TREE BARK AND MOSS AS INDICATORS FOR AIR POLLUTION WITH ORGANOCHLORINE PESTICIDES IN ROMANIA

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Introduction

Over the few decades, the occurrence of organochlorine pollutants in the environment is of great concern due to their persistent and toxic biological effects. Atmospheric transport is one of the main vectors for their transfer away from the sites where they were produced or used.[1] Therefore, atmospheric transport and depositions to soils and vegetation are key factors to study the environmental exposure to persistent organic pollutants (POPs).[2] Several recent studies have used vegetation such as mosses,[1,3] pine needle[3,4] or tree bark[5] for their capacity to bioaccumulate POPs.

Soil, mosses and tree bark are interesting materials for the monitoring studies of atmospheric depositions of organic pollutants since all type of samples are in direct contact with the atmosphere. The uptake of POPs by vegetation is a relevant step by which pollutants enter the terrestrial food web.[6] Forests, in particular, may strongly influence depositions fluxes to the ground.[7] The existence of an organic surface area, together with high soil organic matter content and infrequent biomass harvesting, create conditions for an important storage or "retardation" compartment for POPs in the terrestrial environment.[2]

The main aims of the present study were: 1. to assess the feasibility of using tree bark and moss to monitor air pollution with organochlorine pesticides (OCPs); 2. to investigate relationships between concentrations and profiles of OCPs in soil, tree bark and moss samples collected from the same locations; 3. to measure the patterns of OCP acumulation in moss and tree bark to provide the first data on atmospheric pollution in eastern part of Romania.

Materials and Methods

Sampling

All samples (soil, moss and tree bark) were collected between August-September 2005 from 16 forest locations in Moldavia, the Eastern part of Romania. Sampling sites were located at least 300 m from main roads and populated areas and at least 200 m from any side roads or houses.

Soil. At each sampling site, two sub-samples of surface soil (0-5cm) taken from a square of 10x10 cm were collected within 50 m from the collection sites for moss and tree bark samples. Sub-samples were pooled per location.

Moss. The *Hypnum cupressiforme* moss species, which grows in the forest, was used for the present study. At each sampling site, 4 to 6 sub-samples were taken within an area of 50x50 m. Both epiphytic mosses growing at 0.5-1.5 m height on different trees and epigeic moss in the close vicinity of trees were collected.

Tree bark. Bark samples were collected at 0.5-1.5 m height from trees of similar size and age. At each sampling site, 3 to 6 sub-samples were taken within an area of 50x50 m. On each tree, slices of about 3 mm thickness were detached from the same side of the trunk where the moss was growing, in order to have the same exposure to POPs. The following tree species were sampled: oak, cherry tree, hornbeam, alderwood and ashwood.

Materials

The OCPs under investigation were hexachlorocyclohexane isomers (α-, β-, γ and δ-HCH), DDT and analogues (o,p'-DDE; p,p'-DDE; o,p'-DDD; o,p'-DDT; p,p'-DDD; p,p'-DDT), hexachlorobenzene (HCB), oxychlordane (OxC), *trans*-nonachlor (TN), *trans*-chlordane (TC) and *cis*-chlordane (CC). A number of 21 most abundant polychlorinated biphenyl (PCB) and 2 polybrominated diphenyl ether (PBDE) congeners were also investigated in selected samples. Internal standards (IS) were PCB 143 and ε-HCH. All solvents were of

pesticide grade (Merk, Germany). The acidified silica gel (44% H_2SO_4, *w/w*) was prepared as described by Covaci et al.[8] Empty polyethylene cartridges (25 mL) were purchased from Alltech (Lokeren, Belgium).

Extraction and analysis

Extractions were performed using an ASE system (Dionex, Sunnyvale, CA, USA) using the parameters mentioned by Dragan et al.[9] Two grams of dried soil or 1.5 g dried tree bark or 3 g dried mosses were spiked with 15 ng of each IS. The extract was concentrated in the extractor vials to 2 mL under a nitrogen stream. The clean-up procedure consisted in the purification of the extract on 8 g acidified silica and elution with 15 mL hexane and 10 mL dichloromethane.[8] The purified extract was further concentrated under a nitrogen stream until dryness, resolubilised in 100 µL iso-octane and transferred to a vial for GC/MS analysis.

A 6890 Agilent gas chromatograph (GC) coupled to a 5973 mass spectrometer operated in electron-capture negative ionisation (ECNI) was equipped with a 15m x 0.18mm x 0.20µm AT-5 capillary column (Alltech). One µL was injected in pulsed splitless mode (constant carrier flow 1.0 mL/min, pulse pressure = 10 psi, pulse time = 1.5 min) with the split outlet opened after 1.5 min. Injector and interface temperatures were set at 290 and 300°C, respectively. The temperature program of the oven was set to 90°C, kept for 1.5 min, then with 15°C/min to 180°C, then with 5°C/min to 210°C, then with 3°C/min to 220°C, then to 45°C/min to 300°C, kept for 10 min.

Limits of quantification ranged between 0.05 and 0.6 ng/g for individual compounds and were dependent on the amount of each analyte in the procedural blanks. The method was validated through the use of a certified reference material (CRM 481-PCBs in soil). Recoveries of target analytes and internal standards ranged between 70 and 85%.[9] For samples with concentrations < LOQ, which were minor in number, zero was used for calculations.

Results and Discussion

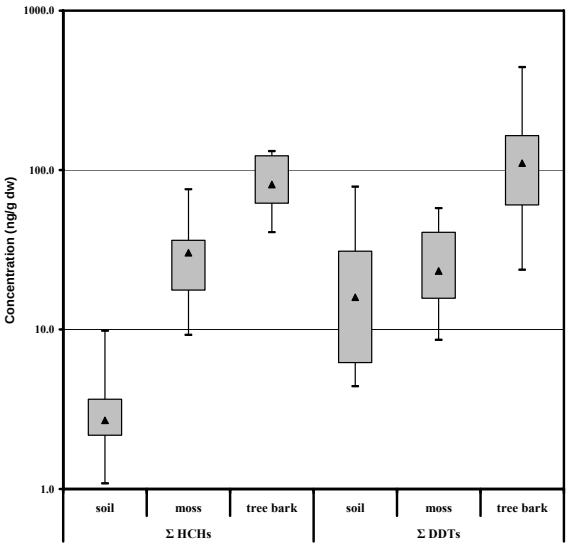
OCPs were the principal pollutants found in the samples. The mean, median, 25- and 75-percentiles, standard deviation and range of concentrations of HCHs, DDTs and chlordanes in moss, tree bark and soil samples are given in Table 1 and Figure 1. Levels of PCBs were under LOQ (< 0.1 and < 2 ng/g dry wt. for individual PCB congeners and sum PCBs, respectively) in the investigated samples. BDE 47 and BDE 99, the two most abundant PBDE congeners, were also found always < LOQ (< 0.05 ng/g per congener for each matrix).

Table 1. Mean, standard deviation and range value of Sum HCHs, Sum DDTs and Sum Chlordanes (ng/g dry wt.) in moss, tree bark and soil samples.

	Moss	Tree bark	Soil
Sum HCHs*			
Mean	31	79	3.1
SD	31	38	1.9
Median	26	81	2
Range	8.9 - 130	12 - 130	1.1 - 9.8
Sum DDTs**			
Mean	28	110	20
SD	22	99	20
Median	21	104	15
Range	5.8 - 95	11 - 440	4.4 - 79
Sum Chlordanes***			
Mean	0.03	0.21	-
SD	0.09	0.18	-
Median	-	-	-
Range	nd - 0.4	nd - 0.6	-

*Sum HCHs = α- + β- + γ- +δ- HCH; **Sum DDTs = o,p'-DDE + p,p'-DDE + o,p'-DDD + o,p'-DDT + p,p'-DDD + p,p'-DDT;
***Sum Chlordanes = OxC + TN + TC + CC; nd – not detected

Figure 1. Box plot distribution (median, 25- and 75-percentiles and range) of sum HCHs and sum DDTs (ng/g dry wt.).



Soil

HCHs were present in a narrow concentration range (1.1 – 9.8 ng/g dry wt for the sum HCHs) at various sampling locations. The low values of the ratio α-HCH/γ-HCH (0.1 – 0.3) suggested the use of pure lindane rather than technical lindane. These indicate that no important pollution point source is present near the sampling sites and that HCHs are originating mainly from long-range air transport processes and through atmospheric deposition of isomers volatilized from agricultural soils[10]. The variations in the composition of HCH isomers in the soil revealed a heterogeneous nature of distribution. The γ-HCH isomer was predominant (64 %) followed by β-, α- and δ-HCH isomers (18, 15, and 4 %, respectively).

The concentrations of sum DDTs ranged between 4.4 – 79 ng/g dry wt in the soil samples. The presence of p,p'-DDE in higher amounts indicates an aerobic degradation of p,p'-DDT in soil or the long-range transport of p,p'-DDE as a result of the transformation of p,p'-DDT after release in the environment. p,p'-DDE and p,p'-DDT were the major contributors to sum DDTs (54 % and 38 %, respectively) followed by p,p'-DDD, o,p'-DDT and o,p'-DDD (5, 2 and <1 %, respectively, Figure 2). The o,p'-DDE metabolite were < LOQ in all soil samples.

Mosses

For moss, the concentration range of HCHs (8.9 – 130 ng/g dry wt) was higher than results reported by Holoubek et al.[3] in moss from Czech Republic (<0.1 – 5.9 ng/g dry wt). β-HCH and γ-HCH (42 and 38 %, respectively) were the major HCH isomers found in the moss samples followed by α-HCH (12 %) and δ-HCH (6 %). This profile could be explained by the high accumulative properties of β-HCH in biological samples and the predominant use of pure lindane in agricultural practices as demonstrated by the profiles of HCH isomers in soil.

Concentrations of sum DDTs in moss ranged between 5.8 – 95 ng/g dry wt. (Table 1). Compared to soil samples, an inversion between the dominance of p,p'-DDE in favour of p,p'-DDT was observed in the moss samples (Figure 2). p,p'-DDT was present at percentages higher than 50 %, followed by p,p'-DDE, o,p'-DDT and p,p'-DDD (24, 12, and 10 %, respectively, Figure 2). The ratio between p,p'-DDT and p,p'-DDE ranged between 1-5.1 for all samples indicating that extensive DDT contamination occurred in the past. Also, these results might suggest the sporadic fresh input of p,p'-DDT in Romania through illegal application of DDT or new use of Dicofol (which may contain up to 11 % DDT).

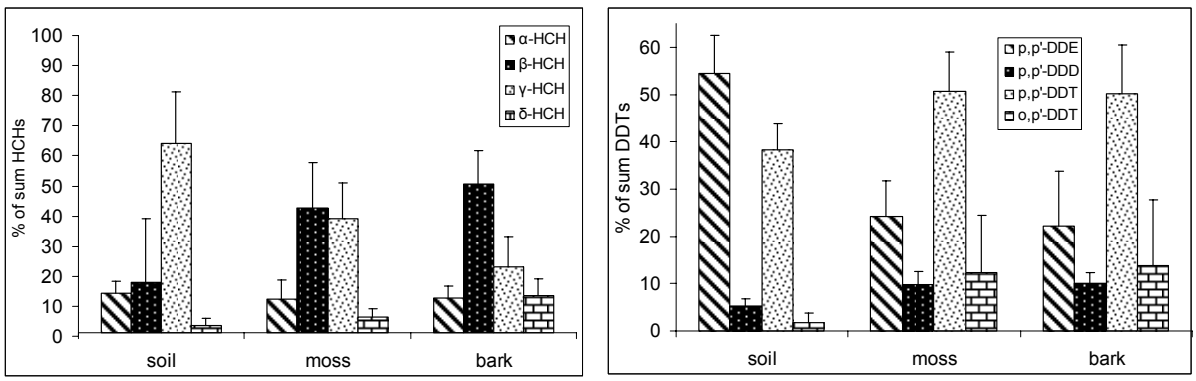


Figure 2. Percent contribution of individual HCH isomers and DDT analogues to the sum HCHs and sum DDTs, respectively.

Tree bark

HCHs and DDTs were detected in all bark samples, confirming that these pollutants are ubiquitous in the eastern part of Romania environment. Concentrations of HCHs and DDTs concentrations varied over a larger range (12 – 130 ng/g dry wt and 11 – 440 ng/g dry wt, respectively). HCHs concentrations in bark samples were the same magnitude with HCHs concentrations in moss samples (Table 1). In contrast, DDTs concentrations were higher in tree bark samples compared to moss. Similar to moss, the predominant HCH isomer was the most bioaccumulative compounds, β-HCH (51 %), followed by γ-HCH, δ-HCH and α-HCH (23, 14, and 12 %,

respectively) (Figure 2). p,p'-DDT was the most abundant in tree bark samples with 50 % followed by p,p'-DDE, o,p'-DDT and p,p'-DDD (22, 14, and 10 %) (Figure 2). For 14 out of 16 sites, the ratio between p,p'-DDT and p,p'-DDE ranged between 1 – 7.1 suggesting, in a similar way as the moss samples, extensive past DDT contamination. OxC, TN, TC and CC, expressed as sum chlordanes, were also detected in the tree bark and moss samples, but at very low levels, suggesting that chlordanes were not used in Romania.

Significant correlations were found between bark and moss concentrations for most DDTs and HCHs (except for α- and β-HCH) (Table 2). This suggests that similar information can be obtained using moss and bark, hypothesis supported also by the resembling profiles of HCHs and DDTs in these two matrices. Except for p,p'-DDE, there were low and statistically not-significant correlations between soil and bark, while correlations were higher and most of them significant between soil and moss (Table 2). Probably this is related to the insignificant interaction between soil and tree bark and to the fact that most of the moss samples were living on the soil.

Table 2. Pearson correlation coefficients obtained for DDT analogues and HCH isomers between the investigated matrices (soil, moss and bark).

Compound	p,p'-DDT	p,p'-DDE	Sum DDTs	α-HCH	β-HCH	γ-HCH	δ-HCH	Sum HCHs
moss-bark	0.71**	0.58*	0.58*	0.45	0.28	0.48*	0.51*	0.33
soil-moss	0.79***	0.72**	0.72**	0.10	0.33	0.48*	0.52*	0.18
soil-bark	0.25	0.49*	0.26	0.12	0.31	0.14	0.01	0.26

*P<0.05; **P<0.01; ***P<0.001

Understanding the accumulation and release of organic pollutants by moss and bark is important to the development of appropriate protocols to biomonitor pollutant's deposition. Since OCPs were not directly applied to the trees, but they were detected in tree bark as well as in moss collected from the same site, it is obvious that their accumulation occurred via atmospheric deposition. OCPs reach the bark mainly through dry deposition and the impact of atmospheric particles. The accumulation of organic pollutants through indirect uptake from soil (via stem flow) has probably a limited impact.[11] The higher concentrations of OCPs in the bark compared to moss are most probably related to the larger surface area of the bark and to its relatively high lipid content (between 1-10%). Therefore, bark is a good passive sampler for organic pollutants even when they are present at low atmospheric concentrations (such as brominated flame retardants[12] or dioxins[13]). Bark may be also used to evaluate spatial distribution of pollutants[14], but also temporal modifications in pollution with POPs in a certain area. Moreover, the sampling of tree bark is inexpensive, easy and can be performed anywhere.

The present study emphasise the value of moss and bark as indicators of pollution with organic compounds over short- and long-term periods. The obtained results are encouraging to continue and to extend the implementation of this biomonitoring technique.

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