Methods for selective determination of persistent organochlorine pesticide residues in water and sediments by capillary gas chromatography and electron-capture detection

O.S. Fatoki*, R.O. Awofolu

Department of Chemistry, University of Fort Hare, Alice 5700, South Africa

Abstract

Different extraction methods were evaluated for the determination of fifteen organochlorine pesticides (OCPs) in water and sediments. Liquid–liquid extraction (LLE) was evaluated for the pesticides analyses in water while Soxhlet extraction (SE) and microwave assisted extraction (MAE) methods were compared in sediment. Of all the extracting solvents used, dichloromethane gave the best results. Percentage recoveries ranged from 71.03±8.15 (dieldrin) to 101.25±2.17% [α-benzenehexachloride (α-BHC)] in water with LLE. In sediments the percentage recoveries with Soxhlet extraction method varied between 88.22±7.85 (endrin) and 109.63±5.10% (β-BHC) and ranged from 74.11±9.82 (2,4 DDT) to 97.50±4.56% (α-BHC) with MAE. The limits of detection for the OCPs ranged from 5.5 to 20.6 ng/l and between 0.6 and 2.1 ng/g, respectively. The LLE and the SE methods were applied to water and sediments samples, respectively, from marine and freshwater sources in the Eastern Cape Province of South Africa that receive runoffs from agricultural lands and effluents from industries. The levels of OCPs ranged from 5.5 (2,4-DDD) to 450±0.10 ng/l (β-BHC) in water samples and from 0.6 (aldrin and 2,4-DDD) to 184±0.12 ng/g (β-BHC) in sediments for triplicate analyses. Some endocrine disrupting OCPs such as DDT, DDE, heptachlor, endosulphan and the chlordanes were detected.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Water analysis; Sediments; Pesticides; Organochlorine compounds

1. Introduction

Organochlorine pesticides (OCPs) have been of great concern due to their persistent nature and chronic adverse effect on wildlife and humans. Despite the ban and restriction on the usage of OCPs in developed countries during the 1970s and 1980s, some developing countries are still using them for agricultural and public purposes because of the low cost and versatility in controlling various insects [1]. The early spectacular success by dichlorodiphenyltrichloroethane (DDT) for malaria eradication in some countries has seen its continuing use in developing countries. Studies have suggested that these compounds may affect the normal function of the endocrine system [2]. The ability of the prevalent isomer of the major and most persistent DDT derivative, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), to bind to the androgen receptor in male rats has been reported [3]. OCPs have also been linked to human breast and liver cancers and to

*Corresponding author.
E-mail address: ofatoki@ufh.ac.za (O.S. Fatoki).

testicular tumors and lower sperm counts in humans [4,5].

DDT and its analogue, DDE, are the archetypes of fat soluble, nonbiodegradable and bioaccumulating compounds. The appearance of DDT in human tissues and its effect on wildlife especially reproduction in pelagic birds [6] triggered its determination in food [7,8], air, water [9,10] and human milk [11–13]. Similar studies have indicated the presence of contamination by OCPs in soil and sediments [14], in wildlife [15,16] and in mussels [17–20] and noticeable concentrations have been found in these regions.

Studies of water monitoring for OCPs in developed European [21–24], Asian [9,25,26] and American [27] countries have shown widespread detection of these pesticides in ground and surface waters where they have been banned for decades. In developing countries such as South Africa, this class of pesticide is believed to be still in use clandestinely under different trade names due to its cheapness. In certain slum areas, it has been used for malaria control. However, there is still a paucity of data on OCPs in South African water environment.

Several methods have been developed and applied for sample preparation, chromatographic separation and detection of OCPs. Common preconcentration methods of water samples include liquid–liquid extraction (LLE) [28,29] and solid-phase extraction (SPE) [22,30–35]. Solid phase microextraction techniques have also been applied [36–38]. The unique physical properties of supercritical fluids extraction (SFE) have attracted considerable attention since the 1980s [39]. SFE is described to provide cleaner extracts, less solvent handling, and equivalent or better recoveries than conventional solvent extraction techniques. Supercritical CO_2 has been the most commonly used fluid for SFE because of its low critical constants ($T_c = 32$ °C, $P_c = 72$ atm; 1 atm = 101.325 Pa), its low toxicity and cost and its ability to extract quantitatively a wide range of relatively nonpolar organics from a variety of matrices [40,41]. However, quantitative extraction of polar and ionic analytes has required the addition of organic modifiers to CO_2 [39]. The use of SFE techniques for the extraction of OCPs from aquatic systems has been widely reported in literature [42–45]. Despite the fact that SFE of OCPs from aqueous samples has

shown remarkable advantages over solvent extraction techniques, there are indications that this technique is not completely successful yet especially for biotic matrices.

The use of gas chromatography (GC) with electron capture detection (ECD) for the detection of OCPs is common because of its high resolution and good sensitivity in the nanogram range [29,34,35,43,46]. Other advantages of this detector include reduced cost of operation and the fact that it requires less technical skill to obtain reliable results. However, gas chromatography–mass spectrometry (GC–MS) [25,37,46,47] is also widely employed for the determination of OCPs in complex matrices. It has better resolution and could give higher sensitivity than the ordinary GC method.

With GC–ECD and/or GC–MS determinations, several columns have been used for the separation of OCPs in the aquatic systems. Tanabe et al. [48] used a fused-silica capillary (30 m×0.25 mm I.D., 0.25 μm film thickness) coated with DB-1 (100% dimethylpolysilaxone) (J&W Scientific). Tolosa et al. [35] used a 25 m×0.25 mm. I.D., 0.32 μm film thickness fused-silica capillary columns coated with SE-54 (Hewlett-Packard Ultra-2) for OCPs analyses in water while Beltran et al. [49] used the same column (25 m×0.20 mm I.D., 0.33 μm film thickness for the analyses of OCPs in waters.

Methylsilicone and methylphenylsilicone columns have also been mentioned in literature as suitable columns for the separation of OCPs with GC–ECD and GC–MS detection. Albanis et al. [47] used 007 Quatrex-Methyl 5% phenylsilicone 30 m×0.32 mm, 0.5 μm for GC–MS determination of OCPs in surface and groundwaters. Vassilakis et al. [46] used the same column (25 m×0.20 mm I.D., 0.1 μm film thickness) for the GC–ECD determination of OCPs in the same type of samples. The methyl–5% phenylsilicone column will be used in the GC–ECD determination of OCPs in this study because of its good resolution and its good retention values for the OCPs [46].

LLE is a common method frequently used for the determination of organic pollutants in water [50,51]. It has frequently been considered to give more reliable data than SPE (the use of commercially available SPE cartridges for sample preparation in OCPs analyses has been shown to give rise to

interferences specially when GC–ECD is employed). Extraneous peaks, which appear in the gas chromatograms, have been attributed to phthalate esters in the housing material of these cartridges [52]. Tan [29] in his study indicated that LLE for sample enrichment of OCPs in environmental water samples would give more repeatable data. In this study, the efficiency and repeatability of the solvent extraction method was evaluated for the determination of 15 organochlorine pesticides in water with different solvents—dichloromethane (DCM), light petroleum and hexane.

SE is an established technique that has been used for the extraction of organic pollutants from marine sediment and soil samples [45,53]. Recently, a novel microwave-assisted extraction (MAE) procedure has been reported as a sample preparation procedure in sediment [54]. Ganzler and co-workers [55,56] were the first to report the use of microwave energy to irradiate solid matrices such as seeds, foods and feeds in the presence of extracting solvents with high dipole moments. OCPs have been extracted from sediment samples using a domestic microwave oven with 5–6 times 30-s exposure to microwave energy [57]. In the present work, we have evaluated both SE and MAE for the 15 OCPs.

LLE and SE methods were tested on water and sediments, respectively, from East London harbor and Buffalo River (BR) in the Eastern Cape province of South Africa. The Buffalo River passes through agricultural areas in the province while the East London harbour receives domestic and industrial effluents from city's sewage works. Water and sediment samples were collected from different monitoring stations along the two rivers.

2. Materials and methods

All glassware was washed with liquid soap and rinsed properly with distilled water, and then with pure acetone. They were then baked in the oven at 100 °C for 24 h. All the solvents used—*n*-hexane, dichloromethane (DCM), petroleum spirit (b.p. 40–60 °C), light petroleum (b.p. 60–80 °C) and acetone were of analytical grade. OCPs standards were purchased from Sigma–Aldrich (Germany). All standard solutions were prepared by dissolving the

pesticide standards in hexane (1000 mg/l). These solutions were further diluted as required.

2.1. Determination of response factors

The response factor (RF) of the standard pesticides relative to the internal standard (I.S.), pentachloronitrobenzene were carried out by injecting 0.001 ml into the GC–ECD system of a mixture of the OCPs together with the I.S. at a concentration range of 60–400 ng/l. The response factor was calculated based on the equation below:

$$\text{Response factor} = \frac{\text{Peak area of the pesticide standard}}{\text{Peak area of the internal standard}}$$

2.2. Liquid–liquid extraction

The validation of the LLE method was carried out by spiking doubly distilled water, passed through the Milli-Q system with OCPs standard mixture at the fortification range of 60 ng/l (α-benzenehexachloride, α-BHC) to 400 ng/l (4,4-DDT) and then extracting with 3×15 ml of each of the extracting solvents tried (hexane, DCM and light petroleum). The extracts were combined, dried with anhydrous sodium sulphate and concentrated to about 2 ml using the Buchi rotary vacuum evaporator for chromatographic clean-up.

Blank extraction of unspiked doubly distilled water (prepared as described above) was carried out using the DCM extraction and chromatographic clean up method as described below, which gave a clean background.

Recoveries of the OCP standards were also investigated in raw water samples from the local Tyume River using same OCPs standards at the same fortification levels as for distilled water samples, to check on the effect of matrix on extraction efficiencies. Recoveries of OCPs in fortified water samples were calculated from the ratio of the amount of OCPs recovered from spiked water samples to the amount added to spike (based on the ratio of the peak areas of the standards to that of the spiked solution with the same concentration). OCP concentrations in raw river water samples were predetermined before spiking and they showed no presence of pesticides of interest. DCM was used as

the only extracting solvent for spiked raw water samples before they underwent the same chromatographic clean-up process as for spiked distilled water.

2.3. Silica gel column chromatography

The chromatographic column (20 cm×8 mm I.D.) was slurry packed with 5.0 g of activated silica gel which was made into a slurry with about 1.2% (v/m) water-adsorbent using distilled water and then stirred well before use. About 0.5 ml 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 petroleum spirit, and prior to the exposure of the sodium sulphate layer to air, the reduced extract from the earlier LLE process was placed in the column and allowed to sink below the sodium sulphate layer. OCPs were then eluted with 2×10 ml portions of the extracting solvent. The eluate was collected, dried with anhydrous sodium sulphate and then evaporated to dryness using the Buchi vacuum rotary evaporator. The I.S. (pentachloronitrobenzene) was added and the residues were reconstituted with 2 ml of the extracting solvent for GC analysis.

2.4. Microwave-assisted extraction

The validation of the MAE method was carried out by spiking dried, sieved and pre-extracted sediment sample with OCP standards at the fortification range of 30 ng/g (α-BHC) to 300 ng/g (4,4-DDT) in a PTFE vessel. This was extracted with the microwave oven (R-340C Sharp domestic microwave) using each of the extraction evaluating solvents (hexane, DCM and light petroleum). Different solvent volume, microwave energy levels and the duration of extraction were evaluated for optimization as presented in Tables 4–6. The extract was filtered into a clean screwcap glass tube and then centrifuged at 100 rps for 1 min. The supernatant was decanted into a 250-ml round-bottomed flask and then concentrated to about 2 ml at 40 °C using vacuum rotary evaporator. The reduced extract is then carried through the column chromatographic clean-up process as described earlier to make ready for GC analysis. Recoveries were calculated as

described previously from the ratio of the amount of OCPs recovered from spiked samples to the amount added to spike. OCP concentrations were predetermined in sediments before spiking and again no pesticides of interest were found. DCM, light petroleum and hexane were used as extracting solvents, respectively.

2.5. Soxhlet extraction

The validation of the SE method was carried out by spiking dried, sieved and pre-extracted sediment sample with OCP standards at the same fortification range as described for MAE in a pre-extracted Whatman extraction thimble and then extracting for 10 h with 120 ml of each of the evaluating solvents (hexane, DCM and light petroleum). The extract was allowed to cool, filtered and then concentrated at 40 °C to about 2 ml on the vacuum rotary evaporator. The reduced extract was then carried through the column chromatographic clean-up process as described above prior to GC analysis. The pre-extracted sediment samples showed no presence of OC pesticides of interest. Recoveries of OCPs in sediment were calculated as described previously for MAE.

2.6. Analyses of environmental water and sediment samples

Water samples were collected in triplicates in clean Winchester bottles from different sites in East London harbor and Buffalo River in January 2002. They were immediately preserved by adding 5 ml of concentrated H_2SO_4 and stored at 4 °C in a refrigerator until analyzed. Sediment samples were collected from about 0–5 cm below the surface from about the same locations as water samples into clean widemouth plastic containers and covered immediately after sampling. They were kept cool during transportation to the laboratory. At the laboratory, they were kept frozen at −18 °C prior to sample preparation. The sediment samples were air-dried in a circulating air in the oven at about 30 °C for 2–3 days and sieved.

A 1-l volume of the acidified water sample was extracted with DCM as described above. The DCM extract was concentrated to about 2 ml and the

residue was made to undergo the silica gel column chromatographic clean-up as described previously to prepare for GC analysis.

A 10-g amount of dried, sieved and pre-extracted sediment sample was weighed into a pre-extracted Whatman extraction thimble and then treated as described previously for SE with DCM as extracting solvent. The reduced extract was then carried through the column chromatographic clean-up process as described above prior to GC analysis.

2.7. Capillary gas chromatographic analysis

Separation and determination of the pesticide residues were carried out with the Perkin-Elmer AutoSystem XL gas chromatograph fitted with an electron-capture detector using a series bonded phase fused-silica capillary column, methyl–5% phenylsilicone (30 m×0.53 mm I.D., 0.2 μm) purchased from Quadrex, New Haven CT, USA. Glass injector liner (8 cm×3 mm), manually packed with silanised glass wool supplied by Perkin-Elmer (Johannesburg, South Africa) was used. The injector and detector temperatures were maintained at 250

and 350 °C respectively. The oven temperature was initially maintained at 120 °C and then programmed at 20 °C/min to 150 °C and finally to 250 °C at 5 °C. The BOC gases, 99.999% ultra pure helium and nitrogen, purchased from Afrox (South Africa) were used as the carrier and make-up gases respectively. The carrier gas flow-rate was 2 ml/min while the make-up gas flow was set at 28 ml/min for optimum performance. A 0.001-ml volume each of the processed samples was injected into the GC in the splitless mode of 1 min (after injection) for analyses (0.001 ml injection was used as against larger volume injection because ECD has a very low upper LOD, and hence is readily overloaded. When this occurs the elution peaks maxima tend to flatten off, and a broad flat peak is shown) [58].

3. Results and discussion

The gas chromatogram of a mixture of the 15 OCPs standards plus the I.S. (pentachloronitrobenzene) is shown in Fig. 1. All the 15 OCPs are well resolved and eluted within a very reasonable time of
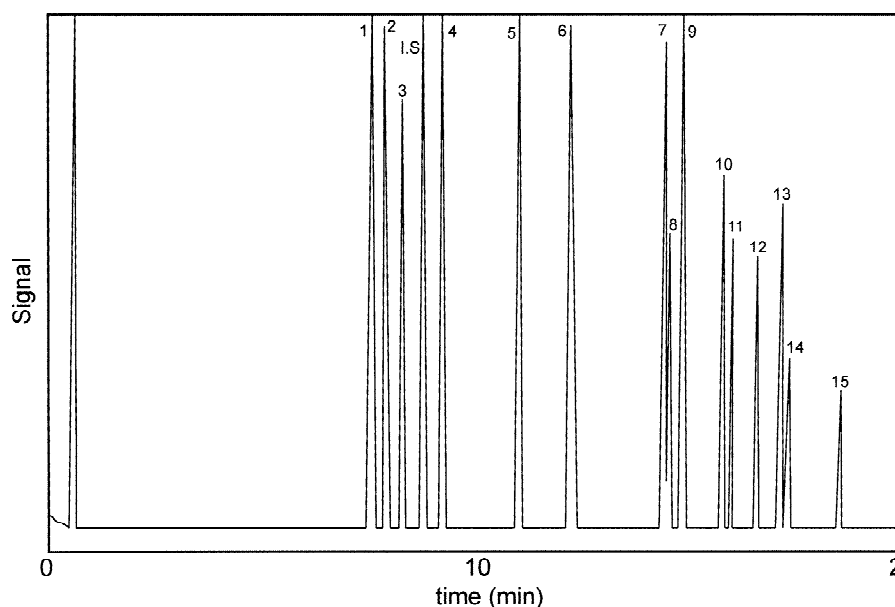

Fig. 1. Gas chromatograph of OCP standards. 1=α-BHC; 2=HCB; 3=β-BHC; I.S., internal standard pentachloronitrobenzene; 4=δ-BHC; 5=heptachlor; 6=aldrin; 7=γ-chlordane; 8=2,4-DDE; 9=endosulfan; 10=dieldrin; 11=2,4-DDD; 12=endrin; 13=4,4-DDD; 14=2,4-DDT; 15=4,4-DDT.

Table 1
Retention times±SD[a] and response factors of OCPs standards

OCPs	Retention time (min)	Response factor
α-BHC	8.18±0.02	1.48±0.07
HCB	8.55±0.04	1.17±0.09
β-BHC	8.97±0.03	1.42±0.09
δ-BHC	9.98±0.04	1.57±0.07
Heptachlor	11.93±0.03	1.24±0.09
Aldrin	13.23±0.01	0.90±0.08
γ-Chlordane	15.57±0.02	1.12±0.11
2,4′-DDE	15.70±0.02	1.93±0.16
Endosulfan I	16.06±0.02	0.88±0.11
Dieldrin	17.04±0.03	0.63±0.19
2,4′-DDD	17.24±0.02	1.67±0.18
Endrin	17.83±0.01	1.81±0.20
4,4′-DDD	18.47±0.02	1.73±0.19
2,4′-DDT	18.63±0.02	1.33±0.23
4,4′-DDT	19.91±0.02	1.28±0.29
Pentachloronitrobenzene	9.49±0.02	

[a] Values are mean of six injections.

about 20 min under the optimized GC conditions. Table 1 shows the retention times and the response factors for the OCPs.

The efficiency of extraction of the 15 OCPs standards from distilled water by LLE with DCM, light petroleum and hexane followed by column chromatographic clean-up is presented in Table 2. The mean percentage recoveries of OCPs with DCM

Table 2
Mean percentage recoveries±SD[a] of OCPs standards added to distilled water by LLE with three solvents (DCM, light petroleum and hexane)

OCPs	DCM	Light petroleum	Hexane
α-BHC	96.15±5.15	96.02±4.90	96.20±1.01
HCB	98.81±3.64	92.01±5.32	98.92±3.55
β-BHC	101.63±2.04	87.50±6.80	81.19±2.60
δ-BHC	99.20±2.79	88.35±5.56	98.67±0.91
Heptachlor	97.03±3.70	85.31±6.35	94.33±1.78
Aldrin	90.09±8.03	76.30±13.38	86.78±1.10
γ-Chlordane	94.92±4.58	72.71±9.70	85.88±1.17
2,4′-DDE	98.45±1.65	90.00±4.25	85.84±1.56
Endosulfan I	92.70±7.76	73.19±8.88	78.19±3.83
Dieldrin	91.61±7.25	68.18±13.8	64.49±3.04
2,4′-DDD	99.80±2.31	89.36±3.88	83.37±1.42
Endrin	97.32±1.85	91.00±4.60	87.78±0.95
4,4′-DDD	102.95±2.84	88.98±2.33	85.07±1.07
2,4′-DDT	99.52±2.73	85.07±5.37	72.99±3.63
4,4′-DDT	97.20±0.62	85.95±5.65	74.71±3.68

[a] Values are mean of triplicate analyses.

as extracting ranged from 90.09±8.03 to 102.95±2.84%. The values using light petroleum as extracting solvent varied between 68.18±13.80 and 96.02±4.90% while those with hexane ranged from 64.49±3.04 to 98.92±3.55%.

DCM appeared to be a better solvent than hexane and light petroleum because the amount of analytes recovered using this solvent was higher than those with hexane and light petroleum with acceptable repeatability. The low SDs obtained (0.62–8.03) were still within the acceptable limits. A SD value range of 5–12 has been reported [34]. The results obtained with DCM extraction were comparable to those reported using the US Environmental Protection Agency (EPA) Test Method 608 for OCPs via solvent extraction with same solvent [28]. Although, a comparatively lower SD was obtained with hexane, the lower recoveries of the analytes when compared to those obtained with DCM, especially the 64.49% recovery for dieldrin with hexane made DCM a better choice.

These solvents have been widely applied in the LLE of OCPs in environmental water samples [28,29,50]. However, they have not been comparatively investigated individually for their capacities as extractants, eluants and dissolution of the dried residue in this sequence for pesticides residue analyses.

The mean percentage recoveries of the 15 OCPs in spiked river water by LLE with DCM ranged from 71.03±8.15 to 101.25±2.17% (Table 3), which were judged acceptable, hence it is the solvent of choice for use in LLE for the analyses of environmental river water samples. The relatively lower recoveries of OCPs from the spiked raw river water (compared with those from spiked distilled water) might be due to matrix effects.

The optimum conditions for the MAE recoveries of OCPs from sediment could be obtained by varying the extracting solvents, extracting temperatures and duration of extraction. Results of our study on MAE procedure with DCM, light petroleum and hexane extractions using different microwave conditions are shown in Tables 4–6. The results with microwave condition A (i.e. 10 ml extracting solvent; low 10% microwave energy for 2 min) gave percentage recoveries that ranged from 77.83±11.80 to 105.99±10.43% with DCM, from 88.32±11.72 to

Table 3
Mean percentage recoveries of OCP standards added to river water by LLE with DCM as the extracting solvent

OCPs	Spiked at (ng/l)	Recovery[a] (%) ±SD	Limits of detection[b] (ng/l)
α-BHC	60	101.25±2.17	18
HCB	200	93.50±11.30	18.6
β-BHC	200	93.37±6.65	7.7
δ-BHC	100	100.13±3.09	15
Heptachlor	140	89.29±2.98	12
Aldrin	160	85.00±3.57	7.5
γ-Chlordane	160	78.13±3.31	20.6
2,4′-DDE	400	83.94±2.96	7.7
Endosulfan I	140	78.69±5.37	18.5
Dieldrin	160	71.03±8.15	5.7
2,4′-DDD	400	83.78±8.42	5.5
Endrin	400	91.46±3.24	14.7
4,4′-DDD	400	80.79±6.45	13.4
2,4′-DDT	400	77.16±9.22	6.0
4,4′-DDT	400	80.42±10.97	18.9

[a] Values are mean of triplicate analyses.
[b] Calculated from the linear regression equation of the calibration curve of each standard pesticide [63].

Table 5
Mean recoveries±SD[a] of OCPs standards added to sediment by MAE condition B[b] with three extracting solvents

OCPs	DCM	Light petroleum	Hexane
α-BHC	97.50±4.56	102.07±2.09	95.84±2.78
HCB	85.09±7.32	99.11±6.29	89.86±5.40
β-BHC	83.52±6.62	98.03±7.29	87.54±7.48
δ-BHC	92.47±3.84	90.38±3.80	84.45±5.40
Heptachlor	92.65±3.30	87.73±18.73	92.12±3.16
Aldrin	90.28±3.85	98.18±6.36	95.18±8.36
γ-Chlordane	83.72±5.61	97.68±4.44	77.92±3.60
2,4′-DDE	87.91±3.96	99.09±6.62	75.45±5.88
Endosulfan I	82.62±6.41	83.88±9.61	48.10±8.46
Dieldrin	80.18±1.13	76.17±11.05	39.35±9.85
2,4′-DDD	84.09±6.07	94.51±11.73	53.29±6.83
Endrin	87.66±6.09	89.23±12.56	66.27±10.53
4,4′-DDD	81.81±6.17	86.23±7.54	45.94±8.87
2,4′-DDT	74.11±9.82	94.92±16.38	57.77±2.47
4,4′-DDT	75.75±6.16	77.26±14.82	36.96±12.13

[a] Values are mean of triplicate analyses.
[b] 15 ml extracting solvent; 30% microwave energy for 4 min duration.

100. 00±6.28% with light petroleum and from 71.08±5.59 to 103.30±4.11% with hexane (Table 4). Results with MAE condition B (i.e. 15 ml extracting solvent; 30% microwave energy for 4 min) showed percentage recoveries varying between 74.11±9.82 and 97.50±4.56% with DCM, between 76.17±11.05 and 102.07±2.09% with light petroleum and between 39.35±9.85 and 95.92±2.78% with hexane (Table 5). MAE condition C (i.e. 20 ml extracting solvent; 50% microwave energy for 6 min) gave percentage recoveries that ranged from 40.20±18.50 to 91.01±5.21% with DCM, from 17.84±25.38 to 88.32±12.16% with light petroleum and from 43.73±33.90 to

Table 4
Mean recoveries±SD[a] of OCPs standards added to sediment by MAE condition A[b] with the three extracting solvents

OCPs	DCM	Light petroleum	Hexane
α-BHC	98.53±4.22	99.96±3.30	99.12±1.25
HCB	96.24±7.34	97.45±6.56	96.50±6.55
β-BHC	87.67±9.10	100.00±6.28	103.30±4.11
δ-BHC	94.71±4.68	94.63±4.73	96.86±3.21
Heptachlor	92.78±6.34	98.68±4.14	96.30±2.91
Aldrin	92.06±6.12	98.89±1.47	99.59±8.31
γ-Chlordane	86.87±7.08	96.49±5.97	91.54±4.48
2,4′-DDE	86.75±5.49	96.49±4.85	89.46±5.44
Endosulfan I	77.83±11.80	88.32±11.72	82.07±6.40
Dieldrin	105.99±10.43	84.33±9.92	71.92±8.81
2,4′-DDD	81.27±9.00	91.26±9.43	83.13±6.76
Endrin	90.88±7.89	100.94±12.75	77.11±10.07
4,4′-DDD	79.34±10.92	90.27±11.64	73.78±11.56
2,4′-DDT	83.53±14.73	98.66±12.27	74.62±5.75
4,4′-DDT	84.85±15.56	96.18±10.05	71.08±5.59

[a] Values are mean of triplicate analyses.
[b] 10 ml extracting solvent; low 10% microwave energy for 2 min duration.

Table 6
Mean percentage recoveries±SD[a] of OCPs standards added to sediment by MAE conditions C[b] with three extracting solvents

OCPs	DCM	Light petroleum	Hexane
α-BHC	91.01±5.21	88.32±12.16	99.96±7.60
HCB	62.80±7.38	79.24±20.93	93.12±15.10
β-BHC	71.81±13.80	44.29±19.10	91.30±9.99
δ-BHC	88.02±5.59	68.51±16.65	85.65±8.68
Heptachlor	85.33±3.76	76.56±21.24	91.00±22.37
Aldrin	71.14±4.32	65.09±31.87	83.42±26.74
γ-Chlordane	68.01±8.08	52.58±37.25	84.05±30.34
2,4′-DDE	72.10±7.58	54.87±38.91	67.01±47.60
Endosulfan I	59.23±13.73	17.84±13.57	62.18±44.78
Dieldrin	43.92±16.02	29.90±17.90	56.75±40.52
2,4′-DDD	60.76±15.07	37.80±26.79	76.12±23.21
Endrin	67.34±4.04	32.80±23.29	63.25±45.54
4,4′-DDD	57.39±18.73	17.94±25.38	49.57±36.01
2,4′-DDT	46.56±16.83	20.59±29.12	78.35±56.23
4,4′-DDT	40.20±18.58	N.D.±0	43.73±33.90

[a] Values are mean of triplicate analyses.
[b] 20 ml extracting solvent; 50% microwave energy for 6 min duration.

99.96±7.60% with hexane (Table 6). Thus, condition A gave the best results, for with the MAE technique the values of the SD are high, especially with MAE conditions B and C. This would make the results less reliable.

The percentage recoveries of OCPs from spiked sediment by Soxhlet extraction method are shown in Table 7. Recoveries of the pesticides ranged from 88.22±7.85 to 109.63±5.10% with DCM, from 74.04±2.11 to 107.26±0.44% with light petroleum and from 63.04±14.13 to 100.98±5.37% with hex-

ane. With this method, DCM gave the best recoveries of the OCPs from sediment. Also, the method appeared generally to give better recoveries than the MAE technique and the results gave better repeatability. Based on these results, SE with DCM as extracting solvent was chosen for OCPs analyses in environmental sediment samples.

The results of the analyses of OCPs in environmental water samples collected from East London harbour and from Buffalo River are shown in Table 8. Fig. 2 showed a representative chromatogram of

Table 7
Mean percentage recoveries±SD[a] of OCPs standards added to sediment by Soxhlet extraction with three extracting solvents

OCPs	DCM	Light petroleum	Hexane
α-BHC	96.09±1.35	97.40±0.80	98.40±2.37
HCB	98.87±11.12	97.87±0.38	97.98±6.37
β-BHC	109.63±5.10	106.32±1.03	100.98±5.37
δ-BHC	98.48±8.19	107.26±0.44	93.94±2.76
Heptachlor	98.88±1.81	97.42±2.41	99.91±2.04
Aldrin	97.12±4.72	97.35±5.18	93.42±5.23
γ-Chlordane	96.89±1.17	94.58±3.23	89.23±4.12
2,4′-DDE	96.03±4.87	92.46±4.12	90.59±7.26
Endosulfan I	97.55±8.65	81.79±3.49	84.19±10.64
Dieldrin	96.17±3.79	79.50±6.69	84.17±13.34
2,4′-DDD	100.61±5.30	85.16±4.61	83.11±14.53
Endrin	88.22±7.85	85.78±2.56	78.46±20.86
4,4′-DDD	97.84±2.74	79.89±7.09	76.03±11.26
2,4′-DDT	99.14±9.09	74.04±2.11	72.22±14.16
4,4′-DDT	90.19±1.80	78.33±19.47	63.04±14.13

[a] Values are mean of triplicate analyses.

Table 8

OCPs levels (ng/l)$\pm$SD[a] in water at different sites collected from the East London harbour (EL) and the Buffalo River BR) at King Williams Town, South Africa in January 2002

OCP	10 January 2002 sampling						
	EL1	EL2	EL3	EL4	BR1	BR2	BR3
α-BHC	40$\pm$0.01	50$\pm$0.01	100$\pm$0.03	18	18	20$\pm$0.02	50$\pm$0.01
HCB	30$\pm$0.02	18.6	90$\pm$0.01	18.6	18.6	100$\pm$0.02	80$\pm$0.03
β-BHC	40$\pm$0.05	210$\pm$0.04	70$\pm$0.02	7.7	200$\pm$0.04	30$\pm$0.01	450$\pm$0.10
δ-BHC	20$\pm$0.03	60$\pm$0.02	30$\pm$0.04	40$\pm$0.03	100$\pm$0.04	80$\pm$0.03	140$\pm$0.05
Heptachlor	70$\pm$0.04	200$\pm$0.03	50$\pm$0.03	12	12	200$\pm$0.01	171$\pm$0.13
Aldrin	40$\pm$0.01	7.5	20$\pm$0.04	7.5	7.5	20$\pm$0.04	120$\pm$0.02
γ-Chlordane	20$\pm$0.03	20.6	20.6	20.6	20.6	100$\pm$0.05	120$\pm$0.03
2,4-DDE	50$\pm$0.06	7.7	7.7	7.7	7.7	100$\pm$0.03	240$\pm$0.07
Endosulfan I	80$\pm$0.01	18.5	18.5	18.5	18.5	100$\pm$0.04	50$\pm$0.02
Dieldrin	50$\pm$0.01	5.7	5.7	5.7	5.7	100$\pm$0.02	60$\pm$0.04
2,4-DDD	100$\pm$0.02	5.5	5.5	5.5	5.5	30$\pm$0.04	180$\pm$0.06
Endrin	80$\pm$0.01	14.7	14.7	14.7	14.7	40$\pm$0.02	30$\pm$0.05
4,4-DDD	13.4	13.4	13.4	13.4	13.4	100$\pm$0.05	210$\pm$0.02
2,4-DDT	6.0	6.0	6.0	6.0	100$\pm$0.02	100$\pm$0.02	260$\pm$0.15
4,4-DDT	18.9	18.9	18.9	18.9	140$\pm$0.04	20$\pm$0.05	160$\pm$0.04

EL1, Orient Pier; EL2, Dry Dock; EL3, West Quay; EL4, S-Berth, BR1, BR2 and BR3, Buffalo River.

[a] Mean of triplicate analyses.

OCPs in water extract from East London Harbour. The identities of the OCPs in samples extracts were confirmed by comparing their retention times with those of the OCPs standards. OCPs concentration ranged from 5.5 to 210$\pm$0.04 ng/l in harbour water samples and from 5.7 to 450$\pm$0.10 ng/l in fresh-
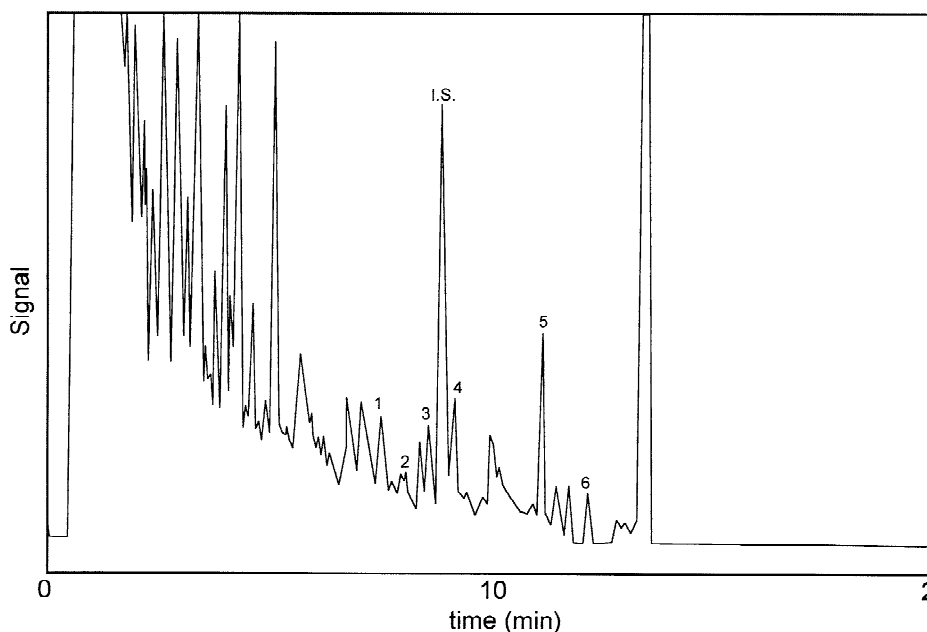

Fig. 2. Representative chromatogram of river water extract (EL2) from East London harbour; 1=α-BHC; 2=HCB; 3=β-BHC; I.S., pentachloronitrobenzene; 4=δ-BHC; 5=heptachlor; 6=aldrin.

water samples (Buffalo River). Levels OCPs in sediment ranged from 0.6 to 134 ± 0.05 ng/g in marine sediment and from 0.6 to 184.0 ± 0.12 ng/g in river sediment (Table 9). β-BCH was found at highest concentration in both the marine and fresh-water samples. Among the OCPs identified at appreciable levels in the marine water samples were α-, β- and γ-BCH, hexachlorobenzene (HCB), chlordane, aldrin, heptachlor, DDE, endosulfan I, DDD, dieldrin and endrin. In the river water all the 15 OCPs analyzed were found at appreciably high levels at most of the sites. Some of the pesticides found in the water sources like chlordane, heptachlor, DDT, DDE and endosulphan found in the river are known to have endocrine and estrogenic disrupting properties [59], which may greatly impact on the biodiversity of the aquatic ecosystem.

The sources of some of the OCPs in the water systems might be from industrial effluents and from diffuse sources such as run-off from agricultural lands. The Buffalo River passes through agricultural areas in the province while the East London harbour receives domestic and industrial effluents from city's sewage works. The presence of DDT and some of its degradation residues in the water systems can be attributed to their wide usage before their banning [60,61]. Since they are persistent enough and de-

grade slowly and easily accumulate in the soil, the transportation of these pesticides both sorbed onto solids, and dissolved by the surface water down to the water sources should actually happen [10].

From the results obtained, the concentration of most of the analyzed OCPs were below the maximum acceptable concentration of 100 ng/l value set by the European Union (EU) for the protection of aquatic environment. However, some elevated levels of about 450, 260 and 240 ng/l were detected in β-BHC, 2,4′-DDT and 2,4′-DDE respectively, in the Buffalo River. These elevated concentrations give cause for concern.

4. Conclusion

This study showed LLE with DCM an accurate and reliable method for OCPs determination in environmental waters. It also showed that despite the numerous claims by both manufacturers and research groups on the usefulness of MAE for sample preparation and trace enrichment in environmental samples, SE with DCM could be a more accurate alternative method for OCP determination in sediments. Several OCPs were detected in the water

Table 9
OCPs levels (ng/g)$\pm$SD[a] in sediments at different sites collected from East London Harbour (EL) and Buffalo river (BR) at King Williams Town, South Africa in January 2002

OCP	10 January 2002 sampling						
	EL1	EL2	EL3	EL4	BR1	BR2	BR3
α-BHC	30 ± 0.02	1.8	1.8	1.8	90.0 ± 0.03	19.1 ± 0.08	23.0 ± 0.03
HCB	45 ± 0.02	1.9	1.9	1.9	34.0 ± 0.02	30.0 ± 0.05	24.6 ± 0.03
β-BHC	134 ± 0.05	0.8	0.8	0.8	81.5 ± 0.04	72.7 ± 0.01	75.3 ± 0.07
δ-BHC	1.5	1.5	1.5	1.5	56.0 ± 0.02	74.3 ± 0.03	177.0 ± 0.05
Heptachlor	1.2	1.2	1.2	1.2	36.7 ± 0.05	95.0 ± 0.01	184.0 ± 0.12
Aldrin	0.8	0.8	0.8	0.8	70.2 ± 0.18	0.8	30.7 ± 0.06
γ-Chlordane	25 ± 0.12	2.1	2.1	2.1	92.0 ± 0.07	2.1	117.0 ± 0.03
2,4-DDE	53 ± 0.04	0.8	0.8	0.8	0.8	0.8	20.6 ± 0.17
Endosulfan I	92 ± 0.15	1.9	1.9	1.9	92.0 ± 0.08	1.9	72.0 ± 0.03
Dieldrin	25 ± 0.06	0.6	0.6	0.6	0.6	0.6	60.0 ± 0.01
2,4-DDD	96 ± 0.03	0.6	0.6	0.6	94.0 ± 0.10	0.6	39.5 ± 0.06
Endrin	80 ± 0.02	1.5	1.5	1.5	100.0 ± 0.05	1.5	38.4 ± 0.02
4,4-DDD	50 ± 0.05	1.3	1.3	1.3	1.3	1.3	47.7 ± 0.01
2,4-DDT	74 ± 0.08	0.6	0.6	0.6	0.6	0.6	109.0 ± 0.04
4,4-DDT	±0.23	1.9	1.9	1.9	110.0 ± 0.02	1.9	33.6 ± 0.06

EL1, Orient Pier, EL2, Dry Dock; EL3, West Quay; EL4, S-Berth, BR1, BR2 and BR3, Buffalo River.
[a] Mean of triplicate analyses.

O.S. Fatoki, R.O. Awofolu / J. Chromatogr. A 983 (2003) 225–236

235

systems including some endocrine disrupting compounds such as DDT, DDE and heptachlor [62].

References

[1] S. Tanabe, H. Iwata, R. Tasukawa, Sci. Total. Environ. 154 (1994) 163.

[2] E. Hileman, Chem. Eng. News, 31 January (1994) 19.

[3] W.R. Keice, R.C. Stone, S.C. Law, L. Earl-Gray, J.A. Kemppainen, E.M. Wilson, Nature 375 (1995) 581.

[4] D.L. Davies, H.L. Barlow, Sci. Am. October (1995) 144–147.

[5] P. Cocco, A. Blair, P. Congia, G. Saba, C. Flore, M.R. Ecca, C. Palmas, Arch. Environ. Health 52 (1997) 299.

[6] US Environmental Protection Agency, DDT: A Review of Scientific and Economic Aspects of the Decision to Ban its Use as a Pesticide, EPA, Washington, DC, 1975.

[7] K. Kannan, S. Tanabe, J.P. Giesy, R. Tatsukawa, Rev. Environ. Contam. Toxicol. 152 (1997) 1.

[8] R. Doong, C. Lee, Analyst 124 (1999) 1287.

[9] H. Iwata, S. Tanabe, N. Sakai, A. Nishimura, Environ. Pollut. 85 (1994) 15.

[10] A. Aydin, T. Yurdin, Water, Air Soil Pollut. 111 (1999) 385.

[11] J.O. Okonkwo, L. Kampita, D.D. Chingakule, Bull. Environ. Contam. Toxicol. 63 (1999) 243.

[12] S. Tanabe, F. Gondaira, A.N. Subramanian, A. Ramesh, D. Mohan, P.L. Kumaran, K. Venogopalan, R. Tatsukawa, J. Agric. Food. Chem. 38 (1990) 899.

[13] A. Schecter, P. Furt, C. Furt, O. Papke, M. Ball, L.C. Dai, H.T. Quynh, N.T. Phoung, A. Beim, B. Vlasov, V. Chongchet, J.D. Constable, K. Charles, Chemosphere 23 (1991) 1903.

[14] D.B. Lee, M.S. Prudente, S. Tanabe, R. Tatsukawa, Toxicol. Environ. Chem. 60 (1997) 171.

[15] A. Ramesh, S. Tanabe, K. Kannan, A.N. Subramanian, P.L. Kumar, R. Tatsukawa, Arch. Environ. Contam. Toxicol. 23 (1992) 23.

[16] S. Tanabe, A.N. Subramanian, R. Ramesh, P.L. Kumaran, R. Tatsukawa, Mar. Pollut. Bull. 26 (1993) 311.

[17] A. Ramesh, S. Tanabe, K. Kannan, A.N. Subramanian, D. Moha, V.K. Venugopalan, R. Tatsukawa, Mar. Pollut. Bull. 21 (1990) 587.

[18] S. Kan-Atireklap, S. Tanabe, J. Sanguansin, M.S. Tabucannon, M. Hungspreugs, Environ. Pollut. 97 (1997) 79.

[19] S. Kan-Atireklap, S. Tanabe, N.T.H. Yen, A.N. Subramanian, Toxicol. Environ. Pollut. 67 (1998) 409.

[20] M. Prudente, H. Ichihashi, S. Kan-Atireklap, I. Watanabe, S. Tanabe, Fish. Sci. 65 (1999) 441.

[21] T.A. Albanis, D.G. Hela, I.K. Konstantinou, T.M. Sakellarides, J. Chromatogr. A 823 (1998) 59.

[22] S. Lacorte, C. Molina, D. Barcelo, Anal. Chim. Acta 281 (1993) 71.

[23] A.R. Fernandez-Alba, A. Aguera, M. Contreras, G. Penuela, I. Ferrer, D. Barcelo, J. Chromatogr. A 823 (1998) 35.

[24] T. Pihlstrom, A. Hellstrom, V. Axelsson, Anal. Chim. Acta 356 (1997) 155.

[25] H. Kobayashi, K. Ohyama, N. Tomiyama, Y. Jimbo, O. Matano, S. Goto, J. Chromatogr. 643 (1993) 197.

[26] K. Ahad, I. Ahmed, S. Aziz, U.K. Balock, A. Mohammad, S. Tahir, Water SA 26 (2000) 409.

[27] F.K.R. Dorothea, D.C.G. Muir, Environ. Sci. Technol. 33 (1991) 3317.

[28] USEPA, Test Method 608, Organochlorine pesticides and PCBs, Environmental Monitoring System Laboratory, US Environmental Protection Agency, Cincinnati, OH, 1984.

[29] G.H. Tan, Analyst 117 (1992) 1129.

[30] T.A. Albanis, D.G. Hela, J. Chromatogr. A 707 (1995) 283.

[31] A.C. Crescenzi, O. Guerreiro, R. Samperi, Environ. Sci. Technol. 31 (1997) 479.

[32] M.V. Russo, G. Goretti, T. Nevigato, Chromatographia 48 (1989) 293.

[33] H.M. Chris, I.D. Brindle, Anal. Chem 62 (1990) 1495.

[34] C.W. Quayle, I. Jepson, I.A. Fowlis, J. Chromatogr. A 773 (1997) 271.

[35] I. Tolosa, J.W. Readman, L.D. Mee, J. Chromatogr. A 725 (1996) 93.

[36] A.A. Boyd-boland, S. Magdic, J.B. Pawliszyn, Analyst 121 (1996) 929.

[37] C. Aguilar, S. Penalver, E. Pocurull, F. Borrull, R.M. Marce, J. Chromatogr. A 795 (1998) 105.

[38] S. Magdic, J.B. Pawliszyn, J. Chromatogr. A 723 (1996) 111.

[39] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A.

[40] E.A. Rochette, J.B. Harsh, H.H. Hill, Talanta 40 (1993) 147.

[41] J.W. Oudsema, C.F. Poole, Fresenius J. Anal. Chem. 344 (1992) 426.

[42] I.J. Barnabas, J.R. Dean, S.M. Hitchen, S.P. Owen, J. Chromatogr. A 665 (1994) 307.

[43] C. Nerin, R. Battle, J. Cacho, J. Chromatogr. A 795 (1998) 117.

[44] J.R. Dean, I.J. Barnabas, S.P. Owen, Analyst 121 (1996) 465.

[45] J.L. Snyder, R.L. Grob, M.E. Macnally, T.S. Oostydyk, Anal. Chem. 64 (1992) 1940.

[46] I. Vassilakis, D. Tsipi and M. Scoullos, J. Chromatogr. A 823 (198) 49–58.

[47] T.A. Albanis, D.G. Hela, T.M. Sakellarides, I.K. Konstantinon, J. Chromatogr. A 823 (1998) 59.

[48] S. Tanabe, M.S. Prudente, S. Kan-atireklap, S. Subramanian, Ocean Coastal Manag. 43 (2000) 819.

[49] J. Beltran, F.J. Lopez, F. Hernandez, Anal. Chim. Acta 283 (1993) 297.

[50] F. Hernandez, I. Morell, J. Bettran, F.J. Lopez, Anal. Chim. Acta 283 (1993) 297.

[51] M.W. Powell, J. Chromatogr. A 697 (1995) 101.

[52] G.A. Junk, M.J. Avery, J.J. Richard, Anal. Chem. 60 (1988) 1347.

[53] Test Methods for Evaluating Solid Waste, EPA SW-846, Method 3540, 3rd ed, US Government Printing office, Washington, DC, 1990.

[54] K.K. Chee, M.K. Wong, H.K. Lee, J. Chromatogr. A 736 (1996) 211.

[55] K. Ganzler, A. Salso, K. Valko, J. Chromatogr. 371 (1986) 299.

[56] K. Ganzler, A. Salgo, Z. Unters. Forsch. 184 (1987) 274.

[57] F. Onuska, K.A. Terry, Chromatographia 36 (1993) 191.

[58] C. Simpson, in: Instrument and Techniques Series: Gas Chromatography, Kogan Page; Barnes and Noble, London; New York, 1970.

[59] A.M. Soto, K.L. Chung, C. Sonnenschein, Environ. Health. Perspect. 102 (1994) 380.

[60] L.P. Van-Dyk, I.H. Wiese, J.E.C. Mullen, Resid. Rev. 82 (1982) 38.

[61] R. Davies, R.M. Randall, Historical and Geological Patterns in Eggshell Thickness of African Fish Eagle, *Halia eetus vocifer*, Meyburg/Chancellor, London, 1989.

[62] S. Safe, Hydroxylated polychlorinated biphenyls (PCBs) and organochlorine pesticides as potential endocrine disruptors, in: M. Metzler (Ed.), The Handbook of Environmental Chemistry. Part L Endocrine Disruptors, Part 1, Vol. 3, Springer-Verlag, Berlin, Heidelberg, 2001, p. 155.

[63] J.C. Miller, J.N. Miller, in: Statistic for Analytical Chemistry, 2nd ed., Ellis Horwood, Chichester, 1998, Chapter 4.