The background of the cover is a photograph of a yellow biohazard container, likely a sharps container, with a black radiation symbol on its side. The container is slightly out of focus, and the lighting is bright, creating some highlights and shadows on its surface.

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ARTICLE

- Identification and quantification of phthalates in water and sediment of Ori Stream, Iwo, Southwestern Nigeria using high performance liquid chromatography** 82
Godwin O. Olutona and Modupe O. Dawodu

Full Length Research Paper

Identification and quantification of phthalates in water and sediment of Ori Stream, Iwo, Southwestern Nigeria using high performance liquid chromatography

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This study provides data on distribution of phthalate esters (PAEs) in the water and sediments in the Ori stream, Iwo, Nigeria. The liquid-liquid extraction method was employed, followed by cleaned up in an alumina column using hexane. Chromatographic separation and quantification were done using Zorbax Eclipse AAA C18 analytical column. The concentrations of DMP, DBP, DEP, and DPP in water ($\mu\text{g/L}$) ranged from 1.29 ± 1.3 in DBP to 938 ± 780 in DMP while in sediment ($\mu\text{g/kg}$) ranged from 12.7 ± 15 in DEP to 152 ± 140 $\mu\text{g/kg}$ in DBP. These concentrations were above the USEPA limits of 3 $\mu\text{g/L}$ as recommended for phthalates in water.

Key words: Phthalates, liquid-liquid extraction, high performance liquid chromatography (HPLC), endocrine disruptors, stream.

INTRODUCTION

Phthalate esters (PAEs) are well known polymer additives and widely used in industries as plasticizers for polymeric products especially cellulose esters and vinyl chloride copolymers (Adeniyi et al., 2011; EU-RAR, 2008); and non-plasticizers such as paints, varnishes, adhesive, cosmetics, insect repellants, insecticides carriers, propellants, building materials, automobile parts and food packaging (EU-RAR, 2008; Staples et al.1997; Gobas et al., 2004; Zheng et al., 2010) with the aim of improving their flexibility, transparency, durability, longevity and altering physical properties like malleability and flame resistant of synthetic products (Arago et al., 2010; Kanchanamayoon et al., 2012). Chemically, phthalates are stable, colour-, odour- and flavorless, soluble in water liquids over a long temperature range

(Clara et al., 2010). Estimate of 8 billion tones of phthalate esters are reported being used globally each year particularly as additives to plastics products, industrial chemicals and parts of several consumer products (Blount et al, 2000).

Due to the additives nature of these contaminants, phthalates exist freely and are not chemically bound to the polymer chain in a freely mobile phase and drain away; hence they can drain away, migrate or evaporate into the environment via domestic and industrial effluents, sewage sludge, storm water, runoffs and indiscriminate dumping of phthalate products (Adeniyi et al., 2011; Fujii et al., 2003; Gomez-Hens et al., 2003; Afshari et al., 2004). These contaminants have been found in air, food, water and soil, organism, tissues and fluid of wild life,

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developmental projects have extended to the area. Due to the rising human needs, a lot of activities such as open markets, motor parks, abattoir, irrigation farming activities, car wash, filling stations, technical school and spiritual bathing taking place along the course of the stream has led the stream to be subjected to various eutrophic, disruptive and thermal stress as it flows through the city and empty itself into Oba river (Akindele et al., 2015).

Sampling

Water and sediment samples were collected from five different sites (St 1-5) as shown in the study area map (Figure 1). Water samples were collected in amber whinchester bottle (2.5 L) to prevent bacteria growth. The water and sediment samples were collected with stainless steel auger and grab samplers. Sediment samples were scooped using a pre-cleaned stainless steel scoop into solvent-rinsed aluminium containers. The samples were stored cold in the refrigerator prior to analysis.

Apparatus and reagent used

All bottles and glassware used in the study were earlier steeped in 10% nitric acid for 48 h, washed with detergent, rinsed three times with tap water before finally rinsed with distilled water then with acetone (99.5%) and n-hexane (99.0%) mixture (1:1 v/v) and then heated in an oven at a temperature of 105°C for 1 h prior to use. The entire reagent used, viz. dichloromethane and n-hexane (GFS chemicals, Inc Columbus); acetone, acetonitrile, ethyl acetate (Park Scientific Ltd. Northampton, United Kingdom); Alumina from Lab Tech Chemicals; Sodium sulphate and sodium chloride (BDH, England). Standard mixture of phthalate ester and n-butyl benzoate were purchased from Sigma Aldrich, South Africa.

Extraction procedures

Liquid-liquid chromatographic extraction method was employed for this experiment. 500 mL of the sample were measured into a separatory funnel and this was spiked with 0.1 mL (100 ppm) of the internal standard. Ten (10 g) of NaCl was used to saturate the sample so as to destabilize the formation of emulsions from the solvent. Three portions of 25 mL dichloromethane were then employed to extract the sample. Three portion extracts from each sample were put together in another separatory funnel and further extracted with three times of 5 mL 0.1 M Sodium carbonate. This was done to remove the free-fatty acid (FFA) from the organic phase that had been extracted by dichloromethane (Fatoki and Ogunfowokan, 1993; Ogunfowokan et al., 2006). The extracts were then dried over anhydrous sodium sulphate. The extracts were evaporated over steam bath and the residue re-dissolved in 1 mL of dichloromethane. Sediment samples were extracted in Soxhlet extractor with dichloromethane. This was then clean-up in alumina column.

Clean up procedure

A column of about 15 cm × 1 cm (i.d) was packed with about 10 g activated alumina prepared in a slurry form in n-hexane. About 0.5 cm³ of anhydrous sodium sulphate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 15 mL of n-hexane and 30 mL ethyl acetate. The eluate was collected, was then concentrated to about 1 mL by evaporation to dryness under a stream of analytical grade nitrogen gas. 1 mL of acetonitrile was added to the reduced extract for HPLC analysis (Fatoki and Ogunfowokan, 1993; Ogunfowokan

et al., 2006). The extract was refrigerated prior to instrumental analysis was conducted.

Recovery experiment

In this study, no standard phthalate esters reference materials were available; hence, recovery analysis was conducted in order to establish the efficiency of the analytical procedures used. Recovery study was carried out by spiking 500 mL organic free water with 0.1 mL (1000 ppm) of phthalate standard mixture and was extracted and treated the same way as the sample.

Stock solution preparation and estimation of response factor

One hundred ppm stock solution of the mixture of the dibutyl (DBP), dimethyl (DMP), diethyl (DEP) and diphenyl (DPP) and n-butyl benzoate (internal standards) was prepared by weighing 1.0 mg each of the DMP, DEP, DBP and DPP and internal standards using weighing boat into a beaker, and make up to mark in 10 mL standard flask with acetonitrile. The stock solution with internal standard was run on high performance liquid chromatography for the determination of response factor which was calculated using the equation below (Fatoki and Noma, 2001).

$$\text{Response Factor} = \frac{\text{Peak Area of Phthalate}}{\text{Peak area of Internal Standard}}$$

Instrumental analysis

High-performance liquid chromatography (HPLC) was done with Agilent HPLC 1260 infinity series available at the Central Science Laboratory of Bowen University, Iwo, which was used in determining the presence and level of phthalate ester in the water and sediment samples. The HPLC conditions were as follows: Zorbax Eclipse AAA C18 column 150 mm × 4.6 mm i.d., particle size of 3.5 µm, 228 nm wavelength, temperature 40°C, injection volume 20 µl and gradient elution condition using acetonitrile and water (90:10 v/v) as mobile phase. Under this condition separation lasted for about 4 min with flow rate of 1 mL/min for the first four minutes then 2 mL/min. Identification of individual phthalate ester was established based on its retention time and quantification was performed by combination of internal standardization and response factor.

RESULTS AND DISCUSSION

The efficiency and reliability of the analytical method employed was determined by the analysis of the response factor, retention time and percentage recovery. The phthalates were eluted from the column in the order of dimethyl- (DMP), diethyl- (DEP), dibutyl- (DBP), and diphenyl phthalates (DPP). The response factor and retention time were indicated in Table 1. The recovery analysis was done to proof the reliability of the analytical method employed. The percentage recoveries ranged from 78.0% in diethyl- and 94.3% in diphenyl-.

The quantitative results of phthalates in water and sediment at the designating sampling locations along the Ori stream are presented in Tables 2 and 3. The concentrations of phthalates obtained in this study

Table 1. Values of response factor, retention time and percent recoveries.

Compound	Response factor	Retention time (min)	% Recovery
DEP	0.27	1.86	78.0
DBP	0.85	2.96	85.5
DMP	0.21	1.62	88.7
DPP	0.47	2.25	94.3
n-Butyl benzoate: IS		1.28	

Table 2. Levels of phthalate esters ($\mu\text{g/L}$) in water samples from Ori stream.

Station	DEP	DBP	DMP	DPP	$\Sigma_4\text{PEs}$
St1 Ori	ND	ND	74.8 \pm 45	ND	75
St 2 Agogo	ND	ND	1980 \pm 62	ND	1980
St 3 Osadep	505 \pm 74	ND	1440 \pm 570	ND	1945
St 4 Technical	ND	6.43 \pm 3.10	81.4 \pm 48	21.3 \pm 13	109
St 5 Oba	ND	ND	1110 \pm 560	6.35 \pm 9.0	1116
Mean	101 \pm 16	1.29 \pm 1.3	938 \pm 780	5.53 \pm 6.5	

Table 3. Levels of phthalate esters ($\mu\text{g/kg}$) in sediment samples from Ori stream.

Station	DEP	DBP	DMP	DPP	$\Sigma_4\text{PEs}$
St 1 Ori	21.2 \pm 19	110 \pm 59	12.9 \pm 8.3	18.0 \pm 1.1	162
St 2 Agogo	10.8 \pm 5.3	213 \pm 130	11.7 \pm 6.5	27.2 \pm 16	360
St 3 Osadep	2.45 \pm 3.5	86.1 \pm 9.9	28.5 \pm 20	24.1 \pm 13	141
St 4 Technical	25.5 \pm 7.5	54.3 \pm 10	118 \pm 13	51.1 \pm 3.1	249
St 5 Oba	3.50 \pm 2.9	295 \pm 160	ND	55.2 \pm 46	354
Mean	12.7 \pm 15	152 \pm 140	34.2 \pm 28	35.1 \pm 26	

showed a number of occurrence of phthalate in both water and sediment of Ori stream.

Table 2 presents the levels of phthalate esters in water samples. The phthalates esters determined in the water > DBP. A quick glance at the concentrations of phthalate esters showed the presence of dimethyl phthalate in all sites. Generally, the levels of DMP are higher than all other phthalate compounds. Dibutyl phthalate was detected only in St 4 (Technical) while diphenyl phthalate were detected in St 4 and 5 (Technical and Oba). The mean levels of phthalate in the stream water ranged from 1.29 $\mu\text{g/L}$ for dibutyl phthalate to 938 $\mu\text{g/L}$ for dimethyl phthalate. The level of dilution between DBP and DMP was above 450 fold. This could be attributed to the weak and van der waal force interaction of phthalate esters which contributes to the slow decomplexation of phthalate-solute complexes observed. The $\Sigma_4\text{PEs}$ concentrations ranged from 75 $\mu\text{g/L}$ at St1 (Ori) to 1980 $\mu\text{g/L}$ at St 2 (Agogo). The highest $\Sigma_4\text{PEs}$ concentrations were found at St2, St 3 (Osadep) followed by St 5(Oba). It could also be observed that the levels of phthalate

ester decreases from the Upstream St 1(Ori) downstream. The presence of phthalate ester in this water body could be due to local contamination from domestic wastes including disposable plastic materials (such as tiles, wire coatings, synthetic leathers, wall papers, wrapping materials and plastic soft drink bottles) and agricultural wastes, from motor park and market area thrown into the water body at St 1(Ori), farming as well as recreational activities occurring along the stream bank. Burning of tyres at abattoir located at St 1(Ori) and refuse incineration could also be a good source of phthalates in this water body. The values reported in this study for the stream are above the USEPA water criteria of 3 $\mu\text{g/L}$ for the survival of fish and other aquatic life in river (USEPA, 1980).

Our results for the stream, are above those reported elsewhere for river, e.g New England Rivers, United States (1-30 $\mu\text{g/L}$) (Hites, 1973); the Delaware River, United States (0.3 – 50 $\mu\text{g/L}$ for DBP and DEHP) (Sheldon and Hites, 1979); River Irwell and Etherow in the Manchester area of United kingdom (0.2 – 33.5 $\mu\text{g/L}$)

Table 4. Comparison of PAEs in sediment measured in this study with those reported from other locations ($\mu\text{g/g}$).

Location	No. of target PAEs	No of sample sites	Range of ΣPAEs	DMP	DEP	DBP	DPP	Sample year	Reference
Ori stream	4	6	0.141-0.36	0.034	0.013	0.152	0.035	2015	This study
Qiantang River, China	16	23	0.585-6.74	0.053	0.018	-	-	2011	Sun et al. (2013)
Gomti River, India	5	30	0-0.364	0.011	0.005	-	-	2008	Mathur et al. (2010)
False Creek Harbour, Canada	13	4	0.004-2.1	0.038	0.021	-	-	2004-06	Mackintosh et al. (2006)
Ogun River, Nigeria	4	6	0.325-2.88	0.214	0.200	-	-	2005	Adeniyi et al. (2011)
Lake of Guangzhou, China	15	16	2.27-74.94	0.039	0.13	-	-	2005	Zeng et al. (2008)
Yellow River, China	5	7	30.52-85.16	2.594	5.049	-	-	2004	Xia et al. (2007)
Yongding River, China	16	12	0.679-2.119	0.009	0.022	-	-	2003	Wang et al. (2006)
Rivers of Taiwan	8	6	2.9-27.1	-	0.200	-	-	2000	Chang et al. (2002)

(Fatoki and Vernon, 1990); River Ronneybyan (0.32-3.10 $\mu\text{g/l}$ and River Svartan (0.39 to 1.98 $\mu\text{g/l}$), Sweden (Thuren 1986), University Teaching Hospital Sewer waste and receiving stream 2.05 to 62.81 $\mu\text{g/L}$ and 0.18 to 9.17 $\mu\text{g/L}$, respectively (Adewuyi, 2012).

Table 3 present the levels of phthalate esters in sediment samples of Ori stream. All the four phthalate esters analyzed were detected at all the sites with exception of DMP which was not detected at St 5 (Oba). The mean values of phthalate esters in the sediment samples ranged from 12.7 $\mu\text{g/l}$ for DEP to 152 $\mu\text{g/l}$ for DBP. The order of prominence of phthalate esters detected were DBP > DPP > DMP > DEP. The levels of DBP and DPP were extremely low in water sample while DEP were extremely low in sediment sample. The $\Sigma_4\text{PAEs}$ concentrations ranged from 141 $\mu\text{g/kg}$ at St 3 (Osadep) to 354 $\mu\text{g/kg}$ at St 5 (Oba). It could also be observed that the sediment record display relatively diversified results which could be an indication of photolysis and biodegradation conditions. Comparative study of PAEs concentration in sediment of Ori stream with those measured in other locations over the world revealed a medium concentrations magnitude (Table 4).

The occurrence of DMP and DEP PAEs in water and sediment of Ori stream might be as a result of their widely use as additives in products, such as perfumes, lotions, cosmetics, toys, adhesives, inks, varnishes (Sun et al., 2013). Similarly, the occurrence of DBP usually used in epoxy resin and cellulose ester containing products such as disposable medical tubes, blood storage bags, toys that found their ways into the water body (Heise and Litz, 2004). The $\Sigma_4\text{PEs}$ levels in surface water were higher than that of the sediments. This may suggest PAEs fresh inputs into the water body and partitioning of organic contaminant in the aquatic ecosystem depends on the physical and chemical parameters of the individual compounds such as sorption coefficient, vapour pressure, solubility, suspended organic matter and sediment characteristics (Zeng et al., 2008). Other factors that may responsible for different levels of PAEs at different sites and aqueous matrices include adsorption, deposition of suspended particles, microbial or photo-chemical degradation and dilution in the partitioning systems of dissolved and sediment-associated phase (Tao et al., 2005). The hydrophobic nature couple with high octanol-water ratio and low aqueous solubility of PAEs tend to

be bounded to particulate and dissolved matter as a result of low flow velocity (Chen et al., 1999). The low flow velocity of the stream enhances the deposition of suspended particles which are easily bonded by organic contaminants than coarse particles (Chen et al., 2006).

Conclusion

This study provides first hand information on the contamination levels of four PAEs in the water and sediments of the Ori stream, Iwo, south-western, Nigeria. The levels of phthalate esters recorded in this study were at levels that raise concern. The state environmental protection agency needs to adopt a constant monitoring plan so that the concentrations of this persistent organic pollutant discharged into the water body are in conformity with international standard.

Conflict of Interests

The authors have not declared any conflict of interests.


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