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Polychlorinated Biphenyls and Organochlorine Pesticides in Vegetation Samples Collected in Croatia

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are ubiquitous environmental pollutants throughout the world. Once released into the environment, they are distributed in all compartments (Ballschmiter 1996). They can be transported via air to long distances and are detected even in polar regions. They have the propensity to accumulate in foodchains (Jones and de Voogt 1999).

The waxy surface of needles and leaves has the ability to absorb airborne lipophilic pollutants and may serve to biomonitor atmospheric contamination. Moss (Bacci et al. 1986; Knulst and Westling 1995; Lead et al. 1996), lichen (Bacci et al. 1986; Gaggi et al. 1985), and the needles of coniferous trees (Eriksson et al. 1989; Reischl et al. 1989; Tremolada et al. 1996; Jensen et al. 1992; Wenzel et al. 1997) are most often used in biomonitoring studies as a way to follow up trends over long periods on global, regional, and local levels.

The lack of information about the distribution of organochlorine compounds in the vegetation in Croatia initiated a pilot analysis of needles and leaves collected from different sites in the country. Samples of different trees were collected at the same site in order to compare the difference in accumulation capacity of various trees. The geographic distribution was screened by the analysis of pine needles taken from different sites

MATERIALS AND METHODS

Samples were taken from six locations shown in Fig. 1. Zagreb, the capital of Croatia, is an industrial town with about 1 000 000 inhabitants and samples were collected at three locations: a landfill site, an industrial zone and a suburb. Medvednica is a mountain (1000 m altitude) on the northern edge of Zagreb and samples were collected at about 700 m alt. from its southern side which is facing the city. Ogulin is located in the mountainous region Gorski Kotar. Križišće is located at the northern part of the Adriatic

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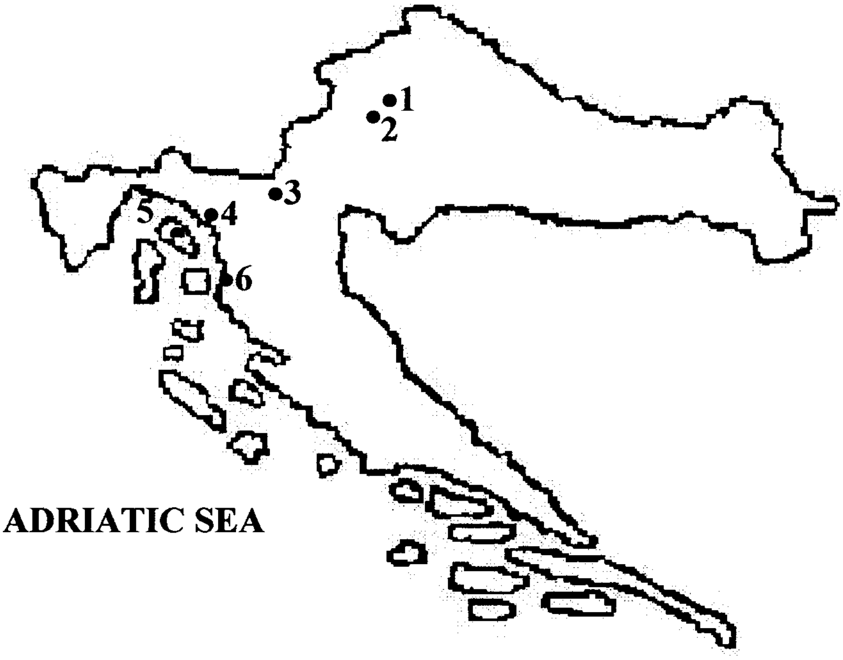

Figure 1. Map of Croatia with indicated sampling sites: 1. Medvednica, 2. Zagreb, 3. Ogulin, 4. Križišće, 5. Krk, 6. Velebit

coast while Krk is the biggest island (412 km^2) in the Kvarner bay of the Adriatic. At Krk, samples were collected from four locations: three are on the coast and one in the centre of the island. Velebit is a mountain (1758 m alt.) in the north part of Kvarner bay and acts as a barrier between the Adriatic coastal region and the inland. Samples from Velebit were collected at about 500 m alt. on its southern face.

Branches were collected from approximately 1.5 m above the ground level. All samples were stored in plastic bags. Needles (generation from the previous year), larch needles and leaves (grown in the year of sampling) were removed from branches and dried at room temperature to constant weight. Dried samples were stored at room temperature until analysis, which was done within at least two months. Samples were collected from pine (*Pinus nigra*), fir (*Abies alba*), spruce (*Picea abies*), larch (*Larix decidua*), thuja (*Thuja ocidentalis*) and lime (*Tilia platyphyllos*). Table 1 lists the collected samples.

The analysis was undertaken for the pesticides HCB, HCH and DDT complex, and for total PCBs which were determined as compared to Aroclor 1260 (Table 2).

Table 1. Samples and sampling sites by years. The number of samples is given in parenthesis.

SITE	SPECIES	SAMPLING YEAR
Križišće	pine (1)	November 1992
Island of Krk		November 1992
- Punat (coast)	pine (1)	
- Baška (coast)	pine (1)	
- Omišalj (coast)	pine (1)	
- Dobrinj (centre of the island)	pine (1)	
Ogulin	fir (2)	June 1993
	fir (1), pine (7), spruce (8), larch (2), thuja (1), lime (1)	May 1994
Zagreb		March 1995
- landfill site	pine (1)	
- industrial zone	pine (1)	
- suburb	pine (1)	
Medvednica	pine (1)	April 1995
Velebit	pine (1)	April 1995

Table 2. Compounds measured in this study.

HCB	Hexachlorobenzene
α-HCH	alpha-hexachlorocyclohexane
β-HCH	beta-hexachlorocyclohexane
γ-HCH	gamma-hexachlorocyclohexane
p,p'-DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
o,p'-DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
p,p'-DDD	1,1-dichloro-2,2-di(4-chlorophenyl)ethane
p,p'-DDE	1,1-dichloro-2,2-di(4-chlorophenyl)ethene
PCBs	Polychlorinated biphenyls

Ten grams of dry ground samples were extracted in three steps with *n*-hexane:acetone (1:1) as follows: 60 mL was added and shaken 5 hrs, then 40 mL was added and shaken 1 hr, and finally 20 mL was added and left for 24 hrs. The solvent layers were separated and the combined extracts were partitioned with distilled water (40 mL). The upper organic phase was separated and purified with sulphuric acid (15 mL, 10 mL and 2 x 5 mL) and on florisil column (2 g anhydrous Na_2SO_4+2 g florisil) prewashed with *n*-hexane (10 mL). Compounds were eluted with *n*-hexane (35 mL). The sample volume was reduced in a rotary evaporator and the solvent evaporated to dryness under a gentle stream of nitrogen.

Solvents, suphuric acid and Na_2SO_4 were from Merck, Darmstadt (pro analysi or for organic trace analysis), while florisil was from Kebo AB, Stockholm (for chromatography, 0.15-0.25 mm). Pesticides and Aroclor 1260 were kindly provided by the US Environmental Protection Agency (purity: 99.5%-100% depending on compound).

Gas chromatographic analysis was done on a Tracor 550 equipped with two ^{63}Ni electron capture detectors and two borosilicate glass columns (2 m x 2 mm) packed with 5% OV-101 or 3% OV-1 on Chromosorb W 0.20 - 0.25 mm. The carrier gas was nitrogen (flow rate 50 mL/min). The injector temperature was 220°C and that of the detector 250°C. Column temperature was 185°C for OCPs and 205°C for PCBs. The concentrations of the organochlorine compounds were calculated by comparison to external standards. Recovery was measured by addition of the studied compounds to ground needles prior to extraction. All results are corrected for recoveries. The determination limits were 0.1 – 0.5 ng/g dry weight depending on the compound.

RESULTS AND DISCUSSION

The samples were collected from six species of trees in order to assess the extent to which accumulation of the pollutants varied. Larch-, thuja-, pine-, spruce- and lime-tree samples were collected from Ogulin in May 1994. Two fir samples were collected from the same site in Ogulin in June 1993.
Table 3 shows the levels found in the 1994 yr. samples. In the six species, concentrations of HCB, HCH isomers and PCBs differ less than one order of magnitude. However, the concentrations of the DDT complex span over a broader range, depending on the species.

The accumulation period of deciduous tree leaves is about six months while for conifer needles it can be many years, depending on the species and the climate zone. The examined conifer needles reflect about one-year accumulation period while larch and lime only about two months. This means that according to the accumulation period, different species reflect different levels. The results shown in Table 3 are the same as reported by Ockenden and co-workers, who have also shown that different species accumulate pollutants differently. Therefore, for the comparison of vegetation pollution levels between sites, samples of the same or taxonomically similar species should be analysed (Ockenden et al. 1998).

Fir tree needles were collected in Ogulin also in 1993. As seen in Table 3, all concentrations of analysed compounds were lower in 1993 than in 1994. This result might indicate an increase in pollution between 1993 and 1994 although we have no information on an increased input into the environment during that year. Whatever the reason, this result implies that for the sake of comparison between sites it is important to collect samples during one given accumulation period.

Table 3. Levels of organochlorine compounds (ng/g dry weight) in different types of trees collected in Ogulin in 1994, and in two fir samples (*) collected in Ogulin in 1993.

	Larch	Pine	Lime	Spruce	Thuja	Fir	Fir*
N	2	7	1	8	1	1	2
HCB	1.2	1.0	2.0	0.8	2.3	0.8	0.2
α-HCH	5.0	1.3	5.4	1.3	1.3	7.1	0.2
β-HCH	1.9	1.2	3.0	0.9	1.8	1.2	0.3
γ-HCH	4.3	5.5	12	3.6	4.8	5.7	0.4
p,p'-DDE	5.2	1.6	15	2.9	5.7	4.5	0.7
p,p'-DDD	1.5	0.2	0.4	1.0	2.7	2.1	0.2
p,p'-DDT	0.9	0.7	0.6	0	0	0	0
o,p'-DDT	2.1	1.1	9.3	0	0	0	0
PCBs	12	10	7.2	16	17	14	1.2

The results for spruce and pine are expressed as medians. N is the number of samples. Zero stands for concentrations below determination limit.

In order to evaluate geographic distribution of PCBs and OCPs, pine needles were chosen as suitable due to their worldwide spread including Croatia and due to the fact that data on pine needles already exist in the literature. Pine needles from Zagreb and from the mountains of Velebit and Medvednica, were collected in March - April 1995, and from Adriatic sampling points in November 1992. The obtained results are given in Table 4.

Concentrations of *p,p'*-DDT in the 1995 yr. samples were all below determination limit. The same holds for *o,p'*-DDT, except for the sample from Velebit, which had however a very low concentration of *o,p'*-DDT. The concentrations of all other compounds ranged from 0.7 to 8.0 ng/g dry weight irrespective of whether the sample was from the city of Zagreb or from the mountains Medvednica and Velebit. Concentrations of β-HCH in the 1992 yr. samples from Križišće and Krk were all below determination limit. The concentrations of all other compounds ranged from 0.2 to 3.0 ng/g dry weight. Concentrations in the pine sample from Ogulin (1994) ranged from 0.2 – 10 ng/g dry weight (Table 3).

The results in Tables 3 and 4 show that the levels of OCPs and PCBs vary according to sampling site and the year of sampling. No consistent difference was observed either between the urban and the rural districts, or between the Adriatic area and the inland. However, some difference seems to exist with respect to the year of sampling. This suggests that in monitoring studies of the geographic distribution of these pollutants, samples from different sites should be collected at the same time of a given year.

Table 4. Levels of organochlorine compound in pine needles (ng/g dry weight) collected at different sites and years.

Sites	HCB	α-HCH	β-HCH	γ-HCH	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	*o,p'*-DDT	PCBs
MARCH – APRIL 1995									
Zagreb									
- landfill site	1.3	5.6	1.8	6.9	2.5	0.9	0	0	7.5
- industrial zone	1.2	7.4	1.1	7.4	2.6	0.7	0	0	6.5
- suburb	1.5	6.9	1.7	7.0	1.8	2.4	0	0	3.8
Medvednica	1.3	4.8	1.2	4.2	2.3	1.6	0	0	/
Velebit	1.5	1.8	3.2	3.2	2.2	2.5	0	0.5	8.0
NOVEMBER 1992									
Križišće	0.5	0.4	0	0.5	1.2	0.3	0.4	0.9	2.2
Island of Krk									
- Punat	0.5	0.2	0	0.7	2.1	0.3	0.5	1.0	3.1
- Baška	0.3	0.2	0	0.5	1.6	0.3	0.5	1.5	2.5
- Omišalj	0.4	0.3	0	0.5	1.2	0.4	0.6	0.7	1.4
- Dobrinj	0.6	0.6	0	0.7	0.8	0.4	0.4	0.8	1.4

Zero stands for concentrations below determination limit.

Ratios of DDE/DDT and α-HCH/γ-HCH are often used as indicators of recent DDT and lindane (γ-HCH) input into the environment (Ballschmiter and Wittlinger 1990; Larson and Okla 1989; Brun et al. 1991). Low ratios, particularly below one, indicate recent input.

Our results showed that DDE/DDT ratios were above 2.0 in all analyzed samples (Tables 3 and 4). This indicates that there had not been a recent input of DDT into the environment and that DDT, which had been released earlier, was converted to DDE. The ratios α-HCH/γ-HCH calculated from the results outlined in Tables 3 and 4 range from 0.2 (pine needles collected in Ogulin in 1994) to 1.3 (fir needles collected in Ogulin in 1994). The ratios in pine needles collected in Zagreb in 1995 are about one, while in samples collected on the island of Krk in 1992 they are between 0.3 and 0.8.

The use of lindane in Europe is not banned, but restricted (Voldner and Li 1995). Our results indicate that recent input of lindane into the environment had probably occurred.

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REFERENCES

Bacci E, Calamari D, Gaggi C, Fanelli R, Focardi S, Morosini M (1986) Chlorinated hydrocarbons in lichen and moss samples from the Antarctic peninsula. Chemosphere 15:747–754

Ballschmiter B (1996) Persistent, ecotoxic, and bioaccumulative compounds and their possible environmental effects. Pure Appl Chem 68:1771–1780

Ballschmiter K, Wittlinger R (1991) Interhemisphere exchange of hexachlorocyclohexanes, hexachlorobenzene, polychlorobiphenyls, and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane in the lower troposphere. Environ Sci Technol 25:1103–1111

Brun GL, Howell GD, O'Neill HJ, (1991) Spatial and temporal patterns of organic contaminants in wet precipitation in Atlantic Canada. Environ Sci Technol, 25:1249–1261

Eriksson G, Jensen G, Kylin H, (1989) The pine needles as a monitor of atmospheric pollution. Nature 341:42–44

Gaggi C, Bacci E, Calamari D, Fanelli R (1985) Chlorinated hydrocarbons in plant foliage: an indicator of the tropospheric contamination level. Chemosphere 14:1673–1686

Jensen S, Eriksson G, Kylin H (1992) Atmospheric pollution by persistent organic compounds: monitoring with pine needles. Chemosphere 24:229–245

Jones KC, de Voogt P (1999) Persistent organic pollutants (POPs): state of the science. Environ Pollut 100:209–221

Knulst JC, Westling HO (1995) Airborne organic micropollutant concentrations in mosses and humus as indicators for local versus long-range sources. Environ Monit Assess 36:75–91

Larsson P, Okla L (1989) Atmospheric transport of chlorinated hydrocarbons to Sweden in 1985 compared to 1973. Atmos Environ 23:1699–1711

Lead WA, Steinnes E, Jones KC, (1996) Atmospheric deposition of PCBs to moss (*Hylocomium splendens*) in Norway between 1977 and 1990. Environ Sci Technol 30:524–530

Ockenden WA, Steinnes E, Parker C, Jones KC (1998) Observation on persistent organic pollutants in plants: implications for their use as passive air samplers and for POP cycling. Environ Sci Technol 32:2721–2726

Reischl A, Reissinger M, Thoma H, Hutzinger O (1989) Accumulation of organic air constituents by plant surfaces: part IV Plant Surfaces: A sampling system for atmospheric polychlorodibenzo-p-dioxin (PCDD) and polychlorodibenzo-p-furan (PCDF). Chemosphere 18: 561–568

Tremolada P, Burnett V, Calamari D, Jones KC (1996) A study of the spatial distribution of PCBs in the UK atmosphere using pine needles. Chemosphere 32:2189–2203

Voldner ER, Li YL (1995) Global usage of selected persistent organochlorines. Sci Total Environ 160/161:201 –210

Wenzel K–D, Weiβlog L, Paladini E, Gantuz M, Guerreiro P, Pulifiato C, Schüürmann G (1997) Immision patterns of airborne pollutants in Argentina and Germany II. Biomonitoring of organochlorine compounds and polycyclic aromatics. Chemosphere 34:2505 – 2518