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RESEARCH ARTICLE

Determination of Phthalate Esters in the Aquatic Environment

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Abstract

The use of solid phase extraction and capillary GLC provides the basis for selective determination of phthalate ester plasticizers in rivers and marine water samples. Of the several solvent ratios (methanol in dichloromethane) that were tried for selective elution of phthalate esters from the C18 solid phase glass catridge, the 50/50 ratio, CH_3OH in CH_2Cl_2 (v/v) gave the best result. The method was tested on river and marine water samples that receive effluent from industries that use phthalate esters. The rivers and marine water samples are grossly polluted as several phthalate esters, for example, dimethyl(DMP), diethyl(DEP), dibutyl(DBP) and diethylhexyl(DEHP) were found present at 0.03 – 2 306 $\pm$ 9.4 $\mu g\ l^{-1}$. A study on uncontaminated water was done to establish bank levels.

Keywords Phthalates, Plasticizers, Solid Phase Gas Chromatography.

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1. Introduction

In the last decade, there has been a growing interest and concern for the study of the impact of man-made chemicals on wildlife and humans. These studies have suggested that synthetic and naturally occurring substances in the environment may affect the normal function of the endocrine systems. These substances are also referred to as endocrine disrupting chemicals (EDCs)[1]. In wildlife, alterations in sexual reproductive behaviour have been reported in areas of contamination with EDCs. For example, malformations in the sexual organs of alligators have been reported in Lake Apopka, Florida, where high concentrations of DDT and its degradation products have been detected[2,3], and feminization of trout in the Great Lakes has been associated with high levels of polychlorinated biphenyls (PCBs) in water samples[4]. Other studies have indicated that many chemicals including phthalate esters may affect development and reproduction, including germ cells, sperm mobility, chryptorchidism and hypospadias, in laboratory animals[5-8].

Phthalate ester plasticizers are widely used in synthetic polymers, especially polyvinyl – chloride commonly used for packaging, storing and preserving food[9], in insect repellant preparations, cosmetics, decorative inks, munitions, and industrial and lubricating oil[10,11]. The ubiquity of phthalate esters has been widely reported in various environmental samples in the developed countries of Europe and America. Their occurrence has been reported in the Greater Manchetser River[12]. They were found in Philadelphia drinking water[13] and in tap water from the Municipal Institute of Environmental Health Sciences, Shinike, Japan[14]. They were found in the water, fish, and other aquatic organisms of the Gulf of Mexico[15] and in sediments, bivalves, and from estuaries of the River Crouch[16]. They are suspected to be carcinogenic[17,18] and lipophilic and they tend to concentrate along the ecological food chains, a process known as bio-amplification[19]. Since humans are usually at the top of the food chain, high concentrations of such toxic substances may occur in the human diet with undesirable results. The reports of their toxicity should make it important to have knowledge of the presence of these compounds in our environment. One of the main routes of exposure is via water as these chemicals find their ways into rivers through effluent discharges, leaching from waste dumps and through diffuse sources.

Several attempts have been made to determine phthalate esters in the aquatic environment by gas liquid chromatography (GLC) with electron capture detector[19-22] and FID[12,23,24]. Other methods include the use of GCMS[25] and differential pulse polarography[26].

A major problem in the analyses of environmental samples is the reduction of background contamination to levels less than the very low (parts per billion or ppb) levels generally present in the samples. This problem of background contamination has been more serious in the trace analysis of phthalates than in the studies of many other pollutants (including the chlorinated hydrocarbons) because phthalates are present in almost all equipment and reagents used in the laboratory. Non-platsic materials like cork, glass wool, Teflon sheets and aluminum foil have been found to contain the most prevalent of the phthalates, di-2-ethylhexyl phthalate (DEHP) which often results in high background levels[15,27].

The aim of this study was to develop a solid phase extraction procedure for the determination of phthalates in water and to reduce background contamination. The method was tested on rivers and on marine water samples that receive effluents from industries that use phthalate esters in the Eastern Cape province of South Africa.

2. Experimental

2.1. Apparatus

A Perkin Elmer Autosystem XL capillary gas chromatograph with a flame ionization detector connected to a workstation powered by Turbochrom 4 was used.

2.2. Reagents

All chemicals used were of analytical reagent grade. All solvents used were further purified by distillation. Standard phthalate esters – dimelthyl(DMP), diethyl(DEP), dibutyl(DBP) and diethylhexyl(DEHP) purchased from Merck – Schuchard (with percentage assay greater than 99%) were used.

2.3. Preparation of Stock Standard Solution and Determination of Response Factors

A stock solution (1 gl^{-1}) of the mixture of esters dimethyl(DMP), diethyl(DEP), dibutyl(DBP) and diethylhexyl(DEHP) in methanol was prepared in one flask. The required volume of ester was calculated from the density of each of the esters. 1 gl^{-1} n-butyl benzoate (a non-aqueous pollutant) in methanol was used as internal standard. The stock solution containing the internal standard was run on the GC. The response factor was calculated from:

Area of the peak of phthalate ester/ Area of the peak of internal standard.

2.4. Determination of the Limit of Detection (LOD) of GC System

The LOD was calculated considering y_b+3S_b for each calibration curve[28] for each phthalate esters (DMP-, DEP-, DBP- and DEHP-), with the range 2.5 – 50 mgl^{-1} (S_b = standard error of the regression line and Y_b is the blank value).

2.5. Quality Assurance Studies

A twelve- (12) port glass micro-extractor tank was used. The column (syringe) barrels were the Envi. C18 (1 g packing) designed for environmental samples and purchased from Supelco. The vacuum in the tank was created using a water-jet pump. Columns were first conditioned by passing deionized water through, followed by about 2 ml CH_3OH.

1 l each of deionized water samples was spiked, respectively, with 1 ml of 500 mgl^{-1} of standard mixtures of dimethyl(DMP), diethyl(DEP), dibutyl(DBP) and diethylhexyl(DEHP). The spiked samples were each passed through the preconditioned C18 columns at a flow rate of 1ml min^{-1}. Thereafter the columns were dried, by blowing nitrogen through them. 2-ml mixture each, of variable portions of CH_3OH in CH_2Cl_2 (10:90, 30:70, 50:50, 70:30 and 90:10,v/v) were used separately to elute the columns. The eluent from each column was dried under vacuum in sample vials. Once dry, 1 ml of 1000 mgl^{-1} n-butyl benzoate in methanol was added as internal standard to each of the residues. 0.1 μl each of the solutions from a 1 μl syringe was injected on the capillary

GC for analysis. Triplicate analyses were performed for each ratio of solvent mixture using the GC conditions as described below.

2.6. Determination of Blank Levels

1 l deionized water was passed through a pre-conditioned column (same as above) at a flow of 1ml/min without the standard phthalate esters. Thereafter the column was dried by blowing nitrogen gas through it. 2 ml of CH_3OH in CH_2Cl_2 (50:50, v/v) were used to elute the column. The eluent was dried under vacuum and 1ml of internal standard in methanol was added to dissolve the residue. 0.1 μl of resulting solution from a 1 μl syringe was run on the GC using similar GC conditions as described below.

2.7. Routine Analyses of Water Samples.

The procedure investigated above was tested on water samples taken from rivers and from the harbour environment in the Eastern Cape. Water samples were collected from about 5 - 20 cm below the surface of rivers - River Buffalo, River Swartkops, Umtata River and Keiskamma River, respectively, from Sandile Dam in Keiskammahoek and from East London and Port Elizabeth harbours to determine their phthalate contents. They were immediately acidified with about 5 ml concentrated H_2SO_4 to preserve the samples and stored at about 4 ^oC until analyzed. 2-l water sample was passed through the C18 glass column at a flow rate of 1 ml/min. Thereafter, they were air-dried to get rid of excess water in the packing and then dried by passing nitrogen gas through the column. Once dry the column was eluted with 2 ml of CH_3OH in CH_2Cl_2 (50:50,v/v). The eluent was collected into a 5-ml sample vial and dried under vacuum. 1 ml of internal standard (n-butyl benzoate) in methanol was added to dissolve the residue. 0.2 μl of resulting solution was analyzed by the GC using similar conditions as described below.

2.8. Gas Chromatographic Analysis

Phthalate esters were determined by Perkin Elmer Autosystem XL capillary chromatograph with FID. The GC conditions were as follows: Column, PTE^{TM}-5 X 30 m X 0.25 (I.D.) X 0.25 μm film, purchased from Sulpeco, injector temperature, 180 ^oC, detector temperature, 280 ^oC, temperature programme - initial temp, 180 ^oC/2min, and

final temperature, 280 ^{o}C/7 min., ramp rate, 12 ^{o}C/min, split ratio, 40:1, He, flow rate, 32 ml/min. Identification of compounds in the aqueous extract was based on comparison of the relative retentions of the phthalate ester standards with those in the sample. Quantitation was done by internal standardization, using n-butyl benzoate.

3. Results and Discussion

The phthalates were eluted from the gas chromatographic column in the order DMP, DEP, DBP and DEHP and the response factor of the detectors are DMP, 0.73, DEP, 0.75, DBP, 0.72 and DEHP, 0.55 (Table 1). The limits of detection were 28.5ngl^{-1} for DMP, 27.2 ngl^{-1} for DEP, 42.7 ngl^{-1} for DBP and 60.6 ngl^{-1} for DEHP. The blank determination showed a clean background with no contamination by phthalate esters (Fig 1).

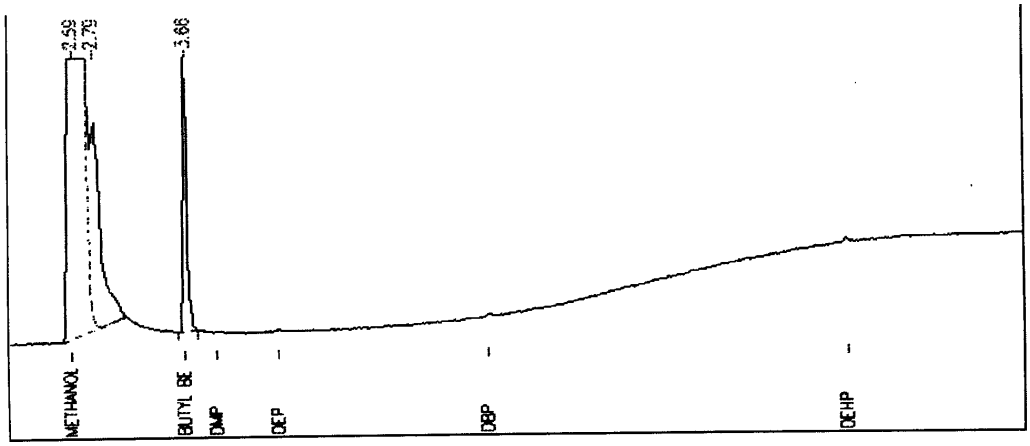

Figure 1 Gas chromatogram showing blank levels of phthalate esters.

The recoveries obtained from spiking experiments ranged from 62% to 94 % for DMP, 59 % to 96 % for DEP, 57 % to 89% for DEP and 42 % to 83 % for DEHP using elution with different ratio of CH_3OH/CH_2Cl_2 (v/v) solvent mixture (Table 2). The recoveries were highest for the phthalate esters when 50/50 ratio (v/v) of CH_3OH in CH_2Cl_2 was used to elute the column. Recoveries varied then between 83 % for DEHP and 96 % for DEP (Table 2) and this ratio of solvent mixture (CH_3OH/CH_2Cl_2) was used for the routine analyses of water samples.

Table 1 Values of response factors (RF) and retention times[a].

Compound	Response Factor	Retention time (min.)
DMP	0.73 ± 0.03	4.36 ± 0.53
DEP	0.75 ± 0.05	5.00 ± 0.50
DBP	0.72 ± 0.06	7.39 ± 0.40
DEHP	0.55 ± 0.04	11.26 ± 0.34
n-Butyl benzoate: IS	----	4.06 ± 0.51

a Data are means ± standard deviation for ten replicate injections of standard phthalate esters. DMP: Dimethyl phthalate; DEP: Diethyl phthalate; DBP: Di -n-butyl phthalate; DEPH: Diethylhexyl phthalate; IS: Internal standard.

Many methods have been described for the clean up of phthalate esters prior to their analysis by gas chromatography[1215,23,29]. The most commonly used solid phases for separation/clean up of environmental samples are deactivated florisil (3% water v/v)[15], alumina and silical gel (5 % water) [12,23] . The solvents used are diethyl either in petroleum[15], and benzene in ethylacelate[12,23]. Large quantities of the eluting solvents, are, however required for each sample, which makes both the elution and concentration time consuming. This may increase the contamination of the samples since the solvents contain impurities. Moreover, the use of benzene as eluting solvent is discouraged because of its toxic effect and the small volume of methanol in dichloromethane (2ml) used in this study to elute the solid phase column, eliminated the problem of disposal of large volume of chlorinated solvent[30], usually encountered when using solvent extraction procedures.

The levels of phthalate esters in the harbours and river water samples are as shown in Table 3 - 5. A representative chromatogram of phthalate esters in harbour water sample is shown in Fig. 2. The levels of phthalates in East London harbour water samples ranged from 0.03 $\mu g l^{-1}$ for DMP to 197.4 $\mu g l^{-1}$ for DEHP (Table 3). The values of phthalate esters for Port Elizabeth harbour water samples varied between 0.03 $\mu g\ l^{-1}$ for DMP and 2 306.8 $\mu g/l^{-1}$ for DEHP (Table 4). The levels in freshwaters ranged from 0.3 $\mu g\ l^{-1}$ for DMP in the Swartkops, Keiskamma and the Buffalo Rivers to 90.4 $\mu g l^{-1}$ for DEHP in Buffalo River (Table 5).

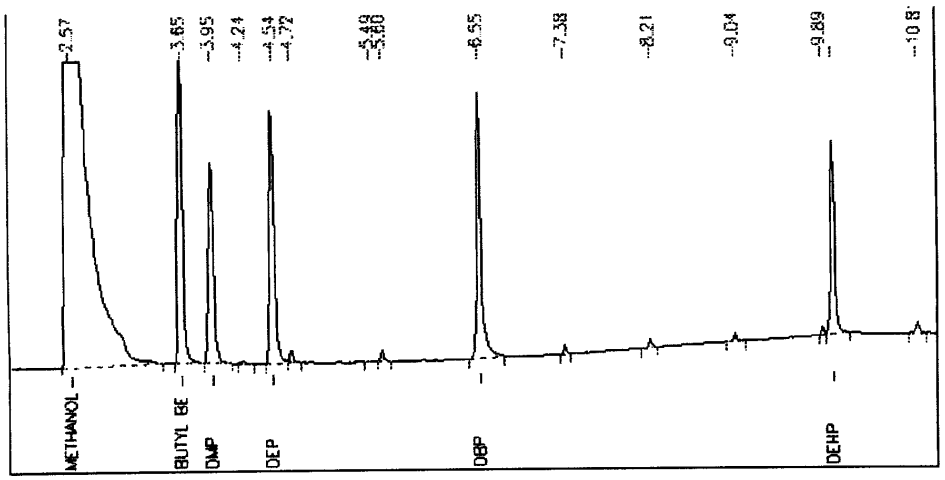

Figure 2 Representative chromatogram of phthalate esters in Port Elizabeth harbour water sample. DMP, DEP, DBP and DEHP are peaks for dimethyl phthalate, diethyl phthalate, dibutyl phthalate and diethylhexyl phthalate, respectively.

Table 2 Mean % recoveries of phthalate esters from spiked water sample by extraction with various ratios of methanol in dichloromethane[a] solvent mixture.

Methanol – Dichloro-methane mixture(v/v)	% DMP	% DEP	% DBP	% DEPH
10:90	62.0 ± 0.1	64.0 ± 0.1	57.0 ± 0.3	42.0 ± 0.4
30:70	69.0 ± 0.1	70.0 ± 0.1	66.0 ± 0.1	49.0 ± 0.4
50:50	**94.0 ± 0.07**	**96.0 ± 0.04**	**89.0 ± 0.06**	**83.0 ± 0.1**
70:30	74.0 ± 0.08	68.0 ± 0.04	71.0 ± 0.07	65.0 ± 0.09
90:10	66.0 ± 0.1	59.0 ± 0.1	60.0 ± 0.08	48.0 ± 0.2

a Data are means ± relative standard deviation for triplicate analysis

In the harbour samples, concentrations of phthalate esters were highest at Site S3 (Ore Berth) with values of esters ranging from 1.4 $\mu g l^{-1}$ for DMP to 2 306.8 $\mu g l^{-1}$ for DEHP. As S3 is a docking site for big ships, the significantly high concentrations of phthalate esters at this site might be due to local contamination from domestic wastes including plastics which were being frequently thrown into the sea from the ships that docked there.

Table 3 Variation of phthalate esters ($\mu g l^{-1}$)[a] in East London harbour water samples taken between May and August 2000.

Sample	Date	Time	DMP	DEP	DBP	DEHP
S1(EL)	18/05/00	11:25	0.03	0.03	15.3±0.8	8.0±0.5
S1(EL)	06/00	12:45	31.7±6.7	30.4±1.7	13.7±1.5	3.8±0.9
S1(EL)	12/06/00	10:10	0.03	5.7±0.9	36.0±2.8	197.4±1.0
S1(EL)	12/07/00	9:10	1.7±1.3	5.4±0.5	15.6±3.3	81.9±10.6
S1(EL)	01/08/00	14:20	0.03	0.03	4.1±0.3	8.2±1.0
S1(EL)	22/08/00	13:40	2.5±0.1	12.8±0.7	14.7±3.4	14.1±0.5
S2(EL)	18/05/00	11:34	20.6±0.9	4.7±1.0	6.3±0.7	3.4±0.2
S2(EL)	12/06/00	10:17	5.5±1.0	4.0±0.9	98.6±5.9	6.3±6.3
S2(EL)	12/07/00	9:35	16.5±0.9	33.1±2.6	121.9±9.7	9.3±3.8
S2(EL)	01/08/00	14:35	0.03	8.2±0.9	27.6±3.4	61.6±3.0
S3(EL)	18/05/00	11:41	8.2±1.2	5.8±1.8	4.1±1.2	0.06
S3(EL)	12/06/00	10:29	4.7±0.2	7.9±1.0	16.0±1.1	9.9±0.9
S3(EL)	12/07/00	9:37	2.9±0.3	6.6±1.0	27.7±1.7	8.0±3.0
S3(EL)	01/08/00	14:43	5.8±0.2	0.03	11.6±0.4	35.4±2.7
S3(EL)	22/08/00	14:10	0.5±0.02	7.3±1.3	2.8±0.4	4.0±0.4

a Data are means ± standard deviation for triplicate analyses. S1(EL), S2(EL) and S3(EL) are samples taken at Orient Pier, Dockyard and West Quay Sites, respectively.

Generally, the levels of phthalate esters in the East London and Port Elizabeth harbours water samples are high. This might be due to local contamination from harbour activities including refuse disposal from ships that dock and from stormwater/streams discharges from urban /industrial areas around the harbours.

Values of phthalate esters are higher at the Port Elizabeth harbour than at the East London harbour. This is expected as Port Elizabeth harbour is a more active port and many large ships dock regularly at the port and they frequently throw their domestic wastes including plastics into the sea.

Table 4 Variation of phthalate esters ($\mu g l^{-1}$)[a] in Port Elizabeth harbour water samples taken between May and November 2000.

Sample	Date	Time	DMP	DEP	DBP	DEHP
S1(PE)	05/00	14:12	0.03	0.03	14.7±1.8	7.4±3.0
S1(PE)	2/06/00	13:15	0.03	4.4±0.9	1.0±0.9	4.5±0.8
S1(PE)	13/06/00	13:16	5.8±1.3	13.2±1.6	10.0±2.8	10.7±3.0
S1(PE)	13/07/00	12:23	88.1±1.0	370.6±15.3	1028.1±13.3	596.8±10.8
S2(PE)	/05/00	14:18	0.03	0.03	11.3±2.4	3.6±0.1
S2(PE)	2/6/00	13:20	0.03	2.1±1.3	2.6±0.7	2.1±1.4
S2(PE)	13/06/00	13:20	61.1±6.3	218.6±10.6	96.7±9.7	25.8±5.8
S2(PE)	13/07/00	12:26	0.03	7.8±0.3	2.6±0.8	14.7±0.2
S2(PE)	10/11/00	09:45	1.4±0.3	8.0±1.0	9.4±0.5	18.1±0.9
S3(PE)	05/00	14:05	350.8±0.2	398.3±1.7	382.2±0.3	334.3±9.6
S3(PE)	02/6/00	13:25	1.4±0.4	5.4±0.6	1.5±1.0	23.0±3.1
S3(PE)	13/6/00	13:27	111.1±10.3	216.5±18.7	435.4±9.1	2306.8±9.4
S3(PE)	13/07/00	12:29	3.2±0.8	11.0±1.0	11.8±0.6	14.8±0.8

a Data are means ± standard deviation for triplicate analyses. S1(PE), S2(PE) and S3(PE) are samples taken at 103 Berth, Slipway and Ore Berth Sites, respectively.

In the freshwater samples, values of phthalate esters were highest in Buffalo River with values of phthalate esters varying between 0.3 $\mu g l^{-1}$ for DMP and 90.5 $\mu g l^{-1}$ for DEHP. Generally, DEHP is the most predominant phthalate esters in the harbour and freshwater samples. This is consistent with the fact that the DEHP is the most widely used phthalate esters[25].

The majority of the phthalate esters in the harbours and rivers water samples are considered due to anthropogenic inputs from various resources which include sewage treatment plants, industries that use phthalate esters, refuse incineration and leaching from disposed plastics wastes from refuse dumps which is a common practise[25].

The values reported in this study for the rivers are higher than the USEPA (U.S. Environmental protection Agency) water criteria of 3 $\mu g l^{-1}$ criteria for the protection of fish and aquatic life in river[31]. There are no EIFAC (Europe) guidelines for phthalate

esters in rivers and no chronic quality criteria of phthalates were available for seawater. Our results for the freshwaters, are higher than those reported elsewhere for rivers polluted with industrial chemicals. For example, New England rivers, US[32], 1-30 μgl^{-1}, the Delaware River, US[33], 0.3 –50 μgl^{-1} for DBP and DEHP and rivers Irwell and Etherow in the Manchester area of the U.K[12], 0.2 – 33.5 μgl^{-1} for the phthalate esters detected in the rivers. They are also higher than those reported for River Ronnebyan (0.32 – 3.10 μgl^{-1}) and River Svartan (0.39 – 1.98 μgl^{-1}), Sweden[19] and for Dutch rivers Rhine, Ijssel and Meuse[24]. They are however lower than the values recorded for phthalate esters in the rivers of Southwestern Nigeria[34].

Table 5 Variation of phthalate esters (μgl^{-1})[a] in major rivers and Sandile Dam, Eastern Cape (May – August 2000).

Sample	Date	Time	DMP	DEP	DBP	DEHP
UR	25/05/00	N.D.	0.03	0.03	0.04	6.6±0.5
SR	13/06/00	14:50	0.03	0.03	11.9±0.5	15.6±0.8
SR	/06/00	14:30	0.03	7.7±0.4	7.7±0.4	37.1±0.8
SR	13/06/00	14:25	0.03	6.9±1.9	0.4±0.1	11.9±1.1
SR	13/07/00	14:10	19.4±0.9	35.6±2.4	75.6±6.7	33.5±3.3
KR	14/06/00	16:38	0.03	0.03	0.04	4.6±0.7
KR	22/08/00	16:45	9.1±0.2	5.7±0.1	6.7±0.9	7.4±0.1
SD	22/08/00	17:12	0.03	0.03	2.1±0.8	12.4±1.8
BR	14/06/00	15:48	0.03	0.03	0.04	7.2±4.0
BR	29/06/00	N.D.	0.03	0.03	3.0±1.1	5.4±2.4
BR	8/7/00	N.D.	1.8±0.01	8.1±0.1	0.7±0.01	6.6±0.2
BR	1/8/00	16:07	2.6±0.3	0.03	2.9±0.2	12.5±2.4
BR	14/08/00	N.D	12.3±2.0	0.03	4.8±0.8	90.5±2.7

a Data are means ± standard deviation for triplicate analyses. UR, SR, KR, SD,and BR are samples taken from Umtata River, Swartkops River, Keiskamma River, Sandile Dam and Buffalo River, respectively. N. D.: Not Determined.

Phthalate esters are chemicals with known endocrine-disrupting properties[5-8]. There is significant concern for their ubiquitous presence in the environment, and

scientists, clinicians and regulatory agencies currently debate their potential for adverse health effects in humans[35,37]. Phthalate esters have been shown to be estrogenic when assayed by the recombinant yeast screen test[38]. Besides their estrogenic activity, phathalates such as DBP have blocking antiandrogen action[39] and Colon et. al.[1] has suggested that these properties of phthalate esters may play a role in the cause of premature breast development in Puerto Rican females. Phthalates have also been shown to show both acute and chronic toxicities to aquatic organisms including fish[40,41] and they have phytotoxic effects on some plants[42].

Phthalate ester plasticizers are loosely linked to the plasticizers and are easily extracted[43]. The most important sources of exposure are ingestion from contaminated formulas, food, and water from contact with plastic wrappings and containers [44-46].

The relatively high levels of phthalates in the rivers are not unexpected because many of these rivers receive effluents from industries and municipal sewage works without treatment or with partial treatment. Presently in South Africa, there is paucity of data on the occurrence and fate of phthalate esters in its aquatic environment, nor have water quality criteria been established for them. The high levels of phthalates recorded in this study raises some concern.

4. Conclusion

Phthalate esters plasticizers were selectively determined in river waters and in marine harbour water samples using the C18 solid phase column. Dimethyl (DMP), diethyl(DEP), dibutyl(DBP) and diethylhexyl (DEMP) were found in the tested river and marine water samples at levels that raise concern.

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