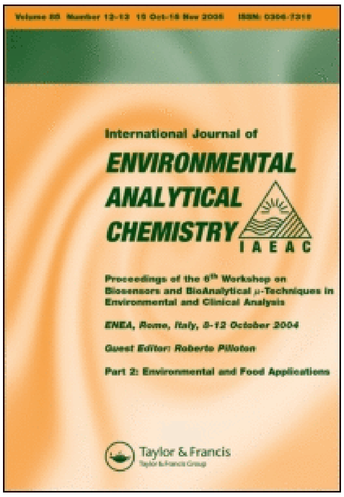

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Evaluation of Extraction Procedures of Organochlorine Pesticides from Natural Waters and Sediments

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EVALUATION OF EXTRACTION PROCEDURES OF ORGANOCHLORINE PESTICIDES FROM NATURAL WATERS AND SEDIMENTS

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This study aims to evaluate different procedures for the extraction of organochlorine pesticides (OCP's) from natural waters and sediments. In the case of extraction from water, a C18 disk solid-phase extraction method was employed. Recovery experiments in the range of 40 to 200 ng/l with selected organochlorine compounds resulted in average recoveries between 80 and 100%. Four different solvents, hexane, ethyl acetate, acetonitrile and methanol, were tested as eluting agents. Best recoveries were obtained with ethyl acetate and hexane. A comparative study of OCP sediment extraction procedures was performed employing sonication, Soxhlet extraction and shake-flask methods. The capacity of these methods to recover OCP's from a sediment sample fortified at 50 ng/g was evaluated using hexane:acetone (1:1 v/v), hexane: acetone (8:2 v/v), acetonitrile and dichlorometane. The three extraction techniques gave similar results and dichloromethane was the most effective solvent. The optimised methods were applied in the analysis of waters and sediments from the "Aiguamolls de l'Empordà" Nature Park, Girona (Spain).

Keywords: Organochlorine pesticides; Sediment; Water; Extraction; Analysis

INTRODUCTION

Organochlorine pesticides (OCP's) have been widely used in agriculture. Although the use of some OCP's has long been prohibited in most countries,

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*Corresponding authors. Fax: 34-972-418150. E-mail: victoria.salvado@udg.es and manuela.hidalgo@udg.es their exceptional properties of persistence and bioaccumulativity have resulted in traces of these substances remaining in the environment to this day[1].

The nature of the distribution of chlorinated contaminants in water, sediments and soils depends on both the properties of the OCP compounds and the physico-chemical characteristics of the ecosystem. The study of their distribution, which is of great interest from an environmental point of view, can be undertaken by the analytical determination of OCP-containing samples.

The determination of organic micropollutants presents certain analytical problems as their trace-level concentrations require the use of preconcentration techniques and the separation of the analytes from the matrix. The most widely used methods for the preconcentration of most types of pesticide from water samples are liquid–liquid extraction (LLE) and solid-phase extraction (SPE) techniques[2]. Nowadays, SPE methods are routinely employed for monitoring pesticides in aquatic environments and international organisations such as the EPA have introduced these methods as an alternative to LLE[3].

Solid-phase techniques have the advantages over LLE of lower cost, greater simplicity, lesser need for solvents and contamination free sample manipulation. SPE also allows the standards laid down in new regulations governing the use and disposal of solvents to be met. For example, only 10 mL of solvent is required to elute the analytes when SPE disks are used. This represents a reduction in volume of between 100 and 1000 when compared to traditional liquid–liquid extraction techniques[4]. The most popular disks for this purpose are octyl- or octadecyl-bonded silica (C8 or C18) disks[5].

A C18 cartridge was evaluated for the extraction of a wide groups of pesticides, including OCP's from surface- and ground-water samples obtaining recoveries between 52 and 102%[6].

The high organic matter content of the sediments together with their direct interaction with the water column make them reservoirs for organochlorine pesticides. Given the highly variable composition of the sediments and their complex matrices, no standard extraction method has yet been established. Amongst the many methods and solvents that have been tested for pesticide extraction are mechanical agitation for different time periods at different temperatures[7], ultrasonic agitation[8], Soxhlet[9], and microwave-assisted extraction[10,11]. The drawback of these techniques is that a post-extraction clean-up process is required to attain good chromatographic detection limits. The use of optimised methods, involving fewer steps, for the separation and enrichment of OCP's would considerably reduce the possibility of analytical error.

The objectives of this study are to appraise the most efficient method of extraction in the determination of organochlorine pesticides found in natural waters and sediments, and to apply the optimised methodology to the analysis of lindane, endrin, aldrin, dieldrin, heptachlor epoxide, 4,4′DDT, 2,4′-DDE and 4,4′-DDD in real samples.

EXPERIMENTAL

Chemicals

The organochlorine pesticides used in this study were dieldrin, 4,4′-DDT, 2,4′-DDE, 4,4′-DDD, γ-HCH, aldrin, endrin and heptachlor epoxide (all supplied by Dr. Ehrenstorfer, Ausburg, Germany).

All solvents used were of analysis grade pesticide residue (Carlo Erba, Italy). Florisil was purchased from Aldrich (Germany). Anhydrous sodium sulphate was of analytical reagent grade (Panreac, Spain).

Solid-phase extraction SPEC disks with a diameter of 74 mm (Ansys Diagnostics Inc., USA) and containing 30 mg of C18 AR bonded phase were held in a 716 SPEC single extraction station with pump (CROMLAB, Spain) for the extraction of OCP's from water samples.

Stock solutions of pesticide mixtures were prepared by dissolving precisely weighted amounts of the different organochlorine pesticides in hexane or acetone. Working standard solutions for GC analysis were diluted with hexane and methanol to obtain spiked samples.

Preparation of spiked samples

MilliQ water was spiked at four concentration levels between 40 and 200 ng/L. The concentrations used to spike waters were in the ratios 1, 2, 4 and 5.

Spiked sediment samples at 50 ng/g were prepared by adding an appropriate standard mixture of organochlorine pesticides to 5 g of wet sediment previously passed through a 0.9 mm sieve. To ensure that the pesticides were well distributed, a reasonable amount of methanol was added to moisten the sediment and careful agitation was performed. The sediment was then dried at room temperature.

Extraction procedure

Solid phase extraction of OCP's from water samples was performed as follows: disks were pre-washed with 10 mL ethyl acetate under vacuum, dried by passing air for 10 min and conditioned with the successive addition of 5 mL of methanol and 5 mL of milliQ water. A 1 L pesticide spiked water sample was passed through the disk and the vacuum was adjusted to obtain a flow rate of approximately 30 mL/min. The disk was then air dried for 10 min. The retained pesticides were eluted with 5 + 5 mL of the different solvents tested: ethyl acetate, methanol, acetonitrile or hexane and the eluate was filtered through anhydrous sodium sulphate. The extract was then evaporated in a rotary evaporator and, finally, 1 mL *n*-hexane was added to dissolve the residue.

The extraction of 10 g of fortified sediment samples was carried out by sonication, mechanical shaking and Soxhlet using acetonitrile, dichloromethane, *n*-hexane : acetone (1 : 1 v/v) and *n*-hexane : acetone (8 : 2 v/v). The sonication procedure consisted in extracting each sample three times for 15 min with 25 mL of solvent in an ultrasonic bath and then combining the extracts. With mechanical shaking, 25 mL of solvent was added to the sediment samples and shaken for 15 h. Soxhlet extraction was performed by extracting the analytes with 300 mL of solvent using a Soxhlet apparatus for 24 h. In all cases the extracts were treated with activated copper for the removal of sulphur and concentrated in a rotary evaporator at 25–30°C. The residue was then dissolved in 5 mL *n*-hexane and cleaned up using a Florisil column previously conditioned with 15 mL of *n*-hexane.

The organochlorine pesticides were eluated from the column by passing 25 mL of *n*-hexane : ethylacetate (14 : 1 v/v) and 25 mL of *n*-hexane : ethylacetate (15 : 10 v/v). After evaporation of the eluates, the final residue was dissolved in 1 mL of *n*-hexane and submitted to GC analysis.

Analysis of real samples

Two or 2.5 L volumes of water were collected monthly between November 1996 and August 1997 at 29 sites within the territory of the "Aiguamolls de l'Empordà" Natural Park. The sampling sites were located at the main supply channels of this area as well as at the salt and fresh water reserves. After filtration with 0.45 µm cellulose nitrate filters, water samples were passed through a C18 pre-conditioned disk following the previously described procedure. The pesticides retained were eluted with 5 + 5 ml of ethyl acetate, which gave as good results as hexane in the recovery experiments and had the additional advantage of also extracting triazines. After elution, the extract was carefully evaporated and the final residue, dissolved in 1 μl of hexane, was analysed by GC-ECD.

Two core sediment samples were collected at an approximate depth of 5 cm from each of six pre-selected sites in spring 1997. After collection, the samples were preserved at −20°C. OCP's were extracted from five gram portions of the two sediment samples with 3 × 30 mL of hexane : dichloromethane (1 : 1 v/v) in an ultrasonic bath. The three fractions obtained were mixed together to form one single sample which was then purified, following the clean-up procedure described before.

GC Analysis

Gas chromatography was performed using a Fisons HRGC 8000 equipped with a split/splitless injector, electron-capture detector and a J&W Scientific DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium and nitrogen were employed respectively as the carrier and auxiliary gases. OCP determinations were carried out with an injector temperature of 270°C and a detector temperature of 300°C. The column temperature program was set at 70°C and held for 1 min before increasing to 180°C at 15°C/min, then 225°C at 5°C/min, and finally to 270 at 3°C/min for 3 min. A 1 μL volume of the sample was injected in the splitless mode.

RESULTS AND DISCUSSION

Recovery studies

Evaluation of extraction efficiency was carried out using water and sediment samples spiked with the pesticides under investigation, as has been described in the experimental section.

The degree to which organic micropollutants may be recovered from water samples depends on sample volume, the sorbents used, the type of water, pesticide concentration, pH, and the process of elution [3]. In our case, the concentration of the pesticides and their specific properties led us to give the greatest importance to the solvent used as the eluting agent. The results obtained for the four solvents tested (methanol, acetonitrile, hexane and ethyl acetate) are presented in Table I as the mean values of four separate analyses.

TABLE I Mean recoveries, *R*%, of tested compounds at different spiking levels in Milli-Q water after extraction using C18 disks and elution with 5 + 5 mL of different solvents

Pesticide	*R% (n = 4)*			
	40 ng/L	*80* ng/L	*160* ng/L	*200* ng/L
Solvent: Ethyl Acetate				
Lindane	118	91	96	77
Aldrin	85	97	93	62
Endrin	–	114	110	93
Dieldrin	–	115	112	94
Heptachlor Epo.	100	76	99	82
4,4′-DDE	110	109	100	82
4,4′-DDD	119	110	110	92
Solvent: Hexane				
Lindane	109	83	91	72
Aldrin	102	84	87	72
Endrin	106	99	107	104
Dieldrin	112	105	104	97
Heptachlor Epo.	86	68	84	70
4,4′-DDE	109	100	95	89
4,4′-DDD	109	98	100	99
4,4′-DDT	100	100	104	91
Solvent: Methanol				
Lindane	61	85	81	56
Aldrin	47	84	82	69
Endrin	–	105	101	90
Dieldrin	113	108	94	89
Heptachlor Epo.	51	73	64	61
4,4′-DDE	102	111	98	72
4,4′-DDD	103	106	92	86
4,4′-DDT	99	110	97	86
Solvent: Acetonitrile				
Lindane	98	108	85	79
Aldrin	90	87	75	71
Endrin	114	–	104	86
Dieldrin	–	–	109	90
Heptachlor Epo.	63	83	75	66
4,4′-DDE	109	109	75	66
4,4′-DDD	100	108	86	90
4,4′-DDT	108	110	86	91

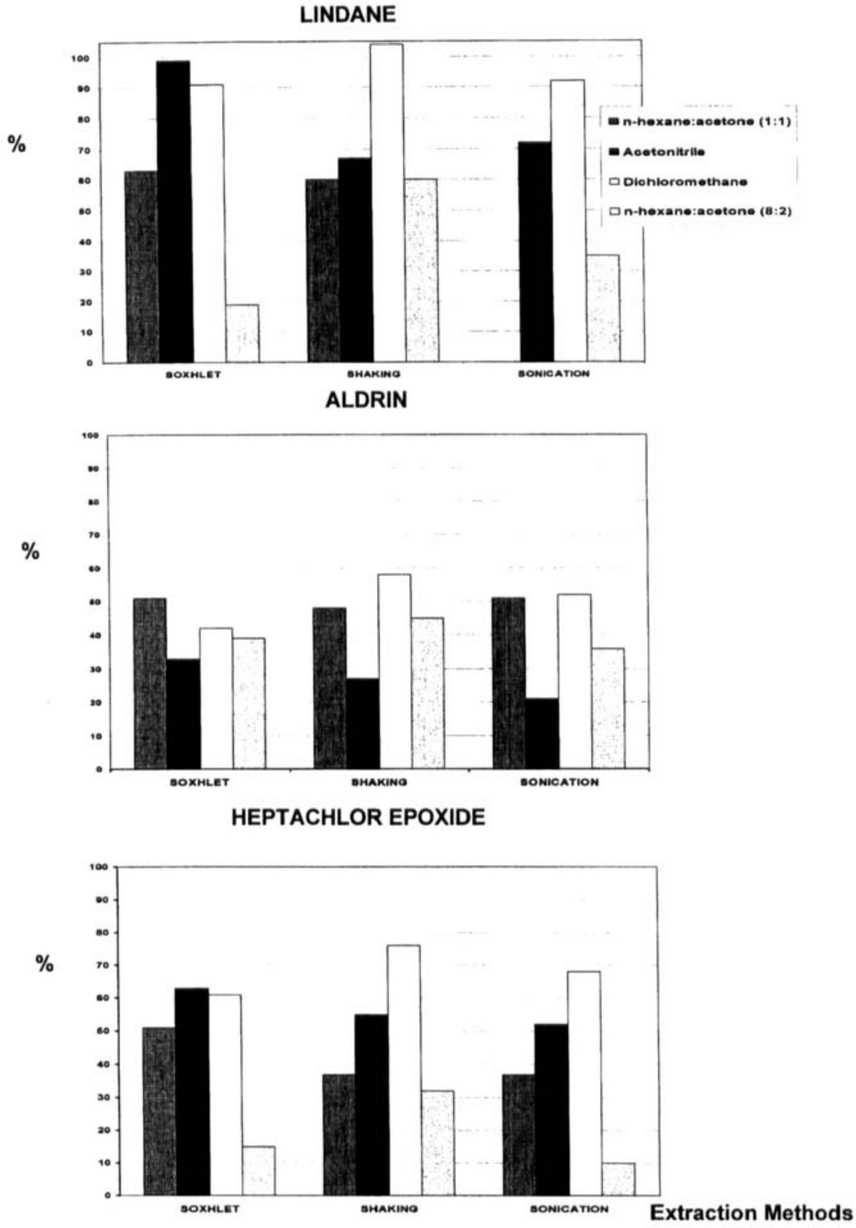
As can be seen in the table, good recoveries (80–110%) for all the pesticides are obtained with hexane and ethyl acetate, but a slight decrease is observed at a spiking level of 200 ng/L (these values are in accordance with the recovery levels established by the AOAC[12] for the spiking levels employed). With the exception of hexane and ethyl acetate, all the solvents proved to be less effective for the highest concentration (200 ng/L). The lowest recovery values correspond to methanol, which is the most polar solvent used in this study. The different polarities of the pesticides, which are deduced from their octanol/water coefficients[13], may be a further factor in explaining variations in recovery rate. Among the compounds analysed the lowest values are obtained for hepthachlor epoxide, which is particularly noticeable when methanol is used. Earlier studies[14] conducted with the same solvents and extraction disks have not given as good results as those of the present study. We believe that this may be explained by the lower concentration of OCP's used in our experiments, which have been carried out with similar levels to those found in natural waters. It should be noted that analytical determination errors are high when spiking levels are very low (i.e. 40 ng/L) given that concentrations are near the limits of quantification. The limit of detection (LOD) of each pesticide, which was between 1–3 ng/L for the OCP's analysed, was determined after the extraction of spiked water samples and chromatographic analysis.

OCP extraction from a 50 ng/g spiked sediment was carried out by three different techniques: shake-flask, sonication and Soxhlet extraction. Four eluting agents were tested with each technique: acetonitrile, dichloromethane and hexane : acetone mixtures, respectively, at ratios of (1 : 1 and 8 : 2 v/v) (Figs. 1–3). Dichloromethane presented the best recovery rates (70–100%) except in the case of aldrin for which none of the eluting agents managed to reach recovery rates of over 60%. Hexane : acetone (8 : 2 v/v) was the least effective in most experiments. However, the results obtained show that there is no significant difference between the three techniques with respect to recovery rate and hence we conclude that the technique for OCP extraction to be favoured should be determined by analysis time and solvent consumption. Sonication assisted solid-liquid extraction was chosen for the analysis of sediment samples collected in the "Aiguamolls de l'Empordà" and a dichloromethane : hexane (1 : 1 v/v) mixture was used as the solvent in the belief that the addition of hexane would improve the efficiency of the extraction, as had been observed in preliminary experiments. The limit of detection (LOD) of each pesticide was between 0.1–0.2 ng/g.

The lowest recovery values were obtained, in the case of extraction from sediments, for aldrin, which has a high $K_{o/w}$ value. This finding, which has been observed in other studies[15], may be explained by the strength of the interaction of aldrin with the sediment matrix.

Real samples

The optimised procedures were applied to the analysis of surface waters and sediments from the "Aiguamolls de l'Empordà," a salt marsh area in the


LINDANE

ALDRIN

HEPTACHLOR EPOXIDE

FIGURE 1 Comparison of recoveries ($n = 2$) of lindane, aldrin and heptachlor epoxide from spiked sediments by using three extraction methods.

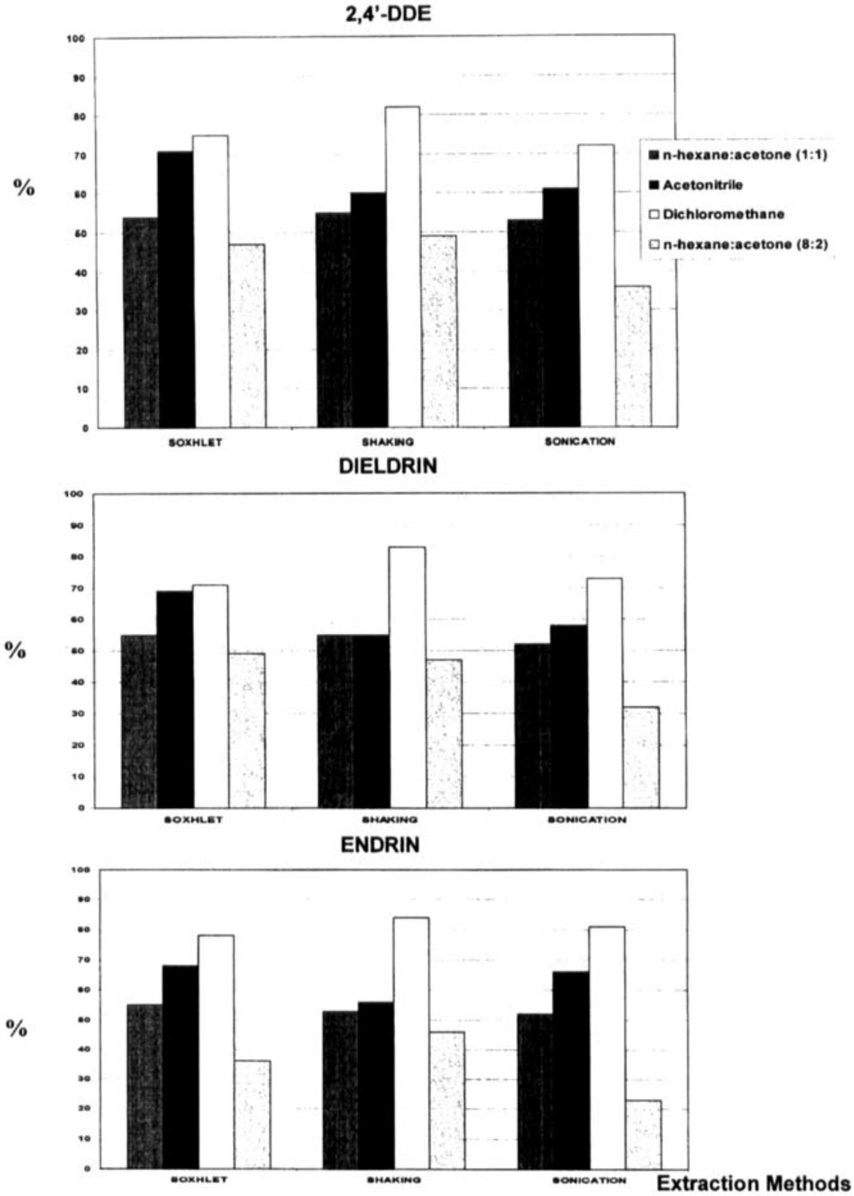


FIGURE 2 Comparison of recoveries ($n = 2$) of 2,4′-DDE, dieldrin and endrin from spiked sediments by using three extraction methods.

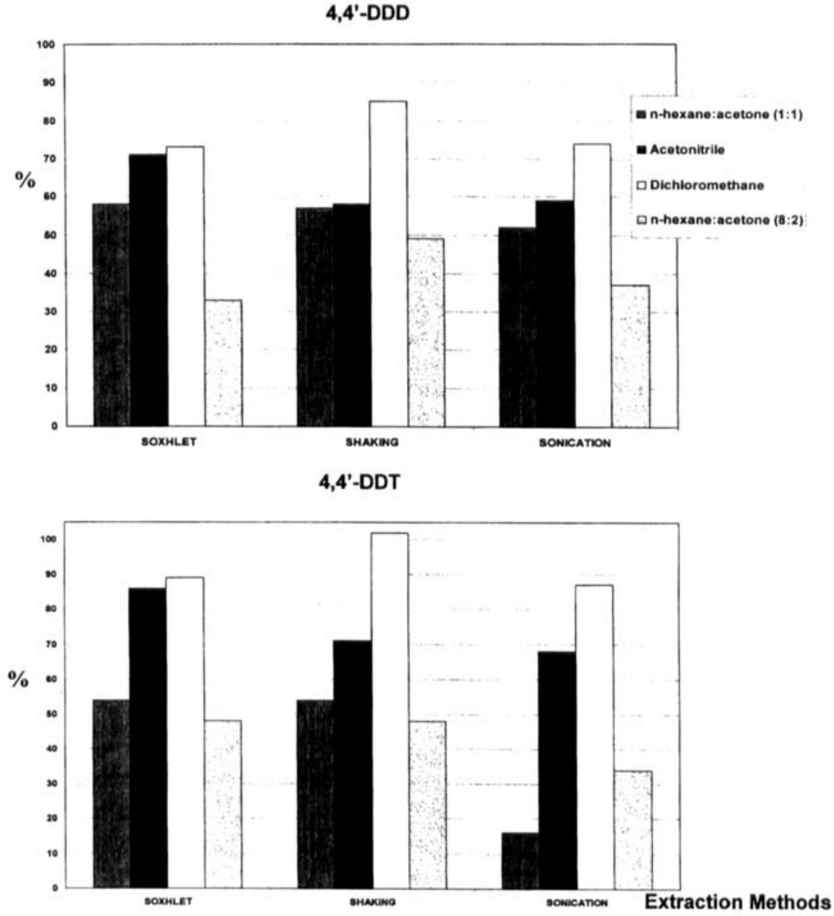


FIGURE 3 Comparison of recoveries ($n = 2$) of 4,4′-DDD and 4,4′-DDT from spiked sediments by using three extraction methods.

province of Girona (North-East Spain)[16]. This natural park, bordered by agricultural land except to the west where it meets the Mediterranean Sea, is one of the few areas in Europe acting as a way station for migratory birds and it is made up of a salt water reserve and a fresh water reserve.

Analysis of these waters allowed us to determine the mean concentrations of organochlorine pesticides (Fig. 4). The higher levels correspond to Lindane and Heptachlor epoxide, which are still used in local farming practices. There was also a significant presence of DDT, DDD, aldrin, endrin and dieldrin. As can be seen in the graph, concentrations of OCP's were slightly different in the fresh water and salt water reserves. The highest concentrations of lindane and heptachlor epoxide were found in the main supply channels. Significant seasonal variations were observed in pesticide levels during a 1-year monitoring period. By way of example, the monthly levels for lindane range from maximum values of 33 ng/L in April and May 1997, corresponding to periods when pesticides are commonly applied to animal feed crops, to almost undetectable levels in November and December 1996. Similar behaviour was observed for heptachlor epoxide reaching the maximum concentration of 86.3 ng/L. The other pesticides detected in surface waters presented maximum concentrations of 80 ng/L for endrin, 79 ng/L for DDT and 53.2 ng/L for DDD after intensive rainfall episodes. The above values were determined after filtration of the water samples and do not take into account the pesticide present in the suspended matter.

The results obtained from the analysis of sediment samples are set out in Fig. 5. The values given are the mean of the two samples collected at each site. We observe that more OCP's were detected in sediments than in water. The presence of organic matter, due to its adsorption capacity, favours the accumulation of OCP's in the sediments. Heptachlor epoxide, lindane and DDT followed by aldrin and endrin, were the most commonly found pesticides in sediments. Analysis and determination of the content of

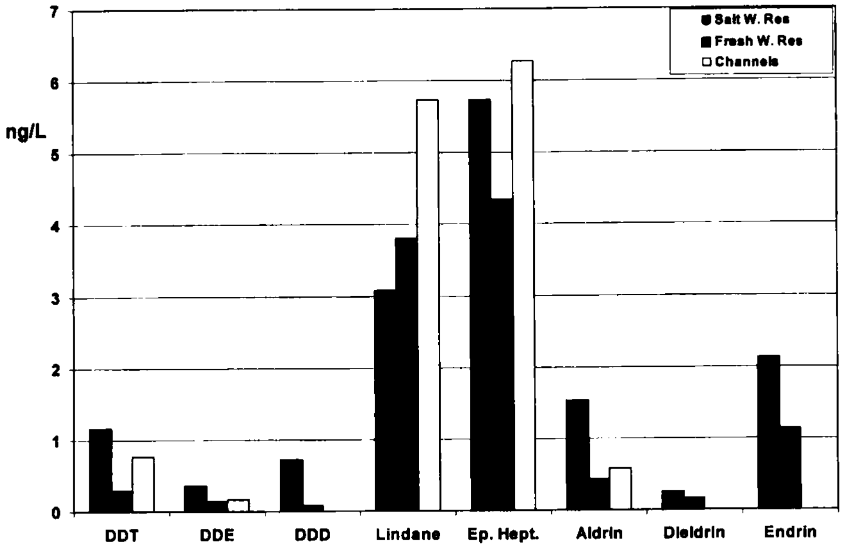


FIGURE 4 Mean levels of concentration of organochlorine insecticides in surface waters of "Aiguamolls d'Empordà" (1996–97).

the organochlorine pesticides in sediments give information about the accumulation of these contaminants over a long period of time, as can be seen by the detection of DDE and DDD – degradation products of DDT, which has long been prohibited. DDT was found at each of the different sampling sites. The highest concentrations of OCP's 2.46 ng/g for lindane, 1.34 ng/g for DDD, 13.1 ng/g for heptachlor epoxide, 3.24 ng/g for aldrin and 2.12 ng/g for endrin were found in the salt water reserve sites demonstrating that salinity contributes to pesticide accumulation in sediments[17].

CONCLUSIONS

In this study we have attempted to seek improvements in the procedures used in the analysis of OCP's in natural waters and sediments, particularly concentrating on the extraction of analytes from samples. We have tested different solvents for the extraction of pesticides from natural waters using C18 extraction disks, and different techniques and solvent in the case of sediments.

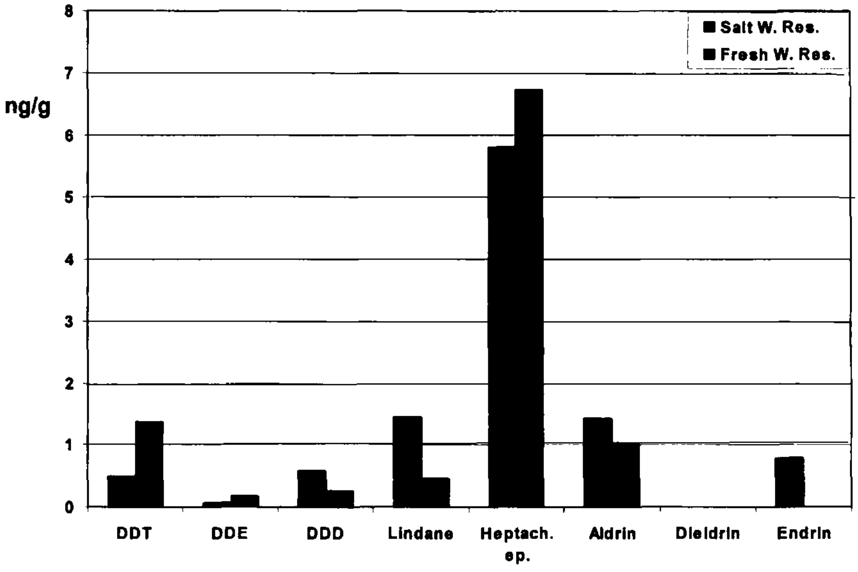


FIGURE 5 Mean levels of concentration of OCP's in sediments of "Aiguamolls de l'Empordà".

Ethyl acetate and hexane proved to be the most suitable of the solvents tested in the extraction of pesticides from waters giving recovery rates in excess of 80%. No significant differences were observed between the ultrasound, soxhlet extraction and shake-flash methods used in extraction from sediments. All techniques were most effective when dichloromethane was used as the solvent.

Modified procedures were employed for the analysis of real samples collected in a protected natural marsh. OCP levels both in sediments and filtered waters were within permitted levels for individual tests although aggregate levels were occasionally exceeded. Lindane and hepthachlor epoxide, whose concentrations varied seasonally, were the most commonly detected pesticides in waters. The greater capacity of sediments to accumulate OCP's resulted in higher levels being found than in waters. Those OCP's which are particularly hydrophobic, heptachlor epoxide, DDT, endrin and lindane, proved to be those which were most widely detected in sediments.

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