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Characterization of Soil and Sediment Samples Collected from the Zadar Area, Croatia, by GC-ECD PCB Analysis and Bioassay

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In recent decades, persistent organic pollutants (POPs) have become a major research issue in order to investigate their ubiquitous environmental occurrence, biochemical and toxic effects, human exposure and health risk assessment (Picer, 2000).

In general, there is scant data on the POP compounds levels in many Central and Eastern European countries. However, it should not been forgotten that this region has very specific environmental pollution problems due to the recent wars. During the Balkan Wars (Croatia, Bosnia and Herzegovina) and operation "Allied Force" in the spring of 1999, the burning or damaging of industrial and military targets in Croatia, Bosnia and Herzegovina, Kosovo and Serbia resulted in the release of a large number of chemicals into the environment.

There are great fears and concrete evidence that significant quantities of polychlorinated biphenyls (PCBs), various flame-retardants, explosives, and their by-products were released into the environment during warfare (Thompson et al., 1993; Picer, 1996). Although there were no large urban centers in the karstic area of the former Yugoslavia involved in warfare, it nonetheless warrants special attention due to its high ecological vulnerability. European Commission experts of the fifth framework Programme (Call Identifier ICFP501A2PR02) have recognized these problems and approved the proposed investigations under Contract APOPSBAL ICA2 - CT2002-10007. One of the most important tasks of this project is not only to recognize the persistent organic pollutant (POP) problems of the war-ravaged areas in former Yugoslavia but also to develop cooperation between scientific institutions from this area and other countries in the region. Some of the advanced results of cooperation among institutions from Croatia and Serbia and Montenegro have been presented (Picer and Hodak Kobasić, 2004).

MATERIALS AND METHODS

In the Zadar area, we have found several points where the soil is seriously contaminated with PCBs, as presented in Figure 1. ETS stands for the 110/35 kV Zadar Electrical Transformer Station which was damaged during the war; TIZ

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stands for the damaged textile factory; BAGAT is the site of a large ruined factory for the production of sewing machines; GAZENICE is a site where building wastes are deposited; WASTE DEPO is a site were various metal wastes for recycling are deposited.

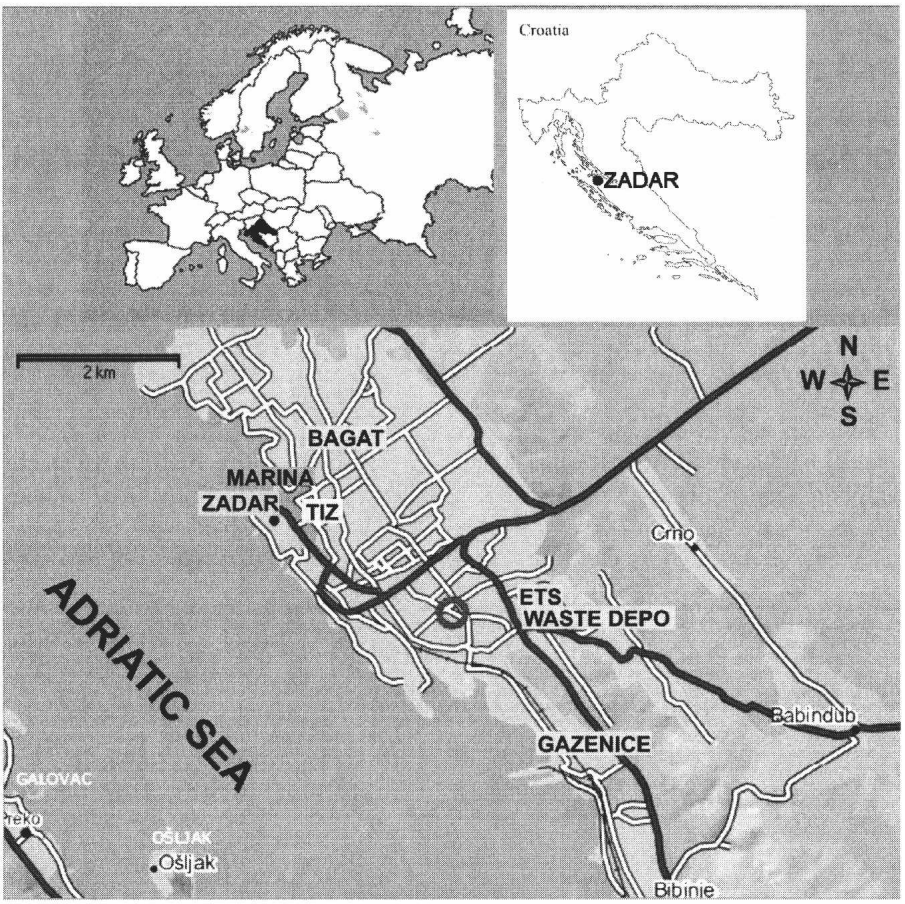

Figure 1. Map showing the location of sampling sites in Zadar, Croatia.

Organic chlorine compounds were analyzed after extraction, purification and concentration using a gas chromatograph equipped with an electron capture detector. Contaminants were extracted from soil and sediment samples using Soxhlet apparatus or accelerated solvent extractor (ASE 200 extractor). Crude extracts of 5 grams of soil sample were sent for bioassay analysis. The rest of extracts were purified using an aluminum oxide column, while chlorinated insecticides were separated from polychlorinated biphenyls with miniature silica gel column. Sulfur was removed from the sediment samples with elementary mercury. A detailed description of the methodology has been published in several papers (Picer and Ahel 1978; Picer and Picer 1993; Picer 2000).

Extracts obtained by an ASE–200 apparatus were evaporated to dryness and then dissolved in a suitable quantity of DMSO (1 ml DMSO to 5 g aliquot of samples), and subjected to EROD analysis.
Bioassay was performed on a primary rat hepatocyte culture. The isolation of rat hepatocytes was performed according to a method originally described by Seglen (1973), with minor modifications. Cells were plated in 96-well collagen-coated culture plates at a density of 25000 cells/well in 0.1 ml of the culture medium supplemented with 10% fetal calf serum. After cell attachment, the medium was removed and fresh serum-free medium containing various concentrations of the samples was added. The hepatocytes were incubated for 48 h and measurement of EROD activity was performed according to the method of Donato et al. (1993). EROD activity in wells was analyzed by a Fluoroscan Ascent FL plate reader (ThermoLabsystems) using 544 nm excitation and a 590 emission filter.
PCB 126 was used as a reference compound in bioassay and bio-TEQ value was calculated according to literature (Zoric et al., 2004).

RESULTS AND DISCUSSION

Table 1 presents the polychlorinated biphenyl contents in soil samples from the transformer station in the Zadar area and from various sub areas of the city of Zadar.

Table 1. Polychlorinated biphenyl content in soil samples from transformer station (E.T.S.) in Zadar area and from other stations around Zadar.

Sampling station	Sampling year	Depth (cm)	No. of samples	PCB total range ($\mu g g^{-1}$ d.w.)	PCB total (geom mean)
E.T.S. Zadar	1996	0 - 10	6	0.2 - 214.4	19.2
	1996	20 - 30	4	13.2 - 124.5	52.8
	2001	0 - 10	5	0.2 - 2.0	0.5
	2002	0 - 5	23	0.02 - 10455.0	1.0
	2002	15 - 20	6	0.02 - 35.3	0.3
	2003	0 - 25	2	2.8 - 1162.5	57.4
	2004	0 - 10	7	2.0 - 208.8	11.7
Other places in Zadar area	2004	0 - 10	12	0.07 - 976.1	3.5

Statistical investigation of the chlorinated hydrocarbon levels in many biotic and abiotic samples from the Adriatic Sea and other environments shows that the standardized coefficients of the kurtosis and the skewness are much higher than 2, which means that these data may depart significantly from normal distribution (Picer and Picer 1995). Consequently, medians and geometric means ought to be more appropriate measures of the central tendency of the investigated pollutant data than arithmetic means (averages). For that reason, besides providing the year and level of collecting samples, Table 1 also presents the minimum, maximum

and geometric means of the levels. Due to the extremely high range of PCB levels found in the investigated soil samples, an arithmetic mean would not represent the central tendency of the PCB levels in the investigated samples.
Results of PCB analysis of the soil from E.T.S. Zadar indicate significant contamination of the area with polychlorinated biphenyls. On Figure 2 are presented levels of the PCBs (Geometric means) determined on the other four main investigation localities in Zadar besides the E.T.S.

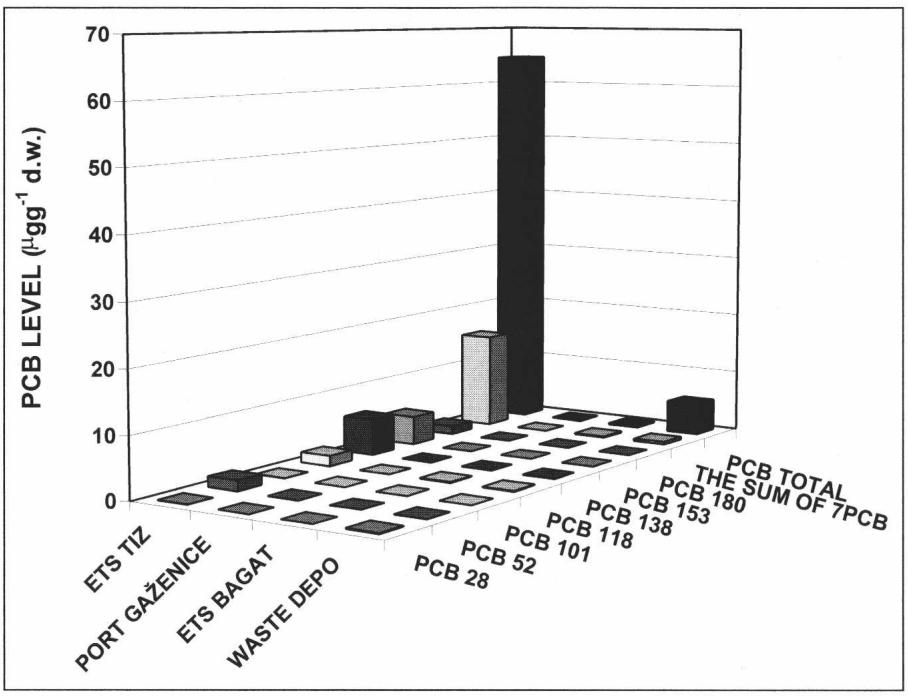

Figure 2. Comparison the levels of the total PCBs, the sum of the 7 key PCB congeners and individual 7 PCB congeners in soil samples collected from various sub areas of the Zadar

In order to contaminate underground water, a contaminant must penetrate from the surface to the ground water level. Potential contamination very much depends on the sorption processes in the aeration zone, porosity and the depth of the ground water.

If contamination occurs on covered karst (karstic fields in general), the possibility of contaminant penetration to underground water is low or even insignificant (depending on the contaminant characteristics and cover layer of soil, i.e. controlled sorption processes). Nevertheless, depending on the terrain situation, lateral migration of contaminants to superficial water streams is not excluded. For that reason, investigation of the distribution of PCBs in the coastal marine environment of Zadar was performed. Previous investigation showed that sediment and fish samples from the Zadar Marina had the highest PCB

contamination in the small bay of Zadar. During 2003 and 2004, the distribution of PCBs in Marina sediment samples and coastal water sediment off the city of Zadar was investigated (Table 2).

Table 2. Polychlorinated biphenyl content in sediment samples from Vruljica Creek, Marina and from other stations in coastal water of Zadar.

Sampling station	Sampling year	Depth (cm)	No. of samples	PCB total range (ng g^{-1} d.w.)	PCB total (Geom. mean)
Vruljica Creek	1997	0 – 2	5	141 - 524	306
	2003	0 – 2	2	21 - 34	27
Marina	2003	0 – 2	5	158 - 1622	663
	2003	2 – 21	4	35 - 2172	503
Coastal water	1997	0 – 2	4	30 - 2203	184
	2004	0 – 2	22	53 - 4050	170
	2004	2 – 5	9	53 - 8815	343

Estimation of bio-TEQ for each sample was based on the calculation of the amount of sample needed to produce a response equivalent to EC_{25} of PCB 126 (Alcock 1998).

All the investigated samples demonstrated high EROD-inducing potency, with bio-TEQ values between 302.46 pgg^{-1} and 44476.19 pgg^{-1}. These results demonstrated good correlation with the GC/ECD analyses, which also revealed high concentrations of PCBs in the samples (Table 3).

Table 3. Correlation between levels of PCBs (sum of 7 key congeners, μg g^{-1}) with TEQ (pg g^{-1}) in the some sediment samples of Marina, and in some soil samples around ETS Zadar and TIZ.

Sampling station	Date of sampling	Sign	Σ7 PCB cong. (μg g^{-1})	TEQ (pg g^{-1})
Marina (sediment)	28.02.2003.	ZDMA53	0.35	4304.15
	28.02.2003.	ZDMA54	0.72	1334.29
	28.02.2003.	ZDMA55	0.21	2157.04
	28.02.2003.	ZDMA56	0.22	1667.86
ETS Zadar (soil)	29.05.2002.	ZD26	15.69	3003.22
	29.05.2002.	ZD28	0.65	532.50
	27.02.2003.	ZD44	4.58	302.46
	27.02.2003.	ZD45	232.50	44476.19
	17.06.2004.	ZDTS1	17.80	2122.73
	17.06.2004.	ZDTS6	208.81	11253.01
TIZ (soil)	29.02.2004.	TIZ2	180.24	7412.70

According to Engwall et al. (1996), background levels in sediment samples from the reference lake in the river system upstream from the site of PCB contamination were about 3 pg bioTEQ/g dry weight, while EC_{25} was about 200 μgg^{-1} dry weights. TCDD, which was used as a reference compound in that study, has 10 times higher potency than PCB 126 in primary rat hepatocytes (Zeiger et al. 2001). Even when this difference in potencies is taken into account, bio-TEQ values obtained for the investigated samples from Zadar are 10–1500 times higher than the background bio-TEQ levels.

On the basis of the present results, it is possible to draw the following conclusions: Four out of five grab samples in the Jazine and Marina harbor area indicate that the PCB content increases with depth. Sedimentation rates in natural marine environments are quite low but in harbor areas with sewage pumping the sedimentation rates can be quite high (several mm/year). Therefore, the top 2 cm could cover the last ten years and the deeper parts (2–10 cm) could cover the time period of warfare damages 12 years ago. Whereas the soil around the destroyed electrical transformer station is obviously contaminated by PCB-containing oils, the most likely sources in the harbor sediment are either PCB-containing ship paints or PCB-containing wastes transported via the sewage system into the harbor basin. Understandably, the increased PCB levels in the Zadar area pose the question of the source of these contaminants. Two locations are in question. The first and perhaps less significant is the Brodanovo location. Is the explicitly high PCB level in a sediment sample mere coincidence or is there a significant source of PCB? Moreover, mussel and fish samples also had increased PCB levels. The second location, the Marina and Vruljica Creek, where increased levels of PCBs were observed in almost all the analyzed samples, is more important.
The bio-TEQ values obtained for the investigated samples from Zadar are 10–1500 times higher than the background bio-TEQ levels. There is no doubt of the existence of PCB contamination source(s) at this position. Is there soil contamination with polychlorinated biphenyls in the area of the 110/35 kV Zadar Electrical Transformer Station where 24 condenser batteries containing PCBs were damaged in warfare? This question requires a definite answer.

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