducted in order to assess the variations and correlations in the concentrations of lead, zinc, iron, copper and cadmium metals in the urine samples of selected primary school pupils with respect to their sex, age and academic performance. Urine samples were collected from 240 pupils in Dar es Salaam, Tanzania and then digested in concentrated acids and analysed using atomic absorption spectrometry (AAS). Lead, zinc, iron and copper were detected in most of the urine samples. Cadmium was not detected in any of the samples. The mean concentrations of lead, zinc, iron and copper in the samples from different classes ranged between 0.27–0.90, 0.59–0.78, 1.56–2.32 and 0.005–0.01 mg/L, respectively in pupils with high academic performance, and 0.37–0.71, 0.56–0.81, 1.79–2.55 and 0.005–0.01 mg/L, respectively in pupils with low performance. The overall mean concentrations of the metals ranged between 0.01–2.04 and 0.01–2.17 mg/L in males and females, respectively. There were no significant differences in most of the concentrations of the heavy metals in pupils' urine samples between the two sexes. The findings indicated some significant positive correlations between the pupils' age and the concentrations of lead and iron in urine samples, while there were no significant correlations for zinc and copper. Some of the concentrations of the heavy metals showed significant negative or positive correlations with the academic performance of the pupils. Therefore, the concentrations of the heavy metals were correlated with age, sex and academic performance in some samples. The concentrations of lead were generally alarming.

Keywords: Heavy metals; children urine; age; sex; academic performance; Tanzania.

Introduction
Heavy metals in water, sediments, air, and other environmental media are of great concern because of their potential long-term effects on human health particularly in developing countries where remedial techniques are very limited (Wilson and Pyatt 2007). The metals enter the environment by both natural means such as weathering of the earth's crust, soil erosion, and anthropogenic means such as mining, industrial discharge, urban runoff, sewage effluents, pest or disease control agents containing metals applied to plants and animals, air pollution fallout and a number of others (Ming-Ho 2005). Heavy metal poisoning can lead to neurological diseases, depression, aggravate conditions such as hyperthyroidism, memory loss, mental retardation in children, poor coordination, muscle pain, anorexia, constipation, vomiting, anaemia, kidney and liver damage, and cancer (Zheng et al. 2007, Jaishankar et al. 2014). Metal toxicity depends upon the absorbed dose, the route of exposure and duration of

exposure, i.e., acute or chronic (Jaishankar et al. 2014).

Children are vulnerable to the health effects associated with elevated metals exposure and can easily be affected by heavy metals because their body systems are still developing (Olsen 2000). In addition, they are proportionally more exposed to toxic chemicals because they drink more water, have higher breathing rates and eat more food per body weight unit compared to adults (Schwartz 2004). Bateson and Schwartz summarised that this disparity arises from physiological, metabolic and behavioural differences between the two populations. Children, while playing, experience rapid and deep breathing, which not only increases the inhaled doses of airborne pollutants but also increases deposition to the lungs. This increased dose is compounded by the slower rate of particle clearance from the lungs (Ginsberg et al. 2005). Children may acquire heavy metals from the normal hands-to-mouth activities with contaminated soil or by actually eating objects and it is a common habit for some children in schools to eat food without washing their hands, especially in areas where there is shortage of water. Depending upon the individual susceptibility of the child based upon age, general health and social supports, the exposure may cause harm ranging from subtle changes in function to death. For example, exposure to high levels of lead is known to affect brain development in children resulting in reduced intelligence quotients (IQ), behavioural changes such as reduced attention span and increased antisocial behaviour, and reduced educational attainment (WHO 2017).

Urine can be used as an indicator of the heavy metal burdens in the body. The advantages in the utilization of urine for biomonitoring include the following: urine is a relatively less invasive sample, relatively stable matrix under certain conditions, easy to collect and can be used in short and long-term investigations of exposure tracings. Urine samples are excellent for showing current exposures as they reflect the levels of heavy metals in the bloodstream during the hours immediately before urination (Adotey et al. 2011).

Many public primary schools in Tanzania, especially in urban and peri-urban areas, are located in the proximity to industrial areas and major roads. A substantial fraction of pupils at public primary schools in Tanzania, particularly pupils attending schools in low income neighbourhoods, may be exposed to elevated levels of air pollution, soil pollution and water pollution (including heavy metals) while at school, in addition to the exposure from their residential areas and other settings. Most public primary schools in Tanzania have no hand washing facilities, no water for hand washing and no reliable water supply (MoEVT 2012).

As a result, the locations and circumstances of the schools may negatively impact the healthy development and academic performance of a large number of school children depending on their age, social behaviour and other factors. The aim of the present study was to determine the relationships between the concentrations of lead, zinc, iron, copper and cadmium in the urine samples with the age, sex and academic performance of the pupils. This is the first study conducted on this subject in Tanzania.

Materials and Methods

Study areas and sampling

The study was conducted at four selected primary schools located in three districts (Temeke, Ilala and Kinondoni Districts) in Dar es Salaam city, Tanzania in April to September 2016. The pupils involved in this study were selected from the following primary schools: Bunju A (Kinondoni), Bunju B (Kinondoni), Chang'ombe (Temeke) and Mtakuja (Ilala). The schools were designated as school 1, school 2, school 3 and school 4, respectively. Schools 1 and 2 were located away from industrial areas, while schools 3 and 4 were located close to industrial areas. There are major and small roads close to these schools. The locations of the study areas (schools) are shown in Figure 1. The pupils attending the schools involved in this study were mostly from low income and mid income families and they lived in nearby areas to the schools. Most of the schools had no reliable water supply. In order to obtain representative samples, the research considered the distribution of sex, age and academic performance categories within each school. Participants were obtained from six classes (class 1/standard 1 to class 6/standard 6) and in each class, 10 pupils were selected based on sex, age and academic performance. Each school provided a total of 60 participants, giving a total sample size of 240 pupils. Information about age and academic performance of the pupils were collected from the records provided by the school administration and academic offices. Academic performance was based on average scores in the examinations in all the studied subjects for the whole schooling duration. High achiever pupils were defined as those with average marks of 60% and above in the examinations, while low achiever pupils were those with average marks of below 60% in the examinations. Table 1 summarises the distribution of the samples of school children who participated in this study based on their age, academic performance and sex. Children aged 5 to 14 years were involved in the study. Among the pupils involved in the study, 129 (53.7%) were males while 111 (46.3%) were females. Among the participants, 127 (52.9%) were pupils with high academic performance (HA) while 113 (47.1%) were with low academic performance (LA). A spot morning urine sample was collected from each participant in a 60-mL sterile polyethylene bottle. The volume of each sample was recorded at the time of sampling. The samples were packed into a plastic container and immediately transferred to the laboratory and prepared for analysis.

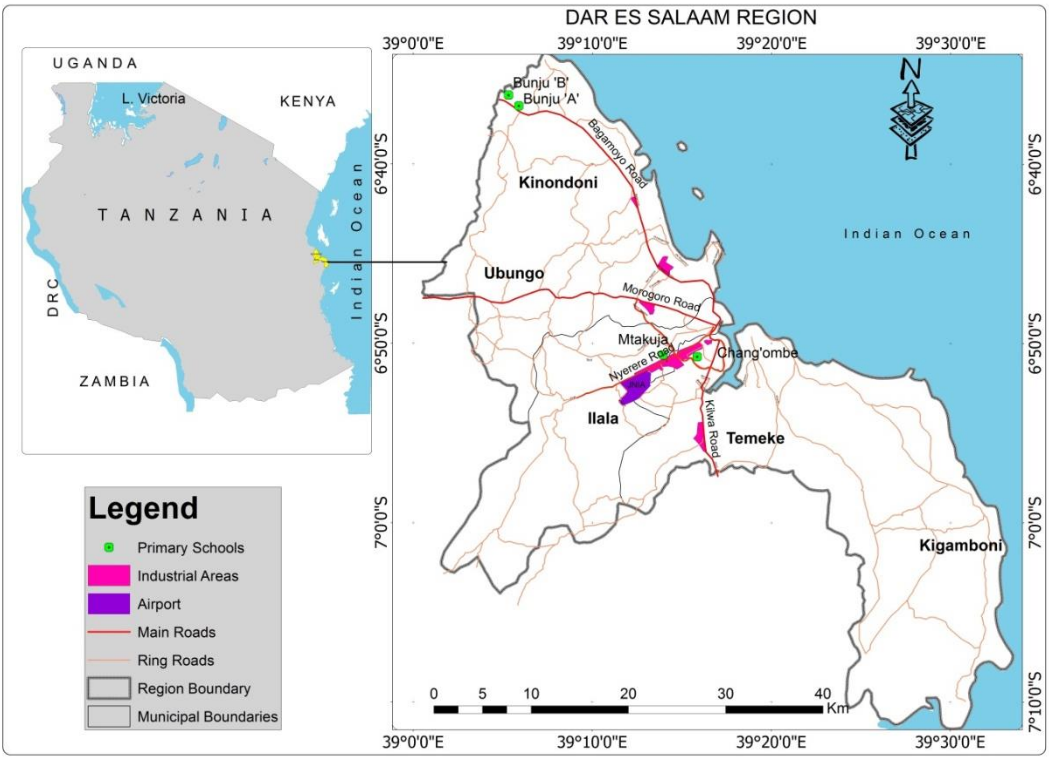

Figure 1: Map showing the school locations, roads and industrial zones.

Table 1: Numbers of pupils selected with respect to age, sex and academic performance

Sampling site	Age (years)										Sex, Academic performance				
											Males		Females		
	5	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*HA*	*LA*	*HA*	*LA*	*Total*
School 1	0	3	7	8	7	11	9	12	3	0	21	9	10	20	*60*
School 2	0	0	4	7	17	10	7	10	5	0	12	16	19	13	*60*
School 3	0	0	13	9	10	8	11	6	3	0	25	13	9	13	*60*
School 4	1	4	5	7	6	12	12	11	1	1	16	17	15	12	*60*
Total	*1*	*7*	*29*	*31*	*40*	*41*	*39*	*39*	*12*	*1*	*74*	*55*	*53*	*58*	*240*

HA = High academic performance; LA = Low academic performance.

Ethical issues

Ethical approvals for the study were obtained from the Ethics Commission of the National Institute for Medical Research (NIMR) of Tanzania and the Research Ethics Committee of the University of Dar es Salaam. The participants and their teachers/parents were given informed consent before the sampling commenced. The participants were informed about their right to decline from participating in the study if they wished. Selection of qualified pupils was purely based on the willingness of the particular pupil to participate in the study. Participating pupils were given unique number codes to ensure confidentiality, and no names appeared on the sample labels and forms.

Laboratory sample storage and treatment

The samples were kept refrigerated at –18 °C for not more than 2 days prior to processing. Refrigeration storage of urine samples without the use of chemical additives is an effective preservation method for analysis of many trace metals (Bornhorst 2005). The treatment procedures described by Adotey et al. (2011) were adopted with minor modifications, and during the treatments, 20 mL of each urine sample were placed into a 150-mL beaker, concentrated nitric acid (10 mL, 70% analytical grade, Fisher Scientific, UK) was added, then covered with a watch glass and heated on a hot plate for 30 minutes. The mixture was allowed to cool to room temperature, then, concentrated nitric acid (10 mL) was added and heated until the mixture was evaporated to about 5 mL and then allowed to cool. Distilled water (2 mL) and 10 mL of hydrogen peroxide (30%, certified ACS grade, Fisher Scientific, UK) were added; the beaker was covered with a watch glass and heated on a hot plate until the effervescence became minimal. The mixture was then allowed to cool and thereafter 10 mL of concentrated HCl (39%, ACS reagent grade, Rochelle Chemicals-South Africa) and 10 mL of distilled water were added and heated until the volume was reduced to about 5 mL. The samples were finally diluted to 20 mL with distilled water after cooling.

Analysis of heavy metals

The concentrations of the heavy metals in urine samples were determined using an atomic absorption spectrophotometer (Thermo Scientific Model iCE 3000) at the Chemistry Department, University of Dar es Salaam. Quantitative analysis was achieved by measuring the absorbances of analytes in standards and sample solutions. Absorbances of lead, cadmium, copper, iron and zinc were measured at energy wavelengths of 283.3 nm, 228.8 nm, 324.7 nm, 248.3 nm and 213.9 nm, respectively (Zolotov and Kuzmin 1990). Standard solutions were prepared by dissolving each of the pure metals in concentrated nitric acid and diluting them to obtain series of concentrations ranging from 0.01 to 2 mg/L for zinc and cadmium solutions, 0.01–15 mg/L for copper and iron solutions and 0.05 to 20 mg/L for lead solution. A series of calibration standards were analysed at the beginning of each sample batch and after 10 to 15 samples. The linear calibration curves were used for the calculation of the concentrations of the metals. The result of each metal was corrected by subtracting the blank value.

Analytical quality assurance

The glassware and sampling bottles were thoroughly washed using detergents and rinsed with clean and distilled water before and after use. Four blank samples were prepared for each batch using distilled water samples (20 mL each) to check for background contamination caused by the reagents used. Recovery tests for checking accuracy and precision involved spiking standard solutions in distilled water samples (20 mL each). The distilled water was tested before use and no traces of the heavy metals were found. The blanks and recovery samples were treated/processed using the same procedures used for the urine samples. The recovery tests were performed in replicates for each of the metals determined. The limit of detection (LOD) for each metal was calculated as the concentration equivalent to three times the standard deviation of the procedural (reagent) blanks. The mean recoveries of lead, cadmium, zinc, copper and iron were 109%, 95%, 99%, 101% and 99.8% with precision of relative standard deviations (%) of 1.8, 4.7, 0.8, 2.1 and 0.95, respectively. The detection limits were 0.001 mg/L for lead, cadmium and zinc, 0.002 mg/L for copper and 0.005 mg/L for iron.

Data analysis

The GraphPad Instat software (GraphPad Software, Inc., San Diego, California USA) was used for the data analysis. Comparison of the heavy metal concentrations in urine samples between males and females was done using an independent samples *t*-test. The differences in the concentrations of the metals in the samples among the schools were checked using One-way ANOVA with Tukey post-test. Pearson correlation coefficients were computed to check for the relationships between the concentrations of the metals in urine and the age and academic performance of the pupils at 95% confidence level (α = 0.05).

Results and Discussion

Distribution of the concentrations of heavy metals in urine samples according to the academic performance and sex of pupils

Lead, zinc, iron and copper were detected in the urine samples while cadmium was not detected in any of the urine samples. The distributions of the concentrations of the heavy metals in the urine samples with respect to the academic performance and sexes of the children are summarised in Table 2 and Table 3. The mean concentrations of lead, zinc, iron and copper in the urine samples of pupils from different classes ranged 0.27–0.90, 0.59–0.78, 1.56–2.32 and 0.005–0.01 mg/L, respectively in pupils with high academic performance (HA), and 0.37–0.71, 0.56–0.81, 1.79–2.55 and 0.005–0.01 mg/L, respectively in pupils with low academic performance (LA). The maximum concentrations of lead, zinc, iron and copper in urine samples were 1.92, 2.55, 5.40 and 0.05 mg/L in HA, and 1.51, 2.38, 9.00 and 0.04 mg/L in LA, respectively. The mean concentrations of lead, zinc, iron and copper in the urine samples of pupils from different schools ranged 0.15–0.87, 0.54–0.94, 1.22–2.98 and ND–0.03 mg/L, respectively in males while in females they ranged 0.14–0.76, 0.57–0.92, 1.32–3.03 and ND–0.03 mg/L, respectively. The overall mean concentrations of lead, zinc, iron and copper in the samples (mg/L) were 0.60, 0.66, 2.04 and 0.01, respectively in males and 0.50, 0.73, 2.17 and 0.01, respectively in females. The maximum concentrations of lead, zinc, iron and copper in urine samples varied from 0.05 to 5.40 mg/L in males and 0.05 to 9.00 mg/L in females.

Table 2: Distribution of concentrations of the heavy metals detected in urine according to academic performance of pupils

Classes	Academic performance		Concentrations of heavy metals (mg/L)				
	Category, sample size (n)	Marks (%) Range, Mean	Parameter	Lead	Zinc	Iron	Copper
Std 1	HA (n = 21)	60–100	Range	ND–0.93	0.20–1.21	0.88–3.17	ND–0.04
		89.7	Mean, Median	0.27, 0.11	0.60, 0.56	1.56, 1.27	0.01, ND
	LA (n = 19)	20–59	Range	ND–1.08	0.27–1.44	0.93–3.75	ND–0.04
		45.3	Mean, Median	0.37, 0.32	0.73, 0.69	2.03, 2.01	0.01, ND
Std 2	HA (n = 22)	60–100	Range	ND–0.91	ND–1.80	ND–3.40	ND–0.04
		89.2	Mean, Median	0.46, 0.62	0.70, 0.63	2.10, 2.33	0.01, ND
	LA (n = 18)	5.0–59	Range	ND–0.87	0.31–1.47	0.80–3.24	ND–0.03
		28.2	Mean, Median	0.44, 0.44	0.81, 0.75	2.03, 1.96	0.005, ND
Std 3	HA (n = 20)	70–100	Range	ND–1.92	0.28–1.47	0.84–3.23	ND–0.04
		91.0	Mean, Median	0.51, 0.48	0.72, 0.69	2.05, 2.08	0.01, ND
	LA (n = 20)	3.0–54	Range	ND–0.98	0.39–1.82	1.19–4.17	ND–0.04
		21.9	Mean, Median	0.56, 0.57	0.80, 0.61	2.41, 2.66	0.01, ND
Std 4	HA (n = 20)	65–94.1	Range	ND–1.33	ND–1.65	1.28–3.21	ND–0.05
		83.8	Mean, Median	0.51, 0.45	0.78, 0.77	2.02, 1.80	0.011, ND
	LA (n = 20)	8.4–54	Range	ND–1.17	0.07–2.38	0.56–3.81	ND–0.042
		28.2	Mean, Median	0.57, 0.58	0.74, 0.48	1.79, 1.60	0.008, ND
Std 5	HA (n = 23)	61.5–90.8	Range	ND–1.42	0.30–0.94	0.55–5.40	ND–0.05
		81.7	Mean, Median	0.68, 0.47	0.59, 0.56	2.23, 2.06	0.007, ND
	LA (n = 17)	6.8–55.8	Range	ND–1.51	0.31–1.21	1.18–3.07	ND–0.04
		33.6	Mean, Median	0.66, 0.51	0.60, 0.56	2.12, 2.05	0.008, ND
Std 6	HA (n = 21)	68–86.4	Range	ND–1.67	ND–2.55	0.57–4.56	ND–0.043
		77.6	Mean, Median	0.90, 1.23	0.68, 0.41	2.32, 2.25	0.005, ND
	LA (n = 19)	13.1–50.8	Range	ND–1.50	0.24–1.00	0.37–9.00	ND–0.041
		27.7	Mean, Median	0.71, 0.56	0.56, 0.60	2.55, 2.37	0.01, ND
All	HA (n = 127)	60–100	Range	ND–1.92	ND–2.55	ND–5.40	ND–0.05
		85.4	Mean, Median	0.56, 0.47	0.68, 0.60	2.05, 1.98	0.01, ND
	LA (n = 113)	3.0–59	Range	ND–1.51	ND–2.38	ND–9.00	ND–0.04
		30.7	Mean, Median	0.55, 0.51	0.71, 0.61	2.16, 2.04	0.01, ND
Detection frequency (%)				78.8	98.8	99.6	22.9

Std = standard; HA = High academic performance; LA = Low academic performance; ND = not detected (below detection limit); Range = minimum–maximum (min–max).

Table 3: Distribution of concentrations of the heavy metals detected in urine according to the sexes of the pupils

Sites / Schools	Sex of pupils, sample size (n)	Concentrations of heavy metals (mg/L)				
		Parameter	Lead	Zinc	Iron	Copper
School 1	Male (n = 30)	Range	ND–1.67	ND–2.38	2.03–5.40	ND
		Mean, Median	0.81, 0.88	0.94, 0.89	2.98, 2.86	ND
	Female (n = 30)	Range	ND–1.92	0.28–2.55	0.84–9.00	ND
		Mean, Median	0.76, 0.83	0.92, 0.79	3.03, 2.93	ND
School 2	Male (n = 28)	Range	ND–0.63	0.07–1.80	0.56–3.64	ND
		Mean, Median	0.15, 0.04	0.63, 0.56	1.71, 1.70	ND
	Female (n = 32)	Range	ND–0.68	0.24–1.82	0.89–4.01	ND
		Mean, Median	0.14, ND	0.81, 0.67	2.05, 1.92	ND
School 3	Male (n = 38)	Range	ND–1.51	ND–1.05	1.20–3.05	ND–0.05
		Mean, Median	0.87, 0.89	0.56, 0.56	2.25, 2.29	0.03, 0.04
	Female (n = 22)	Range	ND–1.43	0.20–1.32	1.27–3.23	ND–0.05
		Mean, Median	0.70, 0.78	0.57, 0.49	2.19, 2.19	0.03, 0.04
School 4	Male (n = 33)	Range	ND–0.83	ND–0.96	ND–2.08	ND
		Mean, Median	0.47, 0.47	0.54, 0.50	1.22, 1.29	ND
	Female (n = 27)	Range	0.29–0.79	0.30–1.68	0.55–2.05	ND
		Mean, Median	0.49, 0.47	0.57, 0.49	1.32, 1.42	ND
All	Male (n = 129)	Range	ND–1.67	ND–2.38	ND–5.40	ND–0.05
		Mean, Median	0.60, 0.56	0.66, 0.60	2.04, 2.03	0.01, ND
	Female (n = 111)	Range	ND–1.92	ND–2.55	ND–9.00	ND–0.05
		Mean, Median	0.50, 0.44	0.73, 0.62	2.17, 2.02	0.01, ND

Statistical analysis using One-way ANOVA with Tukey post test showed that there were significant differences in the concentrations of the metals in the samples among the schools as summarised here; lead: School 3 = school 1 > school 4 > school 2 ($F_{(3, 239)} = 42.744$, $p < 0.0001$); zinc: School 1 > school 2 = school 3 = school 4 ($F_{(3, 239)} = 15.406$, $p < 0.0001$); iron: School 1 > school 3 = school 2 > school 4 ($F_{(3, 239)} = 58.029$, $p < 0.0001$). Copper was only found in samples from school 3. The mean concentrations of the metals detected in the samples were in the order iron > zinc > lead > copper. The highest concentrations of the metals were generally found in the samples from school 1 and school 3 and this could partly be related to the locations of these schools, i.e., close to major roads (both schools) and close to industrial areas (school 3). The probable sources of the metal contaminants in urine samples from the children are due to exposure through contaminated food, water, soil, air, dust and various objects. The industries, major roads and poor sanitation (unhygienic) conditions could be among the major factors contributing to the exposure of the children to the heavy metals (Mahugija et al. 2018).

The concentrations of lead in the urine samples from most of the pupils (78.8%) were alarming as they generally exceeded the reference values in children (0–0.004 mg/L). Urinary excretion of lead of < 0.004 mg/L is not associated with any significant lead exposure. Urinary excretion of lead of > 0.004 mg/L is usually associated with pallor, anaemia, and other evidence of lead toxicity (Paschal et al. 1998, De Burbure et al. 2006, Kosnett et al. 2007). The concentrations of zinc and iron varied from low to moderately high but no reference values or permissible levels were established for their concentrations in urine in children. The concentrations of copper were generally low and could not be expected to be related to health concerns in the children.

Correlations and comparison of concentrations of the heavy metals in urine with age, academic performance and sex of the pupils

The correlations and comparison of concentrations of the heavy metals (lead, zinc, iron and copper) in urine samples with age, academic performance and sex of the pupils are summarised in Tables 4, 5 and 6.

Table 4: Correlations of the concentrations of the heavy metals with age of pupils (N = 60 for each school and N = 240 for all schools; degrees of freedom = 58 and 238, respectively)

Site/Samples/ Age	Lead		Zinc		Iron		Copper	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
School 1	**0.6336***	**< 0.0001***	0.0048	0.9712	0.1703	0.1934	NA	NA
School 2	–0.0934	0.4779	–0.1850	0.1570	**0.5383***	**< 0.0001***	NA	NA
School 3	**0.6439***	**< 0.0001***	–0.1128	0.3910	0.03422	0.7952	–0.2465	0.0576
School 4	**–0.3188***	**0.0130***	–0.05823	0.6585	–0.1931	0.1394	NA	NA
All schools	**0.2577***	**< 0.0001***	–0.0548	0.3980	0.1234	0.0562	NA	NA

* = Significant correlation, *r* = correlation coefficient, *p* = significance level, NA = Not applicable

Table 5: Correlations of the concentrations of the heavy metals with pupils' academic performance (n = 10 in each class per school)

Classes	Performance	Lead		Zinc		Iron		Copper	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Std 1	School 1	–0.2350	0.5134	–0.09708	0.7896	–0.4218	0.2247	NA	NA
	School 2	–0.05901	0.8714	**–0.7121***	**0.0209***	–0.3998	0.2523	NA	NA
	School 3	–0.4412	0.2018	–0.4348	0.2092	**–0.6823***	**0.0297***	0.2922	0.4127
	School 4	–0.5536	0.0969	0.2434	0.4980	–0.6196	0.0561	NA	NA
	All schools	–0.1369	0.3996	**–0.3427***	**0.0304**	**–0.3698***	0.0189*	0.2185	0.1757
Std 2	School 1	–0.01607	0.9649	0.2709	0.4491	–0.1417	0.6961	NA	NA
	School 2	0.1671	0.6444	–0.3096	0.3840	–0.05837	0.8728	NA	NA
	School 3	0.1793	0.6201	–0.2643	0.4606	0.05435	0.8815	0.5633	0.0899
	School 4	–0.2322	0.5185	–0.3009	0.3983	–0.07680	0.8330	NA	NA
	All schools	0.02809	0.8634	–0.2514	0.1176	0.07271	0.6557	0.3101	0.0515
Std 3	School 1	0.1652	0.6483	0.03761	0.9178	–0.08517	0.8150	NA	NA
	School 2	–0.1513	0.6765	–0.4045	0.2463	–0.4999	0.1412	NA	NA
	School 3	–0.2292	0.5242	0.2478	0.4900	–0.2085	0.5633	–0.4833	0.1570
	School 4	–0.2022	0.5754	0.2961	0.4062	0.2159	0.5492	NA	NA
	All schools	–0.03088	0.8500	–0.06219	0.7030	–0.09356	0.5658	–0.07383	0.6507
Std 4	School 1	0.07152	0.8443	–0.4075	0.2425	–0.2150	0.5508	NA	NA
	School 2	0.3369	0.3411	**0.8394***	**0.0024***	**0.7143***	**0.0203***	NA	NA
	School 3	**–0.6741***	**0.0326***	0.2402	0.5039	0.3831	0.2745	0.4222	0.2242
	School 4	–0.2786	0.4358	–0.4159	0.2320	–0.2387	0.5066	NA	NA
	All schools	0.06750	0.6790	0.1424	0.3806	0.1926	0.2339	0.2767	0.0839
Std 5	School 1	–0.6182	0.0568	0.1088	0.7648	–0.2353	0.5129	NA	NA
	School 2	0.02966	0.9352	–0.1222	0.7367	0.1493	0.6805	NA	NA
	School 3	0.2307	0.5213	–0.2299	0.5229	0.02850	0.9377	0.2641	0.4609
	School 4	**–0.6904***	**0.0271***	0.07553	0.8357	–0.6096	0.0613	NA	NA
	All schools	0.1756	0.2785	–0.09869	0.5446	–0.05744	0.7248	0.2046	0.2053
Std 6	School 1	**0.8239***	**0.0034***	0.4785	0.1618	–0.1186	0.7442	NA	NA
	School 2	0.5767	0.0809	–0.05238	0.8857	–0.6190	0.0564	NA	NA
	School 3	0.1566	0.6657	–0.4960	0.1449	–0.04947	0.8921	–0.4075	0.2425
	School 4	–0.02435	0.9468	–0.4104	0.2388	0.04596	0.8997	NA	NA
	All schools	0.3085	0.0528	0.08083	0.6200	–0.03245	0.8424	0.06874	0.6734
All	All schools	0.03899	0.5478	–0.06087	0.3477	–0.06411	0.3226	NA	NA

***** = Significant correlation, *r* = correlation coefficient, *p* = significance level, NA = Not applicable, std = standard.

Table 6: Comparisons of the concentrations of the heavy metals between males and females (n = 10 in each class per school, n = 60 per school)

Site/ Schools	Classes	Lead		Zinc		Iron		Copper	
		t	*p*	*t*	*p*	*t*	*p*	*t*	*p*
School 1	Std 1	0.7700	0.4634	0.4078	0.6941	1.621	0.1438	NA	NA
	Std 2	1.829	0.1047	0.02011	0.9845	1.127	0.2923	NA	NA
	Std 3	0.1014	0.9217	**2.771***	**0.0243***	1.030	0.3330	NA	NA
	Std 4	0.8494	0.4204	0.4159	0.6884	1.981	0.0829	NA	NA
	Std 5	2.187	0.0602	0.8902	0.3993	0.9449	0.3724	NA	NA
	Std 6	1.915	0.0918	1.133	0.2899	0.6053	0.5618	NA	NA
	All (1-6)	0.3789	0.7061	0.1642	0.8702	0.1905	0.8496	NA	NA
School 2	Std 1	0.8502	0.4199	1.469	0.1800	1.616	0.1448	NA	NA
	Std 2	0.9406	0.3744	1.600	0.1483	0.04018	0.9689	NA	NA
	Std 3	0.8069	0.4430	0.03511	0.9729	0.7877	0.4536	NA	NA
	Stf 4	NA	NA	**2.336***	**0.0477***	2.015	0.0787	NA	NA
	Std 5	0.1138	0.9122	1.058	0.3212	1.876	0.0976	NA	NA
	Std 6	0.3100	0.7645	0.2233	0.8289	1.088	0.3081	NA	NA
	All (1-6)	0.3045	0.7618	1.591	0.1171	1.624	0.1098	NA	NA
School 3	Std 1	0.5301	0.6105	0.7871	0.4539	0.4237	0.6829	1.019	0.3379
	Std 2	0.5604	0.5905	0.3251	0.7534	2.226	0.0567	0.7303	0.4860
	Std 3	0.3233	0.7548	1.172	0.2748	0.5059	0.6265	0.7460	0.4770
	Std 4	0.6446	0.5372	1.090	0.3076	0.8130	0.4397	0.5314	0.6096
	Std 5	1.203	0.2635	0.6917	0.5087	0.4496	0.6649	1.215	0.2589
	Std 6	0.8432	0.4236	0.1388	0.8931	0.5625	0.5892	0.8557	0.4171
	All (1-6)	1.412	0.1633	0.2449	0.8074	0.4792	0.6336	0.5244	0.6020
School 4	Std 1	0.1963	0.8493	0.5056	0.6272	0.4695	0.6513	NA	NA
	Std 2	0.04805	0.9629	0.7570	0.4708	0.5489	0.5981	NA	NA
	Std 3	1.317	0.2243	0.3846	0.7105	0.7131	0.4961	NA	NA
	Std 4	0.5027	0.6287	1.214	0.2595	0.8538	0.4181	NA	NA
	Std 5	0.03639	0.9719	**2.683***	**0.0278***	0.7457	0.4772	NA	NA
	Std 6	0.4054	0.6958	0.3164	0.7598	0.1034	0.9202	NA	NA
	All (1-6)	0.5546	0.5813	0.4680	0.6415	0.9841	0.3291	NA	NA
All	All stds	1.644	0.1015	1.573	0.1171	1.015	0.3110	NA	NA

* = Significant difference, *t* = *t*-value, *p* = significance level, NA = Not applicable, std = standard = class.

Correlations of concentrations of the heavy metals in urine and age of pupils

The correlations between the concentrations of the heavy metals (lead, iron, zinc and copper) in urine and the age of the pupils are shown in Figure 2. There were significant positive correlations between the concentrations of lead in urine and the age of the pupils from school 1 (Bunju A) and school 3 (Chang'ombe) (r = 0.6336 to 0.6439, p < 0.0001) and there was an overall significant positive linear correlation between the concentrations of lead in the urine samples and the age of the pupils (r = 0.2577, p = 0.0001). This implied that the concentrations of lead in the urine samples significantly increased with increase in the age of the pupils and this might be due to metal accumulations within their bodies. These findings are similar to the findings of Yuan et al. (2013) in Taiwan who found that the concentrations of lead in urine samples were positively correlated with age in subjects aged above 35 years. A significant negative correlation was found between the concentrations of lead and the age of the pupils from school 4 (r = –0.3188, p = 0.0130) suggesting that pupils of low age were more exposed to lead than those of high age.

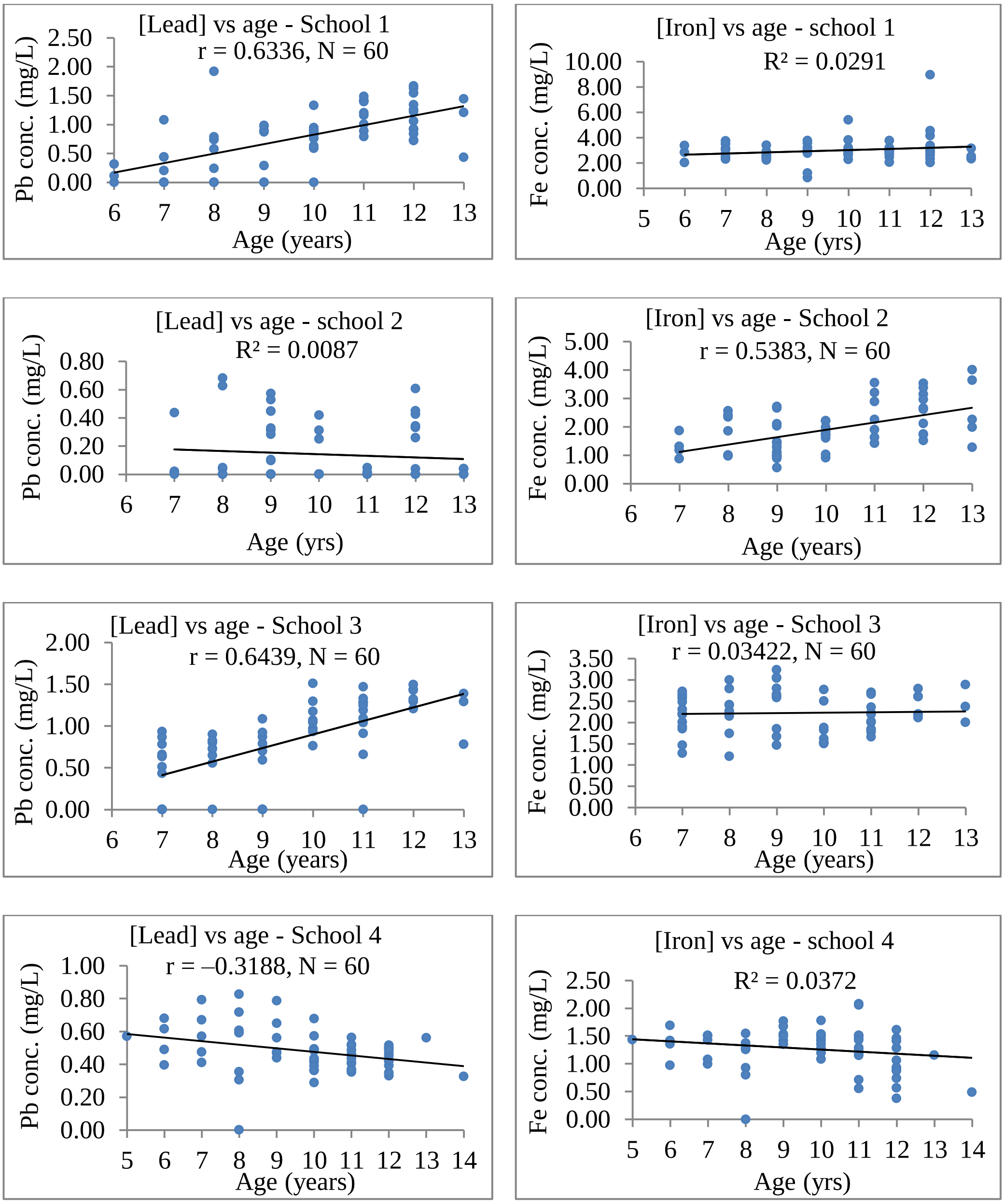

Figure 2: Correlations of lead, iron, zinc and copper concentrations in urine with age of pupils.

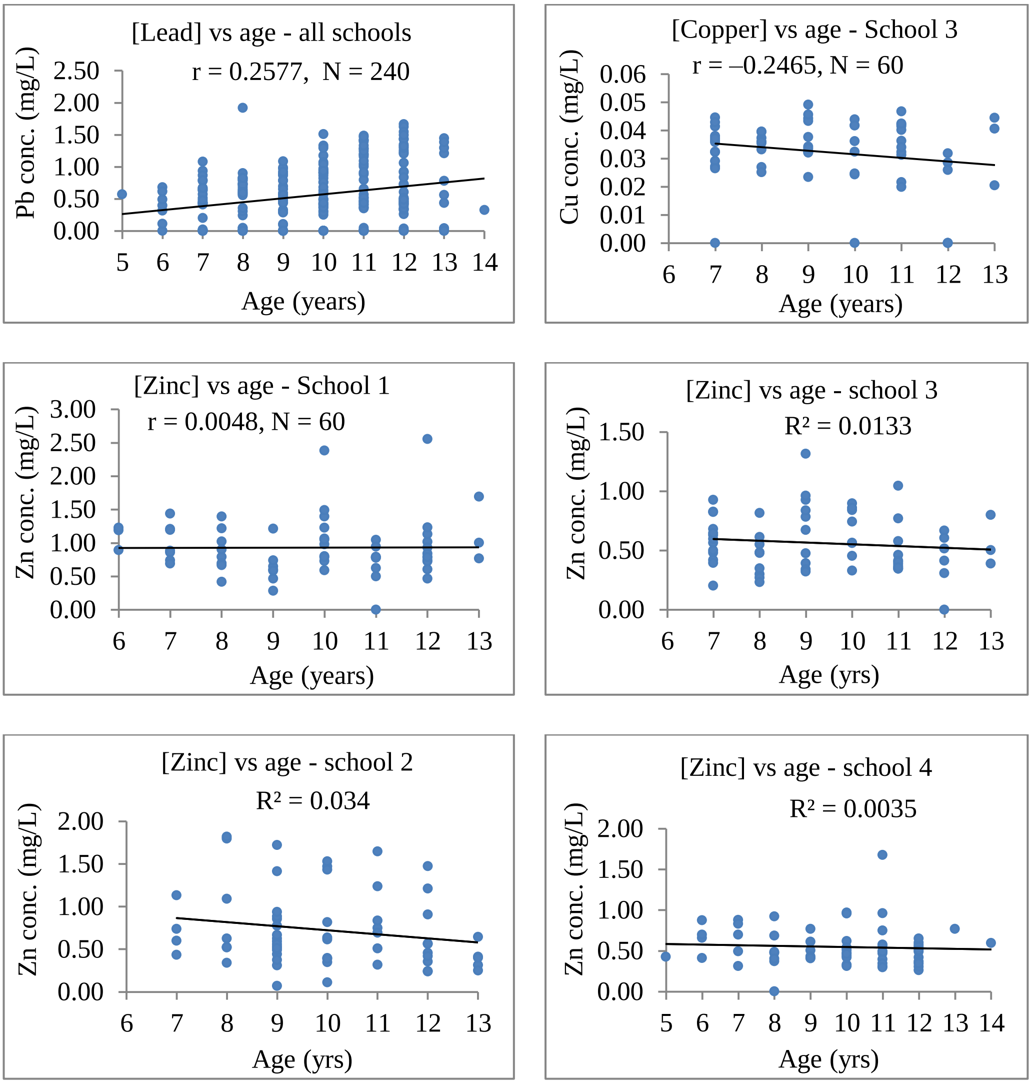

Figure 2 (Ctd): Correlations of lead, iron, zinc and copper concentrations in urine with age of pupils.

Weak negative correlation coefficients between the concentrations of zinc in the urine samples and pupils' age were obtained in the samples form most schools (r = –0.05823 to –0.1850) while a weak positive correlation coefficient was obtained in the samples from one school (r = 0.0048), but the correlations were not significant (p = 0.1570 to 0.9712). This suggested that there was no clear indication on the differences in the levels of zinc across the ages of the pupils included in the study. However, the weak negative correlations in samples from most schools implied that the concentrations of zinc slightly decreased with increase in age, which might be due to slight differences in metabolic rates between pupils of low age and those of high age. Some investigators have shown the tendency for zinc to increase with age (Alarcon et al. 1997, Thurlow et al. 2006). Increased requirements of zinc in the growing school age children may be due to the requirements of pubertal growth spurt, hormonal influences and co-existing micronutrient deficiencies (Thurlow et al. 2006). The findings of the present study differ from the findings obtained by Rodríguez and Díaz (1995) who reported that there was an increase in the levels of zinc in urine samples with increase in age.

A significant positive correlation was found between the concentrations of iron in urine and age of the pupils at school 2 (Bunju B) (r = 0.5383, p < 0.0001). An overall positive correlation coefficient of r = 0.1234 was found between the concentrations of iron in the urine samples and pupils' age, but this correlation was not very significant (p = 0.0562). The findings implied that the concentrations of iron in the urine samples generally increased with increase in age of the pupils and this might be related to metal accumulations. The findings of this study are similar to the findings of Rodríguez and Díaz (1995) in Spain who found an increase in concentrations of iron in urine samples with increase in age.

A weak negative correlation was found between the concentrations of copper in the urine samples and the age of the pupils from school 3 (r = –0.2465) but was not very significant (p = 0.0576). This indicated that there was a general decrease in the concentrations of copper with increase in age, which may be due to differences in metabolic rates among the pupils of different ages.

The findings of this study on copper differ from those of Yuan et al. (2013) who found that the concentrations of copper in urine samples were positively correlated with age in subjects above 35 years. A similar study that evaluated trace metals (arsenic, chromium, cobalt, copper, lead, nickel, manganese and zinc) in a non-directly exposed population of age ranging from 18 to 74 years old in southern Brazil found no significant correlation between the urine concentrations and age (Rocha et al. 2016).

Correlations of concentrations of metals in urine and academic performance of pupils

The concentrations of lead, zinc, iron and copper in the urine samples were compared to check the relationships with the academic performance of the pupils. There were no significant differences in the mean concentrations of the heavy metals in urine between the pupils with high academic performance and those with low academic performance analysed according to their classes (Pb: t = 0.1014–1.013, p = 0.3176–0.9198; Zn: t = 0.1577–1.372, p = 0.1781–0.8755, Fe: t = 0.2686–1.897, p = 0.0655 and Cu: t = 0.02256–1.338, p = 0.1888–0.9821) and overall for all the classes (Pb: t = 0.03652 at 238 degrees of freedom (df), p = 0.9709; Zn: t = 0.6976 at 238 df, p = 0.4861 and Fe: t = 0.8627 at 238 df, p = 0.3892). However, the results showed that most of the concentrations of the heavy metals in the urine samples of the pupils were higher in those with low academic performance than those with high academic performance. The correlation coefficients between the concentrations of the heavy metals in the urine samples and the academic performance of the pupils were negative in most of the samples and positive in some samples. The negative correlations indicated that the concentrations of the metals decreased with increase in academic performance while the positive correlations indicated that the concentrations of the metals increased with increase in academic performance, but were not significant in most of the samples at 95% confidence level ($p > 0.05$). Significant negative correlations between the concentrations of the metals and academic performance ($p < 0.05$) were found in some samples (lead: standard 4 school 3 and standard 5 school 4, $r = –0.6741$ to $–0.6904$, $p = 0.0271$ to 0.0326; zinc: standard 1 school 2, $r = –0.7121$, $p = 0.0209$; and iron: standard 1 school 3, $r = –0.6823$, $p = 0.0297$). Significant positive correlations of the concentrations of the metals with academic performance were found in the following samples: standard 6 school 1 (lead, $r = 0.8239$, $p = 0.0034$) and standard 4 school 2 (zinc and iron, $r = 0.8394$, $p = 0.0024$ and $r = 0.7143$, $p = 0.0203$, respectively). These findings of significant correlations implied that the concentrations of lead, zinc and iron found in the urine samples of some pupils could be related with affecting or influencing the pupils' academic performance. No similar study was found in literature for comparison of the correlations between concentrations of the heavy metals in human urine and academic performance.

Comparison of the concentrations of heavy metals in urine between males and females

The concentrations of the heavy metals in urine samples were compared to establish whether there were significant differences in the concentrations of the heavy metals in urine samples between female and male pupils. Statistically, the results from independent samples *t*-test revealed that there were no significant differences in the concentrations of the heavy metals (lead, iron, zinc and copper) in most of the urine samples between male and female pupils in all the classes and schools ($p > 0.05$). This might be due to the similarities in the behaviours of the pupils and the sources of exposures between the male and female pupils. The concentrations of zinc in the urine samples of pupils in standard 3 school 1, standard 4 school 2 and standard 5 school 4 showed significant differences between males and females ($t = 2.336$ to 2.771, $p = 0.0243$ to 0.0477). The findings of the present study are similar to the previous study conducted by Okonkwo et al. (2001) in Swaziland who found that there was no significant difference in the mean urine lead concentrations for boys and girls, despite the higher values shown by the girls. Also the study by Benes et al. (2002) in Czech Republic found that there was no significant difference between boys and girls in zinc concentrations while for copper there was a significant difference. Most of the findings are also comparable to the findings of Rocha et al. (2016) who found no significant differences for the levels of all the metals (arsenic, chromium, cobalt, copper, lead, nickel, manganese and zinc) in urine of males and females, although that study involved a population of age ranging from 18 to 74 years old.

Conclusion

This study found that there were no significant differences in the concentrations of the heavy metals in most of the urine samples between the boys and girls. The findings indicated significant positive correlations between the concentrations of lead and iron in urine samples and age of the pupils indicating accumulation, while for other metals, there were no significant correlations. On the other hand, there were significant correlations between the concentrations of the heavy metals (Pb, Zn and Fe) in urine and the academic performance of the pupils in some of the samples, and this implied that the concentrations of the heavy metals found in the urine samples of pupils could be associated with affecting or influencing their academic performance. The concentrations of lead were alarming.

Acknowledgements

We are extremely grateful to all the children who took part in this study. The Muslim University of Morogoro (MUM) is highly acknowledged for sponsoring this study. Mr. M. Mayuni (Chemistry Department, University of Dar es Salaam) is sincerely appreciated for technical assistance during the laboratory preparations and analyses.

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