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Sample Purification for Analysis of Organochlorine Pesticides in Sediment and Fish Muscle

B. Pan,[1] W. X. Liu,[1,*] Z. Shi,[1] J. Cao,[1] W. R. Shen,[2] B. P. Qing,[3] R. Sun,[3] and S. Tao[1]

[1]Laboratory for Earth Surface Processes Simulation and Analysis, College of Environmental Sciences, Peking University, Beijing, China
[2]Tianjin Environmental Protection Bureau, Tianjin, China
[3]Tianjin Environmental Monitoring Center, Tianjin, China

ABSTRACT

The methods of sample purification for determinations of organochlorine pesticides (OCPs) in sediment and fish muscle were investigated in this study. A two-step procedure with preliminary cleanup and florisil column fraction was adopted. The working conditions of florisil column were firstly optimized. As to preliminary cleanup, liquid–liquid extraction by mixture of dimethyl formamide (DMF) and hexane and sulphonation by concentrated sulfuric acid were applied respectively and compared for the studied samples. The results indicated that liquid–liquid distribution could effectively separate lipids from fish muscle extracts, while failed in elimination of chlorophyll extracted from the relicts of hydrophytes in the sediment sample. In view of the sample appearance and clarity of gas chromatogram, sulphonation was more suitable to purify the sediment sample, however, the recoveries of the spiked standards were poor. The use of eluting solvent with stronger polarity could improve the corresponding recoveries significantly. Due to complete loss of Dieldrin, Endrin, and Endrin aldehyde after sulphonation, this pretreatment was improper to the three components. Sulfur, as the particular disturbing element in sediment, could be removed sufficiently by

*Correspondence: W. X. Liu, Laboratory for Earth Surface Processes Simulation and Analysis, College of Environmental Sciences, Peking University, Beijing 100871, China; Fax: 86-10-62751938; E-mail: wxliu@urban.pku.edu.cn.

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addition of activated copper powder. The relevant detection limits of the selected methods for OCPs in the studied substrates were also provided.

Key Words: Organochlorine pesticides; Sample purification; Sediment; Fish muscle.

INTRODUCTION

Although many organochlorine pesticides (OCPs) have been forbidden or reduced to apply, large quantities of residual fractions still remain in the natural environment due to their persistence, and cause adverse effects on organisms and human beings. Sediment and biota are the main sinks of OCPs based on actual measurements and simulation results using the multimedia fate model.[1] Therefore, precise analysis of OCPs in these substrates may offer accurate information on the distribution of persistent organic pollutants and provide background data for further bioremediation and ecological risk assessment.

In the course of quantitative determinations, extraction and cleanup pretreatment of raw samples are the fundamental procedures, since the purity of sample input for gas chromatography (GC), commonly employed as the analytical technique of OCPs, would directly affect the accuracy and sensitivity. Many impurities (e.g., triglyceride and humic substances) having similar solubility in the extracting solvents may coexist in the extract of OCPs. The presence of impurities can decrease the analytical performance of instrument via retaining in the injection port and/or in the chromatographic separation column. New developments on sample extraction have been achieved, exemplified by pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE).[2,3] To date, the common cleanup method is the solid–phase extraction (SPE) or column chromatography,[4] in which adsorbed OCPs can be eluted by appropriate solvent whereas the impurities are held in the column filled with polar adsorbents. Among various adsorbents, florisil is utilized frequently. The adsorptive sites on florisil are always partly deactivated by water, and the reported deactivated ratio ranged from 0.5 to 15%.[5] Since the compositions of produced florisil often differ from batch to batch, the specific elution behavior should be examined in each batch before use.[6] Another effective cleanup approach to the complex extract is chemical degradation of the impurities. Addition of strong acid or base, for instance, concentrated sulphuric acid or mixture of potassium hydroxide and alcohol, can saponify the impurities to form products with strong polarity that can be easily washed out by water.[4] To some extent, the method is, however, limited due to the violent reaction resulting in operational risk and mass loss.[7] In addition, liquid–liquid distribution, based on selective dissolution of OCPs, also acts as a surrogate cleanup method.[8,9]

Despite the recent progresses, current knowledge on the purification procedure, especially for the miscellaneous environmental samples, such as natural sediments in the polluted area, is insufficient, and necessary modifications are required in many cases. The main objective of this study was to optimize the sample cleanup conditions for determining OCPs in natural sediment and fish muscle after extraction by PLE, in which the deactivated ratio of florisil and the eluting properties of OCPs concerned were

investigated. Moreover, the effects of two assistant procedures, as the preliminary purification step, are compared between sulphonation and liquid–liquid distribution.

EXPERIMENTAL

Reagent

Dichloromethane (DCM) and hexane were analytical grade and distilled in glassware for purification. Dimethyl formamide (DMF, analytical grade) was saturated with hexane. Anhydrous Na_2SO_4 was activated by heating at 650°C for 4 h. Florisil (particle size of 100 ~ 200 mesh) was roasted at 600°C for 6 h and saved in a closed container, then activated at 130°C for 10 h just before use. The activation was valid within three days. Super purified copper powder was activated in diluted hydrochloride acid everyday and stored in methanol. The mixed standard of OCPs was provided by Agilent Company (Model 8500-5896), including 16 components ranked by the retention time in gas chromatography: (1) α-Hexachlorocyclohexane (HCH), (2) β-HCH, (3) δ-HCH, (4) γ-HCH, (5) Heptachlor, (6) Aldrin, (7) Heptachlor epoxide, (8) Endosulfan I, (9) Dieldrin, (10) *p*,*p*′-Dichlorodiphenyldichloroethylene (DDE), (11) Endrin, (12) Endosulfan II, (13) *p*,*p*′-Dichlorodiphenyldichloroethane (DDD), (14) Endrin aldehyde, (15) Endosulfan sulfate, and (16) *p*,*p*′-Dichlorodiphenyltrichloroethane (DDT). In this study, we paid main attention to HCHs and DDTs since they were predominant pollutants in the local area.

Sample Preparation

Surface sediment in a river was gathered from Huangzhuang located in the wastewater irrigation area of Tianjin. The freeze-dried sample was ground and passed through an 80-mesh sieve. Before extraction, 20 g sediment powder was mixed with 10 g anhydrous Na_2SO_4 and packed into a 34-mL vessel of accelerated solvent extractor (ASE, DIONEX ASE-300). This sediment sample was regarded as the one without de-sulfur pretreatment to study the influence of sulfur impurity.

Rainbow trout (*Salmo gairdneri*) was collected from Shuntong feeding pond far from the downtown of Beijing. After seven days accommodation in the lab, the healthy fish was killed and 2 ~ 3 g of skinned dorsal muscle were ground with 15 g anhydrous Na_2SO_4 in an agate mortar. Like the sediment sample, the mixture was then packed into the vessel of ASE for the next extraction.

The mixed standard solution was added to the samples before extraction.

Extraction

Pressured liquid extraction (PLE) was implemented using ASE. The working conditions were as follows: preheating for 5 min, extraction temperature at 125°C, pressure at 1500 psi, static cycle of 10 min in twice, and purging with N_2 for 60 s.

The extraction solvent was DCM-hexane (v:v = 1:1). The whole procedure for one sample needed about 30 min. Other relevant details could be seen elsewhere.[10]

Cleanup

The extract was concentrated to around 1 mL in a rotary evaporator and then purified by following steps: (i) preliminary cleanup, and (ii) column separation. In this study, the efficiency of cleanup was evaluated with three criteria, namely, the appearance of the treated sample, the quality of GC peak (e.g., shape, sharpness, and baseline) and the recovery of the spiked standard.

Preliminary Cleanup

Prior to column separation, the extract should be pretreated to reduce the load of the separation column. For this purpose, two methods were compared in the preliminary cleanup steps.

Sulphonation with Concentrated Sulfuric Acid

The concentrated extract was transferred into a 250 mL separatory funnel with 20 mL hexane. Twenty milliliters of concentrated sulfuric acid was added to the funnel followed by violent shaking for 1 min. Subsequently, two clear layers would appear. If not, several drops of 4% Na_2SO_4 solution were input to demolish the emulsion layer, the funnel was then agitated gently and continously to avoid boiling inside. The lower H_2SO_4 layer was discarded. Then, 10 and 8 mL of concentrated H_2SO_4 were added, in turn, to the sample. After rinsing with 20 mL 4% Na_2SO_4 solution in twice, the upper hexane layer was rotary evaporated to about 1 mL.

Liquid–Liquid Extraction with DMF

Alternatively, the concentrated extract was moved into a 250 mL separatory funnel using 20 mL hexane. Ten milliliters of DMF, presaturated by hexane, was added into the funnel, which was shaken for 3 min and laid aside until two distinct layers were presented. DMF in the lower layer was released to another funnel. The residue in the first funnel was additionally extracted by DMF twice. All the added DMF was gathered in the second funnel. Afterwards, 100 mL 2% Na_2SO_4 solution and 10 mL hexane were put into the second funnel, which was stirred fully and laid aside for several minutes. Finally, the lower layer in the second funnel was discharged and the upper one rotary evaporated to about 1 mL.

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Column Cleanup

This step was conducted through a multi-layer column packed orderly from the bottom with: a small amount of absorbent cotton extracted in ASE under the identical conditions of sample extraction, 6 g florisil activated at 130°C and partly deactivated by water, 1 cm height anhydrous Na_2SO_4 activated at 650°C.

Deactivated Ratio

As mentioned earlier, the proper deactivated ratio is necessary to be determined in every batch of florisil, owing to the variations in the product. In this study, the following deactivated ratio of florisil was examined to find the favorite condition of OCPs cleanup: 1, 3, 5, 7, 9, 11, 13, and 15% in weight. Before being packed into the column, florisil was shaken completely with a certain amount of water in a conical flask until no block could be seen inside.

Eluting Solvents and Eluting Curves

To separate the analytes of interest sufficiently from the disturbing co-extracts, it is necessary to select the eluting solvents with appropriate polarity. We originally employed the method introduced by Takahashi et al.[11] with a small modification: the extract was poured into the column and firstly eluted by 50 mL hexane, then 50 mL mixed solvent of DCM and hexane (v:v = 2:8) at the rate of 2 mL/min. All the 100 mL solvents were put together and rotary evaporated, then nitrogen blowed to 1 mL for the further GC analysis.

Moreover, the eluting curve, indicating the elution characteristics of analytes, may provide some important information on the modification of the eluting solvents. In this article, every 6-mL of the effluent was collected and determined individually. The eluting curve was illustrated by accumulative recovery vs. accumulative eluting volume.

De-sulfur Procedure

As to natural sediment samples, sulfur, having similar solubility to OCPs and forming the co-extract with OCPs, is a common disturbing element caused a broad overlapping peak in GC to influence the accuracy of quantitative measurements.[12] To eliminate the negative effect, 1 g copper powder (high purity) activated by diluted hydrochloric acid, was mixed with the ground sediment sample and reacted at high temperature and high pressure in ASE. Furthermore, in the separation column, 1 g activated copper powder and 1 cm height anhydrous Na_2SO_4 were filled between the absorbent cotton layer and the florisil layer to enhance the removal of sulfur.

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Table 1. Working conditions in gas chromatography.

Condition	Setting value
Flow rate of carrier gas	1.7 mL/min (high purity N_2)
Injection temperature	220°C
Injection volume	1 μL
Splitless time	0.75 min
Detector	Ni ECD
Detector temperature	280°C
Flow rate of makeup gas	60 mL/min (N_2)
Retention gap	Fused silica, methyl deactivated, 2.5 m, 0.53 mm(i.d.)
Column	HP-5, 30 m × 0.32 mm(i.d.) × 0.25 μm(film thickness)
Initial oven temperature	50°C
Initial programming rate	10°C/min
Second isotherm temperature	150°C
Second isotherm rate	3°C/min
Third isotherm temperature (period)	240°C (10 min)
Quantitative method	External standards, peak area

GC Analysis

Quantification of the studied analytes was finished on a gas chromatography (Agilent 6890 plus) equipped with an electronic capture detector (ECD) and an auto-sampler (Agilent 7683). The analytical conditions of GC were listed in Table 1.

Method Detection Limit (MDL)

The detection limit of the selected method was calculated based on the replicated determinations:

$$\mathrm{MDL} = t_{0.99[n-1]} \times s$$

where t is the threshold value of student t-distribution at the freedom degree of $(n-1)$, n represents the number of replications (7 aliquots in this study), and s denotes the standard deviation. The confidence interval is 99% ($\alpha = 0.01$).

The MDL was acceptable when the measured concentrations of the aliquots were lower than 5-fold of the MDL.[13]

RESULTS AND DISCUSSION

Column Separation

Deactivated Ratio of Florisil

After activation at 130°C for 10 h, florisil is a very strong adsorbent for the OCPs. To avoid the loss of OCPs, some sorption sites should be deactivated by

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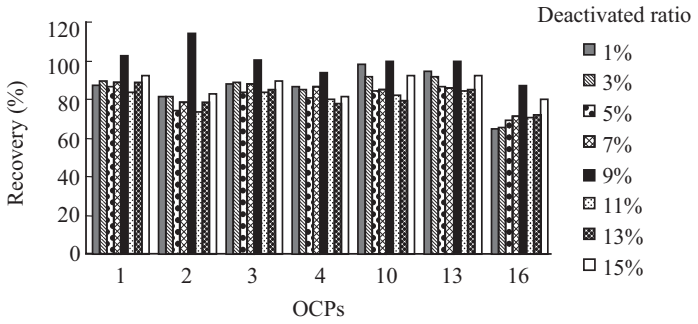

Figure 1. Comparison of deactivated ratio of florisil. OCPs: (1) α-HCH, (2) β-HCH, (3) δ-HCH, (4) γ-HCH, (10) p,p'-DDE, (13) p,p'-DDD, and (16) p,p'-DDT.

adding certain amount of water before loading into the separation column. Based on a former study,[14] the deactivated ratio of florisil was studied among 1, 3, 5, 7, 9, 11, 13, and 15% in this study. The recovery of the spiked standard was depicted in Fig. 1.

As to HCHs and DDTs of interest, the deactivated ratio of 9% is undoubtedly the favorite condition of florisil, and the result is consistent with the previous study.[14]

Eluting Curves

After the preliminary cleanup, the sample was concentrated and transferred into the separation column. Then, a certain amount of solvent with proper polarity eluted the analytes to separate from the disturbing impurities. The eluting behaviors of the studied components were shown in Fig. 2.

Most DDTs and HCHs were eluted in the first 50 mL of hexane (neutral solvent), except γ-HCH (see the panel A in Fig. 2), which flowed off the column only in the polar surroundings. On the other hand, the features of eluting curves of other analytes were quite different (see the panel B in Fig. 2). The curves of Heptachlor, Aldrin, Heptachlor epoxide, Endosulfan I, Dieldrin, Endrin and Endrin Aldehyde, leveled off to a plateau and showed the presence of eluting endpoint. The increasing trend occurred to Endosulfan II and Endosulfan sulfate suggest that two components are still in a desorption stage, and additional extractant may increase their recoveries.

After the changing point of solvent, there was a slightly rising tendency present for all the eluting curves. This situation implies that further effusion can be found when using a solvent with stronger polarity. However, increase in the solvent polarity would probably enhance the possibility of effusion of the disturbing impurities. Therefore, change in extractant should be considered carefully.

Compared with the normal procedure,[15] the recoveries of most components were much lower in the results of 6-mL batch, especially for Dieldrin, Endrin, and Endrin aldehyde. The phenomenon is possibly originated from other losses in the accumulative sampling of 6-mL batch. Nevertheless, the higher recoveries of HCHs with respect to the normal procedure[15] demonstrate that HCHs are liable to

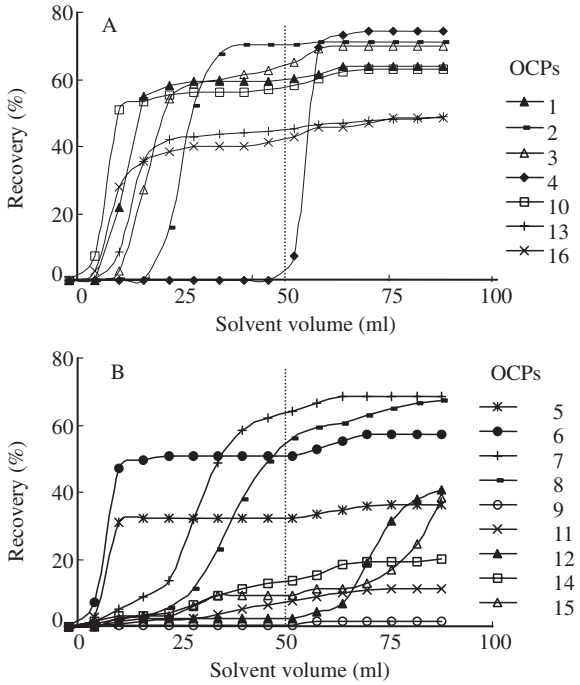

Figure 2. Eluting curves of all the components. OCPs: (1) α-HCH, (2) β-HCH, (3) δ-HCH, (4) γ-HCH, (5) Heptachlor, (6) Aldrin, (7) Heptachlor epoxide, (8) Endosulfan I, (9) Dieldrin, (10) p,p'-DDE, (11) Endrin, (12) Endosulfan II, (13) p,p'-DDD, (14) Endrin aldehyde, (15) Endosulfan sulfate, and (16) p,p'-DDT. Panel A—HCHs and DDTs, and panel B—other components. The dotted lines denote the changing point of solvent.

release from the column, and the main losses of HCHs may occur in the preliminary cleanup.

Cleanup of Fish Muscle Sample

The extract of fish muscle using ASE was turbid due to the presence of lipid, which after freezing aggregated and transformed into white blocks. The remained lipid with high boiling point would make negative influence on the following GC analysis, in the form of polluting the inlet and capillary or confusing the normal gas chromatogram by unexpected effusion. After the preliminary cleanup (sulphonation or liquid–liquid extraction) and column separation, the sample appearance was clear, and plain baseline arose in the corresponding chromatographs. A quantitative comparison between sulphonation and liquid–liquid distribution, in terms of recovery of the spiked standard, was illustrated in Fig. 3.

As manifested in Fig. 3, for the first seven components, the two preliminary cleanup methods did not reveal significant difference at the level of $\alpha = 0.01$, and the related recoveries were around 60%. However, most of the other components with

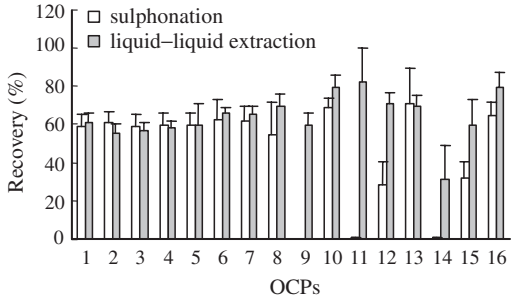

Figure 3. Comparison between sulphonation and liquid–liquid extraction for the fish muscle sample. OCPs: (1) α-HCH, (2) β-HCH, (3) δ-HCH, (4) γ-HCH, (5) Heptachlor, (6) Aldrin, (7) Heptachlor epoxide, (8) Endosulfan I, (9) Dieldrin, (10) p,p'-DDE, (11) Endrin, (12) Endosulfan II, (13) p,p'-DDD, (14) Endrin aldehyde, (15) Endosulfan sulfate, and (16) p,p'-DDT.

longer retention time and stronger polarity exhibited considerable low recoveries at the level of $\alpha = 0.01$, and Endrin and Endrin aldehyde were totally lost in the sulphonation treatment. Accordingly, the liquid–liquid extraction is preferable for the fish muscle sample.

Cleanup of Sediment Sample

De-sulfur Procedure

Based on the normal pretreatment and freezing, some pale yellow acicular crystals of sulfur often occur in the vial loading sediment samples. In some cases, although sulfur impurity is invisible in the sample, the baseline in gas chromatograms is highly elevated and the peaks are overlapped to a large extent (see the upper panel in Fig. 4) probably due to the presence of sulfur. After addition of activated copper powder described in the experimental section, the baseline was plain and the GC peaks of the components could be distinguished clearly (see the lower panel in Fig. 4). The uncertain peak with the retention time at 29 min made no influence on the qualitative and quantitative analysis of the components studied.

Preliminary Cleanup

In view of the analytical recoveries of the components in the sediment sample, sulphonation and liquid–liquid extraction, using the same mixed volume ratio of DCM and hexane, displayed approximate purification efficiency for the first seven components, while the latter method showed higher recoveries for the other components as shown in Fig. 5.

In this study, the color of the sediment extract was pea green. By the liquid–liquid distribution, the pea green substances (i.e., chlorophyll extracted from the

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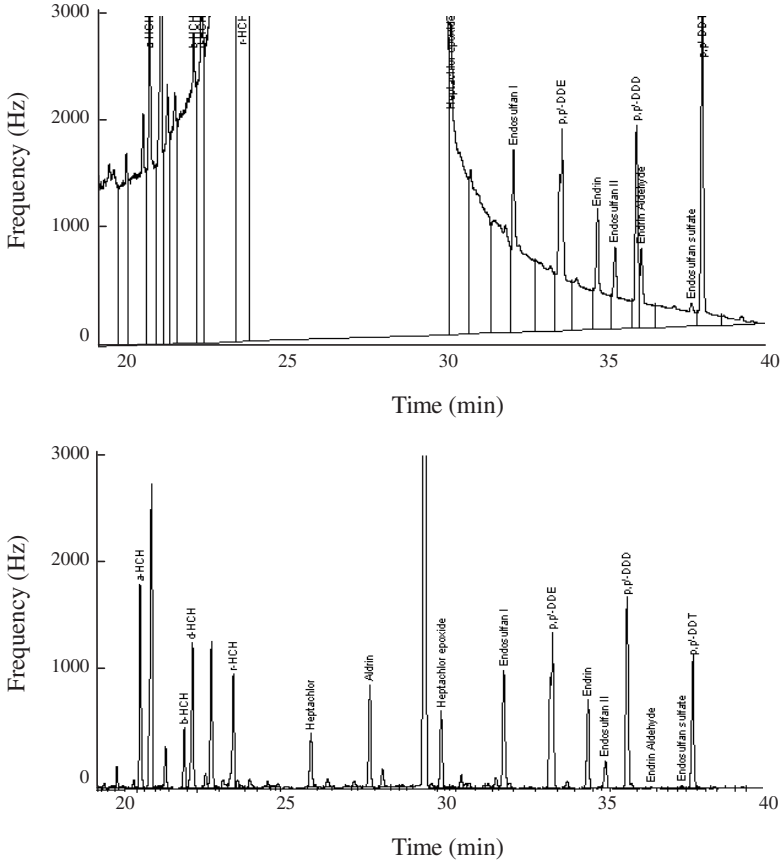

Figure 4. Gas chromatograms of the sediment sample pretreated without (upper panel) and with (lower panel) de-sulfur procedure.

relicts of hydrophytes in the sediment sample) could not be eliminated, and would be subsequently eluted from the separation column. Thereby, considering the appearance of sediment sample after the preliminary cleanup, the liquid–liquid extraction has to be discarded.

The sediment sample experienced sulphonation was colorless and the relevant gas chromatogram was clear, whereas the lower recoveries of the concerned components should be improved. Based on actual tests and discussion in the section of eluting curves, a possible choice is application of a solvent with stronger polarity to the second elution stage after the changing point of solvent. Thus, a mixed solvent of DCM and hexane (v:v = 3:7) was evaluated. As viewed in Fig. 5, the recoveries of the other components improved to different extents, except those of Dieldrin, Endrin and Endrin Aldehyde, which infer a total loss of them during sulphonation. Seidel et al.[7] also reported similar results of Dieldrin and Endrin. As for use of higher ratio of DCM and hexane, since increase in the extractant polarity would probably lead to more effusion of the disturbing impurities, we do not adopt in the present study.

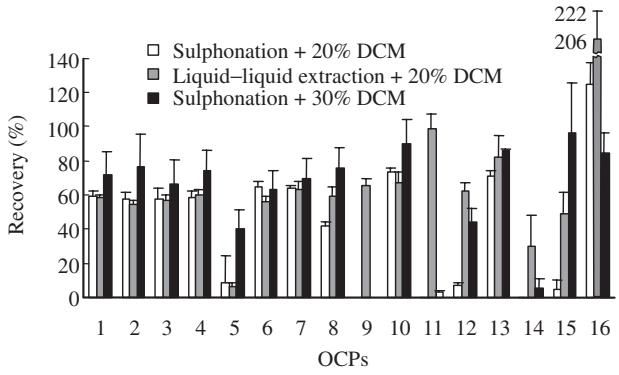

Figure 5. Comparison between sulphonation and liquid–liquid extraction for the sediment sample. OCPs: (1) α-HCH, (2) β-HCH, (3) δ-HCH, (4) γ-HCH, (5) Heptachlor, (6) Aldrin, (7) Heptachlor epoxide, (8) Endosulfan I, (9) Dieldrin, (10) p,p'-DDE, (11) Endrin, (12) Endosulfan II, (13) p,p'-DDD, (14) Endrin aldehyde, (15) Endosulfan sulfate, and (16) p,p'-DDT.

In a sense, it is difficult to find a universal method to satisfactorily purify the natural complex samples containing OCPs with a variety of structures and physicochemical properties. The method presented here is preferably applicable to HCHs and DDTs. As to detection of other specific components, some substitutive methods may be taken into account,[16] such as enzyme-linked immunosorbent assay (ELISA) for Dieldrin.

Selected Methods and Related MDLs

In summary, appropriate cleanup methods were selected for the studied samples as follows.

For the fish muscle sample:

Liquid–liquid extraction → Florisil column fractionation (9% deactivated ratio, 1 cm height anhydrous Na_2SO_4), 50 mL hexane + 50 mL mixture of DCM and hexane (v:v = 2:8) → Concentration → GC analysis

For the sediment sample after the first de-sulfur procedure with activated copper powder in ASE:

Sulphonation → Florisil column fractionation (9% deactivated ratio, 1 g activated copper powder, 1 cm height anhydrous Na_2SO_4), 50 mL hexane + 50 mL mixture of DCM and hexane (v:v = 3:7) → Concentration → GC analysis

Table 2. Recovery and MDL of OCPs determinations in the fish muscle and sediment samples.

		Fish muscle		Sediment	
No.	Components	Recovery (%)	MDL[a] (μg/kg)	Recovery (%)	MDL[a] (μg/kg)
1	α-HCH	61	0.76	79	0.05
2	β-HCH	55	0.66	88	0.13
3	δ-HCH	57	1.12	75	0.16
4	γ-HCH	58	0.75	81	0.98
5	Heptachlor	60	2.34	46	0.43
6	Aldrin	66	1.09	69	0.38
7	Heptachlor epoxide	65	0.60	76	0.09
8	Endosulfan I	70	1.67	82	0.02
9	Dieldrin	59	3.72	—	—
10	*p*,*p*′-DDE	80	1.24	98	0.14
11	Endrin	83	1.37	4	—
12	Endosulfan II	71	0.94	49	0.14
13	*p*,*p*′-DDD	89	1.11	86	0.10
14	Endrin aldehyde	32	5.93	8	—
15	Endosulfan sulfate	60	4.52	80	0.17
16	*p*,*p*′-DDT	79	4.26	91	0.24

[a]The MDL is calculated as the actual concentration in 20 g dry sample of the sediment and in 3 g fresh sample of the fish muscle.

The power of a method to quantify trace pollutants is often evaluated by its detection limit (MDL), being defined as the lowest detected concentration of the objective significantly higher than the analytical blank. In this article, the corresponding MDL was ascertained according to the procedure proposed by Glaser et al.[13] The related results were tabulated in Table 2. Briefly, the corresponding MDLs of HCHs and DDTs varied from 0.66 to 4.26 μg/kg in 3 g fresh sample of the fish muscle and from 0.05 to 0.98 μg/kg in 20 g dry sample of the sediment.

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