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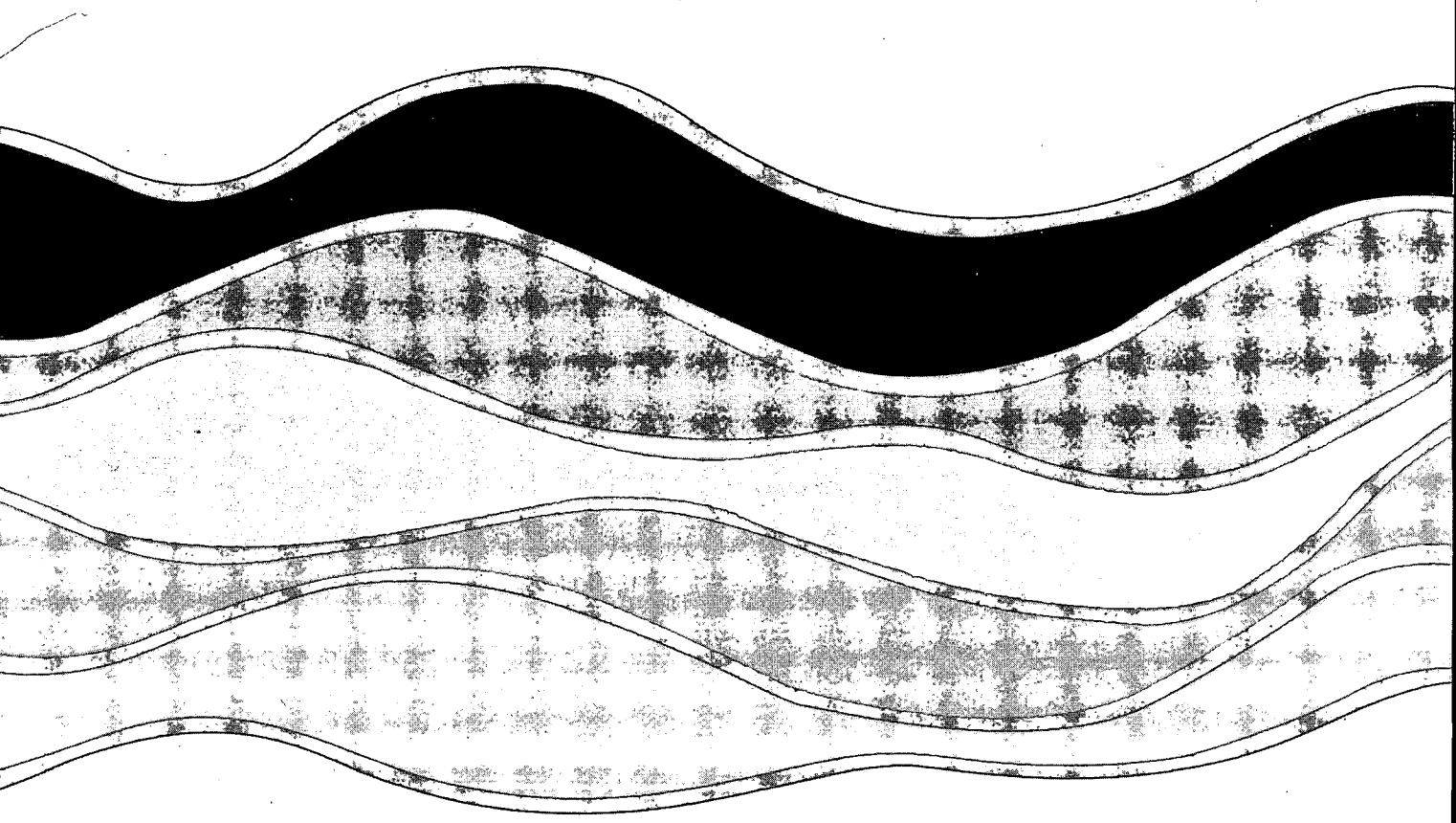

In Situ SUPERCRITICAL CARBON DIOXIDE EXTRACTION AND DERIVATIZATION DETERMINATION OF PHENOLICS FROM SEDIMENTS OF PULP MILL ORIGIN

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NWRI Contribution No. 92-25

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MANAGEMENT PERSPECTIVE

Chlorinated phenolics have long been detected in effluent samples downstream of pulp mills using chlorine bleaching steps. Because of their documented toxicity to fish, these pollutants have been the main target compounds in all pulp mill monitoring programs. Although analytical methods for the determination of phenolics in sediment samples exist, the procedures are generally tedious and use a large quantity of organic solvent. In our new supercritical fluid extraction (SFE) method, we have successfully eliminated the use of potentially hazardous solvents in the extraction stages by using inert carbon dioxide. Meanwhile, this SFE method is extremely efficient since it only takes 10% of the time required by the conventional solvent extraction techniques.

SOMMAIRE À L'INTENTION DE LA DIRECTION

La présence de phénols chlorés est décelée depuis longtemps dans des échantillons d'effluents en aval des usines de pâte à papier effectuant le blanchiment au chlore. En raison de leur toxicité prouvée pour le poisson, ces polluants ont été les principaux composés visés par tous les programmes de surveillance des usines de pâte à papier. Même s'il existe des méthodes de dosage des composés phénoliques dans des échantillons de sédiments, les procédés sont en général fastidieux et exigent une grande quantité de solvant organique. Cette nouvelle méthode d'extraction par fluide supercritique, nous a permis de remplacer, dans des étapes d'extraction, les solvants potentiellement dangereux par du dioxyde de carbone inerte. En attendant, cette méthode d'extraction est très efficace puisqu'elle n'exige que 10% du temps requis par les techniques classiques d'extraction par solvant.

ABSTRACT

A method for the determination of extractable chlorinated phenolics in sediments collected downstream of chlorine-bleaching mills was developed by using a single step *in situ* derivatization technique in conjunction with supercritical carbon dioxide extraction. Phenolics in air dried samples were extracted at 110°C and 37 MPa and simultaneously acetylated under static conditions by acetic anhydride in the presence of triethylamine. The derivatives were then removed from the matrix in the dynamic extraction stage. Various factors affecting the recovery of phenolics in weathered sediment samples were evaluated. While the results obtained by this SFE/derivatization method were comparable to conventional technique such as Soxhlet extraction, the SFE approach required no solvent in the extraction steps and was extremely time efficient (ca. 35 min).

RÉSUMÉ

Une méthode pour le dosage de composés phénoliques chlorés extractibles dans les sédiments prélevés en aval de moulins utilisant un procédé de blanchiment au chlore a été élaborée à l'aide d'une technique de formation *in situ* de dérivés, à une seule étape, utilisée conjointement avec une technique d'extraction au dioxyde de carbone en conditions supercritiques. Les composés phénoliques d'échantillons séchés à l'air ont été extraits à 110°C et 37 MPa, puis ils ont été simultanément acétylés dans des conditions statiques à l'aide d'anhydride acétique, en présence de triéthylamine. Les dérivés ont été extraits de la matrice au cours de l'étape d'extraction dynamique. Divers facteurs modifiant le taux de récupération des composés phénoliques dans les échantillons de sédiments altérés ont été évalués. Alors que les résultats obtenus par la méthode de EFS (extraction par fluide supercritique)/formation de dérivés étaient comparables à ceux de techniques habituelles comme l'extraction Soxhlet, la méthode EFS ne nécessitait pas l'utilisation de solvants au cours des étapes d'extraction et elle était extrêmement efficace sur le plan de la rapidité (env. 35 min.).

INTRODUCTION

Of all the pulp and paper mills operating in Canada, 47 of them use chlorine for bleaching either entirely or in at least one of the multiple bleaching steps. In a 1991 report jointly published by Environment Canada and Health and Welfare Canada [1], it was estimated that Canadian mills used over 610,000 tonnes of chlorine annually to produce over 10 million tonnes of bleached pulp and released over a million tonnes of chlorinated organics to the aquatic environment. Hundreds of compounds were found in the final effluents of the bleached kraft mills, including the chlorinated dibenzofurans and dibenzo-*p*-dioxins, phenolics, resin and fatty acids, and a variety of low molecular weight aliphatic compounds [2,3]. Recent studies carried out by the Pulp and Paper Research Institute of Canada indicated that the undesirable production of the highly toxic furans and dioxins can be greatly minimized by the elimination of the non-chlorinated dibenzo-*p*-dioxin and dibenzofuran in defoamers used in chlorine bleaching mills [4]. Chlorinated phenolics such as catechols, guaiacols, vanillins and syringols in the bleachery effluents are derived from the degradation of lignin during the bleaching process. Although substituting chlorine dioxide for chlorine in the bleaching steps reduces the formation of the total chlorinated phenolics [5], complete elimination of these compounds would require the use of non-chlorine bleaching techniques. Installation of secondary (biological) waste treatment facilities by the pulp mills also removes many toxic

substances including the phenolics from the effluents before they are discharged into the receiving waters.

Many chlorinated phenolics are acutely toxic to fish and their 96-hr LC_{50} values range from 0.3 to 3 mg/L [6]. The octanol-water partition coefficients (K_{ow}) of chlorinated guaiacols and catechols are similar to those of chlorophenols with the same level of chlorine substitution [7], thus, accumulation of the toxic phenolics in the sediments is predicted and has actually been observed [8,9]. Therefore, there is a need to monitor the level of phenolic contamination in sediments created by the bleaching process from the paper mills.

Different approaches to the extraction of phenolics from sediments have been used [10]. Nearly all of them are either time-consuming or use a lot of solvent or both. Recently, we successfully developed a method for the extraction of resin and fatty acids from sediments collected downstream of pulp mills using supercritical carbon dioxide [11]. This supercritical fluid extraction (SFE) method not only provided recovery of the acids equal to or better than the Soxhlet technique, but was also extremely time-efficient and used practically no solvent. Moreover, we have also demonstrated that, an *in situ* extraction and acetylation of penta- and tetra-chlorophenols could be achieved under SFE conditions [12]. This approach further reduced sample preparation time and at the same time enhanced the extractability of polar organic compounds. In this paper, we shall

describe an efficient SFE method for the determination of extractable chlorinated phenolics commonly found in sediments downstream of chlorobleaching mills.

EXPERIMENTAL

Reagents and chemicals

All chlorinated phenolics were obtained from Helix-Biotech Scientific (Vancouver, Canada) and used without further purification. These included 4,5- and 4,6-dichloroguaiacols (45G and 46G), 3,4,5- and 4,5,6-trichloroguaiacols (345G and 456G), 3,4,5,6-tetrachloroguaiacol (3456G), 3,5- and 4,5-dichlorocatechols (35C and 45C), 3,4,5-trichlorocatechol (345C), 3,4,5,6-tetrachlorocatechol (3456C), 6-chlorovanillin (6V), 5,6-dichlorovanillin (56V), and 3,4,5-trichlorosyringol (345S). Stock solutions of each individual compound were prepared in acetone at 1000 μg/mL and kept at -20°C in crimped top vials. A mixture of the above 11 phenolics at 10 μg/mL was also prepared in acetone for spiking and preparation of the acetylated standards.

Triethylamine and acetic anhydride were purchased from Aldrich Chemicals (Milwaukee, WI, USA). The anhydride was triple distilled before use. SFC grade carbon dioxide without helium head pressure was obtained from Scott Specialty Gases (Troy, MI, USA) and Linde (Division of Union Carbide, Oakville, ON, Canada). Silica gel (GC grade 950, 60 - 200 mesh, Fisher Scientific) was activated overnight at 200°C and the 5%

deactivated silica gel was prepared by adding 5 mL of water to 95 g of the activated adsorbent.

Grab sediment samples were collected downstream of several Ontario pulp mills using chlorine bleaching. These samples were air dried at room temperature, crushed, ground and sieved through a 60 mesh screen before they were used in the extraction experiments.

SFE of sediment samples

All supercritical fluid extractions were carried out with carbon dioxide using the Hewlett-Packard 7680A or 7680T extractor module. The two modules have similar capabilities except that, in the case of the 7680T, a series of up to eight thimbles can be prepared and loaded into the extractor for unattended sequential extraction. Prior to the extraction, two layers of Whatman GFC filter paper cut to internal diameter of the extraction thimble were placed at the bottom of the thimble before it was filled with 200 mg of Celite. The filter paper and Celite kept the sediment fines from plugging the fritted thimble cap and also prevented the modifier from leaking out of the thimble. The thimble was then filled with 1 g of sediment, followed by spiking 30 μL of triethylamine to the sample. The thimble contents were mixed for 30 seconds on a vortex mixer before the addition of another 200 mg of Celite. The derivatization reagent, 120 μL of acetic anhydride, was added to the top Celite layer. The thimble was then mixed again for 30

seconds. In a typical extraction, the extractor was set at a temperature of 110°C and a constant pressure of 37 MPa. Sample extraction and derivatization were first performed in the static mode for 10 minutes, followed by a 5-minute dynamic extraction with a flow rate of two mL/min to remove the analytes. During the dynamic extraction stage, the acetylated phenolics were collected on a built-in octadecylsilane (ODS) trap connected to a variable diameter restrictor nozzle which was responsible for the depressurization of supercritical carbon dioxide. The trap temperature was set at 15°C for the extraction stages and 40°C during the rising stage. Finally, the derivatized extract was removed from the trap by two 1-mL rinses of dichloromethane.

Column cleanup

The above dichloromethane rinses were combined and solvent exchanged into 1 mL of iso-octane. The extract was then cleaned up on a 5 cm 5% deactivated silica gel column prepared with a 23 cm Pasteur pipet. After the extract was applied, the column was eluted with 5 mL of 5% dichloromethane in petroleum ether (30-60°C) and the eluate was discarded. The acetyl derivatives of the phenolics were eluted from the column by 10 mL of 1% methanol in dichloromethane. This fraction was subsequently solvent exchanged into 1 mL of iso-octane for final analysis.

Chromatographic analysis

Gas chromatographic analysis of the extract was performed with both electron capture (EC) and mass selective (MS) detectors. The ECD was used for the routine analysis of sediment extracts for all phenolics and the MSD was used for the confirmation of peak identity. The capillary column and chromatographic conditions used for ECD and MSD work were identical to those described in our previous report [13]. In the case of MSD analysis, selected ion monitoring (SIM) of the characteristic $[M-42]^+$ and $[M-42-15]^+$ ions was performed [13].

A mixture of the acetyl derivatives was prepared by an aqueous acetylation of known amounts of the phenolics [13] and appropriate dilutions of this mixture were used as external standards for the quantitation of the samples.

RESULTS AND DISCUSSION

Conventional extraction of phenolics from sediments

Organics from sediments are usually extracted by a solvent or a mixture or solvents at an elevated temperature (e.g. the Soxhlet procedure) or at ambient temperature (e.g. by an ultrasonic or high speed mixing technique). In many cases, acidic compounds

are better recovered from the sediment if a strong acid is present with the solvent system. However, in the cases of sediments with high contents of humic substances such as those samples collected from pulp and paper mills, extraction under acidic conditions produces a large amount of coextractives which may precipitate when the solvent is being evaporated. The precipitate not only changes the homogeneity of the extract if it is to be subsampled but can also adversely affect the derivatization reaction which is often required for the gas chromatographic analysis of the acidic compounds.

Another approach that has been applied to the determination of PCP (pentachlorophenol) in sediment was steam distillation [14]. In our work, we found that some free phenols such as the less chlorinated catechols could not be fully recovered by this technique, presumably due to their higher water solubilities than other chlorophenols. We have also attempted to acetylate the phenolics in the sediment suspended in a potassium carbonate slurry and subsequently steam distilled the acetyl derivatives from the mixture. This method worked well with all chlorinated phenols, guaiacols and syringols but did not work with the chlorinated vanillins and catechols. The latter compounds were not recovered since their acetyl derivatives were completely decomposed during the steam distillation stage. Thus, before the advent of the SFE technique, solvent extraction was the only way to recover all the phenolics from a sediment sample.

Development of a SFE method for chlorinated phenolics in sediment

In the beginning, we were using the *in situ* extraction and derivatization method developed for the determination of PCP and other chlorophenols [12]. Using sediment spiked at 500 ng/g of the phenolics, a one gram aliquot was extracted for five minutes statically and then dynamically with 385 bar supercritical carbon dioxide at a temperature of 80°C in the presence of 30 μL each of triethylamine and acetic anhydride. Although the above *in situ* derivatization condition was also feasible for the extraction of the catechols and guaiacols from sediment samples, the results (Table 1) indicated the recovery of the phenolics was far from complete, particularly for 3456C. An increase in static extraction time from five to 10 min produced a significant improvement on the recovery of all compounds, yet longer dynamic extraction did not help since the derivatization occurred during the static extraction stage. While chlorophenols and chloroguaiacols were easily converted into their acetyl derivatives under SFE conditions, our previous work on the aqueous acetylation of phenolics indicated that complete derivatization of the chlorocatechols required an excess of acetic anhydride [13]. This principle again applied to our present work, since an increase of the amount of anhydride used from 30 to 120 μL produced a recovery better than 85% for each phenolic compound from spiked sediment samples using the SFE technique.

Once we had a method that worked reasonably well with spiked samples, the next phase of development was to optimize this procedure by applying it to naturally

contaminated samples. In the following work, a bulk sediment collected approximately two km downstream of a bleached kraft mill was used as a reference sample. Analysis of effluent samples collected in the same area indicated the site was contaminated by resin and fatty acids as well as the chlorinated phenolics. By following the procedure developed for the spiked samples, all the common phenolics were detected in this reference sample. However, we were also able to recover an additional 30% or more of these phenolics from a second extraction of the same sample, indicating that the extraction conditions were still not optimized for natural samples.

Factors affecting the SFE recovery of phenolics

Among the many factors that can affect the SFE results, the effect of extraction chamber temperature was the first one to be studied. The temperature dependence on the recovery of six major phenolic components in the reference sample, namely, 45G, 45C, 345G, 56V, 345C, 3456G and 3456C, was examined in ten-degree increments from 40 to 120°C. In these experiments, 1 g aliquots of the sample were extracted for 10 minutes in the static mode and for a further 5 min in the dynamic mode at 37 MPa using 30 μL of triethylamine and 120 μL of acetic anhydride for the acetylation reaction. To facilitate the following discussion, recoveries of the above compounds at different temperatures relative to those at 110°C were calculated. At an extraction temperature of 40°C, less than 15% of the pheolics were extracted from the sediment and acetylated. Although the recovery of the catechols was vastly improved when the extraction was carried out at

60°C, the guaiacols and 56V were still poorly recovered (≤40%) at this temperature. Continuous increase in recovery for all phenolics were observed when the extraction temperature was increased to 100°C, where the recovery of catechols reached a maximum. While the recovery of the catechols began to drop at higher extraction temperatures, highest recoveries for 56V and the guaiacols were obtained at 120°C. We were not able to study the recovery of these phenolics at even higher temperatures since 120°C is the maximum extraction chamber temperature that our extractor can reach. Since the optimal recovery of different phenolics were obtained at different extraction temperatures, 110°C was chosen as the extraction temperature since it gave the best overall recovery of all compounds. A graphical summary of the temperature effect on the recovery of phenolics is depicted in Figure 1.

The recovery of the chlorinated phenolics was also studied at four different extraction fluid densities, namely, 0.71, 0.64, 0.55, and 0.50 g/mL. No difference in the phenolics results was observed at the two highest fluid densities, suggesting that a further increase in density (or carbon dioxide pressure) would not result in better extraction efficiency. Although the chlorinated guaiacols and vanillins did not seem to be affected, the recovery of the catechols, particularly 3456C, dropped substantially at fluid densities of 0.55 and 0.50 g/mL and thus extraction with the lower density fluid is not recommended. Extraction times of 10 (static) and five minutes (dynamic) were always used since shorter static time caused a reduction in the recovery while longer static and dynamic extractions did not improve the yield for the reference sample.

The amount of reagents used and the presence of solvents can also affect the derivatization and the recovery of the phenolics. For example, the recovery of guaiacols and catechols was ca. 60 and 15%, respectively, lower if triethylamine was not used in the derivatization. However, there was no significant change in the results when 60 instead of 30 μL of the base was used and there was a slight decrease in recovery when 240 instead of 120 μL of the anhydride was employed. We were also unable to improve the recovery of phenolics by the addition of a modifier such as dichloromethane to the sample. Yet, it was noted that the presence of either methanol or water was detrimental to the derivatization of all phenolics. Less than 25 or 50% of the phenolics could be recovered if 250 μL of methanol or water, respectively, were added to the sample prior to extraction. This result is not unexpected since both methanol and water react with the anhydride causing a deficiency in the reagent for derivatization. Therefore, the *in situ* SFE/acetylation technique should not be applied to a wet sediment sample.

Using the above optimized extraction and derivatization conditions, we were able to recover ca. 80% of the extractable phenolics from a natural sediment sample in the first extraction. An additional 10 to 20% of the phenolics could be recovered if a second extraction of the sample at 110°C with fresh reagents was performed. A third extraction, however, recovered less than 5% of the derivatized products. Therefore, two extractions of the same sample are required for the quantitative recovery of chlorinated phenolics from sediments.

Method evaluation and application

For further evaluation of this *in situ* extraction and acetylation technique, results for the reference sediment (sample A) obtained by SFE were compared with those acquired by conventional techniques such as steam distillation and Soxhlet extraction with acidified acetone (Table 2). As mentioned earlier, only chloroguaiacols were recovered by our modified steam distillation procedure since the derivatives of chlorinated vanillins and catechols decomposed under such conditions. It is obvious from Table 2 that the SFE results, obtained by a single extraction, were very similar to the steam distillation results for chloroguaiacols and were slightly higher than all of the Soxhlet results. In the absence of a certified sediment reference material for total (free and bound) chlorinated phenolics, we were unable to ascertain how close were the SFE results to the total phenolic contents in naturally contaminated sediments. However, our findings already indicated that the SFE technique was at least capable of producing precise and quantitative results for the free or extractable phenolics commonly found in sediments downstream of bleached kraft mill. Unlike the procedures involving methanolic KOH hydrolysis [10], the SFE technique employed here will not convert catechols into guaiacols and produce biased results.

This SFE method has been applied to the determination of chlorinated phenolics in sediment samples of pulp mill origin and some of the results are tabulated in Table 2. Samples B and C were obtained from sites approximately 2 and 5 km,

respectively, downstream of a chlorine-bleaching mill. A GC-ECD chromatogram of the acetylated SFE extract for sample B is shown in Figure 2. Sample D came from the sedimentation basin of another bleached kraft mill and thus it is not surprising to find that its phenolic levels are higher than those in the river sediments. The predominant phenolics in these samples are 45G, 345G, 3456G, 45C, 345C, and 3456C and are consistent with previous findings [8-10].

CONCLUSIONS

An *in situ* extraction and acteylation procedure has been optimized for the determination of the extractable chlorinated phenolics in sediment samples. For the best recovery of all compounds involved in this work, the sample should be air dried prior to supercritical carbon dioxide extraction at 385 bar and a temperature of 110°C. For 1 g of sediment, 30 μL of triethylamine and 120 μL of acetic anhydride were found to produce the best results for the acetylation of phenolics. A second extraction of the sample should be performed if quantitative recovery of the extractable phenolics in sediments is required.

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Table 1. **% Recovery of chlorinated phenolics from spiked sediment samples using the *in situ* extraction and derivatization technique. All extractions were done at 80°C and 37 MPa with 1 g samples.**

Spiking level (ng/g)	500	500	500	50
Amount of Et_3N (μL)	30	30	30	30
Amount of Ac_2O (μL)	30	30	120	120
Static time (min)	5	10	10	10
Dynamic time (min)	5	5	5	5
No. of replicates	3	3	6	6
Recovery	%	%	%	%
45G	80	89	97 ± 5	94 ± 7
45C	67	92	92 ± 4	93 ± 6
345G	78	95	100 ± 7	98 ± 4
56V	54	81	98 ± 5	89 ± 6
345C	50	89	96 ± 8	92 ± 6
3456G	56	87	89 ± 4	96 ± 5
345S	73	90	91 ± 5	87 ± 6
3456C	16	44	84 ± 8	92 ± 7

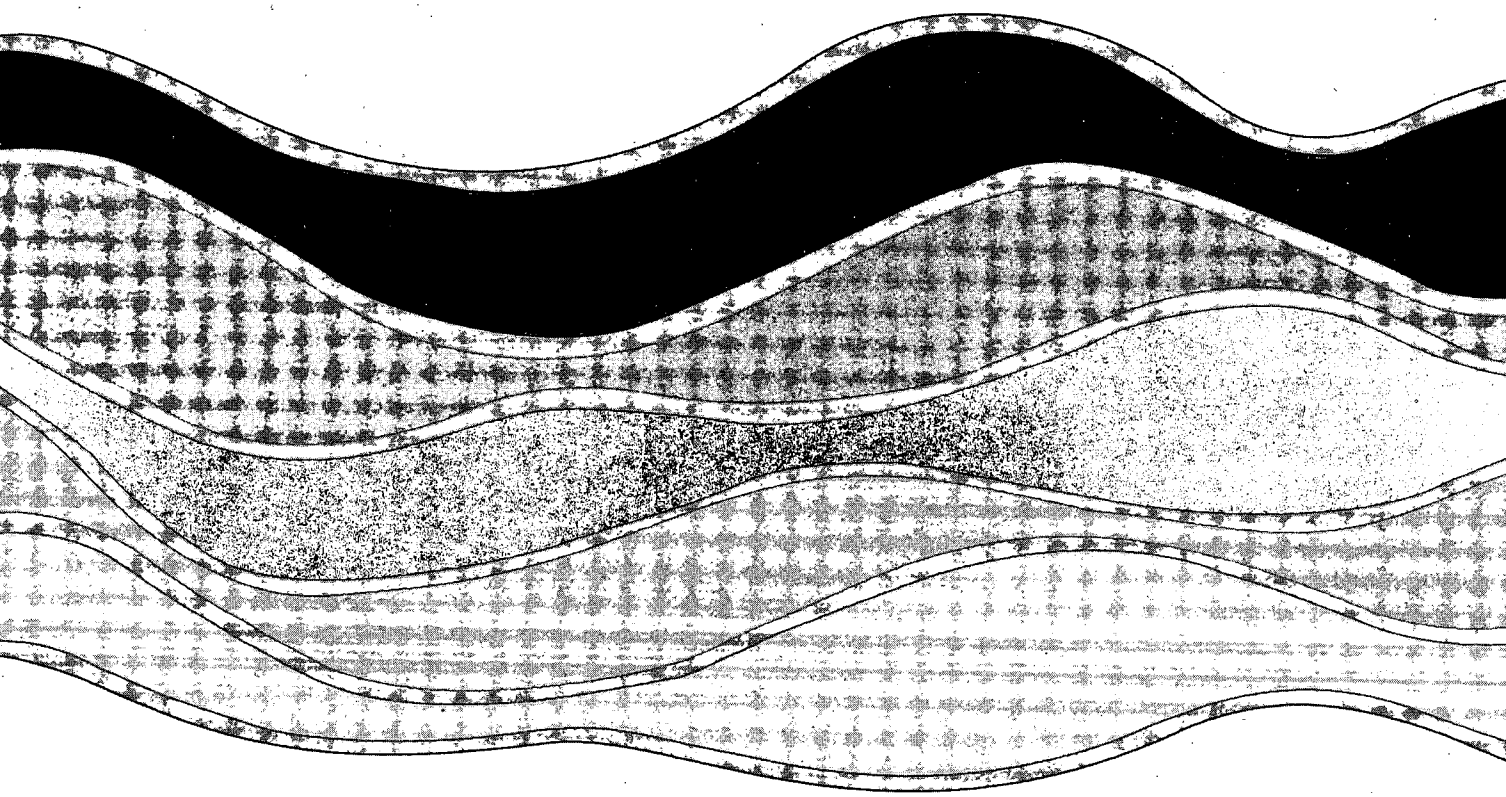
Table 2. **Levels of chlorinated phenolics (ng/g) in sediment samples determined by various techniques and from different locations. All SFE results were based on a single extraction at 110°C and 37 MPa.**

Sample	A	A	A	B	C	D
Extraction method	Steam distillation[a]	Soxhlet[a]	SFE[b]	SFE	SFE	SFE
45G	410	381	396 ± 41	717	284	822
6V	N.D.	65	83 ± 6	505	222	303
45C	N.D.	305	325 ± 28	342	133	428
345G	123	126	131 ± 10	297	82	2258
56V	N.D.	40	44 ± 5	111	45	83
345C	N.D.	1205	1364 ± 75	1264	209	1416
3456G	13	11	15 ± 2	65	27	1502
3456C	N.D.	666	688 ± 42	982	113	2796

[a]Mean of two determinations.

[b]Mean of six determinations and the uncertainty is one standard deviation.

LIST OF FIGURES



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