Journal of Environmental Chemistry and Ecotoxicology

ABOUT JECE

The **Journal of Environmental Chemistry and Ecotoxicology (JECE)** is published bi-monthly (one volume per year) by Academic Journals.

Journal of Environmental Chemistry and Ecotoxicology (JECE) is an open access journal that provides rapid publication (bi-monthly) of articles in all areas of the subject such as ocean acidification, pesticides use and regulation, arsenic induced genotoxicity by curcumin, heavy metals and its environmental effect etc.
The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JECE are peer-reviewed.

Contact Us

Editors

Journal of Environmental Chemistry and Ecotoxicology

Table of Content: Volume 8   Number 7 July, 2016

ARTICLES

Full Length Research Paper

Determination of bisphenol A in exposed bottled water samples to direct sun light using multi walled carbon nanotubes as solid phase extraction sorbent

Leena Omer[1], Hassn Ahmed[2]* and Abdalla Elbashir[1]

[1]Department of Chemistry, Faculty of Science, University of Khartoum, Khartoum1536, Sudan.
[2]Department of Chemistry, Faculty of Science, Qassim University, Qassim 11841, Saudi Arabia.

Polycarbonate plastics (PC) containing bisphenol A (BPA) are used for the production of bottles for storage drinking water. Trace amounts of BPA have been detected in ten different brands of bottled water samples from Khartoum supermarkets. Residues of BPA in bottled water were pre-concentrated using solid-phase extraction and determined by high performance liquid chromatography with fluorescence detector. Recently, multiwalled carbon nanotubes (MWCNTs) have great attention because of their application in many fields. MWCNTs were used as adsorptive material for solid phase extraction which it was packed in mini column prior to pre-concentration step. The results indicate that the mean concentration of BPA in ten bottled water brands was 4.28 ng/ml for those stored at room temperature (25°C) and 11.81 ng/ml for those exposed to direct sunlight (40°C). The concentration of BPA in bottled water exposed to direct sun light was significantly higher than those stored at room temperature. Recovery of BPA from water was 92.5%. Detection limit for BPA were 0.30 ng/ml. Good correlation coefficients (>0.9996) were obtained. The method has been successfully applied for the determination of trace BPA in bottled water samples.

Key words: Bisphenol A, plastic bottled drinking water, solid phase extraction, multiwalled carbon nanotube, high performance liquid chromatography.

INTRODUCTION

Bisphenol A (2,2-di(p-hydroxyphenyl) propane, BPA) has been used for production of polycarbonates, epoxy resins, polysulphones and polycrylate resins (Letcher et al., 2005; Joskow et al., 2006). BPA is obtained by condensation of phenol with acetone in the presence of an ion-exchange resin as a catalyst. Because BPA and its derivatives can be harmful to living organisms, their presence and concentration in food products must be monitored.

Polycarbonate plastics (PC) are characterized by great strength, stability, elasticity, and low density. For these reason they have been widely used for production of food packaging, bottles of water, kitchen utensils, medical equipment (Hao-Chang et al., 2010; Rivas et al., 2009; Schecter et al., 2010). Substances capable of releasing BPA are used to coat the interior surfaces of bottles

such as epoxy resins, plastics and fibers (Sung-Hyun et al., 2010; Joaquim et al., 2010; Munguia-Lopez and Soto-Valdez, 2001; Aldea et al., 2008; Wu et al., 2010). BPA residues have been detected in water stored in packages made of PC. BPA can be released from PC and migrate to the water inside the bottle. This migration is promoted by acidity of the water stored, elevated temperature, mechanical cleaning, and use of detergents for cleaning this packaging (Coulier et al., 2010; Carvalho et al., 2015; Lane et al., 2015).

Since the BPA level reflects the amount of the analyte that has been recently adsorbed, the determination of BPA is useful for assessing occupational and environmental exposure. However, monitoring BPA in bottled water is difficult task due to the low concentration of the analyte. Therefore, the determination of BPA in bottled water samples need a pretreatment step as well as sensitive instrumental technique such as chromatographic technique high performance liquid chromatography (HPLC) equipped with fluorescence detection (John and Irving, 2013). Various methods such as liquid-liquid extraction (LLE) (Jiang et al., 2015; Larki et al., 2015) and solid phase extraction (SPE) (Yang et al, 2013; Gallart-Ayala et al., 2010) have been employed as sample pretreatment procedure for the determination of BPA. Among the aforementioned sample pretreatment procedures, solid-phase extraction based on the use of different types of adsorbents such as C_{18} (Liu et al., 2014), C_8 (Hadjmohammadi et al., 2010) and multi-walled carbon nanotubes (MWCNTs) (Ma et al., 2015).

In order to achieve valuable and effective adsorbents for the SPE of the target analytes BPA, new kind of adsorbent for improving the enrichment performance are needed. In recent years, a novel carbonaceous material multi-walled carbon nanotubes (MWCNTs) was successfully observed to adsorb increasing attentions. On the basis of their peculiar electronic, metallic and structural characteristics, they have been exploited in analytical and other fields.

MWCNTs used as adsorbents for the preconcentration of BPA from bottled water samples for chromatographic analysis (Yaqi et al., 2003), because of high surface area and inner volume, stability, high enrichment factor and mechanical strength. MWCNTs packed in mini-column pre-treated with methanol to concentrate BPA. The strong interaction may be attributed to the unique structure of carbon nanotubes, the hexagonal arrays of carbon atoms in graphene sheets of carbon nanotubes surface have strong interaction with the two benzene rings of BPA. The facts mentioned earlier show that MWCNTs may have great analytical potential as effective solid-phase extraction adsorbent for BPA.

In the present paper, the aim of the present study was to investigate the presence of BPA in ten brands of bottled drinking water under different storage situations in the province of Khartoum, Sudan. The applicability of MWCNTs as packing adsorbents for solid phase extraction was investigated by using BPA as model compound. The analyte were extracted by a MWNTs packed solution, and the analyte attracted on MWCNTs were eluted with suitable amount of methanol. Finally, the elute was analyzed by high performance liquid chromatography with fluorescence detector.

MATERIALS AND METHODS

Apparatus, reagents, and water samples

Bisphenol A was obtained from Acros Organics (NJ). Standard stock solutions (1000 μg/ml) containing this compound were prepared by dissolving an appropriate amount of bisphenol A in methanol. Working standard solutions 1, 10, 20, 40 and 50 ng/ml were prepared by an appropriate dilution of stock solutions with water. Methanol was purchased from ScharalaceChemi SA, Barcelona, Spain. Sodium hydroxide and hydrochloric acid were guarantee grade reagents (Beijing Chemicals Corporation, Beijing, China). MWCNTs with an average external diameter of 30 to 60 nm were kindly provided by Tsinghua-Nafine Nano-Powder Commercialization Engineering Center.

The Mini column (6.0 ×1.0 cm i.d.), employed for packing the MWCNTs was made of polyethylene containing glass wool placed at both ends of mini column aiming to prevent sorbent losses during the system operation.

A MWNTs-packed mini column was prepared by modifying 0.5 g of MWCNTs was packed into the mini column. The 20-μg polypropylene upper and lower frits remained at each end of the column to hold the MWCNTs packing in place. Prior to use, the entire solid-phase extraction assembly was carefully washed with sufficient methanol.

To reduce the organic and inorganic contaminants, all beakers, calibrated flasks, and other glassware used in the experiments were cleaned sequentially with tap water, neutral detergent, and tap water, then soaked in nitric acid for 48 h and cleaned with Mill Q purified water.

Ten brands (labeled 1-10) of commonly consumed bottled water were randomly purchased from local supermarkets in Khartoum, Sudan. A set of ten different brands of bottled water was purchased from among those stored at room temperature (25°C) while the second set (the same brands as those in the first set) was set purchased from among those exposed to direct sun light (39 to 42°C). In each case, water in the bottles was immediately extracted and analyzed. Two water bottles per brand were used in the analysis. The two sets of bottles were stored for 1 week prior to extraction. A sample of water (25 ml) was transferred from each bottle to a separating glass funnel. Then, the solid-phase extraction method was used.

Solid phase extraction procedure

As a pre-concentration step, the MWCNTs packed mini-column was washed by 5 ml of methanol and activated with 5 ml of water. Then, a 25 ml of bottled water sample spiked with BPA was passed through the mini-column at a flow rate of 2 ml/min (Pei et al., 2008). After the sample solution had passed through, the mini-column, it was washed with 5 ml of 10% methanol aqueous solution to remove matrix materials from the column. Then, the BPA retained on the SPE packing was eluted with 2.5 ml of methanol. Finally, 20 μl of methanol elute was injected into the HPLC system for the HPLC determination. By using the standard solutions of BPA, the external calibration curve can be acquired, and then the

Table 1. HPLC Conditions for the Analysis of BPA.

HPLC system	PerkinElmer Flexar FX-10 UHPLC
Injection volume	50 µl
Column	PerkinElmer C8 (150 mm × 4.6 mm, 5 µm)
Mobil phase	Acetonitrile/Water (60/40)
Flow rate	2 ml/min
Detector wavelength	Excitation: 275 nm/Emission: 313 nm
Detector response time	0.1 s
PMT, Em BDW	Super high, wide
Run time	7 min

Table 2. Physical characteristics and pH of bottled water samples.

Bottled water brands	pH	Color
1	7.5	Clear
2	7.2-8.2	Clear
3	7.4	Clear
4	7-8	Light blue
5	6.5-8.5	Light blue
6	8.0	Light blue
7	7,8	Light blue
8	7.5	Clear
9	7.5	Clear
10	7.4	Light blue

concentration of BPA in the eluate can be gotten. Finally, the recoveries of BPA at two situation storage at room temperature and exposed to direct sun light for one week obtained by comparing the concentration of BPA in the elute with the spiked concentration levels.

Instruments

Standards and bottled water samples were analyzed with a PerkinElmer Flexar FX-10 HPLC system including a PerkinElmer Series 200, a Fluorescence detector. The separation was performed on Mini Column.

A 744-pH Meter Metrohm (Switzerland) was used for pH measurement. Millipore SAS 67120 Molsheim system for deionized water (Table 1).

RESULTS AND DISCUSSION

Possible factors that may influence the enrichment include the eluent and its volume, sample volume, pH of the sample and flow rate of the sample. These parameters will place an important role on the concentration of the target analytes.

Effect of sample pH on adsorption

The adsorption of BPA on MWCNTs depends on pH

value for the extraction of BPA. Ten brands of bottled water samples were purchased at a Khartoum city markets, the amount of BPA was determined by HPLC after SPE on MWNTs. Table 2 obtained the physical characteristics and pH of BPA of the ten examined bottled water brands**.**

Effect of eluent concentration and volume

The eluent concentration is one of the most important parameters of desorption of BPA from the mini-column. It was found that methanol was an effective eluent for BPA analyte. And therefore, it was accepted as an eluent. To find the required volume of methanol to elute the analyte from the mini column, eluent volumes up to 10 ml were investigated. The experimental results indicated the quantitative recoveries (95%) could be achieved when the volume of methanol was in the range 2 to 10 ml. Because application of a smaller volume of eluent contributes to obtaining a higher preconcentration factor, 2.5 ml of methanol was adopted as the eluent throughout the experiments.

Effect of flow rate of sample solution

In solid-phase extraction system, the flow rate of sample
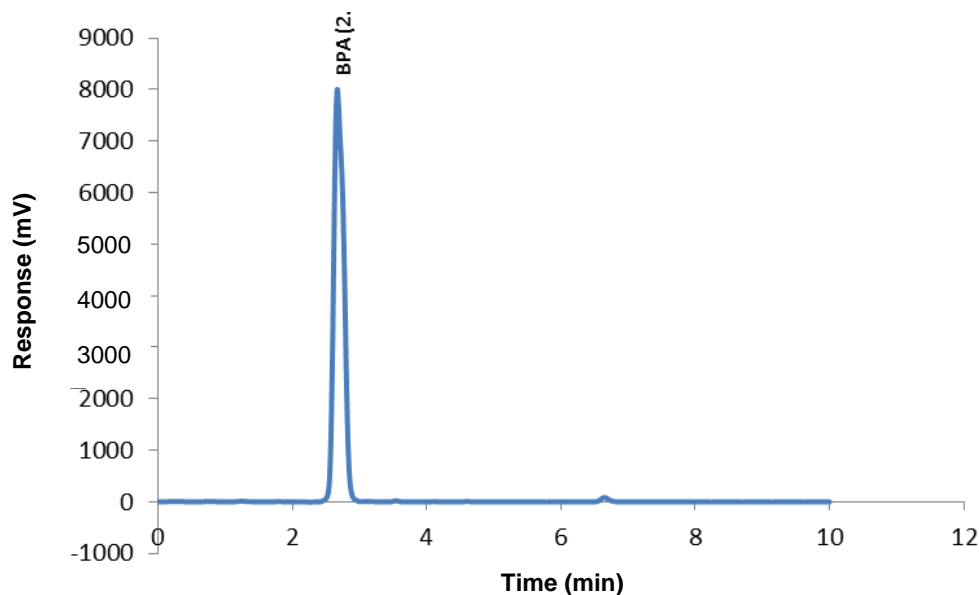
Figure 1. Chromatogram obtained the standard of BPA (1 ng/ml).

solution does not only affect the recovery of analyte, but also controls the analysis time. After optimization of HPLC instrument, determination of BPA was performed at flow rate of 2 ml/min, an example of chromatogram obtained the standard of BPA (1 ng/ml) as shown in Figure 1.

Effect of volume of the sample solution

To obtain reliable and reproducible analytical results and a high concentration factor, it is very important to get satisfactory recoveries for BPA in a large volume sample solutions as possible. So it is necessary to obtain the breakthrough volumes. Following the experimental procedure, the recoveries of the analyte at different volumes were obtained. The effects of sample volumes on the recoveries of the BPA are good recoveries (>92.5%) were obtained for BPA when the volumes of sample solutions were under 50 ml. Although a decline of recoveries was observed for the BPA in 100 ml of sample solutions, the recoveries for the analyte were still above 90%, which was acceptable in trace analysis. A sample volume of 25 ml was selected in the subsequent analysis of several environmental samples.

Comparison studies

For comparative studies, the results obtained in this research were compared with those of other researches. As mentioned in breakthrough volume experiment earlier, the recoveries (>92.5%) were obtained when the volumes of bottled water samples were under 50 ml. In some

cases, it appears that the recoveries obtained in this present work with bottled water samples are better than those obtained in some of other research work (Syed, 2011). In this research, C_{18} were used for the pre-concentration of several BPA aqueous solution and recoveries obtained were 80%. In research of Shan et al. (2014), another adsorbent C_8 was used for the pre-concentration of BPA in water samples at 50 ng/ml and recoveries were 89.2%.

In respect to the extraction recoveries, the aforementioned facts show that multi-walled carbon nanotubes were more effective than or as effective as some other commercially available solid phase extraction adsorbents such as C_{18}, C_8 for the solid phase extraction of BPA. Carbon nanotubes are hollow nanosize tubes that constitute a new structure of graphite carbon consisting of one or several concentric tubules with a helically wound hexagonal honeycomb lattice. The reason why the MWCNT's have stronger interaction with BPA perhaps is that the hexagonal arrays of carbon atoms in graphene sheets in their structures may have strong interaction with the benzene ring in their structures.

Linearity of calibration curves and correlation coefficients

Calibration was performed with mixed standard solutions of the BPA. To determine the range in which response was a linear function of amount injected, standard solutions containing 10, 20, 30, 40 and 50 ppm BPA in the mobile phase were injected. Each solution was injected in twice. Good correlation coefficients (>0.9996)

Table 3. Limits of detection and quantitation and Recovery of BPA from 25 ml Bottled water spiked at 1 ppm after SPE on MWNTs.

Parameter	LOD (ng/ml)	Recovery (%)
BPA	0.30	92.5

Table 4. BPA concentrations in bottled drinking water products.

Examined bottle	Country of origin	Content (ml)	[1]Mean concentration±[2]SD of samples stored at room temperature (ng/ml)	[3]%RSD	[1]Mean concentration±[2]SD of samples exposed to sunlight (ng/ml)	[3]%RSD
1	Sudan	500	3.40±0.15	4.3	8.32±0.33	4.0
2	Sudan	600	4.50±0.18	3.9	7.90±0.36	4.6
3	Sudan	400	4.32±0.14	3.2	10.76±0.30	2.8
4	Sudan	600	3.10±0.13	4.2	9.64±0.32	3.3
5	Sudan	500	6.24±0.17	2.8	10.10±0.31	3.1
6	Sudan	300	3.62±0.14	4.0	12.23±0.24	2.0
7	Sudan	400	3.21±0.12	3.6	16.85±0.20	1.2
8	Sudan	500	4.59±0.10	2.2	14.32±0.23	1.6
9	Sudan	600	5.65±0.11	1.9	12.80±0.27	2.1
10	Sudan	300	4.21±0.13	3.1	15.27±0.23	1.5

[1]Mean concentration (three replicates). [2]Standard deviation. [3]Percentage relative standard deviation.

were obtained. Limits of detection (LOD) were defined as the concentrations giving peak heights five times the standard deviation of the baseline signal. The limits of detection and recovery of BPA from water are shown in Table 3. The compounds were added to the water at a concentration of 1 ppm. The method proposed is characterized by high recovery.

BPA levels in plastic bottled water

Table 4 shows that the mean concentration of BPA in ten bottled water brands was 4.28 ng/ml for those stored at room temperature (25°C) and 11.81 ng/ml for those exposed to sunlight (40°C). The concentration of BPA in bottled water exposed to sun light was significantly higher than those stored at room temperature. Among the bottled water brands stored at room temperature, the difference between their BPA concentrations was not significant, e.g., 1 vs. 6, 2 vs. 8, 3 vs. 10, 4 vs. 7, 5 vs. 9. The same observation applied to those exposed to sun light and stored outdoor.

Because of the trace concentration of BPA and to facilitate the desired sensitivity and selectivity of HPLC measurements, we used SPE as an efficient pre-concentration step and it has several advantages over other separation techniques in view of trace and major concentrations of trace ions present in hazardous samples can be removed with equal ease, higher

enrichment factors, reusability of the adsorbent, absence of emulsion, minimal costs due to low consumption of reagents, ease of automation, environmental friendly and safety with respect to hazardous samples. Accordingly, several solid phase extractants have been employed for enrichment of traces of elements from dilute solutions.

In this respect, SPE has commonly been used as a selective technique for pre-concentration/separation of various inorganic species and enhanced selectivity is achieved because of the discriminatory binding of the analyte to a solid support and accumulated analyte is subsequently eluted with a small volume of solvent. The adsorbents with high surface area are preferred in solid phase extraction of traces of BPA. MWCNTs are adsorbent materials in the pre-concentration step for solid phase extraction method. According to our literature review, until now, the MWNTs had much better extraction capacity for the target, BPA in bottled water samples and more suitable to extract BPA in samples. In a word, MWCNTs would be a valuable and alternative adsorbent for the trace level analysis of BPA, and will be widely used in analytical and environmental fields.

Polycarbonate plastic has been the material of choice for food and beverage product containers for nearly 50 years, because it is lightweight, highly shatter-resistant, and transparent. It is widely used in various food containers and many other everyday items. Many plastic bottled water companies in Sudan use polycarbonate plastic for water bottles. Toyo'oka et al. (2008) found

the concentration of BPA in drinking water bottles made from polycarbonate to be between 3 and 10 ng/ml. While another study did not detect BPA in different plastic containers for beverages, including drinking water (Shao et al., 2005). Our results are in agreement with other studies which reported the presence of plastic residues in water stored in bottles (Tokuanaga et al., 2008; Amiridou and Voutsa, 2011). This could be attributed to the migration of BPA from the bottle material to the water since bottle quality may vary depending on the raw material and technology used in bottled water.

The fact that the bottled water products stored at room temperature had lower levels of BPA demonstrate that migration of BPA from bottle coatings into the water is extremely slow and is negligible. But, the bottled water products which exposed to direct sunlight for one week had relatively high levels of BPA due to the fast migration from their bottle coatings into water during the bottle sterilization process.

High concentrations of BPA levels in different bottled water products in this analysis is due to careless exposure of products to heat (sunlight) during storage and transportation. Also, it could be due to differences in can coatings (type, amount, etc.) and sterilization condition (temperature - duration) used by different canned food products companies.

CONCLUSION AND RECOMMENDATIONS

The proposed method involves concentration of BPA residues in drinking water samples by solid-phase extraction, then analysis by high performance liquid chromatography with fluorescence detection. Based on the experimental results, a simple and appropriate method was developed for the solid phase extraction and HPLC determination of BPA in bottled water samples. Under the optimized conditions, detection of limits of 0.30 ng/ml was achieved for BPA. The accuracy of the proposed method was tested by recovery measurement on spiked sample, the recovery of 92.5% were obtained. The experiment proved that BPA is present in the water samples stored at room temperature at concentrations in range 3.10 to 6.24 ng/ml which is lower than the concentrations of water samples exposed to direct sunlight in range from 7.90 to 16.85 ng/ml.

Here are some recommendations of what need to be done to avoid the risky effects of BPA, drink tap water, rely on BPA-free stainless steel water bottles instead of plastic bottles, and always store the bottles in dry, clear and cool place.

REFERENCES

Aldea AS, Porro C, Ojea G, Lago J, Sobrado C, Vieites JM (2008). Migration of BADGE (bisphenol Adiglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) in canned seafood. Cabado Food Chem. Toxicol. 46(5):1674-1680.

Amiridou D, Voutsa D (2011), Alkylphenols and phathalates in bottled waters. J. Hazard. Mater. 185:281-286.

Carvalho AR, Cardoso VV, Rodrigues A, Ferreira E, Benoliel MJ, Duarte EA (2015). Occurrence and analysis of endocrine-disrupting compounds in a water supply system. Environ. Monit. Assess. 187(3):139.

Coulier L, Bradley EL, Bas RC, Verhoeckx KC, Driffield M, Harmer N, Castle LJ Agric (2010). Analysis of reaction products of food contaminants and ingredients: bisphenol Adiglycidyl ether (BADGE) in canned foods. Food Chem. 58(8):4873-82.

Gallart-Ayala H, Moyano E, Galceran MT (2010). On-line solid phase extraction fast liquid chromatography-tandem mass spectrometry for the analysis of bisphenol A and its chlorinated derivatives in water samples. J. Chromatogr A. 1217(21):3511-358.

Hadjmohammadi MR, Peyrovi M, Biparva P (2010). Comparison of C18 silica and multi-walled carbon nanotubes as the adsorbents for the solid-phase extraction of Chlorpyrifos and Phosalone in water samples using HPLC. J. Sep. Sci. 33(8):1044-1051.

Hao-Chang Su, Xiao-Bing Yang, Li Wang (2010). Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles. Environment International. 36(6):557-562.

Joskow R, Barr DB, Barr JR, Calafat AM, Needham LL, Rubin C (2006). Exposure to bisphenol A from bis-glycidyldimethacrylate-based dental sealants. J. Am. Dent. Assoc. 137(3):353-362.

Joaquim M, José Manuel C, Raquel S, Juana B, Maria Eugenia C, José Juan S (2010). Effect of amines in the release of bisphenol A from polycarbonate baby bottles. 43(5):1283-1288.

Jiang Y, Tang T, Cao Z, Shi G, Zhou TJ (2015). Determination of three estrogens and bisphenol A by functional ionic liquid dispersive liquid-phase microextraction coupled with ultra performance liquid chromatography and ultraviolet detection. Sep. Sci. 38(12):2158-2166.

Lane RF, Adams CD, Randtke SJ, Carter RE Jr (2015). Bisphenol diglycidyl ethers and bisphenol A and their hydrolysis in drinking water. Water Res. 72:331-339.

Larki A, Nasrabadi MR, Pourreza N. Forensic (2015). UV-vis spectrophotometric determination of trinitrotoluene (TNT) with trioctylmethylammonium chloride as ion pair assisted and disperser agent after dispersive liquid-liquid micro extraction. Sci. Int. 251:77-82.

Letcher RJ, Sanderson JT, Bokkers A, Giesy JP, van den Berg M (2005). Effects of bisphenol A-related diphenylalkanes on vitellogenin production in male carp (*Cyprinus carpio*) hepatocytes and aromatase (CYP19) activity in human H295R adrenocortical carcinoma cells. 209(2):95-104.

Liu X, Yu Y, Zhao M, Zhang H, Li Y, Duan G (2014). Solid phase extraction using magnetic core mesoporous shell microspheres with C18-modified interior pore-walls for residue analysis of cephalosporins in milk by LC-MS/MS. Food Chem. 150:206-212.

Rivas MA, Granada A, Monteagudo C, Murcia MA, Olea-Serrano (2009). Dietary exposure assessment of pregnant women to bisphenol-A from cans and microwave containers in Southern Spain. Mariscal-Arcas. Food Chem. Toxicol. 47(2):506-510.

Ma J, Lu X, Xia Y, Yan FJ (2015). Determination of pyrazole and pyrrole pesticides in environmental water samples by solid-phase extraction using multi-walled carbon nanotubes as adsorbent coupled with high-performance liquid chromatography Chromatogr Sci. 53(2):380-384.

Munguia-Lopez EM, Soto-Valdez HJ Agric (2001). Effect of heat processing and storage time on migration of bisphenol A (BPA) and bisphenol A-diglycidyl ether (BADGE) to aqueous food simulant from Mexican can coatings. Food Chem. 49(8):3666-3671.

Pei L, Ehong Z, Qiong D, Dan D (2008). Multiwalled carbon nanotubes micro column preconcentration and determination of gold in geological and water samples by FAAS. 20:0584-8574.

Syed SL (2011). Analysis of bisphenol A leaching from baby feeding bottles. Agilent Technologies, Inc. Bangalore, India.

Schecter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, Birnbaum L (2010). Bisphenol A (BPA) in U.S. food. 44(24):9425-30.

Shan XM, Shen DH, Wang BS, LU BB, Huang FY (2014). Simultaneous Determination of Bisphenols and Alkylphenols in Water by Solid Phase Extraction and Ultra Performance Liquid Chromatography-

tandem Mass Spectrometry. Biomed. Environ. Sci.27(6):471-474.

Shao HJ, Hu J, Wu G, Xue Y, Ma Y, Zhan S (2005). Determination of alkayelphenol and bisphenol A in beverages using liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chim Acta. 530:245-252.

Sung-Hyun N, Young-Min S, Man-Goo K (2010). Bisphenol A migration from polycarbonate baby bottle with repeated use. 79(9):949-952.

Tokuanaga OH, Liu X, TakayMastushima A, Shimohigashi Y (2008). Direct evidence revealing structural elements essential for the high binding ability of bisphenol a to estrogen-realeted receptor gamma environmental Health Perspect. 116(1):32-38.

John WL, Irving WW (2013). High Performance Liquid Chromatography: Fundamental Principles and Practice. Blackie Academic and Professional. 2 September, 978-0-7514-0076-2.

Wu X, Ding L, Li Z, Zhang Y, Liu X, Wang LS Pu (2010). Determination of the migration of bisphenol diglycidyl ethers from food contact materials by high performance chromatography-tandem mass spectrometry coupled with multi-walled carbon nanotubes solid phase extraction. 28(11):1094-1098.

Yang Y, Niu Y, Yang Y, Zhang J, Shao BW, Sheng YJ (2013). An on-line solid phase extraction column switching liquid chromatography tandem mass spectrometry system to analyze bisphenol A and nonylphenol in drinking water. 42(1):127-132.

Yaqi C, Guibin J, Jingfu L (2003). Solid-phase extraction of several phthalate esters from environmental water samples on a column packed with polytetrafluoroethylene turnings. Anal Sci. 19(11):1491-1494.

Full Length Research Paper

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

Nesta Bortey-Sam[1], Shouta M. M. Nakayama[1], Yoshinori Ikenaka[1], Osei Akoto[2], Elvis Baidoo[2], Hazuki Mizukawa[1] and Mayumi Ishizuka[1]*

[1]Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Japan.
[2]Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Previous studies revealed high levels of metals in soils, drinking water, foodstuffs and food animals in several communities in Tarkwa, Ghana. Therefore wild rats were trapped from 16 communities in Tarkwa to estimate the environmental pollution state of metals; determine differences in sex in metal accumulation; and assess the potential risks involved. Concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) were measured in the livers and kidneys of wild rats; 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 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 a biological effect remains 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.

Key words: Wild rats, heavy metal, metalloid, liver, kidney, Ghana.

INTRODUCTION

Anthropogenic activities including artisanal and small-scale gold mining have caused elevated levels of heavy metal 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, immunosuppression, 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).

Tarkwa (05°18′00″N; 01°59′00″W) is a town in the southwest of Ghana, with a population of 90,477 (Ghana Statistical Service, 2010). It is a noted centre for gold and manganese 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 nearly a century of gold mining history and the largest concentration of mining companies in a single district in Ghana and the West African sub-region (Akabzaa and Darimani, 2001).

Studies conducted by Asante et al. (2007), Bortey-Sam et al. (2015a, b, c, d) 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 arsenic (As) and mecury (Hg) than maximum levels by European Commission (EC) (2006), World Health Organization (WHO) (1996; 2011) and United States Environmental Protection Agency (USEPA) (2004; 2012). Bortey-Sam et al. (2015a) indicated that the concentrations of some heavy metals/metalloid 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 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 metals 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 metals and metalloid accumulation; to examine the relationship between body weight of rats and metal; to identify the possible exposure route of these metals to wild rats in Tarkwa; and to examine the potential risks 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 (Samahu), 3.4 (Abekuase) and 5.2 (T-Tamso) km away from the mines, respectively (Figure 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., 2015d). 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.

Sample preparation and metal analysis

Preparations and digestion of liver and kidney samples for heavy metals and metalloid analysis were done according to method described by Bortey-Sam et al. (2015d). 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, HNO_3 (Kanto Chemical Corp., Tokyo, Japan) and 1 ml of (30%) hydrogen peroxide, H_2O_2 (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),
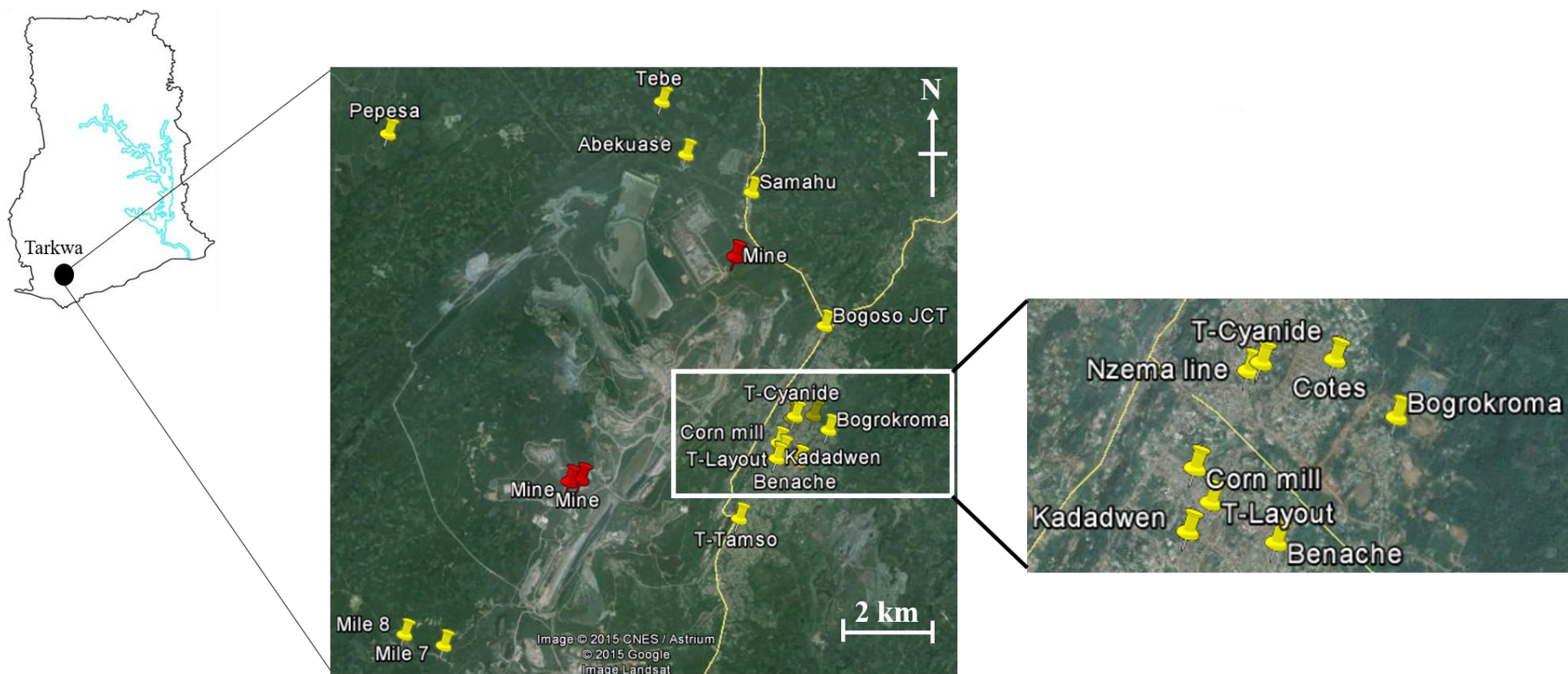
Figure 1. Map showing wild rats sample locations in Tarkwa, Ghana (yellow and red and pins indicate sampled communities and gold mines, respectively).

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 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 weight, and significant level was p value 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

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

Samples		n	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; $p < 0.05$.

(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 following paragraphs discusses As, Pb and Cd which were classified as the first, second and seventh most hazardous substances (Agency for Toxic Substances and Disease Registry (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., 2015a, b, d). The levels were attributed to processing of the ore which involves roasting, and this result in the

production of arsenic trioxide gas which is distributed throughout the study area by air current (Amonoo-Neizer et al., 1995).

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., 2015a, c, d). 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., 2015d), 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

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; $p < 0.05$).

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., 2015d).

Sex differences in heavy metal and metalloid accumulation

Bio-accumulation of heavy metals in animals vary 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 ($p > 0.05$). 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]), respectively. 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; Kowel, 1988; Taguchi and Suzuki, 1981) and these could be the reasons why Cd levels were higher in females than males.

Heavy metals correlation with body weight of wild rats

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

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

Metal	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.

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 weight. This trend suggests that the increased body weight of obese OVX rats may have provided an additional stimulus for bone formation in the weight-bearing long bones (Wronski et al., 1987). The findings of Wronski et al. (1987) were 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 weight of rats.

Possible sources of heavy metal and 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. (2015a, b, c). As shown in Figure 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. (2015d) 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 (Figure 2).

Toxicological significance

The levels of heavy metals and 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. (2015a, d). Recently, electronic wastes are disposed and often burnt at refuse dumps.

Nakayama et al. (2013) studied heavy metals in wild rats 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
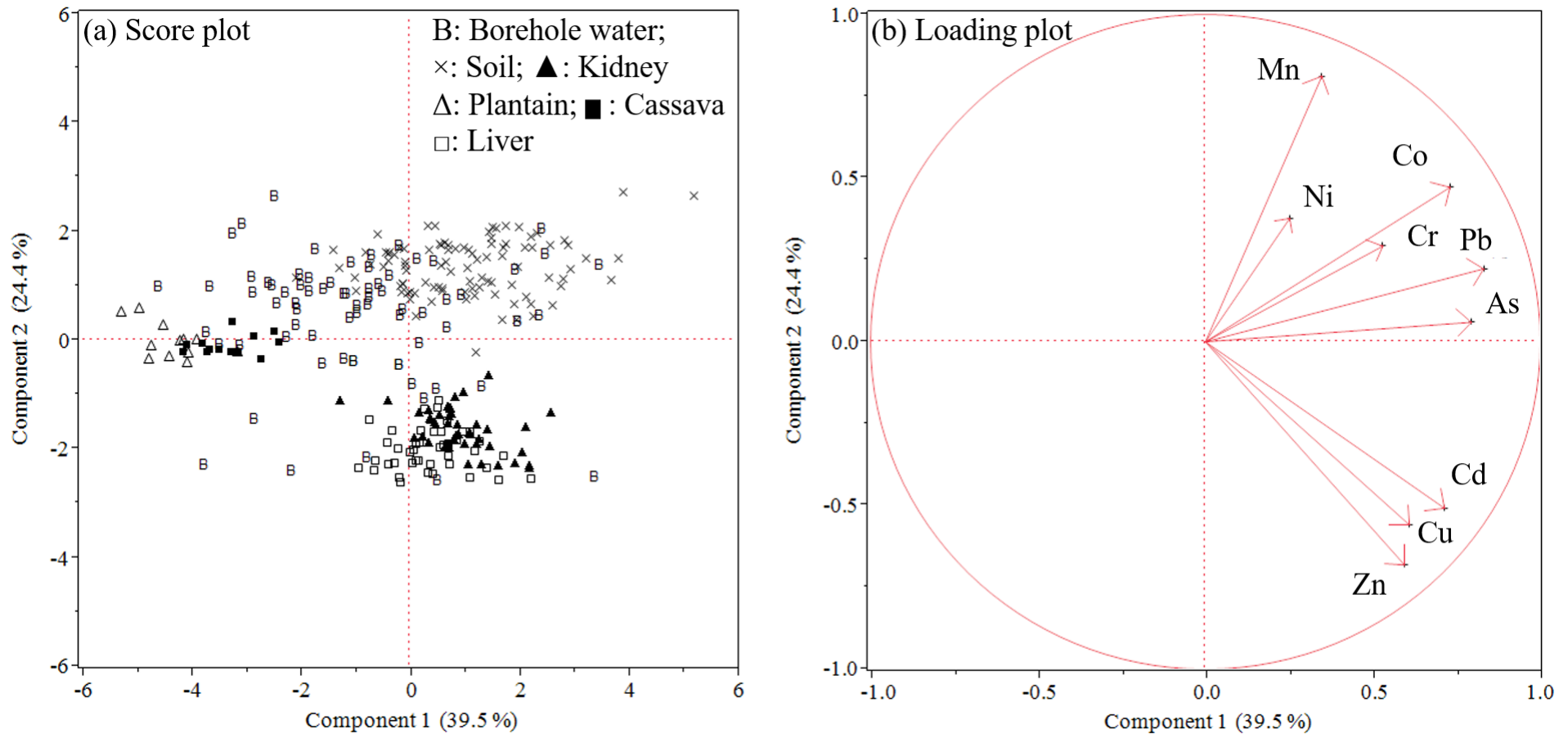
Figure 2. Distribution pattern of heavy metals and a metalloid in cassava, plantain, borehole drinking water, soils, livers and kidneys of wild rats in Tarkwa characterized by PCA.

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).

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 to 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

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 (This study)	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 (This study)	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).

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 wild life.

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 the male rats. The strong positive correlation between body weight 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 caused 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 caused glomerular swelling in 9% of rats. With the rapid increase in mining in Ghana and high concentrations and possible risk of metals to wild life, 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.
3. Set policies to curb the rate of metal pollution in Ghana.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Abou-Arab AAK (2001). Heavy metal contents in Egyptian meat and the role of detergent washing on their levels. Food Chem. Toxicol. 39(6):593-599.
Akabzaa T, Darimani A (2001). Impact of mining sector investment in Ghana: a study of the Tarkwa mining region. Health Impacts 6(4):60.

Alfven T, Elinder CG, Carlsson MD, Grubb A, Hellstrom L, Persson B, Pettersson C, Spång G, Schütz A, Järup L(2000). Low-level cadmium exposure and osteoporosis. J. Bone Miner. Res. 15:1579-1586.
Amonoo-Neizer EH, Nyamah D, Bakiamoh SB (1995). Mercury and arsenic pollution in soil and biological samples around mining towns if Obuasi, Ghana. Water Air Soil Pollut. 91:363-373.
Asante KA, Agusa T, Subramanian A, Ansa-Asare OD, Biney CA, Tanabe S (2007). Contamination status of arsenic and other trace elements in drinking water and residents from Tarkwa, a historic mining township in Ghana. Chemosphere 66:1513-1522.
ATSDR (2013). Agency for Toxic Substances and Disease Registry. Minimum risk levels (MRLs). Available online at http://www.atsdr.cdc.gov/mrls/pdfs/atsdr_mrls_february_2012.pdf
Barhoumi R, Faske J, Liu X, Tjalkens RB (2004). Manganese potentiates lipopolysaccharide-induced expression of NOS2 in C6 glioma cells through mitochondrial-dependent activation of nuclear factor kappaB. Mol. Brain Res. 122:167-179.
Battaglia A, Ghidini S, Campanini G, Spaggiari R (2005). Heavy metal contamination in little owl (Athene noctua) and common buzzard (Buteo buteo) from northern Italy. Ecotoxicol. Environ. Saf. 60:61-66.
Berglund M, Akesson A, Nermell B, Vahter M (1994). Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environ. Health Perspect. 102:1058-1066.
Blagojevic J, Jovanović V, Stamenković G, Jojić V, Bugarski-Stanojević V, Adnađević T, Vujošević M (2012). Age differences in bioaccumulation of heavy metals in populations of the black-striped field mouse, Apodemusagrarius (Rodentia, Mammalia). Int. J. Environ. Res. 6(4):1045-1052.
Bortey-Sam N, Nakayama SM, Akoto O, Ikenaka Y, Baidoo E, Mizukawa H, Ishizuka M (2015a). Ecological Risk of Heavy Metals and a Metalloid in Agricultural Soils in Tarkwa, Ghana. Int. J. Environ. Res. Public Health 12(9):11448-11465.
Bortey-Sam N, Nakayama SM, Akoto O, Ikenaka Y, Fobil JN, Baidoo E, Mizukawa H, Ishizuka M (2015c). Accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa area in Ghana, and associated human health risks. Int. J. Environ. Res. Public Health 12(8):8811-8827.
Bortey-Sam N, Nakayama SM, Ikenaka Y, Akoto O, Baidoo E, Mizukawa H, Ishizuka M (2015b). Health risk assessment of heavy metals and metalloid in drinking water from communities near gold mines in Tarkwa, Ghana. Environ. Monit. Assess. 187(7):1-12.
Bortey-Sam N, Nakayama SM, Ikenaka Y, Akoto O, Baidoo E, Yohannes YB, Mizukawa H, Ishizuka M (2015d). 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). Ecotoxicol. Environ. Saf. 111:160-167.
Brzoska MM, Majewska K, Moniuszko-Jakoniuk J (2004). Mineral status and mechanical properties of lumbar spine of female rats chronically exposed to various levels of cadmium. Bone 34(3):517-526.
Ceruti R, Ghisleni G, Ferretti E, Cammarata S, Sonzogni O, Scanziani E (2002). Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. Environ. Pollut. 117:255-259.
Chen X, Wang G, Li X, Gan C, Zhu G, Jin T, Wang Z (2013). Environmental level of cadmium exposure stimulates osteoclasts formation in male rats. Food Chem. Toxicol. 60:530-535.
Crossgrove J, Zheng W (2004). Manganese toxicity upon overexposure. NMR Biomed. 17:544-553.
Damek-Poprawa M, Sawicka-Kapusta K (2004). Histopathological changes in the liver, kidneys, and testes of bank voles environmentally exposed to heavy metal emissions from the steelworks and zinc smelter in Poland. Environ. Res. 92:72-78.
Daniell HW (1976). Osteoporosis of the slender smoker. Obstet. Gynecol. Surv. 31(11):808-810.
Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA, Wong BA (2006). Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. Toxicol. Sci. 92:201-210.
EC (2006). European Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in

foodstuff. Off. J. Eur. Union.

Finley JW (1998). Manganese uptake and release by cultured human hepato-carcinoma (Hep-G2) cells. Biol. Trace Elem. Res. 64:101-118.

Flanagan PR, McLellan JS, Haist J, Cherian MG, Chamberlain MJ, Valberg LS (1978). Increased dietary cadmium absorption in mice and human subjects with iron deficiency. Gastroenterology 74:841–846.

Florea AM, Busselberg D (2006). Occurrence, use and potential toxic effects of metals and metal compounds. Biometals 19(4):419-427.

Ghana Statistical Service (2010). Population and Housing census. https://www.google.co.uk/#q=Ghana+Statistical+Service%2C+2010.+Population+and+Housing+census+ P 93.

Godowicz B (1988). Cadmium-induced liver and spleen damage in inbred mouse strains. Folia Biol. 36:47-52.

Guerrero-Castilla A, Olivero-Verbel J, Marrugo-Negrete J (2014). Heavy metals in wild house mice from coal-mining areas of Colombia and expression of genes related to oxidative stress, DNA damage and exposure to metals. Mutat. Res Gen. Tox. Environ. 762:24-29.

Gupta UC (1998). Trace element toxicity relationships to crop production and livestock and human health: implications for management. Commun. Soil Sci. Plan. 29:1491-1522.

Hamilton DL, Valberg LS (1974). Relationship between cadmium and iron absorption. Am. J. Physiol. 227:1033–1037.

Hayford EK, Amin A, Osae EK, Kutu J (2008). Impact of gold mining on soil and some staple foods collected from selected mining communities in and around Tarkwa Prestea Area. West African J. Appl. Ecol. 14:1-12.

Homady M, Helmi H, Jiries A, Mahasneh A, AlNasir F, Khleifat K (2002). Survey of some heavy metals in sediments from vehicular service stations in Jordan and their effects on social aggression in prepubertal male mice. Environ. Res. 8 (1):43-49.

Honda R, Tsuritani I, Noborisaka Y, Suzuki H, Ishizaki M (2003). Urinary cadmium excretion is correlated with calcaneal bone mass in Japanese women living in an urban area. Environ. Res. 91:63-70.

Hunter BA, Johnson MS, Thompson DJ (1989). Ecotoxicology of copper and cadmium in contaminated grassland ecosystem. 4. Tissue distribution and age accumulation in small mammals. J. Appl. Ecol. 26:89–99.

Husain A, Al-Rashdan A, Al-Awadhi A, Mahgoub B, Al-Amiri H (1996). Toxic metals in food products originating from locally reared animals in Kuwait. Bull. Environ. Contam. Toxicol. 57:549-555.

Jarup L (2002). Cadmium overload and toxicity. Nephrol. Dial Transplant 17(2):35-39.

Kabata-Pendias A, Pendias H (1992). Trace Elements in Soils and Plants, second ed. CRC Press, London, P 413.

Kowel NE (1988). Urinary cadmium and beta2-microglobulin: correlation with nutrition and smoking history. J. Toxicol. Environ. Health 25:179–183.

Licata P, Naccari F, Lo Turco V, Rando R, Di Bella G, Dugo G (2010). Levels of Cd (II), Mn (II), Pb (II), Cu (II), and Zn (II) in Common Buzzard (Buteo buteo) from Sicily (Italy) by Derivative Stripping Potentiometry. Int. J. Ecol. 1-7.

Lindsay R, Dempster DW, Clemens T, Herrington BS, Wilt S (1984). Incidence, cost, and risk factors of fracture of the proximal femur in the USA. Osteoporosis 1:311-315.

Liu J, Liu Y, Goyer RA, Achanzar W, Waalkes MP (2000). Metallothionein-I/II null mice are more sensitive than wild-type mice to the hepatotoxic and nephrotoxic effects of chronic oral or injected inorganic arsenicals. Toxicol. Sci. 55:460-467.

López-Alonso M, Miranda M, García-Partida P, Cantero F, Hernández J, Benedito JL (2007). Use of dogs as indicators of metal exposure in rural and urban habitats in NW Spain. Sci. Total Environ. 372:668-675.

Ma WC (2011). Lead in mammals. In Beyer & Meador (Eds.), Environmental contaminants in biota: interpreting tissue concentrations (2nd ed.). New York: CRC Press pp. 595-607.

Ma WC, Denneman W, Faber J (1991). Hazardous exposure of ground living small mammals to cadmium and lead in contaminated

terrestrial ecosystem. Arch. Environ. Contam. Toxicol. 20:266-270.

Martinez-Finley EJ, Gavin CE, Aschner M, Gunter TE (2013). Manganese neurotoxicity and the role of reactive oxygen species. Free Radic. Biol. Med. 62:65-75.

Naccari C, Cristani M, Cimino F, Arcoraci T, Trombetta D (2009). Common buzzards (Buteo buteo) bio-indicators of heavy metals pollution in Sicily (Italy). Environ. Int. 35:594-598.

Nakayama SM, Ikenaka Y, Hamada K, Muzandu K, Choongo K, Teraoka H, Mizuno N, Ishizuka M (2011). Metal and metalloid contamination in roadside soil and wild rats around a Pb–Zn mine in Kabwe, Zambia. Environ. Pollut. 159(1):175-181.

Nakayama SM, Ikenaka Y, Hamada K, Muzandu K, Choongo K, Yabe J, Umemura T, Ishizuka M (2013). Accumulation and biological effects of metals in wild rats in mining areas of Zambia. Environ. Monit. Assess. 185(6):4907-4918.

Obiri S (2007). Determination of heavy metals in water from boreholes in Dumasi in the Wassa West District of the Western Region of the Republic of Ghana. Environ. Monit. Assess. 130:455-463.

Pereira R, Pereira ML, Ribeiro R, Gonçalves F (2006). Tissues and hair residues and histopathology in wild rats (Rattus rattus L.) and Algerian mice (Mus spretus Lataste) from an abandoned mine area (Southeast Portugal). Environ. Pollut. 139:561-575.

Pompe-Gotal J, Crnic AP (2002). Cadmium in tissue of roe deer (Capreolus capreolus) in Croatia. Vet. Arh. 72:303-310.

Saville PD, Nilsson BE (1966). Height and weight in symptomatic postmenopausal osteoporosis. Clin. Orthop. Relat. Res. 45:49-54.

Sawicka-Kapusta K, Zakrzewska M, Kowalska A, Lenda B, Skrobacz M (1995). Heavy metal concentrations in small mammals from Borecka forest. Arch. Ochr. Środ. 3(4):229-234.

Scheuhammer AM (1987). The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. Environ. Pollut. 46:263-295.

Soewu DA, Agbolade OM, Oladunjoye RY, Ayodele IA (2014). Bioaccumulation of heavy metals in cane rat (Thryonomys swinderianus) in Ogun State, Nigeria. J.Toxicol. Environ. Health Sci. 6(8):154-160.

Swiergosz R (2001). Cadmium distribution and toxicity in tissues of small rodents. Microsc. Res. Tech. 55:208-222.

Taguchi T, Suzuki S (1981). Influence of sex and age on the biological half-life of cadmium in mice. J. Toxicol. Environ. Health 7:239–249.

Tohyama C, Sugihira N, Saito H (1987). Critical concentration of cadmium for renal toxicity in rats. J. Toxicol. Environ. Health 22:255-259.

Uriu K, Morimoto I, Kai K, Okazaki Y, Okada Y, Qie YL, et al. (2000). Uncoupling between bone formation and resorption in ovariectomized rats with chronic cadmium exposure. Toxicol. Appl. Pharmacol. 164:264-272.

USEPA (United States Environmental Protection Agency) (2004). Framework for Inorganic Metals Risk Assessment; USEPA: Washington, DC, USA.

USEPA (United States Environmental Protection Agency) (2012). Ground water and drinking water http://www.water.epa.gov/drink

Waalkes MP, Ward JM, Liu J, Diwan BA (2003). Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol. Appl. Pharmacol. 186:7-17.

Whelton BD, Peterson DP, Moretti ES, Dare H, Bhattacharyya MH (1997). Skeletal changes in multiparous, nulliparous and ovariectomized mice fed either a nutrient-sufficient or -deficient diet containing cadmium. Toxicology 119:103-121.

WHO (World Health Organization) (1981). Environmental Health Criteria 18. Arsenic. International Programme on Chemical Safety.World Health Organization, Finland.

WHO (World Health Organization) (1996). Trace Elements in Human Nutrition and Health. Geneva.

WHO (World Health Organization) (2011). Guidelines for drinking-water quality (4th ed., http://www.whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf). Geneva

Wronski TJ, Schenk PA, Cintron M, Walsh CC (1987). Effect of body weight on osteopenia in ovariectomized rats. Calcif. Tissue Int. 40(3):155-159.

Short Communication

Study on the effectiveness of five cleaning solutions in removing chlorpyrifos residues in cauliflower (*Brassica oleracea*)

M. F. M. Nowowi[1]*, M. A. M. Ishak[2], K. Ismail[1] and S. R. Zakaria[3]

[1]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA, Perlis Branch, 02600 Arau, Perlis, Malaysia.
[3]Faculty of Applied Sciences, Universiti Teknologi MARA, Pahang Branch, 26400 Jengka, Pahang, Malaysia.

Nowadays, contents of pesticide residues in vegetables had become one of the hot topics closely related to human health. In order to investigate the effectiveness of several cleaning solutions in removing pesticide residues in vegetables, a polluted cauliflower was washed using five types of cleaning solutions which were soda-salt solution, vinegar solution, tamarind juice solution, filtered flour solution, and tap water. Chlorpyrifos was extracted from residues cleaning process and cauliflower itself. Gas chromatography-electron capture detector was used to identify content in residues cleaning process (RCP). Tamarind juice solution had the greatest removal effect, in relation to other of cleaning solutions, with 93.04% removal rate followed by filtered flour solution (17.03%) and vinegar solution (11.42%). However, soda-salt solution and tap water did not have any removal effect in removing chlorpyrifos in cauliflower. Tamarind juice solution was significantly higher than other types of cleaning solutions and tamarind juice solutions itself is a natural fruit juice and non-chemical substance with very easy preparation; therefore, the tamarind juice solution can be used as a general-purpose cleaning solution to remove chlorpyrifos residues in cauliflower.

Key words: Pesticide residues, chlorpyrifos, cleaning solutions, removal effect.

INTRODUCTION

Consuming vegetables and cereals in daily meals is very important. The nutrients that are provided mostly covered all of the reactions in the human body system (Keikotlhaile and Spanoghe, 2011). However, vegetables can be toxic to humans due to pesticide usage (EL-Saeid and Selim, 2013).

Twenty three years ago at Aging City of Anhui Province in China, eleven people have died because of breakfast. After the case investigation, expert found that all the victim had consumed wheat spoiled by organophosphorus pesticides (Hui et al., 2003). Vegetables contamination caused by pesticide residues causes a serious problem to human health. Pesticide residues in vegetables are noxious metabolites produced by usage of pesticide for
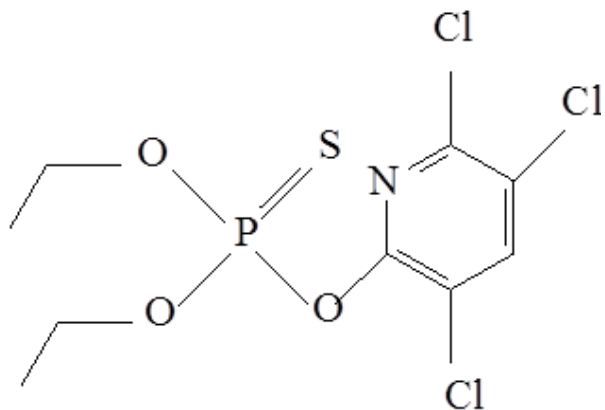
Figure 1. Structure of chlorpyrifos (Ion and Ion, 2012).

long duration in vegetables planting.

The existence of pesticide residues in vegetables can lead to contamination in its nutrient and may give a concern to the consumers regarding the maximum residue limits (MRLs) of vegetables (EL-Saeid and Selim, 2013; Dasika et al., 2012). Based on Food Regulation 1985, Ministry of Health Malaysia has set particular level of MRLs for all pesticides in specific food class to all farmers. Aly et al. (2010) mentioned that chlorpyrifos (O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl)-phosphorothioate) is certified as a wide spectrum pesticide and commonly used to manage various types of pest in agriculture field and livestock (US EPA, 1986) and the structures of chlorpyrifos is as shown in Figure 1. Based on previous studies, there are several methods used to remove chlorpyrifos in vegetables and fruits. The methods include wash treatment using some acid (Osman et al., 2014), hydrostatic pressure process (Iizuka et al., 2013), ozonation (Kusvuran et al., 2012), and horizontal sub-surface flow constructed wetlands (SSFCW) (Agudelo et al., 2010). However, these methods were difficult to be conducted. In order to attain an effective technique in removing chlorpyrifos residues in cauliflower, five types of cleaning solutions were made to examine their effectiveness based on percentage of removal from the concentration of chlorpyrifos on samples before and after cleaning process.

MATERIALS AND METHODS

Preparation of cleaning solution

Five types of cleaning solutions were prepared as follows:

Soda-salt solution: For the soda-salt solution (5%), 5 g of edible soda and 5 g of salt was added into 1 L of water. Then, the solution was stirred until completely dissolved.

Tamarind juice solution: 50 g of tamarind without its seed was weighted. Then, 1 L of water was added to tamarind and it was soaked for 15 min.

Flour solution: For the flour solution, 50 g of flour was weighted. Then, the flour was poured into 1 L of hot water; the solution was stirred until completely dissolved and filtered from the suspension.

Vinegar solution: For the vinegar solution, 100 ml of vinegar was measured. Then, 900 ml of water was added into vinegar, the solution was stirred lightly to dissolve the solutions. If there was residue present in the solutions, the solution was filtered and supernatant was collected.

Preparation of simulated chlorpyrifos pollution in cauliflower

Preparation of chlopyrifos mixture: 2.0 L of water was added into the 0.95 ml of chlorpyrifos. After water was added, the mixture was stirred until dissolved.

Preparation of chlorpyrifos pollution in cauliflower: A cauliflower of 1000 g was bought from the supermarket and washed with tap water and dried with absorbent paper. Then, the cauliflower was soaked in chlorpyrifos mixture for 10 min and stored at room temperature in a dark room for 24 h.

Method of cleaning samples and extraction of pesticide residues

The contaminated cauliflower was divided into five parts, of which three parts each were soaked with tap water, soda-salt solutions, tamarind juice solutions, filtered flour solutions, and vinegar solutions for 10 min separately. Then, the cauliflower was rinsed off with running tap water for 1 min. The residues cleaning process was extracted using Solid Phase Extraction (SPE).

Gas chromatography-electron capture detector (GC- ECD)

Extracted samples were injected onto GC-ECD model 7890A-Agilent (Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, US) for quantitative analysis. The GC-ECD instrument is made up of two parts. The GC portion separates the chemical mixture into pulses of pure chemicals and electron capture detector was equipped with GC. The GC separates chemicals based on their volatility which they evaporate into a gas. The chemicals in the mixture separate based on their volatility. Electron capture detector converted the sample and represents it via chromatogram. Table 1 shows the instrument set up for detection of chlorpyrifos in solutions after cleaning process.

RESULTS AND DISCUSSION

The concentration of the chlorpyrifos in which the cauliflower was soaked was 7.3407×10^{-4} mol/L.

Analysis of residues cleaning process

Cleaning solutions were made and the pH of each solution was checked before undergoing the cleaning process. The residues from cleaning process were analysed to determine the presence of chlorpyrifos. Table 2 tabulates the concentration of chlorpyrifos in the residues cleaning

Table 1. Instrument set up for detection of chlorpyrifos in solutions after cleaning process

Injector temperature	280°C
Detector temperature	300°C
Carrier gas flow rate	20.0 ml min^{-1} (nitrogen)
Column temperature	Initial temperature 165°C for 3 min, increase to 260°C at 3°C min^{-1} with final time of 2 min

Table 2. Concentration of chlorpyrifos in the residues cleaning process.

Types of cleaning solution	pH	Type of acidity	Concentrations of chlorpyrifos (mol/L)
Tamarind juices solution	1.88	Acid	6.8299×10^{-4}
Filtered flour solutions	5.52	Acid	1.2504×10^{-4}
Vinegar solutions	2.02	Acid	8.3828×10^{-5}
Soda-salt solutions	10.82	Base	0.0000
Tap water	6.39	Neutral	0.0000

process which had been analysed using GC-ECD. The concentrations of five RCP were calculated.

From Table 2, mostly acid cleaning solutions have shown the presence of chlorpyrifos in their residues. However, there is no presence of chlorpyrifos in the basic cleaning solutions by GC-ECD.

Comparison of the effect of five cleaning solutions in removing chlorpyrifos in cauliflower with control

The effectiveness of cleaning solution was determined based on their removal rates of chlorpyrifos in cauliflower shown in Table 3 which was determined after the cauliflower had been cleaned using five types of cleaning solutions.

As shown in Table 3, after the cauliflower had been cleaned using five types of cleaning solutions, the removal rates of chlorpyrifos by using tamarind juices solutions is very good compared to others cleaning solutions. While filtered flour and vinegar solutions removal rates are the same which is less than 20%. However, by using soda-salt solution and tap water, there are no removal of chlorpyrifos detected.

The effect of each cleaning solutions in removing pesticide residues

Tamarind juice solution

Based on Table 3, tamarind juices solution has great removal rates. By using tamarind juice, the chlorpyrifos in cauliflower can be removed at high percentages of removal rate is about 93.04%. The usage of tamarind juices solution as a cleaning solution in removing pesticide residues is undiscovered yet. However, the pH of tamarind

juices solution is in acidic medium. In tamarind, there are two types of volatile constituent which contribute to the acidity of the tamarind juices which is furan derivatives and carboxylic acids which is about 44.4 and 38.2%. Based on previous study, chlorpyrifos degradation is very slow in acidic medium. The more the acidic of the solution react with chlorpyrifos, the slower the degradation of chlorpyrifos (Singh et al., 2003). Tamarind juices solutions can be used as universal cleaning solution to remove chlorpyrifos in cauliflower.

Filtered flour solutions

Using filtered flour solutions in cleaning process, the result have shown that the removal rates have big difference compared to tamarind juice solutions. The removal rate is about 17.03%. However, filtered flour solutions have better removal effect compared to vinegar solutions. In term of stability, filtered flour solutions are stable for three days only. After that, filtered flour solution start to release bad odour. Nicotinic acid was presented in large quantity of about 4.60 mg in 100 g of flour due to the manufacturer labelled. Based on consumer medicine information, nicotinic acid was used to reduce cholesterol. Chlorpyrifos have their own metabolites which can produce their own cholesterol after they had been consumed with food or contact with vegetables (Watts, 2013). In addition, filtered flour solutions can be used as cleaning solutions to partially reduce pesticide residues in term of preparation which is very simple.

Vinegar solutions

Vinegar solutions have shown the ability in removing chlorpyrifos residues in cauliflower. The removal rate is

Table 3. Removal effects of chlorpyrifos residues after cauliflower has been washed using five type of cleaning solution.

Cleaning solution	Removal rates of chlorpyrifos (%)
Tamarind juices solution	93.04
Filtered flour solutions	17.03
Vinegar solutions	11.42
Soda- salt solutions	0.00
Tap water	0.00

about 11.42% which is slightly lower than flour solutions. Vinegar solutions consist of acetic acid and water. Based on Howard (2012), 10% of vinegar with 90% of water can be used as a pesticide residues remover. In addition, vinegar solutions can be used as cleaning solution to certain kinds of pesticides. Thus, the use of vinegar solution in removing chlorpyrifos residues is proved; although, the removal rates are less than 20%.

Soda-salt solution and tap water

Compared to other cleaning solutions, soda-salt solution and tap water have no removal effect on chlorpyrifos residues. Based on previous study, it is shown that soda-salt has a good removal effect on other type of pesticides. Yu-shan et al. (2013) proved that soda-salts have very good removal effect on mixed pesticide residues consisting of three types of pesticides which are dimethoate, dicofol, and cyhalothrin. The removal rates of soda-salt solutions in removing these three types of pesticide residues are very good results which are 32.5% of dimethoate, 26.9% of dicofol, and 44.4% of cyhalothrin (Yu-shan et al., 2013). However, in this project, the removing of chlorpyrifos residues cannot be detected due to the degradation of chlorpyrifos in basic medium. Besides that, soda-salt solutions have very high basic pH which is 10.82. From the previous study, the degradation of chlorpyrifos is very fast in basic medium and it was stated that in pH 8.4, the degradation of chlorpyrifos with half-life is about 16 days (Singh et al., 2003). The higher the alkalinity of solutions, the degradation of chlorpyrifos became faster. So it is difficult to detect the presence of chlorpyrifos in the sample.

In soda-salt solutions, there is a factor that had effect on the percentages of removal. After the residues cleaning process was extracted using Solid-Phase Extraction, the sample was stored for long before its analysis. So, the chlorpyrifos in the sample was degraded due to long period of time kept in basic condition.

For tap water, the removal rate is same as soda-salt solution. However, soda-salt solutions can be used as pesticides remover for other kind of pesticides. In tap water, there is no component that can contribute in removing pesticide residues. So, using tap water in removing chlorpyrifos residues in cauliflower is not good.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Agudelo RM, Peñuela G, Aguirre NJ, Morató J, Jaramillo ML (2010). Simultaneous removal of chlorpyrifos and dissolved organic carbon using horizontal sub-surface flow pilot wetlands. Ecol. Eng. 36(10):1401-1408.

Aly N, EL-Gendy K, Mahmoud F, El-Sebae AK (2010). Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pestic. Biochem. Physiol. 97(1):7-12.

Dasika R, Tangirala S, Naishadham P (2012). Pesticide residue analysis of fruits and vegetables. J. Environ. Chem. Ecotoxicol. 4(2):19-28.

EL-Saeid MH, Selim MT (2013). Multiresidue Analysis of 86 Pesticides Using Gas Chromatography Mass Spectrometry: II-Non-leafy Vegetables. J. Chem. 2012:1-10.

Food Regulation (1985). Sixteenth Schedule, Regulation 41, Food Safety Information System of Malaysia (FoSIM), Ministry of Health Malaysia. Available at: http://fsis2.moh.gov.my/UploadFosim/FAR/040810103612F595SIXT EENTH%20SCHEDULE.pdf

Howard G (2012). Simple trick removes pesticides from your vegetables and fruits. Natural health. Available at: http://articles.mercola.com/sites/articles/archive/2012/08/20/vinegar-a s-non-toxic-cleaner.aspx

Hui X, Yi Q, Bu-zhuo P, Xiliu J, Xiao-mei H (2003). Environmental pesticide pollution and its countermeasures in China. AMBIO: J. Hum. Environ. 32(1):78-80.

Iizuka T, Maeda S, Shimizu A (2013). Removal of pesticide residue in cherry tomato by hydrostatic pressure. J. Food Eng. 116(4):796-800.

Ion I, Ion AC (2012). Determination of chlorpyriphos in broccoli using a voltammetric acetylcholinesterase sensor based on carbon nanostructure–chitosan composite material. Mater. Sci. Eng. C 32(4):1001-1004.

Keikotlhaile BM, Spanoghe P (2011). Pesticide residues in fruits and vegetables. InTech. pp. 243-252.

Kusvuran E, Yildirim D, Mavruk F, Ceyhan M (2012). Removal of chloropyrifos ethyl, tetradifon and chlorothalonil pesticide residues from citrus by using ozone. J. Hazard. Mater. 241(242):287-300.

Osman KA, Al-Humaid AI, Al-Redhaiman KN, El-Mergawi RA (2014). Safety methods for chlorpyrifos removal from date fruits and its relation with sugars, phenolics and antioxidant capacity of fruits. J. Food Sci. Tech. 51(9):1762-1772.

Singh BK, Walker A, Morgan JAW, Wright DJ (2003). Effects of Soil pH on the Biodegradation of Chlorpyrifos and Isolation of a Chlorpyrifos-Degrading Bacterium 69(9):5198-5206.

United State Environmental Protection Agency (USEPA) (1986). Ambient Water Quality Criteria for Chlorpyrifos, Report, US, Washington, DC.

Watts M (2013). Chlorpyrifos, Pesticide Action Network Asia and the Pacific (April). 68 p.

Yu-shan SZ, Li XP, Liu HM, Zhang YK, Zhao FF, Yu Q (2013). Study on universal cleaning solution in removing blended pesticide residues in Chinese cabbage. J. Environ. Chem. Toxicol. 5(8):202-207.

Journal of Environmental Chemistry and Ecotoxicology

Related Journals Published by Academic Journals

- African Journal of Pure and Applied Chemistry
- International Journal of Physical Sciences
- Journal of Geology and Mining Research
- African Journal of Mathematics and Computer Science Research
- Journal of Internet and Information Systems
- Journal of Oceanography and Marine Science
- Journal of Petroleum Technology and Alternative Fuels