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Degradation of Polychlorinated Biphenyls in the Rhizosphere of Rape, *Brassica napus* L.

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Abstract The objective of this study was to investigate the rhizosphere effect of rape plants on polychlorinated biphenyls (PCB) dissipation in soils spiked with seven indicator congeners. Depletion of PCB in the rhizosphere was significantly higher in the soil with lower organic matter content. While in the Chernozem soil, 87% of PCB related to bulk soil were found in the 1st mm from roots, only 62%–69% were found in the Fluvisol soil with no significant influence of increased initial PCB concentration. Further from the roots, the concentration of lower chlorinated congeners decreased, which indicates their greater biodegradation in comparison with more chlorinated ones.

Keywords PCB congeners · Soil · Rhizosphere · Phytoremediation

Polychlorinated biphenyls (PCB) have been produced worldwide due to their suitable physical and chemical properties and have been used in hundreds of commercial and industrial applications. Nevertheless, these accumulate in the environment and food chain making PCB a great environmental concern (Baird 2003). Phytoremediation, the use of green plants to remove pollutants from the environment or rendering them harmless, is a promising and environmentally friendly and less expensive approach of soil decontamination (Cunningham et al. 1996). Plants are able to directly extract and remove many persistent organic pollutants from soil but also produce root exudates (amino acids, simple sugars, flavonones, phenolic compounds, enzymes and other organic materials), which enhance microbial and biochemical activities in the immediate vicinity of the roots (rhizosphere) causing favorable environment for more intensive metabolism of contaminants than in the surrounding bulk soil (Hoagland and Williams 1985). It was found that plant (e.g., species, age of the plant, rooting density) and site (e.g., physical and chemical parameters of soil, climatic conditions, nutrient availability) factors are critical ones influencing the development of microbial community and the metabolic pathways in the rhizosphere (Siciliano and Greer 2000). More rapid degradation of several xenobiotics in the rhizosphere of plant species varieties related to bulk soil was revealed (Liste and Alexander 2000; Chekol et al. 2004). However, the fate of PCB in rhizosphere has not much been studied so far. Special chambers—rhizoboxes proposed by Wenzel et al. (2001) allow to separate rhizosphere soil compartment from adjacent plant roots and to slice it into thin layers. Hence a novel design of a rhizobox system overcomes most of the problems related to the separation rhizosphere soil from adjacent roots using conventional techniques such as brushing, shaking and drying. This unique equipment thus enables deeper investigation of contaminant biodegradation in dependence on the distance from the plant roots.

Materials and Methods

Two subsequent experiments were set up: (1) investigating the effect of soil properties on PCB degradation and (2) monitoring of PCB degradation in soil with increased initial PCB concentration. Soil samples with different physico-chemical properties and particle-size distribution

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classified as Chernozem (CH) and Fluvisol (FL) were used for the first experiment (Table 1). Soils were spiked prior to experiment with a precisely defined mixture of seven indicator PCB congeners (IUPAC No. 28, 52, 101, 118, 138, 153, 180; 200 μg of each PCB congener per 1 kg of soil) purchased from Analytika Ltd. (Prague, Czech Republic) dissolved in petroleum benzene. These soils were marked as CH-200 and FL-200A.

The experiment was carried out with oil seed rape (*Brassica napus* L.) grown for 75 days in rhizoboxes placed in an experimental greenhouse. Rape plants produce a well established root system which is substantial for rhizobox experiments and contain lipids in their tissues which have been reported to enhance the uptake potential of chlorinated compounds (Kipopoulou et al. 1999). Ten seeds were introduced into soil–plant compartment with an open slit at the bottom and then placed into the greenhouse. After 15 days of pre-growing, seedlings were tinned to five plants per box and soil–plant compartment was transferred on top of a rhizosphere soil compartment where plant roots are separated by a membrane. Plants were continuously watered using irrigation wicks filled with fiber glass and soil moisture was checked regularly. At the end of the experiment, soil rhizosphere compartment was separated from the membrane and cut under room temperature into six layers (each 1 mm thick) from the side adjacent to rape roots.

In the second step, only the FL was chosen for monitoring PCB degradation in the experiment with increased initial PCB concentrations since it was found to be more suitable for this experiment. Soil was spiked with three different concentrations of individual PCB congeners per 1 kg of soil: 200 (FL-200B), 400 (FL-400) and 600 (FL-600) μg and the experiment was carried out following the same pattern.

After harvest, plants were divided into shoots and roots and washed with deionized water in order to remove all soil particles. Samples were cut to small pieces, homogenized and subsequently stored in a freezer before analytical processing. Soil samples from each layer and bulk soil were also collected and frozen after homogenization.

Table 1 Agrochemical characteristics of the studied soils

Soil type	Particle-size distribution (%)			CEC (cmol kg^{-1})	pH_{KCl}	SOM (%)
	Clay (<0.002 mm)	Loam (0.002–0.02 mm)	Sand (0.02–2 mm)			
CH	31	30	39	21.4	7.1	2.2
FL	10	5	85	6.30	5.5	1.0

CH, Chernozem; FL, Fluvisol; CEC, cation exchange capacity; SOM, soil organic matter

Congener-specific analyses of both soil and plant samples were performed using the modified EPA 1668 method (US EPA 1999). The extractions of both soil and plant samples were done with the pressurized microwave extraction system Milestone Ethos 1 (Milestone, USA). Five grams of samples were weighed into the vessels and 20 mL of acetone/n-hexane mixture 3/7 (v/v) was added to each vessel. The Milestone Extraction Application Note EX-EN-10 temperature program was used. Five milliliter of the extract were taken from cooled vessels into test tubes equipped with ground glass stoppers, mixed with 5 mL water, shaked and allowed to stand for 5 min. The upper hexane layer was sampled for analysis either directly (soil extracts) or after shaking with equal volume of concentrated sulphuric acid (plant extracts). The determination of the seven investigated PCB congeners was carried out using gas chromatography coupled with mass spectrometry detection (GC/MSD, HP 6890N/5975, Agilent Technologies, USA). Detailed description of procedure is presented in Javorská et al. (2009).

One-way analysis of variance (ANOVA) with further Duncan test was performed for the evaluation of the data (Statistica 7.0, StatSoft). The results were evaluated on the basis of homogeneous groups at the level of significance $p < 0.05$.

Results and Discussion

Results showed that neither rape roots nor shoots mass of all treatments were affected by PCB contamination (Table 2) and that plants did not show any stress-related symptoms. These results contrast the study performed by several authors studying plant growing on PCB contaminated soils (Weber and Mrozek 1979; Chekol et al. 2004; Zeeb et al. 2006). Greater sensitivity to PCB that resulted in significantly biomass reduction and toxic exposure of some plants to PCB could be mainly attributed to the relatively higher initial PCB concentration levels in that studies compared to our.

Rape plants exhibited several fold higher concentrations of the sum of PCB congeners (PCB_7) in roots than in shoots (Table 2). It confirms the result of several authors demonstrating that root uptake and translocation of PCB to aboveground biomass of plants is low (Pier et al. 2002; Zeeb et al. 2006; Javorská et al. 2007). Higher concentrations of PCB_7 in roots were observed in rape grown on FL than on CH indicating that bioavailability of the pollutant depends on soil physico-chemical properties (Collins et al. 2006; Javorská et al. 2007). Amount of PCB_7 in both shoots and roots linearly increased with increasing initial PCB concentrations. The presence of PCB_7 in control plants grown on uncontaminated soils could be associated

Table 2 Plant biomass and PCB accumulation

Soil type	Treatment[a]	Mass (g)		PCB_7 concentration (μg kg^{-1} biomass DW)		BAF	
		Roots	Shoots	Roots	Shoots	Roots	Shoots
CH	0	0.46a	4.0a	56.3x	ND	<d.l.	<d.l.
	200	0.55a	4.9a	10,091x	74.4x	7.32x	0.054x
FL A	0	0.53a	4.9a	78.2x	ND	<d.l.	<d.l.
	200	0.48a	4.6a	22,076y	104.5x	16.5y	0.081x
FL B	0	0.49a	4.8a	92.8x	ND	<d.l.	<d.l.
	200	0.51a	5.3a	21,567a	97.3a	17.3a	0.074a
	400	0.58a	5.2a	36,415b	141a,b	15.9a,b	0.062a,b
	600	0.56a	4.3a	50,259c	185b	15.6a	0.058b

[a] Microgram of each PCB congener per kg of studied soil; CH, Chernozem; FL, Fluvisol; PCB_7, the sum of the seven indicator PCB congeners; BAF, bioaccumulation factor; ND, not determined; <d.l., bellow detection limit; data marked with the same letter did not significantly differ at $\alpha = 0.05$

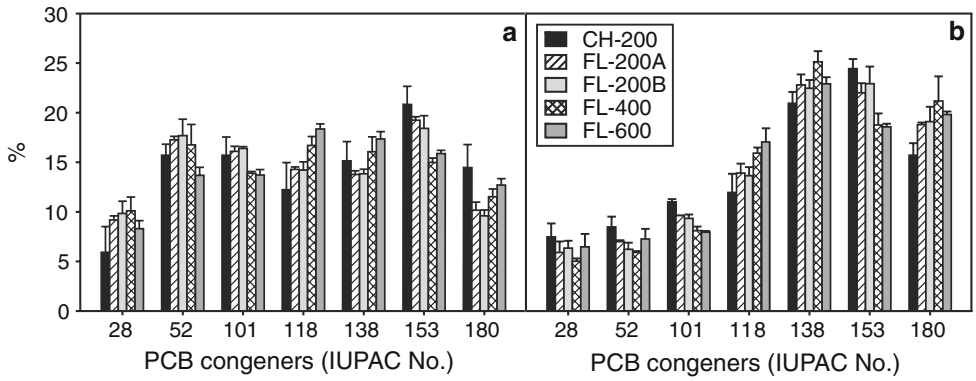

Fig. 1 Concentration of individual PCB congeners in roots (**a**) and shoots (**b**) of rape plant grown on studied soils

with their threshold value in soils. PCB_7 detected in shoots are bellow the detection limit and thus the secondary contamination by PCB deposition volatilized from soil is unlikely. The bioaccumulation factors (BAF) calculated as a ratio of PCB concentrations in plant tissue and soil ($BAF = PCB_{shoot\ or\ root}/PCB_{soil}$) also confirmed that root parts significantly contribute to the total PCB accumulation within the plants (Table 2). BAF were found to be dependent on the exposure level: BAF decreased with increasing initial PCB concentration. This suggests that at higher PCB exposure levels their accumulation may be kinetically limited by redistribution processes within the plant (Tolls and McLachlan 1994). Also higher BAF was found in rapes growing on FL which is in accordance with higher root PCB concentration in these soils compare to CH.

Individual PCB congeners were generally distributed equally in rape roots but shoots showed a higher proportion of highly chlorinated biphenyls (HCB) (Fig. 1). Our findings indicating that also HCB have an ability to be transported from roots to shoots. The hypotheses that plants are able to transport more lipophilic chemicals may be due to the release of crop-specific root exudates with mobilizing properties making these compounds more available for plant uptake and translocation as was demonstrated for PCDD/Fs (Hulster et al. 1994). In our experiment no differences in the distribution of individual PCB congeners were found between both soil types and contamination levels.

A strong linear depletion of PCB_7 with statistical differences almost between each soil layer related to bulk soil was found in FL (Fig. 2a). The PCB concentration decrease in CH was insignificant, with the only statistical differences found between the 1st and the 6th mm. This implicates that physico-chemical properties, mainly soil organic matter (SOM) content, are the more important factors limiting availability and mobility of persistent organic pollutants in soil. Similarly, Joner and Leyval (2003) documented on PAH contaminated soils with higher SOM content low initial PAH dissipation rates with small positive effects of plants. There were no significant differences among initial PCB contents in FL (Fig. 2b). The concentration in the 1st mm was 1.4- to 1.5-fold lower than in the 6th mm. It was confirmed that rhizosphere effects are likely to decrease with increasing distance from the root surface. The positive effect of plant on the rate of PCB degradation was also documented in the study investigating plant growth on

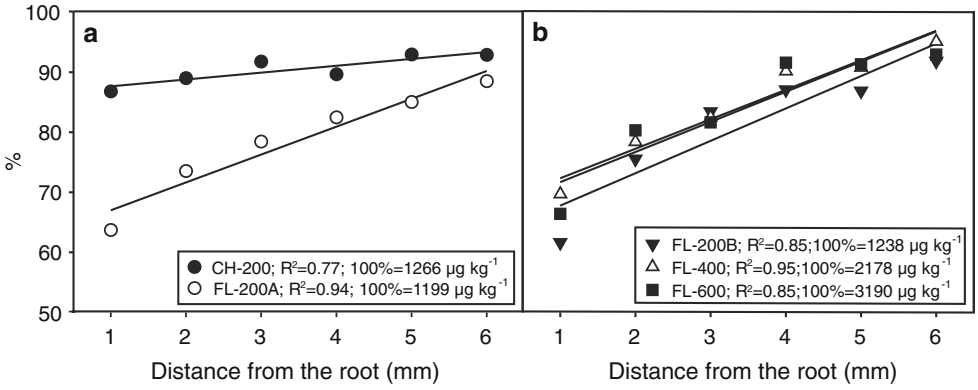

Fig. 2 The relative concentration of PCB_7 in the rhizosphere layers related to the bulk soil after rape harvest in the experiment with different soils (**a**) and in the experiment with increased initial PCB concentration (**b**)

long-term contaminated soils. The highest decline of PCB content was observed in the rhizosphere of tobacco plant, where 66% of initial concentration was found in comparison with bulk (non-vegetated) control soil (Ryšlavá et al. 2003).

Figure 3 shows the distribution of individual PCB congeners in each rhizosphere layer and in bulk soil. While the concentration of PCB congeners was the lowest and almost constant in the 1st mm, further from the roots, mainly lower congeners showed a decrease in concentration indicating their greater biodegradation in comparison with more chlorinated ones. In some cases, increased concentration mainly HCB in the further layers of the rhizosphere soil than in the bulk soil was also documented. Similarly, Liste and Alexander (2000) found increased concentration of PAH in rhizosphere of some plants than in unplanted soils. This could be regarded to the accumulation of such hydrophobic compounds in the rhizosphere due to the mass flow of dissolved chemicals toward the roots induced by plant water uptake (Clothier and Green 1997). Since HCB are more hydrophobic and thus have greater sorption ability to SOM, this mechanism could prevail over the degradation process in the layers further of the roots.

Table 3 shows a percentual balance of PCB_7 related (1) to the residual contents of PCB in rhizosphere/bulk soil; (2)

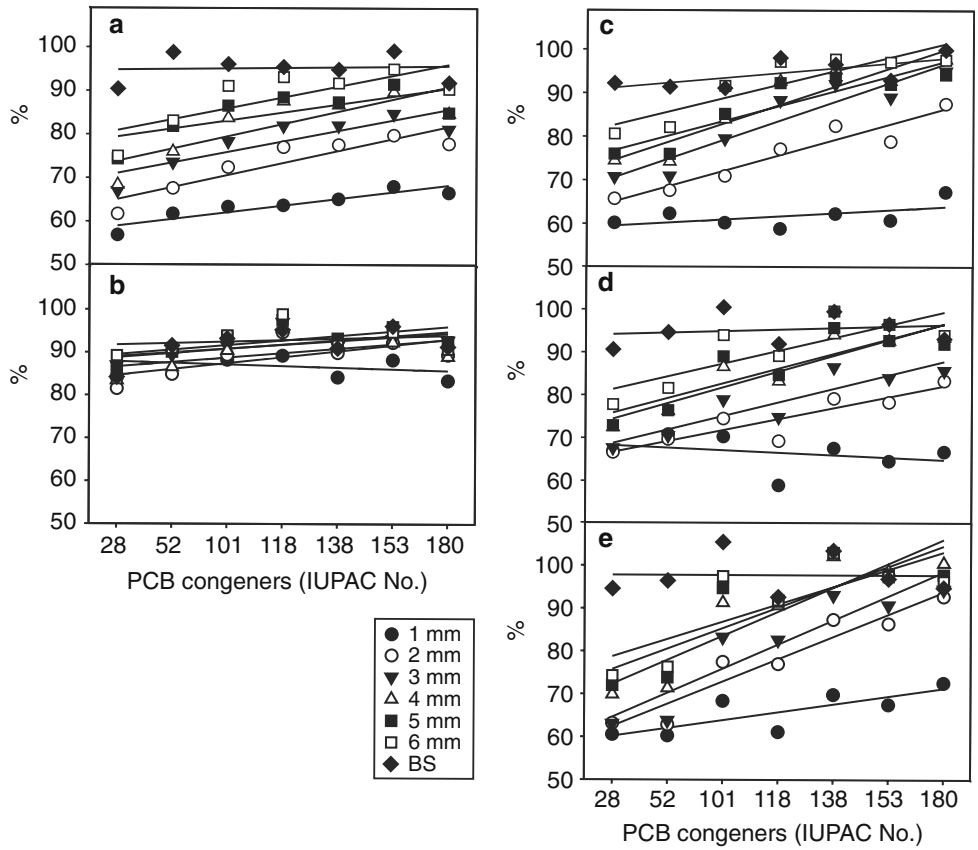

Fig. 3 Relative distributions of individual PCB congeners in rhizosphere layers and in bulk soil (BS) of FL-200A (**a**), CH-200 (**b**), FL-200B (**c**), FL-400 (**d**) and FL-600 (**e**)

Table 3 Simple balance of PCB_7 (%) related to rhizosphere (RS) and bulk soil (BS)

Distribution of PCB_7	Treatment									
	CH-200		FL-200A		FL-200B		FL-400		FL-600	
	RS	BS	RS	BS	RS	BS	RS	BS	RS	BS
PCB_7 in soils	90.2	91.9	77.3	95.4	79.3	94.7	77.9	95.0	80.9	99.1
PCB_7 degradation/fixation	6.0	8.1	15.1	4.6	12.0	5.3	12.3	5.0	10.0	0.9
PCB_7 in plants	3.8	–	7.6	–	8.7	–	9.8	–	9.1	–

100% = amount of PCB_7 (μg kg^{-1}) found after PCB spiking: CH-200 = 1,378, FL-200A = 1,257, FL-200B = 1,307, FL-400 = 2,293, FL-400 = 3,218

to the uptake by rape plants and (3) to the degradation/fixation in the rhizosphere/bulk soil counted from the spiked amount of PCB before experiment establishment. Results showed that rape exudates and microbial processes involved have a positive effect on reduction of PCB concentration mainly in the rhizosphere of FL. In the experiment conducted by Chekol et al. (2004) 38% or less of the initial PCB concentration was recovered from plots planted with grasses (reed canarygrass and switchgrass). However, this experiment was established for a longer period (4 months). Since there were not found any promotive effects of plants on PCB degradation in CH soils, the degradation process in FL was significantly slower in the bulk soil and relatively decreased with increasing contamination levels. Our results indicated a significant influence of soil characteristics on degradation and plant uptake of PCB from contaminated soils.

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