Environmental Pollution 235 (2018) 163–170

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Association between human exposure to heavy metals/metalloid and occurrences of respiratory diseases, lipid peroxidation and DNA damage in Kumasi, Ghana☆

Nesta Bortey-Sam [a], Yoshinori Ikenaka [a, b], Osei Akoto [c], Shouta M.M. Nakayama [a], Kwadwo A. Asante [d], Elvis Baidoo [c], Christian Obirikorang [e], Hazuki Mizukawa [a], Mayumi Ishizuka [a, *]

[a] Laboratory of Toxicology, Department of Environmental Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita Ku, Sapporo, 060–0818, Japan
[b] Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa
[c] Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[d] CSIR Water Research Institute, P. O. Box AH 38, Achimota, Accra, Ghana
[e] Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana

ARTICLE INFO

Article history:
Received 12 May 2017
Received in revised form
2 November 2017
Accepted 3 December 2017

Keywords:
Metals
Urine
Kumasi
Asthma
DNA damage

ABSTRACT

Heavy metals and metalloids contamination in soils, water, food and livers of wild rats have been studied in Kumasi, Ghana and despite the estimated risks to residents, there is no epidemiological study to ascertain these projections. In addition, the World Health Organization and International Agency for Research on Cancer have reported an increase in respiratory diseases and cancers, in Ghana. The study's purpose was therefore to explore the potential associations between metal exposure and occurrences of respiratory diseases, lipid peroxidation and/or DNA damage to different age groups and sexes in Kumasi. Human urine was collected from the general population in urban and control sites in Kumasi and nine metals were measured in each sample. Results showed that although Zn was the most abundant total urinary As concentration was higher in 83% of samples compared to reference values. Urinary concentrations of metals, malondialdehyde (MDA) and 8-hydroxy-2-deoxy-guanosine (8-OHdG) were higher in urban sites compared to the control site. Based on the results obtained, there was no significant correlation between urinary metals and age. However, urinary Cd and MDA were highest in age groups 61–85 and 3–20 years, respectively. Significantly higher levels of urinary Co, As and Cd were detected in female participants. The study revealed that exposure to As was significantly associated with increased odds of asthma (odds ratio (OR) = 2.76; CI: 1.11–6.83) and tachycardia (OR = 3.93; CI: 1.01–15.4). Significant association was observed between urinary metals and MDA and 8-OHdG indicating possibility of lipid peroxidation and/or DNA damage in Kumasi residents.

1. Introduction

Heavy metals and metalloids are among the most toxic substances (ATSDR, 2015) and despite their natural abundance, they are formed mainly from human activities such as mining, smelting, combustion, tannery or fertilizer applications. Humans and animals could be exposed to metals via inhalation, consumption and/or dermal contact (Saoudi et al., 2012). Despite the importance metals (iron, zinc, copper and manganese) play in maintaining normal physiological functions, excessive intake could result in health implications (Magge et al., 2013). In addition, exposure to cadmium, nickel, lead and arsenic could generate reactive oxygen species (ROS) leading to modifications of DNA and lipids (Stohs and Bagchi, 1995). These modifications have been reported to contributes to the incidence of cancers and cardiovascular diseases (Shi et al., 2004). Indicators of DNA damage, oxidative stress and lipid peroxidation

☆ This paper has been recommended for acceptance by David Carpenter.

* Corresponding author. Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita–ku, Sapporo, 060–0818, Japan.
E-mail address: ishizum@vetmed.hokudai.ac.jp (M. Ishizuka).

https://doi.org/10.1016/j.envpol.2017.12.005
0269-7491/

such as 8-hydroxy-2-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) have widely been used to determine the health effects of human exposure to metals (Chen et al., 2005).

Besides DNA damage and lipid peroxidation, various epidemiological studies have also found and reported associations between heavy metal/metalloid (including arsenic, cadmium, copper, manganese, nickel, lead) exposure and the occurrence of respiratory effects such as asthma, rhinitis, wheeze, bronchitis, and allergies (Gehring et al., 2015; Huang et al., 2016).

The growing rate of industrialization (including mining), and resulting increases in economic activity and population growth in Kumasi, Ghana, has led to increased pollution of the environment (Bortey-Sam et al., 2014). Studies of environmental contamination and possible health risks due to metal exposure via medicinal herbs (Nkansah et al., 2016a), geophagic white clay (Nkansah et al., 2016b), food (Nkansah et al., 2016c), dust (Nkansah et al., 2015), soils (Akoto et al., 2016, 2017) and streams (Akoto et al., 2010) within Kumasi metropolis have been reported. Furthermore, levels of zinc, arsenic, copper and nickel in livers of wild rats sampled in Kumasi (Bortey-Sam et al., 2015a) were higher compared to the levels in wild rats sampled around mining sites in Kabwe, Zambia (Nakayama et al., 2013). Despite these reports and estimated risks, there is no study to assess the impact of metal exposure to Kumasi residents.

In Ghana, there are an estimated 16,000 cancer cases annually and also an increase in occurrence of respiratory disease (GLOBACAN, 2008; WHO, 2011). In 2012, the estimated cancer incidence in Kumasi was 11.9 per 100,000 and was higher in females (15.7 per 100,000) than males (7.3 per 100,000) (Laryea et al., 2014). Due to the high cancer incidence and respiratory symptoms in Kumasi (GLOBACAN, 2008; Laryea et al., 2014; WHO, 2011), and unavailability of research on the epidemiology and risks of metal exposure to residents, the objectives of this study were to: explore the potential associations between metal exposure and occurrence of respiratory diseases; assess the relationship between metal exposure and incidence of oxidative stress; find the association between urinary concentrations of metals, MDA, 8-OHdG with age and sex.

2. Materials and methods

2.1. Sampling

Urine is considered the main excretory pathway for metals and a better medium for biomonitoring metal exposure (Smolders et al., 2014). Heavy metals and metalloid concentrations in urine could be an indication of both long and short term exposures (Crinnion, 2010). In view of this, human urine (n = 190; 57 males and 133 females) was collected in the morning from the general population of three urban sites (Atonsu, Manhyia and Tafo) in Kumasi (Fig. 1). Samples were collected into corning tubes (Corning Incorporated, New York, USA) in January to February of 2015. Manhyia is in close proximity to Kejetia (1.1 km apart), Adum (1.5 km apart) and Romanhill (1.2 km apart), where soils were polluted with metals (Akoto et al., 2017). In previous studies, concentrations of metals were highest in the livers of wild rats trapped in Adum compared to other sites in Kumasi (Bortey-Sam et al., 2015a). Tafo is also 2.3 and 2.6 km from Suame and Mbrom, respectively, whose soils were polluted with metals (Akoto et al., 2017).

Moreover, 12 human urine samples (7 males and 5 females) were collected from Kwame Nkrumah University of Science and Technology campus (KNUST) and used as reference/control samples, even though metal exposure via consumption or inhalation was possible. KNUST, a university in Kumasi, has minimal vehicular motion and no industrial activities. In previous studies, heavy metals and metalloid levels in KNUST soils were low compared to recommended levels (Akoto et al., 2016, 2017). In addition, particulate matter and soil samples from KNUST have been used as controls in previous studies of environmental contaminants (Bortey-Sam et al., 2013, 2014; Bortey-Sam et al., 2015b).

For quality control purposes, urine was collected from 4 children in residential areas of KNUST to form a composite. Composite samples were used to give a more representative measure and also to account for any variabilities in heavy metals and metalloid concentrations. Since humans could be exposed to metals through various sources, the sample was measured several times to confirm the concentration.

During the sampling process, participant's information, including age, gender, body weight, height, place of residence, occupation, and personal lifestyle including smoker/non-smoker, were obtained through face-to-face interviews. Further, information on respiratory symptoms related to metals such as asthma, wheeze, tachycardia, bronchitis and rhinitis (Gehring et al., 2015; Huang et al., 2016) were collected. The Ethical/Institutional Review Board of Ghana Health Service (GHS) and Council for Scientific and Industrial Research (CSIR), Accra, Ghana, approved this study. Written and informed consent was obtained from each participant and parents gave consent and completed questionnaires on behalf of their children. The samples collected were kept frozen at the Department of Chemistry, KNUST, Ghana. Later the samples were transported to the Toxicology laboratory of the Graduate School of Veterinary Medicine, Hokkaido University, Japan, and stored at −30 °C until analysis.

2.2. Sample extraction and analysis

2.2.1. Heavy metals and a metalloid

Method described by Yabe et al., (2015) was used for the extraction of heavy metals and a metalloid from the urine samples collected. Briefly, 1 mL of each urine was transferred into a digestion vessel and 5 mL of 60% nitric acid (Kanto Chemical) and 1 mL of 30% hydrogen peroxide (Kanto Chemical) were added. Sample digestion (Speedwave MWS-2; Berghof) was for 52 min and up to 190 °C. The digested samples were transferred into corning tubes and diluted to 10 mL with de-ionized water (Milli-Q). Concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn) in each urine were measured by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, Japan).

2.2.2. Malondialdehyde, MDA (elisa kit)

Concentrations of urinary MDA were measured (based on instructions from manufacturer) using a UV-VIS Spectrophotometer (UV-2600 Shimadzu Corporation, Kyoto, Japan). Briefly, 10 μL of butylated hydroxytoluene (BHT) reagent was transferred into a vial and 250 μL of calibrator (0, 1, 2, 3 and 4 μM) or urine was added. After the addition of 250 μL each of 1 M phosphoric acid and 2-thiobarbituric acid (TBA) reagent, the solution was vortexed vigorously and incubated at 60 °C for 1 h. The mixture was transferred into a cuvette and spectra was recorded from 400 to 700 nm after it was centrifuged at 10,000×*g* for 2–3 min. 3rd derivative analysis was performed at 514 nm.

2.2.3. 8-Hydroxy-2-deoxy-guanosine (8-OHdG)

Extraction and analysis of urine sample for 8-OHdG followed the method described by Bortey-Sam et al., (2017). Briefly, urine (1 mL) was diluted with HPLC grade water (2 mL) after spiking with 25 ng/mL of (15N5) 8-OHdG (internal standard). Prior to sample loading, the Oasis HLB cartridge (3 cc, 60 mg; Waters Corporation, Milford, MA, USA) was primed with I mL each of methanol and water. The solid-phase extraction cartridge was then washed with 3 mL of water and the target analyte (8-OHdG) eluted with 3 mL of water:

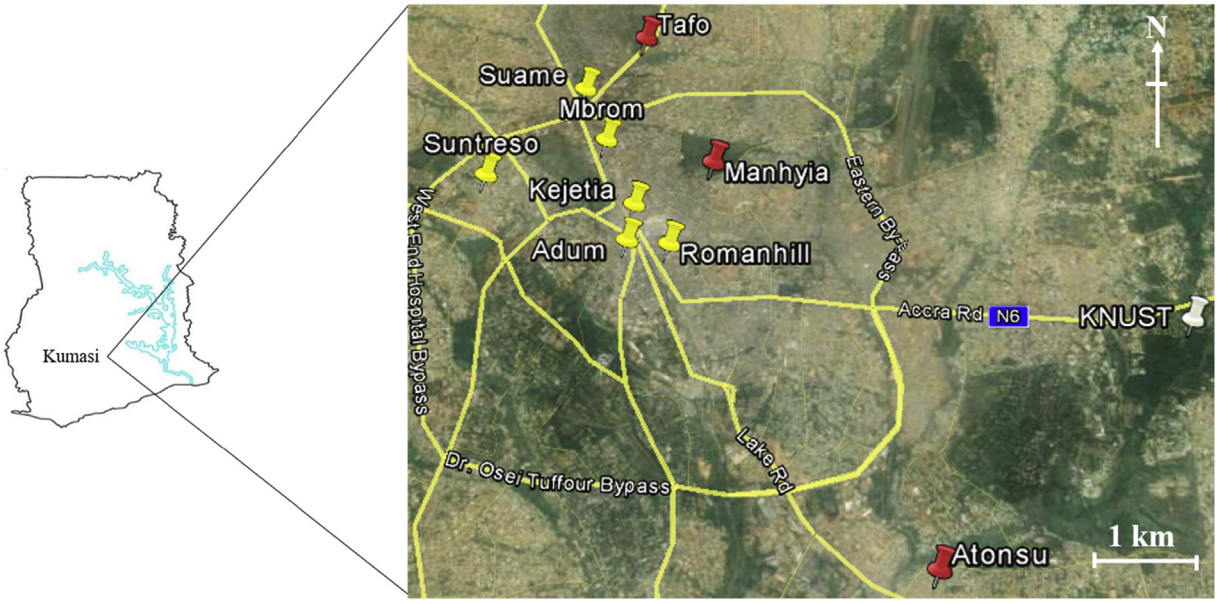

Fig. 1. Map showing human urine sampling locations in Kumasi, Ghana (yellow pins indicate city centre and environs contaminated with metals; red pins indicate human urine sites; white pin indicate KNUST campus). Obtained from (Bortey-Sam et al., 2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

acetonitrile (1:1, v/v). The eluate was evaporated to near dryness under nitrogen gas. The residue was re-dissolved in water (100 μL), and 10 μL injected into the LC–MS/MS. A Phenomenex Gemini 3u C18 110A column (150 mm × 2 mm i.d., 4 μm, Phenomenex, California, USA) with a guard column was used for the separation of 8-OHdG and (15N5) 8-OHdG in urine. Gradient elution was as follows: 0.0–1.0 min, 5% B; 1.01–3.00 min, 50% B; 3.01–6.00 min, 5% B. Multiple reaction monitoring (MRM) in negative ionization mode was used to identify the target analytes at a column temperature of 40 °C. Mobile phases A (0.1% formic acid) and B (100% methanol) were pumped at a flow rate of 250 μL/min (Bortey-Sam et al., 2017).

2.3. Creatinine concentrations in human urine

To compensate for variations in urine dilution, urinary creatinine was used to adjust concentrations of metals, MDA and 8-OHdG. Concentrations of creatinine in urine were determined based on the manufacturer's instructions (Arbor Assays, Michigan, USA). Briefly, 100 μL of DetectX® Creatinine Reagent (Arbor Assays, Michigan, USA) was added to 50 μL of sample, blank (water), or standards into clear plate wells. Prior to 30 min incubation (at room temperature), the sides of the plate was tapped for adequate mixing and the plate was covered with a plate sealer and pressed to seal adequately. The optical density produced from the plate reader well (Muiltiskan GO, Thermo Scientific, Vantaa, Finland) was read at 490 nm. To calculate the creatinine concentrations, a 4PLC built-in software was used and results expressed in g/L. Obtained creatinine concentrations (g/L) (mean; [range]) in human urine in Atonsu (1.57 ± 0.969; [0.0398–4.35]), Manhyia (1.76 ± 1.33; [0.202–5.96]), Tafo (2.30 ± 1.59; [0.0185–7.43]) and KNUST (2.71 ± 1.45; [1.46–5.58]) were used to adjust the respective urinary metal concentrations.

2.4. Quality control and quality assurance

2.4.1. Heavy metals and a metalloid

After every 10 sample analyses, blanks and duplicates were analysed and the Relative Standard Deviation (RSD) obtained for duplicate runs was ≤4%. Calibration curves using standard solutions were run and the linearity obtained for each metal was greater than 0.999. Analytical-reagent grade chemicals and standard stock solutions were used (Wako Pure Chemicals). The detection limits (μg/L) were 0.009 (As), 0.001 (Cd), 0.003 (Co), 0.003 (Cr), 0.008 (Cu), 0.002 (Pb), 0.034 (Mn), 0.003 (Ni) and 0.019 (Zn). Heavy metals and metalloid concentrations in human urine were expressed in μg/g creatinine.

The urine samples of children collected from residential areas of KNUST was used for quality control and recovery tests. The samples were spiked with standard solutions of metals and digested using the method described. The recovery rates of the spiked urine ranged from 95 (Pb) - 98% (Cd). Concentrations of metals in urine sample used for this purpose were below the respective limits of detection (LODs) and differences between concentrations before and after spiking was used to calculate the recoveries.

2.4.2. MDA and 8-OHdG

For 8-OHdG, (15N5) 8-OHdG was used as internal standard and spiked into urine prior to sample preparation and extraction. Internal standard method with a five-point calibration (1, 5, 10, 50 and 100 ng/mL) was used for quantification. The average linearity of the calibration standards for both MDA and 8-OHdG were greater than 0.99. The LOD and limit of quantification (LOQ) for 8-OHdG were 0.0196 and 0.6 ng/mL, respectively, and average recovery ([15N5] 8-OHdG) was 86 ± 9.8%. After every 10 samples, spiked solvent blanks (with 8-OHdG only) and duplicate samples were analysed and average internal standard recovery for spiked solvents blanks was 104 ± 8.7%. The %RSD for duplicate samples were less than or equal to 10% (MDA and 8-OHdG). LOD and LOQ for MDA were 0.205 and 0.63 μM, respectively.

2.4.3. Creatinine

Quantitation was performed based on a seven–point calibration (0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 mg/dL) and the average linearity of the calibration standard was greater or equal to 0.9996. LODs and LOQs were calculated based on 3SD/S and 10SD/S, respectively (SD is the standard deviation of five replicate

measurements of the target analyte and S is the slope of the calibration curve). LOD and LOQ of creatinine were 0.00151 and 0.00505 g/L, respectively. Duplicate samples were run after every batch of 10 samples and the %RSD was 2.99 ± 2.30. Blank samples were also run after every 11 samples.

2.5. Data analysis

IBM SPSS v 20 (SPSS Inc., Illinois, USA) was used for statistical analyses and the normality of the data was tested using Kolmogorov–Smirnov (K–S) and Shapiro-Wilks tests. A value of LOD/2 was assigned to metals/metalloid concentrations below their respective LODs. The central tendency of the analyte concentrations was illustrated with the geometric mean concentrations (Ott, 1990). To compare urinary concentrations of metals from the study areas, ANOVA and Tukey tests were performed, after data was normalized by log transformation, and a p value less than 0.05 was considered significant. Pearson's correlation of logged data was used to determine the association between metals, MDA, 8-OHdG and age. The distribution of metals/metalloid, MDA and 8-OHdG between male and female participants was done using Student's T-Test and statistical significance was at p less than 0.05. Odds ratios (ORs) at 95% confidence interval (CI) was used to determine the association between exposure to As and occurrences of respiratory symptoms. This was derived using logistic regression model. Arsenic was treated as a continuous variable in the logistic regression. Regression models were adjusted for covariates such as age and sex. Statistical significance was set at $p < .05$ and performed with JMP 10 statistical software (SAS Institute).

3. Results and discussion

3.1. Urinary levels of heavy metals and a metalloid

The order of the geometric mean concentrations (adjusted by urinary creatinine; GM_{creat}) of heavy metal and metalloid from all study sites in Kumasi was Zn (335 ± 340) > As (49.8 ± 52.2) > Cu (14.7 ± 28.9) > Ni (2.36 ± 6.33) > Cr (0.825 ± 7.32) > Pb (0.716 ± 7.59) ≥ Co (0.712 ± 3.57) > Mn (0.276 ± 2.35) ≥ and Cd (0.240 ± 2.22) μg/g creatinine. The urinary concentrations of all metals measured varied significantly (p <0.01) (K-S and S-W's tests). The results of GM_{creat} indicated that Co, Cu, Zn and As were detected in all samples (100%) while the detection rate for urinary Cr was 78%, Mn (89%), Ni (79%), Cd (99%), Pb (76%), MDA (95%) and 8-OHdG (59%).

Urinary concentrations of Ni and Cr (except Manhyia) were significantly higher (p = .0002–.0145) in participants who lived in Atonsu compared to other sites including KNUST (Table 1). Additionally, urinary concentrations of As and Cd in participants who lived in urban sites were significantly higher (p = .0001–.01) compared to KNUST participants (Table 1). Although not significant (p = .0798–.838), concentrations of Mn, Co and Cd (significantly lower in KNUST) in urine were higher in Atonsu participants while highest levels of Pb and Cu (significantly lower in KNUST) were detected in Tafo participants. The high urinary metals could be due to high metal exposure to residents. Previous studies reported that although metal concentrations in Atonsu soils were below recommended levels, the soils were enriched with Zn, Cd, Cr and Pb (Akoto et al., 2017). Tafo on the other hand is close to communities (Suame and Mbrom) polluted with metals and filled with light scale industries (Akoto et al., 2017).

Metals and metalloid concentrations detected in urine of participant's from the present study were compared to studies conducted in the US (Caldwell et al., 2009; CDC, 2005), Czech Republic (Benes et al., 2002), Spain (Aguilera et al., 2010), France (de Burbure et al., 2006), Italy (Alimonti et al., 2000) China (Huang et al., 2016; Lu et al., 2016) and Democratic Republic of Congo (Banza et al., 2009) (Table S1). The results (Table S1) showed that all metal measured in this study were comparable or lower than previous studies with the exception of urinary As concentrations which were higher in this study. From Table S2, the unadjusted urinary concentrations (ng/mL) of metals (except Co) from all sites were on the average (11.3 (Zn) - 83.3% (As)) higher compared to the unadjusted reference values suggested by the Canadian Health Measure (Saravanabhavan et al., 2016); Fourth National Report on Human Environmental Chemicals, USA (Crinnion, 2010) and Human Biomonitoring Commission, Germany (Schulz et al., 2009).

In addition to water and food consumption, soil and dust are other possible ways of exposure to metals/metalloids especially children via hand-to-mouth practices (Berglund et al., 2011). Residents of various countries including Ghana, consume large amounts of geophagic white clay for religious, cultural, nutritional, and medicinal reasons as well as in response to famine and pregnancy-related cravings (Mathee et al., 2014; Nkansah et al., 2016b). Also, exposure to metals could be through the intake of medicinal herbs (Nkansah et al., 2016a) frequently used in the treatment of various ailments. Lower levels of urinary metals in KNUST participants could be due to the low vehicular movement and industrial activities, and point source of metal pollution was low.

The high levels of urinary As in participants could be attributed to the gold mining activities in some parts of Kumasi. Residents could be exposed because of the composition of the ore containing the gold. After blasting the gold bearing rock, miners roast the ore and this leads to the production and distribution of arsenic trioxide gas (Amonoo-Neizer et al., 1996). In addition, exposure to organic As was associated with consumption of sea foods such as shellfish (Aguilera et al., 2010), although the recommended total urinary As concentration was estimated to be 27 ng/mL by the Canadaian Health Measures (Saravanabhavan et al., 2016). Highest urinary concentrations of As (336, 297 and 234 μg/g creatinine) were detected in urine of participants who complained of asthma, diabetes, rhinitis and tachycardia, symptoms which have been associated with As exposure (Maull et al., 2012; Parvez et al., 2008, 2010, 2011; Saha et al., 1999).

3.2. Association between urinary metals, MDA, 8-OHdG with age

As shown in Table 2, there was no significant association (p = .424–.928) between urinary metal concentrations and age from KNUST participants and participants who lived in urban areas. Previous studies reported a positive correlation between urinary Cd levels and age while a negative association was observed between other metals and age (Banza et al., 2009). Results of this study further showed that urinary MDA was highest in age group 3–20 years. The effects of metal exposure is more pronounced in children than adults because their immune and nervous systems are not fully developed (Olsen, 2000). Moreover, the breathing rate of children is higher and their consumption rate per body weight is also higher than adults (Schwartz, 2004).

Although significant correlation (p = .443) between age and urinary Cd concentrations was not observed (Table 2), levels were highest in ages 61–85 years compared to the other age groups (Table 3). This is possibly because concentrations of Cd in urine indicates long-term accumulation and consequently higher in the elderly (Paschal et al., 2000).

3.3. Association between urinary metals, MDA, 8-OHdG with sex

As shown in Table 4, significantly higher levels (p = .0089–.017) of Co, As and Cd were detected in urine of female participants

Table 1
Creatinine adjusted metal concentrations (μg/g creatinine) in human urine, collected in 2015, from four sites in Kumasi, Ghana.

Sample site	n		Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	MDA	8-OHdG
Atonsu	82	GM_{creat}	1.50^{a}	0.375^{a}	0.855^{a}	4.20^{a}	15.7^{a}	386^{a}	67.3^{a}	0.289^{a}	0.615^{a}	54.9^{a}	1.02^{a}
		SD	2.21	2.94	4.00	6.30	25.6	291	61.1	0.980	8.27	68.2	1.99
Manhyia	51	GM_{creat}	0.961^{ab}	0.220^{a}	0.734^{a}	1.19^{b}	16.1^{a}	350^{a}	43.5^{b}	0.227^{a}	0.636^{a}	40.1^{ab}	0.876^{a}
		SD	1.17	2.01	1.17	5.66	13.6	409	31.6	0.254	0.897	55.9	1.82
Tafo	57	GM_{creat}	0.442^{c}	0.259^{a}	0.569^{a}	1.76^{b}	16.7^{a}	290^{a}	42.8^{b}	0.231^{a}	0.962^{a}	32.7^{b}	0.911^{a}
		SD	3.84	1.45	4.36	6.58	23.2	357	45.5	3.861	9.601	48.7	3.78
KNUST	12	GM_{creat}	0.180^{bc}	0.115^{a}	0.481^{a}	0.524^{b}	5.67^{b}	180^{a}	15.9^{c}	0.074^{b}	0.312^{a}	23.7^{ab}	0.779^{a}
		SD	0.132	0.310	0.359	0.970	2.88	80.9	18.1	0.046	0.302	11.9	0.270

n: number of samples; GM_{creat}: geometric mean concentration adjusted by creatinine; SD: standard deviation; different letters (a, b and c) within a column indicates significant differences ($p < 0.05$).

Table 2
Correlation analysis between urinary concentrations (μg/g creatinine) of metals, MDA, 8-OHdG and age of participants in Kumasi, Ghana.

Variables	Age/years	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	MDA	8-OHdG
Urban sites												
Age/years	1	−0.0643	−0.0585	-0.337^{*}	−0.0174	0.0698	0.0493	0.0466	0.11	−0.0314		
MDA	−0.181	0.532^{**}	0.381^{**}	0.669^{**}	0.592^{**}	0.660^{**}	0.585^{**}	0.777^{**}	0.593^{**}	0.586^{**}	1	
8-OHdG	0.0812	0.171	0.217	0.218	0.223	0.360^{*}	0.224	0.488^{**}	0.267	0.108	0.453^{**}	1
KNUST (Control site)												
Age/years	1	0.0352	−0.142	−0.305	−0.077	−0.250	−0.205	−0.217	−0.254	−0.707		
MDA	0.0451	0.357	0.339	0.271	0.311	0.332	0.348	0.371	0.263	0.182	1	
8-OHdG	−0.567	0.490	−0.013	0.411	0.077	0.340	0.242	0.270	0.224	0.221	0.340	1

*: Indicates significance at $p < .05$.
** : Indicates significance at $p < .01$.

Table 3
Age differences in urinary concentrations (μg/g creatinine) of metals, MDA, and 8-OHdG among participants in Kumasi, Ghana.

Age groups	n		Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	MDA	8-OHdG
3–20	36	GM_{creat}	1.66^{a}	0.400^{a}	1.24^{a}	3.26^{a}	20.3^{a}	425^{a}	51.6^{a}	0.296^{a}	1.18^{a}	50.5^{a}	0.557^{a}
		SD	1.02	0.43	4.59	4.74	17.2	572	51.2	0.619	8.18	98.9	0.558
21–40	102	GM_{creat}	0.801^{a}	0.249^{a}	0.700^{ab}	2.36^{a}	14.2^{a}	297^{a}	49.3^{a}	0.224^{a}	0.664^{a}	43.0^{a}	1.07^{a}
		SD	3.39	2.75	2.82	5.61	24.9	256	51.1	3.08	7.87	48.2	3.48
41–60	33	GM_{creat}	0.554^{a}	0.279^{a}	0.472^{b}	2.99^{a}	17.4^{a}	336^{a}	63.9^{a}	0.299^{a}	0.758^{a}	42.2^{a}	1.12^{a}
		SD	2.57	0.653	0.414	7.12	27.3	238	57.8	0.269	1.39	70.3	2.21
61–85	31	GM_{creat}	1.25^{a}	0.421^{a}	0.572^{ab}	2.59^{a}	17.6^{a}	528^{a}	82.7^{a}	0.400^{a}	0.728^{a}	47.9^{a}	0.604^{a}
		SD	1.76	0.330	8.69	5.93	6.93	291	36.6	2.02	16.7	36.9	0.539

n: number of samples; GM_{creat}: geometric mean concentration adjusted by creatinine; SD: standard deviation; different letters (a and b) within a column indicates significant differences ($p < .05$) among age groups.

compared to males. However, urinary MDA and 8-OHdG were comparable (Table 4) and this is similar to outcomes obtained by Lu et al., (2016). Although not significant ($p = .054–.383$), urinary concentrations of Cr, Mn, Cu and Zn were also higher in females than males which is similar to results obtained by Banza et al., (2009). The significantly higher urinary Cd in females was similar to results obtained in other studies (Berglund et al., 2011; Castano et al., 2012; Paschal et al., 2000; Vahter et al., 2007). Iron deficiency has mainly been related to gender differences in urinary Cd excretion. This influences high levels of duodenal divalent transporter which results in the rise in transport and absorption of Cd (Berglund et al., 2011; Paschal et al., 2000; Vahter et al., 2007).

Sex differences in Cu levels have been reported and females recorded higher concentrations than males (Benes et al., 2002). The higher urinary Cu levels in women could be attributed to hormonal changes that occur in puberty (Wapnir, 1998). Additionally, significantly higher urinary Mn was detected in women than men and this trend could be attributed to biological differences in the way females

Table 4
Sex differences in urinary metal, MDA and 8-OHdG concentrations (μg/g creatinine) in Kumasi, Ghana.

Sex	n		Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	MDA	8-OHdG
Female	138	GM_{creat}	0.903^{a}	0.331^{a}	0.812^{a}	2.24^{a}	16.5^{a}	362^{a}	56.1^{a}	0.283^{a}	0.687^{a}	42.3^{a}	0.962^{a}
		SD	2.84	2.67	3.92	11.5	22.6	334	57.9	2.692	8.46	60.1	2.86
Male	64	GM_{creat}	0.678^{a}	0.187^{a}	0.547^{b}	3.00^{a}	14.2^{a}	291^{a}	40.8^{b}	0.175^{b}	0.838^{a}	45.0^{a}	0.910^{a}
		SD	2.13	0.529	2.80	7.63	21.5	215	36.3	0.314	5.08	59.4	2.33

n: number of samples; GM_{creat}: geometric mean concentration adjusted by creatinine; SD: standard deviation; different letters (a and b) within a column indicate significant differences (Student's T-Test; $p < 0.05$).

and males handle Mn (Berglund et al., 2011). Lindberg et al. reported that women had significantly higher dimethylarsinic acid (DMA) concentrations than men, because they (women) can more efficiently methylate arsenic than men (Lindberg et al., 2008). Although not significant ($p = .371$), urinary concentrations of Pb were higher in males than females. In a study by Berglund et al., higher levels of Pb were detected in urine of men than women (Berglund et al., 2011). This results was opposite from previous studies where women excreted higher urinary Pb than men (Castano et al., 2012). These in addition to other factors (such as differences in exposure levels, lifestyle etc.) could explain the sex difference in urinary excretion of metals although the mechanisms underlying these sex differences remain unknown (Howe et al., 2016).

3.4. Association between metal exposure and occurrence of respiratory symptoms

OR was performed for all metals, however, the study focused on As since it was the most toxic substance (ATSDR, 2015) and the second most abundant metal detected in human urine in Kumasi. In addition, concentrations of urinary As in this study was higher than previously conducted studies in China, Congo, Spain and USA (Table S1). Moreover, urinary As was on average 83% higher (from all study sites) compared to recommended values.

The study revealed that exposure to As was significantly ($p = .041–.043$) associated with increased odds of asthma (OR = 2.76, CI: 1.11–6.83) and tachycardia (OR = 3.93, CI: 1.01–15.4) in Kumasi residents (Table 5). In a study by Huang et al., urinary concentrations of As were significantly and positively associated with the occurrence of asthma when the metalloid was considered as a continuous variable and when divided into quantiles (Huang et al., 2016). Similarly, As exposure has been associated with (i) occurrence of lung dysfunction (ii) increased mortality due to respiratory diseases (Parvez et al., 2011). Additionally, inhalation of As dust or fumes during milling of ores or mining (which is a common practice in Kumasi) often resulted in chronic cough, laryngitis, bronchitis and rhinitis (Saha et al., 1999). In other cohort studies, chronic cough, chest sounds, shortness of breath, blood in sputum and other respiratory symptoms were observed due to As exposure (Parvez et al., 2010). Additionally, positive association between urinary As and serum Clara cell protein (CC16; a novel biomarker of respiratory illness) was found, with urinary As levels inversely related with lung function (Parvez et al., 2008).

Environmental factors have been associated with the occurrence of asthma (Huang et al., 2016) and as a results, living in metal contaminated areas could increase the risks of respiratory disease among residents.

3.5. Human health risk implications

As shown in Table 2, there was no association ($p = .180–.972$) between urinary concentrations of metals, MDA and 8-OHdG in KNUST participants. However, in urine of participants at urban sites, significant association ($p = <0.0001–0.0063$) was noted between metals and MDA. Additionally, concentrations of urinary As also correlated significantly ($p = .0003$) with 8-OHdG. This trend indicates the possibility of lipid peroxidation or DNA damage although these products have also been associated with the presence of cardiovascular diseases, atherosclerosis, diabetes and cancers (Wu et al., 2004). Aflanie et al. concluded that human exposure to As and Cd could increase MDA levels and cause oxidative stress and inflammation (Aflanie et al., 2015). Lu et al. also reported a correlation between As and 8-OHdG (Lu et al., 2016). Arsenic could bind to thiols and this has been considered crucial in increasing 8-OHdG levels (Valko et al., 2007). In previous reports, urinary As was associated with diabetes (Maull et al., 2012). Several metals (e.g. As, Cd, Pb) are also known to impair kidney function and increase different cancer risks, while others could affect the nervous and cardiovascular systems (e.g., As, Cd, Mn, Pb) (EFSA, 2011; Straif et al., 2009; WHO, 2008). In previous studies, oxidative stress correlated with allergic inflammatory diseases (Bartsch and Nair, 2004), obesity and atherosclerosis (Kobayashi et al., 2011; Wu et al., 2004) and these were some symptoms participants complained of during the face-to-face interview. 10% of participants in this study were diabetics and 2% had arthritis. Of the 33 participants whose body mass indexes were calculated, 11 were overweight and 7 were obese.

Table 5
Adjusted odds ratios (OR; 95% CI) for the presence or absence of respiratory symptoms in Kumasi residents due to arsenic exposure.

Metalloid	Clinical symptom	OR	CI		*p* value
As	Asthma	2.76	1.11	6.83	.043
	RTI	2.42	0.075	7.86	.618
	Tachycardia	3.93	1.01	15.4	.041
	Rhinitis	0.848	0.145	4.97	.855
	Dyspnea	1.09	0.344	3.47	.880

OR: Odds ratio; CI: 95% Confidence Interval; RTI: respiratory tract infection; The models were adjusted for age and sex.

3.6. Limitations of the study

The major limitation to this study was the small sample size which could have resulted in wide CI in the regression analysis. Additionally, participants were not medically examined and this could have resulted in false information.

4. Conclusions

Urinary metal/metalloid concentrations were studied in Kumasi residents, and although Zn was the most abundant, urinary As was higher in 83% of participants compared to reference values. The study revealed that urinary concentrations of metals, MDA and 8-OHdG were higher in urban sites participants compared to the control site. Females excreted significantly higher levels of Co, As and Cd than males. As exposure was significantly associated with increased odds of asthma and tachycardia. Although no relationship was found between urinary metals and age, Cd and MDA levels were highest in age groups 61–85 and 3–20 years, respectively. Exposure of Kumasi residents to heavy metals and a metalloid increased the occurrences of lipid peroxidation and/or DNA damage.

Acknowledgements

We are highly grateful to all the participants, hospital administration and staff, the Ghana Health Service and Council for Scientific and Industrial Research, Ghana, for facilitating this study. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No. 24405004 and No. 24248056), Y. Ikenaka (No. 26304043, 15H0282505, 15K1221305), S. Nakayama, H. Mizukawa and the foundation of JSPS Core to Core Program (AA Science Platforms) and Bilateral Joint Research Project (PG36150002 and PG36150003). We also acknowledge the financial support by The Mitsui & Co., Ltd. Environment Fund and Sumitomo, and Soroptimist Japan Foundation We are grateful to Mr. Takahiro Ichise and Ms. Mio Yagihashi (Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University) for technical support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.12.005.

References

Aflanie, I., Muhyi, R., Suhartono, E., 2015. Effect of heavy metal on malondialdehyde and advanced oxidation protein produtcs concentration: a focus on arsenic, cadmium, and mercury. J. Med. Biogr. 4, 332–337.

Aguilera, I., Daponte, A., Gil, F., Hernandez, A.F., Godoy, P., Pla, A., et al., 2010. Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialised area of ria of huelva (sw Spain). Environ. Int. 36, 563–569.

Akoto, O., Bruce, T.N., Darko, G., 2010. Chemical and biological characteristics of streams in the owabi watershed. Environ. Monit. Assess. 161, 413–422.

Akoto, O., Nimako, C., Asante, J., Bailey, D., 2016. Heavy metals enrichment in surface soil from abandoned waste disposal sites in a hot and wet tropical area. Environ. Prog. 3, 747–761.

Akoto, O., Bortey-Sam, N., Ikenaka, Y., Nakayama, S.M.M., Baidoo, E., Yohannes, Y.B., et al., 2017. Contamination levels and sources of heavy metals and metalloid in surface soils in the kumasi metropolis, Ghana. J. Health Pollut. 7, 28–39.

Alimonti, A., Petrucci, F., Krachler, M., Bocca, B., Caroli, S., 2000. Reference values for chromium, nickel and vanadium in urine of youngsters from the urban area of rome. J. Environ. Monit.: JEM 2, 351–354.

Amonoo-Neizer, E., Nyamah, D., Bakiamoh, S., 1996. Mercury and arsenic pollution in soil and biological samples around the mining town of obuasi, Ghana. Water Air Soil Pollut. 91, 363–373.

ATSDR, 2015. Priority List of Hazardous Substances. ATSDR (Agency for Toxic Substance and Disease Registry), Division Of Toxicology & Human Health Sciences.

Banza, C.L., Nawrot, T.S., Haufroid, V., Decree, S., De Putter, T., Smolders, E., et al., 2009. High human exposure to cobalt and other metals in katanga, a mining area of the democratic republic of Congo. Environ. Res. 109, 745–752.

Bartsch, H., Nair, J., 2004. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Canc. Detect. Prev. 28, 385–391.

Benes, B., Spevackova, V., Smid, J., Cejchanova, M., Kaplanova, E., Cerna, M., et al., 2002. Determination of normal concentration levels of cd, pb, hg, cu, zn and se in urine of the population in the Czech Republic. Cent. Eur. J. Publ. Health 10, 3–5.

Berglund, M., Lindberg, A.L., Rahman, M., Yunus, M., Grander, M., Lonnerdal, B., et al., 2011. Gender and age differences in mixed metal exposure and urinary excretion. Environ. Res. 111, 1271–1279.

Bortey-Sam, Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Yohannes, Y.B., Baidoo, E., et al., 2015a. Exposure levels of polycyclic aromatic hydrocarbons (pahs) and heavy metals in wild rats in kumasi, Ghana. 7th Int. Toxicol. Symp. Afr. 2.

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Yohannes, Y.B., Baidoo, E., et al., 2015b. Levels, potential sources and human health risk of polycyclic aromatic hydrocarbons (pahs) in particulate matter (pm10) in kumasi, Ghana. Environ. Sci. Pollut. Res. 22, 9658–9667.

Bortey-Sam, N., Akoto, O., Ikenaka, Y., Nakayama, S.M.M., Ishizuka, M., 2013. Determination of benzo[a]pyrene levels in ambient air and the source of polycyclic aromatic hydrocarbons using a diagnostic ratio method in Ghana. Jpn. J. Vet. Res. 61, S72–S74.

Bortey-Sam, N., Ikenaka, Y., Nakayama, S.M., Akoto, O., Yohannes, Y.B., Baidoo, E., et al., 2014. Occurrence, distribution, sources and toxic potential of polycyclic aromatic hydrocarbons (pahs) in surface soils from the kumasi metropolis, Ghana. Sci. Total Environ. 496, 471–478.

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Asante, K.A., Baidoo, E., et al., 2017. Oxidative stress and respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (pahs) in kumasi, Ghana. Environ. Pol. 228, 311–320.

Caldwell, K.L., Jones, R.L., Verdon, C.P., Jarrett, J.M., Caudill, S.P., Osterloh, J.D., 2009. Levels of urinary total and speciated arsenic in the us population: national health and nutrition examination survey 2003-2004. J. Expo. Sci. Environ. Epidemiol. 19, 59–68.

Castano, A., Sanchez-Rodriguez, J.E., Canas, A., Esteban, M., Navarro, C., Rodriguez-Garcia, A.C., et al., 2012. Mercury, lead and cadmium levels in the urine of 170 Spanish adults: a pilot human biomonitoring study. Int. J. Hyg Environ. Health 215, 191–195.

CDC, 2005. National Center for Environmental Health. Third National Report on Human Exposure to Environmental Chemicals. Nceh pub, Atlanta, ga. No. 05-0570; 2005.

Chen, C.Y., Qu, L.Y., Li, B., Xing, L., Jia, G., Wang, T.C., et al., 2005. Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2 '-deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. Clin. Chem. 51, 759–767.

Crinnion, W.J., 2010. The cdc fourth national report on human exposure to environmental chemicals: what it tells us about our toxic burden and how it assists environmental medicine physicians. Alternative Med. Rev. 15, 101–108.

de Burbure, C., Buchet, J.P., Leroyer, A., Nisse, C., Haguenoer, J.M., Mutti, A., et al., 2006. Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ. Health Perspect. 114, 584–590.

EFSA, 2011. Statement on tolerable weekly intake for cadmium. Efsa j 9 (2), 1975.

Gehring, U., Beelen, R., Eeftens, M., Hoek, G., de Hoogh, K., de Jongste, J.C., et al., 2015. Particulate matter composition and respiratory health: the piama birth cohort study. Epidemiology 26, 300–309.

GLOBACAN, 2008. Country Fast Stats, Ghana. Lyon, France: Iarc; 2008. International Agency for Research on Cancers.

Howe, C.G., Liu, X.H., Hall, M.N., Slavkovich, V., Ilievski, V., Parvez, F., et al., 2016. Associations between blood and urine arsenic concentrations and global levels of post-translational histone modifications in bangladeshi men and women. Environ. Health Perspect. 124, 1234–1240.

Huang, X.J., Xie, J.G., Cui, X.Q., Zhou, Y., Wu, X.J., Lu, W., et al., 2016. Association between concentrations of metals in urine and adult asthma: a case-control study in wuhan, China. PLoS One 11.

Kobayashi, S., Susa, T., Tanaka, T., Wada, Y., Okuda, S., Doi, M., et al., 2011. Urinary 8-hydroxy-2'-deoxyguanosine reflects symptomatic status and severity of systolic dysfunction in patients with chronic heart failure. Eur. J. Heart Fail. 13, 29–36.

Laryea, D.O., Awuah, B., Amoako, Y.A., Osei-Bonsu, E., Dogbe, J., Larsen-Reindorf, R., et al., 2014. Cancer incidence in Ghana, 2012: evidence from a population-based cancer registry. BMC Canc. 14, 362.

Lindberg, A.L., Ekstrom, E.C., Nermell, B., Rahman, M., Lonnerdal, B., Persson, L.A., et al., 2008. Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. Environ. Res. 106, 110–120.

Lu, S.Y., Ren, L., Fang, J.Z., Ji, J.J., Liu, G.H., Zhang, J.Q., et al., 2016. Trace elements are associated with urinary 8-hydroxy-2'-deoxyguanosine level: a case study of college students in guangzhou, China. Environ. Sci. Pollut. Res. 23, 8484–8491.

Magge, H., Sprinz, P., Adams, W.G., Drainoni, M.L., Meyers, A., 2013. Zinc protoporphyrin and iron deficiency screening: trends and therapeutic response in an urban pediatric center. J. Pediatr. 167, 361–367.

Mathee, A., Naicker, N., Kootbodien, T., Mahuma, T., Nkomo, P., Naik, I., et al., 2014. A cross-sectional analytical study of geophagia practices and blood metal concentrations in pregnant women in johannesburg, South Africa. SAMJ: S. Afr. Med. J. 104, 568–573.

Maull, E.A., Ahsan, H., Edwards, J., Longnecker, M.P., Navas-Acien, A., Pi, J.B., et al., 2012. Evaluation of the association between arsenic and diabetes: a national toxicology program workshop review. Environ. Health Perspect. 120, 1658–1670.

Nakayama, S.M.M., Ikenaka, Y., Hamada, K., Muzandu, K., Choongo, K., Yabe, J., et al., 2013. Accumulation and biological effects of metals in wild rats in mining areas of Zambia. Environ. Monit. Assess. 185, 4907–4918.

Nkansah, M.A., Fianko, J.R., Mensah, S., Debrah, M., Francis, G.W., Slawin, A.M.Z., 2015. Determination of heavy metals in dust from selected nursery and kindergarten classrooms within the kumasi metropolis of Ghana. Coke Chem. 1.

Nkansah, M.A., Hayford, S.T., Borquaye, L.S., Ephraim, J.H., Ng, C.A., 2016a. Heavy metal contents of some medicinal herbs from kumasi, Ghana. Cogent Environ. Sci. 2.

Nkansah, M.A., Korankye, M., Darko, G., Dodd, M., 2016b. Heavy metal content and potential health risk of geophagic white clay from the kumasi metropolis in Ghana. Toxicol. Rev. 3, 644–651.

Nkansah, M.A., Opoku, F., Ackumey, A.A., 2016c. Risk assessment of mineral and heavy metal content of selected tea products from the ghanaian market. Environ. Monit. Assess. 188, 1–11.

Olsen, J., 2000. Prenatal exposures and long-term health effects. Epidemiol. Rev. 22, 76–81.

Ott, W.R., 1990. A physical explanation of the lognormality of pollutant concentrations. J. Air Waste Manag. Assoc. 40, 1378–1383.

Parvez, F., Chen, Y., Brandt-Rauf, P.W., Bernard, A., Dumont, X., Slavkovich, V., et al., 2008. Nonmalignant respiratory effects of chronic arsenic exposure from drinking water among never-smokers in Bangladesh. Environ. Health Perspect. 116, 190–195.

Parvez, F., Chen, Y., Brandt-Rauf, P.W., Slavkovich, V., Islam, T., Ahmed, A., et al., 2010. A prospective study of respiratory symptoms associated with chronic arsenic exposure in Bangladesh: findings from the health effects of arsenic longitudinal study (heals). Thorax 65, 528–533.

Parvez, F., Chen, Y., Yunus, M., Zaman, R.U., Ahmed, A., Islam, T., et al., 2011. Associations of arsenic exposure with impaired lung function and mortality from diseases of the respiratory system: findings from the health effects of arsenic longitudinal study (heals). Epidemiology 22, S179.

Paschal, D.C., Burt, V., Caudill, S.P., Gunter, E.W., Pirkle, J.L., Sampson, E.J., et al., 2000. Exposure of the u.S. Population aged 6 years and older to cadmium: 1988-1994. Arch. Environ. Contam. Toxicol. 38, 377–383.

Saha, J., Dikshit, A., Bandyopadhyay, M., Saha, K., 1999. A review of arsenic poisoning and its effects on human health. Crit. Rev. Environ. Sci. Technol. 29, 281–313.

Saoudi, A., Zeghnoun, A., Bidondo, M.L., Garnier, R., Cirimele, V., Persoons, R., et al., 2012. Urinary arsenic levels in the French adult population: the French national nutrition and health study, 2006-2007. Sci. Total Environ. 433, 206–215.

Saravanabhavan, G., Werry, K., Walker, M., Haines, D., Malowany, M., Khoury, C., 2016. Human biomonitoring reference values for metals and trace elements in blood and urine derived from the canadian health measures survey 2007-2013. Int. J. Hyg Environ. Health 220, 189–200.

Schulz, C., Angerer, J., Ewers, U., Heudorf, U., Wilhelm, M., Human Biomonitoring Commission of the German Federal Environment A, 2009. Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German environmental survey on children 2003-2006 (geres iv). Int. J. Hyg Environ. Health 212, 637–647.

Schwartz, J., 2004. Air pollution and children's health. Pediatrics 113, 1037–1043.

Shi, H., Hudson, L.G., Liu, K.J., 2004. Oxidative stress and apoptosis in metal ion-induced carcinogenesis. Free Radic. Biol. Med. 37, 582–593.

Smolders, R., Koch, H.M., Moos, R.K., Cocker, J., Jones, K., Warren, N., et al., 2014. Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 1: Metals. Toxicol. Lett. 231, 249–260.
Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18, 321–336.
Straif, K., Benbrahim-Tallaa, L., Baan, R., Grosse, Y., Secretan, B., El Ghissassi, F., et al., 2009. A review of human carcinogens-part c: metals, arsenic, dusts, and fibres. Lancet Oncol. 10, 453–454.
Vahter, M., Akesson, A., Liden, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ. Res. 104, 85–95.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84.
Wapnir, R.A., 1998. Copper absorption and bioavailability. Am. J. Clin. Nutr. 67, 1054S–1060S.
WHO, 2008. Guidelines for Drinking Water Quality, Recommendations (Web Version), vol. 1. World health organization.
WHO, 2011. Noncommunicable diseases country profiles. Behav. Environ. Risk Factor.
Wu, L.L., Chiou, C.C., Chang, P.Y., Wu, J.T., 2004. Urinary 8-ohdg: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin. Chim. Acta J. Int. Fed. Clin. Chem. 339, 1–9.
Yabe, J., Nakayama, S.M., Ikenaka, Y., Yohannes, Y.B., Bortey-Sam, N., Oroszlany, B., et al., 2015. Lead poisoning in children from townships in the vicinity of a lead-zinc mine in kabwe, Zambia. Chemosphere 119, 941–947.