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ORIGINAL ARTICLE

Biomonitoring of Heavy Metals in Blood and Urine of African Children from OwerriMetropolis, Eastern Nigeria

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KEYWORDS

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Trend

ABSTRACT: Childhood illnesses have been linked to elevated heavy metals in children's bodies. Such studies are lacking in developing countries despite the fact that African children could be most exposed to heavy metals. This study determines the concentrations of heavy metals in blood and urine of children in selected schools within Owerri metropolis. Consent and due process were followed to obtain blood and urine samples from 60 children. Samples were digested with nitric and hydrochloric acids for 6 hours at 90^{o} C and analyzed using Perkin Elmer 400 A analyst atomic absorption spectrometer. Mean concentrations in blood was Pb (4.517±1.599 mgl^{-1}); Cd (1.04±0.671 mgl^{-1}); Ni (5.612±1.237 mgl^{-1}); Mn (7.198±4.705 mgl^{-1}) and Cr (0.288 ±0.412 mgl^{-1}). In urine; Pb (1.912±1.219 mgl^{-1}); Cd (0.39±0.138 mgl^{-1}); Ni (3.988±1.230 mgl^{-1}); Mn (3.099±0.990 mgl^{-1}) and Cr (20.773 ±10.449 mgl^{-1}) were generally lower than concentrations in blood. Maximum metals concentrations in blood were higher than values for USA Academy of pediatrics. Except at WBP, Cr was highest concentrations within each school. Order of prevalence (%) was Pb> Ni > Cr >Mn> Cd in blood and Cd >Mn>Pb> Ni > Cr in urine. Variability revealed moderate to high with Cr (143%) as highest for blood while Pb (63.76%) was highest in urine. There was low relationship between metal in blood and urine as R^2 values ranged between manganese (0.006) to nickel (0.216). The prominent trend of metal was Mn - Cr as highest and lowest concentrations respectively. Metal diagnostic ratios revealed very undesirable values for Cd (4.05) and Mn (3.545) in blood. Currently no standards for metals in blood and urine for African children, metal concentrations in the present study were elevated. Government agencies and policy makers need to act in time to reduce the potential danger in the near future.

INTRODUCTION

African children are continuously being exposed to many toxic heavy metals ubiquitous in our environment [1, 2]. These pollutants can be indentified and quantified but their effects to children vary [2]. Variability amongst children in absorption, metabolism, distribution, excretion of pollutants can be much [3-6]. Several metals can act on a single

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organ leading to complications as the child grows and organs develop [6]. Childhood exposure to toxins and pollutants makes children vulnerable, according to UN Special Rapporteur on hazardous substances [7]. For many years, their exposure leaves them defenseless compared to adults. Numerous health effects are linked to childhood exposure to toxins include cancer, developmental disorders, learning disabilities and respiratory related disease and autism [8]. According to the World Health Organization (WHO), more than 1.7 million children under the age of five died in 2012 from modifiable environmental factors. In his report, Tuncak called these deaths "the tip of the iceberg", because disabilities and diseases associated with exposure to toxins and pollutants do not manifest themselves until years later [7]. Recently, childhood cancers have increased to 20 percent over a 20-year period, and various types of diabetes are now seen in children that were previously only observed in adults [9]. Asthma in early puberty and birth defects has also increased dramatically. Most of these cannot be explained by lifestyle choices or genetics especially being rampant during periods of rapid industrialization. It is the obligation of States to prevent children from being exposed to toxins and pollutants, and the corresponding responsibility for businesses. Research reports recommendations for States and businesses to better protect children from exposure to toxic chemicals. These include States to take into account the best interests of children when designing and implementing laws and policies for toxins and pollutants; and businesses to prevent exposure to toxins and pollutants throughout their activities and as part of their human rights due diligence.

Toxic metals are endangering and poisoning our children's brains. Recent researchers [10-11], have reported that heavy metals exposure is directly associated with biomarkers of effects than measurement of external pollutants. Biomarkers and heavy metals are equally better in providing estimates of health risk before onset of diseases [12]. A study [13] revealed that biomarkers can reveal exposure and early health effects of heavy metals. Many of these heavy metals have their effects and fate abundantly found in urban environment. Literature reveal that exposure to multiple heavy metals even at low concentrations could have detrimental health effects.Heavy metals are capable of causing both acute and chronic toxicity in children. Acute toxicity refers to ability of heavy metals to cause injury to a school from a single exposure over a short duration [14]. On the other hand chronic toxicity occurs when children are left exposed to pollutant over a long period of time [15]. Heavy metals occur in small doses repeatedly usually years before effects are manifested. Heavy metals normally occur in small amounts but their effects over years have been known to include the mutagenic, teratogenic and carcinogenic effects.

Data, nor studies of heavy metals in blood and urine samples of our school children anywhere in Nigeria were not found.However a close study was conducted by Akan [16] for children (not school) (aged 1 -10 years) in Maiduguri, Borno State, Nigeria. Presence of heavy metals in children body and playground is currently not known in most third world countries because of the absence of sufficient data which could be used by regulatory agencies. This study will provide the basis for future studies involving children and pollutants in our environment. Information is lacking regarding the health effects of potential health effects of mixtures of chemicals in children. Urban children are a high risk group, the essence of this study cannot be overemphasized.

Children are the future of any nation, if any country will enjoy continuity such a nation must adequately address and preserve the health of her children [17]. If this seems like an exaggeration the fact remains that parents are ready to sacrificed their lives for their children in countless examples. Therefore, awareness of the harm caused by heavy metals to children is critical in steps towards prevention. Environmental Justice Principles is anchored on the fact that all people and communities are entitled to equal environmental protection and public health laws and regulations. In Owerri Municpal, the children of public school could be heavily exposed to pollutants both from constructions activities communication activities to industrial activities. A wealth of literature has documented indiscriminate sitting of filling station, schools and children pantics, within Owerri. These will no double inflict

exposure effects on children. Thus the situation needs to be known and proper policies put in place.

The reverence for children here can be observed in the names of children in Igbo culture in names such as "Nwakaego", it is a boring elaboration of the obvious to eulogize the importance of children. But it suffices to state that for any nation to continue to exist; her children must enjoy growth and development to the full. Biomonitary is modern and gives information that is most reliable when adequate reference population and background range is available. Though data of these pollutants in Nigeria schools is absent, it is difficult to put children's exposure levels in perspectives without biomonitory. Heavy metal toxicity impacts brain development and causing a number of neuro developmental disabilities including attention-deficit hyperactivity disorder, autism, dyslexia and other cognitive damage [18].

Literature reveals that children have not been given attention as regards exposure to heavy metals from their environment. Most researchers have limited their studies to lead and only a few have used more accurate instruments than the Atomic absorption spectrophotometer while determining total heavy metals in whole blood and morning urine [19]. In Nigeria, children are more exposed to heavy metals because of factors such as low literacy and less enforcement of environmental laws. Beyond the question of costs and benefits is an ethical issue that society must also consider; children have the right to realize their full potential and when a society allows its children to be exposed to pollutants unchecked it is directly denying the children of that right [20].

The aim of this research was to carry out analysis of lead (Pb) cadmium (Cd) nickel(Ni) manganesse (Mn) and chromium (Cr) metals children blood and urine of children using preselected school playgrounds, In order to realize the aim some specific objectives were listed out as follows: to determine heavy metal in children's blood and urine**;** to determine basic trends to aid understanding of the current conditions of contamination of multiple elements and correlate data to determine the linkages in information obtained. This research therefore provides basic information while serving as a reference for similar studies in the future.

MATERIALS AND METHODS

Study location: Owerri metropolis, lies within latitude 5.48^{o} North and longitude 7.03^{o} Imo State, southeast Nigeria. This area falls within the heart of the humid tropical region. Owerri metropolis lies within one of the three local government areas (LGAs) that make up Owerri city, the capital of Imo state of Nigeria set in the heart of the Igboland. Playgrounds were designated with the letters of the words in the name of the school. Out of 25 government schools within Owerri metropolis, 9 were selected and used in this study (Table 1). School playgrounds were selected to reflect spatial variability and traffic/commercial influence associated with each zone, as well as differences in land use within an urban setting [17]. Literature reveals that Owerri metropolis is at the receiving end of enormous polluting waste of toxic metals from nearby auto mechanic villages [21].

Table 1. Names of schools, coordinates, and close land use to school.

S/N	CODE / Name of school	Latitude			Longitude			ELEV	Land use close to school
		DD	MM	SS	DD	MM	SS		
1	CSO- Central school I	05	29	00.4	007	01	48.8	73	ResidentialEstate
2	HEO –Housing Estate Aladimma P/S	05	29	50.7	007	02	44.6	70	Motor park
3	IKS- Ikenegbu P/S	05	29	27.0	007	02	23.2	74	Police station
4	MNO-Model P/S	05	28	56.4	006	59	59.4	77	EkeonunwaMarket
5	TSO-Township P/S	05	29	05.3	007	02	23.2	73	Motor park and Banks
6	UPS-Urban P/S I	05	28	59.8	007	02	01.5	73	River Nworie

7	WSP-Waterside P/S	05	28	52.8	007	01	44.9	70	Residential and market
8	WBP-World Bank P/S	05	29	09.3	007	00	19.9	82	IMSU and High traffic
9	SCP-Shell Camp P/S	05	29	58.0	007	01	37.0	73	Busy junction

P/S : primary school,

Participant demographics

A total of 60 children residing in Owerri metropolis were recruited as follows; 30 boys and 30 girls. Child participation was limited to those aged 4-10 years. Literature reveals that there is no significant difference between blood concentrations of heavy metals for both men and women and for both boys and girls [22-23]. In a study of metals in Korea, Ha [24] proposed that there is no relationship between sex and metal concentrations in urine amongst Korean children.

Human subjects'interactions

Approval was obtained from the Imo State University and permission obtained from the guardian with written informed consent obtained from each guardian for their child's participation, along with the child's assent. Participants were met either in their home or at the school or playground. Only children who reside in Owerri and attend the schools were considered.

Biological samples

From children, blood and urine samples were collected once for analysis in the month of June 2018. Participants were instructed to collect a mid-stream urine sample in the morning into properly labeled acid-washed 120 mL BD Vacutainer urine cups and royal blue tops (urine reflects exposure to inorganic). Intravenous blood samples were collected following sterile procedures by a licensed phlebotomist from the antecubital fossa into BD Vacutainer trace metals tubes [25] and whole blood digested directly. All samples were stored at -0°C in a blocked freezer till analyzed at chemistry laboratory of Imo State University. Samples were analyzed within 6 hours after collection to avoid problems of storage and deterioration [26].

Determination of total metal content

To 5 ml of blood sample placed in a Pyrex glassware, 10 ml concentrated nitric acid was added and mixture heated to 90^{o} C for 6 hrs and left to evaporate dryness, cooled at room temperature before repeating procedure. The dried sample was then digested with 10 ml concentration HNO_3 then 10 ml of 3M HCl. The digests were warmed and then redissolved the metal salts with 20 ml of 2 M HCl, then centrifuged at 30000 rpm for 15 minutes and clear supernatant volume was made up to the mark in a 25 ml volumetric flask. Then using A Analyst Perkin Elmer 400 Atomic absorption spectrometer and metals were quantified in all samples. Same procedure was repeated for urine samples [27].

Statistical analysis

Statistical analyses were performed in Microsoft excel. Primary comparisons of interest were to determine differences between schools and amongst children and compared with benchmark values as seen throughout literature. Coefficient of variation (CV %) was calculated using the equation (1) by [17].

$$CV\ \% = \frac{StandardDeviation}{mean} X\ 100 \qquad (1)$$

CV % was used to estimate variation in heavy metal contents from the different schools. Variation ranking was : CV % less than 20 as little variation; CV % between 20 to 50 as moderate variation and CV % greater than 50 as high variation. ANOVA was employed in comparing mean metal concentrations among the playgrounds and statistically significant differences was described when $P < 0.05$. Metals concentrations were assessed normally by comparing with cut off values and standards found elsewhere in literature while prevalence in percent distribution of metals amongst schools was determined

based on number of children with metal in question. Metal diagnostic ratio (MDR) were estimated using equation (2)

$$MDR = \frac{Blood-metalconcentration}{Urine-metalconcentration} \qquad (2)$$

In order to retain maximum interpretability, metal data was analyzed and reported without transformations.

Quality control and quality assurance

Analytical grade chemicals and reagents were purchased from Fin Lab Owerri and used without further purification. These include nitric acid (HNO_3) 6.5% v/v HCl, sodium sulphate (Na_2SO_4 and potassium hydrogen carbonate, $KHCO_3$) which were purchased from Merck through Fin Lab agents while double distilled water was used for heavy metals analysis; working standard of metal for references was sourced from Fluka (Bucs, Switzerland) and preparation for use was by diluting a concentrated stock solution of 1000 mg/dm^3 in 0.25 moldm^3 of HNO_3.

RESULTS AND DISCUSSION

The result for the mean heavy metal concentration in the blood and urine samples of children from different schools is presented in Table 1. The concentration of heavy metals in bloods ranged from 2.83 to7.61 mg/l for Pb, 0.39 to 2.51 mgl^{-1} for Cd, 4.21 to 8.06 mgl^{-1} for Ni, 2.46 to 16.2 mgl^{-1} for Mn, 0.042 to 1.06 mgl^{-1} for Cr while metal concentrations in the urine ranged from 0.71 to 4.2 mgl^{-1} for Pb, 0.13 to 0.62 mgl^{-1} for Cd, 2.72 to 6.84 mgl^{-1} for Ni, 1.88 to 4.57 mgl^{-1} for Mn, and 0.97 to 36.25 mgl^{-1} for Cr. The decreasing order of mean metal concentrations studied in blood as follow: Mn> Ni >Pb> Cd > Cr while in urine the order was: Cr > Ni >Mn>Pb> Cd. Calculated prevalence indicates that the most abundant metal in the blood was Pb (100 %), Ni (55.22 %), Cr (33.33 %), Mn (22.22 %) and Cd (11.11%). In urine sample order of decreasing prevalence was Cd (88.88%), Mn (33.33%), Ni (11.11.33%), while Cr (0.00%) showed no abundance.

Comparing mean concentration of heavy metals in all children in this study with the threshold limit of USA, Academy of Pediatrics, (2003) revealed high level of blood lead in all schools. Meanwhile, we recorded elevated concentration for urine-lead at only two schools viz, CSO (3.66 mg/l) and TOS (4.2 mg/l). A study by Akan [16] reported lower concentrations of Pb in blood and urine for children (children aged 1- 10 years) in Maiduguri, Nigeria. Lower concentration for urinary lead (0.017 to 0.040 mgl^{-1}) was also reported for school children in Manzini, Swaziland, South Africa [28]. Children in Owerri metropolis are therefore at risk of lead poisoning. High level of lead in children blood can severely affect their mental and physical development [29]. The high lead concentration in the blood can be related to high exposure of children to traffic exhausts, lead-based paint and lead-contaminated dust in older buildings within Owerri.

In comparison with mean values reported in other countries our results for urine-lead (Pb) was generally higher than value reported earlier for school children in Dar es Salaam, Tanzania [30], but lower than values of blood-lead for children in Cairo, Egypt [11,29]. Our value was also lower compared to values reported in studies conducted in India (children aged 3–12 years) [31], Uruguay (children aged 2–14 years) [32], France (children aged 8–12 years old) [33], Canada (children aged 6–11 years) [34] and Brazil (children aged 6–8 years) [35] cities with blood-lead levels of 93.2, 94, 34.8-39.5, 10.2 and 24 mgl^{-1}. These studies opined on the close proximity to traffic could be the main cause of high blood-lead levels in children. However more recent studies were not found and given the phasing out of leaded fuel there is a need for lead blood studies in this cities and elsewhere to determine the current levels of lead in children. This could be important in identifying other major sources of Pb in blood and hence mitigation. Currently no agreed threshold values for Pb with many researchers proposing different values [27].

Cadmium mimics zinc, causing disruption of enzymatic mechanism which has been known to stunt growth. It is widely used in industry so Cd is found in elevated levels in food and water and atmosphere where it is attached to particulates [36]. Reference value for whole blood–Cd is 0.3-1.2 µgl^{-1} while the mean Cd (1.04±0.671 µgl^{-1}) is comparable. Given that the half-life of Cd is about 20 years, the mean value in our work could be a concern.

When adsorbed in the blood Cd binds with albumin. A low molecular weight protein called metallothionein (MT) binds to Cd to enable absorption in the proximal tubule where Cd-MT degrades to give free Cd ions. Hopefully, this is the amount detectable in urine and of course it's a fraction of the absorbed amount. Free cd ions are capable of accumulating in the cortex of the kidney causing damages. Though most urine-Cd concentrations in this study were less than 2.5 μgl^{-1}, considered safe for Cd exposure [37], new diseases may have lower values due to changing diets and the environment. For instance "ouch ouch" a condition characterized by severe osteomalacia and osteoporosis has been linked to Cd in rice but equally found in aging patients with osteoporosis but safe limits of Cd for this condition are yet to be known [38-39].

The USA, Academy of Pediatrics [13] recommended a threshold limit (>5mgl^{-1}) for nickel (Ni) in children's blood and urine. Our findings showed that only urine-nickel levels didn't conform to this standard except at WBP (6.84 mgl^{-1}) while data for blood was generally > 5 mgl^{-1}. Nickel and its compounds are air lipophilic thereby able to accumulate in major organs of the body with fats. It was thus no surprise that Ni concentration in blood and urine were low. However Verla [17], identified that of Ni concentration in dust within these schools environment were significant. Since 09% of Ni is excreted in excreta and only a small fraction in urine, it is understandable that urine-Ni concentrations can be low. Nickel can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs [20]. Hence our data for Ni is a public health concern.

Manganese is an essential micronutrient with USEPA daily recommended intake of 0.14 $mgkg^{-1}$ [40]. It is absorbed majorly through human respiratory track where 100% inhaled is absorbed into the blood stream which opens into the CNS. Mn accumulates in the CNS, bone, liver and kidney where it causes various kinds of damaging effects [12]. Though maximum threshold for Mn (4-15 μgl^{-1}) was exceeded only for children at TOS (Mn:16.2 μgl^{-1}), the overall mean value of Mn (7.2 μgl^{-1}) remains a concern due to bioaccumulation. Due to many relatively stable valences of Mn the metal can cause harm to children.

Our data showed that mean Cr concentration in children's blood at WSP (0.18 mgl^{-1}), and WBP (1.06 mgl^{-1}) exceeded the threshold limit of 0.052 - 0.156 mgl^{-1} set by USA, Academy of Pediatrics [13]. The overall mean of Cr (0.288 mgl^{-1}) for all children studied in Imo state was greater than USA, Academy of Pediatrics [13] but less than cut off values found in literature [8]. The entire data for urine-Cr level was within the standard range of 0 – 40 mgl^{-1}. Children at the aforementioned schools viz MNO, SCP and WBP may be exposed to chromium contaminated dust, water, or other external sources. Another reason could be wide individual variation in metabolism and rapid depletion of body burden of chromium. Chromium (III), an essential micronutrient helps in sugar, protein and fat metabolism in humans [41] (Table 2).

Table 2. Mean heavy metal concentration in whole blood and morning urine.

	Metals in whole blood					**Metals in morning urine**				
	Pb (mgl^{-1})	**Cd (mgl^{-1})**	**Ni (mgl^{-1})**	**Mn (mgl^{-1})**	**Cr (mgl^{-1})**	**Pb (mgl^{-1})**	**Cd (mgl^{-1})**	**Ni (mgl^{-1})**	**Mn (mgl^{-1})**	**Cr (mgl^{-1})**
HEO	4.06	0.65	6	4.15	0.08	1.62	0.62	3.78	2.56	22.58
MNO	4.6	0.39	6.15	4.02	0.046	1.24	0.45	3.24	2.18	24.29
SCP	3.2	1.29	4.93	2.46	0.042	0.85	0.13	4.1	4.57	31.4
CSO	3.66	0.6	4.24	2.84	0.065	3.66	0.46	3.28	3.28	10.6
TOS	6.24	0.75	6.53	16.2	0.076	4.2	0.43	3.46	4.16	21.26
WSP	7.61	0.68	6.01	8.51	0.18	1.84	0.45	2.72	4.2	17.84
WBP	5.28	2.51	8.06	8.6	1.06	1.22	0.38	6.84	1.88	0.97
LKS	2.83	0.85	4.21	5.46	0.084	0.71	0.31	3.57	2.68	21.77
UPS	3.17	1.64	4.38	12.54	0.96	1.87	0.28	4.88	2.38	36.25

Mean	4.517	1.04	5.612	7.198	0.288	1.912	0.39	3.986	3.099	20.773
Max	7.61	2.51	8.06	16.2	1.06	4.2	0.62	6.84	4.57	36.25
Min	2.83	0.39	4.21	2.46	0.042	0.71	0.13	2.72	1.88	0.97
SD	1.599	0.671	1.237	4.705	0.412	1.219	0.138	1.230	0.990	10.449
CV	35.19	64.51	22.04	65.37	143.05	63.76	35.38	30.85	31.95	50.30
M_b/M_u	1.812	4.05	1.178	3.545	0.029	0.552	0.247	0.849	0.282	34.198
USA, 2003	2.5	0.3– 2.3µgdl^{-1}	> 5 µgdl^{-1}	4-15 µgdl^{-1}	0.052 -0.156 µgdl^{-1}	1-3 µgl^{-1}	0.1-0.3 µgl^{-1}	>5.0 µgl^{-1}	0.8– 3.95 µgl^{-1}	0 – 40 µgl^{-1}
Cut off	2**	0.15 **	2.62*	18.3**	1.86*	NF	2.5	NF	NF	NF
Prevalence (%)	100	11.11	55.55	22.22	33.33	22.22	88.88	11.11	33.33	0.00

USA, 2003: USA, Academy of Pediatrics, 2003 [13], cut off values in blood (µg/l), * refers to [42]; ** refers to [43], NF: Not found in literature.

Three of the metals found in elevated concentrations in children's blood are carcinogens namely Pb (4.517±1.599 mgl^{-1}), Cd (1.04±0.671 mgl^{-1}) and Cr (0.288±0.412 mgl^{-1}). Cd has been linked to lung cancer, prostate cancer and cancer of testes by such mechanisms as oxidative stress induction, DNA repair inhibition, apoptotic tendencies and aberrant gene expression. Furthermore the Institute of Medicine, 2002 has fingered Chromium (VI) to be carcinogen while [44] maintains that there could be adverse health effects if chromium (III) becomes excessive in the body.

We analyzed the variations of heavy metals using the coefficient of variations (CV) model. The results revealed high variations in blood and urine. The blood heavy metals level varied from 22.04 % (Ni) to 143.05 % (Cr) while for urine heavy metals level varied from 31.95 % (Mn) to 63.76 % (Pb). Therefore Cr exhibited high variation in blood whereas high variation was observed for Pb in children's urine. The statistical analysis using one-way ANOVA revealed significant differences in the mean concentrations of the metals in blood [$P < 0.05$; degree of freedom (44); $F_{critical}$ (2.61) $<F_{ratio}$ (14.71)] while between schools revealed no significant differences [$P > 0.05$; degree of freedom (44); $F_{critical}$ (2.21) $>F_{ratio}$ (0.69)]. For urine, similar analytical results were obtained, i.e significant differences between metals [$P < 0.05$; degree of freedom (44); $F_{critical}$ (2.61) $<F_{ratio}$ (27.72)] while between schools revealed no significant differences [$P > 0.05$; degree of freedom (44); $F_{critical}$ (2.21) $>F_{ratio}$ (0.23)]. The studied schools are within the same geographical location and thus experiencing similar anthropogenic disturbance. This could be the reason for the "no significant differences" between them [45].

Correlation analysis was conducted at 5 % significance level to determine how the metal in blood and urine relates with one another (Table 3). The correlation values of heavy metals concentrations in the children's blood were generally low and positive except for Pb/Cd (-0.094) and Pb/Cr (-0.015). However, we observed significant positive association between Pb/Ni (0.65), Pb/Mn (0.50) and Cd/Cr (0.88) in blood. These strong positive correlations found between the heavy metals studied indicate eventual interactions between them [18]. This can probably indicate increase in their toxic effects in children even at a low environmental level of exposure. Meanwhile, in urine metals concentrations exhibited generally negative association except for Pb/Cd (0.36), Pb/Mn (0.337) and Mn/Cr (0.235) though low and insignificant. However in referring to the complex nature of toxic metals analysis researchers propose that in urine, high excretion is usually associated with recent exposure, while low excretion is assigned to low exposure or decreased ability to detoxify a particular metals [46]. Equally worthy of note is that high value of excretion may cause a saturation in which another metal is less excreted. These two complexities may explain why a linear relationship in this work had low R2 values

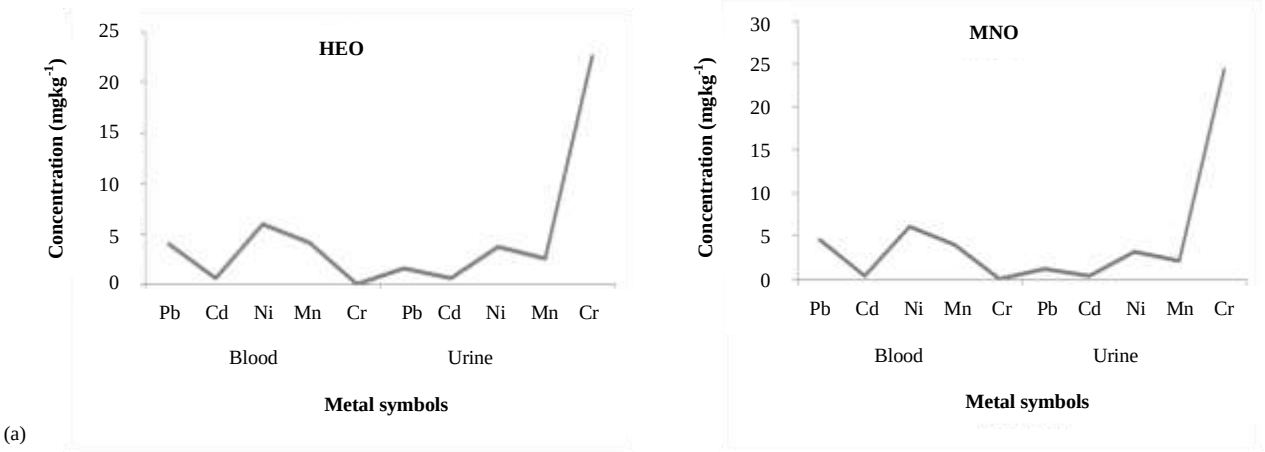
and may not be sufficient to describe metals in urine of children.

Table 3. Correlation matrix for metals in blood and urine.

	Pb	Cd	Ni	Mn	Cr
			Blood		
Pb	1				
Cd	-0.094	1			
Ni	0.646	0.383	1		
Mn	0.456	0.257	0.297	1	
Cr	-0.015	0.883	0.324	0.420	1
			Urine		
Pb	1				
Cd	0.361	1			
Ni	-0.285	-0.266	1		
Mn	0.337	-0.288	-0.519	1	
Cr	-0.184	-0.397	-0.333	0.235	1

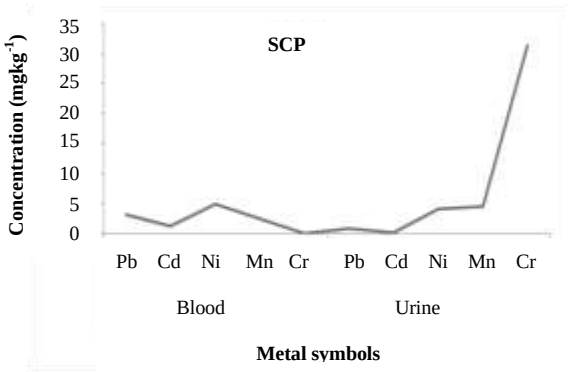
Trends in metals concentrations

Trends in metals concentrations in blood and urine of children from studied schools are presented in Figures 1(a-j). The trends showed that Cr concentration was generally high in children's urine from the schools except at WBP. At HEO the trend of metal concentrations showed that Ni was highest in blood while Cr was highest in urine and highest concentration amongst all five metals. Ni and Cr followed the same trend at MNO both for blood and urine. Though metals concentrations were generally high at SCP, it was observed that Ni was highest and Cr lowest for both blood and urine. Metals concentrations were significantly higher for children at CSO than other schools but Ni and Cr followed the same trends as observed for HEO, MNO, SCP and CSO. There was a shift from the trend above at TOS where Mn was highest in blood while Cr was lowest whereas Cr was highest and Cd lowest in urine. The trend in TOS was repeated in WSP. A different trend was observed for WBP where Mn and Cr showed the two extremes in blood respectively while Ni and Cr showed highest and lowest concentrations in urine.

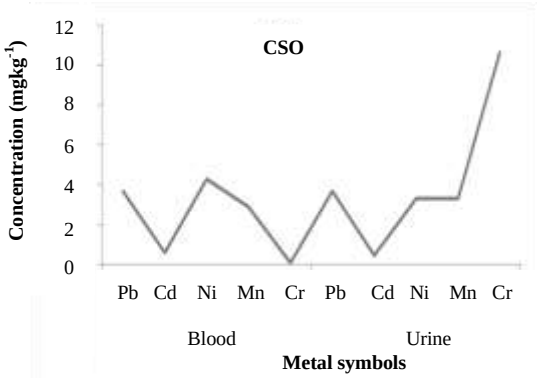


(a)

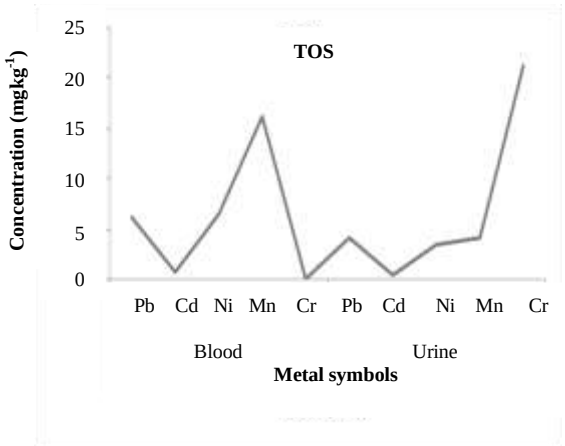
Figure 1a-1i. Trends of heavy metals in blood and urine of children.



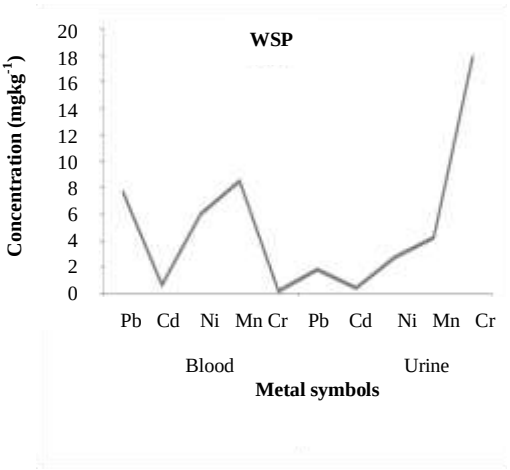
(c)



(d)



(e)



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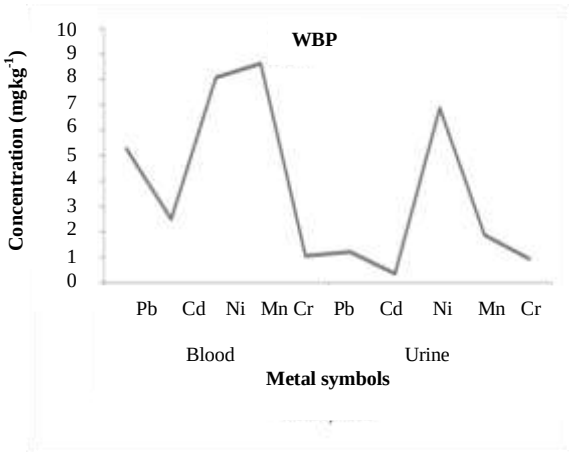

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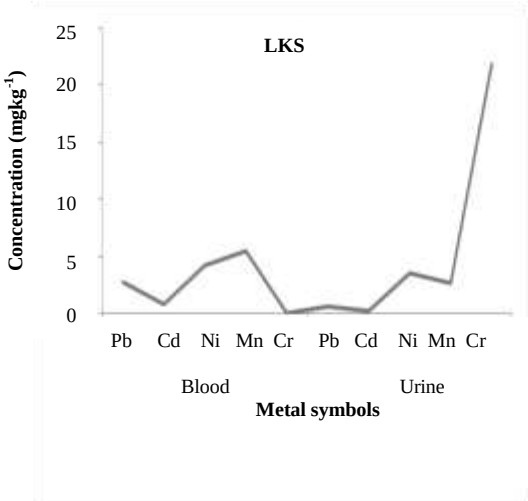


(h)

Figure 1. Continued

(i)

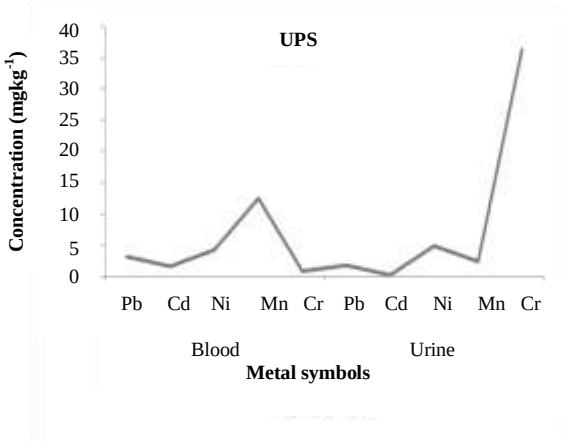


Figure 1. Continued

There was much similarity in the trends of metal concentrations in LKS and UPS which emphasized the Mn-Cr trend observed in many schools within Owerri. Whereas the Mn-Cr trend was dominant in blood, the Cr-Cd trend was dominant in urine. Overall two prominent trends where observed as Ni-Cr and Mn-Cr. These trends may exist as a result of some form of association between metals at major source of exposure to the children which has been identified as dust [17] from playground rather than water or food.

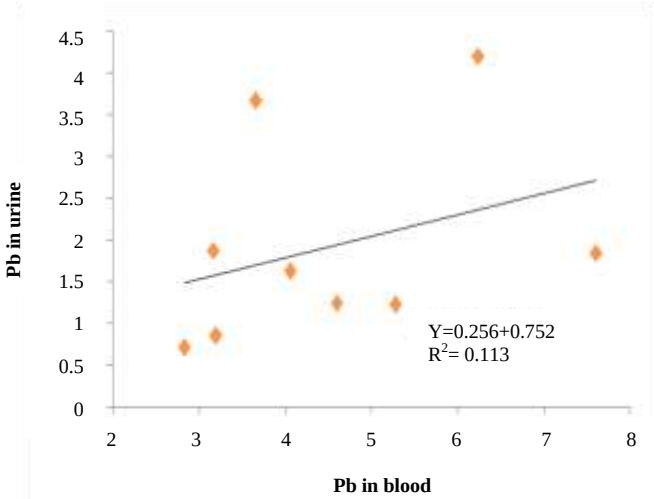
Diagnostic metal ratios

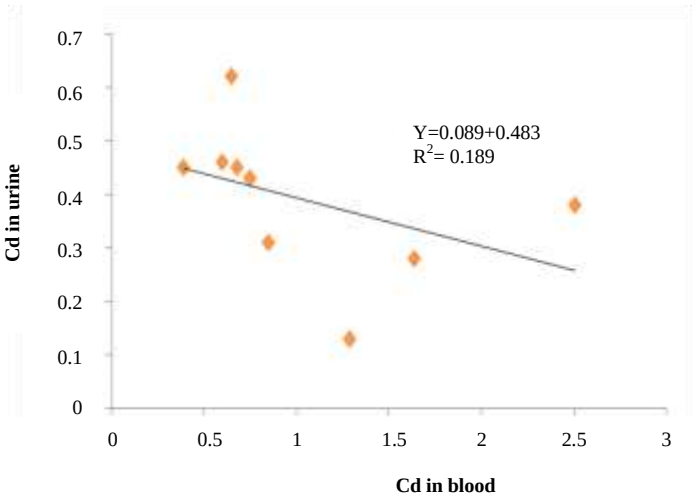
Ratios of metal concentrations in blood to metal concentrations in urine were computed (using equation 2) and reported in Table 1. Large values of ratios are indicative of low excretion of the metal in urine and thus a dangerous pointer, while low values of ratios are a pointer of good removal of metals in urine. Recall that many excretion routs exist for metals. Though ratios for Pb, Ni were greater than 1, it could be said to approach an unsafe level for blood. The ratios for Cd (4.05), Mn (3.545) in blood was considered high and therefore harmful conditions prevail. On the other hand ratios for urine/blood metals concentrations were less than 1, indicating a harmless situation, except for Cr/Cr in urine –blood which was 34.198 indicating an unhealthy situation.

According to WHO [47], 80% of Cr is excreted through urine while other excretory routes are the bile and sweat glands. Cr is not known to accumulate in the body [48-49], hence values reported in this work are in agreement with these concepts.

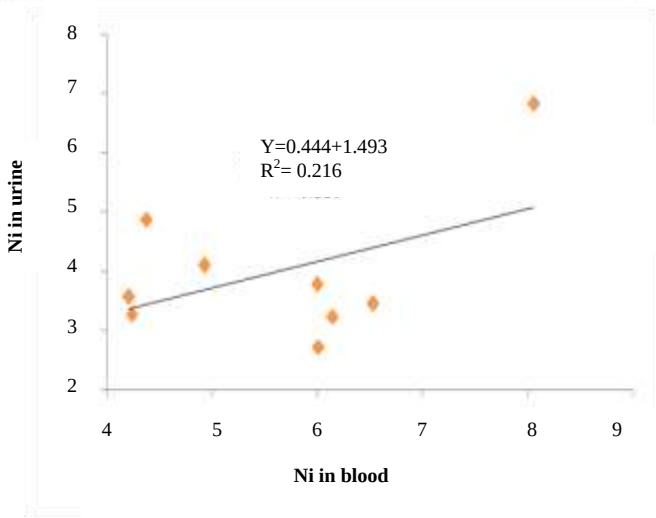
Regression analysis

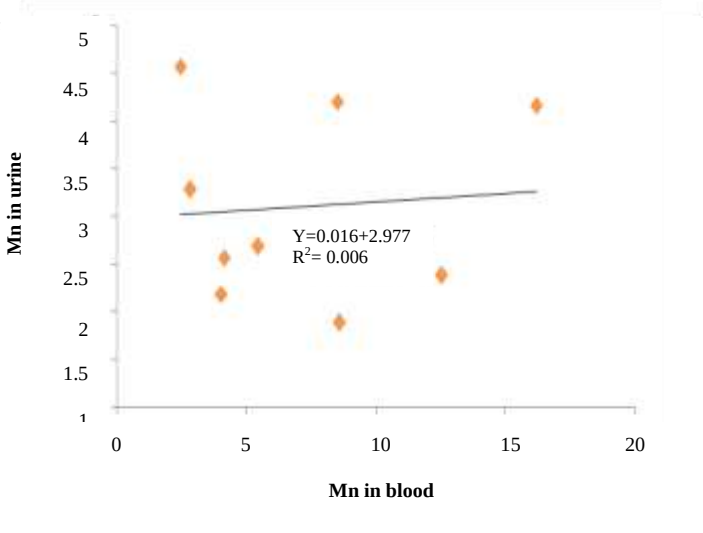
Linear regression analysis was done to evaluate relationship between the concentration of heavy metals in blood and urine of children in Owerri metropolis. The relationship was presented in Figure 2a-2e showing the R2 values and regression equations. The linear regression provides important information on association between the metal variables in the two medium (i.e blood and urine).The extent of association is measured on a scale 1 (perfect positive relationship), 0 (no relationship) and -1 (perfect negative relationship) [50]. The regression, although positive is poorly linear with r values less than 0.5. Therefore, suggesting that the concentrations of heavy metals in blood are not totally responsible for the metals in urine.However, the order of association was in Ni (0.216) > Cd (0.189)>Pb (0.1130 > Cr (0.035) >Mn (0.006).





(b)





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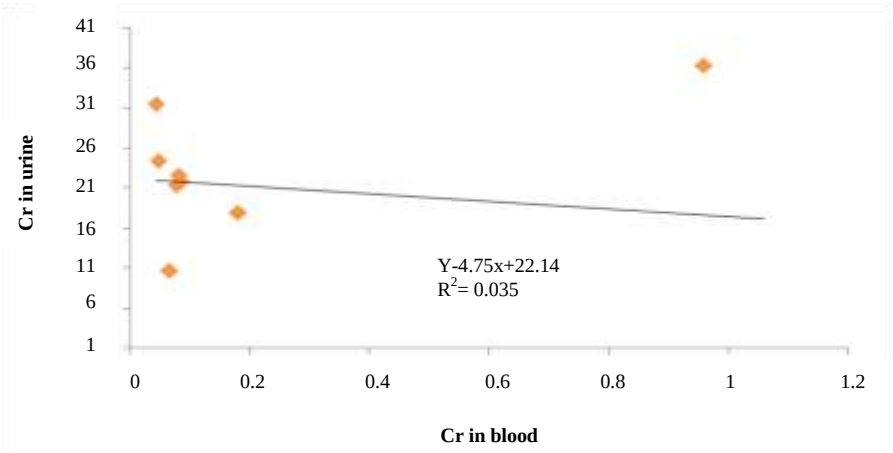

(e)

Figure 2a-2e. Regression plots for heavy metals in blood and urine of children.

These results are similar that obtained by Adams [51] in which non linear regression was opined to better describe relationship amongst metals in children. In the current work trends such as Ni-Cr, Ni-Pb was observed in blood (Table 2). Though R^2 values were low, Adams and co-workers [51] categorized non linear and linear relationships in which Ni-Pb featured and predicted five effects on children namely: irritability, lethargy, stereotypy, hyperactivity and inappropriate speech. Since these low r2 values are indicative of such effect, it could be explained that the distribution of metals in our body is complex is govern by such factors as metabolism, organ type, transportation and adsorption just to mention a few [52-54].

CONCLUSIONS

This study has successfully determined five metal concentrations in blood and urine of children from school playgrounds within Owerri, Nigeria. When compared with cut off values and standards elsewhere the general information suggest immediate action to prevent potential danger. The potential danger arose from children exposure to traffic exposure and mainly contaminated dust from playgrounds. The presence of Pb, Cd and Cr, in concentrations that are significant is a concern due to their carcinogenic potentials. Therefore metal concentrations in blood and urine of children in Owerri showed that the body burdens were above normal despite the absence of standard references for the study area. There is an urgent call for concerted effort involving relevant specialists develop cut off values for Nigeria which will assist efforts towards policies and laws to specifically protect our children. Two major limitations of the current study was lack of finance to recruit a larger sample size and the use of total heavy metal rather than speciation method. However the present results could be clinically relevant in that it is a call for more detailed work and a reference to future research.

Authors' contributions

AW designed the project and wrote draft. Did the first drafts and outline after laboratory results were obtained. SM and LKC collected the blood and urine samples while AW did the digestion in the laboratory prepared the consent letter and meet the head teachers and parents, and helped perform the analytical determinations, acquired and analyzed data and contributed to the writing of the manuscript and revision. ACM proof read the manuscript and added to the discussion. EN arranged references and was instrumental in obtaining consent from parents and children. She equally helps in digestion of samples in the laboratory. ECE assisted in sample digestion in the laboratory, data analysis and in writing of the manuscript.

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