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Persistent Organochlorine Pollutants in Soils from Belgium, Italy, Greece, and Romania

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Persistent organochlorine pollutants (POPs) have become a major issue of research in order to investigate their ubiquitous environmental occurrence, biochemical and toxic effects, human exposure and health risk assessment (Holoubek 1999). Some pollutants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have been produced intentionally in a wide variety of commercial applications. However, due to their persistence, they can be found in the environment even decades after being banned. Soil has been identified as a repository for these toxic chemicals from where they can be released into water or air.

Different procedures are reported for persistent organochlorine pollutants (POPs) extraction from the soil. While the Soxhlet extraction represents the classical methodology (Luque de Castro and Garcia-Ayuso 1998) for the extraction of lipophilic compounds from solid samples, new methodologies using microwave-assisted (Lopez-Avila et al., 1995; Cicero 2000), accelerated solvent extraction (Fisher 1997) or supercritical fluid extraction (Bøwadt et al., 1995) have been developed. However, the efficiency of these procedures is sometime inferior to the Soxhlet procedure or they require costly equipment.

In this paper an evaluation of the hot Soxhlet extraction of POPs from soil was conducted using an industrial soil certified for PCBs. The method was further used for the determination of selected POPs in soils from different European countries (Belgium, Italy, Greece, and Romania).

MATERIALS AND METHODS

The following PCB congeners (IUPAC numbering) were targeted for analysis: 28, 52, 99, 101, 118, 138, 149, 153, 156, 170, 180, and 187. Additionally, we included hexachlorobenzene (HCB), α–, β– and γ–HCH hexachlorocyclohexane isomers (the sum expressed as HCHs), o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT (the sum expressed as DDTs) as the major organochlorine pesticides found in environmental samples. PCB 46 and PCB 143 were used as internal standards and 1,2,3,4-tetrachloronaphthalene (TCN) as recovery standard. All individual standards at a concentration of 10 ng/μl in iso-octane were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany).

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Dilutions were made in iso-octane to cover the range of POPs expected in the samples. All solutions were stored at -20°C. Certified reference material CRM 481, an industrial soil certified for PCBs ((IUPAC Nos. 101, 118, 149, 153, 156, 170 and 180) was purchased from BCR (Brussels, Belgium). Acetone, hexane, dichloromethane and iso-octane were of pesticide grade (Merck, Darmstadt, Germany). All solvents were tested for interferences using concentration from 15 ml to 50 µl and analysis by GC-ECD. Analytical grade concentrated sulphuric acid 95-97% was purchased from Merck. Anhydrous sodium sulphate (Merck) for residue analysis, basic aluminium oxide 70-230 Mesh and silica gel 60-200 Mesh (Merck) were used after heating overnight at 120°C.

A Soxhlet extractor B-811 (Buchi, Switzerland) was used for the extraction of organic residue from soil. The system offers four modes of extraction (standard, warm, hot Soxhlet and continuous extraction mode). Each consists of three steps: extraction, rinsing and concentration, which can be performed in stages for gradual solvent evaporation to near dryness. During hot Soxhlet extraction mode, the solvent is distilled into the extraction chamber, while the upper heating element is turned on. The solvent is always kept above a fixed level by means of an optical sensor. This insures equilibrium between the rate of fresh solvent entering the extraction chamber and solvent leaving the chamber. Thus, the sample is permanently in contact with hot solvent.
Multiple soil cores (n=2-5, 5 cm deep) were collected from each location (urban, rural, industrial and waste incineration sites). Samples were homogenized by sifting through a steel mesh (~2 mm grid size), dried at room temperature and sealed in air-tight polyethylene containers for storage at room temperature until analysis.

Portions of 100 mg for CRM 481 and 2g of soil samples were spiked with internal standards (PCB 46 and 143) and extracted for 2h using hot Soxhlet manifold with 50 ml hexane:acetone=3:1, v/v. The extract was concentrated to 5 ml in the extractor and was further purified on a acidified silica:deactivated alumina cartridge. After elution with hexane and concentration under a nitrogen stream, TCN (recovery standard) was added prior to GC analysis. Method limits of detection ranged between 0.1-0.25 ng/g soil. Recoveries of target analytes and internal standards ranged between 70 and 85%. The procedure was validated through regular analysis of blanks, fortified soil and certified material, CRM 481.
A Hewlett Packard (Palo Alto, CA, USA) 6890 GC-µ ECD was equipped with a 25m x 0.22mm x 0.25µm, HT-8 capillary column (SGE, Zulte, Belgium). One µl was injected in pulsed splitless mode (pulse pressure = 25 psi, pulse time = 1 min) with the split outlet opened after 1 min. Injector and detector temperatures were set at 275°C and 320°C, respectively. The temperature program of the oven was set to 90°C for 1 min, then with 15°C/min to 180°C, kept for 1 min, then to 250°C by 3°C/min and further by 15°C/min to 290°C, kept for 6 min.
A Hewlett Packard (Palo Alto, CA, USA) 6890 GC was connected via direct interface with a HP 5973 mass spectrometer. A 25m x 0.25mm x 0.25µm, DB-1 (J&W Scientific, Folsom, CA, USA) was used with helium as carrier gas at a

constant flow of 1.0 ml/min. One µl was injected in pulsed splitless mode (pulse pressure = 20 psi, pulse time = 1.0 min) with the split outlet opened after 1.0 min. Injector and interface temperatures were set at 270°C and 280°C, respectively. The temperature program of oven was starting from 90°C, kept for 1 min, then with 15°C/min to 275°C, kept for 10 min. The mass spectrometer was operated in electron impact ionization and selected ion monitoring mode. Three most abundant ions were monitored for each level of chlorination for PCBs or for each pesticide. Retention time, masses and relative abundance of the ions were used for identification. All samples were analyzed on both columns, the lowest value for each compound was further considered for calculations.

RESULTS AND DISCUSSION

Different solvent mixtures were compared for the hot Soxhlet extraction (HSE) by analyzing a soil with considerable native PCB concentrations. The method efficiencies were evaluated by comparing the amount of PCB 153 and sum of PCBs (sum of 12 congeners) found in this soil (Table 1). Hot Soxhlet extraction gave higher concentrations of PCB 153 with lower standard deviations than the sonication extraction.

The yield of Soxhlet extraction is influenced by solvent composition (Table 1). The amount of extracted analytes as well as the amount of interfering material extracted increases with the polarity of the mixture used (extraction with acetone:hexane=1:1, v/v showed more interference peaks on ECD chromatogram). Acetone-hexane (1:3, v/v) was the best mixture for extraction by HSE (highest concentrations for PCB 153 and sum PCBs). This mixture also lead to an easy evaporation step through azeotropic distillation (Smedes et al., 1997). Hydrochloric acid pretreatment of soil (as proposed by Smedes et al., 1997) didn't increase the amount of PCB 153 found in the soil.

To check method recoveries, a soil sample (previously found to contain very low amounts of POPs) was fortified by addition of 20 ng of each analyte to four 2 g portions of soil. Average recoveries ranged from 70% to 85% after correcting for the amounts in the soil blanks. Blanks were carried out on extracting a thimble filled with sodium sulfate and treating the extract as a sample. Blank values for all compounds were below quantification limits.

The Soxhlet system has been applied in hot and standard extraction mode to determine the optimum extraction time of PCB from the certified soil. Two hours was a convenient extraction time to obtain high yields, while longer extraction times failed to increase PCB concentrations (Table 2). Recoveries ranged between 73 and 84%. Compared with standard Soxhlet mode, the use of hot Soxhlet reduced the extraction time from 8 hr or more (Smedes et al., 1997) to 2 hr. This probably results from the permanent contact with hot solvent.

PCB concentrations in the certified soil (CRM 481) are in the ppm range and do not reflect concentrations in the environment which are in the ppb range. Because recoveries of analytes at two levels could be different, the validation of the method in the high range of concentrations doesn't indicate if the method is efficient at low concentrations. This hypothesis was tested by 1:20 dilution of CRM 481 (200 mg soil homogenized with 3800 mg anhydrous Na_2SO_4). Recoveries from the certified values (Table 2) were higher in this case and ranged between 83 and 96% due to better method performance at low concentrations and to the increased dispersion of the certified soil. However, higher relative standard deviations were obtained due to adsorption and incomplete homogenization of the material.

Table 1. Solvent evaluation for sonication and Soxhlet extraction of native soil sample (n=3).

		PCB 153 (ng/g soil)	RSD (%)	Sum PCBs (ng/g soil)	RSD (%)
Sonication (30 min)	H:D=4:1	2.4	22	9.4	35
Hot Soxhlet (2hr)	H	4.9	15	22.3	19
	H:D=4:1	5.6	8	24.0	13
	H:D:A=3:1:1	6.7	18	28.2	16
	H:A=1:1	6.6	20	30.1	18
	H:A=3:1	**8.1**	**14**	**34.1**	**16**
HCl + Hot Soxhlet (2 hr)	H:D:A=3:1:1	6.8	16	29.2	15
	H:A=3:1	7.9	13	33.9	18

H-hexane, D-dichloromethane, A-acetone

The method was applied for the determination of selected POPs in soils from Belgium (BE), Italy (IT), Greece (GR) and Romania (RO). Concentrations of various pollutants vary greatly between countries (Table 3). Samples from Romania (details in Covaci 2001) showed similar concentrations of HCB and PCBs, but higher concentrations of DDTs and HCHs than in other countries.

HCB concentrations were lower than 5.5 ng/g soil and similar in all countries. With exception of some Romanian rural sites with values up to 89.5 ng/g soil, low concentrations of HCHs (< 2 ng/g) were found in all sites (Table 3). Similar γ-HCH/Σ HCH ratios (around 0.8) were found in all countries except for Romania (ratio of 0.48) suggesting the use of different mixtures (pure lindane versus technical lindane). Concentrations of HCHs and ratios of γ-HCH/Σ HCH were similar at rural and urban sites.

Table 3. Concentrations of selected POPs (ng/g soil) from Belgium (BE), Italy (IT), Greece (GR) and Romania (RO). Concentrations below detection limit were set at half of the detection limit.

Analyte	BE n=16		IT n=6		GR n=2	RO* n=46	
	mean (SD)	range	mean (SD)	range	mean	mean (SD)	range
HCB	0.3 (0.3)	0.1-1.4	1.3 (2.2)	0.1-5.2	0.1	0.6 (1.2)	0.1-5.5
ΣHCH	0.9 (0.4)	0.6-2.0	1.1 (0.8)	0.6-2.0	0.9	10.0 (19.7)	0.6-89.5
γ-HCH/Σ HCH	0.82 (0.08)	0.66-0.94	0.82 (0.05)	0.75-0.81	0.80	0.48 (0.21)	0.21-0.94
ΣDDT	6.8 (6.7)	0.6-22.4	26.2 (20.8)	1.8-60.4	24.1	96.0 (126)	3.5-561.4
pp-DDT/Σ DDT	0.35 (0.13)	0.17-0.58	0.32 (0.11)	0.22-0.50	0.53	0.41 (0.14)	0.18-0.66
Σ PCB	14.5 (11.4)	3.8-39.3	11.0 (8.3)	3.8-91.0	3.5	24.6 (35.0)	1-134
PCB 153/Σ PCB	0.20 (0.02)	0.17-0.22	0.20 (0.02)	0.17-0.22	0.20	0.21 (0.03)	0.13-0.26

*-detailed results in Covaci 2001

Concentrations of DDTs from Belgian and Italian soils were low (Table 3) and in the same range as other European soils (Harrad et al., 1994; Wilcke et al., 1998), while DDT concentrations in some rural Romanian soils were higher (up to 560 ng/g soil). Three Romanian samples exceeded the official norms for DDTs of 500 ng/g soil (Monitorul Oficial 1997). Concentrations of DDTs were statistically higher at rural sites ($p<0.05$) in all countries except Greece. Similar p,p'-DDT/Σ DDT ratios (up to 0.35) were found in Italian and Belgian soil, suggesting a past DDT exposure. Ratios from Greece and Romania are higher (up to 0.66) suggesting a more recent use. Similar p,p'-DDT/Σ DDT ratios were calculated form rural and urban sites in each country.

PCB 28, 52, 99 and 156 values were sometimes under the detection limit (0.1 ng/g soil) while all other congeners were detected at all sites. PCB concentrations (sum of congeners 101, 118, 138, 149, 153, 170, 180 and 187) were similar in all countries, with slightly lower values (3.5 ng/g soil) in Greece and higher in Romania (up to 134 ng/g soil). PCBs concentrations at rural sites were significantly lower than in urban soils collected mostly from parks. PCB

Table 2. Recoveries of certified PCBs from CRM 481 with hot Soxhlet extraction.

Congener n°	Certified value (µg/g soil)	Standard Soxhlet 8 hr (n=3)	Hot Soxhlet mean ± SD, µg/g soil			
			2 hr (n=3)	3 hr (n=3)	4 hr (n=3)	2 hr (diluted CRM)* (n=3)
101	37 ± 3	28 ± 3 (75.7%)**	27.5 ± 1.5 (74.3%)	29.1 ± 3 (78.6%)	29.5 ± 3.2 (79.7%)	31.4 ± 4.2 (84.9%)
118	9.4 ± 0.7	8.9 ± 0.7 (94.5%)	8.7 ± 0.4 (92.5%)	8.7 ± 0.5 (92.5%)	8.9 ± 0.5 (94.5%)	9.0 ± 0.8 (95.7%)
149	97 ± 7	77 ± 6 (79.4%)	71 ± 4 (73.2%)	73 ± 4 (75.3%)	75 ± 3 (77.3%)	82 ± 9 (84.5%)
153	137 ± 7	116 ± 6 (84.7%)	115 ± 3 (83.9%)	117 ± 5 (85.4%)	118 ± 6 (86.1%)	125 ± 10 (91.2%)
156	7.0 ± 0.5	5.6 ± 0.5 (80%)	5.4 ± 0.7 (77.1%)	5.5 ± 0.9 (78.6%)	5.5 ± 0.7 (78.6%)	5.8 ± 0.7 (82.9%)
170	52 ± 4	41 ± 4 (78.8%)	38 ± 5 (73.1%)	40 ± 5 (76.9%)	40 ± 6 (76.9%)	45 ± 5 (86.5%)
180	124 ± 6	100 ± 7 (80.6%)	95 ± 8 (76.6%)	96 ± 10 (77.4%)	95 ± 8 (76.6%)	108 ± 12 (87.1%)

*- 1:20 dilution of CRM 481 with anhydrous Na_2SO_4

**-percentage of the certified value

concentrations were similar in Italian and Romanian urban sites. The highest PCB concentrations were found in soils from industrial sites (1 from Belgium and 2 from Romania). Similar PCB 153/Σ PCB ratios were found in all countries.
Due to slow degradation, profiles of different pollutants in soil are closely related to the technical mixtures used: Aroclors or similar mixtures for PCBs (high percentage of PCBs 101 and 149 found in soil), technical DDT (high percentage of pp-DDT and op-DDT) and pure lindane (high γ-HCH/Σ HCH ratios). However a broad range of γ-HCH/Σ HCH and pp-DDT/Σ DDT could be explained by different exposure in time and by use of different mixtures.

Generally, with exception of some contaminated sites (rural for DDTs and industrial for PCBs), soils contained relatively low concentrations of organochlorines with an increased load in urban soils collected from parks.

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