



Title	Environmental contamination in Ghana : Environmental fate and risk assessment of heavy metals and polycyclic aromatic hydrocarbons
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Citation	北海道大学. 博士(獣医学) 甲第12397号
Issue Date	2016-09-26
DOI	10.14943/doctoral.k12397
Doc URL	http://hdl.handle.net/2115/67184
Type	theses (doctoral)
File Information	Nesta_Bortey-Sam.pdf



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**Environmental contamination in Ghana: Environmental fate and risk
assessment of heavy metals and polycyclic aromatic hydrocarbons**

(ガーナにおける環境汚染：金属と多環芳香族炭化水素の環境動態と
リスクアセスメント)

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September 2016

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Abbreviations

ADD	Average Daily Dose
ANOVA	Analysis of Variance
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
BaP	Benzo(a)Pyrene
BeP	Benzo(e)Pyrene
BMI	Body Mass Index
CC	City centre
Cd	Cadmium
Chry	Chrysene
Co	Cobalt
Cr	Chromium
CSF	Cancer Slope Factor
Cu	Copper
CV	Coefficient of Variation
CYP450	Cytochrome P450
dw	dry weight
EC	European Commission
ECO	Ecological Soil Screening
EDIs	Estimated Daily Intakes
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agricultural Organization
Flu	Fluorene
FSA	Food Safety Agency
GDP	Gross Domestic Product
GM	Geometric mean

GSD	Geometric Standard Deviation
GIS	Geographic Information System
GPS	Global Positioning System
Hg	Mercury
HI	Hazard Index
HNO ₃	Nitric acid
HRs	Hazard ratios
ICP-MS	Inductively coupled plasma-mass spectrometer
IRS	Indoor Residual Spraying
IARC	International Agency for Research on Cancer
IS	Internal Standard
K-S	Kolmogorov-Smirnov statistical test
LC-MS	Liquid Chromatography-Mass Spectrometer
LOD	Limit of Detection
Log	Logarithm (base 10)
LOQ	Limit of Quantification
MAC	Maximum Allowable Concentration
n	number of samples
Nap	Naphthalene
nd	Not detected or below detection limit
Ni	Nickel
NTP	National Toxicology Program
OH-PAHs	Hydroxy Polycyclic aromatic hydrocarbon
OM	Organic matter
p	Level of Significance
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PCA	Principal Component Analysis
PCD	Pollution Control Department

Phe	Phenanthrene
Pyr	Pyrene
r	Correlation coefficient
RfD	Oral Reference Dose
RSD	Relative Standard Deviation
SD	Standard deviation
SF	Slope Factor
SRM	Standard Reference Material
SG	Specific Gravity
SOM	Soil Organic Matter
SW	Shapiro-Wilk's statistical test
USEPA	United States Environmental Protection Agency
V	Vanadium
WC	Water Content
WHO	World Health Organization
ww	Wet weight
Zn	Zinc

Preface

Contamination of the environment by polycyclic aromatic hydrocarbons (PAHs) and heavy metals/metalloids are serious problems worldwide because of their toxicity, carcinogenicities and persistence in the environment (Lim et al., 2008; Senthil Kumar et al., 2008; Wei and Yang, 2010; Varol, 2011; Yayılı-Abanuz, 2011; Mireles et al., 2012; Flowers et al., 2002; Thorsen et al., 2004; Cornelissen et al., 2006). PAHs and metals occur widely in the environment and although formed by natural processes, are frequently produced as a result of anthropogenic activities such as industrialization, mining, smelting, combustion, vehicular emissions and are elevated around major cities/city centres (Ravindra et al., 2008; Manoli et al., 2000; Yang et al., 2013; Liu et al., 2013). In many countries including USA, China and Japan human exposure to PAHs and metals have been associated with cancers, cardiovascular diseases, chromosomal aberrations, translocations, Itai-Itai disease (Orjuela et al., 2012; Xu et al., 2010; Fan et al., 2012; Grandjean et al., 2010; Ikeda et al. 2004).

The Agency for Toxic substance and Disease Registry (ATSDR, 2013) has classified various metals and PAHs among the top 10 most hazardous substances with health risk implications including carcinogenicity. In humans and animals, PAHs are metabolized by cytochrome P450 enzymes and excreted in urine, feces or bile. One of the major metabolites is monohydroxylated PAH (OH-PAHs) (Burczynski et al., 1998). Studies have also shown that PAH metabolites have strong correlation with atherosclerosis and cardiovascular diseases (Xu et al., 2010).

Similarly, high Pb levels in blood have been associated with various health problems including death (Blacksmith Institute, 2011). Recently, residents of Michigan, USA, were exposed to Pb through drinking with an increase in children's blood Pb levels (Hanna-Attisha et al., 2016). In 2010, more than 400 hundred children died and hundreds confirmed to be at risk due to high levels of Pb exposure in Zamfara State, Nigeria (Blacksmith Institute, 2011).

Additionally, (May 2016), ex-gold miners in Johannesburg, South Africa reported having contracted silicosis, an incurable lung disease, after years of working in the mines ([BBC News; http://www.bbc.com/news/world-africa-36285033](http://www.bbc.com/news/world-africa-36285033)).

The economic and population growth rates in Africa including Ghana have seen tremendous increase over the past few years with continuous increase in Gross Domestic Product (GDP) ([Ghana Statistical Services, 2010](#)). The growing rate of industrialization is gradually leading to contamination and deterioration of the environment, and pollution is likely to reach disturbing levels ([Bortey-Sam et al., 2014; Walsh, 2000; Olaifa et al., 2004](#)).

In Ghana, Kumasi ($6^{\circ}40'N\ 1^{\circ}37'W$) is one of the most industrialized and economically significant cities and has been subjected to heavy anthropogenic influences as a result of rapid economic development and urbanization. The human population has drastically increased, the number of cars has doubled during the past decade and many gas stations are located in this region leading to greater fuel combustion rate. These could lead to more fuel leakages, smoke production from exhaust of automobiles and high levels of PAHs in the environment. In addition, garbage, paper, cloth and wood are burnt in open environments in Kumasi ([Bortey-Sam et al., 2014](#)) and all these are potential sources of contamination with PAHs ([Ravindra et al., 2008](#)). Domestic emissions of PAHs are from the use of traditional fuels such as charcoal (i.e. using coke ovens to convert wood into charcoal) and crop residues for cooking, water heating, etc. ([Bortey-Sam et al., 2014](#)). In Kumasi, contamination of particulate matter (PM10), soils and livers of wild rats with PAHs have been documented ([Bortey-Sam et al., 2013; 2014; 2015; 2015b](#)), however, there is no epidemiological study to assess the risk to humans.

Like many countries, mining is a major backbone of Ghana's economy. However, this activity has caused lots of environmental problems in parts of Ghana including Tarkwa ($05^{\circ}18'00"N; 01^{\circ}59'00"W$). Despite several mine spillages, complaints from residents about possible environmental contamination and health concerns, there has been no comprehensive study to assess the risks due to metal exposure in Tarkwa. According to the District Medical

Officer of Health, mining in Ghana has caused various diseases including: vector-borne diseases such as malaria, schistomiasis and onchocerciasis; respiratory tract diseases, especially pulmonary tuberculosis (TB), and silicosis; skin diseases; eye diseases, especially acute conjunctivitis; and mental cases ([Akabzaa and Darimani, 2001](#)).

In these regards, my thesis focuses on assessing the risks due to PAHs and metal exposure in Kumasi and Tarkwa.

In **Chapter 1 of section 1**, I used cattle urine as a biological indicator to assess the levels of PAH contamination in urban (Offinso) and rural (Kumasi) sites in Ghana

(*Bortey-Sam et al. 2016. Excretion of polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in cattle urine in Ghana. Environmental Pollution, in press*).

In **Chapter 2**, I determined the health risk due to PAHs exposure to Kumasi residents (*Bortey-Sam et al. 2016. Oxidative stress due to exposure to polycyclic aromatic hydrocarbons (PAHs) in residents of Kumasi, Ghana. Environmental International, ready for submission*).

In **Chapter 1 of section 2**, I assessed the ecological risks of metals in agricultural soils in Tarkwa.

(*Bortey-Sam et al. 2015. Ecological risk of heavy metals and a metalloid in agricultural soils in Tarkwa, Ghana. International Journal of Environmental Research and Public Health, 12(8): 8811-8827*).

Chapter 2 of section 2 focuses on risks due to heavy metal and metalloid exposure to Tarkwa residents through borehole drinking water.

(*Bortey-Sam et al. 2015. Health risk assessment of heavy metals and metalloid in drinking water from communities near gold mines in Tarkwa, Ghana. Environmental Monitoring and Assessment, 187(7): 1-12*).

Section 2, chapter 3; as a follow up, I focused on determining the accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa area, and associated human health risks through consumption.

(Bortey-Sam et al. 2015. *Accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa area in Ghana, and associated human health risks. International Journal of Environmental Research and Public Health*, 12(9): 11448-11465).

In **chapter 4 of section 2**, I assessed the human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana by estimating the daily intakes and target hazard quotients (THQs).

(Bortey-Sam et al. 2015. *Human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana: Estimation of the daily intakes and target hazard quotients (THQs). Ecotoxicology and Environmental Safety*, 111: 160-167).

Finally, since rats continuously pick food and water from the ground which could be contaminated with metals, I studied the accumulation of metals in livers and kidneys of wild rats around gold-mining communities in Tarkwa (**Chapter 5 of section 2**).

(Bortey-Sam et al. 2016. *Heavy metals and metalloid accumulation in livers and kidneys of wild rats around gold-mining communities in Tarkwa, Ghana. Journal of Environmental Chemistry and Ecotoxicology, in press*)

Expected Outcome/Results

This study is expected to deliver preliminary data, provide baseline information on the risk of PAHs and metal exposure in Kumasi and Tarkwa, Ghana. The outcome will provide valuable information contributing to the concept of “One Health” i.e. to attain optimal health for people, animals and the environment. Results will also provide valuable and baseline information for the Environmental Protection Agency, Ghana, the Ghana Health Service and other environmental policy makers in Ghana to review the environmental quality guidelines.

Section 1

Polycyclic aromatic hydrocarbons

General introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of carcinogenic, genotoxic and ubiquitous semivolatile organic compounds (SVOCs) (IARC, 2006). Although natural processes are potential sources of PAHs, these compounds are mostly associated with anthropogenic activities such as combustion of fuels or wood or industrial/vehicular emissions. High levels are generally observed in densely populated areas, posing adverse effects on human health. Exposure to PAHs is mainly by inhalation of contaminated air or ingestion of soil, food and drinking contaminated water (Barranco et al. 2004; Dissanayake et al. 2004). Due to health concerns, great efforts have been devoted to the study of PAHs in cities in recent years. Human cancers such as skin, lung, and bladder cancers have been associated with PAHs (Flowers et al. 2002; Thorsen et al. 2004; Cornelissen et al. 2006; Mastral and Callén 2000; Magi et al. 2002; Szolar et al. 2002; Schubert et al. 2003).

Based on fuel leakages, smoke production from vehicles, growth in industrialization, burning of paper, wood and cloth in open environment, and the frequent use coke ovens to convert wood in charcoal, previous studies assessed the levels, sources and potential risks of 22 PAHs in particulate matter (PM_{10}) and soils in Kumasi (Source: Masters degree thesis of Bortey-Sam Nesta from Kwame Nkrumah University of Science and Technology (KNUST), Ghana, and Bortey-Sam et al. 2014; 2015).

The concentrations of 22 individual PAHs (AccuStandard, New Haven, USA) including 16 USEPA priority pollutants (naphthalene (Nap), acenaphthylene (Acl) acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IDP), dibenz[a,h]anthracene (DBahA), benzo[g,h,i]perylene (BghiP), perylene (Peryl), benzo[e]pyrene (BeP), methylene phenanthrene (Methy-Phe), 1-methyl phenanthrene (Me-Phe) and retene (Ret)) were measured in each PM_{10} and soil samples.

Levels, sources and potential risks of PAHs in PM₁₀ in Kumasi, Ghana

Since PAHs are ubiquitous in the environment and human and animal exposure could be through breathing contaminated air, Bortey-Sam et al. (2015) collected 32 particulate matter (PM₁₀) samples from pristine site (Kwame Nkrumah University of Science and Technology, KNUST) and the city centre of Kumasi 1) to determine the concentrations of 21 PAHs; 2) to identify the possible sources of these PAHs; and 3) to determine the toxic potential and possible health risk implications associated with them.

From the 21 PAHs analyzed, the more volatile compounds (Nap, Acl, Ace and Fle) were not discussed at each sampling location because these lower ring molecules are present in the gas phase, especially at high ambient temperatures (20–38°C), which was observed throughout the sampling period. Moreover, their (Nap, Acl, Ace and Fle) concentrations were either undetected or low, which was also observed in studies by Oanh et al. (2000) and Salam et al. (2011).

The sum of concentrations of 17 PAHs (Σ_{17} PAHs) in air samples (PM₁₀) in KNUST ranged from 0.51–16 ng/m³ with mean and median values of 3.3 ± 3.3 and 2.6 ng/m³ respectively. Meanwhile, Σ_{17} PAHs in air samples in city centre (CC) ranged from 19–38 ng/m³ with mean and median concentrations of 30 ± 4.9 and 30 ng/m³, respectively.

The mean concentration of Σ_{17} PAHs in CC was 9 times greater than in KNUST. This could be attributed to the fact that sampling in CC was performed in a traffic area suffering high exhaust emission and oil seep from vehicles, and hosting handicraft and small industrial activities (e.g., corn milling).

Most abundant PAHs in air in KNUST and CC

The most abundant PAHs in air (PM₁₀) in KNUST were Phe (21%), BghiP (16%), Ret (15%), and Pyr (11%) (Table 1). Chr was the least abundant with concentration below

detection limit in almost all samples ([Table 1](#)). Ret (one of the most abundant PAHs in air in KNUST) is a molecular marker of wood combustion ([Shen et al. 2012](#)). PAHs profile developed by Harrison et al. ([1996](#)) and Ho et al. ([2002b](#)) on the types of emission sources suggested that Phe and Pyr in KNUST were typical diesel vehicle markers whereas BghiP, the 2nd most abundant, was indicative of gasoline vehicle emission.

[Table 1](#): Abundance of PAHs in air samples in KNUST and CC

PAHs	Ring No.	KNUST	CC	
		% Abundance	% Abundance	p value
Phenanthrene	3	21	2.6	0.56
Anthracene	3	3.6	0.91	0.15
Methylene phenanthrene	4	3.3	0.20	0.67
1-methyl phenanthrene	3	1.2	0.03	0.29
Fluoranthene	4	8.1	1.3	0.07
Pyrene	4	11	1.7	0.30
Retene	3	15	1.2	0.64
Benz[a]anthracene	4	1.5	0.96	0.001
Chrysene	4	0.0	0.49	0.001
Benzo[b+j+k]fluoranthene	5	0.9	11	0.001
Benzo[e]pyrene	5	1.5	14	0.001
Benzo[a]pyrene	5	0.6	12	0.001
Perylene	5	1.2	3.1	0.001
Dibenz[a,h]anthracene	5	6.9	1.6	0.001
Indeno[1,2,3-cd]pyrene	6	7.8	19	0.001
Benzo[g,h,i]perylene	6	16	29	0.001

Bold values: means data are statistically significant (ANOVA)

(Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2015a](#))

As shown in [Table 1](#), air samples in KNUST were dominated by 3-(44%), 6-(24%), 4-(21%), and 5-ring PAHs (11%). Because only PAHs in the particulate phase were collected, it is expected that some of these 3-ring PAHs will also be in the gas phase and concentrations could be underestimated, especially in KNUST, where 3-ring PAHs showed high contribution. During combustion at low temperatures, the low molecular weight (LMW) PAH compounds (< 4 rings) are abundant ([Lake et al. 1979](#)), whilst at high temperature

combustion the high molecular weight (HMW) PAH compounds (≥ 4 rings) are dominant (Laflamme and Hites 1978). According to that, ~55% of PAHs in KNUST were released by high temperature combustion sources.

The most abundant PAHs in air in CC were BghiP, IDP, BeP and BaP (HMW PAHs) (Table 1, $p < 0.01$). Their abundances were 29%, 19%, 14%, and 12% respectively (Table 1). Me-Phe was the least abundant PAH there (0.01 ± 0.04 ng/m³). This pathway has been reported as indicative of vehicle emission (Harrison et al. 1996; Oanh et al. 2000; Jamhari et al. 2014).

Moreover, in CC the PAHs profile was dominated by 6-(48%) ($p < 0.01$), 5-(42%) ($p < 0.01$), 3-(5%) ($p > 0.01$), and 4-ring PAHs (4.5%) ($p < 0.01$) (Table 1). The high percentage of HMW PAHs (95%) confirmed high temperature processes, such as combustion of fuels in engines, as prevailing sources (Mostert et al. 2010; Tobiszewski and Namiesnik 2012).

PCA was applied to PAHs profiles in KNUST and CC. The PCA revealed that the first principal component (component 1) accounted for 47% of the variation while component 2 accounted for 19% (Fig. 1). As observed from the score plot (Fig. 1a), there was a clear separation between the two sampling sites (K and C). That could depend on the high levels of PAHs in CC compared to KNUST. From the loading plot (Fig. 1b), most of the PAHs (both LMW and HMW) were highly associated/clustered on one side of the component. Another interesting feature observed along the component (Fig. 1) was the clear separation between LMW and HMW PAHs, (Fig. 1b) with the HMW PAHs highly distributed in the CC of Kumasi (Fig. 1).

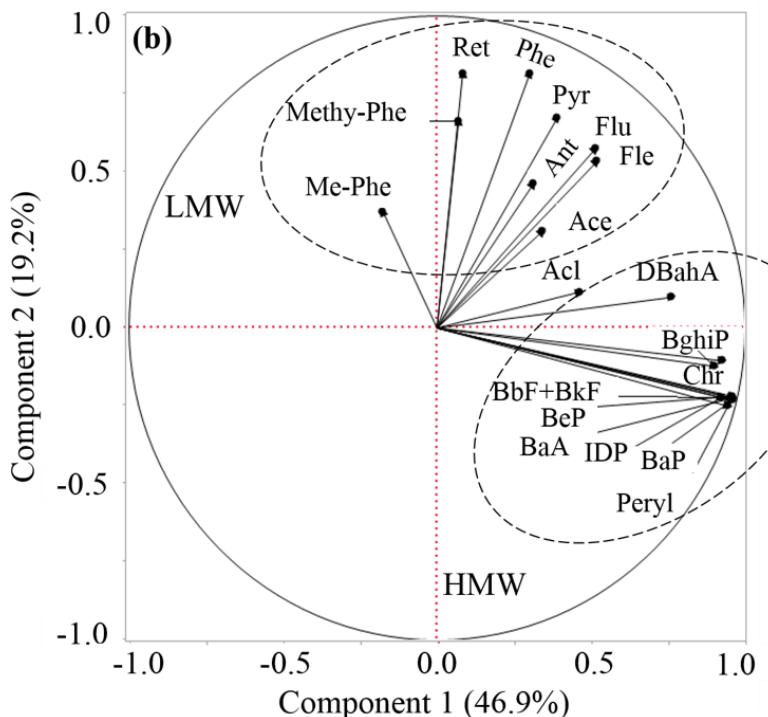
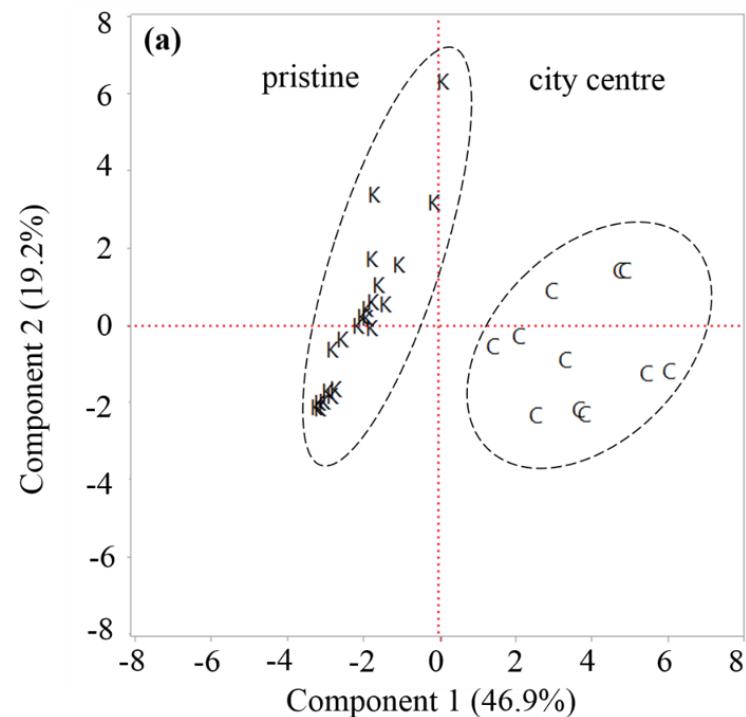


Fig. 1 Distribution patterns of PAHs in PM_{10} in KNUST and CC characterized by PCA; (a) score plot (b) loading plot
 (K: pristine site; C: city centre; LMW: low molecular weight PAHs; HMW: high molecular weight PAHs)
 (Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2015a](#))

Levels of BaP in air in KNUST and CC

The component of PAHs which is typically of greatest interest in terms of potential cancer hazard is BaP. This compound had been known to exhibit a high carcinogenic activity in animals and has been listed as a proven human carcinogen ([WHO, 2000](#)). Concentrations of BaP in air in KNUST ranged from below detection (from most sampling dates) to 0.08 ng/m³. The mean \pm SD and median concentrations of BaP in KNUST were 0.02 \pm 0.03 and 0.01 ng/m³ respectively ([Table 2](#)). Since no environmental standard is available for Ghana, the United Kingdom (UK), Swedish Standards and European Legislation were adopted to assess the quality of air. The UK air quality standards, Swedish guideline and European Legislation, values for BaP are equal 0.25, 0.1 and 1 ng/m³ (for the total content in the PM₁₀ fraction averaged over a calendar year), respectively ([Dimashki et al. 2001; Bostrom et al. 2002; Directive 2004/107/EC](#)).

Table 2: Mean concentrations, standard deviations of PAHs (ng/m^3), BaP equivalence (BaPeq) in air in KNUST and CC

PAHs	Sampling Sites		BaPeq	
	KNUST ($n = 20$)	CC ($n = 12$)	KNUST	CC
	Mean \pm SD	Mean \pm SD	TEF \times Mean conc.	TEF \times Mean conc.
Phe	0.70 ± 0.49	0.79 ± 0.38	$7.0\text{E}-04$	$7.9\text{E}-04$
Ant	$0.12 \pm 0.27^*$	$0.27 \pm 0.31^*$	$1.2\text{E}-03$	$2.7\text{E}-03$
Flu	$0.27 \pm 0.19^*$	$0.39 \pm 0.14^*$	$2.7\text{E}-04$	$3.9\text{E}-04$
Pyr	$0.39 \pm 0.35^*$	0.50 ± 0.16	$3.9\text{E}-04$	$5.0\text{E}-04$
BaA^a	0.05 ± 0.02	0.29 ± 0.08	$5.0\text{E}-03$	$3.0\text{E}-02$
Chr^a	0.01 ± 0.01	0.15 ± 0.06	$5.0\text{E}-05$	$1.5\text{E}-03$
BbF^a + BjF + BkF^a	0.03 ± 0.02	3.2 ± 0.98	$3.0\text{E}-03$	$3.2\text{E}-01$
BeP	$0.05 \pm 0.05^*$	4.1 ± 1.1	$5.0\text{E}-04$	$4.0\text{E}-02$
BaP^a	$0.02 \pm 0.03^*$	3.7 ± 1.1	$2.0\text{E}-02$	$3.7\text{E}+00$
Peryl	$0.04 \pm 0.05^*$	0.94 ± 0.32	$4.0\text{E}-05$	$9.4\text{E}-04$
DBahA^a	$0.23 \pm 0.12^*$	0.50 ± 0.16	$2.3\text{E}-01$	$5.0\text{E}-01$
IDP^a	$0.26 \pm 0.35^*$	$5.8 \pm 1.2^*$	$3.0\text{E}-02$	$5.8\text{E}-01$
BghiP	$0.53 \pm 1.1^*$	8.6 ± 2.3	$1.0\text{E}-02$	$9.0\text{E}-02$
$\sum_{\text{carc}} \text{PAHs}$	0.6 ± 0.5	14 ± 3.6	0.28	5.2
$\sum_{16} \text{USEPA PAHs}$	3.0 ± 3.5	25 ± 7.9	0.30	5.3
$\sum_{14} \text{PAHs}$	2.7 ± 3.0	29 ± 8.7	0.30	5.3

PAHs Carc^a: BaA, Chr, BkF, BbF, BaP, DBahA and IDP according to IARC (2006); Bold: indicates the 16 USEPA priority PAHs; *: indicates statistical significance with p value less than 0.05 (Shapiro–Wilks test for normality); n: number of samples

(Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and Bortey-Sam et al. 2015a)

The mean concentration of BaP in air in KNUST ($0.02 \pm 0.03 \text{ ng}/\text{m}^3$) was 5, 12, and 50 times below the BaP standard set by the Swedish, UK, and European Legislation, respectively. Similarly, individual concentrations of BaP in air in KNUST were all below the recommended guideline values. Low concentrations of BaP and Σ_{17} PAHs were recorded in air in KNUST because the sampling site was located within an area with low vehicular movement, low industrial and human activities and therefore PAHs from point sources were negligible.

BaP was the fourth most abundant PAH in air in CC ($p < 0.01$) and concentration ranged from 1.6–5.6 ng/m³. The mean concentration of BaP in CC (3.7 ± 1.1 ng/m³) was 187 times higher than in KNUST (0.02 ± 0.03 ng/m³). The BaP concentrations in CC was 37, 15, and 4 times higher, respectively, than the air quality standards of Sweden, UK, and European Legislation. Therefore, the CC air was classified as heavily polluted.

Determination of sources of PAHs in air

Chemical mass balance (CMB) model

In general, the source contributions were similar at the two sites. For instance, the exhaust contributions from diesel and gasoline engines were high in both cases. 15.3% of PAHs in KNUST was contribution from coal related sources i.e. coke oven and 84.7% from traffic related sources (Fig. 2). Of this 84.7%, 42.5% were emissions from diesel engines and the other 42.2% from roadway traffic (Fig. 2).

Similarly 85% of PAHs in CC were from traffic related sources out of which 78.4% was contribution from gasoline engines and 6.3% from roadway traffic (Fig. 3). The overall CMB results clearly showed that gasoline and diesel engines were the two major sources of PAHs in air (PM₁₀) in the Kumasi metropolis.

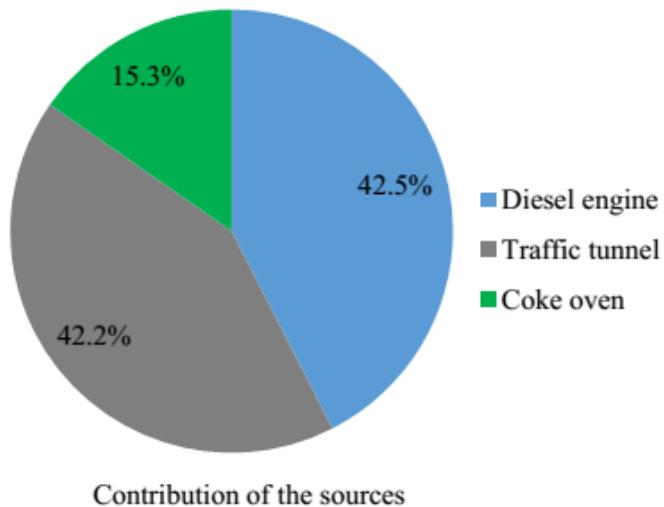


Fig. 2 Chemical mass balance source apportionment of PAHs in air (PM_{10}) in KNUST
 (Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2015a](#)).

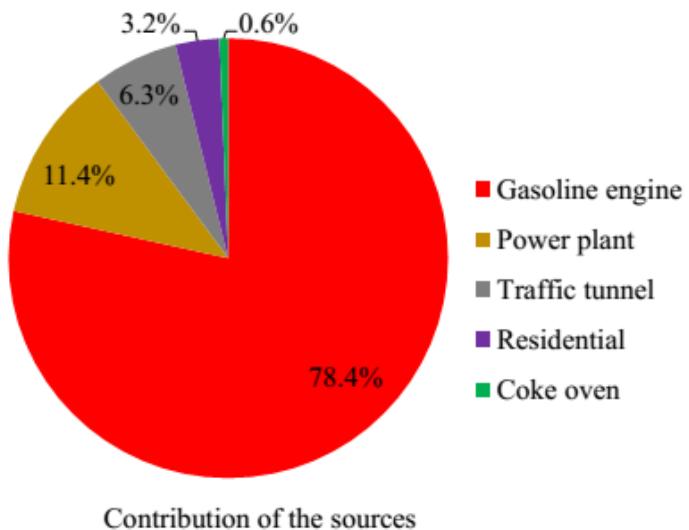


Fig. 3 Chemical mass balance source apportionment of PAHs in air (PM_{10}) in CC
 (Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2015a](#)).

Air toxicity assessment (CC)

In Kumasi (CC), PAHs including human carcinogenic compounds (BaA, BbF, BkF, BaP, Chr, DBahA, and IDP) ([IARC, 2006](#)) were at high concentrations ([Tables 1 and 2](#)). The mean concentrations of these compounds were IDP (5.8 ± 1.2), BaP (3.7 ± 1.1), BbF + BjF + BkF (3.2 ± 0.98), DBahA (0.5 ± 0.16), BaA (0.29 ± 0.08) and Chr (0.15 ± 0.06) ng/m³ ([Table 2](#)).

WHO ([2000](#)) stated that the BaP equivalent concentration producing an excess lifetime cancer risk of 1/10,000 is approximately 1.2 ng/m³. The mean concentrations of BaP alone in CC (3.7 ± 1.1 ng/m³) exceeded this threshold. The toxic equivalent concentration (BaPeq) of carcinogenic PAHs in air was 0.3 ngBaPeq/m³ in KNUST and 5.2 ngBaPeq/m³ in CC ([Table 2](#)). Thus BaPeqs in CC were, on the average, 18 times higher than in KNUST. According to BaPeqs of individual PAHs, BaP was the predominant contributor to toxicity in CC. To assess the health risk to humans, adults and children were assumed to be exposed to airborne PAHs through inhaling PM₁₀. The estimates were made by considering lifetime average daily dose (LADD) and the corresponding incremental lifetime cancer risk (ILCR).

The LADD for PAH ranged from 2.5E–9 to 2.1E–6 for adults (average 4.4E–7) and 6.9E–8 to 5.8E–6 for children (average 1.3E–6) ([Table 3](#)). According to that, ILCR associated to carcinogenic PAHs ([IARC 2006](#)) ranged from 2.2E–10 to 5.4E–6 (adults) and 6.1E–10 to 1.5E–5 (children). The mean ILCR for adults (1.2E–6) and children (3.3E–6) ([Table 3](#)) were within the acceptable limit of 10⁻⁶ to 10⁻⁴ as acknowledged by regulatory agencies ([USEPA 2005a](#)).

Table 5: LADD and ILCR of PAHs in air in CC

PAHs	LADD		ILCR	
	Adult	Child	Adult	Child
Phe	1.9E-07	5.3E-07		
Ant	6.6E-08	1.8E-07		
Methy-Phe	1.5E-08	4.2E-08		
Me-Phe	2.5E-09	6.9E-09		
Flu	9.4E-08	2.6E-07		
Pyr	1.2E-07	3.4E-07		
Ret	8.6E-08	2.4E-07		
BaA	6.9E-08	1.9E-07	4.2E-08	1.2E-07
Chr	3.5E-08	9.9E-08	2.2E-10	6.1E-10
BbF + BjF +BkF	7.6E-07	2.1E-06	4.6E-08	1.3E-07
BeP	9.8E-07	2.8E-06		
BaP	8.9E-07	2.5E-06	5.4E-06	1.5E-05
Peryl	2.3E-07	6.3E-07		
DBahA	1.2E-07	3.3E-07	7.2E-07	2.0E-06
IDP	1.4E-06	3.9E-06	8.4E-07	2.4E-06
BghiP	2.1E-06	5.8E-06		
\sum PAHs	7.1E-06	2.0E-05		
Minimum	2.5E-09	6.9E-09		
Maximum	2.1E-06	5.8E-06		
Average	4.4E-07	1.3E-06	1.2E-06	3.3E-06

Bold: indicates 16 USEPA priority PAHs

(Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2015a](#))

Occurrence, distribution, sources and toxic potential of PAHs in Kumasi soils

Based on the levels and high concentrations of BaP in PM₁₀ and because some PAHs are known to settle in soil, Bortey-Sam et al. (2014) collected 129 soils from 36 communities in Kumasi: to determine the concentrations of 22 PAHs including the 16 that are listed by the United States Environmental Protection Agency (USEPA) as priority pollutants in surface soil samples from the Kumasi metropolis; to identify the possible sources of PAHs in surface soils from the Kumasi metropolis; to develop distribution maps of PAHs throughout the city using Geographic Information System (GIS) and; to evaluate the extent of pollution and toxic potential of PAHs in soils from Kumasi.

Concentrations/distributions of PAHs in soils in Kumasi

The mean concentrations of total PAHs in 129 soils from 36 communities in the Kumasi metropolis ranged from 14.78 at Ahinsan (a community) to 2,084 ng/g dw at Adum (around city centre), with mean value 442.5 ± 527.2 ng/g dw. Kurtosis, skewness and K–S tests for normality showed a significant variation in the concentrations of PAHs in surface soils from the communities for all the measured analytes (Z value > 1.96 ; $p < 0.01$).

PAHs concentrations spread throughout the communities with the highest concentrations exhibited in the city centre. Higher levels of BaP and sum of 16 USEPA priority pollutants were observed from the city centre of Kumasi, and this could be due to the high or heavy vehicular load, congestion and combustion rate within the region. In the surface soils from Kumasi, Flu recorded the highest PAH concentration followed by its isomeric Pyr, interestingly these two showed the same distribution pattern ($p < 0.01$) with extremely high concentrations in communities such as Suame, Mbrom, Adum, Suntreso, Romanhill and Sofoline. Suame exhibited the highest for both Flu and Pyr at concentrations of 381.3 ± 54.57 and 355.1 ± 49.42 ng/g dw, respectively. The distribution for BaA is not extremely different from the 2 mentioned above, since very high concentrations were also recorded at the same

sampling communities as Flu and Pyr. The distributions of BbF + BkF, BeP and BaP with same molecular weight were nearly identical and higher concentrations were recorded in Romanhill and Adum (city centre).

BaP was the 6th most abundant PAH determined in soils from the study area. The mean concentrations of BaP in Kumasi soil ranged from 0.5939 ± 0.3284 (Ahinsan) to 259.5 ± 38.37 ng/g dw (Adum). However, the mean value of BaP throughout the 36 communities was 32.59 ± 51.90 ng/g dw. Kurtosis and K-S normality tests indicated a significant variation in the distribution of BaP through the communities ($p < 0.01$).

Source identification CMB model

CMB model was also applied for source identification per each sampled community and the source profiles used were classified as either coal related (power plant, residential or coke oven) or traffic related (gasoline engine, diesel engine or traffic tunnel). In general, the contributions appeared similar in most cases. Fuel contributions from diesel and gasoline engines were dominant from the most polluted sites in Kumasi.

In Adum, the largest apportioned contributor was gasoline engine recording 42%, 25% from diesel engines and 33% of the total PAHs sources was from coke oven. From Romanhill, the CMB data showed that 81% of the PAHs sources were from fuel combustion, and gasoline engines accounted for 39% of the total and the rest was from diesel engines (42%). Also in Mbrom, the source of PAHs was attributed to fuel combustion. This accounted for 72% of the sources and diesel engines was dominant with 46% of the total. From Fig. 4, 70% of PAHs in the surface soils from Suame was from diesel (36%) and gasoline (34%) engines. There was no difference in source contributions from Suntreso as 84% of the total contribution was attributed to gasoline engines.

The overall CMB results clearly showed that gasoline and diesel engines were the two major sources of PAHs in soil samples from the most contaminated communities in the Kumasi metropolis. These results are in agreement with the known PAH sources at this location. That is, PAHs input in the Kumasi metropolis was significantly influenced by fuel combustion (diesel and gasoline engines) as was also indicated by the diagnostic ratio methods ([Bortey-Sam et al. 2014](#)).

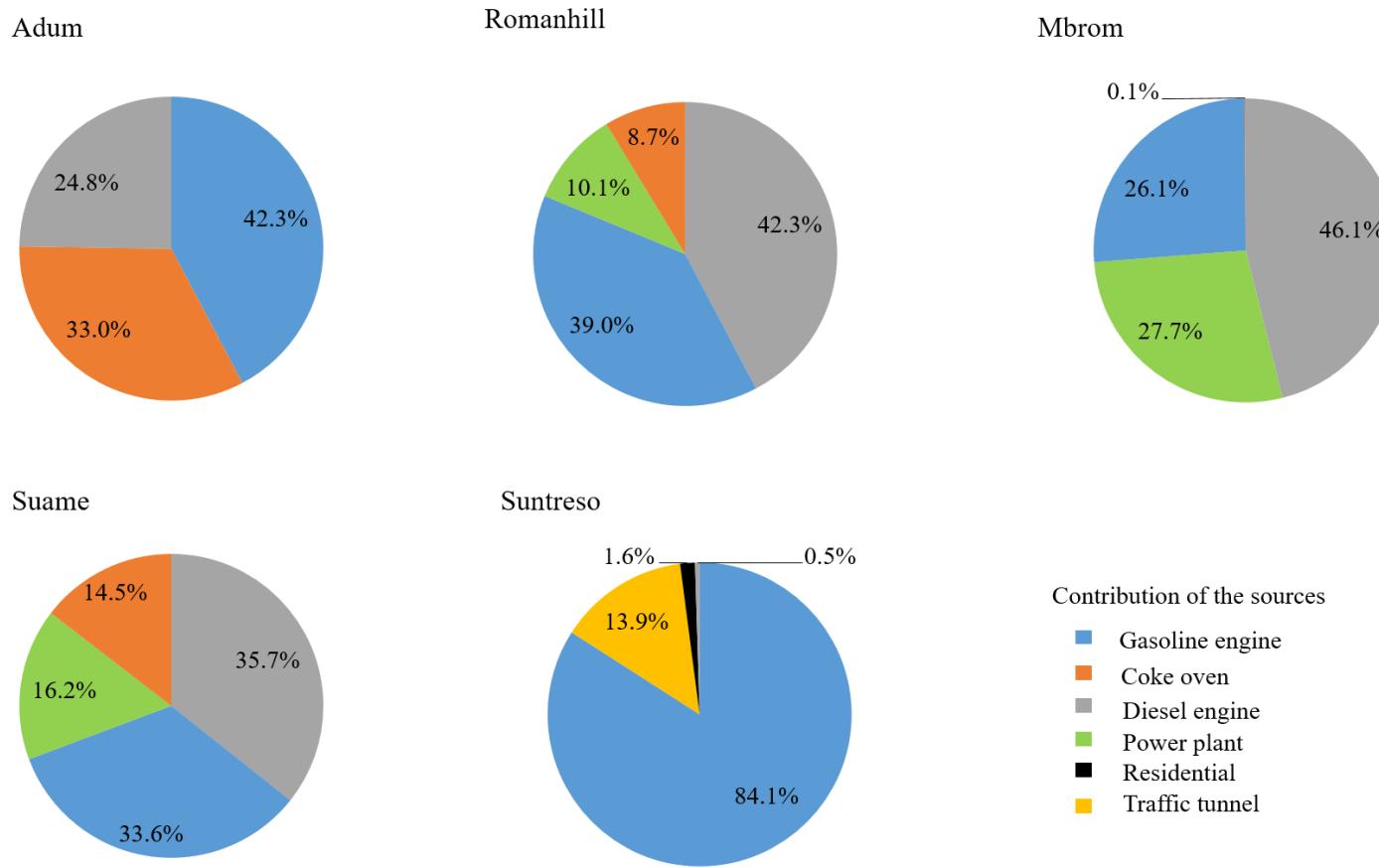


Fig. 4. Chemical mass balance model for sources of PAHs in surface soils from the severely polluted communities in the Kumasi Metropolis

(Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2014](#)).

Kumasi soil toxicity assessment

Maliszewska-Kordybach (1996) has classified pollution levels of PAHs in soils into 4 categories based on the 16 USEPA priority pollutants, namely unpolluted (< 200 ng/g), weakly polluted (200–600 ng/g), polluted (600–1000 ng/g) and severely polluted (> 1000 ng/g). By this classification, 51% of soil samples from the communities in this study were considered unpolluted with PAHs (< 200 ng/g). The least polluted community was Ahinsan. About 30% of the communities had their soils weakly polluted with PAHs while 5% of the communities in the study area were polluted with PAHs. The severely polluted communities representing 14% of the communities were Adum, Mbrom, Suame, Romanhill and Suntreso.

The mean concentrations of total PAHs (16 USEPA PAHs) recorded from these severely polluted communities were 1,708 (Adum), 1,441 (Mbrom), 1,435 (Suame), 1,379 (Romanhill) and 1,045 ng/g dw (Suntreso) respectively. The high levels of PAHs in soil from these communities could come from the high vehicular traffic and the high human population who may use firewood or charcoal for domestic heating. The high concentrations of total PAHs in the surface soils from these severely polluted communities could pose high environmental and human health risks (Ping et al. 2007). The study indicated that in these communities (Adum, Romanhill, Mbrom, Suame and Suntreso), levels of PAHs compounds including the human carcinogenic compounds (BaA, BbF, BkF, BaP, DBahA, Chr and IDP) (IARC, 2006) were high.

Health risk assessment of carcinogenic PAHs cannot be related only to overall concentration. Rather, each PAH has a different carcinogenic potential. Health risk assessment associated with PAHs uptake is often estimated on the basis of the BaP concentrations. BaP is the highest carcinogenic contributor in almost every study (Halek et al. 2008). The development and establishment of a toxicity equivalency factors (TEFs) are used in the assessment of mixtures containing PAHs. The TEFs were used to quantify the carcinogenic potential of other PAHs relative to BaP and to estimate benzo[a]pyrene-equivalent concentration (BaPeq) (Nadal et al. 2004). The list of TEFs (Table 6) compiled

by Tsai et al. (2004) was adopted to calculate BaPeq. Therefore, the carcinogenic potencies of individual PAHs have to be considered by multiplying their concentration with the appropriate TEF.

The Toxicity Equivalent Concentrations (TEQ's) which are the sum of average BaPeq concentration in soil, $TEQ = \sum(C \times TEF)$, from KNUST Botanical Gardens (pristine) and 5 most polluted communities were 1.245 BaPeq ng/g and 188.2 BaPeq ng/g respectively (Table 6). These implies that carcinogenic potency of PAHs load in these 5 severely polluted communities were higher (approximately 150 times higher than from KNUST Botanical Garden) and BaP contributed 70% of the total toxicity level.

Table 6: List of TEF and total Benzo(a)Pyrene–equivalent concentration (BaPeq).

PAHs	TEF values	KNUST mean \pm SD	5 polluted communities mean \pm SD	BaPeq ng/g	BaPeq ng/g
				KNUST	5 most polluted communities
BaP	1	0.85 \pm 0.82	132 \pm 80	0.85	132
DBahA	1	0.10 \pm 0.12	13 \pm 4.1	0.10	13
BbF+BkF	0.1	1.5 \pm 1.3	141 \pm 96	0.16	14
BaA	0.1	0.33 \pm 0.09	135 \pm 44	0.033	14
IDP	0.1	0.68 \pm 0.43	113 \pm 55	0.068	11
Chr	0.01	0.42 \pm 0.22	71 \pm 19	0.0042	0.71
Nap	0.001	1.0 \pm 0.28	9.2 \pm 6.0	0.0010	0.0092
Ace	0.001	0.02 \pm 0.01	7.9 \pm 9.8	0.000020	0.0079
BghiP	0.01	0.62 \pm 0.94	128 \pm 57	0.0062	1.3
BeP	0.01	1.3 \pm 1.1	129 \pm 91	0.013	1.3
Pyr	0.001	1.4 \pm 0.46	235 \pm 74	0.0014	0.24
Flu	0.001	1.1 \pm 0.43	258 \pm 77	0.0011	0.26
Ant	0.01	0.18 \pm 0.14	24 \pm 9.6	0.0018	0.24
Phe	0.001	1.4 \pm 0.24	112 \pm 64	0.0014	0.11
Peryl	0.001	0.83 \pm 0.65	44 \pm 26	0.00083	0.040
Fle	0.001	0.23 \pm 0.04	5.6 \pm 3.4	0.00023	0.0056
Acl	0.001	0.12 \pm 0.03	12 \pm 5.3	0.00012	0.012
Sum				1.2	188

List of TEF values was compiled by [Tsai et al. \(2004\)](#)

(Source: Data for this figure was obtained from Masters degree thesis of Bortey-Sam Nesta and [Bortey-Sam et al. 2014](#))

Exposure levels of PAHs in wild rats in Kumasi

Due to the toxic potential of PAHs in PM₁₀ and soils in Kumasi and especially the city centre, rats were used as sentinels to measure the environmental pollution state because they are mammals that share many processes with humans and are appropriate for use to answer many research questions. They tend to pick up food and water from the ground which could be contaminated with PAHs. The objective was therefore to determine the exposure levels of wild rats in Kumasi to 16 USEPA PAHs.

Of the 16 PAHs analysed, 6 were detected in rat livers. Ten PAHs, namely Acl, Fle, BaA, Chr, BbF, BkF, BaP, IP, DBahA, and BghiP, were not detected in any of the samples. The 6 compounds detected were considered for \sum PAHs. The \sum PAH concentrations in liver ranged from 0.05 in Bomso (a community) to 1.64 ng/g dw in the city centre, respectively. The minimum (0.05 ng/g dw) and maximum (1.64 ng/g dw) concentration of \sum PAHs were both in female rats that weighed ~42 and 300 g, respectively.

The abundance of PAHs (ng/g ww) in livers of wild rats collected decreased in the order: Phe (0.51 ± 0.08) > Pyr (0.39 ± 0.20) > Ace (0.19 ± 0.01) > Nap (0.15 ± 0.01) > Flu (0.11 ± 0.11) and > Ant (0.07 ± 0.07). The levels of Phe and Pyr were significantly higher than other PAHs. Nap and Flu were detected in 80% and 78% of the samples, respectively. In surface soils in Kumasi, Pyr was the second most abundant PAH ([Bortey-Sam et al. 2014](#)), and rats could have been exposed (to Pyr and other PAHs) from the soil through dermal contact or through ingestion during feeding or drinking.

DISCLAIMER: (Under recommendation of Supervisors in the Laboratory of Toxicology, Hokkaido University, Japan, the general introduction including figures and tables of Section 1 were solely obtained from thesis by Nesta Bortey-Sam submitted in partial fulfilment of a Master of Science degree in Environmental Chemistry from the Kwame Nkrumah University of Science and Technology, Ghana; and published data: Bortey-Sam et al. 2013; 2014; 2015a; 2015b).

Chapter 1
Excretion of polycyclic aromatic hydrocarbon metabolites
(OH-PAHs) in cattle urine in Ghana

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are pollutants formed during incomplete combustion of organic materials. They are found in vehicle exhaust, wood and cigarette smoke, and also in grilled foods. Human and animal exposure to PAHs occur mainly through inhalation of contaminated air or ingestion of soil, food and/or drinking contaminated water ([Barranco et al., 2004; Dissanayake and Galloway, 2004](#)).

According to the Agency for Toxic Substances and Disease Registry's ([ATSDR, 2013](#)) priority list of hazardous chemicals, PAHs were classified as the 9th most hazardous chemical. In humans and animals, PAHs are metabolized by cytochrome P450 enzymes and excreted in urine. One of the major metabolites is monohydroxylated PAH (OH-PAHs) ([Burczynski et al., 1998](#)) and urinary levels of 1-hydroxypyrene has been used as biomarker of PAHs exposure ([Bouchard and Viau, 1999; Jongeneelen, 2001](#)).

However, since ratios of different PAHs may vary depending on the source and personal enzymatic capacity, concentration profiles of multiple OH-PAHs biomarkers are necessary and required to assess the environmental exposure risk. Urinary metabolites of naphthalene, fluorene and phenanthrene are also commonly used as biomarkers to assess the exposure level and environmental risk ([Li et al., 2008; Fan et al., 2012; 2012b](#)).

The assessment of health risk to humans exposed to PAHs is primarily based on results from animal studies, which indicated that PAHs can produce carcinogenic and mutagenic effects. Recent studies also indicated that some PAH metabolites have strong correlation with atherosclerosis and cardiovascular diseases ([Xu et al., 2010](#)), and exposure of rats and mice to naphthalene caused nasal and bronchiolar tumors, respectively ([NTP, 1992; 2000](#)).

As a developing country, the economic and population growth rates in Ghana have seen tremendous increases over the past few years. The growing rate of industrialization is gradually leading to contamination and deterioration of the environment and pollution is likely to reach disturbing levels ([Bortey-Sam et al., 2014](#)). Studies by [Bortey-Sam et al.](#)

(2013; 2014; 2015) in particulate matter (PM10) and soils indicated that the city centre of Kumasi, Ghana has been polluted with PAHs when compared with recommended levels, and fuel and wood/grass combustion were the dominant sources. The total benzo(a)pyrene equivalent concentration and estimated carcinogenicity of PAHs in PM10 and soil from the city centre was approximately 18 and 150 times higher, respectively, as compared to a pristine site. Rats were therefore used as sentinels to measure the environmental pollution state, and higher levels of PAHs were detected in the livers of wild rats in the city centre of Kumasi. Naphthalene was detected in 80% of those samples, and levels of phenanthrene and pyrene (the first and second most abundant, respectively) were significantly higher than other PAHs measured ([Bortey-Sam et al., 2015b](#)).

Based on the high levels and cancer potency of PAHs in PM10, soils and the levels found in livers of wild rats ([Bortey-Sam et al. 2013; 2014; 2015; 2015b](#)), cattle urine was collected because cattle is known to excrete large amount of PAH metabolites due to high intake of the parent compound through feed or inhalation ([Saengtienchai et al., 2014](#)). PAHs in the atmosphere are known to settle in soil ([Rey-Salgueiro et al. 2008](#)) and this could increase the levels of exposure in Kumasi, Ghana, because these free-range cattle also pick food and/or water from the ground. Urinary levels of PAHs could be widely used as biological indicator of exposure ([Jongeneelen, 2001](#)), and there is limited/no data from literature that addresses the excretory levels of OH-PAHs in cattle in Ghana. The objectives of the present study were therefore: to determine the concentrations of OH-PAHs in cattle urine in Kumasi (urban) and Offinso (rural), Ghana; find the association between urinary OH-PAHs concentrations and sex; and to estimate cattle's exposure to PAHs from the different sites.

Materials and methods

Sampling

In August 2014, urine samples of healthy cattle (West African Shorthorn) were randomly collected from 5 communities in Kumasi and Offinso, both in the Ashanti Region of Ghana. Offinso is about 33 km from the city centre of Kumasi ([Fig. 1](#)). Samples were collected from Oforikrom and Santasi in Kumasi (urban), which are 5.1 and 3.5 km from the city centre, respectively ([Fig. 1](#)), where previous studies reported high levels of PAHs in PM10, soils and livers of wild rats ([Bortey-Sam et al., 2013; 2014; 2015; 2015b](#)). On the other hand, the three sites in Offinso (Twumasesen Estate, Saboa and Kokote) selected for cattle urine sampling ([Fig. 1](#)) are in rural and agricultural areas where bush burning is rampant and the use and sometimes abuse of pesticides such as carbaryl (1-naphthyl-N-methylcarbamate), which could be metabolized to 1-hydroxy naphthalene, was possible ([Meeker et al., 2007; Orjuela et al., 2012](#)).

In addition, due to the lack of background urine and interferences during OH-PAHs quantification, 500 mL of cattle urine (blank stock) was collected from Hokkaido University School farm. Hokkaido University is a public university located in Sapporo, Japan, and because of the low vehicular movement and industrial activities around the farm, PAHs exposure from point sources were assumed to be negligible. However, because cattle could be exposed to PAHs through feed and/or inhalation, the sample collected was measured several times to confirm levels of OH-PAHs.

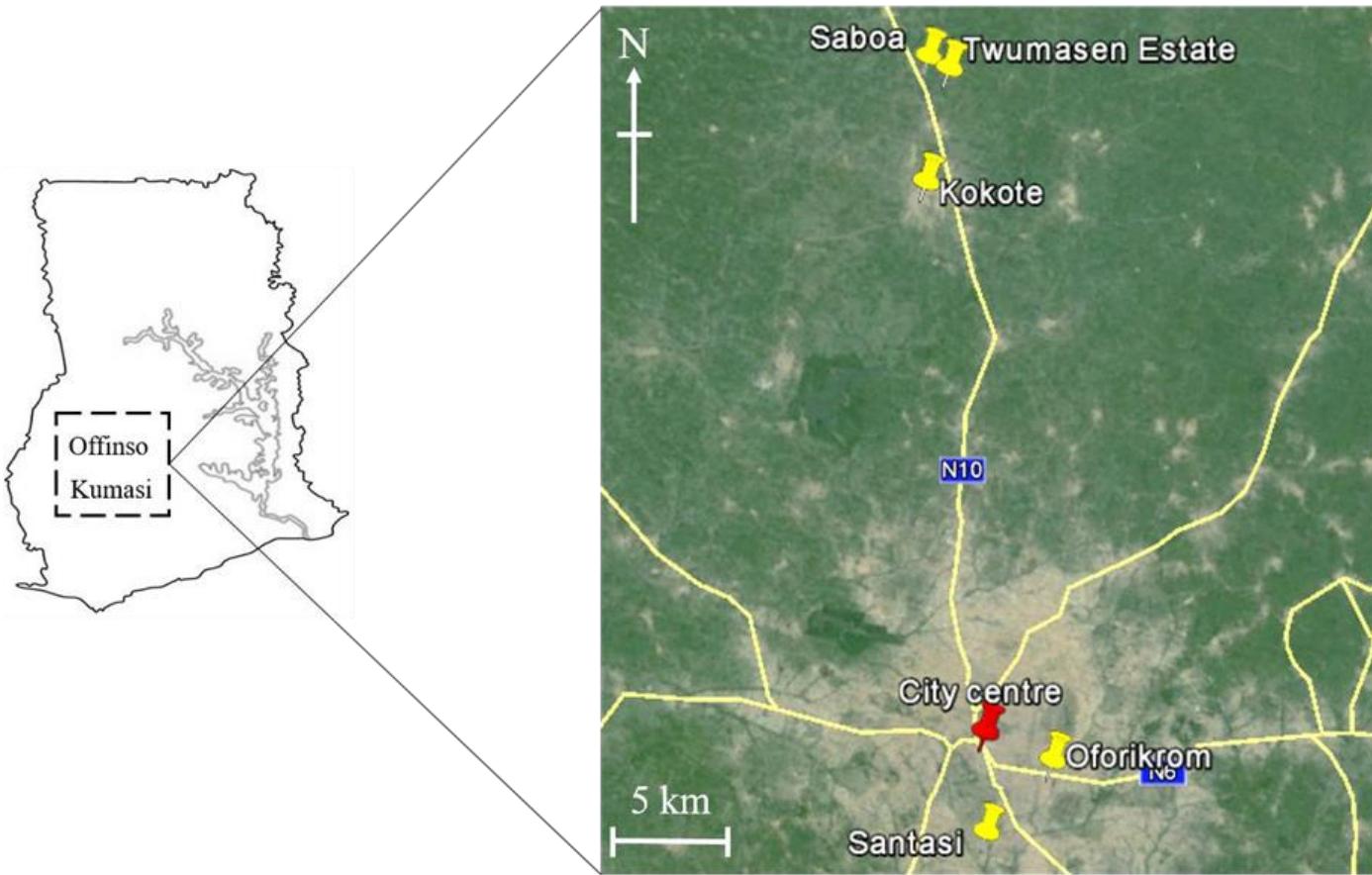


Fig. 1 Map showing cattle urine sampling locations in the Ashanti Region, Ghana (yellow pins indicate sampled locations and red pin indicate city centre in Kumasi)

From the sample sites in Ghana, spot urine ($n = 95$; with 30 males and 65 females) were collected, transferred into labelled corning tubes (Corning Incorporated, New York, USA) and stored at -20°C in the Department of Chemistry, Kwame Nkrumah University Science and Technology (KNUST), Ghana. Of the 5 sites, only ages of some cattle in 2 sites (Twumasesen Estate and Saboa) were obtained from the herdsmen. The average ages (ranges) of cattle were 2.9 ± 1.0 years (1-4.5years) in Twumasesen Estate and 4.2 ± 2.9 years (1-12 years) in Saboa, respectively. Samples were later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan where they were stored at -30°C until analysis (quarantine number for importing is 26 douken 383).

Sample extraction and analysis

Twenty microlitres each of β -glucuronidase (bovine liver, type B-1; 1240 U/mg; Sigma Aldrich) and arylsulfatase (limpets Type V; 34 units/mg; Sigma Aldrich) enzymes, and 5 mL of 0.1 M sodium acetate buffer (pH 5.6) were added to 5 mL urine sample after spiking with three PAH internal standards (13C6-2-OHFluorene, 3-OHPhenanthrene-d9, and 13C6-1OHPyrene). The pH of samples were adjusted to 5.5 using 1 M acetic acid (Wako Pure Chemicals, Osaka, Japan) and incubated overnight at 37°C . The samples were diluted with 4 mL of Milli-Q water and extracted twice (liquid-liquid extraction) with 10 mL each of n-pentane (Kanto Chemical Corp., Tokyo, Japan) by shaking for 1 h. To reduce the interference of sulfur metabolites, the combined extracts were washed with 2 mL of 1 N AgNO_3 solution (Wako Pure Chemicals, Osaka, Japan), concentrated to 50-100 μL , redissolved to 0.5 mL using methanol and filtered (0.20 μm DISMIC-13JP membrane filter, ADVANTEC, Toyo Roshi Kaisha Ltd., Japan) prior to instrumental analysis. All sample preparation steps were performed in darkness (by covering tubes completely with aluminum foil) to avoid possible photodegradation of target analytes. A total of 13 OH-PAHs; 2-hydroxynaphthalene (2-OHNap), 2-hydroxyfluorene (2-OHFlu), 3-hydroxyfluorene (3-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-

hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 1-hydroxypyrene (1-OHPyr), 6-hydroxychrysene (6-OHChry), 3-hydroxybenzo(e)pyrene (3-OHBeP) and 9-hydroxybenzo(a)pyrene (9-OHBaP), were analyzed in each sample. The standards (purity \geq 98%) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and Toronto Research Chemical Inc. (Brisbane Road, North York, Canada). Difficulties were often associated with the separation of 1-OHPhe and 9-OHPhe, for this reason, the sum of these isomers was used as an abbreviation, 1-9-OHPhe. Similarly, 2-3-OHFlu was used as sum of 2-OHFlu and 3-OHFlu. All results were adjusted by specific gravity and expressed in ng/mL.

A Shimadzu 8030 triple quadrupole mass spectrometer, upgraded to 8040 with UF lens, (ESI MS-MS; Shimadzu, Kyoto, Japan), equipped with a Prominence UFC system (Shimadzu, Kyoto, Japan) was used for analysis. Chromatographic separation was achieved using an Agilent Eclipse PAH column (150 mm \times 2.1 mm, 3.5 μ m). The mobile phases were methanol:water (2:3, v:v) (A) and methanol (B), pumped at a flow rate of 250 μ L/min. The mobile phase gradient was maintained as follows: 0.0–2.0 min, 5% B; 2.0–20 min, 40% B; 20–25 min, 40% B; 25–30 min, 95% B; 30–35 min, 95% B; 35–35.01 min, 5% B. Target compounds were determined by multiple-reaction monitoring (MRM) in the negative ionization mode.

Specific gravity (SG) of cattle urine

To compensate for variations in urine dilution, urinary OH-PAH concentrations were adjusted by specific gravity (SG). Urinary SG was measured by a hand refractometer (ATAGO, PAL-095, Tokyo, Japan). Obtained mean and ranges of SG in urine of cattle in Oforikrom (1.013; [1.004-1.029]), Santasi (1.035; [1.03-1.041]), Twumases Estate (1.035; [1.028-1.04]), Saboa (1.036; [1.026-1.049]) and Kokote (1.037; [1.029-1.042]) were used to adjust urinary OH-PAHs concentrations as illustrated by [Nermell et al \(2008\)](#). The correction formula applied to each urine concentration was as follows:

$$\text{SG_corrected concentration} = \text{urinary OH - PAH concentration} \times \frac{(\text{SG}_{\text{target}} - 1.0)}{(\text{SG}_{\text{sample}} - 1.0)}$$

Where, SG_{target} is the mean specific gravity of cattle urine per community; SG_{sample} is the specific gravity of a particular sample.

Quality control and quality assurance

A mixture of three 13C-isotopically labelled OH-PAHs (13C6-2-OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) was spiked into urine samples as internal standard prior to sample preparation and extraction. 13C6-2-OHFlu was used for quantification of metabolite of naphthalene and three metabolites of fluorene. 3-OHPhe-d9 was used for quantification of the five metabolites of phenanthrene and 13C6-1-OHPyr was used for quantification of 1OHPyr, 6-OHChry, 3-OHBeP and 9-OHBaP.

Quantitation was performed using internal standard method (five-point calibration; 1, 5, 10, 50 and 100 ng/mL), and average correlation coefficients (r^2) for the calibration curves in cattle urine were greater or equal to 0.99. The standard solutions (spiked with internal standards) for the calibration curves were prepared in urine in order to normalize differences in interferences between standards and samples. Concentrations of OH-PAHs in urine sample used for this purpose were below the limits of detection (LOD) and differences between this and sample concentration was used in this study. Analytical methods were checked for precision and accuracy. Limits of quantification (LOQs) were calculated based on 10SD/S (SD is the standard deviation of the response of seven replicate standard solution measurements and S is the slope of the calibration curve). LOQs (ng/mL) of OH-PAHs were, 0.29 (2-OHNap), 0.24 (2-3-OHFlu), 0.60 (9-OHFlu), 0.23 (2-OHPhe), 0.71 (1-9-OHPhe), 1.16 (3-OHPhe), 0.15 (4-OHPhe), 0.87 (1-OHPyr), 0.73 (6-OHChry), 0.54 (3-OHBeP) and 0.32 (9-OHBeP), respectively. Internal standard recoveries (13C6-2OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) ranged from 89 ± 5.8–96 ± 11.4% (Table 1).

Table 1: Quality assurance and control (QA/QC) for OH-PAHs analysis in cattle urine

Compound name	LOD (ng/mL)	LOQ (ng/mL)	ISTD Recovery (%)	Spiked solvent blanks (%)	Matrix spikes (%)
2-OHNaphthalene	0.0898	0.295			
9-OHFluorene	0.181	0.603			
2-3-OHFluorene	0.0745	0.248			
<i>13C6-2-OHFluorene</i>			94	96	96
1-9-OHPhenanthrene	0.214	0.714			
2-OHPhenanthrene	0.0696	0.232			
3-OHPhenanthrene	0.348	1.16			
4-OHPhenanthrene	0.0437	0.145			
<i>3-OHPhenanthrene-d9</i>			96	98	95
1-OHPyrene	0.259	0.865			
<i>13C6-1-OHPyrene</i>			89	92	85
6-OHChrysene	0.220	0.733			
3-OHBenzo(e)Pyrene	0.162	0.542			
9-OHBenzo(a)Pyrene	0.0959	0.319			

Italicized compounds are internal standards (ISTD); LOD: Limit of detection; LOQ: Limit of quantification

For every batch of 10 samples, a solvent blank, a spiked solvent blank (internal standards spiked into solvent), a matrix spike (internal standards spiked into blank urine), and duplicate sample were analyzed. The average recoveries in spiked solvents blanks ranged from $92 \pm 4.6\%$ – $98 \pm 8.3\%$, and that for matrix spikes was $85 \pm 9.1\%$ – $96 \pm 7.1\%$. Blanks were run periodically and contained no detectable amount of target analyte. The coefficients of variation of OH-PAH in duplicate samples were less than 15%.

Data analysis

Data analysis was performed using IBM SPSS v 20 for windows (SPSS Inc., Illinois, USA). Kolmogorov–Smirnov (K–S) and Shapiro-Wilks tests were used to determine the normality of data and were considered statistically significant if p value was less than 0.05. Concentrations of OH-PAH below their respective LOQs were replaced with a value of LOQ/2. Geometric mean concentrations were used to represent the central tendency of OHPAH in this study ([Wayne, 1999](#)). ANOVA and Tukey analyses of log transformed data were used to compare concentrations of OH-PAH in cattle urine from the study areas and differences were considered statistically significant with p value < 0.05 . Student's T-Test was also used to compare distribution of OH-PAHs between male and female cattle; and, urban and rural sites.

Results and discussion

Excretory levels of OH-PAH in cattle urine

As shown in [Table 2](#), there was no significant difference ($p > 0.05$) between urinary OH-PAHs concentrations in cattle from urban and rural sites except for 2-OHPhe and 4-OHPhe. Data for 3-OHPhe was not included because concentrations from urban sites and 2 rural sites were below the LOQ ([Table 3](#)). The significant differences ($p < 0.05$) could indicate differences in cattle's exposure to PAHs from urban and rural sites. [Table 3](#) shows the distribution of OH-PAHs in cattle urine from the 5 sample sites.

Table 2: Specific gravity adjusted urinary OH-PAHs concentrations (ng/mL) in cattle from urban and rural sites in Kumasi and Offinso, Ghana

Site	<i>n</i>		2-OHNap	2-3-OHFlu	2-OHPhe	1-9-OHPhe	4-OHPhe	1-OHPyr
Urban	17	GM _{SG} ± SD	2.29 ± 3.40 ^a	0.919 ± 0.462 ^a	0.245 ± 0.171 ^a	1.96 ± 1.14 ^a	1.09 ± 0.841 ^a	1.52 ± 0.873 ^a
Rural	78	GM _{SG} ± SD	2.91 ± 6.37 ^a	1.24 ± 1.97 ^a	0.552 ± 0.598 ^b	2.03 ± 1.17 ^a	1.88 ± 2.01 ^b	1.27 ± 0.824 ^a

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

Table 3: Specific gravity adjusted OH-PAHs concentrations (ng/mL) in cattle urine

Sample site	<i>n</i>	Location	2-OHNap	2-3-OHFlu	1-9-OHPhe	2-OHPhe	3-OHPhe	4-OHPhe	1-OHPyr	
Oforikrom	8	urban	GM _{SG} ± SD	4.15 ± 4.37 ^b	0.99 ± 0.62 ^b	1.67 ± 1.21 ^b	0.17 ± 0.10 ^c	nd	0.73 ± 0.41 ^c	1.37 ± 1.18 ^{ab}
Santasi	9	urban	GM _{SG} ± SD	0.61 ± 0.23 ^{cd}	0.75 ± 0.35 ^b	2.26 ± 1.14 ^{ab}	0.32 ± 0.20 ^{bc}	nd	1.14 ± 0.33 ^{bc}	1.33 ± 0.80 ^{ab}
Twumasen Estate	31	rural	GM _{SG} ± SD	0.69 ± 0.57 ^d	0.31 ± 0.13 ^c	1.73 ± 0.58 ^{ab}	0.29 ± 0.26 ^c	nd	1.50 ± 0.86 ^b	0.99 ± 0.73 ^b
Saboa	40	rural	GM _{SG} ± SD	1.24 ± 0.67 ^c	0.80 ± 0.79 ^b	2.07 ± 1.43 ^{ab}	0.41 ± 0.16 ^b	nd	1.27 ± 0.91 ^{bc}	1.16 ± 0.74 ^b
Kokote	7	rural	GM _{SG} ± SD	21.9 ± 6.51 ^a	6.74 ± 1.41 ^a	3.12 ± 0.79 ^a	2.26 ± 0.51 ^a	2.1±0.57	7.49 ± 1.73 ^a	2.29 ± 1.28 ^a

n: number of samples; nd: below limits of quantification (LOQ); different letter (a, b, c and d) within a column indicate significant difference (*p* < 0.05) among communities; GM_{SG}: geometric mean concentration adjusted by specific gravity; SD: standard deviation

From the results, 2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr were detected. 9-OHFlu and the high molecular weight, HMW (\geq 4 rings) PAHs (6-OHChry, 3-OHB(e)P and 9-OHB(a)P) were, however, not detected in the cattle urine and could be due to low detection sensitivity (Campو et al., 2008) and/or because the HMW PAHs, such as BaP, are mainly excreted through feces (Burgaz et al. 1992; Li et al., 2008).

Specific gravity adjusted geometric mean concentrations (GM_{SG}) showed that 2-OHNap (2.77 ± 5.91 ng/mL; $p < 0.01$) was the most abundant OH-PAH in cattle urine from all study sites followed by 1-9-OHPhe (2.02 ± 1.16 ng/mL) $>$ 4-OHPhe (1.74 ± 1.87 ng/mL) $>$ 1-OHPyr (1.22 ± 0.87 ng/mL) $>$ 2-3-OHFlu (1.08 ± 1.75 ng/mL) $>$ 2-OHPhe (0.489 ± 0.555 ng/mL) $>$ and 3-OHPhe (0.278 ± 0.553 ng/mL) (Fig. 2).

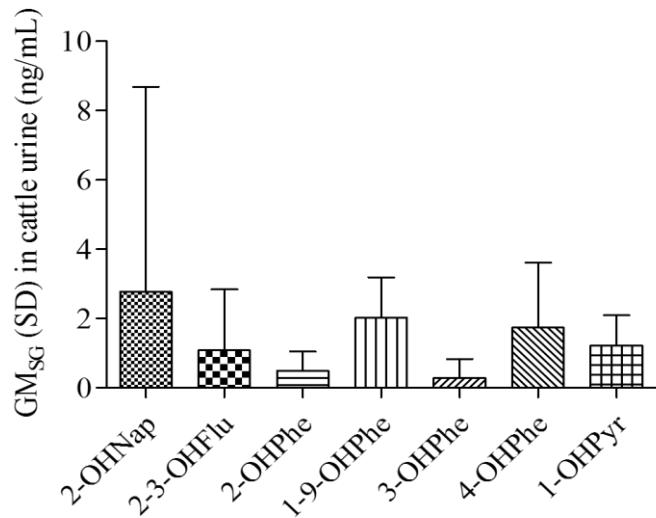


Fig. 2 Geometric mean concentrations (adjusted by specific gravity) of OH-PAHs in cattle urine from 5 sample sites in Kumasi and Offinso, Ghana

The GM_{SG} concentration (ng/mL) of the sum of OH-PAHs (2-OHNap, 2-3-OHFlu, 1-9OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe and 1-OHPyr) decreased in the order, Kokote (44.7 ± 10.4) $>$ Oforikrom (7.87 ± 7.41) $>$ Saboa (6.98 ± 3.86) $>$ Santasi (6.68 ± 2.701) $>$ and Twumasesen Estate (5.23 ± 1.55). High urinary concentrations of 2-OHNap, 2-3-OHFlu, 2-OHPhe, 3-OHPhe and 4-OHPhe were detected in Kokote (Table 3) indicating high exposure of cattle to the parent PAHs within the sample site. Kokote is a rural area filled with many

farmlands, with high agricultural and burning activities compared to the other sites, and the levels of OH-PAHs could mean that there were different or specific sources of PAHs exposure to cattle in the area.

2-OHNaphthalene

The GM_{SG} of 2-OHNap in cattle urine in Kokote (21.9 ± 6.51 ng/mL) was significantly higher ($p < 0.05$) compared to the other sites followed by Oforikrom (4.15 ± 4.37 ng/mL) (Table 3). However, the least GM_{SG} concentration for 2-OHNap was recorded in Santasi (0.61 ± 0.23 ng/mL). Kokote is mainly an agricultural area with many farmlands, and the use and sometimes abuse of pesticides containing naphthalene was possible. Although 1-OHNap could be derived from both naphthalene and carbaryl, 2-OHNap is derived only from naphthalene (Orjuela et al., 2012). The high levels of 2-OHNap could be due to exposure through ingestion and/or inhalation, although it has been proposed as a biomarker of inhalation (Kim et al. 2000). Naphthalene is ubiquitous in ambient air with high volumes in vehicular traffic, cigarette smoke (ATSDR, 2005) and is elevated when mothballs or stoves burning biomass fuels are used (Griego et al., 2008; Riojas-Rodriguez et al., 2011). Urinary levels of 2-OHNap are markers of vehicular traffic (Li et al., 2010) and mothball exposure (Owa et al., 1993). Naphthalene is most likely the primary ingredient of mothballs in Ghana (Soghoian et al., 2012), and is frequently used to drive away insects both in and outdoors. These practices could contribute to 2-OHNap being the most abundant metabolite in cattle urine since exposure through ambient air was also possible because of its volatile nature.

1-OHPyrene

The highest GM_{SG} concentrations of 1-OHPyr were detected in cattle in Kokote (2.29 ± 1.28 ng/mL) and Oforikrom (1.37 ± 1.18 ng/mL). Levels in Kokote were significantly higher ($p < 0.05$) compared to other sites except Oforikrom and Santasi (Table 3). GM concentrations (not adjusted by SG) of 1-OHPyr in this study (1.02 ng/mL [Twumasesn Estate] to 2.41 ng/mL [Kokote]) were generally higher compared to study by [Saengtienchai et al. \(2014\)](#) using cattle from Japan and Thailand (non-adjusted). A study by [Ferrari et al. \(2002\)](#) on determination of 1-OHPyr in bovine urine from three farms located near rural and urban areas recorded non-adjusted average concentrations of 0.66 ng/mL (urban area); 1.52 ng/mL (rural) and 5.09 ng/mL (highway). Similar to the present study, [Ferrari et al. \(2002\)](#) indicated higher levels of 1-OHPyr in rural areas compared to urban and suggested that other important sources besides traffic could contribute to the PAHs burden of animals. Results of non-adjusted 1-OHPyr concentrations from the present study was higher compared to study by [Ferrari et al. \(2002\)](#) except for levels recorded in cattle raised on farms located in the vicinity of a highway (5.09 ng/mL).

The levels in the present study could be due to vehicular activities or traffic. At high temperature combustion (that is during vehicular emissions) the HMW PAH compounds are dominant ([Laflamme and Hites, 1978](#)). Previous studies by [Bortey-Sam et al. \(2014; 2015\)](#) in PM10 and soils indicated pyrene as the eighth and second most abundant PAH in Kumasi, respectively, and combustion of fuel (74%) and wood/grass (23%) were the dominant sources in the region. In Ghana, some farms are generally located close to major roads with high vehicular activities or traffic ([Tay and Biney, 2013](#)), and exposure to domestic and grazing animals could be through inhalation, or picking food or water from the ground.

OHPheanthrenes and OHFluorenes

The distribution of 2-OHPhe, 4-OHPhe and 2-3-OHFlu were significantly higher ($p < 0.05$) in Kokote than the other sites (Table 3). Urinary levels of 1-9-OHPhe in Kokote was, however, significantly higher ($p < 0.05$) than levels found in Oforikrom. 3-OHPhe levels in cattle urine from all sites were below the LOQ except Kokote (Table 3). In this study, the most dominant OHPhe isomer from all sites was 1-9-OHPhe, which is similar to results obtained by [Fan et al. \(2012\)](#) in humans. However, in Kokote, 4-OHPhe was most abundant (Table 3). In human urine, [Thai et al. \(2016\)](#) and [Levine et al. \(2015\)](#) found 1-OHPhe as most dominant while [Guo et al. 2013](#) also reported 3-OHPhe as the most dominant of four phenanthrene metabolites. These variations could be due to differences in metabolic pathway among species. The possible source of phenanthrene and fluorene exposure to cattle in Kokote could be due to inhalation during combustion at low temperatures such as wood or grass combustion since the low molecular weight, LMW (< 4 rings) PAH compounds are abundant during low temperature combustion ([Lake et al., 1979](#)). Because Kokote is mainly agricultural area with many farmlands, resident farmers frequently practice bush burning. Another possible source could be due to ingestion of soil or water since the cattle graze freely and the soil from which they pick food or water may be bound to PAHs from burning activities. Previous study by [Tay and Biney \(2013\)](#) indicated that agricultural soils in Accra, Ghana were dominated by LMW PAHs through which domestic animals could be exposed. PAHs tend to adsorb tightly to organic matter in soil rendering them less susceptible to biological and chemical degradation ([Hatzinger et al., 1997](#)) and in general, LMW PAHs are more water soluble than HMW PAHs ([Nam et al., 2008](#)).

Association between urinary OH-PAHs concentrations and sex

Gender differences have been used in various studies to predict differences in OH-PAHs concentrations in human (Sul et al. 2012; Levine et al., 2015; Bartolomé et al., 2015; CDC, 2015). Study by Thai et al. (2016) in human urine showed no association between sex and urinary OH-PAHs concentrations. In this study, 2-OHNap was significantly higher ($p < 0.05$) in male cattle ($GM_{SG} = 4.43 \pm 7.16 \text{ ng/mL}$) compared to females ($GM_{SG} = 2.01 \pm 5.12 \text{ ng/mL}$) (Table 4). Kim et al. (2013) suggested that rates of intake, accumulation, and excretion of chemicals differ in male and female cattle, although ADME (absorption, distribution, metabolism, and excretion) data would be needed to support that assertion. Differences could also be due to different rearing systems from the various sites.

Table 4: Specific gravity adjusted urinary OH-PAHs concentrations (ng/mL) in male and female cattle in Kumasi and Offinso, Ghana

Sex		2-OHNap	2-3-OHFlu	1-9-OHPhe	2-OHPhe	3-OHPhe	4-OHPhe	1-OHPyr	Σ OHPAHs
Male	GM _{SG} ± SD	4.43 ± 7.16 ^a	1.36 ± 1.94 ^a	1.71 ± 1.07 ^a	0.534 ± 0.650 ^a	0.364 ± 0.658 ^a	1.91 ± 2.22 ^a	1.19 ± 0.981 ^a	11.5 ± 14.6 ^a
Female	GM _{SG} ± SD	2.01 ± 5.11 ^b	0.950 ± 1.65 ^a	2.16 ± 1.18 ^b	0.468 ± 0.510 ^a	0.237 ± 0.499 ^a	1.65 ± 1.69 ^a	1.22 ± 0.827 ^a	8.71 ± 10.0 ^a

GM_{SG}: geometric mean concentration adjusted by specific gravity; SD: standard deviation; Σ OHPAHs: sum of OHPAHs; different letters (a and b) within a column indicate significant differences (Student's T-Test; $p < 0.05$)

Several non pharmacogenetic factors such as age, gender, species, disease factors or exposure to environmental pollutants might contribute to the expression and regulation of hepatic P450 in man, laboratory species and domestic animals (Guengerich, 2002; Nebbia, 2001). In a study by Dacasto et al. (2005), male piedmontese cattle showed significantly higher CYP3A-dependent drug metabolizing enzymes, erythromycin *N*-demethylase (ERDEM), ethylmorphine *N*-demethylation (ETDEM) and testosterone 6 β -hydroxylation (6 β -OHT), activities compared to females, with the exception of testosterone 2 β -hydroxylase, 2 β -OHT, (whose enzymatic activity was yet lower in females). On the other hand, no gender-difference was noticed in limousin cattle.

In human, Sul et al. (2012) observed significantly higher levels of urinary 2-OHNap in men than females, and suggested that gender were predictors of urinary 2-OHNap concentrations. 1-9-OHPhe on the other hand was significantly higher in female cattle ($GM_{SG} = 2.17 \pm 1.18$ ng/mL) than males ($GM_{SG} = 1.71 \pm 1.07$ ng/mL) (Table 4), while $\sum OHPhe$ s in women were low compared to men (Bartolomé et al., 2015). These differences could be due to variations in metabolism, levels and route of exposure to PAHs. The urinary levels of 1-OHNap, 2-OHNap, $\sum OHNap$, 1-OHPhe, 9-OHPhe, $\sum OHPhe$, 1-OHPyr, and $\sum OHPAHs$ among women were all significantly or marginally higher than those among men workers (Guo et al. 2014). In consistency, Guo et al. (2014) found that, when exposed to similar levels of PAHs, women had significantly higher micronuclei frequencies than men. Emerging evidence also indicates that women may be at greater risk of lung cancer than men, probably because the elevated activity of CYP1A1 enzymes in women can produce higher levels of DNA adducts, and women have lower DNA repair capacity than men (Mollerup et al., 2006; Uppstad et al., 2011).

Conclusions

Cattle urine samples were collected from both urban (Kumasi) and rural (Offinso) sites in the Ashanti Region, Ghana, and GM_{SG} concentration of OH-PAHs indicated that, 2-OHNap was the most abundant followed by; 1-9-OHPhe > 4-OHPhe > 1-OHPyr > 2-3OHFlu > 2-OHPhe > 3-OHPhe. The results of the present study showed that cattle in Kokote (rural area) were exposed to significantly higher levels of PAHs than the other sites, and naphthalene-containing-mothballs might have contributed significantly to 2-OHNap levels detected in cattle urine. There was no significant difference between urinary OH-PAHs concentrations in cattle in urban and rural sites except for 2-OHPhe and 4-OHPhe. Similar to urban areas, rural sites could also be polluted with PAHs. Levels of 2-OHNap was significantly higher in male cattle compared to females, while the opposite was for 1-9-OHPhe.

Chapter 2

Oxidative stress due to exposure to polycyclic aromatic hydrocarbons (PAHs) in residents of Kumasi, Ghana

Introduction

In chapter 1, cattle urine was used as a “biological indicator” to assess the human risk in Kumasi and high levels of urinary OH-PAHs were detected with 2-OHNap as the most abundant (Bortey-Sam et al., 2016). In this chapter, I focused on effects of PAHs exposure to Kumasi residents. In humans, PAHs are metabolized by cytochrome P450 enzymes to generate active semiquinones (Penning et al., 2007) which are free radical intermediates and can go through redox cycling and generate reactive oxygen species (ROS). The ROS can then cause oxidative modification of DNA and lipids in the body (Palackal et al. 2002). The DNA adduct, 8-hydroxy-2'-deoxyguanosine (8-OHdG), is a useful biomarker of DNA damage to assess human exposure to carcinogenic compounds and malondialdehyde (MDA) as an indicator for oxidative stress and lipid peroxidation (Basu and Marnett, 1983).

Epidemiological studies have reported associations between PAHs exposure and urinary MDA and 8-OHdG in children, workers, as well as general population in several countries (Lee et al., 2012; Fan et al., 2012a; Kuang et al., 2013). 8-OHdG is very stable, excreted in urine without being metabolized (Valavanidis et al., 2009) and measurement could offer a specific and quantitative biomarker indicating oxidative DNA damage caused by ROS which is a risk factor for cancer and cardiovascular disease (Shen and Ong, 2000).

It is estimated that by 2020, cancer could kill 10.3 million people worldwide, with a 50 to 75% increase in cancer mortality in sub-Saharan Africa (Ferley et al., 2004). The number of cancer cases in Ghana has drastically increased (WHO, 2010; 2011) and GLOBOCAN estimated 16,600 cases of cancer annually in Ghana, yielding an age standardized rate of 109.5 cases per 100,000 persons (IARC GLOBOCAN, 2008). In addition, the rate of respiratory diseases doubled in Ghana from 2004-2008, and 6,500 Ghanaians died from air pollution/year (WHO, 2010; 2011); and PAHs could play a significant role. The commonest sites for cancers reported among both sexes in Kumasi were breast (24%), cervix uteri (20%), ovary (7.9%), liver (6.4%) and prostate (4.0%) (Laryea et al., 2014). PAHs have been shown to accumulate in breast tissues and led to breast cancer in humans (Korsh et al. 2015;

[Mordukhovich et al., 2016](#); [White et al., 2016](#)). The estimated cancer incidence for 2012 in Kumasi was 11.9 per 100,000. Among males and females, the cancer incidences were 7.3 and 15.7 per 100,000, respectively. The age-standardised incidence rates (ASR) for males was 10.9/100,000 and 22.4/100,000 for females ([Laryea et al., 2014](#)).

With the high cancer incidence and toxic potential of PAHs and its metabolites in Kumasi ([Laryea et al., 2014](#); [Bortey-Sam et al., 2014; 2015a; 2016](#)), and due to the lack of epidemiological studies on the risks of PAHs exposure to Kumasi residents, the objectives of the present study were to: assess the possible health effects due to PAHs exposure to residents of Kumasi; find the association between urinary concentrations of OH-PAHs, MDA and 8-OHdG with age and sex.

Materials and methods

Sampling

In January to February 2015, human urine samples ($n = 190$; 57 males and 133 females) were collected from 3 hospitals in Kumasi (Atonsu, Manhyia and Tafo hospitals), into labelled corning tubes (Corning Incorporated, New York, USA). Manhyia and Tafo hospitals are 5.1 and 3.5 km from the city centre of Kumasi, respectively (Fig. 1). Variations in temperature both annually and daily are quite small and the minimum temperature is around 23°C (73°F) (Bortey-Sam et al., 2015a). The coldest/wettest season is June to September.

Due to the lack of background urine and interferences during OH-PAHs quantification, 400 mL of human (children) urine (blank stock) was collected from 4 participants in residential areas of the Kwame Nkrumah University of Science and Technology (KNUST) campus to form a composite. KNUST is a public university located in Kumasi, Ghana, and because of the low vehicular movement, industrial and human activities, PAHs exposure from point sources were assumed to be negligible (Bortey-Sam et al., 2015a). However, because humans could be exposed to PAHs through feed and/or inhalation the sample collected was measured several times to confirm levels of OH-PAHs.

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. Additionally, the presence or absence of symptoms of PAHs exposure such as persistent cough, persistent headache, asthma, wheeze, respiratory tract infection (RTI), tachycardia, rhinitis/nasal congestion or inflammation and dyspnea were recorded. The study was performed in accordance with the guidelines and approval of the Ethical/Institutional Review Board of Ghana Health Service (GHS) and Council for Scientific and Industrial Research (CSIR), Accra, Ghana, after informed consent was obtained from each participant. Parent(s) gave written or informed consent and completed questionnaires on behalf of their child/children. Samples collected were stored at -20°C in the Department of Chemistry, KNUST, Ghana and later transported to the Laboratory of

Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan, where they were stored at –30°C until analysis.

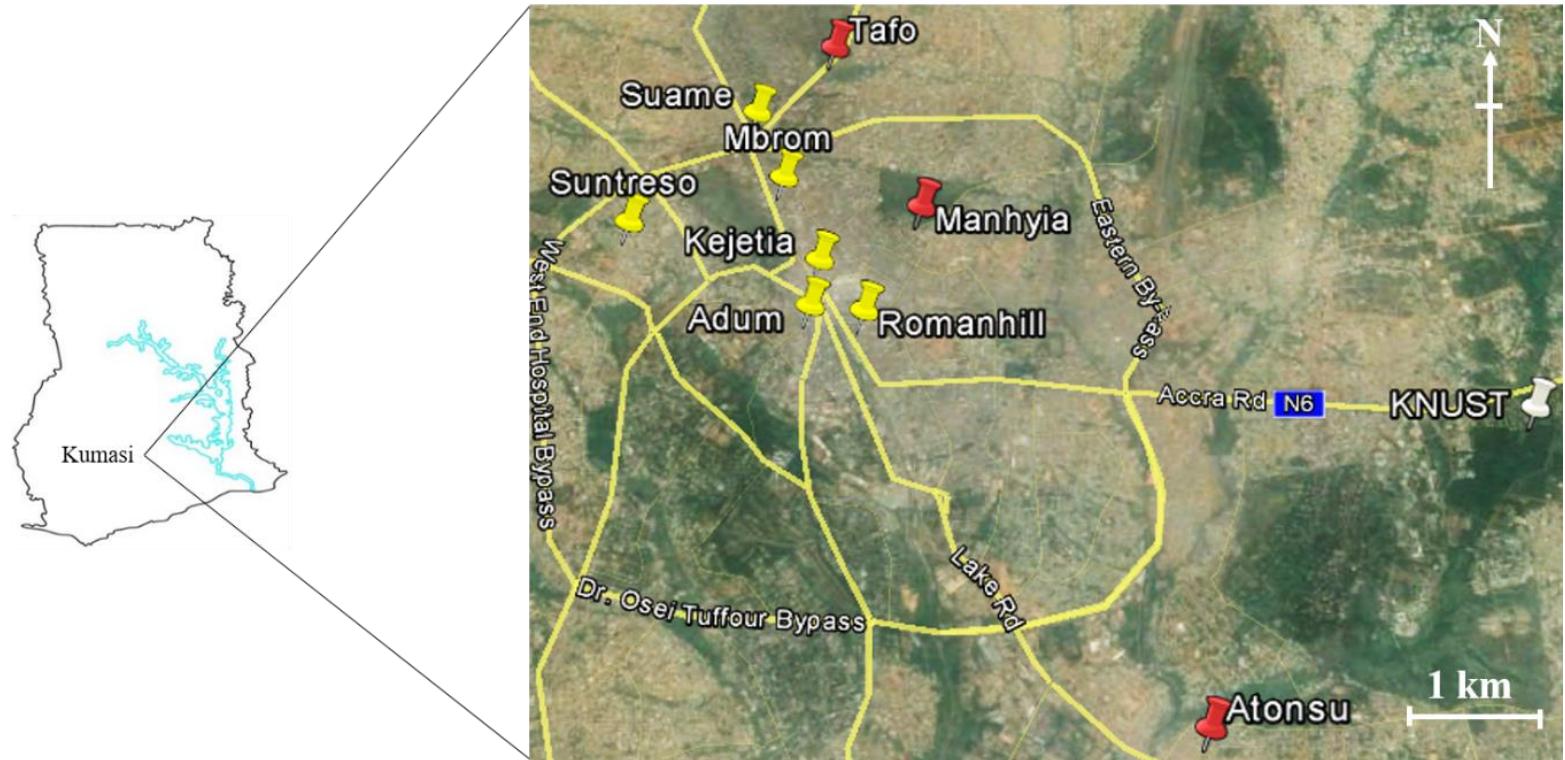


Fig. 1 Map showing human urine sampling locations in Kumasi, Ghana (yellow pins indicate city centre contaminated with PAHs; red pin indicate human urine sites; white pin indicate KNUST campus)

Sample extraction and analysis

OH-PAHs

Extraction of OH-PAHs from human urine was done according to method described by [Bortey-Sam et al. \(2016\)](#). Briefly, 20 µL each of β-glucuronidase (bovine liver, type B-1; 1240 U/mg; Sigma Aldrich) and arylsulfatase (limpets Type V; 34 units/mg; Sigma Aldrich) enzymes, and 5 mL of 0.1 M sodium acetate buffer (pH 5.6) were added to 5 mL urine sample after spiking with three PAH internal standards (13C6-2-OHFluorene, 3-OHPhenanthrene-d9, and 13C6-1-OHPyrene). The pH of sample was adjusted to 5.5 using 1 M acetic acid (Wako Pure Chemicals, Osaka, Japan) and incubated overnight at 37 °C. The sample was diluted with 4 mL of Milli-Q water and extracted twice (liquid-liquid extraction) with 10 mL each of n-pentane (Kanto Chemical Corp., Tokyo, Japan) by shaking for 1 h. To reduce the interference of sulfur metabolites, the combined extracts were washed with 2 mL of 1 N AgNO₃ solution (Wako Pure Chemicals, Osaka, Japan), concentrated to 50-100 µL, re-dissolved to 0.5 mL using methanol and filtered (0.20 µm DISMIC-13JP membrane filter, ADVANTEC, Toyo Roshi Kaisha Ltd., Japan) prior to instrumental analysis. All sample preparation steps were performed in darkness (by covering tubes completely with aluminium foil) to avoid possible photodegradation of target analytes. A total of 13 OH-PAHs; 2-hydroxynaphthalene (2-OHNap), 2-hydroxyfluorene (2-OHFlu), 3-hydroxyfluorene (3-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 1-hydroxypyrene (1-OHPyr), 6-hydroxychrysene (6-OHChry), 3-hydroxybenzo(e)pyrene (3-OHBeP) and 9-hydroxybenzo(a)pyrene (9-OHBaP), were analyzed in each sample. The standards (purity ≥ 98%) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and Toronto Research Chemical Inc. (Brisbane Road, North York, Canada). Difficulties were often associated with the separation of 1-OHPhe and 9-OHPhe, for this reason, the sum of these isomers was used as an abbreviation, 1-9-OHPhe. Similarly, 2-3-OHFlu was used as

sum of 2-OHFlu and 3-OHFlu. All results were adjusted by specific gravity and expressed in ng/mL.

A Shimadzu 8030 triple quadrupole mass spectrometer, upgraded to 8040 with UF lens, (ESI MS-MS; Shimadzu, Kyoto, Japan), equipped with a Prominence UFLC system (Shimadzu, Kyoto, Japan) was used for analysis. Chromatographic separation was achieved using an Agilent Eclipse PAH column (150 mm × 2.1 mm, 3.5 µm). The mobile phases were methanol:water (2:3, v:v) (A) and methanol (B), pumped at a flow rate of 250 µL/min. The mobile phase gradient was maintained as follows: 0.0–2.0 min, 5% B; 2.0–20 min, 40% B; 20–25 min, 40% B; 25–30 min, 95% B; 30–35 min, 95% B; 35–35.01 min, 5% B. Target compounds were determined by multiple-reaction monitoring (MRM) in the negative ionization mode ([Bortey-Sam et al., 2016](#)).

Malondialdehyde, MDA (Elisa kit)

Concentrations of urinary MDA were measured according to the manufacturer's instructions using a UV-VIS Spectrophotometer (UV-2600 Shimadzu Corporation, Kyoto, Japan). Briefly, 250 uL of calibrator (0, 1, 2, 3 and 4 µM) or urine sample was added to 10 uL of butylated hydroxytoluene (BHT) reagent in a vial. 250 uL each of acid reagent (1 M phosphoric acid) and 2-thiobarbituric acid (TBA) reagent were added and the mixture was vortexed vigorously then incubated at 60 °C for 1 hr. After centrifugation at 10000 x g for 2-3 min, the reaction mixture was transferred into a cuvette and spectrum was recorded from 400-700 nm. 3rd derivative analysis was performed at 514 nm.

8-hydroxy-2-deoxy-guanosine (8-OHdG)

One millilitre aliquot of each urine sample was diluted with 2 ml of water (HPLC grade) after spiking with 25 ng/mL of the stable isotope labelled internal standard, (15N5) 8-OHdG.

The diluted sample was subjected to solid-phase extraction using Oasis HLB cartridge (3cc, 60 mg; Waters Corporation, Milford, MA, USA) that had been primed with 1 mL each of methanol and water. After sample loading, the cartridge was sequentially washed with 3 mL of water. 8-OHdG was eluted with 3 mL of water: acetonitrile (1:1, v/v), and evaporated to near dryness under nitrogen gas. The residue was re-dissolved in 100 µL of water, and 10 µL was injected into the LC–MS/MS. Chromatographic separation of 8-OHdG and (15N5) 8-OHdG in urine were performed using a Phenomenex Gemini 3u C18 110A column (150 mm × 2 mm i.d., 4 micron, Phenomenex, California, USA) with a guard column. 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. Target compounds were determined by MRM in negative ionization mode 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.

Specific gravity (SG) of human urine

To compensate for variations in urine dilution, urinary OH-PAH, MDA and 8-OHdG concentrations were adjusted by specific gravity (SG). Urinary SG was measured by a hand refractometer (ATAGO, PAL-095, Tokyo, Japan). Obtained mean and ranges of SG in urine of cattle in Atomsu hospital (1.019; [1.003-1.045]), Manhyia hospital (1.020; [1.002-1.039]), Tafo hospital (1.003; [1.028-1.044]), and KNUST campus (1.021; [1.011-1.029]) were used to adjust urinary OH-PAHs concentrations as illustrated by [Nermell et al \(2008\)](#). The correction formula applied to each urine concentration was as follows:

$$\text{SG_corrected concentration} = \text{urinary OH - PAH concentration} \times \frac{(\text{SG}_{\text{target}} - 1.0)}{(\text{SG}_{\text{sample}} - 1.0)}$$

Where, SG_{target} is the mean specific gravity of cattle urine per community; SG_{sample} is the specific gravity of a particular sample.

Quality control and quality assurance

OH-PAHs

A mixture of three ^{13}C -isotopically labelled OH-PAHs (13C6-2-OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) was spiked into urine samples as internal standard prior to sample preparation and extraction. 13C6-2-OHFlu was used for quantification of metabolite of naphthalene and three metabolites of fluorene. 3-OHPhe-d9 was used for quantification of the five metabolites of phenanthrene and 13C6-1-OHPyr was used for quantification of 1-OHPyr, 6-OHChry, 3-OHBeP and 9-OHBaP.

Quantitation was performed using internal standard method (five-point calibration; 1, 5, 10, 50 and 100 ng/mL), and average correlation coefficients for the calibration curves in human urine were greater or equal to 0.99. The standard solutions (spiked with internal standards) for the calibration curves were prepared in urine in order to normalize differences in interferences between standards and samples. Concentrations of OH-PAHs in urine samples used for this purpose were below the limits of detection (LOD) and differences between this and sample concentration was used in this study. Analytical methods were checked for precision and accuracy. Limits of quantification (LOQs) were calculated based on $10\text{SD}/S$ (SD is the standard deviation of the response of seven replicate standard solution measurements and S is the slope of the calibration curve). LOQs (ng/mL) of OH-PAHs were, 0.29 (2-OHNap), 0.24 (2-3-OHFlu), 0.60 (9-OHFlu), 0.23 (2-OHPhe), 0.71 (1-9-OHPhe), 1.16 (3-OHPhe), 0.15 (4-OHPhe), 0.87 (1-OHPyr), 0.73 (6-OHChry), 0.54 (3-OHBeP) and 0.32 (9-OHBeP), respectively. Internal standard recoveries (13C6-2-OHFlu, 3-OHPhe-d9, and 13C6-1-OHPyr) ranged from 92 ± 6.5 – $97 \pm 10.3\%$.

For every batch of 10 samples, a solvent blank, a spiked solvent blank (internal standards spiked into solvent), a matrix spike (internal standards spiked into blank urine), and duplicate samples were analyzed. The average recoveries in spiked solvents blanks ranged from $93 \pm 5.2 - 98 \pm 7.6\%$, and that for matrix spikes was $90 \pm 8.9 - 97 \pm 6.4\%$. Blanks were run periodically and contained no detectable amount of target analyte. The coefficients of variation (CV) of OH-PAH in duplicate samples were less than 15%.

MDA and 8-OHdG

Similarly, for 8-OHdG, (15N5) 8-OHdG was used as internal standard and spiked into urine samples prior to sample preparation and extraction. Quantification was performed using internal standard method (five-point calibration; 1, 5, 10, 50 and 100 ng/mL). The average correlation coefficients for the calibration curves were greater 0.99 for both MDA and 8-OHdG. The LOQ for 8-OHdG was 0.6 ng/mL and average recovery ([15N5] 8-OHdG) was $72 \pm 9.8\%$. For every batch of 10 samples, spiked solvent blanks (8-OHdG only) and duplicate samples were analysed and average internal standard recovery for spiked solvents blanks was $104 \pm 8.7\%$. The CV's for duplicate samples were less than or equal to 10% (MDA and 8-OHdG). LOQ for MDA was 0.63 uM.

Data analysis

Data analysis was performed using IBM SPSS v 20 for windows (SPSS Inc., Illinois, USA). Kolmogorov-Smirnov (K-S) and Shapiro-Wilks tests were used to determine the normality of data and were considered statistically significant if *p* value was less than 0.05. Concentrations of OH-PAH below their respective LOQs were replaced with a value of LOQ/2. Geometric mean concentrations (\pm geometric standard deviation) were used to represent the central tendency of OH-PAH in this study (Wayne, 1999). ANOVA and Tukey analyses of log transformed data were used to compare concentrations of OH-PAH in human

urine from the study areas and differences were considered statistically significant with p value < 0.05 . Pearson's correlation of logged data was used to determine the association between OH-PAHs, MDA, 8-OHdG and age. Student's T-Test was also used to compare distribution of OH-PAHs, MDA and 8-OHdG between male and female participants, and considered statistically significant if p value was less than 0.05. To examine the relationship between PAHs exposure and occurrence of asthma, respiratory symptoms, persistent cough and headache, odds ratios (ORs) were derived from the logistic regression models at 95% confidence interval (CI). Prior to OR calculation, receiver operating characteristic (ROC) was used to set the threshold values for each OH-PAH which could give most accurate discrimination between the positive and negative symptoms based on the questionnaire administered and the responses in a measurement of concentration of each OH-PAH. Logistic regression analysis was performed to acknowledge the association between the high exposure to each OH-PAH (over the threshold) and the number of clinical or respiratory symptoms. Statistical significance level of the tests was set at 0.05 and statistical analyses were performed JMP 10 statistical software (SAS Institute).

Results and discussion

Urinary levels of OH-PAHs

As shown in [Table 1](#), 2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 4-OHPhe and 1-OHPyr were detected in human urine collected in Kumasi. Urinary 9-OHFlu and the high molecular weight, HMW (≥ 4 rings) PAHs (6-OHChry, 3-OHB(e)P and 9-OHB(a)P) were not detected. This trend could be due to low detection sensitivity and/or because the HMW PAHs, such as BaP, are mainly excreted through feces ([Campo et al., 2008](#); [Li et al., 2008](#)).

K-S and S-W's tests for normality showed a significant variation ($p < 0.01$) in all urinary OH-PAHs measured. The detection frequencies (%) of urinary OH-PAHs and oxidative stress biomarkers in this study were 98 (2-OHNap), 87 (2-3-OHFlu), 77 (2-OHPhe), 43 (1-9-OHPhe), 2 (3-OHPhe), 80 (4-OHPhe), 39 (1-OHPyr), 95 (MDA) and 59 (8-OHdG). 3-OHPhe was not included in data and discussion because of the low detection frequency.

Table 1: Specific gravity adjusted OH-PAHs concentrations (ng/mL) in human urine

Sample sites	<i>n</i>		2-OHNap	2-3-OHFlu	2-OHPhe	1-9-OHPhe	4-OHPhe	1-OHPyr	Σ OHPhe	Σ OHPAHs	MDA [#]	8-0HdG
Atonsu hospital	82	GM	5.62 ^a	0.521 ^b	0.181 ^a	0.172 ^{ab}	0.122 ^a	0.145 ^{ab}	0.647 ^a	8.09 ^a	0.958 ^a	1.63 ^a
		GSD	4.24	3.99	3.86	3.77	5.63	4.64	2.84	3.35	2.26	2.26
		Minimum	nd	nd	nd	nd	nd	nd	0.103	0.511	nd	nd
		Maximum	117	8.88	2.81	2.65	2.70	2.49	7.73	120	7.56	14.4
Manhyia hospital	51	GM	8.53 ^a	1.06 ^a	0.170 ^a	0.231 ^a	0.204 ^a	0.208 ^a	0.899 ^a	11.6 ^a	0.736 ^a	1.30 ^a
		GSD	3.97	3.48	4.76	4.30	4.17	4.16	2.70	3.50	2.09	2.62
		Minimum	nd	nd	nd	nd	nd	nd	0.162	1.16	nd	nd
		Maximum	224	6.54	2.27	4.04	7.19	4.54	14.2	230	8.38	7.94
Tafo hospital	56	GM	5.95 ^a	0.390 ^{bc}	0.172 ^a	0.128 ^{ab}	0.131 ^a	0.120 ^{ab}	0.643 ^a	8.05 ^a	0.809 ^a	1.91 ^a
		GSD	4.06	4.15	4.02	3.03	4.61	4.01	2.37	3.24	2.05	2.83
		Minimum	nd	nd	nd	nd	nd	nd	0.159	0.645	nd	nd
		Maximum	152	4.32	2.84	1.58	2.06	1.34	5.68	155	3.27	15.6

n: number of samples; nd: below limits of quantification (LOQ); GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; [#] indicates concentrations in micro molar (uM); different letters (a, b, c and d) indicates significant differences (*p* < 0.05) among sites

The results of specific gravity adjusted geometric mean concentrations ($GM_{SG} \pm GSD$) showed that 2-OHNap (6.01 ± 4.21 ng/mL) was the most abundant urinary OH-PAH in humans from all study sites followed by 2-3-OHFlu (0.526 ± 4.16 ng/mL) > 2-OHPhe (0.171 ± 3.99 ng/mL) \geq 1-9-OHPhe (0.160 ± 3.70 ng/mL) > 4-OHPhe (0.140 ± 4.79 ng/mL) and \geq 1-OHPyr (0.139 ± 4.44 ng/mL). The GM_{SG} ($\pm GSD$) concentration (ng/mL) of the sum of OH-PAHs, \sum OHPAHs (2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 2-OHPhe, 4-OHPhe and 1-OHPyr) decreased in the order, Manhyia (11.6 ± 3.51) > Atonsu (8.09 ± 3.35) \geq Tafo (8.06 ± 3.24) (Table 1). Participants who lived within high-traffic areas could be easily affected by traffic-related airborne PAHs (Fan et al., 2012a).

Urinary 2-OHNaphthalene concentrations

The GM_{SG} ($\pm GSD$) of urinary 2-OHNap in participants decreased in the order Manhyia (8.53 ± 3.97 ng/mL) > Tafo (5.95 ± 4.06 ng/mL) \geq Atonsu (5.62 ± 4.24 ng/mL) (Table 1). Highest level of 2-OHNap (224 ng/mL) was detected in urine of a 26 year old female participant in Manhyia hospital who reported symptoms of persistent cough, headache, tachycardia, nasal congestion and inflammation, all of which are symptoms of naphthalene exposure. In a study by Bortey-Sam et al. (2016) urinary 2-OHNap was the most abundant OH-PAHs in cattle in Kumasi and Offinso, Ghana, and levels were attributed to vehicular emissions and use of naphthalene-containing-mothballs in water purification. The high levels in participants could be due to exposure through ingestion and/or inhalation, although 2-OHNap has been proposed as a biomarker of inhalation (Kim et al., 2000). Naphthalene is ubiquitous in ambient air, contained in cigarette smoke with high volumes in vehicular traffic (ATSDR, 2005) and is elevated in indoor air when mothballs or stoves burning biomass fuels or wood are used (Riojas-Rodriguez et al., 2011). Urinary levels of 2-OHNap are markers of vehicular traffic (Li et al., 2010) and mothball exposure (Owa et al., 1993) and correlate significantly with naphthalene vapour levels in personal air monitors (Li et al., 2010). Exposure could also be through consumption of water “purified” with naphthalene-containing-mothballs. Naphthalene is the primary ingredient of mothballs in Ghana

(Soghoian et al., 2012), is frequently used in water purification (Soghoian et al., 2012), and drive away insects both in and outdoors (Bortey-Sam et al., 2016). In a study by Soghoian et al. (2012), 24% of lay person interviewed in a survey reported using ‘camphor’ (mothballs) routinely to purify water for drinking and bathing. Several people also gave specific recipes for making this ‘camphor water’ (mothballs), which they described as having a pleasing smell as well as internal cleansing abilities when used as a wash, gargle, drink or as an enema. Additionally, two physicians reported interviewing patients with haemolytic anaemia after ingestion of one or more whole ‘camphor’ mothballs to self-treat stomach ache, measles and diarrhoea. Exposure through ambient air is also possible because of its volatile nature as it is frequently used as insecticide and freshener in clothes.

Indoor exposure to naphthalene affects more than half of the world’s population and represents a potentially important environmental contributor to the global burden of disease (Jia and Batterman, 2010; Riojas-Rodriguez et al., 2011). Of the 190 urine samples collected, 51% were above 5.8 ng/mL (SG adjusted) which was associated with chromosomal aberrations including translocation in children exposed to naphthalene, and are pre-cancerous changes in adults (Orjuela et al. 2012).

Urinary 1-OHPyrene concentrations

1-OHPyr is the most commonly used OH-PAH biomarker and has been used to compare exposure to PAHs between occupational and non-occupational populations (Srogi, 2007). The GM_{SG} (\pm GSD) of urinary 1-OHPyr detected were 0.208 ± 4.16 ng/mL (Manhyia), 0.145 ± 4.65 ng/mL, (Atonsu) and 0.120 ± 4.01 ng/mL (Tafo) (Table 1). The highest urinary concentration of 1-OHPyr (4.54 ng/mL) was from a 22 year old cyesis participant who visited Manhyia hospital. The levels in the present study could be due to exposure through vehicular activities or traffic. At high temperature combustion (that is during vehicular emissions) the HMW PAH compounds are dominant (Laflamme and Hites, 1978). In previous studies, pyrene was the eighth and second most abundant PAH in PM10 and soils, respectively, in

Kumasi and combustion of fuel was the dominant source ([Bortey-Sam et al. 2013; 2014; 2015a](#)). Additionally, pyrene was the second most abundant in livers of wild rats in Kumasi ([Bortey-Sam et al., 2015b](#)).

Urinary OHPhenanthrenes and OHFluorenes concentrations

The distribution of 2-OHPhe, 4-OHPhe and 1-9-OHPhe were not significantly different ($p > 0.05$) among participants from the hospitals ([Table 1](#)). 1-9 and 4-OH-Phes were most abundant (4.04 and 7.19 ng/mL, respectively) from a 22 year old participant who visited Manhyia hospital with ulcer and also complained of dyspnea. Urinary levels of 2-3-OHFlu in Manhyia hospital was significantly higher ($p < 0.05$) than levels found in the other hospitals participants ([Table 1](#)). In terms of abundance, the GM concentrations of OHPhe isomers did not show any significant difference ($p > 0.05$) although the order was 2-OHPhe (0.171 ng/mL) \geq 1-9-OHPhe (0.160 ng/mL) \geq 4-OHPhe (0.140 ng/mL). In a study by [Fan et al. \(2012a\)](#), the most dominant OHPhe isomer was 1-9-OHPhe. Similarly, [Thai et al. \(2016\)](#) and [Levine et al. \(2015\)](#) found 1-OHPhe as most dominant while [Guo et al. \(2013\)](#) also reported 3-OHPhe as most dominant of four phenanthrene metabolites. These variations could be due to differences in metabolism or levels of exposure ([Bortey-Sam et al., 2016](#)).

The possible source of phenanthrene and fluorene exposure in Kumasi residents could be due to inhalation during combustion at low temperatures such as wood or grass combustion since the low molecular weight, LMW (< 4 rings) PAH compounds are abundant during low temperatures combustion ([Lake et al., 1979](#)). Residents in Ghana frequently practiced bush burning including wood/grass combustion, and exposure to phenanthrenes and fluorenes through these were possible. In PM10 and soils in Kumasi, wood/grass combustion was the second dominant source of PAHs after fuel combustion ([Bortey-Sam et al., 2013; 2014; 2015a](#)). Additionally, in the livers of wild rats in Kumasi, phenanthrene was the most abundant and was significantly higher ($p < 0.05$) than other PAHs measured ([Bortey-Sam et al., 2015b](#)).

Association between urinary concentrations of OH-PAHs, MDA, 8-OHdG with age

Gender and age differences have been used in various studies to predict differences in OH-PAHs concentrations in humans ([Sul et al., 2012](#); [Levine et al., 2015](#); [Bartolomé et al., 2015](#)). As shown in [Table 2](#), there was no association between urinary OH-PAHs concentrations and age, which is similar to studies by [Thai et al. \(2016\)](#). The non-linear relationship suggests adult-specific behaviours or exposure sources to PAHs that are not experienced by the other age groups ([ABS, 2013](#)).

Table 2: Correlation between urinary concentrations (ng/mL) of OH-PAHs, MDA, 8-OHdG and age of participants in Kumasi, Ghana

	2-OHNap	2-3-OHFlu	2-OHPhe	1-9-OHPhe	4-OHPhe	1-OHPyr	Σ OHPhe	Σ OHPAHS	MDA	8-OHdG	Age (yrs)
2-OHNap	1										
2-3-OHFlu	0.554**	1									
2-OHPhe	0.492**	0.634**	1								
1-9-OHPhe	0.432**	0.673**	0.741**	1							
3-OHPhe	0.164*	0.062	0.229**	0.239**							
4-OHPhe	0.424**	0.659**	0.623**	0.732**	1						
1-OHPyr	0.451**	0.524**	0.638**	0.695**	0.726**	1					
Σ OHPhe	0.503**	0.726**	0.879**	0.883**	0.854**	0.767**	1				
Σ OHPAHS	0.966**	0.674**	0.604**	0.552**	0.545**	0.546**	0.639**	1			
MDA	0.359**	0.223**	0.399**	0.200**	0.146*	0.136	0.216**	0.362**	1		
8-OHdG	0.120	0.119	0.074	0.062	0.149*	0.088	0.123	0.138	0.088	1	
Age (yrs)	0.159	0.158	0.163	0.087	0.074	0.051	0.132	0.189	0.009	0.138	1

* Indicates significance at $p < 0.05$; ** Indicates significance at $p < 0.01$

For example, in Ghana ([WHO, 2006](#)) like Australia ([ABS, 2013](#)), smoking rates were higher in adolescents (13-15%) and adults ($\geq 15\%$) than among the other age groups. Because smoking is a major source of PAH exposure among non-occupationally exposed population ([Srogi, 2007](#)), different smoking rates by different age groups could have contributed to the age-dependent profile of OH-PAH concentrations seen in this study ([Thai et al., 2016](#)). Of the 141 participants who answered, 13 smoked while 128 including children did not, in this study. Additionally, according to NHANES 1999–2002 data, income level and to lesser extent education attainment, were significant determinants of urinary excretion of PAH metabolites ([Suwan-ampai et al., 2009](#)).

Among the various age groups ([Table 3](#)), there was no significant difference in all measured urinary concentrations of OH-PAHs MDA and 8-OHdG except for 1-9-OHPhe which was significantly higher ($p < 0.05$) in age group 61-85 compared to 41-60. The Σ OHPAHs, although not significant, was highest in age groups 21-85, with MDA and 8-OHdG highest in age groups 21-60 ([Table 3](#)). The lower concentrations at this age (3-20 years) could reflect lower exposure to PAHs and thus potentially reduce the health effects among the group as suggested by [Perera et al. \(2014\)](#). This results is consistent with the 2012 Cancer Summary data from the Kumasi Cancer Registry (KsCR) ([Population-based cancer registry, PBCR](#)), where the incidence rate of cancer in Kumasi is stable or low from 0-19 years, and gradually increases with age and the peak incidence being at ages 40-64 (males) and 30-69 (females), then decreases around age 70 (KsCR ([Population-based cancer registry 2012; Laryea et al., 2014](#)).

Similarly, in a study by [Thai et al. \(2016\)](#), urinary concentrations of OH-PAHs were low in infants (0-4), children (5-14) and the elderly (over 60 years) than in adolescents and adults (15-59 years).

Table 3: Age differences in urinary concentrations (ng/mL) of OH-PAHs, MDA, and 8-OHdG among participants in Kumasi, Ghana

Age groups		<i>n</i>	2-OHNap	2-3-OHFlu	2-OHPhe	1-9-OHPhe	4-OHPhe	1-OHPyr	Σ OHPhe	Σ OHPAHs	MDA [#]	8-OHdG
Ages 3-20	GM	36	4.99 ^a	0.501 ^a	0.145 ^a	0.134 ^{ab}	0.113 ^a	0.141 ^a	0.576 ^a	6.94 ^a	0.813 ^a	1.01 ^a
	GSD		3.84	3.75	3.81	3.93	3.38	3.31	2.61	3.32	4.23	1.99
	Minimum		0.709	nd	nd	nd	nd	nd	0.137	1.16	nd	0.206
	Maximum		62.6	3.46	1.08	1.42	0.922	0.922	3.46	63.1	8.38	2.01
Ages 21-40	GM	94	5.28 ^a	0.552 ^a	0.170 ^a	0.174 ^{ab}	0.147 ^a	0.143 ^a	0.716 ^a	7.69 ^a	0.914 ^a	1.83 ^a
	GSD		4.04	4.61	4.16	3.93	5.72	5.53	2.88	3.33	2.01	2.52
	Minimum		nd	nd	nd	nd	nd	nd	0.102	0.511	nd	nd
	Maximum		224	8.88	2.81	4.04	7.19	4.54	14.2	230	7.56	12.9
Ages 41-60	GM	29	9.88 ^a	0.652 ^a	0.251 ^a	0.125 ^b	0.114 ^a	0.137 ^a	0.654 ^a	12.8 ^a	0.928 ^a	2.29 ^a
	GSD		4.97	2.83	3.04	3.73	5.30	3.23	2.31	3.50	1.97	2.93
	Minimum		nd	nd	nd	nd	nd	nd	0.137	0.644	nd	nd
	Maximum		152	2.85	2.84	0.575	0.772	0.622	5.68	155	6.59	15.6
Ages 61-85	GM	31	9.10 ^a	0.745 ^a	0.240 ^a	0.369 ^a	0.216 ^a	0.224 ^a	1.03 ^a	12.7 ^a	0.751 ^a	0.959 ^a
	GSD		4.96	4.56	4.18	3.16	5.73	4.40	3.07	4.20	1.58	1.53
	Minimum		nd	nd	nd	nd	nd	nd	0.182	0.549	nd	nd
	Maximum		117	6.54	1.67	2.02	2.49	2.49	5.24	125	1.45	1.81

n: number of samples; nd: below limit of quantification; GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; [#] indicates concentrations in micro molar (uM); different letters (a, b, c and d) indicates significant differences ($p < 0.05$) among age groups

Association between urinary concentrations of OH-PAHs, MDA, 8-OHdG with sex

For sex differences, significantly higher ($p < 0.05$) urinary concentrations of OH-PAHs (2-OHNap, 2-3-OHFlu, 1-9-OHPhe, 4-OHPhe, 1-OHPyr, Σ OHPhe and Σ OHPAHs) were observed in females compared to males (Table 4). Although gender variation for 8-OHdG agreed with the fact that micronuclei rate (an indicator of genetic damage) was higher for females (Yang et al. 2015), urinary concentrations of MDA and 8-OHdG in this study were higher ($p > 0.05$) in male participants than females (Table 4). The differences could be impacted by many factors, including lifestyle/behaviours not captured in this study or differences in genetic factors impacting metabolism of these compounds, such as polymorphism in acetylators genes (Levine et al., 2015; Al-Daghri et al., 2013; Hansen et al., 2008). In addition to other exposure sources, this could be due to the fact that the daily tobacco smoking, which contains other contaminants other than PAHs, was 4 times higher in males (7) than females (1.7) (WHO, 2011) and percent of tobacco use in persons 15 years of age or older in Ghana was 13 times higher in males (9.5) than females (0.7) (WHO, 2010). Females could also be exposed to higher levels of PAHs because they usually burned firewood/charcoal for cooking and/or cooked (fried, grilled, smoked, etc.) for their families. These activities have been associated with PAHs exposure in previous studies (Motorykin et al., 2015).

2-OHNap was 3 times higher in females than males (Table 4). This trend is similar to studies by Orjuela et al. (2012) and Guo et al. (2014), in which the urinary levels of 1-OHNap, 2-OHNap, Σ OHNap, 1-OHPhe, 9-OHPhe, Σ OHPhe, 1-OHPyr, and Σ OHPAHs were all significantly or marginally higher among women than men workers. However, a study by Thai et al. (2016) in human urine showed no association between sex and urinary OH-PAHs concentrations. When exposed to similar levels of PAHs, women had significantly higher micronuclei frequencies than men (Guo et al., 2014). Emerging evidence also indicates that women may be at greater risk of lung cancer than men, because the elevated activity of CYP1A1 enzymes in women can produce higher levels of DNA adducts, and women have lower DNA repair capacity than men (Mollerup et al., 2006; Uppstad et al., 2011). In contrast,

[Sul et al. \(2012\)](#) and [Bartolomé et al. \(2015\)](#) observed significantly higher levels of urinary 2-OHNap and \sum OHPheS, respectively, in men than females, and suggested that gender were predictors of urinary 2-OHNap concentrations ([Sul et al., 2012](#)). These differences could be due to variations in levels and route of exposure to PAHs. Additionally, several non-pharmacogenetic factors such as age, gender, disease factors or exposure to environmental pollutants might contribute to the expression and regulation of hepatic P450 in human ([Guengerich, 2002](#); [Nebbia, 2001](#)).

Table 4: Distribution of SG adjusted urinary OHPAHs, MDA and 8-OHdG concentrations (ng/mL) in males and females in Kumasi, Ghana

Sex		<i>n</i>	2-OHNap	2-3-OHFlu	2-OHPhe	1-9-OHPhe	4-OHPhe	1-OHPyr	Σ OHPhe	Σ OHPAHs	MDA [#]	8-OHdG
Males	Geo mean	57	3.09 ^a	0.320 ^a	0.140 ^a	0.114 ^a	0.0998 ^a	0.0932 ^a	0.522 ^a	4.65 ^a	0.933 ^a	1.78 ^a
	Geo SD		3.55	4.01	3.45	3.89	4.38	4.30	2.44	2.88	2.74	2.63
	Minimum		nd	nd	nd	nd	nd	nd	0.102	0.549	nd	nd
	Maximum		73.6	4.81	1.89	1.58	2.49	2.49	5.24	75.0	8.38	12.9
Females	Geo mean	133	8.27 ^b	0.666 ^b	0.188 ^a	0.188 ^b	0.165 ^b	0.169 ^b	0.774 ^b	11.1 ^b	0.808 ^a	1.58 ^a
	Geo SD		4.08	4.01	4.23	3.51	4.90	4.36	2.66	3.37	2.08	2.46
	Minimum		nd	nd	nd	nd	nd	nd	0.137	0.511	nd	nd
	Maximum		224	8.88	2.84	4.04	7.19	4.54	14.2	230	7.56	15.6

n: number of samples; nd: below limit of quantification; GM: geometric mean concentration adjusted by specific gravity; GSD: geometric standard deviation; different letters (a and b) indicate significant differences (Student's T-Test; *p* < 0.05); [#] indicates concentrations in micro molar (uM)

Association between PAHs exposure and respiratory/clinical symptoms

Based on ROC, threshold values obtained were used to determine the odds ratio (OR) ([Table 5](#)). Odds ratio (OR) analysis was used to find/predict the association between PAHs exposure and occurrence of respiratory symptoms, asthma, persistent cough and headache. As shown in [Table 5](#), exposure to PAHs (naphthalene, fluorene and phenanthrene) at concentrations above the threshold significantly increases the odds of occurrence of persistent cough (OR = 2.5, CI: 1.33-4.70), persistent headache (OR = 1.89, CI: 1.66-3.36), RTI (OR = 46.2, CI: 2.88-742), tachycardia (OR = 3.65, CI: 1.50-8.88) and dyspnea (OR = 4.11, CI: 1.75-9.65) among Kumasi residents. Additionally, in both sexes, the results showed a significant relationship ($p < 0.05$) between naphthalene exposure and occurrence of tachycardia (OR = 3.36, CI: 1.39-8.1), dyspnea (OR = 3.07, CI: 1.27-7.43), persistent cough (OR = 2.68, CI: 1.43-5.05) and persistent headache (OR = 1.82, CI: 1.02-3.26) ([Table 5](#)). 2-OHNap concentration over 16.6 ng/mL increased the occurrence of asthma in both sexes though there was no significant association (OR = 5.11; CI: 0.82-31.5).

Gender differences revealed that, in females, occurrence of persistent headache (OR = 2.51, CI: 1.04-4.29) and dyspnea (OR = 3.30, CI: 1.21-8.99) at urinary 2-OHNap concentrations above the respective thresholds were significantly higher ($p < 0.05$) than males ([Table 5](#)). However, occurrence of persistent cough in males are more associated with urinary 2-OHNap concentrations above 8.39 ng/mL (OR = 4.44, CI: 1.05-18.6) compared with females (OR = 2.39, CI: 1.11-5.04).

Living in high-traffic areas and/or exposure to diesel exhaust particles have been associated with increased respiratory symptoms and impaired lung function in humans ([Dockery et al. 1989; Rudell et al. 1996; Gehring et al. 2002; Wyler et al. 2000; Northridge et al. 1999](#)). The high occurrences and odds of respiratory symptoms, asthma, persistent cough and headache among Kumasi residents could be due to high exposure to PAHs from fuel, wood/grass combustion and also exposure through the use of naphthalene-containing-mothballs in drinking water purification, treatment of various ailment, drive away insects and/or freshener in clothes.

Table 5: OR (95% CI) for the presence or absence of clinical/respiratory symptoms in Kumasi residents due to PAHs exposure

OHPAH	Clinical symptom	Threshold	Both sexes				Male				Female				
			OR	CI	CI	p value	OR	CI	CI	p value	OR	CI	CI	p value	
2-OHNap	Persistent cough	8.39	2.68	1.43	5.05	0.0018	4.44	1.05	18.6	0.03	2.368	1.11	5.04	0.02	
	Persistent headache	7.55	1.82	1.02	3.26	0.04		0.461	0.149	1.42	0.17	2.5115	1.04	4.29	0.03
	Asthma	16.5	5.11	0.82	31.5	0.05						3.355	0.538	20.9	0.17
	Wheezing	7.08	1.18	0.07	19.2	0.9						0		0.10	
	Respiratory tract infection	3.11				0.22						20.33	1.42	290.6	0.002
	Upper respiratory congestion	8.88	0.31	0.03	2.71	0.26		2.61	0.213	31.9	0.43	0		0.08	
	Tachycardia	14.1	3.36	1.39	8.1	0.004		8.83	0.61	127	0.06	2.176	0.828	5.718	0.10
	Rhinitis/NCI	3.11										2.715	0.507	14.54	0.22
	Dyspnea	6.6	3.07	1.27	7.43	0.0098		2.38	0.51	11.06	0.25	3.308	1.217	8.99	0.01
$\sum \text{OHPHes}$	Persistent cough	1.16	2.09	1.09	4	0.023	0.73	0.219	2.43	0.60	2.03	0.958	4.298	0.06	
	Persistent headache	1.17	1.46	0.785	2.73	0.228		5.05	0.924	27.6	0.04	0.5368	0.263	1.093	0.08
	Asthma	1.21	10.5	1.14	96.2	0.0107						3.23	0.518	20.12	0.18
	Wheezing	0.186				0.65						0.0677	0.00387	1.187	0.01
	Respiratory tract infection	1.49	7.35	0.649	83.1	0.06						1.453	0.127	16.56	0.76
	Upper respiratory congestion	0.892	7.95	0.91	69.5	0.02									
	Tachycardia	1.42	4.76	1.95	11.5	0.0002		26.5	2.36	296.6	0.0004	3.153	1.182	8.41	0.01
	Rhinitis/NCI	0.855	6.43	1.32	31.1	0.009		9.2	0.758	111	0.04	3.207	0.598	17.18	0.15
	Dyspnea	1.45	3.4	1.45	8	0.003		11.75	2.1	65.7	0.0012	2.32	0.844	6.38	0.09
$\sum \text{OHPAHs}$	Persistent cough	11.7	2.5	1.33	4.7	0.003	3.61	0.898	14.5	0.06	1.917	0.918	3.999	0.08	
	Persistent headache	8.92	1.89	1.06	3.36	0.029		0.3809	0.1145	1.267	0.10	1.735	0.859	3.5	0.12
	Asthma	19.7	2.41	0.39	14.9	0.328						3.65	0.58	22.61	0.14
	Wheezing	10.1				0.205						0		0.10	
	Respiratory tract infection	125	46.2	2.88	742	0.0001						30.75	1.91	494.3	0.0003
	Upper respiratory congestion	13.6	0			0.07		9.2	0.758	111.6	0.04	0		0.12	
	Tachycardia	13.5	3.65	1.5	8.88	0.002		2.722	0.242	30.5	0.40	2.44	0.928	6.43	0.06
	Rhinitis/NCI	13.2	2.85	0.776	10.4	0.10						3.1	0.578	16.61	0.16

Dyspnea	19.7	4.11	1.75	9.65	0.0006		5.375	1.109	26.04	0.025		5.2	1.861	14.56	0.0008
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OR: Odds ratio; CI: 95% Confidence Interval; Threshold values were expressed in ng/mL; $\sum\text{OHPHes}$: sum of OHPHes;
 $\sum\text{OHPAHs}$: sum of OH-PAHs; NCI: nasal congestion or inflammation

Human health risk implications

As shown in [Table 2](#), urinary concentrations of 2-OHNap, 2-3-OHFlu, 2-OHPhe, 1-9-OHPhe, 4-OHPhe, Σ OHPhe and Σ OHPAHs increased significantly ($p < 0.05$) as MDA increases. However, there was no significant association between 8-OHdG and OH-PAHs studied except for 4-OHPhe ($p < 0.05$). This trend is quite similar to previous studies where urinary 8-OHdG and/or MDA gradually increased as the environmental exposure levels to PAHs increased ([Kuang et al., 2013](#); [Li et al., 2015](#); [Yang et al., 2015](#)), although strong correlation between urinary Σ OHPAHs and 8-OHdG was not established in non-occupational population ([Fan et al., 2012b](#)).

MDA has potential to react with nucleic acid bases to form DNA adducts, create DNA interstrand crosslinks, and even generate DNA protein cross-links ([Del Rio et al., 2005](#)). These reaction products have been demonstrated to be involved in the onset and development of a series of adverse health effects including cardiovascular diseases, diabetes, and cancers ([Dierckx et al., 2003](#); [Wu et al., 2004](#)). Similarly, urinary 8-OHdG has also been indicated as a risk factor for atherosclerosis, diabetes, and various cancers ([Wu et al., 2004](#)). Increased oxidative stress levels reportedly correlated with many chronic degenerative diseases such as allergic inflammatory diseases ([Bartsch and Nair, 2004](#)), obesity and atherosclerosis ([Kobayashi et al., 2011](#); [Wu et al., 2004](#)) which were some of the symptoms obtained from participants in this study during the face-to-face interview. For instance, the questionnaire revealed that 10% of participants were diabetics and 2% had arthritis. Of 33 participants that we calculated body mass index, 33% (11) were overweight and 21% (7) were obese.

In addition, the high prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the Ghanaian population may increase the risk of toxic effects from ingestion of mothballs or other naphthalene-containing products ([Soghoian et al., 2012](#)).

Conclusions

Urinary 2-OHNap was the most abundant PAH metabolites excreted in humans in the Kumasi metropolis of Ghana and naphthalene exposure through the frequent use of naphthalene-containing-mothballs in drinking water purification, drive away insects, freshener in clothes have contributed significantly. The present study showed a significant sex difference with higher levels of urinary OH-PAHs in women than men. Exposure to PAHs significantly increases the odds of occurrence of persistent cough, persistent headache, RTI, tachycardia and dyspnea among Kumasi residents. Gender differences revealed that the occurrence of persistent headache and dyspnea due to naphthalene exposure were significantly higher in females than males. There was no association between urinary OH-PAHs concentrations and age, with MDA and 8-OHdG highest in age groups 21-60. The high prevalence of G6PD deficiency in the Ghanaian population and the significantly positive association between urinary MDA, 8-OHdG and OH-PAHs concentrations could suggest lipid peroxidation/cell damage or chronic degenerative diseases including cardiovascular diseases to Kumasi residents through PAHs exposure.

Summary of Section 1

Contamination of rural and urban environments with PAHs from fuel, wood or grass combustion and the frequent use of naphthalene-containing-mothballs for various purposes in Ghana could cause health related problems. For both cattle and human, urinary concentrations of 2-OHNap was the most abundant PAH metabolites and for cattle, levels were higher in male compared to females. On the other hand, levels of 2-OHNap and other metabolites were higher in female participants than males, while MDA and 8-OHdG were higher in males. Cattle in Kokote, a rural and agricultural area, were exposed to significantly higher levels of PAHs than from the other sites. For human participants, the highest concentrations of the sum of OH-PAHs were from those who visited the Manhyia hospital, which is just about 5 km from the city centre where concentrations and risks of PAHs in environmental samples were significantly higher. The present study revealed that exposure to PAHs significantly increases the odds of occurrence of persistent cough, persistent headache, RTI, tachycardia and dyspnea among Kumasi residents. The high levels of 2-OHNap and the high prevalence of G6PD deficiency in the Ghanaian population could increase the risk of toxic effects from the ingestion and exposure to mothballs or other naphthalene-containing products. Σ OHPAHs, 2-OHNap, 2-3-OHFlu, and -OHPhe showed a significantly positive correlation with MDA and 4-OHPhe with 8-OHdG, indicating possible lipid peroxidation/cell damage in some participants. There was no association between urinary OH-PAHs concentrations with age; while MDA and 8-OHdG were highest in age group 21-60.

Section 2

Heavy metals and metalloid

General introduction

Contamination of the environment with heavy metal/metalloids is an issue of worldwide concern because of the several health related problems ([Varol, 2011](#); [Yaylalı–Abanuz, 2011](#); [Mireles et al., 2012](#)). Metals are found naturally in the environment, although anthropogenic activities including mining have caused elevated levels.

The mining industry of Ghana accounts for 5% of the country's GDP and minerals make up 37% of total exports, of which gold contributes over 90% of the total mineral exports. Ghana was Africa's largest gold producer, producing 80.5 tonnes in 2008 and is also a major producer of bauxite, manganese and diamonds. Ghana has 23 large-scale mining companies producing gold, diamonds, bauxite and manganese, and, there are also over 300 registered small scale mining groups and 90 mine support service companies ([Mining Industry of Ghana](#)).

In Ghana, mercury is applied in gold mining because it provides a cheap and effective means to extract gold. However, due to present regulations on its use ([Mercury Act, 1989](#)), it is no longer used in large-scale operations although small-scale gold miners continue to apply it illicitly. The large-scale operators complied with new laws and regulations controlling the use of mercury, and switched to usage of cyanide instead. The difficulty in obtaining the metal due to present regulations and cost has forced small-scale operators to recover the mercury and reuse it ([Hogarh et al., 2016](#)). This practice directly exposes them to mercury, especially from dermal contact (during the field visit *galamsey* operators directly handled mercury in their palm) ([Hogarh et al., 2016](#)). The continuous application of mercury, however, raises serious environmental health concerns. Inorganic mercury may cause various neurotoxic effect and damages to human organs such as the kidney ([Langford and Ferner, 1999](#)).

Tarkwa is located in the south-west of Ghana and is one of the major mining towns in Ghana. The rapid industrialization and mining activities over the past decades has brought significant environmental problems with metals and metalloids (Amonoo-Neizer et al., 1995). A major aspect of the fate of these contaminants in the environment concerns their settling into soils and migration into water bodies, especially via runoffs. These could in turn contaminate food crops as the water is used for irrigation purposes. Accumulation of metals (in soil) could also degrade soil quality; reduce yield and quality of agricultural products. More importantly, the metals bioaccumulate in the edible parts of crops and thus negatively impact the health of human, animals and the ecosystem (Khan et al., 2008; Nagajyoti et al., 2010). Food consumption has been identified as the major pathway of human exposure, accounting for >90% compared to other ways of exposure such as inhalation and dermal contact (Loutfy et al., 2006).

Acute and chronic intake of heavy metals and metalloids above their safe threshold in humans and animals have damaging effects and can cause non-carcinogenic and carcinogenic effects such as neurologic involvement, liver disease/cancer (John and Andrew, 2011; Lai et al., 2010; Zheng et al., 2007; (Kapaj et al., 2006; Lin et al., 2013). In 2015, lifeless bodies of two kids were retrieved after they fell into a pit dug in search of precious minerals in Ghana. The mothers of the kids said while engaged in galamsey (illegal mining), they left the kids to idle, playing around open pits (Ghana News; November, 2015. Available online: <http://myjoyonline.com/news/2015/November-9th/two-kids-die-after-falling-into-galamsey-pit.php>).

Due to continuous growth of mining, mine leakages and the lack of thorough study in Tarkwa, Ghana, this section focuses on determining the distribution, sources and risks of heavy metals and a metalloid in the ecosystem.

Chapter 1

**Ecological risk of heavy metals and a metalloid in agricultural
soils in Tarkwa, Ghana**

Introduction

Heavy metal and metalloids pollution in the environment has become an important issue worldwide due to the abundance of sources, their environmental persistence, and potential toxicity to ecological receptors (Lim et al., 2008; Kumar et al., 2008; Wei and Wang, 2010; Varol, 2011; Yalali-Abanuz, 2011). The accumulation of heavy metals in soils is affected by many environmental variables, including parent material, soil properties, as well as by human activities and point sources. The rapid development and industrialization including mining activities over recent decades in Ghana's Tarkwa region has brought significant environmental problems. For example, a number of researchers have documented widespread contamination in this region with toxic metals, such as arsenic and mercury, in water, soil, plant, food and humans (Smedley and Kinniburgh, 2002; Asante et al., 2007; Obiri, 2007; Akoto et al., 2014, Bortey-Sam et al., 2015c).

Many metals bioaccumulate in the edible parts of crops and thus negatively impact the health of human, animals and the ecosystem (Khan et al., 2008; Nagajyoti et al., 2008). Amongst the many potential metals that may contaminate agricultural and ecological systems, arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) are perhaps the most important (Alloway, 2013). Many of these elements, especially As and Hg, are found in rather high concentrations in mining areas of Ghana (Smedley and Kinniburgh, 2002; Asante et al., 2007; Obiri, 2007; Bortey-Sam et al., 2015c; Hayford et al., 2008).

Studies conducted by Hayford et al. (2008) on the impact of gold mining in soil collected around mining communities in Tarkwa showed high levels of some toxic metals including As and Hg. However, there is limited or no data from literature on the potential ecological risk of heavy metals and a metalloid in agricultural soils in Tarkwa, Ghana. The objectives of this study were therefore to increase understanding of the ecological risk that may be posed by metals contamination in the Tarkwa region of Ghana. Specifically, the study aimed to determine the concentrations of heavy metals and a metalloid in agricultural soils in 19

communities in Tarkwa; to identify the relationship between heavy metals and soil properties; to identify the potential sources of the metals; and to estimate the potential risk of heavy metals and a metalloid in agricultural soils to the ecological system in Tarkwa.

Materials and methods

Study area

Tarkwa ($05^{\circ}18'00''\text{N}$; $01^{\circ}59'00''\text{W}$) is a town in the southwest of Ghana, located about 120 miles west of the capital city, Accra. As of 2010, Tarkwa was estimated to have a population of 90,477 ([Ghana Statistical Service 2010](#)). It is a noted centre for gold and Mn mining. Tarkwa mine, which is a large open-cast gold mine, is situated to the northwest of the town, and Nsuta manganese mine is situated to the east. Tarkwa has a long history of gold mining and perhaps the greatest concentration of mining companies including illegal mining (galamsey) activities in the West African sub-region ([Akabzaa and Darimani 2001](#)).

Sample collection and analysis

In June 2012, a total of 142 soil samples (0–10 cm top layer) were randomly collected from 19 communities in Tarkwa. These sites were selected because of the agricultural activities and also to represent a wide area of the town. Global positioning system (GPS) was used to locate the sampling locations/positions and some sites in Badukrom, Wangarakrom and T-Tamso were approximately 3, 3.4 and 5.2 km away from the mines, respectively. Communities such as Pepesa, Mile 10 and Techiman were farthest away from the mines with average distances of 11, 13 and 14 km, respectively ([Fig. 1](#)).

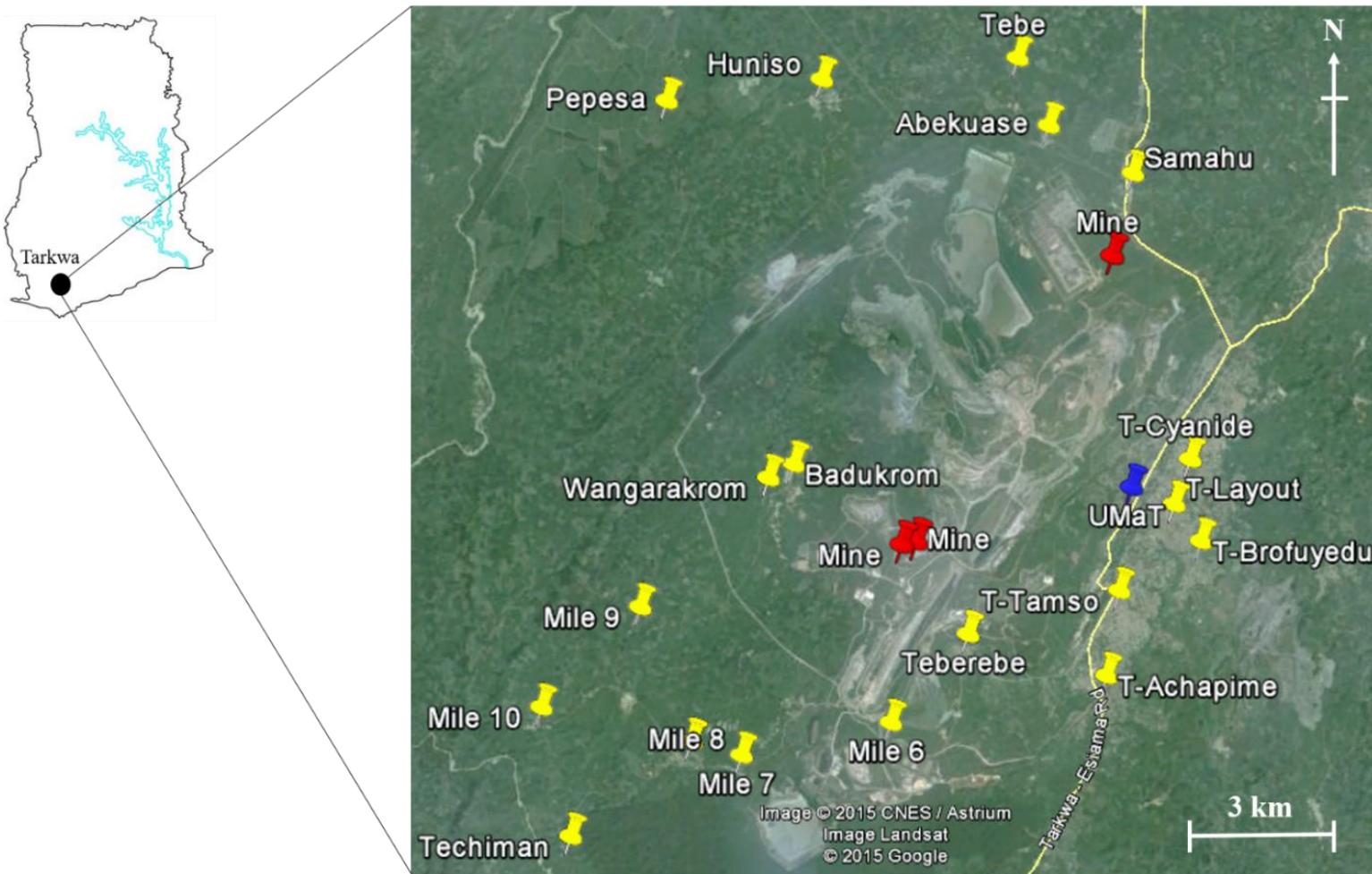


Fig. 1 Map showing surface soil sampling locations in Tarkwa, Ghana (yellow, red and blue pins indicate sampled communities, gold mines and reference site (UMaT) respectively)

In addition, due to the lack of background concentrations in agricultural soils in Tarkwa, Ghana, 5 soil samples were collected from the University of Mines and Technology (UMaT) campus for data comparison (reference values), and to evaluate the extent of metal pollution in this study. UMaT is a public university located in Tarkwa, Ghana, and because of the low vehicular movement and industrial (mining) activities, heavy metals from point sources were assumed to be negligible. Samples were collected using a stainless steel scoop and stored in labeled corning tubes (Corning Incorporated, New York, USA) ([Bortey-Sam et al., 2014](#)). The soil samples obtained were stored at -20°C in the Department of Chemistry, KNUST, Ghana and later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan, where they were stored at -30°C until analysis. A map showing the sampling locations is presented in [Fig. 1](#).

Prior to chemical analyses, the soil samples were air dried at room temperature and passed through a 2 mm sieve to remove coarse debris ([Bortey-Sam et al., 2014](#)). Approximately 0.5 g of soil sample was weighed into a prewashed digestion vessel. The samples were digested (Speedwave two, Berghof, Germany) using 10 ml of 60% nitric acid (Kanto Chemical Corporation, Tokyo, Japan). The microwave unit was calibrated to a temperature of 200°C and digestion was allowed for 45 min at 180 psi. After cooling, samples were filtered into corning tubes (Corning Incorporated, New York, USA) using ashless filter paper 5B (Advantec, Tokyo, Japan). The solution was standardized to 50 ml using distilled, deionised water. Method blanks were prepared using the same procedure.

Concentrations of As, Cd, Co, Cr, Cu, Ni, Pb and Zn were measured by an Inductively Coupled Plasma–Mass Spectrometer (ICP–MS; 7700 series, Agilent technologies, Tokyo, Japan) and expressed in mg/kg dry weight (dw). On the other hand, concentration of total Hg (Hg) in soil sample was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury Analyzer, MA–3000, Nippon Instruments Corporation, Tokyo, Japan), after preparation of the calibration standard.

Quality control and quality assurance

For quality control, blanks were analyzed after every 10 sample analyses. The instrument was calibrated using standard solutions of the respective metals (to establish standard curves before metal analysis). All chemicals and standard stock solutions were of analytical-reagent grade (Wako Pure Chemicals, Osaka, Japan). The detection limits (ng/g) of As, Cd, Co, Cr, Cu, Ni, Pb, and Zn were 0.002, 0.001, 0.0001, 0.007, 0.004, 0.004, 0.001, 0.046, respectively. For metals, reference materials SRM 1944 (New York/ New Jersey Waterway Sediment) and BCR-320 (Channel Sediment, IRMM, Belgium) were used for method validation. Replicate analyses of these reference materials showed good accuracy with recovery rates ranging from 80%–115%. Recovery rates (%) of Hg for the three certified reference materials (BCR-320R, SRM 1944, and DOLT-4) ranged from 92–103. The detection limit of Hg in soil samples was 2.0 pg total Hg.

The water content of each soil sample was measured after 12 h of drying in an oven at 105 °C. Soil organic matter (SOM) content was determined by loss of weight on ignition at oven temperature of 600°C for 5 h. pH was measured in a soil deionized water suspension (soil: water, 1:2.5 by volume) by a calibrated pH meter.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (IBM SPSS Inc., Chicago, USA). Kolmogorov–Smirnov and Shapiro–Wilk's tests were used to determine the normality of data and was considered statistically significant if p value was less than 0.05. Statistical analyses were carried out after data were log transformed (normalized). Principal component analysis (PCA) is a statistical method used to determine components that are linear combinations of the original variables and was performed using JMP 10 statistical software (SAS Institute). In order to identify the important parameters which affect the chemistry of soil and to investigate the possible sources of different metals, Pearson's correlation matrix and PCA were used, respectively. The principal components based on log transformed data were extracted with eigenvalues >1 through a varimax rotation. Spearman and Pearson's correlation were used to determine the relationship between concentrations of metals and distance from the mines, and was considered significant if p value was less than 0.05.

Results and Discussion

Concentrations of heavy metals in soils

[Table 1](#) shows the mean (\pm SD) concentrations of As, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn in soil in 19 communities in Tarkwa. From [Table 1](#), the mean concentrations of eight heavy metals and a metalloid decreased in order of Zn > Cr > Pb > Cu > As > Ni > Co > Hg > Cd. The high variability in concentration was illustrated by the Shapiro-Wilks and Kolmogorov-Smirnov (K-S) tests, showing an abnormal distribution of raw data for all the heavy metals ([Table 1](#); $p < 0.0001$). This variability could be due to the large sampling area of 19 communities. Industrial activities including mining could be associated with heavy metals discharge in some areas and could explain this variability.

The average concentrations of metals in the 19 communities were generally below the corresponding ecological-soil screening levels (ECO-SSL) for plants, soil invertebrates and mammalian wildlife established by the United States Environmental Protection Agency ([USEPA, 2003; 2004](#)) ([Table 1](#)). However, some communities/sample sites showed higher values than the USEPA ECO-SSL ([USEPA, 2003; 2004](#)) and [Kabata-Pendias and Sadurski \(2004\)](#) recommended levels ([Table 1](#)). For instance, two sites in Badukrom and Wangarakrom had higher Hg concentrations than the Maximum Allowable Concentrations (MAC) of 0.5–5 mg/kg in agricultural soils ([Kabata-Pendias and Sadurski, 2004](#)). These results indicated a possible influence of artisanal and small-scale gold mining activities in the study area since Hg is used to amalgamate gold from ore. Further, such influence is consistent with the high coefficients of variation (CV) found for most of the measured heavy metals, (CV values ranged from 50% [Cu]–147% [Ni]; [Table 1](#)) ([Manta et al., 2002; Hani and Pazira, 2011](#)).

As shown in [Table 1](#), the highest mean concentrations of Pb and Hg were in T-Layout and Badukrom, respectively, while highest mean concentrations of As, Cd, Co, Cr, Cu, Ni and Zn were in T-Tamso. The high levels of metals and a metalloid in soil in T-Tamso could be attributed to the proximity of some sample sites to the mines.

Table 1: WC%, SOM%, soil pH and mean concentrations (\pm SD; mg/kg dw) of heavy metals and a metalloid in soil in Tarkwa

Sample sites	n		WC	SOM	soil pH	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
Teberebe	8	Mean	1.1	2.5	7.6	2.6	0.038	3.0	35	8.9	0.072	4.5	6.1	39
		SD	0.48	1.5	0.11	1.2	0.017	2.4	20	6.5	0.044	2.9	1.7	17
Mile 6	7	Mean	0.94	2.2	7.5	2.2	0.020	2.1	30	9.3	0.018	2.6	3.2	12
		SD	0.65	1.9	1.1	1.2	0.010	1.1	16	6.8	0.018	1.2	1.7	11
Mile 7	8	Mean	0.91	1.8	7.3	1.0	0.011	0.67	13	2.8	0.030	1.1	1.5	9.7
		SD	0.31	1.1	1.1	0.51	0.010	0.45	17	1.4	0.011	0.64	0.81	3.3
Mile 8	7	Mean	0.88	1.8	7.1	1.3	0.022	0.74	9.9	2.8	0.19	1.5	2.3	23
		SD	0.53	0.72	1.0	0.72	0.011	0.68	4.2	1.6	0.26	0.91	0.75	13
Techiman	8	Mean	0.66	1.8	6.2	1.0	0.020	1.6	11	5.5	0.17	3.1	2.7	32
		SD	0.78	1.0	1.2	0.44	0.021	1.7	5.0	4.0	0.14	3.0	1.6	45
Mile 9	8	Mean	0.50	2.1	5.8	1.8	0.011	0.80	15	2.5	0.033	1.6	2.0	11
		SD	0.19	2.0	0.31	1.5	0.010	0.64	15	1.9	0.025	1.1	1.0	7.8
Mile 10	8	Mean	0.44	1.3	7.1	0.96	0.020	0.72	27	2.2	0.13	1.0	2.1	29
		SD	0.19	0.34	0.051	0.43	0.016	0.53	34	1.0	0.090	0.21	0.23	25
Wangarakrom	8	Mean	0.66	2.8	6.9	5.4	0.021	1.4	15	3.7	1.9	3.2	2.5	19
		SD	0.33	1.0	0.021	6.3	0.018	1.6	5.6	2.2	1.1	3.0	0.90	11
Badukrom	8	Mean	2.5	2.0	6.9	12	0.013	0.37	9.6	5.9	2.4	1.3	2.4	27
		SD	3.7	1.2	0.030	10	0.010	0.12	4.9	8.2	1.7	0.57	0.98	30
Samahu	7	Mean	2.1	2.4	7.1	4.5	0.030	1.2	38	5.8	0.11	2.4	8.3	36
		SD	2.3	1.7	0.049	4.3	0.026	0.84	39	5.0	0.12	1.8	11	29
Abekuase	9	Mean	2.1	2.5	7.0	3.2	0.024	1.2	9.2	3.8	0.050	1.8	3.8	38
		SD	1.1	1.1	0.084	1.0	0.023	1.2	4.8	4.2	0.021	1.2	2.7	47
Tebe	9	Mean	1.9	2.9	6.9	2.8	0.011	1.8	12	5.6	0.051	2.1	4.1	18
		SD	1.2	1.7	0.074	1.4	0.010	3.2	9.3	5.5	0.035	1.9	2.0	18
Huniso	7	Mean	0.99	1.5	7.2	1.5	0.052	0.76	8.0	4.1	0.13	1.3	13	86
		SD	0.85	0.84	0.11	0.79	0.034	0.32	3.7	2.8	0.12	0.53	15	69

Pepesa	10	Mean	1.9	1.9	7.3	4.9	0.042	0.89	12	6.9	0.20	1.9	5.5	78
		SD	0.79	0.81	0.10	8.1	0.034	0.69	4.2	7.6	0.24	1.2	3.3	73
T-Cyanide	7	Mean	1.2	2.4	7.5	2.7	0.081	1.4	23	8.6	0.18	3.2	16	49
		SD	0.75	0.24	0.087	1.2	0.014	0.81	16	5.0	0.036	1.3	4.6	21
T-Layout	6	Mean	1.4	1.1	7.4	2.7	0.11	1.4	16	7.1	0.11	3.0	27	78
		SD	1.1	0.78	0.062	1.4	0.14	0.70	10	7.4	0.10	1.7	37	108
T-Brofuyedu	5	Mean	0.95	1.9	7.3	8.6	0.058	1.3	18	7.7	0.061	2.9	6.0	32
		SD	0.03	1.8	0.021	10	0.027	1.4	14	7.2	0.034	2.7	2.2	11
T-Achapime	6	Mean	1.5	1.9	7.2	1.4	0.046	0.66	12	4.9	0.73	1.9	6.4	45
		SD	0.94	0.37	0.056	0.13	0.0012	0.04	0.01	0.63	0.59	0.17	0.68	1.8
T-Tamso	6	Mean	1.2	3.2	7.5	27	0.43	9.2	77	16	0.42	28	14	118
		SD	0.42	1.3	0.23	13	0.20	4.4	46	9.2	0.014	14	3.8	85
Minimum			0.44	1.1	5.8	0.96	0.010	0.37	8.0	2.2	0.018	1.0	1.5	9.7
Maximum			2.5	3.7	7.6	27	0.43	9.2	77	16	2.4	28	27	118
Median			1.1	2.2	7.3	2.7	0.024	1.3	15	5.8	0.11	2.5	5.5	32
Average			1.3	2.4	7.2	4.4	0.052	1.8	21	6.2	0.32	3.7	7.2	39
SD			0.61	0.69	0.42	5.1	0.067	1.8	15	3.1	0.36	5.5	6.2	27
CV			47	29	5.8	116	111	101	73	50	113	147	85	70
Tarkwa (<i>n</i> = 142)			0.0–11	0.0–10	4.3–8.8	0.3–37	nd–0.58	0.11–14	2.0–199	0.5–44	nd–6.7	0.3–38	0.4–78	1.1–232
Skewness			3.4	1.3	-1.6	4.1	5.6	2.4	4.00	2.3	8.0	4.1	4.3	2.6
Kurtosis			16	2.4	6.8	19	37	6.8	19	9.3	67	21	21	7.8
K-S/S-Wilk <i>p</i>			< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Reference values [#] (<i>n</i> = 5)			1.2	6.8	7.3	5.8	0.39	3.6	33.18	21	0.24	6.7	52	72
USEPA ^b						18–46	0.36–140	13–230	–	49–80	1*	38–280	56–120	79–160
World range ^c						1.0–15	0.07–1.1	0.1–20	5–120	6.0–60		1–200	10.0–70	17–125

n: number of samples; * Indicates Maximum Allowable Concentration (MAC) of Hg in agricultural soils by [Kabata-Pendias and Sadurski \(2004\)](#); ^b indicates USEPA Ecological-Soil Screening Levels for plants, soil invertebrates and mammalian wildlife [USEPA \(2003; 2004\)](#); ^c indicates recommended levels of heavy metals in soil by [Kabata-Pendias and Pendias 1992](#); [#] indicates reference values (UMaT). Bold values indicates higher concentrations than, [USEPA \(2003; 2004\)](#) and [Kabata-Pendias and Pendias \(1992\)](#); nd: not detected.

Correlation between heavy metals and soil properties

Correlation between heavy metals and selected soil properties was analyzed by Pearson's correlation matrix ([Table 2](#)). Soil properties play an important role in the mobility and bioavailability of heavy metals, thus influencing their distribution in soils ([Khan et al., 2008](#); [Hernandez et al., 2003](#)). This role is generally illustrated by good correlations between heavy metal concentrations and pH, as well as SOM ([Gjoka et al. 2011](#); [Lu et al. 2012](#)). However, only weak correlations were found in some studies ([Manta et al., 2002](#)). In the present study, significant correlations were observed between As, Cd, Co, Cr, Cu, Ni, Pb and SOM ($r = 0.18\text{--}0.51$, $p < 0.05\text{--}0.0001$), indicating that SOM has substantial influence on the total contents of these metals in soil. Similar result was reported by [Gjoka et al. \(2011\)](#). However, no significant correlations were found between pH and heavy metals, which is similar to the results by [Manta et al. \(2002\)](#) and [Al-Khashman and Shawabkeh \(2006\)](#).

Table 2: Pearson's correlation matrix of heavy metals and soils properties in Tarkwa

	WC%	SOM%	soil pH	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
WC%	1											
SOM%	0.33	1										
soil pH	0.04	-0.06	1									
As	0.03	0.27**	-0.01	1								
Cd	-0.02	0.18*	0.09	0.48***	1							
Co	0.06	0.40***	0.11	0.27**	0.35***	1						
Cr	0.08	0.51***	0.03	0.54***	0.27***	0.31***	1					
Cu	0.02	0.47***	0.11	0.37***	0.41***	0.59***	0.50***	1				
Hg	-0.07	-0.05	-0.06	0.45***	0.06	-0.09	-0.03	0.08	1			
Ni	0.01	0.49***	0.06	0.50***	0.56***	0.76***	0.57***	0.68***	-0.03	1		
Pb	0.04	0.34***	0.05	0.09	0.44***	0.28**	0.16*	0.37***	-0.02	0.36***	1	
Zn	-0.00	0.07	0.03	0.23*	0.62***	0.13	0.10	0.34***	0.12	0.25***	0.39***	1

*** Indicates $p < 0.0001$; ** Indicates $p < 0.001$; * Indicates $p < 0.05$

Lack of significant correlation between soil properties and heavy metals could be attributed to a continuous input (Lu et al. 2012; Chen et al. 2008) since the release and transport of heavy metals are complex processes (Grant and Shepherd, 2008). Another possible explanation could be variations in soil type, fertilizer use, and cultivation system within the sampling area (Lu et al., 2012; Grant and Shepherd, 2008). No significant correlation was found between Hg and the other metals ($p > 0.05$) except for As, indicating a specific source for As and Hg. The sources for Hg could be geochemical and/or anthropogenic (Suresh et al., 2012), since it is used in the amalgamation of gold (Amonoo-Neizer et al., 1995). On the other hand, significant positive relationships ($p < 0.0001$) were observed between As and Cd ($r = 0.48$), Cd and Zn ($r = 0.62$), Cd and Pb ($r = 0.44$), Ni and Cr ($r = 0.57$), and Pb and Zn ($r = 0.39$). In addition, significantly weak relationships ($p < 0.05$ or 0.01) were found between Zn and As ($r = 0.23$), and Zn and Ni ($r = 0.25$) (Table 2). The significantly positive correlations among these elements suggested, to some extent, a common source (Al-Khashman and Shawabkeh, 2006).

Sources of metals in soil identified by PCA

In this study, three principal components (PC1, PC2, and PC3) were extracted (with eigenvalues >1) accounting for 72.8% of the total variances. As shown in Fig. 2, PC1, the most important component, explained 42.9% of the total variance and was characterized by high loadings of Co, Cu, Cr, Ni, and Pb. The input of these metals could mainly result from atmospheric deposition, as a consequence of an increase in industrial activities such as mining and smelting processes (Streets et al., 2005; Manno et al., 2006; Chen et al., 1999). The concentrations of Cr, Co and Pb in the study area could also be attributed to the weathering of the Tarkwanian rock system. The Tarkwanian rock system contains high concentration of Cr, Co and Pb. Other sources of Cr, Co and Pb in the study area is the occasional discharge of acid industrial wastes or mine drainage which increases Cr, Co and Pb levels in surface soils in the study area (Obiri, 2007).

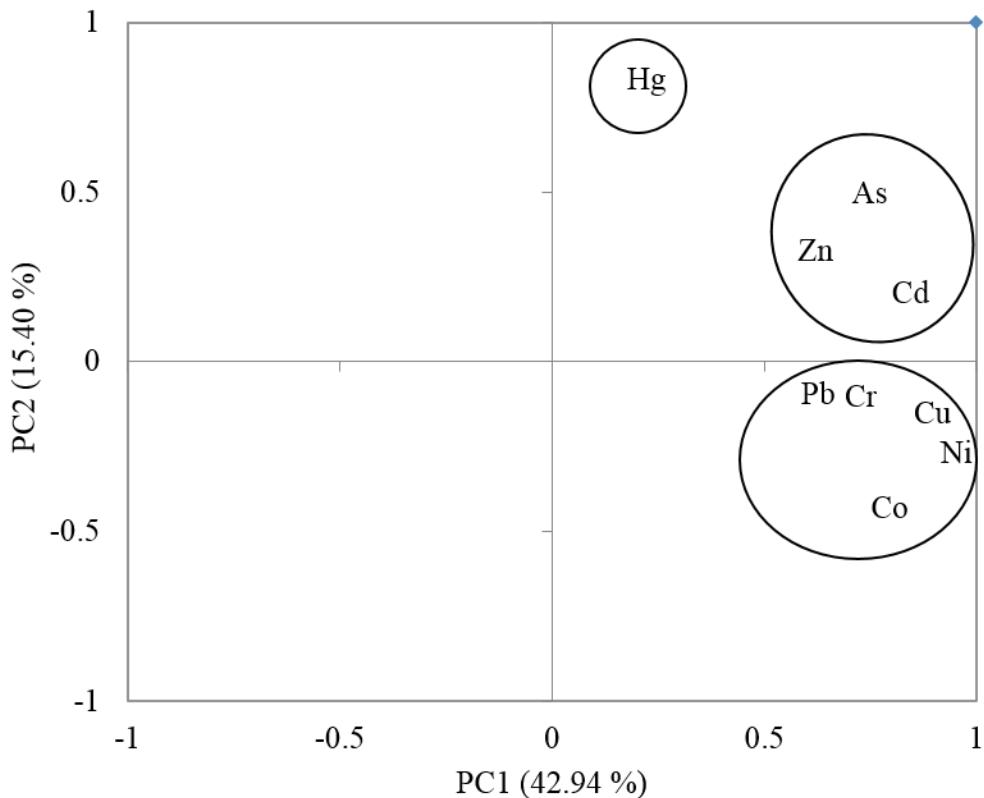


Fig. 2 Distribution pattern of metals in agricultural soils in Tarkwa characterized by PCA

PC2 explained 15.4% of the total variances (Fig. 2) and was dominated by high loadings of As, Cd, and Zn. Similar to PC1, PC2 also represents anthropogenic contamination, probably resulting from irrigation with sewage water (Nicholson et al., 2003; Zhang et al., 2006) and the use, and sometimes abuse, of phosphate fertilizers and organic manures (Hani and Pazira, 2011; Chen et al., 2008; Jiao et al., 2012). Huge amount of phosphate (Luo et al., 2009) causes considerable additions of As and Cd. The use of livestock manure adds As and Cd to agricultural soils (Jiang et al., 2010). In addition, blasting of the gold bearing rock is the most common method of obtaining the ore. The miners engage in surface and subsurface mining (BGL, 2002; Hakanson, 1980). The levels of As in the soils could also be due to the nature of the gold bearing ore which is mineralized pyrites and arsenopyrates. Processing of the ore involves roasting and these results in the production of arsenic trioxide gas which is distributed throughout the study area by air current. As is toxic and due to its non-

biodegradable nature, it could accumulate in surface soil and water ([Amonoo-Neizer et al., 1995](#)).

Cd is soft, ductile and is obtained as a by-product from the smelting of Zn ores. It is also found in chalophile as a mineral called greenockite, CdS. Cd in soils from the study area may come from the mining and processing of Zn and chalophilic metals ([Obiri, 2007](#)). The presence of Zn in the environment is associated with mining and smelting, which pollutes the air, water and soil, and ultimately undergoes oxidation to release Zn²⁺ ions ([Obiri, 2007](#)). Thus, PC2 could be regarded as representing mainly the contribution of mining and use of fertilizers/manure. This is in agreement with study by [Asante et al. \(2007\)](#) which indicated that there could be other sources of As contamination in Tarkwa other than mining activities.

PC3 explained 14.5% of the total variances and was totally dominated by high loading of Hg. The levels of Hg in soils from the sample area could be problematic, as concentration exceeded the maximum values permitted in agricultural soils ([Kabata-Pendias ad Sadurski, 2004](#)). In Ghana, amalgamation using Hg (popularly known as "galamsey"), is the preferred gold recovery method employed by almost all artisanal gold miners because it is a very simple, inexpensive and an easier to use technique ([Amonoo-Neizer et al., 1995](#)). The high levels of Hg in soils could therefore be due to contamination from the mining processes.

Assessment of potential ecological risk

The potential ecological risk (RI) is a commonly used indicator to express a comprehensive assessment of the harmful effects of heavy metals and a metalloid in the environment, including soils and sediments. The RI was calculated using the following equations ([Hakanson, 1980; Zhu et al., 2008](#)):

$$C_f^i = C^i / C_n^i$$

$$C_{\text{deg}} = \sum C_f^i$$

$$E_r^i = T_r^i \times C_f^i$$

$$RI = \sum E_r^i$$

Where C_f^i is the pollution coefficient of a metal which can reflect the pollution character of the investigated region but cannot reveal the ecological effects. C^i is the measured values of heavy metals in surface soils. C_n^i is the reference values of the heavy metals in soil/sediments. The concentrations of metals (mg/kg dw) in soil samples collected from UMaT were used as reference ([Table 1](#)). The C_f^i of each metal was calculated and classified as either low ($C_f^i \leq 1$), middle ($1 < C_f^i \leq 3$) or high ($C_f^i > 3$) ([Chen et al., 2005](#)).

C_{deg} represents the integrated pollution level in the environment, and is expressed as the sum of C_f^i for all examined metals. The four pollution levels may be distinguished as: $C_{deg} < 5$, low pollution; $5 \leq C_{deg} < 10$, medium pollution; $10 \leq C_{deg} < 20$, high pollution; and $C_{deg} \geq 20$, very high pollution [46]. E_r^i is the monomial potential ecological risk factor of the individual heavy metal and T_r^i is the metal toxic factor (based on the standardized heavy metal toxic factor). Referring to Hakanson ([Hakanson, 1980](#)), we used the following T_r^i values: Hg = 40; Cd = 30; As = 10; Cu = Pb = Ni = 5, Cr = 2, and Zn = 1. RI is defined as the sum of E_r^i for all heavy metals and has been grouped into four categories by [Zhu et al. \(2008\)](#) as shown in [Table 3](#).

Table 3: Categories of E_r^i and RI ([Hakanson, 1980](#); [Zhu et al., 2008](#))

E_r^i	Ecological risk level of single factor pollution	RI value	General level of potential ecological risk
$E_r^i < 40$	Low risk	$RI \leq 50$	Low risk
$40 \leq E_r^i < 80$	Moderate risk	$50 < RI \leq 100$	Moderate risk
$80 \leq E_r^i < 160$	Considerable risk	$100 < RI \leq 200$	Considerable risk
$E_r^i < 320$	High risk	$RI > 200$	High risk
$E_r^i \geq 320$	Very high risk		

The C_f^i values for the measured heavy metals ranged from As (0.16–4.6), Cd (0.026–1.1), Cr (0.24–2.3), Cu (0.11–0.80), Hg (0.080–10), Ni (0.15–4.2), Pb (0.030–0.53) and Zn (0.13–1.6). This suggested a low to high pollution level (Table 4; [Chen et al., 2005]). The range of C_{deg} was 1.2–17, with an average of 4.5. From Table 4, average C_{deg} (4.5) indicated low pollution for most soil samples (75%). However, the C_{deg} for Wangarakrom (11), Badukrom (13) and T-Tamso (17) indicated high pollution of toxic metals, especially from As and Hg (Table 4).

Table 4: C_f^i and C_{deg} of heavy metals and a metalloid in surface soils in Tarkwa

Sample sites	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	C_{deg}
Teberebe	0.46	0.10	1.0	0.42	0.30	0.67	0.12	0.54	3.6
Mile 6	0.39	0.044	0.91	0.44	0.08	0.40	0.063	0.18	2.5
Mile 7	0.18	0.030	0.42	0.13	0.11	0.16	0.030	0.13	1.2
Mile 8	0.22	0.060	0.30	0.13	0.78	0.23	0.044	0.33	2.0
Techiman	0.18	0.052	0.34	0.26	0.70	0.46	0.053	0.45	2.4
Mile 9	0.32	0.030	0.46	0.12	0.14	0.24	0.040	0.16	1.5
Mile 10	0.16	0.051	0.84	0.11	0.55	0.15	0.041	0.40	2.3
Wangarakrom	0.93	0.055	0.47	0.17	8.1	0.48	0.051	0.26	11
Badukrom	2.2	0.041	0.29	0.28	10	0.20	0.050	0.38	13
Samahu	0.79	0.064	1.1	0.28	0.44	0.36	0.16	0.50	3.7
Abekuase	0.55	0.062	0.28	0.18	0.19	0.27	0.074	0.53	2.1
Tebe	0.49	0.026	0.38	0.27	0.19	0.32	0.080	0.25	2.0
Huniso	0.26	0.13	0.24	0.20	0.56	0.20	0.26	1.2	3.0
Pepesa	0.84	0.11	0.37	0.33	0.84	0.28	0.11	1.0	3.9
T-Cyanide	0.47	0.21	0.72	0.41	0.73	0.47	0.31	0.69	4.0
T-Layout	0.47	0.28	0.49	0.34	0.48	0.46	0.53	1.08	4.1
T-Brofuyedu	1.4	0.15	0.56	0.36	0.25	0.43	0.12	0.45	3.8
T-Achapime	0.25	0.12	0.36	0.23	3.0	0.28	0.12	0.62	5.0
T-Tamso	4.6	1.1	2.3	0.80	1.7	4.2	0.27	1.6	17
Minimum	0.16	0.026	0.24	0.11	0.080	0.15	0.030	0.13	1.2
Maximum	4.6	1.1	2.3	0.80	10	4.2	0.53	1.6	17
Average	0.79	0.14	0.63	0.29	1.4	0.53	0.14	0.57	4.5

Bold indicates high C_f^i and C_{deg} values (i.e. middle to high pollution) based on: (a) low ($C_f^i \leq 1$), middle ($1 < C_f^i \leq 3$) or high ($C_f^i > 3$) (Chen et al., 2005). (b) $C_{deg} < 5$, low pollution; $5 \leq C_{deg} < 10$, medium pollution; $10 \leq C_{deg} < 20$, high pollution; and $C_{deg} \geq 20$, very high pollution (Loska and Wiechula, 2003).

Hakanson (1980) and Zhu et al. (2008) defined five categories of E_r^i (Table 3) and four categories of RI. As shown in Table 5, the maximum E_r^i values for As (46) and Hg (400) were higher than those of the other metals. This result suggested a moderate to very high risk of As (T-Tamso) and Hg (Badukrom), respectively, to the ecological system especially plants, soil invertebrates and/or mammalian wildlife. Similarly the E_r^i of Hg from T-Achapime (120) and Wangarakrom (324) indicated considerable to high ecological risk (Tables 3 and 5). The E_r^i difference between As/Hg and the other metals resulted from their high toxic factors, T_r^i (Suresh et al., 2012) and high concentration at some sites possibly due to their proximity to the mines (Fig. 1) or illegal mining activities. In fact, the CV of Hg from the sampling communities was 113% (Table 1), indicating high Hg concentrations in some communities.

Table 5: E^i_r and RI of heavy metals and a metalloid in surface soils in Tarkwa

Sample sites	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	RI
Teberebe	4.5	2.9	2.1	2.1	11	3.3	0.59	0.54	28
Mile 6	3.8	1.3	1.8	2.2	3.1	1.9	0.31	0.18	14
Mile 7	1.8	0.90	0.84	0.67	4.5	0.81	0.15	0.13	9.9
Mile 8	2.2	1.6	0.60	0.67	31	1.1	0.22	0.33	37
Techiman	1.7	1.5	0.68	1.3	28	2.3	0.27	0.45	36
Mile 9	3.2	0.89	0.92	0.59	5.5	1.2	0.20	0.16	12
Mile 10	1.6	1.5	1.6	0.53	21	0.75	0.21	0.40	28
Wangarakrom	9.3	1.6	0.94	0.87	324	2.4	0.25	0.26	339
Badukrom	22	1.0	0.58	1.4	400	1.0	0.24	0.38	427
Samahu	7.8	1.9	2.3	1.3	17	1.8	0.80	0.50	34
Abekuase	5.4	1.8	0.56	0.92	7.5	1.3	0.37	0.53	18
Tebe	4.9	0.79	0.77	1.3	7.6	1.6	0.39	0.25	17
Huniso	2.5	3.9	0.49	0.99	22	0.98	1.3	1.2	33
Pepesa	8.4	3.1	0.73	1.6	33	1.4	0.53	1.0	51
T-Cyanide	4.7	6.1	1.4	2.0	29	2.3	1.5	0.69	48
T-Layout	4.7	8.3	0.98	1.7	19	2.2	2.6	1.0	40
T-Brofuyedu	14	4.4	1.1	1.8	10	2.1	0.58	0.45	35
T-Achapime	2.5	3.5	0.73	1.1	120	1.4	0.62	0.62	131
T-Tamso	46	33	4.7	4.0	69	21	1.3	1.6	182
Minimum	1.6	0.79	0.49	0.53	3.1	0.75	0.15	0.13	9.9
Maximum	46	33	4.7	4.0	400	21	2.6	1.6	427
Average	7.8	4.2	1.2	1.4	61	2.6	0.66	0.57	80
Median	4.7	1.9	0.92	1.3	21	1.8	0.39	0.50	35

Bold E^i_r and RI indicates moderate to high risk of heavy metals and/or metalloid

The RI (range, 9.93–427; mean, 80.4) suggested a low to high risk of heavy metals in the ecological system (plants, soil invertebrates and/or mammalian wildlife) in Mile 7 and Badukrom, respectively. Referring to the classification suggested by Zhu et al. (2008) (Table 3), soil samples in 10% of the communities could be classified as causing high potential ecological risk to plants, soil invertebrates and/or mammalian wildlife, and another 10% causing considerable potential ecological risk. However soil samples in 5% of the communities could be classified as causing moderate potential ecological risk, while 75% could be classified as causing low potential ecological risk (Tables 3 and 5). As and Hg, on

average, made up 10 and 75% of the RI values, respectively. Overall the RI of heavy metals in agricultural soils in Tarkwa represented moderate ecological risk. The concentrations of As, Cu, Ni and Pb from the sample sites negatively correlated ($p < 0.05$) with the average distance (km) from the mines (Table 6). The results further suggested that mining activities have played significant roles in the levels, distribution and risk of metals within the study area, especially, the communities closer to the mines.

Table 6: Pearson's correlation matrix of heavy metal concentrations and average distance from the mines

	average distance
average distance	1.0
As	-0.65**
Cd	-0.38
Co	-0.26
Cr	-0.36
Cu	-0.57**
Hg	-0.19
Ni	-0.49*
Pb	-0.53*
Zn	-0.28

** Indicates $p < 0.01$; * Indicates $p < 0.05$

Conclusions

The average concentrations of eight metals and a metalloid in agricultural soils in Tarkwa, Ghana decreased in an order of Zn > Cr > Pb > Cu > As > Ni > Co > Hg > Cd. The C_{deg} for Wangarakrom (11), Badukrom (13) and T-Tamso (17) indicated high pollution of toxic metals, especially from As and Hg. The maximum E^i_r values for As (46) and Hg (400), suggested moderate to very high ecological risk in T-Tamso and Badukrom, respectively. The potential ecological risk indices and potential toxicity response indices of heavy metals and a metalloid indicated low (Mile 7) to high risks (Wangarakrom and Badukrom). Based on the estimates of C_f^i , C_{deg} , E^i_r , and RI, the investigated soils was within low to high contamination and risk of heavy metals to the ecological system especially plants, soil invertebrates and/or mammalian wildlife. This represented moderate potential ecological risk in the study area and mining activities have played a significant role.

Chapter 2

**Health risk assessment of heavy metals and metalloid in
drinking water from communities near gold mines in Tarkwa,
Ghana**

Introduction

Surface water in most mining communities in Ghana have become unfit for human consumption due to chemical contamination from gold mining and processing activities (e.g. cyanide heap leach methods) (Smedley, 1996; Smedley et al., 1996; Asante et al., 2007; Obiri, 2007). Artisanal and small-scale gold mining activities could lead to spillage and run-off into rivers, ponds, streams, wells, borehole drinking water, and these could results in heavy metal and/or metalloid exposure upon consumption, especially to residents in Tarkwa and the surrounding villages (Obiri, 2007). One other concern is that groundwater may also be contaminated with metals and metalloids due to the chemical nature of the aquifer host rock in the area (Obiri, 2007).

Heavy metals and metalloids, such as arsenic, mercury and cadmium, have drawn great attention in the world, and are widely distributed in the environment, including sources of drinking water, through which they could seriously damage the liver, kidney, digestive system and nerve system of human (Wcislo et al., 2002; Li et al., 2008; Volety, 2008; Liu et al., 2009; Zhang et al., 2012; Montuori et al., 2013) and can even cause cancer (Steinemann 2000). Arsenic can affect nerve system, skin and artery and is carcinogenic (WHO, 2001); cadmium is also a carcinogenic metal and resides inside the body for a half-life of 38 years; copper, on the other hand, is a required microelement, but affects human organs upon excessive absorption (Kramer et al., 2001).

Study by Asante et al. (2007) showed high concentrations of arsenic and manganese in borehole, well and river/stream water in Tarkwa. Despite the wide and numerous studies of heavy metal and/or metalloid concentrations in various environmental and biological samples in Ghana, there is limited or no data from literature that addresses the potential health risk of these pollutants in drinking water in Tarkwa, Ghana. The objectives of this study were therefore to determine the concentrations of heavy metals and metalloid in borehole drinking water from 18 communities in Tarkwa; and to estimate their average daily dose (ADD), hazard quotient and index (HQ and HI) and cancer risk through consumption.

Materials and methods

Sampling and pre-treatment

In June 2012, 105 borehole drinking water samples were collected from 18 communities in Tarkwa, Ghana and the pH was measured using a pH meter. Global positioning system (GPS) was used to locate the sampling positions ([Fig. 1](#)). The water samples (collected) were stored in labeled corning tubes (Corning Inc. New York, USA), transported to the laboratory and kept frozen at the Chemistry Department of the Kwame Nkrumah University of Science and Technology, KNUST, Ghana. The samples were later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan, where they were stored at -30°C until analysis. A map of the sampling locations/communities is shown in [Fig. 1](#).

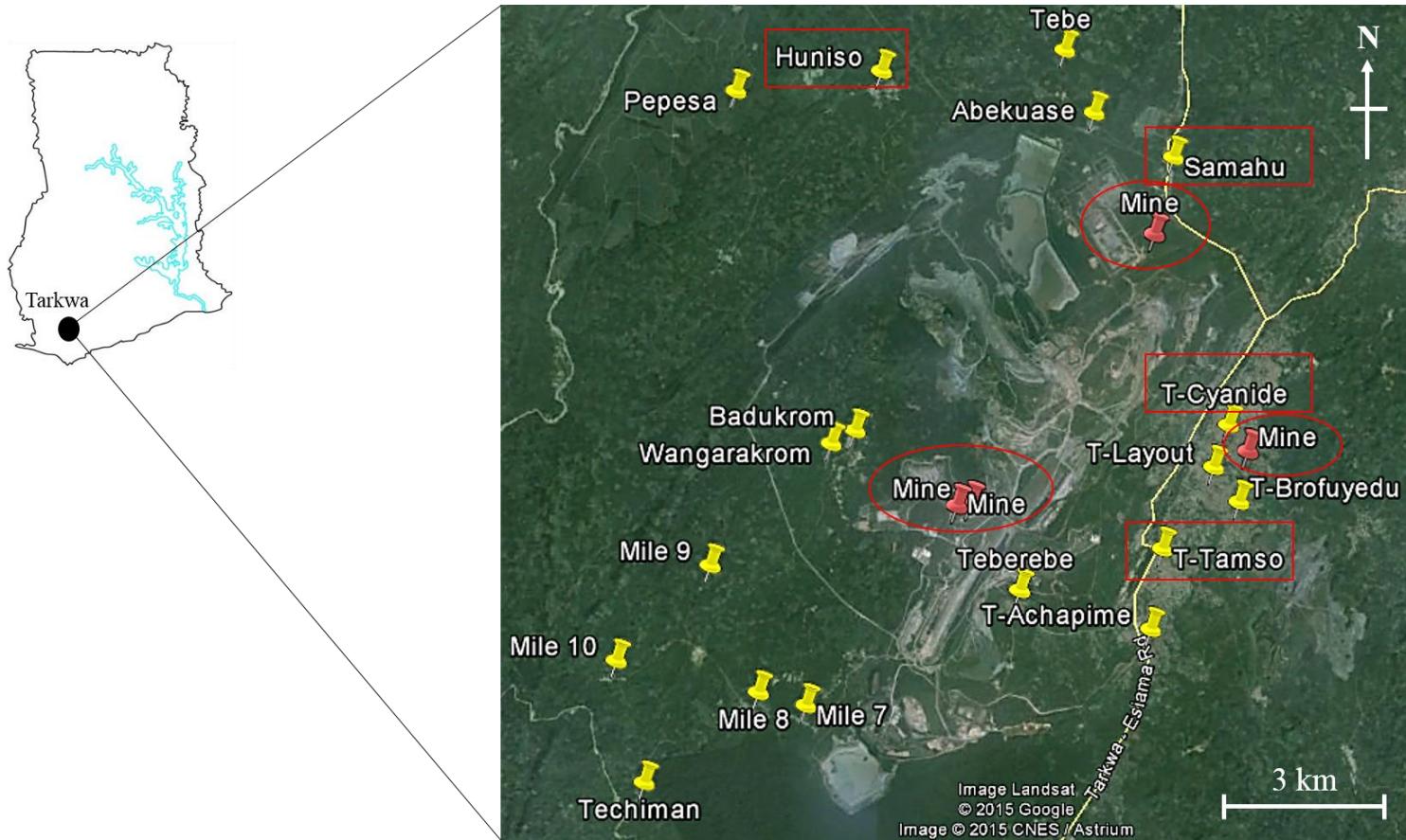


Fig. 1 Map showing borehole water sampling communities and mining sites in Tarkwa (Yellow pins indicate sampled communities and red pins indicate gold mines)

Sample preparation and analysis

Briefly, 10 mL of borehole drinking water was acidified with 0.1 mL of (60%) nitric acid, HNO₃, (Kanto Chemical Corp., Tokyo, Japan) to pH less than 2, and stored at 4°C prior to heavy metals and metalloid analysis. The concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn) were measured in each sample using an Inductively Coupled Plasma–Mass Spectrometer (ICP–MS; 7700 series, Agilent technologies, Tokyo, Japan).

Analysis of total Mercury (Hg)

The concentration of total mercury (Hg) was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury analyzer, MA–3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of the calibration standard. Prior to analysis, additive B (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) was added to the ceramic sample boats before and after borehole water, to prevent evaporation.

Analytical performance

For quality control, blanks and duplicates (samples) were analyzed after every 10 sample analyses. All samples were analyzed three times by the ICP–MS and the Relative Standard Deviation (RSD) was $\leq 4\%$. The instrument was calibrated using standard solutions of the respective metals (to establish standard curves before metal analysis) and correlation coefficients of the obtained standard curves were greater than 0.999. All chemicals and standard stock solutions were of analytical–reagent grade (Wako Pure Chemicals Industries, Ltd., Osaka, Japan). The limits of detection (LOD), calculated as 3 times the standard deviation of 10 blank solutions measurements, divided by the slope of the calibration curve ($\mu\text{g/L}$) were 0.009 (As), 0.001 (Cd), 0.003 (Co), 0.003 (Cr), 0.008 (Cu), 0.013 (Fe), 0.002

(Pb), 0.034 (Mn), 0.003 (Ni) and 0.019 (Zn). The LOD of Hg was 2.0 pg of total Hg. Concentrations of heavy metals and metalloid were expressed in µg/L.

Risk assessment

Risk assessment is a function of the hazard and exposure, and is defined as the process of estimating the probability of occurrence of any given magnitude of adverse health effects over a specified time period. The health risk assessment of each heavy metal or metalloid is usually based on quantification of the risk level and is expressed in terms of a carcinogenic or non–carcinogenic health risk ([USEPA, 2009](#)). The two principal toxicity risk factors evaluated are the slope factor (SF) for carcinogen risk characterization and the reference dose (RfD) for non–carcinogen risk characterization ([USEPA, 1997; 1999](#)). The toxicity indices of some heavy metals and metalloid are shown in [Table 1](#) ([USEPA IRIS, 2011](#)). The estimations of the magnitude, frequency and duration of human exposure to each heavy metal or metalloid in the environment are typically reported as average daily dose (ADD) ([USEPA, 1992](#)), as shown in the equation below;

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT}$$

where ADD is the average daily dose (mg/kg/day), C is the geometric mean concentration (mg/L) of heavy metal or metalloid, IR is the water intake rate (3.45 and 2.0 L/day for adults and children respectively) ([Apambire et al., 1997; Roychowdhury et al., 2003](#)), EF is the exposure frequency (365 days/year), ED is the exposure duration (70 year (adults) and 10 years (children)), BW is the average body weight (60 kg (adults) and 25 kg (children)), and AT is the average time (25550 days i.e. 70 years × 365 days/year (adults) and 3650 days i.e. 10 years × 365 days/year (children)) ([Wongsasuluk et al., 2014; Roychowdhury et al., 2003](#)).

The health risk from groundwater consumption was assessed in relation to its chronic (non–carcinogenic) as well as carcinogenic effects, based on the calculation of ADD and defined toxicity values for each heavy metal or metalloid. The non–carcinogenic risk was calculated as the hazard quotient (HQ), and shown as follows;

$$HQ = ADD/RfD$$

where ADD and RfD are in mg/kg/day.

Table 1: The toxicity responses to heavy metals and metalloid as the oral reference dose (RfD) and oral slope factor (SF) ([USEPA IRIS, 2011](#))

Heavy metals/metalloid	Oral RfD (mg/kg/day)	Oral SF (mg/kg/day) ⁻¹
As	3.00E-04	1.50
Cd	5.00E-04	0.380
Co	2.00E-02	n.a
Cr	3.00E-03	41.0
Cu	4.00E-02	n.a
Hg	3.00E-04	n.a
Pb	3.50E-03	n.a
Mn	1.40E-01	n.a
Ni	2.00E-02	n.a
Zn	3.00E-01	n.a

n.a: not available

For the risk assessment of a mixture of chemicals, the individual HQs are combined to form the hazard index (HI). If the value of HQ and/or HI exceeds 1, there could be potential non–carcinogenic effects on health, while HQ and/or HI < 1 means residents are not likely to experience any health risks as a result of exposure ([USEPA, 2001; ECETOC, 2001](#)). The

carcinogenic risk was calculated as the product of ADD (mg/kg/day) and SF (mg/kg/day)⁻¹ and the scale for characterization has been provided in Table 2, along with the chronic risk assessment ([USEPA, 1999](#)).

Data analysis

Statistical analyses were performed using IBM SPSS 20 (SPSS Inc., Illinois) statistical software after data were normalized by log transformation. Concentrations of heavy metals or metalloid below their respective LODs were replaced with a value of LOD/2. Geometric mean concentrations were used to represent the central tendency of heavy metals and metalloid in this study ([Wayne, 1999](#)). Spatial distributions were performed using ArcGIS 10.1 (ESRI Co, Redlands, USA).

Results and Discussion

pH

The pH of drinking groundwater varied within each borehole and ranged (across 18 communities) from 4.80–6.96 with an average of 6.20 ± 0.629 , which is below the recommended pH of 6.50–8.50 in drinking water by the USEPA (2009). The groundwater pH found in this study agrees and is comparable with results from Obiri (2007) who previously reported that pH of borehole water in Dumasi (a gold mining community) in the Wassa District of Ghana ranged from 4.50 ± 0.200 – 7.60 ± 0.01 , with mean value of 6.40. Similarly, a study by Asante et al. (2007) showed low pH values in boreholes and wells in Tarkwa with an average of 5.60, which is acidic for drinking. This phenomenon of low pH in borehole drinking water in this study suggests high leachate of heavy ions, high draw down rate and high sorption levels of ions from several mining companies and operations in Tarkwa (Obiri, 2007).

Concentrations of heavy metals and metalloid

Heavy metals and metalloid were measured in borehole drinking water collected from 18 communities in Tarkwa, and the mean concentrations are presented in Table 2 along with the drinking groundwater standards of the European Union (EU, 1998), Pollution Control Department (PCD, 2000; 2004), World Health Organization (WHO, 2011) and the US Environmental Protection Agency (USEPA, 2012).

The geometric mean (GM) concentrations [range] and sequence of heavy metals and metalloid from the sampled communities were Mn (91.9 [10.3–943])>Zn (21.0 [1.21–117])>Fe (18.3 [1.16–1397])>Cu (2.39 [0.277–32.8])>Ni (1.28 [0.220–17.4])>Co (0.537 [0.0436–23.7])>As (0.277 [0.0461–1.51])>Pb (0.272 [0.0117–5.06])>Cd (0.0199 [nd–0.161])>Cr (nd) and Hg (nd) µg/L. Concentrations of Cr and Hg were below detection limit in all samples.

GM concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in borehole drinking water in the communities were low compared to the USEPA, PCD, WHO and EU guideline values ([EU, 1998](#); [PCD, 2000; 2004](#); [WHO, 2011](#); [USEPA, 2012](#)). However, GM concentrations of Co, Fe and Mn were higher than recommended values in 5, 11 and 61% of the sampled communities, respectively ([EU, 1998](#); [WHO, 2011](#); [USEPA, 2012](#)).

The high content of Fe and Mn in drinking water from boreholes in some communities in Tarkwa could have resulted from dissolution of Fe and Mn in the Tarkwanian rock system in addition to the acid mine drainage which is a common occurrence in the area ([Obiri, 2007](#)). As shown in [Table 3](#), borehole water in Huniso, Pepesa, Samahu, T–Cyanide and T–Tamso indicated high concentrations of some metals (i.e. Fe, Mn, As, Cd, Co, Zn and Pb) and low pH values (4.80–6.16).

Table 2: Scales for chronic and carcinogenic risks assessment ([USEPA, 1999](#))

Risk level	HQ/HI	Chronic risk	Calculated cases of cancer occurrence	Cancer risk
1	< 0.1	Negligible	< 1 per 1,000,000 inhabitants	Very low
2	$\geq 0.1 < 1$	Low	> 1 per 1,000,000 inhabitants	Low
			< 1 per 100,000 inhabitants	
3	$\geq 1 < 4$	Medium	> 1 per 100,000 inhabitants	Medium
			< 1 per 10,000 inhabitants	
4	≥ 4	High	> 1 per 10,000 inhabitants	High
			< 1 per 1,000 inhabitants	
5			> 1 per 1,000 inhabitants	Very high

HQ: Hazard Quotient; HI: Hazard Index

Comparison of heavy metal and metalloid concentrations with previous studies

Previous studies reported that mining activities have resulted in heavy metals and metalloids contamination of water, soil, plant, food, and humans in Obuasi and Tarkwa, which are important mining areas in Ghana (Amasa, 1975; Smedley, 1996; Smedley et al., 1996; Smedley and Kinniburgh, 2002; Asante et al., 2007; Bortey-Sam et al., 2015c).

At least 10% of Ghana's rural borehole wells have As concentrations exceeding 10 µg/L (Mead, 2005). Because some heavy metals and metalloids including inorganic As are carcinogenic (WHO, 2001), human exposure through consumption of contaminated drinking water and food in these areas could cause serious health problems. Concentrations of As in borehole drinking water in this study (Table 3) was comparable with studies by Smedley et al. (1996); Asante et al. (2007) and Wongsasuluk et al. (2014). Although elevated concentrations were found in groundwater in Obuasi, and many samples exceeded the WHO guideline value, concentrations of As in most samples in Bolgatanga, Ghana which is also gold mining area, were below detection limit (Smedley, 1996). The variation of As levels in groundwater in mining areas could be related to pH in aquifer. Higher concentrations of As were detected in groundwater with pH > 6.70 (Smedley, 1996; Asante et al., 2007). In the present study, low concentrations of As in groundwater could also be due to the low pH (pH across the 18 communities ranged from 4.80–6.96 with an average of 6.20 ± 0.629).

The levels of As in borehole water may be due to the nature of the gold bearing ore which is mineralized pyrites and arsenopyrates. Processing of the ore involves roasting and these results in the production of arsenic trioxide gas which is distributed throughout the study area by air current. Inorganic As easily dissolves and enters underground and surface water (Amonoo-Neizer et al., 1996; Obiri, 2007) and this could be the possible reason or source of As in borehole water in Tarkwa.

The average concentrations of Fe (269 µg/L) and Cd (0.0457 µg/L), in the communities, was low compared to similar studies by Obiri (2007); Kacmaz and Nakoman (2010) and Wongsasuluk et al. (2014) in Dumasi (Ghana), Manisa (Turkey), and Ubon Ratchathani

province (Thailand), respectively. Mean concentrations of Co (2.55 µg/L) and Cd (0.0457 µg/L) in borehole water in this study were comparable with results obtained by [Asante et al. \(2007\)](#) in Tarkwa. However, the average concentrations of Zn (50.2 µg/L), Cu (11.4 µg/L) and Pb (1.29 µg/L) were higher than similar studies conducted by [Obiri \(2007\)](#) and [Asante et al. \(2007\)](#), in Dumasi (Ghana) and Tarkwa (Ghana) respectively, but Pb and Cu concentrations in this study were comparable with a study by Kacmaz and Nakoman ([2010](#)) in Manisa, Turkey. Concentrations of Zn in this study were low compared to study by [Neves and Matias \(2008\)](#) in Portugal.

The mean concentration of Ni in this study (ranging from 0.300– 20.1 µg/L) was comparable with a similar study conducted in mining areas in Jharkhand, India ([Giri et al., 2012](#)). However, levels of Mn in borehole water in this study was higher than the study by [Kacmaz and Nakoman \(2010\)](#) in Turkey, but low compared to similar studies by [Asante et al. \(2007\)](#) and [Neves and Matias \(2008\)](#) in Ghana and Portugal respectively.

Distribution maps of heavy metals and a metalloid

The spatial analysis of geographic information statistics, used for pollution studies can not only help us understand the distribution of heavy metals or metalloids, but also reveal the impact factors ([Zhang et al., 2014](#)) ([Fig. 2](#)). As shown in [Fig. 2](#), the hotspots of high metal concentrations were identified in the distribution maps and pollution was mainly accumulated around Samahu and Huniso. Distribution maps of As highlighted Samahu as the significantly polluted zone ([Fig. 2](#)). On the other hand, the distribution maps showed that Cd, Co, Mn and Ni were high in Huniso, while Cu, Fe, Pb and Zn concentrations were high in Abekuase, Mile 9, Pepesa and Huniso, and Teberebe, respectively ([Fig. 2](#)). Distribution map of Mn ([Fig. 2](#)) indicated higher concentrations than the standard of 50 µg/L set by [EU \(1998\)](#) and [USEPA \(2012\)](#) in 61% of the communities. The high concentrations of Mn in drinking water in Tarkwa could be attributed to the several legal and illegal mining activities within the study area ([Akabzaa and Darimani, 2001; Obiri 2007](#)).

Table 3: pH and mean concentrations ($\mu\text{g/L}$) of heavy metals and metalloid in borehole drinking water in Tarkwa

Sample sites		pH	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Teberebe ($n = 3$)	Mean	6.03	0.180	0.0966	0.670	nd	33.6	2.37	nd	55.7	1.94	0.462	278
	SD	0.353	0.0748	0.0794	0.801	nd	45.7	0.705	nd	65.6	0.320	0.419	71.1
Mile 7 ($n = 3$)	Mean	6.96	1.40	nd	0.493	nd	0.279	3.34	nd	515	0.460	0.0210	1.62
	SD	0.191	0.295	nd	0.690	nd	0.0422	4.09	nd	275	0.571	0.00492	1.51
Mile 8 ($n = 3$)	Mean	6.44	0.127	0.0182	0.519	nd	0.338	16.8	nd	51.7	0.985	0.0654	6.10
	SD	0.284	0.0826	0.0125	0.266	nd	0.164	13.2	nd	42.1	0.328	0.0461	3.78
Techiman ($n = 3$)	Mean	6.51	0.144	0.0681	0.573	nd	2.23	29.0	nd	128	1.49	0.130	11.1
	SD	0.422	0.102	0.0978	0.526	nd	1.97	16.3	nd	85.3	0.540	0.127	9.95
Mile 9 ($n = 3$)	Mean	6.14	0.574	0.0104	0.405	nd	14.3	2336	nd	82.6	1.44	0.221	58.1
	SD	0.475	0.695	0.0100	0.0943	nd	14.2	987	nd	8.93	1.29	0.144	25.1
Mile 10 ($n = 3$)	Mean	6.21	0.0562	0.0220	0.444	nd	4.07	1.24	nd	30.7	1.41	0.243	26.5
	SD	0.0848	0.0454	0.0271	0.625	nd	2.00	0.627	nd	18.9	1.08	0.0800	7.70
Wangarakrom ($n = 4$)	Mean	6.61	0.585	0.121	0.843	nd	7.12	1.98	nd	768	1.25	0.418	35.3
	SD	0.127	0.0252	0.120	0.342	nd	4.02	1.19	nd	461	0.658	0.355	32.9
Badukrom ($n = 3$)	Mean	6.58	0.660	0.0116	0.645	nd	0.484	282	nd	151	0.785	0.0117	15.4
	SD	0.0282	0.429	0.0102	0.315	nd	0.495	249	nd	23.4	0.507	0.00180	17.3
Samahu ($n = 10$)	Mean	6.16	3.73	0.0305	0.426	nd	1.83	47.8	nd	288	1.58	0.0557	32.0
	SD	0.378	2.01	0.0210	0.279	nd	0.783	53.2	nd	60.8	0.700	0.0780	25.9
Abekuase ($n = 14$)	Mean	6.73	0.553	0.139	0.590	nd	48.3	125	nd	44.1	4.62	2.48	118
	SD	0.355	0.452	0.0510	0.556	nd	21.0	104	nd	25.3	2.82	3.94	90.3
Tebe ($n = 11$)	Mean	6.44	0.577	0.0211	0.512	nd	14.7	29.3	nd	46.1	1.74	0.675	54.0
	SD	0.228	0.483	0.0124	0.536	nd	13.6	23.9	nd	44.8	1.45	0.934	43.3
Huniso ($n = 10$)	Mean	4.96	1.10	0.173	29.7	nd	36.9	8.15	nd	1020	20.1	5.89	133
	SD	0.962	1.09	0.0670	23.9	nd	29.7	3.99	nd	492	10.3	3.51	74.9
Pepesa ($n = 14$)	Mean	5.68	0.399	0.0319	5.15	nd	4.42	48.4	nd	236	4.31	6.42	39.0
	SD	0.602	0.319	0.0184	11.7	nd	6.84	76.2	nd	117	4.05	3.41	21.8
T-cyanide ($n = 3$)	Mean	4.99	0.322	0.0418	2.45	nd	1.63	8.97	nd	150	1.27	0.590	19.3

	SD	0.742	0.0988	0.0153	2.88	nd	1.39	7.39	nd	136	0.812	0.745	11.0
T-Brofuyedu (<i>n</i> = 3)	Mean	6.72	0.0885	nd	0.157	nd	0.608	15.6	nd	10.9	0.304	0.0774	5.55
	SD	0.367	0.0290	nd	0.0325	nd	0.280	1.02	nd	5.21	0.0474	0.000487	5.59
T-Layout (<i>n</i> = 5)	Mean	5.92	0.162	0.0245	1.06	nd	1.97	6.31	nd	114	1.08	0.451	10.3
	SD	0.461	0.101	0.0134	0.988	nd	2.54	1.47	nd	62.3	1.01	0.214	7.74
T-Achapime (<i>n</i> = 5)	Mean	6.86	0.283	0.0215	0.499	nd	1.38	2029	nd	559	0.827	0.508	8.69
	SD	1.27	0.267	0.0126	0.559	nd	0.474	1080	nd	338	0.349	0.189	5.07
T-Tamso (<i>n</i> = 5)	Mean	4.80	0.494	0.0434	5.18	nd	59.4	26.1	nd	244	4.13	4.02	49.9
	SD	0.586	0.171	0.0217	1.47	nd	30.1	13.5	nd	91.7	0.739	3.41	0.593
USEPA		6.5–8.5	10	5		100	1300	300	2	50		15	5000
PCD			10	3		50	1000		1		20	10	5000
WHO			10	3	20	50	2000	300	6		70	10	3000
EU			10			50		200		50	20		

n: number of boreholes sampled in each community; nd: not detected; USEPA (2012); PCD (2000; 2004); WHO (2011); EU (1998) Bold indicates lower pH levels and higher metal concentrations than recommended

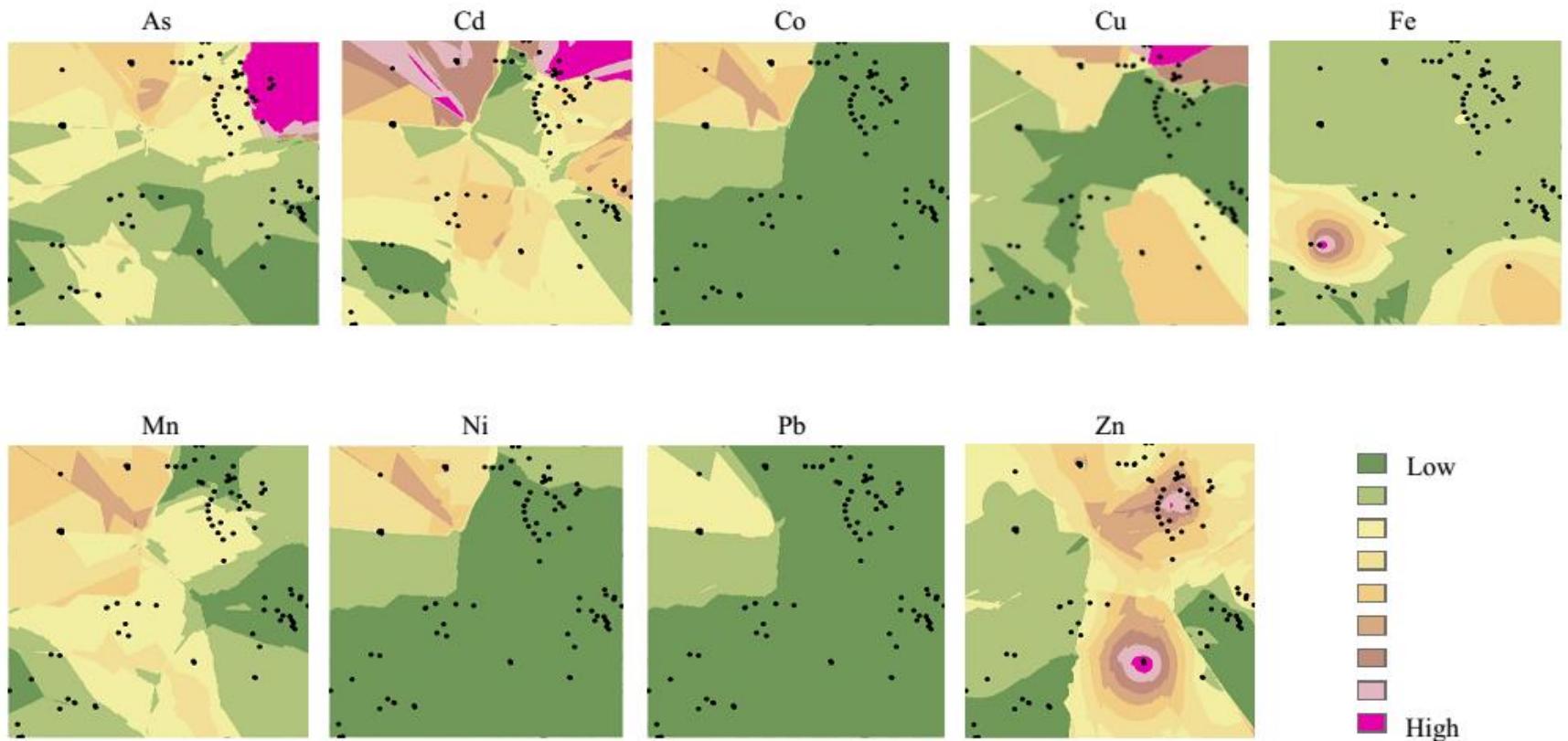


Fig. 2 Distribution maps of heavy metals and metalloid in borehole drinking water collected from 105 spots in Tarkwa (black dots indicate sample spots)

Human health risk assessment

Concentrations of heavy metals in borehole groundwater in this study were higher than the EU (1998), PCD (2000; 2004), WHO (2011) and USEPA (2012) drinking water standards in some communities ([Table 3](#)). Human health risk assessment for adults and children showed HQ suggesting an acceptable level of non–carcinogenic adverse health risk ([Tables 4 and 5](#)) but, if we take into consideration the additive effect of heavy metals and a metalloid, then the oral HI results for children raises concerns about the non–carcinogenic adverse health effects of drinking water in Huniso ([Table 5](#)). As shown in [Tables 4 and 5](#), HI for adults and children in Huniso were 0.781 (low risk; USEPA, 1999; [Tables 2](#)) and 1.08 (medium risk; USEPA, 1999; [Tables 2](#)), respectively, and therefore remedial action and control methods are required in this community. Children are especially vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consume more per unit of body weight as adults ([ENHIS, 2007](#)).

Table 4: Non-carcinogenic risk (HQ and HI) of heavy metals and metalloid in borehole drinking water in Tarkwa (Adults)

Sample sites	As	Cd	Co	Cu	Mn	Ni	Pb	Zn	HI
Teberebe	0.0331	0.00904	0.00103	0.0134	0.0127	0.00554	0.00584	0.0181	0.0987
Mile 7	0.266	0.000200	0.000200	0.000400	0.130	0.000630	0.000240	0.000230	0.398
Mile 8	0.0204	0.00123	0.00136	0.000450	0.0173	0.00272	0.000830	0.00101	0.0453
Techiman	0.0232	0.00334	0.000810	0.00214	0.0170	0.00413	0.00140	0.00158	0.0537
Mile 9	0.0572	0.000750	0.00115	0.00780	0.0338	0.00324	0.00286	0.0103	0.117
Mile 10	0.00885	0.00125	0.000130	0.00549	0.00563	0.00131	0.00389	0.00499	0.0315
Wangarakrom	0.112	0.00905	0.00232	0.00938	0.225	0.00335	0.00550	0.00509	0.372
Badukrom	0.111	0.000530	0.00174	0.000480	0.0618	0.00201	0.000190	0.00181	0.181
Samahu	0.289	0.00260	0.00108	0.00245	0.0656	0.00426	0.000420	0.00337	0.369
Abekuase	0.0759	0.00583	0.00970	0.0217	0.0142	0.00578	0.0188	0.0146	0.157
Tebe	0.0744	0.00154	0.000820	0.00710	0.00853	0.00351	0.00559	0.00641	0.108
Huniso	0.110	0.0185	0.0681	0.0412	0.387	0.0499	0.0832	0.0226	0.781
Pepesa	0.0518	0.00325	0.00457	0.00266	0.0487	0.00874	0.0312	0.00434	0.155
T-Cyanide	0.0603	0.00387	0.00388	0.00187	0.0472	0.00326	0.00435	0.00338	0.128
T-Brofuyedu	0.0165	0.000360	0.000450	0.000830	0.00422	0.000870	0.00127	0.000750	0.0252
T-Layout	0.0200	0.00367	0.00346	0.00218	0.0616	0.00380	0.00939	0.00266	0.107
T-Achapime	0.0405	0.00147	0.000880	0.00193	0.0822	0.00227	0.00806	0.00152	0.139
T-Tamso	0.0919	0.00480	0.0146	0.0472	0.0968	0.0118	0.0529	0.00957	0.329

Table 5: Non-carcinogenic risk (HQ and HI) of heavy metals and metalloid in borehole drinking water in Tarkwa (Children)

Sample site	As	Cd	Co	Cu	Mn	Ni	Pb	Zn	HI
Teberebe	0.0460	0.0126	0.00143	0.0186	0.0176	0.00771	0.00812	0.0252	0.137
Mile 7	0.370	0.000280	0.000270	0.000560	0.181	0.000880	0.000330	0.000330	0.554
Mile 8	0.0283	0.00171	0.00189	0.000630	0.0241	0.00379	0.00116	0.00140	0.0630
Techiman	0.0323	0.00465	0.00112	0.00298	0.0237	0.00575	0.00195	0.00220	0.0747
Mile 9	0.0796	0.00104	0.00160	0.0108	0.0470	0.00450	0.00398	0.0143	0.163
Mile 10	0.0123	0.00173	0.000170	0.00764	0.00784	0.00182	0.00541	0.00694	0.0439
Wangarakrom	0.156	0.0126	0.00323	0.0131	0.313	0.00466	0.00765	0.00708	0.517
Badukrom	0.155	0.000730	0.00242	0.000670	0.0860	0.00280	0.000270	0.00251	0.251
Samahu	0.402	0.00361	0.00150	0.00341	0.0913	0.00592	0.000580	0.00469	0.513
Abekuase	0.105	0.00811	0.00135	0.0302	0.0197	0.00804	0.0262	0.0203	0.219
Tebe	0.104	0.00214	0.00115	0.00988	0.0119	0.00488	0.00777	0.00891	0.150
Huniso	0.153	0.0257	0.0947	0.0573	0.539	0.0695	0.115	0.0315	1.08
Pepesa	0.0721	0.00452	0.00636	0.00371	0.0677	0.0122	0.0434	0.00604	0.216
T-cyanide	0.0838	0.00539	0.00540	0.00260	0.0657	0.00453	0.00606	0.00471	0.178
T- Brofuyedu	0.0230	0.000500	0.000620	0.00115	0.00586	0.00121	0.00177	0.00104	0.0351
T-Layout	0.0278	0.00510	0.00481	0.00303	0.0857	0.00528	0.0131	0.00371	0.148
T-Achapime	0.0563	0.00205	0.00122	0.00268	0.114	0.00316	0.0112	0.00211	0.193
T-Tamso	0.128	0.00668	0.0203	0.0656	0.134	0.0164	0.0736	0.0133	0.458

The carcinogenic risk of Cd for adults through drinking borehole water in the sampled communities, ranged from 3.80E–08 (very low cancer risk; [Tables 2 and 6](#)) to 3.51E–06 (low cancer risk; [Tables 2 and 6](#)) at Mile 7 and Huniso, respectively, with an overall average of 7.52E–07 (very low cancer risk; [USEPA, 1999; Tables 2 and 6](#)). Similarly, the cancer risk of Cd for children ranged from 5.35E–08 (very low cancer risk; [Tables 2 and 6](#)) to 4.88E–06 (low cancer risk; [Tables 2 and 6](#)) with an overall average of 1.04E–06 (low cancer risk; [USEPA, 1999; Tables 2 and 6](#)).

However, the cancer risk levels of As for adults through drinking borehole water in the communities ranged from 3.98E–06 (low cancer risk) to 1.30E–04 (high cancer risk) at Mile 10 and Samahu, with an average of 3.65E–05 (medium cancer risk; [USEPA, 1999](#)) ([Tables 2 and 6](#)). Similarly, the cancer risk levels of As for children, through drinking water, ranged from 5.53E–06 (low cancer risk) to 1.81E–04 (high cancer risk) at Mile 10 and Samahu, with an average of 5.08E–05. The risk values due to As exposure in Mile 7 were 1.19E–04 (adults) and 1.66E–04 (children), and indicated high cancer risk ([Tables 2 and 6; USEPA, 1999](#)). Based on the [USEPA \(1999\)](#) assessment, average cancer risk values of As indicated three (adults) and five (children) cases of neoplasm in a hundred thousand inhabitants.

Within Slovakia, [Rapant and Krcmova \(2007\)](#) reported that the estimated carcinogenic risk caused by As in groundwater corresponds to more than one case of neoplasm occurrence per 1000 inhabitants in each area. [Saipan and Ruangwises \(2009\)](#) showed that the population of Ronphibun, Thailand, could experience adverse health impacts since the carcinogenic health risk index was ascertained to be 1.26E–03.

Table 6: Carcinogenic risk of As and Cd in borehole drinking water in Tarkwa (Adults and Children)

Sample sites	Adults		Children	
	Cancer risk (As)	Cancer risk (Cd)	Cancer risk (As)	Cancer risk (Cd)
Teberebe	1.48E-05	1.71E-06	2.07E-05	2.39E-06
Mile 7	1.19E-04	3.84E-08	1.66E-04	5.35E-08
Mile 8	9.16E-06	2.33E-07	1.27E-05	3.25E-07
Techiman	1.04E-05	6.35E-07	1.45E-05	8.84E-07
Mile 9	2.57E-05	1.41E-07	3.58E-05	1.97E-07
Mile 10	3.98E-06	2.36E-07	5.53E-06	3.29E-07
Wangarakrom	5.04E-05	1.71E-06	7.01E-05	2.39E-06
Badukrom	5.03E-05	1.00E-07	7.01E-05	1.39E-07
Samahu	1.30E-04	4.93E-07	1.81E-04	6.86E-07
Abekuase	3.41E-05	1.10E-06	4.74E-05	1.54E-06
Tebe	3.34E-05	2.91E-07	4.65E-05	4.05E-07
Huniso	4.95E-05	3.51E-06	6.88E-05	4.88E-06
Pepesa	2.33E-05	6.17E-07	3.24E-05	8.59E-07
T-Cyanide	2.71E-05	7.35E-07	3.77E-05	1.02E-06
T-Brofuyedu	7.42E-06	6.85E-08	1.03E-05	9.53E-08
T-Layout	8.99E-06	6.96E-07	1.25E-05	9.69E-07
T-Achapime	1.82E-05	2.79E-07	2.53E-05	3.89E-07
T-Tamso	4.13E-05	9.12E-07	5.75E-05	1.26E-06
Minimum	3.98E-06	3.84E-08	5.53E-06	5.35E-08
Maximum	1.30E-04	3.51E-06	1.81E-04	4.88E-06
Average	3.65E-05	7.52E-07	5.08E-05	1.04E-06

In addition, the local people living in the study area of this report, which is located in a tropical zone, generally had to work on their farms or fields under relatively high temperatures and humidity with strong sunlight exposure; consequently, their physical requirement for water is quite high. The estimated water intake was at an average rate of 3.45 (adults) and 2.0 (children) L/day ([Apambire et al., 1997](#); [Roychowdhury et al., 2003](#); [Wongsasuluk et al., 2014](#)) to the usual average of 2 L/day (adult) ([USEPA, 1980](#)). People in countries with colder weather than in Ghana would be expected to have lower water intake rate, such as 1.50 L/day; average level reported in Korea ([Lim et al., 2008](#)). Thus, the human health risk assessment does not only depend on the heavy metal or metalloid concentration in drinking water, but also on the water consumption rate ([Wongsasuluk et al., 2014](#)).

In accordance, Peplow and Edmonds (2004) found that the average risk of cancer from exposure to As from abandoned mines was approximately two fold higher for adults engaged in occupations with high sunlight exposure. Study by Pokkamthanam et al. (2011) showed that As in water consumed by Bandlaguda adults may cause both adverse non-carcinogenic and carcinogenic health risks since the mean water consumption was quite high (4.50 ± 2.40 L/day). The high water consumption could be due to requirements caused by the participants or inhabitants occupation and/or climate. Therefore, farmers, children and other workers, (factory workers, laborers, welders, carpenters, miners in tropical countries) should be made more aware of heavy metal and/or metalloid contamination, even at low concentrations, because of their high intake rate.

Conclusions

This study evaluated the health risk of As, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni and Zn in drinking water from 18 communities in Tarkwa, Ghana. The concentrations of heavy metals in borehole drinking water in some communities were higher than the drinking water standards by EU (1998), PCD (2000; 2004), WHO (2011) and USEPA (2012). HI in Huniso for adults and children were 0.781 (low risk) and 1.08 (medium risk), respectively, and therefore remedial action and control methods are required in this community. The average cancer risk values of As for adults ($3.65\text{E}-05$) and children ($5.08\text{E}-05$) through drinking borehole water in the study area indicated three (adults) and five (children) cases of neoplasm in a hundred thousand inhabitants. However, concentrations of these heavy metals and metalloid in the groundwater were within acceptable limits, revealing the importance of the actual consumption rates in estimating health risk factors. The local people drinking from groundwater should be educated about the potential adverse effects of drinking directly from the boreholes. In order to reduce the estimated carcinogenic risk and non–carcinogenic HIs, inhabitants should (be advised to) treat their water or find alternative sources of drinking. Local authorities should be made aware of such health risks and provide potable water. The results of this study could be used; to communicate and manage the risk to the local people who generally drink borehole water; and to prevent adverse health risks from groundwater contamination. In general, this study adds to the results of others that illustrate the greater need for risk awareness and communication about heavy metal and metalloid contamination in borehole groundwater, especially around mining areas in tropical zones.

Chapter 3

Accumulation of heavy metals and metalloid from agricultural soils to foodstuffs around Tarkwa area in Ghana, and associated human health risks

Introduction

There has long been concerns about heavy metals and metalloids pollution because of their stability and non-biodegradability in environmental media as well as their toxicity to plants, animals and humans (Zhuang et al., 2009). Soil is the primary reservoir for heavy metals in the atmosphere, hydrosphere and biota, and thus plays a fundamental role in the overall metal cycle in nature (Cao et al., 2010). Heavy metals in soil pose potential threats to the environment and can cause human health problems through various absorption pathways such as inhalation, absorption through dermal pores, or ingestion (diet through the soil–food chain) (Komarek et al., 2008).

Vegetables/foodstuffs play important roles in our daily diet as economic crops. However, in Ghana, various human activities such as mining, industrial processing (smelting), automobile exhausts and applications of organic manure/fertilizers are causing elevated heavy metal concentrations in environmental media and absorption by food crops (Akoto et al., 2014; Asante et al., 2007; Bortey-Sam et al., 2015c; Obiri 2007; Smedley and Kinniburgh, 2002).

Vegetables/foodstuffs take up heavy metals and/or metalloids by absorbing them from contaminated soils, as well as from deposits on parts of the crop exposed to air from polluted environments (Wang et al., 2005). The main pathway by which plants accumulate heavy metals is through root uptake from soils (Uzu et al., 2010). Some heavy metals present in the soil solution are adsorbed onto the roots, and then becomes bound to carboxyl groups of mucilage uronic acid, or directly to the polysaccharides of the rhizoderm cell surface (Seregin and Ivanov, 2001). Once adsorbed onto the rhizoderm roots surface, they may enter the roots passively and follow translocating water streams. Indeed, the highest concentrations of some heavy metals can be found in root apices, where root cells are young and have thin cell walls (with the exception of root cap cells) (Tung and Temple, 1996).

Heavy metals (particular lead) may enter the roots through ionic channels. The amount of heavy metals that moves from soil to penetrate into plants can be measured by “the transfer

factor”; this factor is defined as the ratio that exists between the concentrations of metals in the plant and those in soil. This transfer factor could be different for different plant species and will change as soil physical and chemical properties are altered. Generally, plants having a transfer factor greater than 1 are categorized as hyperaccumulators, whereas those with transfer factor less than 1 are termed as non-accumulators ([Arshad et al., 2008](#)).

Heavy metals in leaf and tuber crops as a consequence of soil and atmospheric contamination by mining and related activities showed concentrations of cadmium, zinc and lead were detected in: leaves of cassava growing near the Enyigba base metal deposit in Nigeria ([Chukwuma, 1995](#)); tubers of cassava in the environs of the Arufu base metal deposit in Nigeria ([Nganje et al., 2010](#)); as well as in the tubers of cassava, sweet potato and yam in other districts in the same country ([Onyedika and Nwosu, 2008](#)). Concentrations of arsenic and zinc in cassava tubers were also investigated in areas where gold was mined in Obuasi, Ghana ([Amonoo-Neizer et al. 1995](#)) and Dunkwa-on-Offin, Ghana ([Golow and Adzei, 2002](#)). Cassava cultivated in areas affected by mining were found to contain higher concentrations of heavy metals/metalloids when compared with those grown in uncontaminated areas ([Kribek et al., 2014](#)).

Chronic intake of heavy metals above their safe threshold in humans and animals have damaging effects and can cause non–carcinogenic hazards such as neurologic impairment, headache and liver diseases ([USEPA, 2000](#)). Dietary cadmium intake due to consumption of environmentally contaminated rice and other foods was associated with an increased risk of postmenopausal breast cancer ([Itoh et al., 2014](#)). Acute and chronic exposure to arsenic could also cause numerous human health problems. These include dermal, respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, neurological, developmental, reproductive, immunological, genotoxic, mutagenic and carcinogenic effects ([Lin et al., 2013](#)). Despite the economic benefits of industry, the negative impacts on humans and the environment may cause additional costs not factored into income projections. Especially for the ubiquitous and non–biodegradable heavy metals, the negative effects persist for several decades and even longer ([Fantke et al., 2012](#)).

In Tarkwa, studies conducted on the impact of gold mining in soil, drinking water and foods collected around mining communities in Tarkwa showed high levels of some toxic metals including arsenic and mercury ([Asante et al., 2007](#); [Bortey-Sam et al., 2015c; 2015d](#); [Hayford et al., 2008](#); [Esumang et al., 2007](#)). Inspite of these, there is limited or no data from literature that addresses the accumulation of heavy metals and metalloids from soil to foodstuffs and the potential health risk to humans through consumption.

The objectives of the present study were therefore to assess the extent of heavy metals and metalloid accumulation from soils to foodstuffs commonly grown in agricultural areas in Tarkwa; to study the relation between bioconcentration factor (BCF), soil pH and soil organic matter (SOM); and to evaluate the potential human health risks of toxic metal exposure to residents in Tarkwa through consumption of foodstuffs.

Materials and methods

Study area

Topography of Tarkwa, shows plateaus with heights between 600 and 1050 meters, and south of the town lays a vast stretch of terrain which is suitable for agricultural crop production. Large plantations of orange, rubber and palm are therefore a major feature of this area. Tarkwa and its environs lie within the transition zone between the tropical rain forest and the coastal shrub vegetation. In Tarkwa, the average rainfall is 850 mm per year, and the maximum is recorded between June and October. Planting and harvesting of food crops are mostly done during this period and cassava, plantain, yam, cocoyam, oranges and vegetables are the staples grown in this area ([Hayford et al., 2008](#)).

Soil and foodstuff sampling and processing

In June 2012, a total of 60 agricultural soils and 65 foodstuffs were randomly collected from 13 communities in Tarkwa. Global positioning system (GPS) was used to geo-reference the sampling positions ([Fig. 1](#)).

Soil samples (0–10 cm top layer) were collected using a stainless steel, and stored in labeled corning tubes (Corning Incorporated, New York, USA). Three to eight agricultural soils were collected from different farms in some community. Two kinds of foodstuffs viz, *M. esculenta* (cassava) and *Musa paradisiaca* (plantain) were collected during this period. In Ghana, cassava and plantain are important components of human diet and constitute the staples in the region. Cassava and plantain are grown and consumed in many places, particularly in the rural areas of Ghana. In some community, foodstuffs were collected from different farms and stored in polyethylene zipbags. The samples obtained were stored at –20°C in the Department of Chemistry, KNUST, Ghana and later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan where

they were stored at -30°C until analysis. A map of the sampling area and points is presented in Fig. 1.

Prior to chemical analysis, the soil samples were air-dried at room temperature and passed through a 2 mm sieve. Soil pH was measured in a soil deionized water suspension (soil: water, 1:2.5 by volume) by a calibrated pH meter. The water content of each soil sample was measured after 12 h of drying in an oven at 105°C . Soil organic matter (SOM) content was determined by loss of weight on ignition at oven temperature of 600°C for 5 h. For foodstuff samples, the edible parts were rinsed with deionized water and dried at 45°C .

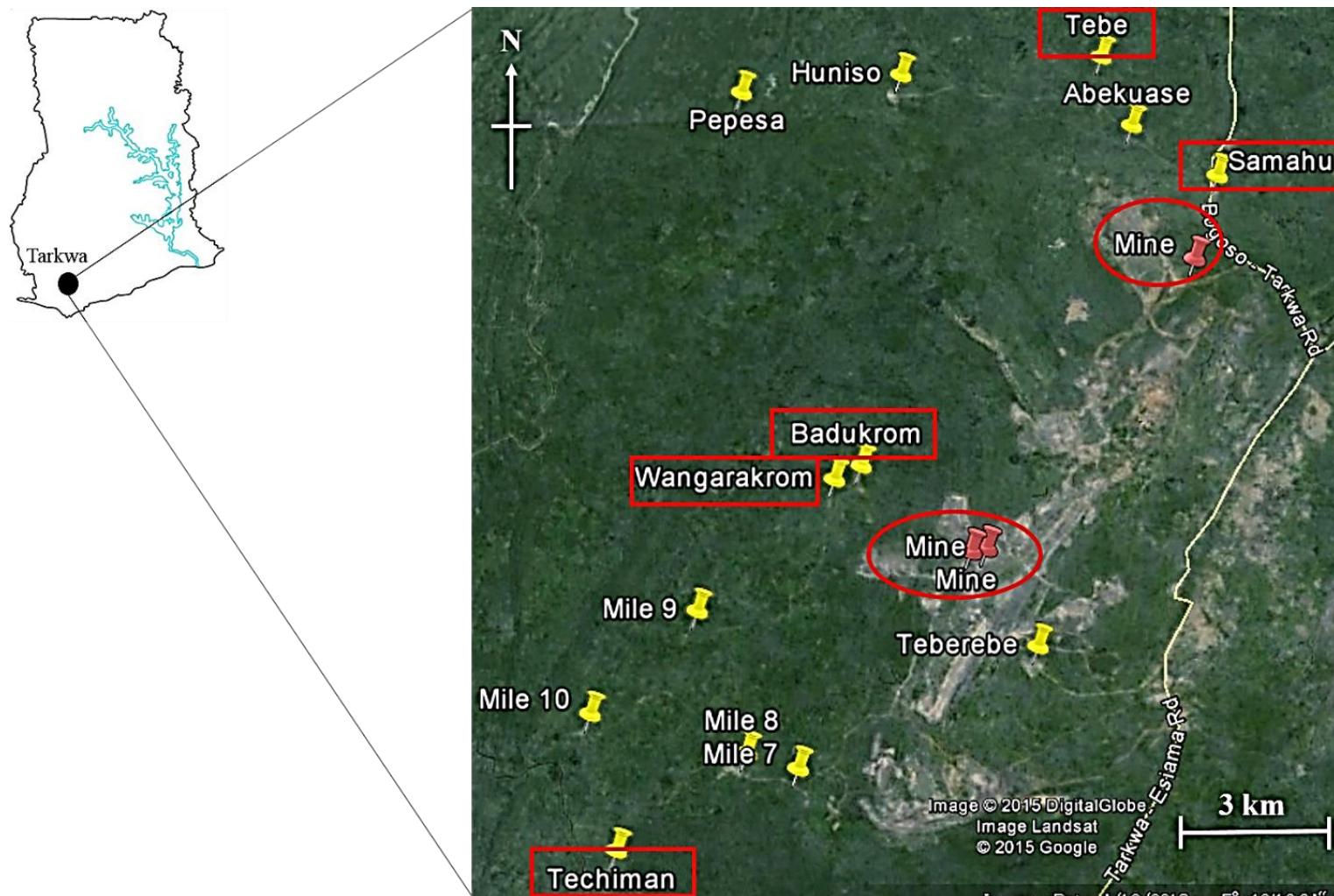


Fig. 1 Map showing agricultural soils and foodstuffs sampling locations in Tarkwa (Yellow pins indicate sampled communities and red pins indicate gold mines)

Heavy metal analyses.

Digestion of heavy metals and metalloid in soil samples was done according to a method described by [Bortey-Sam et al. \(2015e\)](#). Briefly, approximately 0.5 g of soil sample was weighed into prewashed digestion vessel and digested (Speedwave two, Berghof, Germany) using 10 mL of 60% HNO₃ (Kanto Chemical Corporation, Tokyo, Japan). After cooling, samples were filtered into corning tubes (Corning Incorporated, New York, USA) using ashless filter paper 5B (Advantec, Tokyo, Japan). The solution was standardized to 50 mL using distilled, deionised water. Similarly, 0.5 g of foodstuff was weighed and digested in a mixture of HNO₃:H₂O₂ (5:1, v/v; 60%:35%) (Kanto Chemical Corporation, Tokyo, Japan) and the solution was made to 10 mL using distilled, deionised water. Blanks were prepared using the same procedure.

Concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) were measured with an inductively coupled plasma–mass spectrometer (ICP–MS; 7700 series, Agilent technologies, Tokyo, Japan) and results were expressed in mg/kg dry weight (dw). The concentration of total mercury (Hg) was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury analyzer, MA–3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of the calibration standards.

Quality control and quality assurance

For quality control, blanks and duplicates (samples) were analyzed after every 10 sample analyses. The instrument was calibrated using standard solutions of the respective metals (to establish standard curves before metal analysis). The correlation coefficients (r^2) of the calibration curves were greater than 0.999. All chemicals and standard stock solutions were of analytical–reagent grade (Wako Pure Chemicals Industries, Ltd., Osaka, Japan). The

detection limits (ng/g) of the heavy metals were 0.002 (As), 0.001 (Cd), 0.0004 (Co), 0.003 (Cr), 0.007 (Cu), 0.003 (Ni), 0.001 (Pb), and 0.046 (Zn).

For metals, reference materials SRM 1944 (New York/ New Jersey Waterway Sediment) and BCR–320 (Channel Sediment, IRMM, Belgium) were used for method validation. Replicate analysis of these reference materials showed good accuracy and recovery rates ranged from 80–115%. The detection limit of Hg in samples was 2.0 pg total Hg and recovery rates for the three certified reference materials (BCR–320R, SRM 1944, and DOLT–4) ranged from 92–103%.

Foodstuff samples were analyzed three times by the ICP–MS and Hg analyzer and the Relative Standard Deviations (RSD) were $\leq 5\%$.

Data analyses

Bioconcentration factor (BCF)

Recently, the application of enrichment factor in the form of transfer factor, BCF, and plant uptake factor has been expanded to research in soil, water system, sediment, as well as assessment of heavy metal pollution in environmental geochemistry (Wang et al., 2006). In soil research, BCF is defined as the ratio of the content of a particular element in plant to that in soil (mg/kg dw; average water contents in foodstuffs were 58% (cassava) and 62% (plantain)). BCF is an important quantitative indicator of crop contamination and has commonly been used for estimating metal transfer from soil to plants (USEPA, 2005). BCF–based research showed that the extent of metal enrichment in vegetables is highest in leaf vegetables, followed by tubers and fruit vegetables (Pandey and Pandey, 2009). Regarding metal concentrations, Cd and Pb commonly occur at high levels in leafy vegetables, while Zn content in tubers is higher than other metals (Ngole 2011).

The bioavailability and toxicity of metals in soil are significantly influenced by pH conditions. Soil pH is considered to be one of the most important factors that influence the transfer of metals from soil to plants, and higher pH values have been found to reduce the bioavailability and toxicity of some metals (Cd and Pb) (Wang et al., 2006; Kim et al., 2004; Tudorean et al., 2004). The mechanism for this phenomenon can contribute to an increase in solubility and ion competition. As soil pH decreases, concentrations of Fe^{2+} , Mn^{2+} , Zn^{2+} and Ca^{2+} increases in soil solution. This enhances the competition of free ions and reduces the adsorption to soil particles (Tudorean et al., 2004). Additionally, soil pH is the greatest determinant of the solubility and mobility of metals such as Cr, Pb and Zn in sandy soil (Speir et al., 2003). Due to the close relation between soil pH and heavy metal properties, correlation analysis of pH values and heavy metal accumulation is commonly used in research on bioconcentration of heavy metal from soil to crops (Chang et al., 2014).

Foodstuff consumption-associated health risk assessment

Potential human health risks of toxic metal exposure to residents of Tarkwa, through consumption of foodstuff were assessed using target hazard quotient (THQ) (USEPA, 2002). THQ is defined as the ratio of the body intake dose of a pollutant to the reference dose. If THQ is > 1 , there could be potential health risk associated with the pollutant. On the other hand, if THQ is < 1 , there will be no obvious risk. The THQ was calculated using the equation:

$$THQ = \frac{EFr \times ED \times FIR \times MC}{RfD \times BW \times AT} \times 10^{-3}$$

Where MC is the geometric mean (GM) concentration of a particular metal in foodstuff (mg/kg ww), FIR is the daily intake by residents of Ghana (cassava: 601 g/day and 400 g/day for adults and children, respectively; plantain: 369 g/day and 200 g/day for adults and children, respectively) (FAO, 2014). Daily intake of cassava and plantain by children were

assumed. EFr is the exposure frequency (365 d/yr) ([USEPA, 2002](#)), ED is the exposure duration (60 years for adults and 10 years for children) ([Bennet et al., 1999](#)), BW is the average body weight of local residents (60 kg for adults and 32.7 kg for children) ([Bortey-Sam et al., 2015c; Wang et al., 2005](#)), AT is the average exposure time for non-carcinogens (21,900 and 3,295 days for adults and children, respectively), and RfD is the reference dose (RfD for As, Cd, Hg and Pb were 3E-04, 1E-03, 3E-04 and 3.5E-03 mg/kg/d, respectively) ([USEPA, 2002](#)).

Statistical analysis

Statistical analyses were performed using IBM SPSS 20.0 statistical software (SPSS Inc., Illinois, USA) after data were normalized by log transformation. ANOVA and Tukey analyses were used to compare concentrations of metals in soil, cassava and plantain (mg/kg dw), and differences were considered statistically significant with *p* value < 0.05. Concentrations of heavy metals or metalloid below their respective LODs were replaced with a value of LOD/2. In this study, GM concentrations were used to represent the central tendency of heavy metals and metalloid in the study area ([Wayne, 1990](#)). The relationships between BCF of heavy metals in foodstuffs, soil pH and SOM were examined by Pearson's correlation and were considered statistically significant if *p* value was less than 0.05. Principal component analysis (PCA) based on log transformed data was done, to determine the distribution pattern of metals in foodstuffs. PCA was done using JMP statistical software v. 10 (SAS Institute). The principal components were extracted with eigenvalues > 1 through varimax rotation.

Results and discussion

Heavy metal distribution in agricultural soils and foodstuffs

Total concentrations of As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn in agricultural soils showed large variations in Tarkwa (Table 1; [29]). The GM concentrations of metals from the thirteen communities decreased in the order, Zn (37) > Cr (16) > Cu (5.1) > Pb (4.8) > As (3.8) > Ni (2.2) > Co (1.3) > Hg (0.49) and Cd (0.026) mg/kg dw (Table 1). Compared with ATSDR standard values ([ATSDR, 2008](#)), the mean concentrations of Hg in soil exceeded the limit (1 mg/kg) in Badukrom and Wangarakrom. Regarding regional distribution of metals in soil, concentrations of As and Hg in Badukrom and Wangarakrom were relatively higher than those in the other sampling communities around Tarkwa.

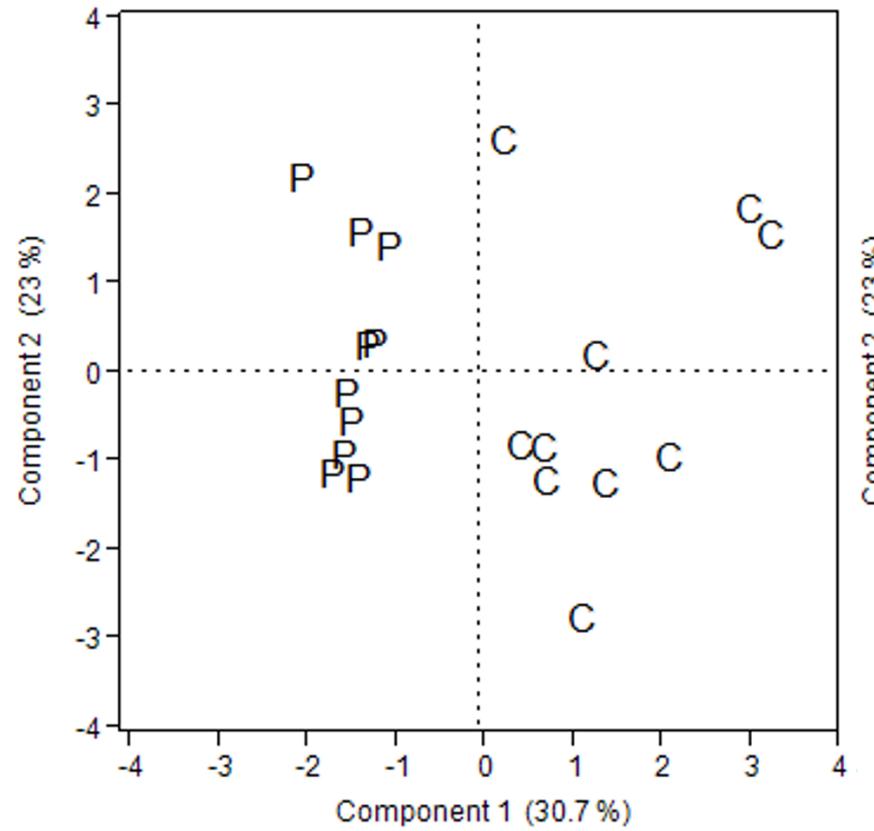
As compared to soils, foodstuffs contained significantly lower concentrations of heavy metals ($p < 0.01$; Table 1) with obvious variations among the different species). The GM concentration (mg/kg ww) of metals in cassava decreased in the order: Zn (7.6) > Ni (3.7) > Cu (2.1) > Pb (0.18) > Cr (0.050) > Co (0.024) > As (0.0090) > Cd (0.0070) and Hg (0.0040) ([Table 1](#)).

Table 1: Concentrations (geometric mean, SD, minimum, maximum) of heavy metals and metalloid in soil (mg/kg dw) and foodstuffs (mg/kg ww) in Tarkwa

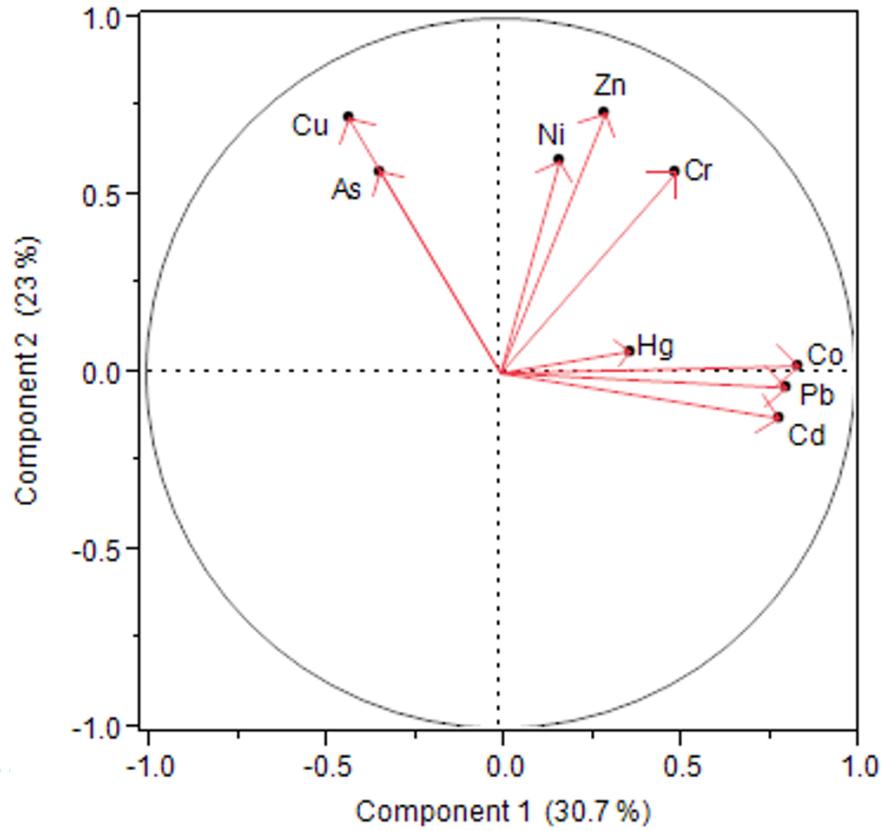
Sample type	Sample number		As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
Soil [#]	60	Geometric Mean	3.8 ^a	0.026 ^a	1.3 ^a	16 ^a	5.1 ^a	0.49 ^a	2.2 ^a	4.8 ^a	37 ^a
		SD	3.4	0.013	0.74	10	1.9	0.85	1.0	3.5	24
		Median	2.9	0.022	1.2	12	5.5	0.13	1.9	3.8	32
		Minimum	1.0	0.010	0.37	8.1	2.8	0.030	1.1	1.6	9.8
		Maximum	13	0.052	3.0	38	8.9	2.4	4.5	13	86
Cassava*	33	Geometric Mean	0.0090 ^b	0.0070 ^b	0.024 ^b	0.050 ^b	2.1 ^b	0.0040 ^a	3.7 ^b	0.18 ^b	7.6 ^b
		SD	0.0050	0.0050	0.013	0.054	0.83	0.0030	0.68	0.17	2.2
		Median	0.0070	0.0050	0.024	0.038	2.3	0.0030	3.6	0.14	6.8
		Minimum	0.0030	0.0020	0.010	0.010	1.0	0.0010	2.7	0.017	4.7
		Maximum	0.017	0.016	0.051	0.20	3.3	0.010	5.4	0.45	12
Plantain*	32	Geometric Mean	0.012 ^b	0.0010 ^b	0.0054 ^b	0.025 ^b	3.4 ^b	0.0010 ^a	3.6 ^b	0.0070 ^b	7.3 ^b
		SD	0.0060	0.0010	0.0030	0.014	1.0	0.0010	0.33	0.0060	1.9
		Median	0.011	0.0010	0.0050	0.023	3.4	0.0010	3.7	0.0050	6.6
		Minimum	0.0060	nd	0.0017	0.0090	1.9	0.0010	2.9	0.0022	4.8
		Maximum	0.026	0.0020	0.011	0.056	5.1	0.0045	4.1	0.020	10
anova p value			< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	≤ 0.05	< 0.01	< 0.01	< 0.01

nd: not detected; Different letters (a and b) between samples indicates significant difference (Tukey's test; $p < 0.05$)

[#]: Data for heavy metals in agricultural soils could be obtained from [Bortey-Sam et al. \(2015e\)](#).



a



b

Fig. 2 Distribution patterns of metals in foodstuffs characterized by PCA (C: Cassava; P: Plantain)

Similarly, GM concentrations (mg/kg ww) of metals in plantain decreased in the order, Zn (7.3) > Ni (3.6) > Cu (3.4) > Cr (0.025) > As (0.012) > Pb (0.0070) > Co (0.0054) > Cd (0.0010) and Hg (0.0010) ([Table 1](#)).

As shown in [Fig. 2](#), As and Cu were highly distributed in plantain while Cd, Co, Cr, Hg, Pb, Ni and Zn were distributed in cassava. This trend in metal distribution could be due to the fact that cassava is a root tuber, and accumulation of metals in the edible part from contaminated soil was highly possible due to the mining operations within the study area. However, the higher distribution of As in plantain could be due to atmospheric deposition. Processing of the ore involves roasting, which results in the production of arsenic trioxide gas which is distributed throughout the study area by the wind ([Obiri, 2007](#)).

Of the cassava samples collected, 30% contained higher concentration of Pb (0.37–0.45 mg/kg ww) when compared to the Codex Alimentarius Commission (CAC) standard values (0.3 mg/kg). However, concentrations of As, Cd, Cr and Hg in all samples were below the CAC ([FAO/WHO, 2001](#)) standard values (0.05, 0.2, 0.5 and 0.01 mg/kg, respectively). Regarding regional distribution, cassava samples with high concentrations of Pb were mainly from Techiman, Wangarakrom, Samahu and Tebe ([Table 3](#)). Among common pollutants that affect plants, Pb is one of the most toxic and frequently encountered ([Grover et al., 2010](#)). Pb continues to be widely used in many industrial processes and occurs as a contaminant in all environmental compartments (soils, water, the atmosphere, and living organisms). Environmental Pb contamination results both from its persistence and from its present and past numerous sources. These sources may include smelting, combustion of leaded gasoline, or fertilizers application ([Grover et al., 2010; Islam et al., 2008; Sammut et al., 2010](#)).

Previous study on the levels of toxic metals in *Xanthosoma sagittifolium* (cocoyam) and *Colocasia esculenta* (water cocoyam), in Tarkwa showed higher concentrations than in this study. From the results of their study, the levels of As, Cd and Hg in cocoyam and water cocoyam were higher than those of the WHO recommended levels ([Essumang et al., 2007](#)). These root tubers absorb toxic chemicals from soil as a result of the mining operations

([Essumang et al., 2007](#)). Similarly, [Hayford et al. \(2008\)](#) reported that, concentrations of heavy metals in cassava and plantain were higher than values proposed by the FAO, Expert Committee on Food Additives of the WHO and ATSDR in mining communities around the Tarkwa–Prestea area. The differences in concentrations between this study and the study by [Hayford et al. \(2008\)](#) could be due to fact that the uptake and allocation of metals in plants is influenced by life history traits and climate, stage, age, nutritional status, disease and rhizosphere interactions with micro-organisms ([Kribek et al., 2014](#)). Another possible reason could be due to differences in sample points.

Bioaccumulation of heavy metals from soil to foodstuffs

Bioconcentration of heavy metals and metalloids from soil to plants depend on soil characteristics, metal chemistry and also on the plant species. BCF values of the metals studied in different foodstuffs were calculated (on dry weight basis) and data are provided in [Table 2](#). In all the foodstuffs tested, the mean BCF of Ni (cassava: 2.0 ± 0.87 and plantain: 2.2 ± 0.96) was highest and that of Cr was lowest (cassava: 0.0035 ± 0.0036 and plantain: 0.0021 ± 0.0011). As shown in [Table 1](#), concentrations of Ni in soil and foodstuff differed considerably ($p < 0.05$) and levels in foodstuffs were significantly higher compared to those in soil ($p < 0.05$). The average BCF of metals and metalloid in the different foodstuffs are shown in [Table 2](#).

Since a greater BCF value indicates a higher transfer potential of metals in vegetables/foodstuffs ([Cui et al., 2004](#)), the above results indicated that Ni had higher capacity of absorption into food crops from soil than the other heavy metals studied. The study further showed that there was no significant correlation between SOM, soil pH and BCF values of heavy metals in the foodstuffs ($p > 0.05$). Thus soil pH and SOM had minor or no significant effect on the accumulation of metals in foodstuffs. This study is consistent with other study ([Dayton et al., 2006](#)) which indicated that pH had no significant effect on the accumulation of metals (Pb) from soil to vegetables.

Potential human health risk associated with consumption of foodstuffs

The potential human health risk of As, Cd, Hg and Pb to local residents associated with cassava and plantain consumption was assessed using THQ index and data are shown in [Table 3](#). For both children and adults, the THQs of Pb in cassava in Techiman, Wangarakrom, Samahu and Tebe (only children) were greater than 1.0 ([Table 3](#)). This meant that residents could be exposed to significant health risks associated with cassava consumption. Children were especially vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consumed more (twice of the amount) of food per unit of body weight compared to adults ([ENHIS, 2007](#)). On the other hand, the THQs of As, Cd, Hg and Pb in plantain were less than 1. The high THQs found in cassava, and especially Pb, could be due to the greater consumption rate, because human health risk assessment does not only depend on the toxic metal concentration in food, but also on the consumption rate ([Wongsasuluk et al., 2014](#)).

The people of Ghana including the residents living in the study area of this report, generally had high requirement for cassava. The daily intakes of cassava and plantain were at an average of 601 g/day and 369 g/day, respectively ([FAOSTAT, 2014](#)) and prolonged consumption of these toxic metals could lead to accumulation and cause toxicity, and therefore there was a clear need for proper treatment before consumption. Furthermore, continuous monitoring of As, Cd, Hg and Pb residues (in foodstuffs, vegetables, fruits, etc.) in the vicinities of gold mines in Tarkwa is recommended in the interest of consumers.

Table 2: Average soil pH, organic matter, water contents and bioconcentration factors (BCF) of heavy metals and metalloid from agricultural soils to foodstuffs in Tarkwa

	Sample site	n	Soil pH	SOM	WC	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
C	Mile 7	3	7.3	1.8	0.91	0.0058	1.1	0.019	0.004	0.64	0.067	3.2	0.13	0.70
	Mile 8	3	7.1	1.8	0.88	0.0043	0.25	0.035	0.001	0.49	0.021	2.3	0.020	0.34
	Techiman	3	6.3	1.8	0.66	0.0034	0.24	0.005	0.001	0.19	0.015	1.1	0.16	0.15
	Wangarakrom	3	6.9	2.8	0.66	0.0031	0.57	0.018	0.013	0.68	0.002	1.1	0.15	0.45
	Badukrom	3	6.9	2.0	2.5	0.00034	1.2	0.11	0.001	0.55	0.001	2.8	0.055	0.22
	Samahu	3	7.1	2.4	2.1	0.0016	0.24	0.041	0.002	0.45	0.020	1.8	0.049	0.34
	Abekuase	3	7.0	2.5	2.1	0.0048	0.14	0.009	0.007	0.87	0.15	2.9	0.013	0.22
	Tebe	3	6.9	2.9	1.9	0.0038	0.39	0.013	0.003	0.41	0.070	1.6	0.073	0.55
	Huniso	3	7.3	1.5	0.99	0.010	0.20	0.031	0.004	0.36	0.006	2.8	0.001	0.073
	Pepesa	3	7.3	1.9	1.9	0.0011	0.041	0.036	0.002	0.38	0.049	1.8	0.004	0.083
	Teberebe	3	7.6	2.5	1.1	0.0036	0.11	0.0035	0.001	0.11	0.023	0.59	0.003	0.14
	Average	7.1	2.2	1.4	0.004	0.40	0.029	0.0035	0.47	0.039	2.0	0.060	0.29	
	SD	0.34	0.46	0.67	0.003	0.39	0.030	0.0036	0.22	0.045	0.87	0.061	0.20	
	Minimum	6.3	1.5	0.66	0.00034	0.041	0.0035	0.0010	0.11	0.0014	0.60	0.0012	0.07	
	Maximum	7.6	2.9	2.5	0.010	1.2	0.11	0.013	0.87	0.15	3.2	0.16	0.70	
P	Mile 7	3	7.3	1.8	0.91	0.0085	0.12	0.0062	0.0024	1.3	0.033	3.7	0.0052	1.1
	Mile 9	3	5.8	2.1	0.50	0.0030	0.011	0.0043	0.00057	0.91	0.0061	2.3	0.0011	0.49
	Mile 10	3	7.2	1.3	0.44	0.0093	0.024	0.0068	0.00073	1.5	0.023	3.8	0.0012	0.21
	Techiman	3	6.3	1.8	0.66	0.0075	0.031	0.0044	0.0023	0.93	0.0053	1.2	0.0018	0.32
	Wangarakrom	4	6.9	2.8	0.66	0.0026	0.070	0.0024	0.0022	0.99	0.0023	1.1	0.0060	0.35
	Badukrom	3	6.9	2.0	2.5	0.00093	0.011	0.015	0.0014	0.39	0.00041	2.8	0.00096	0.17
	Abekuase	3	7.0	2.5	2.1	0.0035	0.024	0.0060	0.0031	0.89	0.018	1.9	0.0019	0.21
	Tebe	3	6.9	2.9	1.9	0.0092	0.13	0.0024	0.0044	0.84	0.014	1.6	0.0048	0.46
	Huniso	3	7.3	1.5	0.99	0.0077	0.005	0.015	0.0020	0.73	0.0054	2.5	0.00031	0.074

Pepesa	4	7.3	1.9	1.9	0.0033	0.030	0.0020	0.0012	0.28	0.0040	1.5	0.00053	0.082
Average		6.9	2.1	1.3	0.0056	0.045	0.0065	0.0021	0.88	0.011	2.2	0.0024	0.35
SD		0.49	0.53	0.76	0.0032	0.044	0.005	0.0011	0.37	0.011	0.96	0.0021	0.29
Minimum		5.8	1.3	0.44	0.00093	0.0051	0.0020	0.00057	0.28	0.00041	1.1	0.00031	0.07
Maximum		7.3	2.9	2.5	0.0093	0.13	0.015	0.0044	1.4	0.033	3.8	0.0060	1.1

C: Cassava; P: Plantain; *n*: number of samples collected; SOM: Soil organic matter (%); WC: Soil water content (%)

Table 3: Target hazard quotients of toxic metals via foodstuff consumption in Tarkwa

		Adult				Child			
	Sample site	As	Cd	Hg	Pb	As	Cd	Hg	Pb
C	Mile 7	0.21	0.13	0.07	0.60	0.28	0.17	0.090	0.81
	Mile 8	0.19	0.05	0.13	0.13	0.26	0.07	0.18	0.18
	Techiman	0.12	0.05	0.09	1.3	0.16	0.07	0.12	1.7
	Wangarakrom	0.57	0.12	0.11	1.1	0.77	0.17	0.14	1.4
	Badukrom	0.15	0.16	0.12	0.39	0.20	0.22	0.16	0.52
	Samahu	0.25	0.06	0.07	1.2	0.34	0.08	0.10	1.6
	Abekuase	0.52	0.03	0.25	0.14	0.71	0.05	0.34	0.19
	Tebe	0.37	0.04	0.12	0.86	0.50	0.05	0.16	1.2
	Huniso	0.50	0.10	0.03	0.05	0.67	0.14	0.036	0.064
	Pepesa	0.19	0.02	0.32	0.06	0.26	0.02	0.44	0.079
P	Teberebe	0.33	0.04	0.05	0.05	0.44	0.06	0.07	0.066
	Mile 7	0.19	0.0086	0.021	0.014	0.21	0.0095	0.023	0.015
	Mile 9	0.11	0.00082	0.016	0.0039	0.13	0.00090	0.018	0.0043
	Mile 10	0.18	0.0029	0.014	0.0045	0.20	0.0032	0.016	0.0049
	Techiman	0.16	0.0039	0.018	0.0091	0.18	0.0042	0.020	0.010
	Wangarakrom	0.29	0.0093	0.092	0.027	0.32	0.010	0.10	0.029
	Badukrom	0.25	0.00091	0.021	0.0042	0.27	0.0010	0.022	0.0045
	Abekuase	0.23	0.0036	0.018	0.013	0.26	0.0039	0.020	0.014
	Tebe	0.54	0.0080	0.014	0.035	0.60	0.0087	0.015	0.038
	Huniso	0.24	0.0016	0.014	0.0074	0.26	0.0018	0.016	0.0082
P	Pepesa	0.33	0.0076	0.016	0.0052	0.36	0.0084	0.018	0.0057

C: Cassava; P: Plantain; THQ: Target hazard quotient; Bold indicates high THQ

Conclusions

The GM concentration of heavy metals and metalloid in foodstuffs collected from communities in Tarkwa decreased in the order: Zn > Ni > Cu > Pb > Cr > Co > As > Cd and Hg (cassava) and, Zn > Ni > Cu > Cr > As > Pb > Co > Cd and Hg (plantain). The transfers of these heavy metals and a metalloid from soil to the foodstuffs were assessed and bioconcentration factor indicated that Ni had higher capacity of absorption into food crops from soil than the other metals studied in Tarkwa. THQ of Pb showed that there could be potential health risk to residents (Techiman, Wangarakrom, Samahu and Tebe) through consumption of cassava. Children were especially vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consumed more (twice of the amount) of food per unit of body weight compared to adults ([ENHIS, 2007](#)).

Chapter 4

**Human health risks from metals and a metalloid via
consumption of food animals near gold mines in Tarkwa,
Ghana: Estimation of the daily intakes and target hazard
quotients (THQs)**

Introduction

Heavy metals and a metalloid (arsenic) are ubiquitous in the environment either naturally or anthropogenically, and their concentrations were elevated promptly through waste disposal, smelter stacks, atmospheric deposition, mining, fertilizer and pesticide use, and the application of sewage sludge in arable land (Cui et al., 2005). Mining and processing metal ore can be a significant source of metal and metalloid contamination in the environment (Navarro et al., 2008; Singh et al., 2005). The increased occurrence of metal and metalloid pollution in the environment has been associated with anthropogenic activities as effluents and emissions from mines and smelters often contain elevated concentrations of toxic metals including arsenic, cadmium, mercury and lead (Tubaro and Hungerford, 2007).

Food consumption has been identified as the major pathway of human exposure, accounting for >90% compared to other ways of exposure such as inhalation and dermal contact (Loutfy et al., 2006). In a review by Yabe et al. (2010), contamination of food animals, fish, soil, water, and vegetables with heavy metals has reached unprecedented levels over the past decade in some parts of Africa. As a result, human exposure to toxic metals has become a major health risk.

Chronic intake of heavy metals above their safe threshold in humans and animals have damaging effects and can cause non–carcinogenic hazards such as neurologic involvement, headache and liver diseases (John and Andrew, 2011; Lai et al., 2010; Zheng et al., 2007). Dietary cadmium intake due to consumption of environmentally contaminated rice and other foods was associated with an increased risk of postmenopausal breast cancer (Itoh et al., 2014). Acute and chronic exposure to arsenic could also cause numerous human health problems. These include dermal, respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, neurological, developmental, reproductive, immunological, genotoxic, mutagenic and carcinogenic effects such as liver cancer (Kapaj et al., 2006; Lin et al., 2013). Excess Pb is known to reduce the cognitive development and intellectual performance in children and to increase blood pressure and cardiovascular disease incidence in adults (EC,

2006). Hg is a neurotoxic poison that causes neurobehavioral effects, especially on psychomotor coordination, neuroendocrine, renal damage and gastrointestinal toxicity (SAC/MOHC, 2005).

The extraction and processing of gold in Tarkwa, has given rise to various environmental related diseases and accidents. The objectives of this study were therefore: to determine the concentrations of heavy metals and a metalloid in offal and muscle of free-range chicken, goat and sheep; to reveal the species differences in accumulation features of metals among free-range chicken, goat and sheep and; to estimate the daily intake (EDI) and target hazard quotient (THQ) (toxic metals) through consumption of these food animals in Tarkwa.

Materials and methods

Sampling and pre-treatment

All procedures used in this experiment were according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee and the local veterinarian policy in the study area. In June 2012, liver, kidney and muscle samples were collected at slaughterhouses from both male and female local goat and sheep ($n = 10$ species each) aged three years and above, and stored in labeled plastic bags in cooler boxes. 10 free-range chickens were also bought from townships surrounding the two major gold mines. All the townships were close to the mine, and some households were within 1 km of the mine. The live adult chickens were transported to the laboratory for dissection after exsanguination. Liver, kidney, muscle and gizzard samples were collected. Samples were transported to the laboratory and kept frozen at the Chemistry Department of the Kwame Nkrumah University of Science and Technology, KNUST, Ghana before being transported and analyzed for metal concentrations at the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

Analysis of total Mercury (Hg)

The concentration of total mercury (Hg) was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury analyzer, MA-3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of calibration standards. Recovery rates of Hg for the certified reference material, DOLT-4 (Dogfish liver, the National Research Council, Canada) ranged from 92% to 103% ($94.3 \pm 4.2\%$). The detection limit (Hg) was 2.0 pg of total Hg.

Sample preparation and metal analysis

Approximately 0.5 g of individual samples were dried in an oven at 40⁰C and digested in a closed microwave extraction system, Speed Wave MWS–2 microwave digestion (Berghof, Germany). Briefly, the dried samples were placed in prewashed digestion vessels followed by acid digestion using 5 mL of (65%) nitric acid, HNO₃ (Kanto Chemical Corp., Tokyo, Japan) and 1 mL of (30%) hydrogen peroxide, H₂O₂ (Kanto Chemical Corp., Tokyo, Japan). The digestion vessels were capped and placed into a 10-position turntable conditions followed by a ramped temperature programme: ramp to 160⁰C (5 min hold); and increase to 190⁰C (15 min hold). After cooling, samples were transferred into plastic tubes and diluted to a final volume of 10 mL with milli Q water. A reagent blank was prepared using the same procedure. An Inductively Coupled Plasma–Mass Spectrometer (ICP–MS; 7700 series, Agilent technologies, Tokyo, Japan) was used for quantification. The instrument was calibrated using standard solutions of the respective metals (to establish standard curves before metal analysis). All chemicals and standard stock solutions were of analytical–reagent grade (Wako Pure Chemicals, Osaka, Japan). The detection limits ($\mu\text{g/L}$) of chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) were 0.003, 0.025, 0.154, 0.0004, 0.024, 0.007, 0.226, 0.002, 0.001 and 0.001, respectively. Concentrations of metals were expressed in mg/kg wet weight (mg/kg ww).

Quality assurance and quality control

For heavy metals, replicate blanks and reference materials, DORM–3 (Fish protein, the National Research Council, Canada) and DOLT–4 were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy (relative standard deviation, RSD, $\leq 3\%$) and recovery rates ranged from 80% to 115%.

Data analysis

Estimated daily intake (EDI) of toxic metals

The estimated daily intake (EDI) of toxic metals (As, Cd, Hg, Pb and Mn) was dependent on both the metal concentration in offal or muscle and the amount of consumption of the respective food. The EDI of metals was determined by the following equation:

$$EDI = \frac{MC \times FDC}{BW}$$

where MC is average concentration of metal in food ($\mu\text{g/g}$, on fresh weight basis); FDC represents the average food daily consumption of offal and muscle in this region (g/person/d); BW is the average body weight. It was assumed that the local inhabitants consumed an average liver, muscle and gizzard of 150 g/day and 100 g/day for adult (60 kg in BW) and children (30 kg in BW) respectively. For kidney, it was assumed that inhabitants consumed 10 g/day and 8 g/day for adults and children respectively ([Ihedioha and Okoye, 2013](#)), because the size of kidneys are generally smaller and is not the favorite compared with liver, muscle, or gizzard. The metal intakes were compared with the tolerable daily intakes for metals recommended by the [WHO \(2000\)](#), [FSA \(2006\)](#), and [FAO/WHO \(2010\)](#).

Target Hazard Quotient (THQ)

The health risks from consumption of offal and muscle of chicken, goat and sheep by the local inhabitants were assessed based on the THQ. The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects. The method of estimating risk using THQ was provided in the U.S. EPA Region III risk-based concentration table ([USEPA IRIS, 2007](#)) and in [Wang et al., \(2005\)](#), and is based on the equation below:

$$THQ = \frac{EFr \times ED \times FIR \times MC}{RfD \times BW \times AT} \times 10^{-3}$$

where THQ is target hazard quotient; EFr is exposure frequency (365 days/year); ED is exposure duration (70 years); FIR is food ingestion rate (g/person/d); MC is average concentration of metal in food ($\mu\text{g/g}$, on fresh weight basis); RfD is the oral reference dose (mg/kg/d); BW is the average body weight, adult (60 kg); children (30 kg); AT is the average exposure time (365 days/year \times number of exposure years, assuming 70 years in this study). Oral reference doses were based on 3E-04, 1E-03, 5E-04, 4E-03, 0.14 mg/kg/d for As, Cd, Hg, Pb and Mn, respectively ([USEPA IRIS, 2007](#)).

Statistical analysis

Statistical analyses were performed using IBM SPSS 20.0 statistical software (SPSS Inc., Illinois, USA) after data were normalized by log transformation. ANOVA and Tukey analyses between organs of the same species and between organs of different species were used to compare metal concentrations in the liver, kidney, muscle and gizzard and differences were considered statistically significant with p value < 0.05 . Principal component analysis (PCA) based on log transformed data was done, to determine the distribution pattern of metals in organs, using JMP statistical software v. 10 (SAS Institute). The principal components were extracted with eigenvalues > 1 .

Results and discussion

Levels of heavy metals and a metalloid

Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, and Pb in the liver, kidney muscle and gizzard of free-range chickens, as well as metal and metalloid levels in liver, kidney and muscle of goat and sheep from Tarkwa were determined ([Table 1](#)). [Table 1](#) shows that the mean Cd concentrations were in the range of nd–0.73 mg/kg ww in all samples while the mean concentrations of Pb were in the range of nd–0.39 mg/kg ww; Cr, 0.01–0.24; Mn, 0.146–3.57; Fe, 6.62–542; Co, nd–0.11; Ni, 0.01–0.55; Cu, 0.348–105; Zn, 5.12–65.8; As, nd–0.14 and Hg, nd–0.12 mg/kg ww. Cu and Zn were detected in 100% of all offal and muscle samples for all food animals studied. All other metals were detected in varying percentages in the samples studied.

Ni can cause respiratory problems and is a carcinogen. The permissible limit of Ni in food according to [WHO \(2000\)](#) standard is 0.5 mg/kg. In this study concentrations of Ni ranged between 0.01–0.55 mg/kg ww, in chicken liver and muscle, respectively. As shown in [Table 1](#), the highest mean concentration of Ni was found in the local chickens while Zn levels in goat muscle exceeded the permissible limit of 50 mg/kg in meat ([USDA, 2006; EC, 2006](#)).

Arsenic

As was detectable in 100%, 83%, 80% and 52% of all gizzard, liver, kidney and muscle samples, respectively. Concentrations of As in muscle and offal samples of free-range chicken, goat and sheep ranged from nd to 0.14 ± 0.11 mg/kg ww in goat muscle and chicken kidney ([Table 1](#)). The distribution of As in liver, kidney and muscle of chicken was found to be higher and differed statistically with that of goat and sheep ($p < 0.05$). However, there were no significant differences when As levels in goat offal and muscle were compared with sheep ([Table 1](#), $p > 0.05$). Mean concentration of As in the kidney (0.14 ± 0.11 mg/kg ww) and gizzard (0.08 ± 0.05 mg/kg ww) of free-range chicken from Tarkwa were found to be

higher than the [USDA \(2006\)](#); [EC \(2006\)](#) and [SAC/MOHC, 2005](#) standard of 0.05 mg/kg ww in food. The high levels of As in the free-range chicken may be due to the nature of the gold bearing ore which is mineralized pyrites and arsenopyrates. Processing of the ore involves roasting and these results in the production of arsenic trioxide gas which is distributed throughout the study area by air current. As is a toxic substance and due to its non-biodegradable nature, it could accumulate in surface soil and water. It could also enter the food chain through plant assimilation ([Amonoo-Neizer et al., 1995](#)). This could be the possible reason for the high As concentrations in free-range chickens from Tarkwa as they continuously pick food particles and water from the ground.

Table 1: Mean (\pm SD) and ranges of metal concentrations (mg/kg ww) in various organs of free-range chicken (C), goat (G) and sheep (S)

Sample		Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb
CG(<i>n</i> =10)	Mean \pm SD	0.06 \pm 0.08	0.64 \pm 0.46	81.4 \pm 87.2	nd	0.10 \pm 0.04	1.92 \pm 0.81	30.3 \pm 3.5	0.07 \pm 0.05	0.08 \pm 0.09	0.01 \pm 0.01	0.38 \pm 1.17
	Range	0.01-0.27	0.20-1.75	37.1-321.4	nd-0.01	0.06-0.19	0.74-3.19	24.3-34.9	0.02-0.16	nd-0.30	0.01-0.03	nd-3.70
CK(<i>n</i> =10)	Mean \pm SD	0.24 \pm 0.40	2.44 \pm 0.55 ^a	94.4 \pm 33.2	0.03 \pm 0.01	0.07 \pm 0.14	2.82 \pm 0.37	28.4 \pm 3.1	0.14 \pm 0.11 ^a	0.72 \pm 0.81 ^a	0.12 \pm 0.08 ^a	0.25 \pm 0.31 ^a
	Range	0.01-1.35	1.65-3.59	59.7-150.5	0.01-0.07	nd-0.46	2.01-3.38	25.2-35.6	0.02-0.37	0.16-2.26	0.02-0.28	0.02-1.05
GK(<i>n</i> =10)	Mean \pm SD	0.01 \pm 0.00	0.77 \pm 0.25 ^b	88.8 \pm 72.8	0.07 \pm 0.03	0.14 \pm 0.07	2.89 \pm 0.46	20.0 \pm 3.9	0.01 \pm 0.01 ^b	0.06 \pm 0.04 ^b	0.03 \pm 0.02 ^b	0.03 \pm 0.04 ^b
	Range	nd-0.02	0.50-1.35	39.2-278.5	0.01-0.12	0.05-0.27	2.42-3.76	15.0-27.5	nd-0.04	0.01-0.13	0.01-0.07	0.01-0.14
SK(<i>n</i> =10)	Mean \pm SD	0.01 \pm 0.00	1.14 \pm 0.27 ^b	49.1 \pm 15.4	0.03 \pm 0.02	0.09 \pm 0.04	3.170 \pm 0.45	22.2 \pm 4.1	0.01 \pm 0.01 ^b	0.15 \pm 0.15 ^b	0.07 \pm 0.08 ^{ab}	0.03 \pm 0.01 ^b
	Range	nd-0.01	0.87-1.70	24.8-80.6	0.01-0.06	0.04-0.15	2.58-3.90	18.1-28.1	nd-0.03	0.01-0.43	nd-0.23	0.01-0.06
CL(<i>n</i> =10)	Mean \pm SD	0.02 \pm 0.01	3.50 \pm 0.92 ^a	542.3 \pm 604.0	0.03 \pm 0.01	0.01 \pm 0.02	3.67 \pm 0.67	65.8 \pm 50.6	0.07 \pm 0.07 ^a	0.22 \pm 0.18 ^a	0.11 \pm 0.07 ^a	0.13 \pm 0.14 ^a
	Range	nd -0.04	1.57-4.84	72.0-1629.3	0.02-0.05	nd-0.06	2.72-4.74	32.5-182.2	0.01-0.21	0.05-0.56	0.05-0.25	0.01-0.41
GL(<i>n</i> =10)	Mean \pm SD	0.02 \pm 0.01	2.42 \pm 0.71 ^b	89.9 \pm 47.7	0.11 \pm 0.05	0.20 \pm 0.05	60.3 \pm 44.7	47.1 \pm 26.6	nd ^b	0.02 \pm 0.01 ^b	0.01 \pm 0.01 ^b	0.02 \pm 0.04 ^b
	Range	0.01-0.04	1.06-3.41	44.3-212.6	0.04-0.21	0.15-0.29	3.3-140.7	21.9-103.3	nd-0.01	0.01-0.04	nd-0.05	0.01-0.13
SL(<i>n</i> =10)	Mean \pm SD	0.02 \pm 0.01	3.57 \pm 1.02 ^a	126.3 \pm 71.3	0.07 \pm 0.03	0.33 \pm 0.13	105.8 \pm 74.3	43.6 \pm 9.6	0.01 \pm 0.01 ^b	0.05 \pm 0.02 ^b	0.03 \pm 0.03 ^b	0.06 \pm 0.03 ^{ab}
	Range	0.01-0.04	1.50-4.92	55.4-294.1	0.04-0.12	0.22-0.68	7.9-224.3	31.7-63.3	0.01-0.05	0.02-0.09	nd-0.09	0.02-0.11
CM(<i>n</i> =10)	Mean \pm SD	0.05 \pm 0.01	0.14 \pm 0.08 ^a	6.6 \pm 1.6	nd	0.55 \pm 0.30	0.34 \pm 0.03	5.1 \pm 1.7	0.04 \pm 0.03 ^a	nd ^a	0.01 \pm 0.01 ^a	0.01 \pm 0.03 ^a
	Range	0.02-0.09	0.09-0.38	4.7-9.5	nd	0.24-1.29	0.28-0.41	3.8-10.0	nd-0.13	nd	0.01-0.02	nd-0.10
GM(<i>n</i> =10)	Mean \pm SD	0.05 \pm 0.08	0.6 \pm 0.81 ^a	31.7 \pm 17.0	0.02 \pm 0.05	0.14 \pm 0.05	1.50 \pm 0.76	51.3 \pm 17.6	nd ^b	nd ^a	nd ^b	nd ^a
	Range	0.01-0.28	0.21-2.83	19.2-76.8	nd-0.15	0.08-0.23	0.91-3.38	17.0-78.2	nd-0.01	nd-0.02	nd	nd-0.01
SM(<i>n</i> =10)	Mean \pm SD	0.03 \pm 0.03	0.71 \pm 0.55 ^a	37.8 \pm 10.9	0.01 \pm 0.00	0.46 \pm 0.16	2.09 \pm 1.19	45.9 \pm 20.8	0.01 \pm 0.01 ^b	0.01 \pm 0.01 ^a	nd ^b	0.01 \pm 0.00 ^a
	Range	0.01-0.09	0.16-2.02	22.7-58.9	nd-0.01	0.15-0.66	0.84-4.85	27.0-83.4	nd-0.02	nd-0.03	nd-0.02	nd-0.01

n: number of samples; CG: chicken gizzard; CK: chicken kidney; GK: goat kidney; SK: sheep kidney; CL: chicken liver; GL: goat liver; SL: sheep liver; CM: chicken muscle; GM: goat muscle; SM: sheep muscle. Different letters (a and b) between columns in the same organ indicates statistical difference (Tukey's test; $p < 0.05$). nd: not detected.

Cadmium

Mean level of Cd in chicken kidney (0.73 ± 0.81 mg/kg ww) was within the recommended residual concentrations of 0.5 to 1.0 mg/kg ww in offal for human consumption (EC, 2006). Cd was detectable in 14% of all muscle, 100% of all liver and kidney, 90% of chicken gizzard. The concentrations of Cd in livers and kidneys of chicken were significantly higher compared to those of goat and sheep (Tukey test; $p < 0.05$). The higher frequencies of Cd in liver and kidney (100%) are likely due to their special functions; liver as storage organ and kidney as excretory organ (Jarup and Akesson, 2009; Wang et al., 2013).

There have also been suggestions that animals exposed to Cd accumulate it in their kidneys because of the presence of free protein–thiol groups which leads to a strong fixation of heavy metals (Pompe–Gotal and Crnic, 2002). The likely unidentified sources of Cd which the animals could come in contact with include municipal waste, electroplating, plastic and paint waste. Other sources include leachates from nickel–cadmium based batteries and cadmium plated items which are so carelessly discarded by battery chargers and users in Ghana. Recently, electronic wastes are disposed and often burnt at refuse dumps.

Mercury

Hg was detectable in 38% of all muscle, 69% and 97% of all liver and kidney, respectively, and 100% of chicken gizzard. The levels of Hg in free–range chicken from Tarkwa were higher compared to that of goat and sheep. The result of this study indicated that chicken kidney (0.12 ± 0.08 mg/kg ww) and liver (0.11 ± 0.07 mg/kg ww) contained the highest concentrations of Hg followed by sheep kidney (0.07 ± 0.07 mg/kg ww). The concentrations of Hg in liver and muscle of free–range chicken from Tarkwa were found to be higher and differed statistically with that of goat and sheep (Tukey test; $p < 0.05$). However, there were no significant differences when concentrations of Hg in goat offal and muscle were compared with sheep (Tukey test; $p > 0.05$). The lowest and highest mean concentrations of Hg in

chicken were 0.01 ± 0.01 mg/kg ww in muscle and 0.12 ± 0.08 mg/kg ww in kidney, respectively; goat (nd– 0.03 ± 0.02 in muscle and kidney); sheep (nd– 0.07 ± 0.07 in the muscle and kidney) (Table 1).

The EC (2006); and USDA (2006) permissible limit for Hg is 0.05 mg/kg and the Standardization Administration of China/Ministry of Health of China established maximum limits of Hg in food to be 0.01 to 0.05 mg/kg (SAC/MOHC, 2005). The high levels of Hg especially in free-range chicken could be problematic, as concentration levels exceeded the maximum values permitted in food. In Ghana, amalgamation is the preferred gold recovery method employed by almost all artisanal gold miners because it is a very simple, inexpensive and an easier to use technique. These activities by local and small scale miners are widespread, and Hg is introduced into the environment (via amalgamation process popularly known as "galamsey") (Amonoo-Neizer et al., 1995). The high levels of Hg in offal of free-range chicken could therefore be due to contamination from soil, feed or water.

Lead

Pb was detectable in all samples of liver, kidney, 80% of chicken gizzard and 38% of muscle. The result of this study indicated that chicken gizzard (0.39 ± 1.17 mg/kg ww) contained the highest concentrations of Pb followed by chicken kidney (0.26 ± 0.31 mg/kg ww) and liver (0.13 ± 0.14 mg/kg ww). Tukey's test was used to compare the levels of Pb in the three food animals, and results showed no statistical significance in liver and muscle when chicken was compared with goat and sheep ($p > 0.05$). The levels of Pb in organs of free-range chickens may emanate mainly from contamination of soil, feeds and/ or water sources. This is because, to be toxic, Pb must be in a form which will be retained in the gizzard, thus resulting in continuous absorption over a prolonged period (Salisbury et al., 1958). In another study by Kendall et al. (1996), analyses of field and laboratory data indicated that two scenarios can be envisioned when Pb is ingested: (1) the ingested grit/pellets containing Pb might be eliminated (via regurgitation or passage through the

gastrointestinal tract) before any significant dissolution or absorption of Pb occurs; (2) the grit/pellets may be partially or totally dissolved in the gizzard, in which case causes Pb accumulation resulting in the occurrence of a range of toxicologic effects.

In this study, concentration of Pb in some organs of chicken exceeded the [EC \(2006\)](#); and [USDA \(2006\)](#) standard of 0.5 and 0.1 mg/kg ww for Pb in offal and muscle respectively ([Table 1](#)). Generally, the low levels of Pb in the offals and muscles of the mentioned food animals could be due to the fact that, Pb accumulates in bone and the metal concentration increases with age ([Caggiano et al., 2005](#); [Rubio et al., 1998](#)).

Manganese

The role of Mn in neuropsychiatric disorders is also documented ([Bowler et al., 2006](#); [Crossgrove and Zheng, 2004](#); [Klos et al., 2006](#); [Saha and Zaman, 2012](#)). Mn was detectable in 100% of all offal and muscle samples. This study showed that mean concentrations of Mn ranged from 0.15–3.51 (chicken); 0.62–2.43 (goat) and 0.71–3.57 mg/kg ww (sheep) ([Table 1](#)). The concentration of Mn in kidney of free-range chicken was found to be higher and differed statistically with that of goat and sheep ($p < 0.05$). However, there was no significant difference when Mn levels in the muscle of the three food animals were compared ([Table 1](#), $p > 0.05$).

In the liver, the levels of Mn between free-range chickens and sheep were statistically insignificant with mean values of 3.50 ± 0.92 and 3.57 ± 1.02 mg/kg ww, respectively ([Table 1](#), $p > 0.05$). The mean concentrations of Mn in offal and muscles of all three food animals were above the [WHO \(1996\)](#) reference standard of 0.5 mg/kg except in chicken muscle ([Table 1](#)). The high Mn levels in these food animals could be due to the fact that the sampling site at Tarkwa was close to the Nsuta Mn mine (which is a famous site for Mn mining).

This study was compared with similar studies in different countries ([Table 2](#)). Results from this study were found to be: comparable with study by [Husain et al. \(1996\)](#), in goat and sheep livers and kidneys, in Kuwait; comparable (except Cu, Zn and Cd) and higher than similar studies by [Uluzlu et al. \(2009\)](#) and [Villar et al. \(2005\)](#) in the liver of poultry from Turkey and Philippines respectively. Cd and Pb levels from this study were lower than studies conducted by [Okoye and Ugwu \(2001\)](#), Nigeria; [Abou-Arab et al. \(2001\)](#), Egypt; and [Caggiano et al. \(2005\)](#), Italy; in the livers and kidneys of goat and sheep from industrial and urban areas.

Study done by [Yabe et al. \(2013\)](#) near a Pb and Zn mine in Kabwe, Zambia indicated that the levels of Cd and Pb in the livers and kidneys of free-range chickens were higher compared to this study but levels in muscle were comparable. On the other hand, the levels of Pb and Cd in chicken gizzard from this study were higher than the study by [Yabe et al. \(2013\)](#). From [Table 2](#), the mean concentrations of Zn in this study were comparable in all organs except for gizzard when compared to the study by [Yabe et al. \(2013\)](#).

Distribution of metals

ANOVA was used to compare the accumulation and distribution of metals in the organs of free-range chicken, goat and sheep. The metal distribution in offal and muscle of chicken was statistically significant ($p < 0.05$) except for Cr, As and Pb. There was also statistical significance ($p < 0.01$) in the distribution of Mn, Co, Cu, Zn and Cd when the organs of goat were compared. On the other hand metal distribution in organs of sheep was significant except for As ($p > 0.05$). The liver and kidneys are target tissues for monitoring metal contamination in animals because both organs function in removing toxic metals from the body ([Abou-Arab, 2001](#); [Husain et al., 1996](#)). Similarly, organs of all species in this study showed that the liver and kidney contained the highest levels of metals ([Table 1](#)).

Distribution of metals in offal and muscle samples in the food animals was analyzed using PCA. PCA results ([Fig. 1](#)) showed a clear separation between chicken (C), grouped on one side, and the ruminants, goat (G) and sheep (S), clustered on another side in both offal and muscle. Interestingly, As, Cd, Hg, Mn and Pb made one cluster or group in the liver and kidney of free-range chicken ([Fig. 1](#)). Chicken muscle also showed similar distribution pattern, with As, Hg and Pb clustered together ([Fig. 1](#)). This could be attributed to the difference in their feeding habits and/ or the different levels of metallothionein (MT) from the sampled food animals (species differences in hepatic MT content). Living organisms (including mammals and non-mammals), have evolved a suite of mechanisms that control and respond to the uptake and accumulation of both essential and nonessential heavy metals. These mechanisms include the chelation and sequestration of heavy metals by particular ligands.

One of the best-characterized heavy metal-binding ligands is the MTs ([Cobbett and Goldsborough, 2002](#)). MT is a low molecular weight protein in various tissues and is involved in the detoxification of metals ([Kagi and Vallee, 1960; Margoshes and Valle, 1957](#)). According to a study by [Bryan-Henry et al. \(1994\)](#), the hepatic MT levels in these three food animals studied decreased in order of livestock > non-mammals; i.e. goat > sheep > chicken.

Table 2: Published data of mean metal levels (mg/kg dw) in organs of different animal groups from different regions

Study/Country	Pollution Factor	Animal	Organ	Cr	Co	Ni	Cu	Zn	As	Cd	Hg	Pb
Yabe et al., 2013*, Zambia	mining	C (broiler)	L	0.06	0.05	0.03	56.80	28.60		0.00		0.06
		C(free-range)	L	0.07	0.09	0.07	3.40	30.90		1.60		4.15
			K	0.08	0.16	0.08	2.12	24.70		3.50		7.62
			M	0.08	0.01	0.02	nd	4.16		0.01		0.23
Uluozlua et al., 2009*, Turkey	markets		G	0.10	0.04	0.03	0.13	30.50		0.02		0.23
			L	0.04	0.02	0.01	12.10	22.50	0.06	2.24		0.12
Demirbas, 1999*, Turkey	control		L				2.95	28.10		0.04	0.08	0.09
Villar et al., 2005, Phillipines			L							0.03		0.08
Husain et al., 1996, Kuwait	wholesalers	G	L							0.05		0.13
			K							0.44		0.43
Okoye and Ugwu, 2010, Nigeria	Industrial & urban		L				134.02	120.44		0.35		0.65
Abou-Arab, 2001*, Egypt	Industrial & urban		L				46.6	32.6		0.26		0.46
			K				2.4	34.1		0.91		0.68
			M				1.2	41.4		0.04		0.08
Husain et al., 1996, Kuwait	wholesalers	S	L							0.04		0.13
			K							0.30		0.15
Caggiano et al., 2005, Italy	urban		L							0.33		1.50
			K							6.71		2.00
			M							0.16		1.60
Abou-Arab, 2001*, Egypt	Industrial & urban		L				48.6	36.8		0.26		0.42
			K				3	34.2		0.82		0.54

C: chicken; G: goat; S: sheep; L: liver; K: kidney; M: muscle; G: gizzard.* means mg/kg ww.

Dietary intake of metals and target hazard quotient

The estimated daily intakes (EDI) of toxic metals for adults in the vicinity of Tarkwa mine through consumption of offal and meat of free-range chicken, goat and sheep are presented in [Table 3](#). The highest EDIs of As ($0.19 \mu\text{g/kg bw/day}$), Pb ($0.96 \mu\text{g/kg bw/day}$), Cd ($0.55 \mu\text{g/kg bw/day}$) and Hg ($0.29 \mu\text{g/kg bw/day}$), through consumption were from the gizzard and liver of chicken ([Table 3](#)). Meanwhile, the highest EDI of Mn ($8.92 \mu\text{g/kg bw/day}$) was through consumption of sheep liver ([Table 3](#)). The calculated EDIs of As, Cd, Hg, Pb and Mn in offal and muscle samples of the 3 food animals from Tarkwa were low compared to the [WHO \(2000\)](#), [FSA \(2006\)](#) and [FAO/WHO \(2010\)](#) tolerable daily intakes ([Table 3](#)). Children are especially vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consume more (twice of the amount) of food per unit of body weight as adults ([ENHIS, 2007](#)). As a result intakes of these toxic metals through food could be higher for children from Tarkwa.

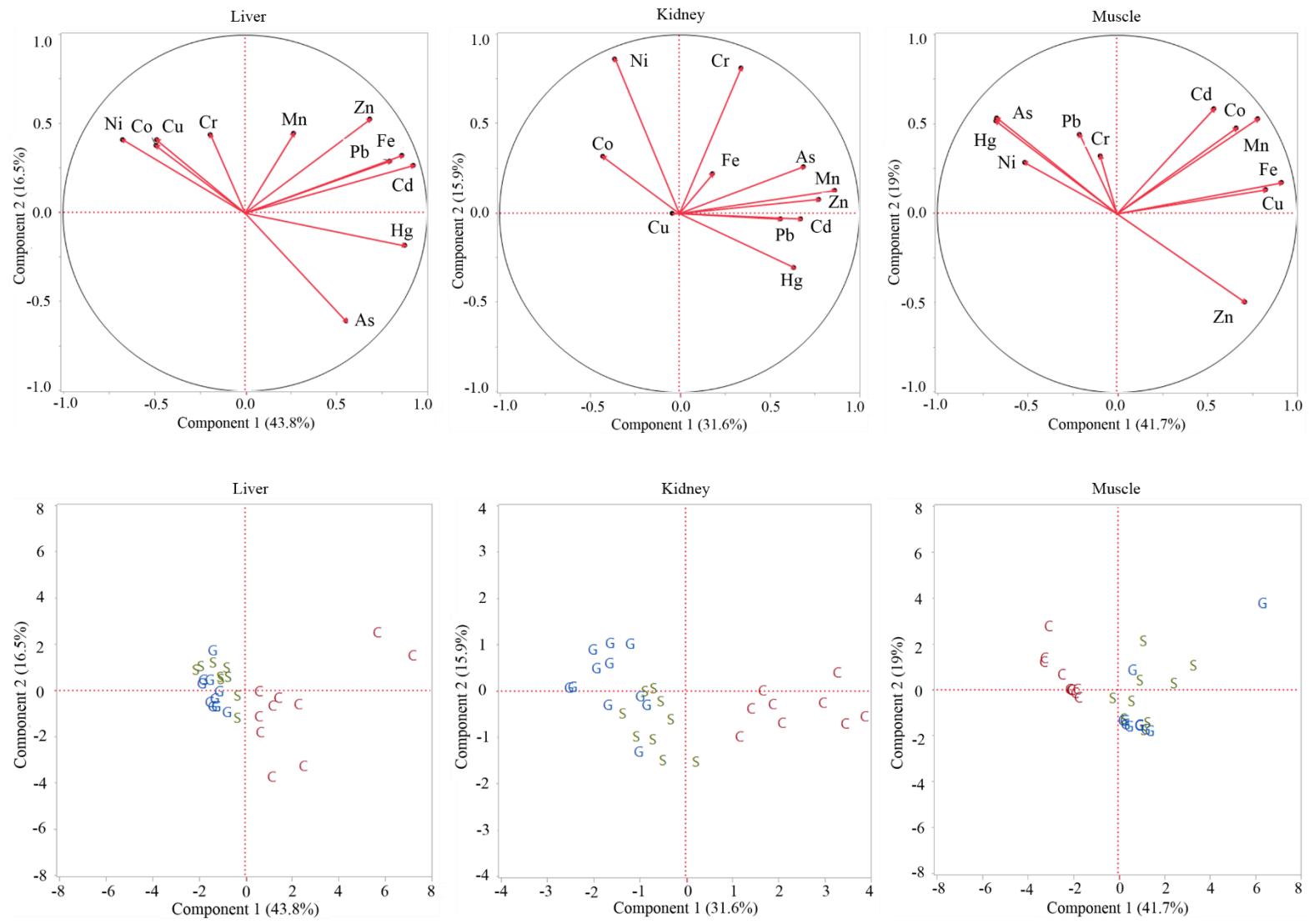


Fig. 1 Distribution patterns of metals in food animals characterized by PCA (C: chicken; G: goat and S: sheep).

Table 3: Estimated Daily Intake ($\mu\text{g}/\text{kg bw/day}$) of As, Cd, Hg, Pb and Mn

Sample name	As	Cd	Hg	Pb	Mn
Chicken Gizzard	0.19	0.21	0.03	0.96	1.61
Chicken Kidney	0.02	0.12	0.02	0.04	0.41
Chicken Liver	0.19	0.55	0.29	0.34	8.77
Chicken Muscle	0.12	0.003	0.03	0.03	0.37
Goat Kidney	0.002	0.01	0.005	0.01	0.13
Goat Liver	0.02	0.05	0.03	0.07	6.07
Goat Muscle	0.01	0.01	0.002	0.01	1.55
Sheep Kidney	0.002	0.03	0.01	0.01	0.19
Sheep Liver	0.04	0.13	0.07	0.14	8.92
Sheep Muscle	0.02	0.02	0.01	0.01	1.77
TDI by FAO/WHO/FSA	2.0	1.0	0.71	3.57	200

FAO: Food and Agricultural Organization; WHO: World Health Organization; FSA: Food Safety Agency.

The health risk from consumption of offal and muscle from the three food animals were assessed based on the THQ. THQ value less than 1 means the exposed population is unlikely to experience obvious adverse effects (USEPA, 2000). Although the calculated individual THQ were below 1.0 (Fig. 2), attention should be paid particularly to some high individual THQ values approaching 1.0 (As Cd, and Hg). The THQs of As in chicken gizzard and liver which are largely consumed were higher in children (0.85 and 0.84 respectively) than in adults (0.63 in both cases). Similarly, the THQ for Cd in the liver of chicken was 0.74 for children compared with 0.55 for adults (Fig. 2).

As shown in Fig. 2, THQ for Hg in the liver of chicken was 0.76 and 0.57 in children and adults, respectively. In spite of this, caution must be taken since perennial intake of these contaminated food animals is likely to induce adverse health effects arising largely from As, Cd, Hg and Pb exposure. Meanwhile, the THQ for Mn was very low among all the three food animals (data not shown) ranging from 0.001–0.06 and 0.001–0.008 in adults and children, respectively. The THQ for As, Cd, Hg and Pb showed that contributions of chicken gizzard and liver to toxic metal exposure in adults and especially children could be significant.

Prolonged consumption of these toxic metals in the offal and muscle may lead to accumulation and cause toxicity and therefore there is a clear need to avoid consumption of these contaminated food animals, as well as restrict them from roaming and scavenging for food and/or water near the mining areas. Furthermore, continuous monitoring of As, Cd, Hg and Pb residues (in these food animals) in the vicinities of gold mines in Tarkwa is recommended in the interest of consumers.

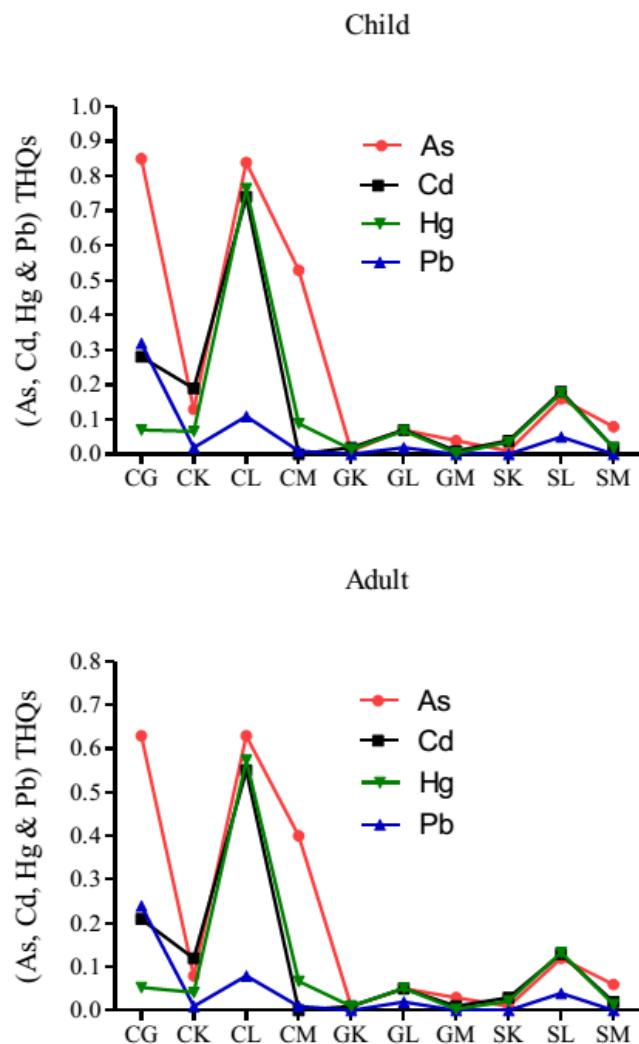


Fig. 2 Target Hazard Quotients (THQs) of As, Cd, Hg and Pb in children and adults (CG: chicken gizzard; CK: chicken kidney; CL: chicken liver; CM: chicken muscle; GK: goat kidney; GL: goat liver; GM: goat muscle; SK: sheep kidney; SL: sheep liver; SM: sheep muscle)

Conclusions

Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, and Pb in the liver, kidney muscle and gizzard of free-range chickens, goat and sheep from Tarkwa were determined. From the results, mean concentrations (mg/kg ww) of metals in all food animals studied ranged Cd: nd–0.73, Pb: nd–0.39, Cr: 0.01–0.24, Mn: 0.15–3.57, Fe: 6.62–542.31, Co: nd–0.11, Ni: 0.01–0.55, Cu: 0.35–105.8, Zn: 5.12–65.89, As: nd–0.14 and Hg: nd–0.12. The dietary intakes of As, Cd, Hg, Pb and Mn from the offal and muscle of free-range chicken, goat and sheep in Tarkwa, Ghana were low when compared to the [WHO \(2000\)](#), [FSA \(2006\)](#) and [FAO/WHO \(2010\)](#) tolerable daily intakes. Children are especially vulnerable to acute, sub-acute and chronic effects of ingestion of chemical pollutants, since they (children) consume more (twice of the amount) of food per unit of body weight as adults ([ENHIS, 2007](#)). As a result, intakes of these four toxic metals through food could be higher for children from Tarkwa. THQ of As, Cd and Hg showed that there could be potential health risk to inhabitants through consumption of larger quantities of contaminated offal of free-range chicken.

Chapter 5
**Heavy metals and metalloid accumulation in livers and kidneys
of wild rats around gold-mining communities in Tarkwa, Ghana**

Introduction

Anthropogenic activities including artisanal and small-scale gold mining have caused elevated levels of heavy metals and metalloids in the environment (Naccari et al., 2009; Licata et al., 2010). This activity could lead to spillage and run-off into rivers, ponds, streams, wells, and borehole drinking water (Obiri, 2007), and further result in heavy metal and/or metalloid exposure to humans and animals through various pathways. Heavy metals and metalloids have a wide range of health effects including mutagenicity, carcinogenicity, teratogenicity, immuno suppression, poor body condition, and impaired reproduction in humans and animals (Scheuhammer, 1987; Florea and Busselberg, 2006). All of these make them a serious threat to living organisms (Battaglia et al., 2005; López-Alonso et al., 2007; Naccari et al., 2009).

Studies conducted by Asante et al. (2007), Bortey-Sam et al. (2015c; 2015d; 2015e; 2015f) and Hayford et al. (2008) on the impact of gold mining in soil, drinking water, foodstuffs and food animals (free-range chickens, goat and sheep) collected around mining communities in Tarkwa showed high levels of some toxic metals including As and Hg than maximum levels by EC (2006), WHO (1996; 2011) and USEPA (2004; 2012). Bortey-Sam et al. (2015e) indicated that the concentrations of some heavy metals/metalloids in agricultural soils in some communities in Tarkwa, exceeded the ecological-soil screening levels (USEPA, 2004) recommended for mammalian wildlife, which includes wild rats and mice. However, levels of metals (0.24 [Hg] to 72 mg/kg dw [Zn]) detected in soils collected from the University of Mines and Technology (uMaT) campus (reference site) were low compared to world range for unpolluted soils by Kabata-Pendias and Pendias (1992).

Although wildlife is rich and diverse, with a large number of mammals, reptiles and insects in the study area, rats were used as sentinels to measure the environmental pollution state because they are mammals that share many processes with humans and are appropriate for use to answer many research questions. They tend to pick food and water from the ground which could be contaminated with various pollutants including metals. In studies by

[Nakayama et al. \(2011; 2013\)](#), wild rats in mining areas in Zambia, were used as sentinels for heavy metal accumulation, and results showed that rats accumulated metals that had likely originated from mining activities. Similarly, [Guerrero-Castilla et al. \(2014\)](#) used wild mice around coal mining areas in Columbia to study the exposure and health effects of heavy metals from the mining processes.

Despite the wide and numerous studies of heavy metal concentrations in various samples in Tarkwa, Ghana, there is limited or no data from literature on its accumulation in wild rats. The objectives of this study were to determine the accumulation of heavy metals and a metalloid in livers and kidneys of wild rats in Tarkwa; to determine sex differences in heavy metal and metalloid accumulation; to examine the relationship between body weight of rats and metal accumulation; to identify the possible exposure route of these metals to wild rats in Tarkwa; and to examine the potential risks that heavy metals could pose to wild rats.

Materials and methods

Sampling

All procedures used in this experiment were according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee and the local veterinarian policy of the study area. In June, 2012, live wild rats (*Rattus norvegicus* or *R. rattus*) were captured using gauze cage traps with food as bait, in residential, commercial and farming areas (within 16 communities) in Tarkwa (n = 46). Rat species were morphologically identified. Some of the sampled communities were 2 km (Samahu), 3.4 km (Abekuase) and 5.2 km (T-Tamso) away from the mines (Fig. 1). After anesthesia overdose, rats were euthanized, and body weight and sex were determined (25 females and 21 males). The livers and kidneys are target organs for monitoring metal contamination in animals because both organs function in removing toxic metals from the body (Abou–Arab, 2001; Husain et al., 1996). Studies of three animal species (free-range chickens, goat and sheep) in Tarkwa showed that the livers and kidneys contained the highest levels of metals (Bortey-Sam et al., 2015c). For these reasons, livers and kidneys were collected from each rat and stored at -20°C. Samples were kept in a freezer at the Chemistry Department of the Kwame Nkrumah University of Science and Technology, KNUST, Ghana and later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan, where they were stored in -30°C until analysis.

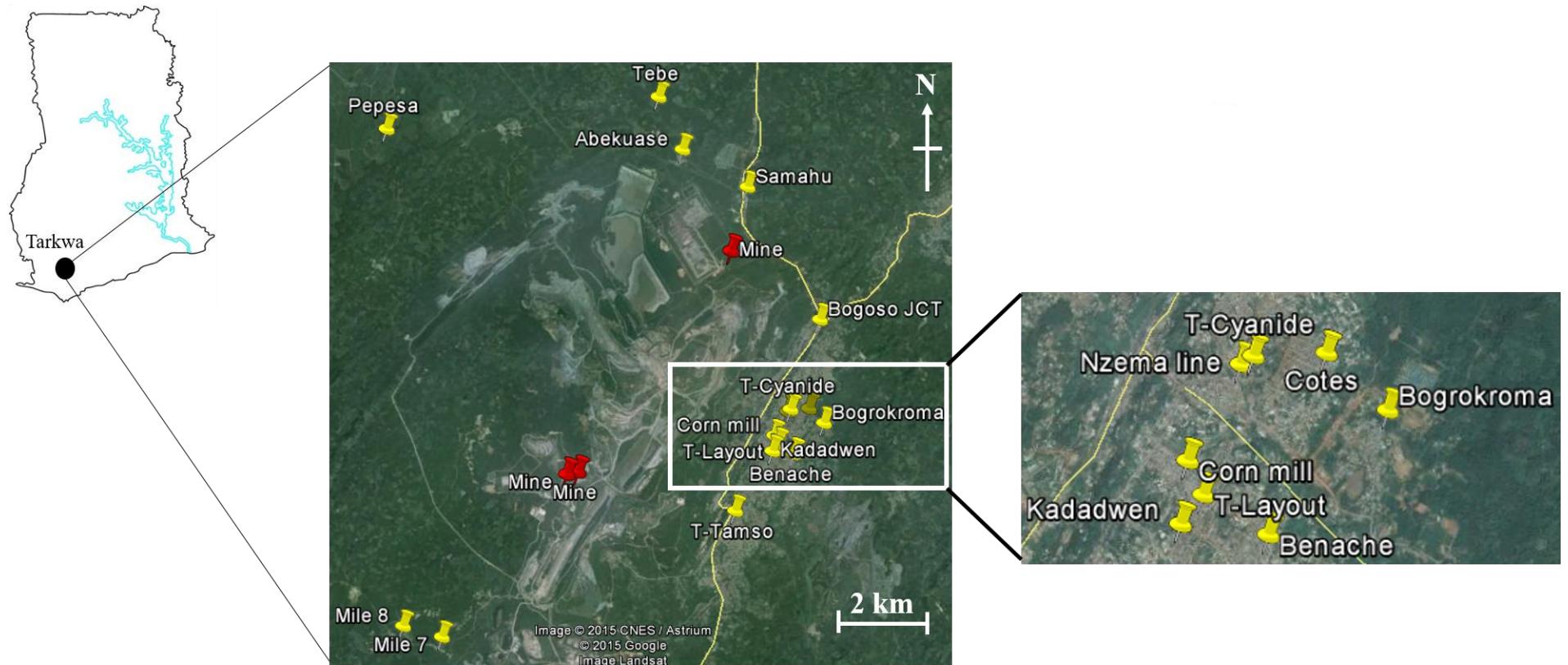


Fig. 1 Map showing wild rats sample locations in Tarkwa, Ghana (yellow and red pins indicate sampled communities and gold mines, respectively)

Sample preparation and metal analysis

Preparations and digestion of liver and kidney samples for heavy metals and metalloid analysis were done according to the method described by [Bortey-Sam et al. \(2015c\)](#). Approximately 0.5 g of individual samples were dried in an oven at 40 °C and placed in prewashed digestion vessels. Samples were digested (for 52 min) (Speed Wave MWS–2 microwave digestion, Berghof, Germany) using 5 ml of (65%) nitric acid, HNO₃ (Kanto Chemical Corp., Tokyo, Japan) and 1 ml of (30%) hydrogen peroxide, H₂O₂ (Kanto Chemical Corp., Tokyo, Japan). After digestion, cooled samples were transferred into corning tubes (Corning Incorporated, New York, USA) and diluted to a final volume of 10 ml with milli Q water. A reagent blank was prepared using the same procedure.

An Inductively Coupled Plasma–Mass Spectrometer (ICP–MS; 7700 series, Agilent technologies, Tokyo, Japan) was used for quantification. The instrument was calibrated using standard solutions of the respective metals (to establish standard curves before metal analysis). All chemicals and standard stock solutions were of analytical–reagent grade (Wako Pure Chemicals, Osaka, Japan). The detection limits (ng/g) of chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) were 0.003, 0.025, 0.154, 0.0004, 0.024, 0.007, 0.226, 0.002, 0.001 and 0.001, respectively. Concentrations of metals were expressed in mg/kg dry weight (mg/kg dw).

Quality assurance and quality control

For heavy metals and a metalloid, replicate blanks and reference materials, DORM–3 (Fish protein, The National Research Council, Canada) and DOLT–4 (Dogfish liver, The National Research Council, Canada) were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy (relative standard deviation, RSD, ≤ 3%) and recovery rates ranged from 80 to 115%.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (IBM SPSS Inc., Chicago, USA). Kolmogorov-Smirnov (K-S) and Shapiro-Wilk's (S-W) tests were used to determine the normality of data and was considered statistically significant if p value was less than 0.05. Statistical analyses were carried out after data were log transformed (normalized). Student's T-test was used to compare distribution of metals between livers and kidneys, and differences were considered statistically significant with p value < 0.05 . Pearson's correlations were used to determine the relationship between concentrations of metals and body weights, and significant level was at p value was less than 0.05. Principal component analysis (PCA) based on log transformed data was done, to determine the distribution pattern and possible route of heavy metals exposure to wild rats, using JMP statistical software v. 10 (SAS Institute). The principal components were extracted with eigenvalues > 1 .

Results and discussion

Levels of heavy metals and a metalloid

Mean concentrations of heavy metals and a metalloid in livers of wild rats in Tarkwa, decreased in the order; Zn > Cu > Mn > As > Pb > Ni > Cr > Co = Cd; and the order in the kidney was Zn > Cu > Mn > Pb > Ni > As > Cd > Co = Cr (Table 1). All metals measured were detected in 100% of liver and kidney samples.

K-S and S-W's tests for normality showed a significant variation ($p < 0.001$) in metal distribution in livers and kidneys of wild rats in Tarkwa. Distribution of Cd, Co, Cr, Mn, Ni, Pb, and Zn between livers and kidneys differed significantly (Student's T-test; $p < 0.05$) (Table 1). The paragraphs below discusses about As, Pb and Cd which were classified the first, second and seventh most hazardous substances (ATSDR, 2013), and Mn because Tarkwa is also noted for Mn mining.

Mean concentration of As was higher in livers (2.59 ± 2.81 mg/kg dw) than kidneys (1.91 ± 2.33 mg/kg dw) (Table 1). The liver is a major target organ of As carcinogenesis (Waalkes et al., 2003) and could be the reason for the higher As levels. As is toxic and most hazardous substance (ATSDR, 2013) and due to its non-biodegradable nature, it could accumulate in soil, food and water (Amonoo-Neizer et al., 1995), through which wild rats could be exposed since they pick food and water mainly from the ground. Levels of As in soils, drinking water and organs of free-range chickens raised health risk concerns for both humans and animals in some communities in Tarkwa with food and water picking being the dominant sources in chicken (Bortey-Sam et al., 2015c; 2015d; 2015e). The levels were attributed to processing of the ore which involves roasting and these results in the production of arsenic trioxide gas which is distributed throughout the study area by air current (Amonoo-Neizer et al., 1995).

Table 1: Mean concentrations (\pm SD) and ranges of heavy metals and a metalloid (mg/kg dw) in the livers and kidneys of wild rats in Tarkwa, Ghana

Samples		<i>n</i>	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Liver	Mean	46	2.59 ^a	0.198 ^a	0.198 ^a	0.147 ^a	18.9 ^a	5.50 ^a	0.869 ^a	1.05 ^a	263 ^a
	SD		2.81	0.321	0.207	0.0908	14.0	2.86	0.522	2.23	95.2
	Minimum		0.0699	0.0213	0.0784	0.0166	7.59	2.54	0.219	0.0532	112
	Maximum		12.6	1.45	1.21	0.406	71.8	19.1	2.11	10.7	524
Kidney	Mean	46	1.91 ^a	1.41 ^b	0.382 ^b	0.375 ^b	14.3 ^a	4.09 ^b	2.02 ^b	3.97 ^b	117 ^b
	SD		2.33	3.57	0.333	0.327	4.57	2.79	1.85	8.15	41.7
	Minimum		0.102	0.0039	0.0741	0.0773	9.17	1.53	0.172	0.044	64.3
	Maximum		14.0	21.1	1.62	1.72	39.5	16.8	7.85	41.6	284

n: number of samples; SD: standard deviation; different letters (a and b) between groups indicates significant difference (Student's T-Test; $p < 0.05$)

As shown in Table 1, the mean levels of Cd in the kidneys (1.42 ± 3.57) was seven times higher ($p < 0.05$) compared to the liver (0.198 ± 0.321) of wild rats in Tarkwa. There have been suggestions that animals exposed to Cd accumulate it in their kidneys because of the presence of free protein-thiol groups which leads to a strong fixation of the metal (Pompe-Gotal and Crnic, 2002). Cd concentrations in blood, urine and kidney have been recognized as good indicators of exposure (Brzoska et al., 2004). Cd could increase excretion of calcium and reduce the generation of active vitamin D in kidney. Consequently, calcium uptake and absorption in gastrointestinal gut are decreased (Chen et al., 2013). Bone lesions, apart from kidney damage, are the main health consequences of chronic exposure to Cd. Osteopenia, osteomalacia and osteoporosis with pathological fractures have been reported in Cd-exposed humans (Jarup, 2002; Alfven et al., 2000; Honda et al., 2003) and experimental animals (Whelton et al., 1997; Uriu et al., 2000).

Levels of Pb were higher ($p < 0.05$) in kidneys (3.97 ± 8.15 mg/kg dw) than livers (1.05 ± 2.23 mg/kg dw) (Table 1). In previous studies, high levels of Pb was found in *Manihot esculenta* (cassava), soils and chickens from some communities around mining areas in Tarkwa, which could cause health risk to residents and especially children (Bortey-Sam et al., 2015c; 2015e; 2015f). The levels of Pb in organs of free-range chickens in Tarkwa emanated from contamination of soil, feeds and/or water sources (Bortey-Sam et al., 2015c), and these could be the same route through which wild rats were exposed.

Livers (5.50 ± 2.86) accumulated higher ($p < 0.05$) levels of Mn than kidneys (4.09 ± 2.79) (Table 1). This is because, the liver is key for maintaining Mn homeostasis (Finley, 1998), and among organs with highest Mn levels (Dorman et al., 2006) as it produces two of the main plasma transport proteins of Mn-albumin and transferrin (Crossgrove and Zheng, 2004). Excess Mn causes neurotoxicity, production of reactive oxygen species and disturbance of mitochondrial dynamics (Barhoumi et al., 2004; Martinez-Finley et al., 2013). In the offal and muscles of free-range chickens, goat and sheep in Tarkwa, the mean concentrations of Mn were above the WHO (1996) maximum levels (0.5 mg/kg) except in

chicken muscle, and levels were attributed to proximity of the sample sites to the Mn mine ([Bortey-Sam et al., 2015c](#)).

Sex differences in heavy metal and metalloid accumulation

Bioaccumulation of heavy metals in animals varies according their sex, size and/or age ([Hunter et al., 1989](#), [Sawicka-Kapusta et al., 1995](#), [Damek-Poprawa and Sawicka-Kapusta, 2004](#)). Although we could not determine the ages of wild rats in this study, the results of sex differences in the accumulation of metals showed no statistical variation ($p > 0.05$) except Ni in kidneys ([Table 2](#)). However, levels of As, Cd and Zn were higher in the livers of female rats compared to males, while in livers of male rats, Cu and Mn were higher than in those of female rats ($p > 0.05$). Co, Cr, Ni and Pb levels were similar in livers of both sexes ([Table 2](#)). This trend was similar for the kidneys except for Ni which was higher in male rats compared to females ([Table 2](#)). Study of [Blagojevic et al. \(2012\)](#) in skull of mice from two localities in Serbia revealed that no gender dependent variation was detected for Fe, Mn, Co, Cd, Zn, Ni, Pb and Cu.

Although not significant ($p > 0.05$), average Cd levels in livers (0.12 mg/kg dw) and kidneys (0.65 mg/kg dw) of females were two times higher than in males (liver [0.07 mg/kg dw] and kidney [0.29 mg/kg dw]). Absorption of Cd is through the gastrointestinal tract (GIT), however, this can be affected by several factors, such as age, sex, nutritional status, and preceding Cd burden. Among these, young age, iron deficiency, and being female are reported to accelerate the absorption of Cd through the GIT in both humans and animals ([Berglund et al., 1994](#); [Flanagan et al., 1978](#); [Hamilton and Valberg, 1974](#); [Kowal, 1988](#); [Taguchi and Suzuki, 1981](#)) and these could be the reasons why Cd levels were higher in females than males.

Table 2: Sex differences in accumulation of heavy metals and a metalloid (mg/kg dw) in the livers and kidneys of wild rats in Tarkwa, Ghana

Organ	Sex		As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Livers	Male	Mean	0.573 ^a	0.066 ^a	0.132 ^a	0.185 ^a	19.8 ^a	5.45 ^a	0.921 ^a	0.237 ^a	256 ^a
		SD	0.586	0.027	0.045	0.078	23.2	6.65	0.607	0.217	103
		Minimum	0.070	0.021	0.088	0.071	8.95	2.54	0.219	0.053	164
		Maximum	1.92	0.098	0.210	0.294	71.8	19.1	1.72	0.666	433
	Female	Mean	1.08 ^a	0.117 ^a	0.163 ^a	0.198 ^a	15.3 ^a	4.60 ^a	0.894 ^a	0.292 ^a	301 ^a
		SD	2.53	0.181	0.194	0.106	7.27	1.21	0.605	1.34	111
		Minimum	0.113	0.040	0.078	0.094	7.59	2.60	0.269	0.093	177
		Maximum	12.6	1.45	1.21	0.406	30.9	6.66	2.12	10.7	524
Kidneys	Male	Mean	0.645 ^a	0.292 ^a	0.332 ^a	0.410 ^a	18.1 ^a	5.21 ^a	4.82 ^a	1.24 ^a	122 ^a
		SD	0.603	0.283	0.316	0.247	9.55	3.53	1.99	1.69	68.7
		Minimum	0.103	0.004	0.074	0.128	11.6	1.53	1.82	0.044	64.3
		Maximum	1.89	0.867	1.62	0.816	39.5	16.8	7.85	5.17	284
	Female	Mean	0.905 ^a	0.650 ^a	0.244 ^a	0.513 ^a	13.7 ^a	4.32 ^a	2.52 ^b	1.53 ^a	140 ^a
		SD	1.32	1.15	0.172	0.508	1.85	1.70	1.33	2.69	45.6
		Minimum	0.159	0.107	0.082	0.077	10.2	2.29	0.939	0.305	81.6
		Maximum	14.0	21.1	0.519	1.72	16.4	6.89	5.03	41.6	284

SD: standard deviation; different letters (a and b) between male and female rats within the same organ indicates significant difference (Student's T-Test; $p < 0.05$)

Heavy metals correlation with body weights of wild rats

Levels of Ni in the livers and kidneys negatively correlated ($p < 0.05$) with body weights of rats (Table 3). Ni is a carcinogen and overexposure could cause decreased body weights and damage to the heart and liver (Homady et al., 2002). On the other hand, body weights correlated positively ($p < 0.05$) with Cd (both livers and kidneys) and Co (livers only).

In exposure studies of Brzoska et al. (2004) and Chen et al. (2013), increase in the body weights of rats was noted when exposed to Cd. Wronski et al. (1987) demonstrated that increased body weight provides a partial protection against the development of osteopenia in the long bones of ovariectomized (OVX) rats.

Nevertheless, the protective effect of obesity against osteopenia in OVX rats is only partial and that marked osteopenia develops in the long bones of OVX rats regardless of body weights. This trend suggests that the increased body weights of obese OVX rats may have provided an additional stimulus for bone formation in the weight-bearing long bones (Wronski et al., 1987). The finding of Wronski et al. (1987) was consistent with reports of diminished bone loss in obese postmenopausal women (Saville and Nilsson, 1966; Daniell, 1976; Lindsay et al., 1984). These trends could explain the significant positive correlation ($p < 0.05$) between Cd levels in livers and kidneys and body weights of rats.

Possible sources of heavy metals and a metalloid in wild rats

PCA was used to trace the possible route of heavy metals and a metalloid exposure to wild rats in Tarkwa. Soil, bore hole drinking water and foodstuff (cassava and *Musa paradisiaca* [plantain]) data on heavy metals in communities in Tarkwa, used for PCA was obtained from Bortey-Sam et al. (2015d, 2015e; 2015f). As shown in Fig. 2, component 1 (PC1) makes up 40% of the PCA and score plot has high loadings of livers, kidneys (of wild rats), soils and borehole drinking water, with some highly associated with all the studied metals. This suggests that exposure of wild rats to metals were through soils and borehole drinking water,

which is similar to conclusion of Bortey-Sam et al. (2015c) on the sources of metals in free-range chickens in Tarkwa. This is obvious because like chickens, these wild rats also pick food and water from the ground which contains heavy metals and / or metalloids. There was a strong association between the livers and kidneys with borehole drinking water from Tarkwa, which was also highly associated with Cu, Cd and Zn ([Fig. 2](#)).

Toxicological significance

The levels of heavy metals and a metalloid in this study were compared with studies by [Nakayama et al. \(2013\)](#), [Soewu et al. \(2014\)](#) and [Guerrero-Castilla et al. \(2014\)](#) on the accumulation and biological effects (oxidative stress) of metals in wild rats and mice around mining and industrial communities in Zambia, Nigeria and Colombia, respectively. Among the livers and kidneys, levels of As, Ni, Zn and Cd (observed only in kidneys) were higher in this study compared to studies by [Soewu et al. \(2014\)](#), [Nakayama et al. \(2013\)](#) and [Guerrero-Castilla et al. \(2014\)](#) ([Table 4](#)). Exposure of wild rats to Ni, Zn and Cd in Tarkwa could have resulted from mining/smelting, municipal waste and/or the use and sometimes abuse of phosphate fertilizers and organic manures. Other sources may include leachates from Ni–Cd based batteries and Cd plated items which are so carelessly discarded by battery chargers and users in Ghana as indicated by [Bortey-Sam et al. \(2015c; 2015e\)](#). Recently, electronic wastes are disposed and often burnt at refuse dumps.

[Nakayama et al. \(2013\)](#) studied heavy metals in wild rats in Zambia and reported to cause toxicological effects, since the accumulated metals caused induction of metallothionein (MT) in the livers and kidneys. Similarly, levels of metals in exposed mice from coal mining areas, in Colombia, caused a significant increase ($p < 0.05$) in mRNA expression of genes related to oxidative stress, metal transport and DNA damage ([Guerrero-Castilla et al., 2014](#)).

Table 3: Pearson's correlation between heavy metals and body weight of wild rats in Tarkwa, Ghana

	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	body weight/g
As	1	0.518*	0.516*	-0.003	0.152	0.215	-0.553*	0.531*	0.704**	0.318
Cd	0.333	1	0.393	0.167	-0.195	0.255	-0.631**	0.704**	0.480	0.599*
Co	0.225	0.531*	1	0.355	0.553*	0.066	-0.166	0.112	0.394	0.444
Cr	0.398	0.058	0.511*	1	0.204	0.562*	0.313	0.128	0.097	-0.315
Cu	0.324	-0.153	0.217	0.328	1	0.298	0.283	0.004	0.305	0.045
Mn	0.420	-0.506*	0.045	0.359	0.733**	1	0.219	0.590*	0.395	-0.374
Ni	-0.349	-0.526*	0.199	0.212	0.319	0.380	1	-0.418	-0.469	-0.725**
Pb	-0.061	-0.049	0.167	-0.264	-0.017	0.064	0.090	1	0.653**	0.253
Zn	0.076	0.075	-0.394	-0.357	0.032	-0.068	-0.022	-0.236	1	0.449
body weight/g	0.350	0.864**	0.538*	0.076	-0.149	-0.391	-0.580*	0.078	-0.047	1

*: Correlation is significant at the 0.05 level. **: Correlation is significant at the 0.01 level; Bold indicates correlations between heavy metals and body weight in kidneys of wild rats otherwise for livers

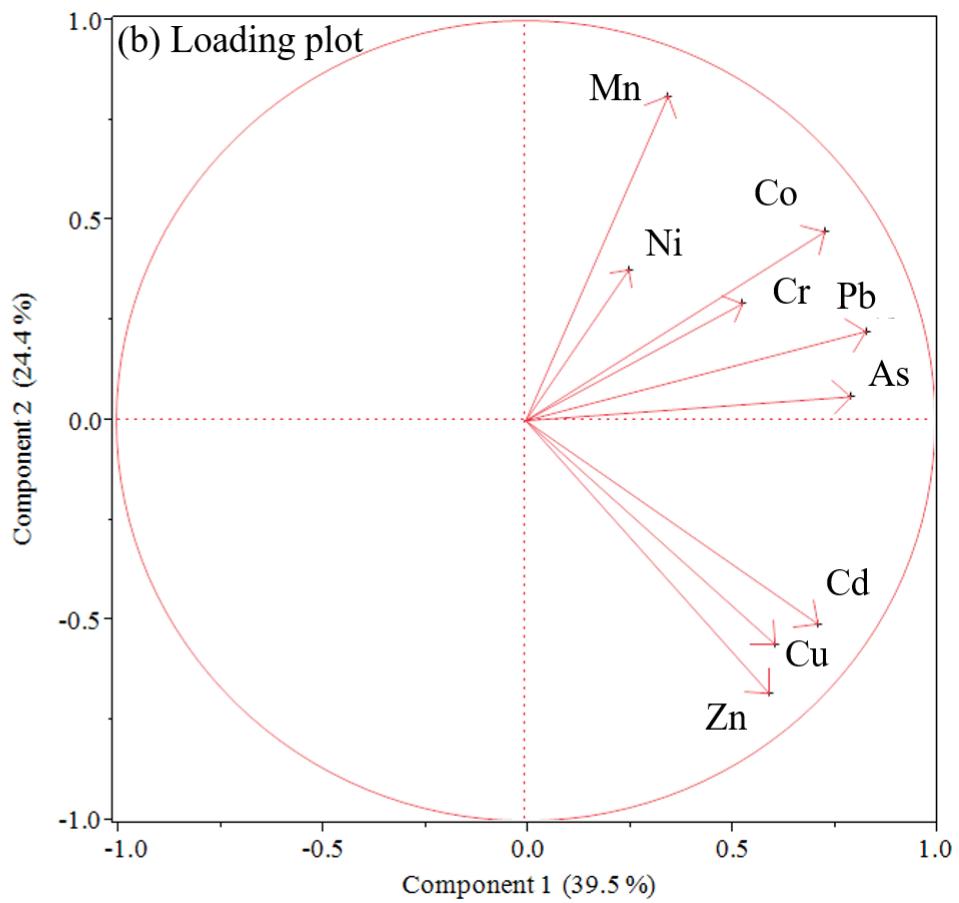
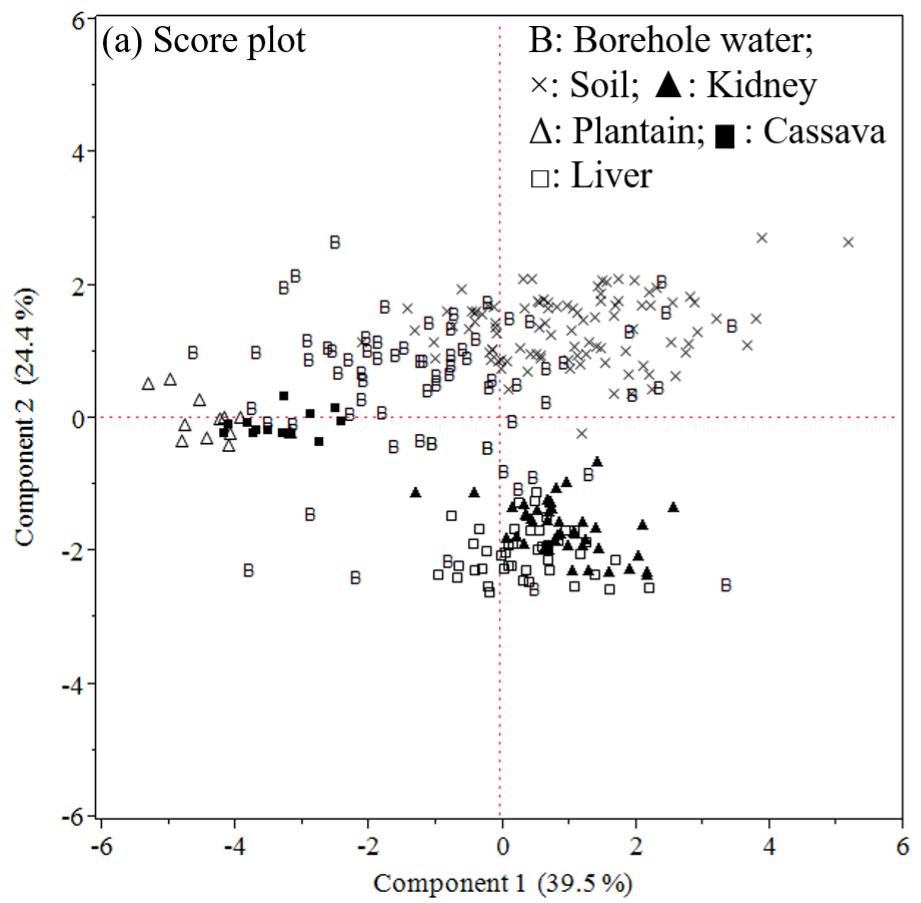


Fig. 2 Distribution pattern of heavy metals and a metalloid in cassava, plantain, borehole drinking water, soils, livers and kidneys of wild rats in Tarkwa characterized by PCA

Concentrations of Cd in both organs of this study, were below the critical renal intoxication level (119 mg/kg dw) which leads to subclinical symptoms for small mammals (Tohyama et al., 1987; Ma et al., 1991). Similarly, a critical liver concentration of 20–30 mg/kg leads to hepatocyte damage (Godowicz, 1988; Swiergosz-Kowalewska, 2001). The toxicity of As in mammals was found to be related with levels above 3 mg/kg in the liver and kidney (Gupta, 1998) and animal data suggest that As exposure may have chronic effects on the kidneys (WHO, 1981). Liu et al. (2000) recorded that glomerular swelling is one of the degenerative changes that usually occur in mice chronically exposed to As. These changes were also observed in livers and kidneys of mice at concentrations of 1.79 ± 0.946 and 3.89 ± 0.817 mg/kg dw, respectively (Pereira et al., 2006). In the present study, concentrations of As in both organs were higher in 47% and 9% of livers and kidneys, respectively, compared with levels observed by Pereira et al. (2006) and could cause glomerular swelling in 9% of rats.

Levels of Pb in the livers and kidneys of wild rats in this study were higher than study by Guerrero-Castilla et al. (2014) but comparable with levels in Kabwe, Zambia, which was a Pb-Zn mine area (Nakayama et al., 2013) (Table 4). Ma (2011) reported that kidney Pb level > 15 mg/kg dw caused structural and functional kidney damage, while concentrations > 120 mg/kg dw caused body weight loss in adults rats. In histopathological studies, changes in the kidney, such as Pb intranuclear inclusion bodies and karyocytomegaly in the proximal tubular cells, were detected in wild brown rat (*R. norvegicus*) at kidney Pb concentrations > 2.5 mg/kg dw (Ceruti et al., 2002). In this study, the average concentration of Pb in kidneys (3.97 mg/kg dw) exceeded this histopathological threshold (2.5 mg/kg dw), and was higher in 29% of wild rats in Tarkwa. Moreover, 3 kidney samples (6%) exceeded the structural and functional kidney damage level (> 15 mg/kg dw) (Ma, 2011).

The high levels of metals detected in the livers and kidneys of wild rats in Tarkwa could cause health risk to mammalian wildlife.

Table 4: Comparison of mean concentrations (mg/kg dw) of heavy metal and a metalloid with other studies

Organ	Sample site/country	Site description	n	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Livers	Tarkwa, Ghana (<i>This study</i>)	Gold and Mn mining	46	2.59	0.198	0.198	0.147	23.0	5.50	0.869	1.05	263
	Kabwe, Zambia*	Pb and Zn mining	20	0.5	0.12	0.22	0.28	10.2		0.11	1.19	151
	Chingola, Zambia*	Co and Cu mining	13	0.07	0.05	1.72	0.15	29.5		0.05	0.41	115
	Lusaka, Zambia*	University campus	18	0.57	0.03	0.37	0.78	14		0.69	0.14	141
	La Jagua, Colombia#	Coal mining		0.35	2.36			22.2			0.18	2.2
	La Loma, Colombia#	Coal mining		0.24	0.55			13.8			0.35	16.7
	Omo forest, Nigeria **#	Undisturbed site	4		0.00		0.77	8.55	6.16		0.77	103
	Mosinmi ecotome **#	Oil and gas industries	4		0.58		0.00	8.05	16.2		0.00	87.1
	Ibese ecotome **#		4		0.00		2.16	11.9	8.64		0.00	77.1
	Agbara ecotome **#	Industrial	4		0.00		0.80	11.9	11.5		0.00	114
Kidneys	Tarkwa, Ghana (<i>This study</i>)	Gold and Mn mining	46	1.91	1.41	0.382	0.375	14.3	4.09	2.02	3.97	117
	Kabwe, Zambia*	Pb and Zn mining	20	0.52	0.64	0.26	0.93	11.5		0.64	5	91
	Chingola, Zambia*	Co and Cu mining	13	0.28	0.71	1.88	0.82	23		0.63	2.85	109
	Lusaka, Zambia*	University campus	18	1.72	0.14	0.98	0.75	14		0.59	0.39	0.39
	Omo forest, Nigeria **#	Undisturbed site	4		0.00		1.44	13.5	5.76		1.45	93.2
	Mosinmi ecotome **#	Oil and gas industries	4		0.00		1.08	13.8	11.0		0.96	95.7
	Ibese ecotome **#		4		0.00		2.04	13.9	6.72		0.00	86.4
	Agbara ecotome **#	Industrial	4		0.32		2.16	15.3	5.76		0.00	97.0

n: number of samples; * indicates study by Nakayama et al. 2013 in wild rats; # indicates study by Guerrero-Castilla et al. 2014 in wild house mice and concentrations were in mg/kg fresh weight; **# indicates study by Soewu et al. 2014 in cane rats (wild grass cutters).

Conclusions

Wild rats in Tarkwa, a mining community in Ghana, have been exposed to heavy metals and a metalloid through borehole drinking water and soils; and livers accumulated higher levels of As than kidneys but the reverse was for Cd and Pb. In both organs As, Cd and Zn levels were higher in female than male rats. The strong positive correlation between body weights and concentrations of Cd in livers and kidneys of wild rats reflects a mechanism of protection against the development of osteopenia in the long bones although biological effects remain a concern. Concentration of Pb in kidneys could cause intranuclear inclusion bodies and karyocytomegaly in the proximal tubular cells in 29% of wild rats in Tarkwa; and structural and functional kidney damage in 6%. Concentrations of As in kidneys of these wild rats could cause glomerular swelling in 9% of rats.

With the rapid increase in mining in Ghana, and high concentrations and possible risk of metals to wildlife, it is recommended that the government considers the following:

(1) educate the public on environmental pollution and management, (2) continuous screening and monitoring of heavy metals and metalloids in the study area and (3) set policies to curb the rate of metal pollution in Ghana.

Summary of Section 2

Mining in Tarkwa, Ghana, has caused contamination of the environment with heavy metals and a metalloid. This study revealed high risks of metals to the ecological system especially plants, soil invertebrates and/or mammalian wildlife in Tarkwanian communities especially from As, Pb and/or Hg contamination. Exposure of wild rats through soils and drinking water indicated that the livers accumulated higher levels of As than kidneys but the reverse was for Cd and Pb. In both organs, As, Cd and Zn were higher in female than male rats. The study further revealed intra nuclear inclusion bodies and karyocytomegaly in the proximal tubular cells in 29% of wild rats in Tarkwa; and structural and functional kidney damage in 6% due to Pb exposure. Additionally, As levels in kidneys of these wild rats could cause glomerular swelling in 9%. The overall results of this study revealed that exposure to metals through soils, drinking water and consumption of cassava and free-range chickens in Tarkwanian communities could cause both cancer and non-cancer risks to residents especially children, and mining has played a significant role.

Summary

The gross domestic product, industrialization, population and economic growth in Africa including Ghana, has seen tremendous growth over the past years. Despite the benefits, the negative impacts on humans, animals and the environment may cause additional costs not included in sales expenditures. Especially for the ubiquitous and non-biodegradable contaminants, the negative effects persist for several decades and even longer. Among the pollutants heavy metals and PAHs constitute a major health hazard in many African countries. Humans and animal exposure of these contaminants are through inhalation, ingestion and/or dermal contact.

Kumasi as the second largest and industrialized city in Ghana and has been subjected to heavy anthropogenic influence. Additionally, Tarkwa, is a city known to be rich in mineral resources such as gold and manganese. Unfortunately, industrialization to boost the economy has led to continuous release of metals and PAHs in the environment which could affect human and animal life. Assessing the risks due to exposure to these pollutants is needed to enable environmentalists, health services and policy makers help curb the deleterious effects to residents and prevent future health hazards. Based on this, I assessed the risks to PAHs and metals in biological and environmental samples in Ghana's Tarkwa and Kumasi regions as below:

In chapter 1 of section 1, cattle urine was collected from Kumasi (urban) and Offinso (rural), Ghana: to determine concentrations of urinary OH-PAHs; and find their association with sex; and to estimate exposure of cattle to PAHs from the different sites. From the results, GM_{SG}, showed that 2-OHNap was the most abundant OH-PAH in cattle urine from all study sites, and naphthalene-containing-mothballs might have contributed significantly to these levels. There was no significant difference between urinary OH-PAHs concentrations in cattle from urban and rural sites except for 2-OHPhe and 4-OHPhe, and similar to urban areas, rural sites could also be polluted with PAHs. GM_{SG} of 2-OHNap in cattle urine in Kokote (a rural area), was significantly higher compared to the other sites followed by Oforikrom (urban). The high concentrations of urinary 2-OHNap, 2-3-OHFlu, 2-OHPhe, 3-OHPhe and 4-OHPhe in Kokote indicated high PAHs exposure to cattle in this area or different/specific source of

PAHs exposure. GM_{SG} of 2-OHNap was significantly higher in male cattle compared to females while 1-9-OHPhe was significantly higher in females.

With the high cancer incidence, concentrations and cancer potency of PAHs in PM10, soils, livers of wild rats and high concentrations of OH-PAHs in cattle urine in Kumasi, human urine were collected to assess the health effects associated with PAHs exposure; and identify any association between OH-PAHs, MDA, 8-OHdG with age and sex in chapter 2 of section 1. Results of the present study revealed that exposure to PAHs significantly increases the odds of occurrence of persistent cough ($OR = 2.5$, CI: 1.33-4.70), persistent headache ($OR = 1.89$, CI: 1.66-3.36), respiratory tract infection ($OR = 46.2$, CI: 2.88-742), tachycardia ($OR = 3.65$, CI: 1.50-8.88) and dyspnea ($OR = 4.11$, CI: 1.75-9.65) among Kumasi residents. For gender differences, occurrence of persistent headache ($OR = 2.51$, CI: 1.04-4.29) and dyspnea ($OR = 3.30$, CI: 1.21-8.99) in females were significantly higher ($p < 0.05$) than males. GM_{SG} indicated 2-OHNap as the most abundant OH-PAH and highest level of urinary 2-OHNap was detected in a female participant in Manhyia hospital, who reported symptoms of persistent cough, headache, tachycardia, nasal congestion and inflammation, all of which are symptoms of naphthalene exposure. 1-9, 4-OH-Phenanthrenes, and 1-OHPyrene were most abundant from a participant who visited Manhyia hospital with ulcer and also complained of dyspnea. Σ OHPAHs, 2-OHNap, 2-3-OHFlu, and -OHPhe showed a significantly positive correlation with MDA and 4-OHPhe with 8-OHdG, indicating possible lipid peroxidation/cell damage in some participants. There was no association between urinary OH-PAHs concentrations with age; while MDA and 8-OHdG were highest in age group 21-60. The present study showed a significant sex difference with higher levels of urinary OH-PAHs in females than males.

In chapter 1 of section 2, heavy metals and a metalloid were analyzed in agricultural soils in 19 communities in Tarkwa to assess the potential ecological risk. Potential ecological risk (RI) indices indicated low (Mile 7) to high risks (Wangarakrom; Badukrom) of metals and soil organic matter could have played a substantial role on the total contents of these metals in soil. The investigated soils fall within low to high contamination and risk of heavy metals to the ecological system especially plants, soil invertebrates and/or mammalian wildlife. This

represented moderate potential ecological risk in the study area, and mining activities have played a significant role.

As a follow up, in chapter 2 of section 2, concentrations of heavy metals and metalloid in borehole drinking water from 18 communities in Tarkwa were measured to assess the health risk associated with its consumption. Mean concentrations of heavy metals ($\mu\text{g/L}$) exceeded recommended values in some communities and the HI results raise concerns about the non-carcinogenic adverse health effects of drinking groundwater in Huniso. The average risk values of As for adults and children through drinking borehole water in the communities indicated medium cancer risk, but high cancer risk in some communities such as Samahu and Mile 7. Based on the USEPA assessment, the average cancer risk values of As for adults ($3.65\text{E-}05$) and children ($5.08\text{E-}05$) indicated three (adults) and five (children) cases of neoplasm in a hundred thousand inhabitants. The results of this study showed that residents in Tarkwa who use and drink water from boreholes could be at serious risk from exposure to these heavy metals and metalloid.

Chapter 3 of section 2, assesses the extent of heavy metals and metalloid accumulation from agricultural soils to foodstuffs (viz, (cassava) and plantain) around thirteen neighboring communities within Tarkwa; and estimated the human health risk associated with consumption of these foodstuffs. From the results, 30% of cassava samples collected, contained higher concentrations of Pb when compared to Codex Alimentarius Commission standard values. Bioconcentration factor indicated that Ni had higher capacity of absorption into food crops from soil than the other heavy metals. For both children and adults, the target hazard quotient (THQ) of Pb in cassava in communities such as Techiman, Wangarakrom, Samahu and Tebe (only children) were greater than 1, which is defined as an acceptable risk value. This indicated that residents could be exposed to significant health risks associated with cassava consumption.

In chapter 4 of section 2, I estimated the daily intakes of heavy metals/metalloid and health risks implications due to consumption of different organs of different animal species (free-

range chickens, goat and sheep) near gold mines in Tarkwa. Principal component analysis of the results showed a clear separation of metal accumulation between the various animal species. Interestingly, As, Cd, Hg, Mn and Pb made one cluster in the offal of chicken. Chicken muscle also showed similar distribution with As, Hg and Pb clustered together. The daily intakes of As, Cd, Hg, Pb and Mn in these food animals were low compared to the provisional tolerable daily intake guidelines. The THQs although less than one, indicated that contributions of chicken gizzard and liver to toxic metal exposure in adults and especially children could be significant.

In chapter 5 of section 2, wild rats were used to estimate the environmental pollution state of metals; in addition, I determined the sex differences in metal accumulation; and assessed the potential risks involved. From the results, livers accumulated higher levels of As than kidneys but the reverse was for Cd and Pb. In both organs, As, Cd and Zn levels were higher in female than the male rats. There was a strong positive correlation between body weight and Cd concentrations in livers and kidneys of wild rats which reflects a mechanism of protection against the development of osteopenia, although biological effects remain a concern. Pb levels in the kidneys could cause intra nuclear inclusion bodies and karyocytomegaly in the proximal tubular cells in 29% of wild rats in Tarkwa; and structural and functional kidney damage in 6%. Concentrations of As in kidneys of these wild rats could cause glomerular swelling in 9% of rats. Principal component analysis of the results showed that wild rats in Tarkwa were exposed to heavy metals and a metalloid through borehole drinking water and soils.

Acknowledgements

I am very grateful to God for enabling me reach a point like this in my career, having bestowed upon me such abundant grace to go through this program.

I would like to express my deepest appreciation to my supervisor Prof. Mayumi Ishizuka and Prof. Yoshinori Ikenaka (Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University) for the opportunities, guidance, support and persistent help throughout my studies, this dissertation would not have been possible if it wasn't for them. I also express my deepest gratitude to Dr. Osei Akoto (Chemistry Department, KNUST, Ghana) and Dr. Shouta M.M. Nakayama (Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University) for their unreserved support, opportunity, guidance, constructive suggestions, and above all for their encouragement throughout the entire period of my research work.

I would like to acknowledge and express my gratitude to the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan; The Leading Program of Graduate School of Veterinary Medicine, The Japan Society for the Promotion of Science, Grant-in-Aid and the foundation of JSPS Core to Core Program, The Mitsui & Co., Ltd. Environment Fund, the Akiyama Life Science Foundation and the Nihon Seimei Foundation for the financial support throughout my study. My heartfelt gratitude also goes to the Graduate School of Veterinary Medicine, Hokkaido University for accepting me to pursue my PhD study.

I would like to thank all my colleagues (past and present members) in the Laboratory of Toxicology for their encouragement, generosity and support. In particular, I am very grateful to Drs. Yared Beyene Yohannes and Balazs Oroszlany for their time, generosity, advice and guidance in every aspect.

I thank Prof. Toshio Tsubota (Laboratory of Wildlife Biology and Medicine, Graduate School of Veterinary Medicine, Hokkaido University) and Prof. Kei Nomiyama (Center for

Marine Environmental Studies, Ehime University) for their valuable and critical comments on this thesis. I also thank Prof. Norikazu Isoda (Risk Analysis and Management, Research Centre for Zoonosis Control, Hokkaido University) for his guidance on calculations and interpretations of odds ratio.

I am 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. Many thanks to Dr. Elvis Baidoo (Chemistry Department, KNUST, Ghana) for his help during sampling.

I thank my parents and siblings for their endless love, encouragement and support, without you I would not be where I am today. My heartfelt appreciation and love goes to my wife, Joyce Amoah to whom this thesis is dedicated. My deepest affection and love goes to the Boss.

Finally, I would like to acknowledge all who endeavored to assist me one way or the other, God bless you all.

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Environmental contamination in Ghana: Environmental fate and risk assessment of heavy metals and polycyclic aromatic hydrocarbons

(ガーナにおける環境汚染：金属と多環芳香族炭化水素の環境動態とリスクアセスメント)

要旨 (Abstract in Japanese)

ガーナでは近年、産業化が急速に増大しており、それに伴う環境汚染が深刻化している。そこで、ガーナの都市の1つであるクマシと金鉱山地域であるタクワにおける多環芳香族炭化水素類 (PAHs) と金属レベルの測定とリスク評価を実施した。ウシおよびヒトの尿中 PAH 代謝産物 (OH-PAH) を分析した結果、いずれも高濃度で検出され、とくに 2-OHNap が最も高値であった。ナフタレンは水浄化、殺虫剤または清浄剤目的として使用される mothballs に含有されており、この使用が原因であると考えられた。一方、クマシにおいて、各患者の尿中 PAH 代謝物濃度と各種健康症状を疫学的に解析した結果、PAH 曝露により、咳 (OR = 2.5, CI: 1.33-4.70)、頭痛 (OR = 1.89, CI: 1.66-16.0)、呼吸器疾患 (OR = 46.2, CI: 2.88-742)、頻脈 (OR = 3.65, CI: 1.50-8.88)、呼吸困難 (OR = 4.11, CI: 1.75-9.65) が高いオッズで検出された。尿中 2-OHNap が最も高く検出されたのはマンヒア病院の女性労働者で、長期間の咳、頭痛、めまい、頻脈、筋肉痛、鼻詰まり、炎症などの症状が認められており、これら全てはナフタレンの毒性影響と一致していた。人の尿中 OH-PAHs 濃度は男性に比べての女性で高値であり、OH-PAHs 濃度と脂質酸化ストレスのバイオマーカーであるマロンジアルデヒドや 8-水酸化-2-デオキシグアノシンとの正の相関も認められた。タクワにおける金属およびメタロイドを分析したところ、飲料水や食料品、ニワトリなどの家畜や土壤などを介した曝露が明らかとなり、居住者、とくに子どもにおける腫瘍を含む健康悪影響が心配された。さらに、野生ネズミの肝臓と腎臓中の金属濃度を分析したところ、メスのネズミにおける As、Cd、Zn 濃度はオスと比べて高値であった。野生ネズミの体重と臓器中 Cd レベルの間に強い正の相関が認められ、骨減少に対する防御メカニズムであると考えられた。本研究で検出されたタクワの野生ネズミの腎臓中 Pb 濃度より毒性影響を推察したところ、29%のネズミで近位尿細管細胞の核内封入体や核・細胞巨大化が、6%のネズミで構造および機能的な腎臓障害が、また、9%のネズミでは糸球体腫脹がそ

の毒性学的基準レベルを超過していた。主成分分析を行った結果、タクワの野生ネズミは掘削孔の飲み水や土壤を介して金属に暴露されていることが明らかとなった。