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Extraction and clean-up methods for improvement of the chromatographic determination of polychlorinated biphenyls in sewage sludge-amended soils: elimination of lipids and sulphur

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Abstract

Different extraction and clean-up methods for the determination of polychlorinated biphenyls (PCBs) in sewage sludge-amended soil were investigated. Soxhlet extraction with hexane–acetone (41:59) and simultaneous elimination of sulphur, with recently precipitated copper, and Florisil cartridge clean-up provided the best results. High-resolution GC with electron-capture detection achieved accurate identification and quantification of the PCBs. Initial pollutant concentrations in soil amended with sewage sludge ranged from 0.5 to 94 ng/g for PCB congeners. The weathering of the PCBs in sludged soil was monitored for a 168-day period. The results indicated that PCBs were persistent.

1. Introduction

Wastewater catchments receive polychlorinated biphenyls (PCBs) from two main sources: fluid spillages, such as transformer oils or dielectrics, and urban runoff inputs that flush the organics deposited on the ground surface from burning systems. As a result of their very low aqueous solubility, PCBs are efficiently removed from the water during sedimentation in the wastewater treatment. This results in the formation of sewage sludges with PCB concentrations of ca. 1–10 mg/kg [1,2]. A significant proportion of the generated sewage sludge is applied to land as an organic fertilizer or amendment. Current regulations restrict the total amount of sludge in soil according to the content of heavy metals; only the USA and a few European countries have proposed enforceable restrictions to indicate the amount of PCBs that can be applied to soil during sewage sludge application [3–7]. Since some PCB congeners are known or suspected carcinogens, the fate of these compounds in the soil environment is critical to assess their potential hazard risk [5,8,9]. However, there is little information about the persistence or loss of PCBs in sewage sludge-amended soil and their possible biodegradation [5,10,11].

A prerequisite to quantifying the evolution of PCBs in sludged soils is to develop sensitive and effective methods to measure their concentration. The analysis consists in the extraction of the lipids and the elimination of fats and sulphur to isolate PCBs. Soxhlet extraction of soils has

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been extensively used for the isolation of non-polar organics. Soils need to be thoroughly wetted to obtain an efficient extraction, thus the surface tension of the solvent across the pores of a dry particle prevents the diffusion of the liquid. As non-polar solvents (e.g., n-hexane) are too immiscible with water to penetrate the wet material, the use of medium-polarity or binary solvents is recommended, such as dichloromethane or hexane–acetone (41:59) [12].

Co-extracted lipids may interfere in the final determination of PCBs, because contamination can overload a high-resolution gas chromatographic (HRGC) column and create negative peaks or an erratic response when electron-capture detection (ECD) is used. There are several methods to remove co-extracted lipids, such as saponification and adsorption chromatography (silica, Florisil and alumina). Although better elimination of fats is obtained by saponification, the more chlorinated PCBs are prone to loss of chlorine, especially with high temperatures and prolonged basic reactions [8,12,13]. On the other hand, sulphur is present in sewage sludge, and is soluble enough in organic solvents that the extract must be treated before HRGC–ECD determination to avoid large co-eluted peaks. Removal of sulphur is possible with tetrabutylammonium sulphite–sodium sulphite, mercury or copper, which require the surface to be clean and reactive [8,11,12].

This paper discusses the use of different solvents in Soxhlet extraction, viz., hexane, dichloromethane and hexane–acetone (41:59), and Soxhlet plus saponification extraction, compares the capability of different clean-up cartridges in order to separate PCBs from lipids, viz., silica, Florisil, neutral alumina and acidic alumina, and different methods for sulphur elimination, viz., recently precipitated copper (added to the Soxhlet cartridge), activated copper bars in the concentrated extract before clean-up and activated copper bars in the fraction before injection. HRGC–ECD was used. With the method developed, the weathering of PCBs in sewage sludge-amended soil was also studied for a 168-day period in a laboratory experiment.

2. Experimental

2.1. Experiment design and details

Anaerobically digested sewage sludge was obtained from DARGISA, a sewage aerobic treatment plant in Girona (Spain). The sludge was air-dried and ground to less than 0.4 mm before soil addition. Limey soil from Bellaterra (Barcelona, Spain) was ground and sieved to less than 2 mm.

High doses of sewage sludge (15%) were mixed with soil and were placed in 4-kg polyethylene containers. Three different treatments were adopted in duplicate: (a) control sewage sludged soil amended with 15% sewage sludge (CON A, CON B), in which the PCB concentration was 1.6 μg/g; (b) sludge-amended soil to which Aroclor 1221, 1254 and 1260 were added at twice the control concentration (PCB 2A, PCB 2B); and (c) sludged soil to which PCBs were added at ten times the control concentration (PCB 10A, PCB 10B). Experimental soils were watered when necessary to maintain a moisture content of 20% in order to ensure half the water-holding capacity, which was appropriate to have the optimum microbiological activity. Soils were sampled after 0, 22, 50, 78, 106, 137 and 168 days. Homogeneous samples were guaranteed by sampling and mixing through all the container depth. The samples were preserved with formaldehyde (0.4%) and were stored in the dark at 4°C.

2.2. Reagents and materials

Acetone, dichloromethane and hexane (Pestipur) were obtained from SDS (Peypin, France). Methanol of HPLC grade and isooctane for residue analysis were supplied by Merck (Darmstadt, Germany). Deionized water was produced with a Millipore Milli-Q system from Waters (Milford, MA, USA). Anhydrous sodium sulphate (analytical-reagent grade) was supplied by Merck and treated at 300°C for 12 h before analysis. Copper sulphate and zinc powder (both of analytical-reagent grade) from Panreac (Bar-

celona, Spain) were also treated at 300°C for 12 h. Potassium hydroxide and formaldehyde (35–40%) (purissimum grade) were obtained from Panreac. Sulphuric acid (96%) and nitric acid (60%), both of analytical reagent grade) were obtained from Montplet and Esteban (Barcelona, Spain). Copper bars were made from commercially available 1-mm diameter copper wire. Sep-Pak silica, Florisil, neutral alumina and acidic alumina cartridges were supplied by Waters.

1,2,3,4-Tetrachloronaphthalene (TCN) from ICN (Costa Mesa, CA, USA) was used as an internal standard for PCB determination. Aroclor 1221, 1254 and 1260 were supplied by Chem Service (Birkenhead, UK) and individual PCB congeners (28, 52, 101, 149, 118, 153, 105, 138, 163, 128, 156, 180 and 170) by the Community Bureau of Reference (Brussels, Belgium). All PCBs were used in appropriate dilutions in isooctane.

2.3. Soxhlet extraction

About 1 g of sewage sludge or 10 g of sludge-amended soil, thoroughly mixed with 10 g of anhydrous sodium sulphate, was Soxhlet extracted with 200 ml of hexane, dichloromethane or hexane–acetone (41:59) for 6 h at a rate of 4–6 cycles/h. The extract was dried over 0.5 g of anhydrous sodium sulphate. The decanted extract was evaporated at 40°C in a rotary evaporator under reduced pressure to near dryness, dissolved in 1 ml of hexane and re-evaporated to less than 1 ml before clean-up.

2.4. Soxhlet and saponification extraction

Soxhlet extraction was carried out with 200 ml of hexane, dichloromethane or hexane–acetone (41:59) for 6 h. The solvent was concentrated to 5 ml in a rotary evaporator under reduced pressure. A 100-ml volume of 0.5 *M* potassium hydroxide in methanol was added and the mixture was refluxed for 4 h in a water-bath at 80°C. After cooling, 20 ml of Milli-Q-purified water were added and extraction was performed with hexane (3×50 ml). The combined organic extracts were dried over 0.5 g of anhydrous sodium sulphate. The decanted extract was evaporated at 40°C in a rotary evaporator under reduced pressure to near dryness, dissolved in 1 ml of hexane and re-evaporated to less than 1 ml before clean-up.

2.5. Silica and Florisil cartridge clean-up

A silica or Florisil cartridge was rinsed with 5 ml of hexane–dichloromethane (80:20) and 10 ml of hexane before use. The extract was transferred on to the cartridge and eluted with 10 ml of hexane. The eluate was evaporated under reduced pressure to near dryness and replaced with 1 ml of isooctane. Exactly 50 μl of TCN (1.5 μg/ml) were added.

2.6. Neutral alumina or acidic alumina cartridge clean-up

A neutral alumina or acidic alumina cartridge was rinsed with 5 ml of hexane–dichloromethane (80:20) and 10 ml of hexane before use. The extract was transferred on to the cartridge and eluted sequentially with 10 ml of hexane and 5 ml of hexane–dichloromethane (80:20). The second eluate was evaporated to near dryness, dissolved in 1 ml isooctane and re-evaporated to less than 1 ml. Exactly 50 μl of TCN (1.5 μg/ml) were added.

2.7. Sulphur elimination

Recently precipitated copper in Soxhlet extraction

A 125-g amount of copper sulphate was dissolved in 300 ml of Milli-Q-purified water acidified with 5 drops of sulphuric acid, then 30 g of zinc powder were slowly added. The precipitated copper was filtered and rinsed with Milli-Q-purified water until neutrality and then sequentially with acetone and hexane. The copper was placed in the bases of the Soxhlet cartridge.

Activated copper bars before clean-up

Copper bars 0.5 cm long were cut and immersed in 30% nitric acid for ca. 30 s. The bars were cleaned sequentially with Milli-Q-purified water (until pH7), acetone and hexane and finally dried with a stream of nitrogen.

At least ten bars of recently activated copper were added to the concentrated lipidic extract before clean-up. After 12 h, the decanted extract was submitted to clean-up.

If sulphur was still evident in the chromatographic profile, five more bars of recently activated copper were added and, after 12 h, the extract was injected again.

Activated copper bars before injection

Copper bars 0.5 cm long were cut and immersed in 30% nitric acid for ca. 30 s. The bars were cleaned sequentially with Milli-Q-purified water (until pH7), acetone and hexane and finally dried with a stream of nitrogen.

At least ten bars of recently activated copper were added to the solution to be injected. After 12 h, 2 μl were injected into the HRGC–ECD system. If sulphur was not completely removed, five more bars of recently activated copper were added and, after 12 h, the extract was injected again.

2.8. Chromatographic instruments and conditions

A DB-1701 (14% cyanopropylphenyl–86% methylsilicone) column (60 m × 0.25 mm I.D., 0.25 μm) from J & W Scientific (Folsom, CA, USA) was used. The chromatographic system consisted of an HP-5890 A gas chromatograph equipped with an ^{63}Ni-electron capture detector and a Model 7673 automatic injector, both from Hewlett-Packard. Quantification was effected with a Maxima 820 chromatography workstation from Waters. The carrier gas was hydrogen at 3.8 ml/min with a splitting ratio of 1:13. The auxiliary gas was argon–methane (95:5) at 60 ml/min. The temperature programme was 80°C (held for 3.1 min), increased at 50°C/min to 190°C (5 min), at 1°C/min to 223°C/min (4 min) and at 4°C/min to 260°C (held isothermal for 15 min). The injector and detector temperatures were 250 and 350°C, respectively. Injections of 2 μl were made in the splitless mode (3 min) [14].

3. Results and discussion

3.1. HRGC–ECD

HRGC–ECD provided a linear response from 0.5 ng/ml to 20 ng/ml for CBs 28, 105, 128 and 156, from 0.5 to 100 ng/ml for CBs 118, 180 and 170, from 5 to 100 ng/ml for CB 52; and from 5 to 250 ng/ml for CBs 101, 149, 153, 138 and 163. Therefore it was necessary to dilute the final extracts of samples PCB2 and PCB10 to 2 ml and 10 ml, respectively, in order to have concentrations in the linear range. The detection limit, calculated with a signal-to-noise ratio of 3 on the basis of a standard solution, ranged from 0.2 to 0.5 ng/ml.

Dilutions of the congeners at their real proportions in the sample were prepared as standards, with the addition of TCN (internal standard), and had concentrations above and below those in the sample.

The precision of the ratio of the CBs peak height to TCN peak height within one day ranged from 1.3 to 7.0%. The reproducibility from day-to-day of the CBs-to-TCN peak-heights ratio produced only a slight decrease in precision (4.5–10.1%).

3.2. Extraction solvent and saponification

A control sample of 15% sewage sludge-amended soil was Soxhlet extracted with hexane, dichloromethane and hexane–acetone (41:59), the extracts were submitted to Florisil clean-up and sulphur was removed before injection. The results for CBs recommended by the BCR are given in Table 1.

There were no significant differences between the PCB concentrations obtained with the different solvents. As hexane is too non-polar and dichloromethane is halogenated, the use of hexane–acetone (41:59) is recommended.

Fig. 1 shows the result of hexane–acetone

Table 1
PCB results in 15% sewage-sludge amended soil using different Soxhlet extraction solvents

Isomer No.	Concentration (ng/g)		
	Hexane	CH_2Cl_2	Hexane–acetone (41:59)
28	0.9	1.0	1.0
52	1.9	1.9	1.9
101	3.8	3.6	3.8
149	6.2	5.8	6.2
118	1.6	1.6	1.6
153	8.2	7.8	8.0
105	3.2	3.4	3.2
138 + 163	9.4	8.7	9.4
128	0.6	0.6	0.6
156	0.5	0.5	0.5
180	8.2	7.8	8.0
170	4.0	3.8	4.0

(41:59) Soxhlet extraction with and without saponification of a sewage sludge sample. As stated previously [8,12,13], highly chlorinated PCBs were lost during the basic treatment, and a saponification is therefore not suitable.

3.3. Clean-up methods

The effectiveness of the clean-up methods was assessed with the fractionation of a standard solution of PCBs in isooctane (0.90 μg/ml of Aroclor 1254 and 0.83 μg/ml of Aroclor 1260), which also contained other presumably sewage sludge pollutants. The cartridge were eluted with increasing volumes of hexane and hexane–dichloromethane (80:20) in order to achieve the elution of PCBs with one of the eluents. The subsequent fractions were analysed by HRGC–ECD.

PCBs were eluted from the silica and Florisil cartridges in the first fraction, whereas PCBs were eluted in the second fraction with both neutral and acidic alumna. Fats, mainly fatty acids, were retained in the cartridges. Although PCBs were separated from aliphatic hydrocarbons, linear alkylbenzenesulphonates and polycyclic aromatic hydrocarbons only with the alumina clean-up methods, these organics did not interfere in HRGC–ECD and, moreover, the alumina purification require more time and solvents than the silica or Florisil clean-ups. Hence only silica and Florisil purifications were tried with a hexane–acetone extract of a sewage sludge-amended soil. The results are given in Table 2.

The PCB concentrations obtained were not statistically different between clean-up methods. However, Florisil provided a better precision with relative standard deviations below 11%.

3.4. Sulphur removal

Three samples of 15% sewage sludge-amended soil were submitted to hexane–acetone (41:59) Soxhlet extraction, Florisil clean-up and the above-described sulphur removal methods. The sulphur peak interfered with the initial peaks of the chromatogram, including that of the internal standard. In Fig. 2, the results for a sample without any treatment and with a first step in the sulphur elimination just before injection are shown.

The sulphur removal methods provided PCB results without any significant difference (Table 3). The methods with copper bars usually required a further addition of copper after the injection, when the presence of sulphur still interfered in the chromatogram. The method with recently precipitated copper generally provided the elimination of sulphur without further treatment, hence this was the preferred method.

In conclusion, Soxhlet extraction with hexane–acetone (41:59), including recently precipitated copper in the cartridge, and a clean-up with Florisil microcolumn was the recommended method.

The precision, repeatability and reproducibility were studied with the analysis of a 15% sludged soil sample. The accuracy of the method was tested by a standard addition analysis. Two different levels of addition were made in order to increase the pollutant concentration in the sewage sludged soil by two and five times. The

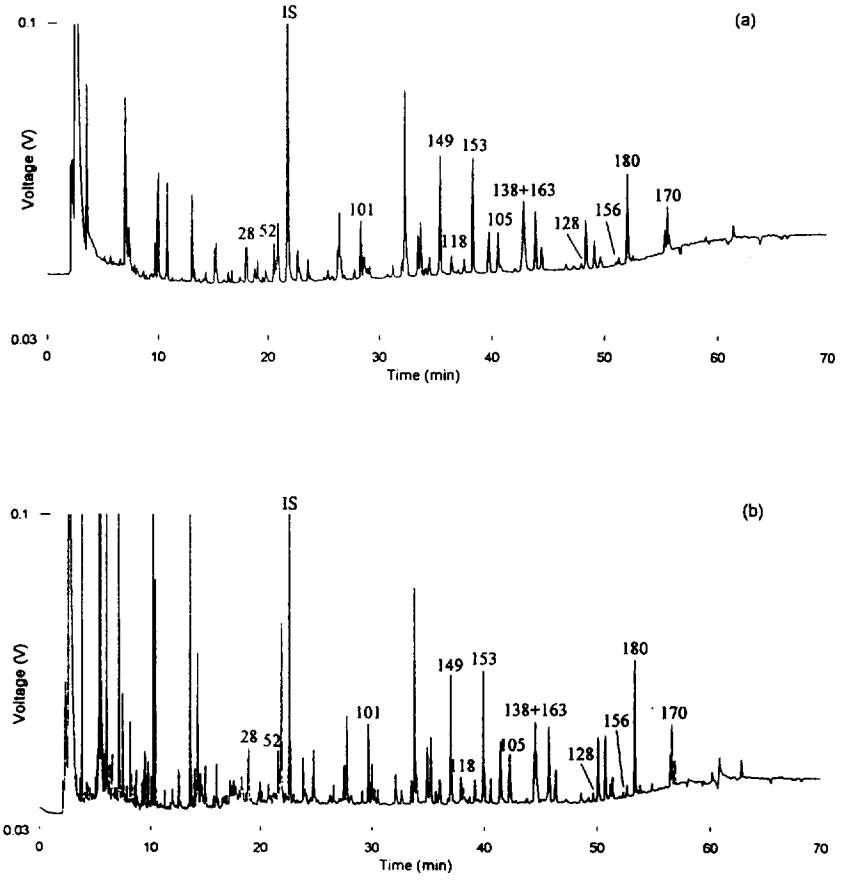

Fig. 1. HRGC–ECD of PCBs in a sewage sludge sample submitted to hexane–acetone (41:59) Soxhlet extraction, (a) with and (b) without saponification. Identified PCBs are recommended by the Community Bureau of Reference: 28, 52, 101, 149, 118, 153, 105, 138 + 163, 128, 156, 180 and 170; internal standard, TCN.

results are given in Table 4. The recoveries were quantitative except for CB 52, which had an abnormal value (177%) because of some interferences in the chromatogram.

3.5. Fate of PCBs in sewage sludge-amended soil

Fig. 3 shows the HRGC–ECD results at days 0 and 168 for sample extracts taken from treatment PCB2 A. Significant variations in the concentrations of CBs 28, 118, 105, 128, 156 and 170 in sewage sludge-amended soil were achieved over the 168-day experimental period, according to an analysis of variance at the $\alpha = 0.01$ level of confidence. The CB 52, 149 and 180 concentrations also varied, but according to a level of confidence of $\alpha = 0.05$. CBs 101, 153 and 138 + 163 were persistent ($\alpha = 0.05$).

Congeners 28, 118, 105 and 170 were clearly lost and CBs 128 and 180 showed a decreasing trend. On the other hand, the concentration of congeners 52 and 149 increased during the study. Fig. 4 shows the evolution of CBs 28, 128 (both decrease), 52 (increase) and 153 (persistent) during the experiment.

Table 2
PCB results in 15% sewage sludge-amended soil using different clean-up methods

Isomer No.	Silica (3 replicates)		Florisil (3 replicates)	
	Mean concentration (ng/g)	R.S.D. (%)	Mean concentration (ng/g)	R.S.D. (%)
28	0.8	6	0.9	2
52	1.7	17	1.6	11
101	2.7	6	2.8	7
149	4.3	7	4.6	3
118	1.4	16	1.4	4
153	5.4	12	5.6	3
105	2.1	10	2.2	7
138 + 163	6.1	17	6.2	3
128	0.4	10	0.4	7
156	0.4	47	0.4	6
180	4.8	17	5.1	4
170	2.5	22	2.7	1

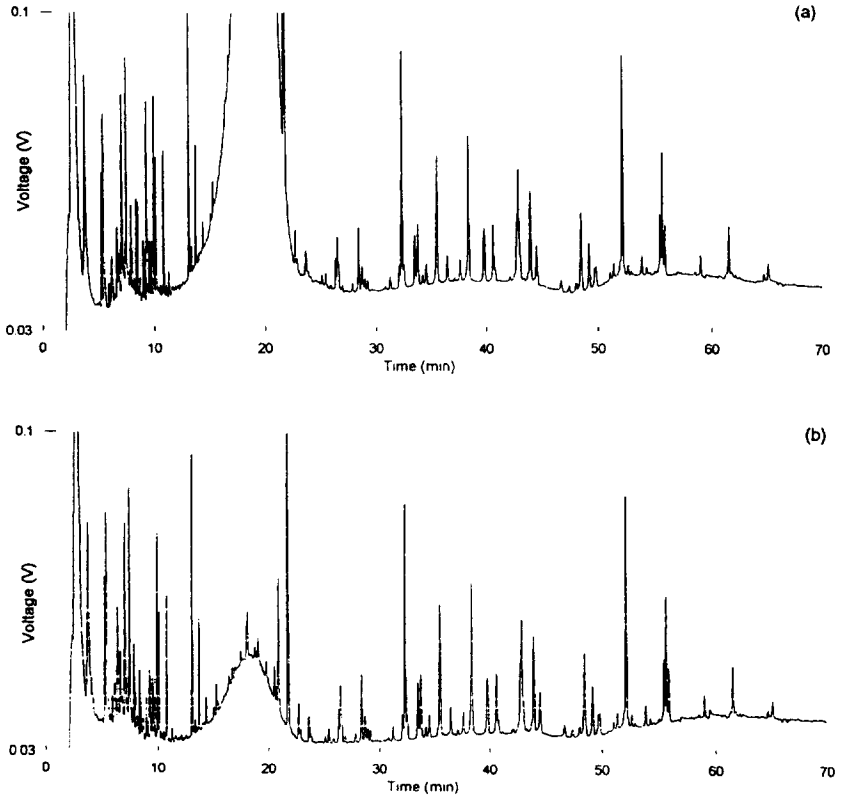

Fig. 2. HRGC–ECD of PCBs from a sample of sewage sludge-amended soil submitted to hexane–acetone (41:59) Soxhlet extraction and Florisil clean-up, (a) without sulphur removal and (b) with a first step in the sulphur elimination just before injection.

Table 3
PCB results in 15% sewage-sludge amended soil using different methods for the removal of sulphur

Isomer No.	Concentration (ng/g)		
	Cu added to Soxhlet	Cu before clean-up	Cu before injection
28	0.7	0.7	0.9
52	1.5	1.3	1.6
101	2.9	2.5	2.8
149	4.7	3.9	4.6
118	1.2	1.1	1.4
153	5.6	4.7	5.6
105	2.2	1.8	2.2
138 + 163	6.3	5.1	6.2
128	0.4	0.3	0.4
156	0.3	0.2	0.4
180	4.9	3.6	5.1
170	2.2	1.7	2.7

4. Conclusions

Soxhlet extraction [hexane–acetone (41:59)], recently precipitated copper in the Soxhlet cartridge and Florisil clean-up are suitable for the isolation of PCBs from sewage sludge-amended soil. HRGC–ECD permits the determination of PCBs. Losses of CBs 28, 118, 105 and 170 were clear from sewage sludge-amended soil after 168 days, whereas CBs 128 and 180 showed only slight losses. Congeners 52 and 149 increased during this period and there were no variations in CBs 101, 153 and 138 + 163.

Table 4
Validation of the procedure for the determination of PCBs in sewage-sludge amended soils

Isomer No.	Repeatability (4 replicates)		Reproducibility (5 replicates)		Accuracy: recovery (%)
	Mean concentration (ng/g)	R.S.D. (%)	Mean concentration (ng/g)	R.S.D. (%)	
28	1.0	5	1.2	32	110
52	1.4	5	1.5	8	177
101	3.0	9	3.0	9	69
149	5.0	12	4.8	9	68
118	1.2	10	1.3	17	119
153	5.7	10	5.7	9	69
105	2.5	11	2.4	10	116
138 + 163	6.3	10	6.3	9	128
128	0.4	11	0.4	1	113
156	0.3	12	0.3	16	101
180	4.5	12	4.6	11	118
170	2.0	13	2.0	10	115

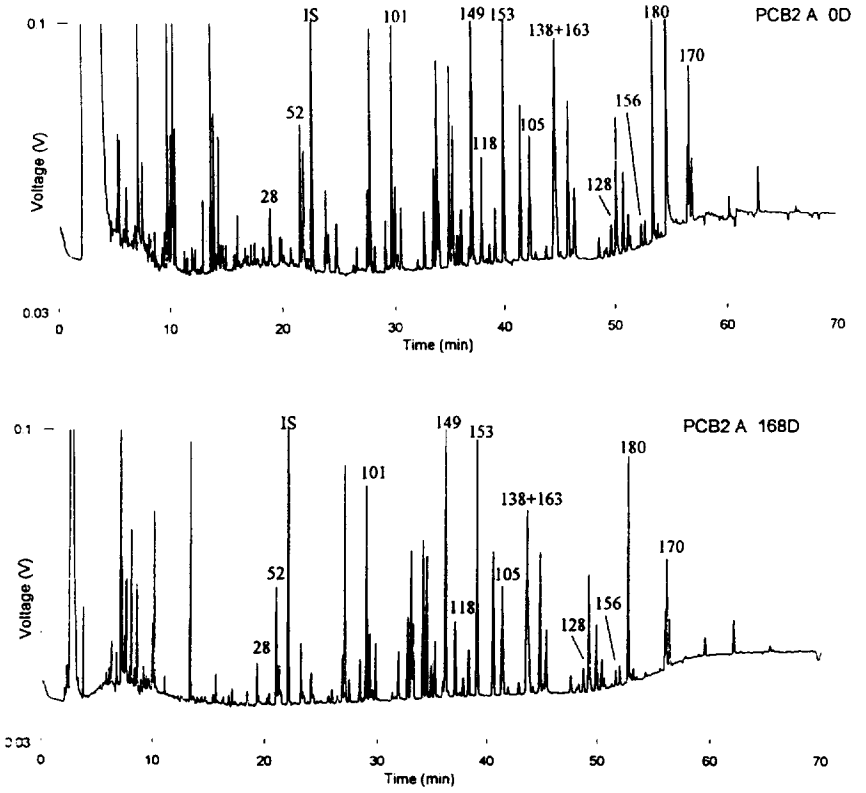

Fig. 3. HRGC–ECD results at days 0 and 168 for sample extracts taken from treatment PCB2 A.

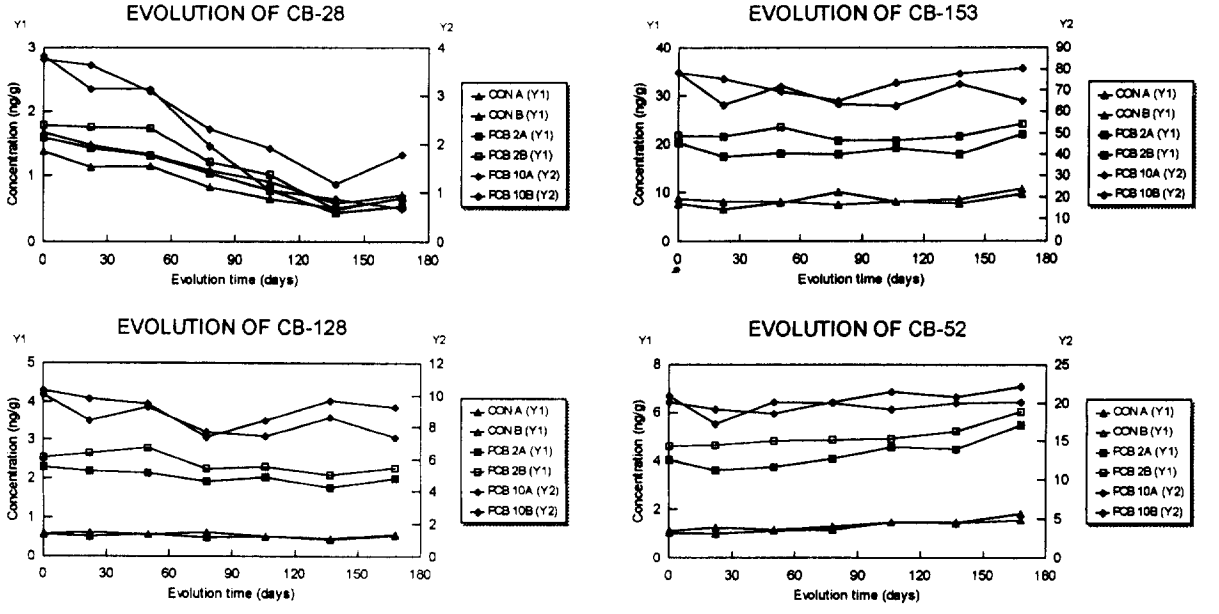

Fig. 4. Variation of CB 28, 128, 52 and 153 concentration in sewage sludge-amended soil for each experiment versus time elapsed from the start of the experiment.

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