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Distribution of Organochlorine Compounds in Pine Needles Collected at Urban Sites in Croatia

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, bioaccumulative, toxic compounds prone to long-range atmospheric transport. In spite of bans and restrictions of their use, PCBs and OCPs are still present in the environment. Vegetation has been used in many studies to assess the environmental fate and human exposure to PCBs and OCPs, and it has widely been accepted that conifer needles are reliable biomonitors due to their high lipid content (Wenzel et al. 1997; Weiss et al 2000; Holoubek et al. 2000; Blais et al. 2003). In this study, six indicator PCBs and several OCPs were analysed in pine needle samples collected in urban sites in Croatia. Earlier data obtained from the same sites made it possible to see temporal trends in the distribution and concentration of the studied compounds.

MATERIALS AND METHODS

Twenty seven *Pinus strobus* or *Pinus nigra* needle samples were collected at 18 sites indicated on the map in Figure 1. At each site one sample was taken, except for Zagreb (site 1) and Krk (site 17) where samples were taken at 7 and 4 locations within these sites respectively (1a – 1g and 17a – 17d). During January – March 1998 samples were collected at sites 1 – 15 and 18 while in December 2000 samples were collected at sites 16 and 17.

Branches were collected at approximately 1.5 m above the ground level and stored in plastic bags. Needles were dried at room temperature to constant weight.

The following organochlorine compounds were measured: HCB (hexachlorobenzene), α-, β-, γ-HCH (alpha-, beta-, gamma-hexachlorocyclohexane), DDE (1,1-dichloro-2,2-di(4-chlorophenyl)ethene), DDD (1,1-dichloro-2,2-di(4-chlorophenyl)ethane, DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane and six indicator PCB congeners (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180).

The method of Franich and co-workers (1993) was modified by Herceg (1999). Dried ground needles (15 g) were mixed with dichloromethane (50 mL) shaken for 3 min and left standing for 24 hrs. The extracts were decanted and additional

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50 mL of dichloromethane was added to the needles and shaken for 3 min. Extracts were combined and filtered through a dichloromethane prewashed filter paper (Whatman No. 1). Extract volumes were reduced using rotary evaporator and evaporated to dryness under a gentle stream of nitrogen to give waxy solid.

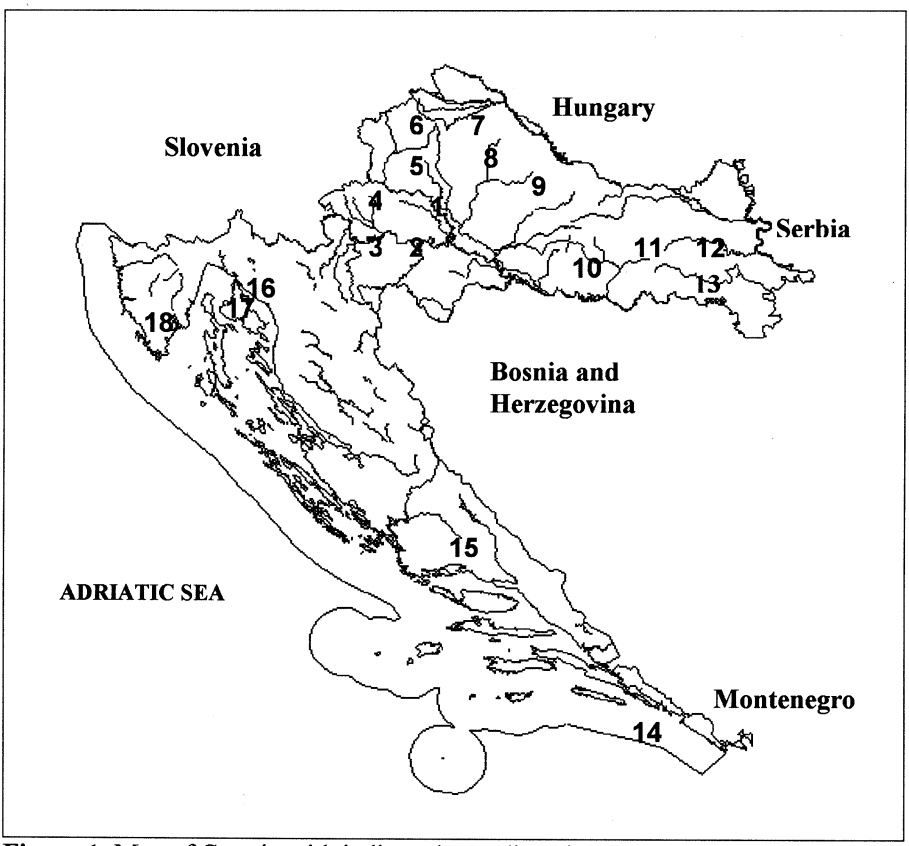

Figure 1. Map of Croatia with indicated sampling sites.

1–Zagreb [locations: a-Borongaj, b-Jakuševec, c-Ksaver, d–Markuševačka Trnava, e-Opatovina, f-Novaki, g-Odra]; 2-Jastrebarsko; 3-Karlovac; 4-Kamanje; 5-Krapina; 6-Bednja; 7–Čakovec; 8-Ludbreg; 9-Koprivnica; 10-Požega; 11-Našice; 12-Vinkovci; 13-Županja; 14-Dubrovnik; 15-Kaštel Sućurac; 16-Križišće; 17–Krk [locations: a-Omišalj, b-Baška, c-Punat, d-Dobrinj]; 18–Plomin

As the needles have a complex wax composition, a multi-step clean-up was required. Waxy solid extract was dissolved in hot toluene:hexane (3:17; 7 mL) and then precipitated by cooling to 0 °C. This was repeated once, and the two supernatants were pooled. The clean-up was continued by saponification under alkaline conditions (potassium hydroxide 1 mol/L in water:ethanol 1:1, 5 mL). The upper phase was separated and evaporated to dryness at 40 °C under a gentle

stream of nitrogen and redissolved in hot acetonitrile (2 mL). Wax was precipitated by cooling to 0 °C and this step was repeated. Two combined acetonitrile solutions were evaporated to dryness at 40 °C under gentle stream of nitrogen. The residues were dissolved with 4% diethyl-ether in *n*-hexane (0.5 mL) and loaded onto a multilayer silica column and purified by adsorption chromatography. The column was filled from the base as follows: silica gel (1 g), 33%-0.1 mol/L NaOH-silica gel (2 g), silica gel (1 g), 44%-H_2SO_4-silica gel (4 g) and silica gel (2 g). The acidic and basic silica layers were prepared by mixing apropriate amounts of sulphuric acid with silica gel or of sodium hydroxide with silica gel and homogenised in a rotary mixer for 8 hrs. The compounds were eluted with 4% diethyl-ether in *n*-hexane (80 mL). The eluate was reduced to dryness and redissolved in *n*-hexane for gas chromatographic analysis.

Solvents, *n*-hexane, acetonitrile and toluene were from "Merck KGAa", Darmstadt, Germany while dichloromethane, diethyl-ether and sulphuric acid (min. 96%) (p.a.) were from "Kemika", Zagreb, Croatia. The silica gel used for chromatography (Kieselgel 60, 0.063-0.200 mm "Merck", Darmstad, Germany) was prewashed with dichloromethane and heated 24 hrs at 180 °C.

Gas chromatography (HRGC-ECD) was done on "ATI UNICAM" 610 SERIES with ^{63}Ni electron capture detector. The compounds were separated on two capillary columns: 1) 60 m × 0.25 mm, SPB-5 film thickness 0.25 μm, flow 14 mL/min, temp. programme 100 °C, then 4 °C/min to 240 °C, 50 min isothermally 2) 30 m × 0.25 mm, SPB-1701 film thickness 0.25 μm, flow 15 mL/min, temp. programme 110 °C, then 4 °C/min to 240 °C, 50 min isothermally. Carrier gas was nitrogen. The injector and detector temperature were 250 °C and 270 °C respectively. Qualitative and quantitative analyses were done by comparison with external standard. Each sample was analysed on both columns. Only compounds identified on both columns were evaluated. The detection limit was 0.4 ng/mL for each compound.

RESULTS AND DISCUSSION

Method recovery and reproducibility were determined by adding a known amount of analysed compounds to dichloromethane extracts of dried ground needles. The levels of added compounds were similar to levels at which they are present in native samples (range: 0.8-2.8 ng/g dry weight depending on the compound). Between 1998 and 2002 thirteen samples were analyzed. These results are presented in Table 1.

The recoveries were between 34% and 54%. This is fairly low recovery, probably due to the multiphase clean-up which was absolutely necessary. The reproducibility for all compounds was below 50%. Considering the low levels of compounds present in the pine needles, it was concluded that the method is suitable for monitoring studies.

Table 1. Method recovery and reproducibility

Compound	Recovery (%)	RSD (%)
HCB	39	49
α-HCH	49	45
β-HCH	36	42
γ-HCH	39	46
DDE	56	27
DDD	35	37
DDT	36	50
PCB-28	57	39
PCB-52	50	38
PCB-101	47	32
PCB-138	47	43
PCB-153	34	29
PCB-180	36	31

RSD-relative standard deviation

Levels and geographic distribution of organochlorine compounds were investigated in samples collected in urban sites during January-March 1998. Samples were collected at all sites indicated in Fig. 1, except on the island Krk (site 17) and in Križišće (site 16). In order to investigate concentration distribution trend in Zagreb (site 1) as the largest city in Croatia (Population: about one million habitants), we decided to collect seven samples from seven locations, six of which were residental [north (c-Ksaver), north east (d-Markuševačka Trnava), west (e-Opatovina), south (g-Odra), east (a-Borongaj and f-Novaki)] and one industrial near waste dump (b-Jakuševac). Kaštel Sućurac (site 15), Plomin (site 18) and Dubrovnik (site 14) are located at the Adriatic coast.

All compounds were found in all samples (Table 2). Organochlorine pesticides ranged between 0.05 and 5.93 ng/g dry weight and PCBs 0.12 – 8.31 ng/g dry weight. Concentration medians for organochlorine pesticides appear in following order γ-HCH>DDE>HCB>β-HCH>α-HCH>DDT>DDD, and for PCBs as follows: PCB-28>PCB-101>PCB-138>PCB-52>PCB-153>PCB-180. Table 2 shows no particularly high or particularly low levels of any of the analysed compounds at any single site. However, the levels of certain compounds do stick out such as β-HCH in Kamanje (site 4), Požega (site 10) and Zagreb (site 1) (Zagreb area median). Concentration medians for organochlorine pesticides in Zagreb appear in the following order: γ-HCH>β-HCH>HCB>DDE>α-HCH>DDT> DDD, and for PCBs: PCB-101>PCB-28>PCB-138>PCB-52>PCB-153 > PCB-180. In general, no marked difference in the levels and distribution of compounds was observed between samples collected in Zagreb and those collected at other sites.

DDE/DDT and α-HCH/γ-HCH ratios are often used to indicate a fresh input of

Table 2. Organochlorines in pine needles (ng/g dry weight) collected in 1998. *Herceg Romanić and Krauthacker 2000b

	HCB	α-HCH	β-HCH	γ-HCH	DDE	DDD	DDT	PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180	α-/γ-HCH	DDE/DDT
Zagreb-Borongaj *	1.38	0.44	0.57	1.59	0.35	0.22	0.72	1.74	5.09	1.96	1.76	0.74	0.56	0.28	0.49
Zagreb-Opatovina	0.70	0.33	2.10	1.13	0.66	0.22	0.14	2.52	0.34	1.79	0.56	0.50	0.16	0.29	4.89
Zagreb-Novaki	1.00	1.15	2.84	5.25	0.84	0.29	0.33	1.33	0.78	1.45	0.92	0.54	0.12	0.22	2.55
Zagreb-Odra	0.69	0.39	2.03	2.17	1.45	0.21	0.41	1.64	0.56	2.32	1.65	0.79	0.24	0.18	3.58
Zagreb-Trnava	0.64	0.63	3.01	3.80	0.72	0.32	0.23	3.71	0.42	2.96	1.74	0.41	0.35	0.17	3.15
Zagreb-Ksaver *	0.83	0.97	1.14	3.63	0.64	0.44	2.62	1.58	2.80	2.72	0.69	0.74	0.48	0.27	0.24
Zagreb-Jakuševec*	1.55	1.27	0.96	4.91	0.72	0.50	2.65	0.68	6.63	1.63	1.23	1.30	0.54	0.26	0.27
Jastrebarsko	0.64	0.99	0.29	0.60	0.95	0.41	1.18	1.88	0.25	1.22	0.63	0.48	0.15	1.66	0.80
Karlovac *	0.83	0.58	0.68	1.46	0.43	0.19	0.43	1.72	7.25	1.44	1.36	0.80	0.61	0.40	1.00
Kamanje	0.49	0.42	1.77	1.45	0.86	0.37	0.29	2.35	0.58	1.84	0.79	0.56	0.14	0.29	2.94
Ludbreg *	1.04	0.47	0.47	0.98	0.48	0.15	0.45	1.07	3.72	1.10	0.81	0.51	0.49	0.48	1.07
Bednja	1.05	0.91	0.31	1.01	1.43	0.09	0.32	2.86	0.86	3.13	1.94	0.94	0.19	0.90	4.52
Krapina	1.57	0.99	0.51	0.22	1.42	0.55	0.55	2.50	0.77	1.97	1.22	1.07	0.20	4.48	2.60
Čakovec	1.84	0.27	0.6	1.30	2.56	0.25	0.39	5.87	2.68	3.25	2.39	1.27	0.34	0.21	6.56
Koprivnica	1.07	0.27	0.36	2.04	0.67	0.09	0.35	2.23	0.68	1.10	1.05	0.40	0.19	0.13	1.94
Našice*	0.81	0.47	0.49	1.49	0.38	0.19	0.23	1.70	3.28	1.08	1.60	1.16	2.83	0.32	1.65
Požega *	0.85	0.37	1.62	1.95	1.00	0.27	0.67	3.50	8.31	2.10	3.17	2.05	2.61	0.19	1.49
Županja	0.92	0.07	0.59	1.34	1.78	0.10	0.22	3.20	0.49	1.75	1.29	0.94	0.27	0.05	8.08
Vinkovci *	0.85	0.55	1.36	3.81	0.97	0.31	0.78	0.82	5.03	1.24	0.88	0.77	0.42	0.14	1.24
Dubrovnik	0.42	0.05	0.26	0.26	1.16	0.37	1.05	1.57	1.33	2.17	1.24	0.87	0.40	0.20	1.11
Kaštel Sućurac	0.63	0.26	0.13	2.18	1.03	0.11	0.19	3.38	1.96	1.69	0.96	0.47	0.34	0.12	5.52
Plomin	0.61	1.31	0.40	5.93	2.38	0.35	0.72	3.57	0.93	4.08	2.13	1.42	0.24	0.22	3.29
Median of all samples	0.84	0.47	0.59	1.54	0.90	0.26	0.42	2.06	1.13	1.82	1.23	0.78	0.34	0.24	2.58
Zagreb area median	0.83	0.63	2.03	3.63	0.72	0.29	0.41	1.64	0.78	1.96	1.23	0.74	0.35	0.26	3.15

DDT or γ-HCH in environment. In our samples, the α-HCH/γ-HCH ratios were below 0.9 which indicates a recent input of γ-HCH ; but it is known that the use of lindane (γ-HCH) is restricted but not banned (Breivik et al. 1999). Higher α-HCH/γ-HCH ratios were observed in samples from Krapina (4.48; site 10) and Jastrebarsko (1.66; site 11). DDE/DDT ratios close to or below 1 were also observed in Vinkovci (1.24; site 12), Dubrovnik (1.11; site 14), Zagreb (0.49; site 1a-Borongaj), Zagreb (0.24; site 1c-Ksaver), Zagreb (0.27; site 1b-Jakusevec), Jastrebarsko (0.8; site 2), Karlovac (1.0; site 3), Ludbreg (1.07; site 8). This implies an input of DDT in the atmosphere. Low DDE/DDT ratios were also found in samples of snow, rain and air collected at Zagreb area in 1990/92. and 1997. resp. (Fingler et al. 1994; Herceg Romanić and Krauthacker 2000a).
It should be noted that inland of Croatia is characterised by continental climate and the Adriatic coast is characterised by Mediterranean climate. In other words, annual ambient temperatures are higher at the Adriatic coast. It is known that higher temperature influence on volatilisation of previously deposited OCPs and PCBs from Earth's sorbents to the ambient air and for this reason it can be expected that the level of compounds analysed in pine needles collected from the Adriatic coast would be lower than in samples from the inland areas. However, our results show that the level of organochlorine compounds in pine needles collected in Croatia are not influenced by differences in the climate, but the distribution of individual compounds is rather influenced by long range transport (DDT) and the proximity of pollution sources (mainly agricultural activities in the case of lindane).
If our results are compared with literature (Kylin et al. 1994; Wenzel et al. 1997; Ockenden et al. 1998; Holoubek et al. 2000) it is evident that the levels in pine needles samples collected in Croatia could be characterised as typical for urban areas.

Temporal trends in concentrations are based on data obtained from samples collected at the same site and from the same pine tree in 2000 (this study) and 1992 (Krauthacker et al. 2001). Samples were collected in Križišće (site 16) and at four locations on the island Krk (site 17): a-Omišalj (coast), b-Baška (coast), c-Punat (coast), d-Dobrinj (centre of island). Križišće are located at northern Adriatic and Krk is island (412 km^2) in the Kvarner bay of the northern Adriatic.

Comparison of results obtained in 1992 and 2000 is presented in Table 3. All analysed OCPs were found in all samples collected in 2000 and their concentration ranged between 0,03 and 1,2 ng/g dry weight. The comparison of concentration medians in 2000 and 1992 indicated a slight decreasing trend with some exceptions. First, in 2000 β-HCH was higher than in 1992 and second, DDE/DDT ratio in samples from Križišće and Krk-Omišalj decreased (it was 3 in Križišće in 1992 and 0.5 in 2000; it was 2 in Krk-Omišalj in 1992 and 0.7 in 2000). Table 3 shows that DDE and DDT levels are low and that DDE levels slightly dropped between 1992 and 2000. Levels of DDT remained unchanged or increased slightly, which implies DDT input. As mentioned in above, low DDE/DDT ratios were detected in samples in Zagreb in 1990/92 and 1997

(Fingler et al. 1994; Herceg Romanić and Krauthacker 2000a) and in some pine needle samples collected in 1998 in inland Croatia and Dubrovnik (Table 2). There has been no evidence of DDT use in this region over the past two or three decades. The only explanation for decrease in the DDE/DDT ratio is the air transport of DDT from countries where it has been used or produced and emitted in the environment. There have been reports of fresh DDT input. Di Guardo and co-workers (2003) published data about DDT levels in spruce needles collected close to chemical plant which produced DDT in Italy in 1999. The sum of six DDT isomers and metabolites reached the peak near the plant (40-50 ng/g dry weight) and was falling with the distance from the source. Another possibility is long range transport of DDT from Africa where it is used for malaria vector control (Voldner and Li 1995).

Table 3. Levels of organochlorine pesticides in pine needles (ng/g dry weight) at same site in 2000 (this paper) and in 1992 (Krauthacker et al. 2001).

Site	Year	HCB	α-HCH	β-HCH	γ-HCH	DDE	DDD	DDT
Križi-	2000	0.14	0.60	1.1	0.10	0.40	0.20	0.90
šće	1992	0.50	0,40	0	0,50	1.20	0.30	0.40
Krk	2000	0.03	0.03	0.20	0.10	0.70	0.20	0.20
Dobrinj	1992	0.60	0.0	0	0.70	0.80	0.40	0.40
Krk	2000	0.30	0.10	1.20	0.10	1.00	0.20	0.30
Punat	1992	0.50	0.20	0	0.70	2.10	0.30	0.50
Krk	2000	0.20	0.50	1.10	1.10	0.50	0.40	0.70
Omišalj	1992	0.40	0.30	0	0.50	1.20	0.40	0.60
Krk	2000	0.10	0.05	0.20	0.30	0.30	0.20	0.10
Baška	1992	0.30	0.20	0	0.50	1.60	0.30	0.50
Median	2000	0.10	0.08	1.05	0.10	0.50	0.20	0.30
	1992	0.50	0.30	0	0.50	1.20	0.30	0.50

0 – below detection limit

Our data and other published data (Harrad et al. 1994) suggest that the levels are decreasing very slowly or not at all. Random increase in PCB and OCPs levels might have been caused by a fresh input and/or long range transport, and due to persistence of this compounds, PCB and OCPs will exist and re-cycle in the environment for many years.

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