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Original Research

Distribution of Polychlorinated Biphenyl Congeners in Root Vegetables

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Abstract

A concentration of seven indicator polychlorinated biphenyl (PCB) congeners (IUPAC No. 28, 52, 101, 118, 138, 153, and 180) in root vegetables was investigated. Plants were grown on equally spiked soils with different physico-chemical properties. The concentration of PCB in all screened plants was higher in roots than in shoots, with a 1.5-3.9-fold higher content in vegetables grown on the Fluvisol compared to the Chernozem. Parsley was more efficient in extracting PCB from soils, followed by carrot and red beet. The majority of PCB accumulated in peels and ranged between 75.3% (red beet) to 93.6% (carrot), regardless of soil type. Lower chlorinated biphenyls were more abundant in surface root layers (peel, cortex). The core of all screened plants showed an almost even distribution of congeners with a higher abundance of hexachlorinated biphenyls.

Keywords: PCB congeners, bioconcentration, carrot, parsley, red beet

Introduction

Polychlorinated biphenyls (PCB) belong to a class of synthetic chemicals with pronounced persistence against chemical/biological degradation, environmental mobility, tendency for bioaccumulation in human and animal tissues, and significant impacts on human health and the environment, even at extremely low concentrations [1]. Despite the ban on production and use of PCB many years ago, they still remain ubiquitous among all environmental compartments [2, 3]. The hydrophobicity of PCBs has resulted in their rapid accumulation in sediments and soils; they also can be present in organic fertilizers, such as compost derived from rough materials of origin (sewage sludge, organic fraction of municipal solid wastes, etc.). Thus, contamination of agricultural fields and accumulation in crops can substantially contribute to human exposure through food consumption [4-7]. Root is the most important pathway for PCB entry from soil and therefore root vegetables have to be estimated as the worst case candidates for PCB uptake from soils [8]. It has been widely reported that hydrophobic compounds such as PCBs can enter plant tissue principally through the volatilization from the soil rather than translocation from root to shoot [9-14]. While several studies have demonstrated for various plant species that roots are means of plant contamination by PCB [15-20]. It is apparent that routes of PCB contamination in plants remain a contradictory phenomenon and thus still represent a research area that should receive more attention. The majority of studies investigated plant uptake potential for PCB from commercial mixtures that might obscure potential correlation between physico-chemical properties of individual PCB congeners and their observed concentration in plant tissues. Therefore, the present study was designed to overcome these limitations through quantification of individual congeners within their uptake and translocation in plants. Seven indicator congeners (IUPAC No. 28, 52, 101, 118, 138, 153, and 180) have been chosen on the basis of their persistence in the food chain and their tendency to bioaccumulate; they have also been predominantly present in most PCB-mixtures and in environmental samples [21].

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The aim of the study was to assess concentration of PCBs in total and congener profiles of seven indicator PCB congeners in root vegetables (carrot, parsley, and red beet) to simultaneously evaluate the effect of soil characteristics on PCB uptake by the studied plants and to determine the concentration gradient in root compartments (peel, cortex, and core).

Materials and Methods

Soil Characteristics, Spiking and Cultivation

Two different agricultural soils were used for a pot experiment established in an outdoor weather-controlled hall: (i) Chernozem (CH) containing 36% clay, 28% loam, and 36% sand with pH 7.4 and organic carbon content (C_{ox}) of 1.26%, and (ii) Fluvisol (F) containing 12% clay, 7% loam, and 81% sand with pH 4.5 and C_{ox} of 0.57%. Five kg of soil (per pot) was fertilized (0.75 g N, 0.16 g P, and 0.4 g K per pot) and spiked with seven indicator PCB congeners (IUPAC No. 28, 52, 101, 118, 138, 153, 180; 100 μg of each PCB congener per 1 kg of soil) purchased from Analytika, Ltd. (Czech Republic) dissolved in petroleum benzene.

The experiment was carried out with carrots (*Daucus carota* L.), parsley (*Petroselinum crispum* (Mill.) A. W. Hill), and red beet (*Beta vulgaris* L. var conditiva). Three replicates were set up per each treatment. After germination, ten seedlings of carrot and parsley and five seedlings of red beet were kept in each pot. Plants were manually watered with deionized water every day and after three months they were harvested. After harvest, shoots were separated from roots and all plant parts were washed with deionized water to remove traces of soil. One half of the harvested roots were subsequently divided into peel (peeled with a kitchen peeler; layer app. 1 mm thick), cortex and core for carrot and parsley roots or split only in peel and core for red beet; the other part was sampled intact. All plant samples were cut, homogenized, and air dried. Individual soil samples were collected from each pot after harvest and also thoroughly homogenized and air dried prior to analyses.

Preparation of Plant and Soil Samples

Congener-specific analyses of samples were performed using the modified EPA 1668 method briefly described below [22].

Approximately 5 g of a finely ground plant sample was weighed and 50 mL of n-hexane (Merck, Germany) was added. Samples were sonicated for 30 min and subsequently shaken for 1 h. The extract was transferred into a 100 mL volumetric flask and cleaned with 10 mL of concentrated sulphuric acid. Twenty mL of clear extract was evaporated in a gentle nitrogen stream to near dryness, filled up to 1 mL with n-hexane and analyzed.

An amount of 15 g of soil subsample was weighed and mixed with 20 mL of n-hexane. Samples were sonicated for 15 min and shaken for 1 h. After settling, a part of the hexane extract was cleaned with concentrated sulphuric acid (Chromservis, Czech Republic). After the phase separation, the part of the upper phase was transferred to a GC-vial for further analysis.

PCB Analyses

Analyses were performed with an Agilent Technologies gas chromatograph equipped with a mass spectrometry detector (GC/MSD; 6890N/5975). A HP5-MSI capillary column (J&W Scientific, USA) was used for separation (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The injector temperature was kept at 250ºC with the injection volume of 1.0 μL in the splitless mode. The oven temperature was maintained at 70ºC for 2 min, than increased to 150ºC at the rate of 25ºC min^{-1}, to 200ºC at 3ºC min^{-1}, to 270ºC at 8ºC min^{-1}, to 290ºC at 25ºC min^{-1}, and held for 5 min at 290ºC. Data acquisition was carried out in SIM mode with two characteristic ions. Quantification was performed using the external standard technique. The calibration was performed by means of linear regression analysis on standard mixtures Mix 3 (Dr. Ehrenstorfer, Germany) and Mix 2 (Restek, USA) at the beginning of each run. The recoveries of analytes were determined using the Surrogate Standard Mix 9 (Tetrachloro-m-xylene and Decachlorobiphenyl; Dr. Ehrenstorfer, Germany). The accuracy of analyses was verified by certified reference material METRANAL™2 (river sediment, Analytika, Ltd., Czech Republic).

Statistical Analyses

One-way analysis of variance (ANOVA) with consequent 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 of $p<0.05$.

Results and Discussion

The concentration of PCB in total and congener profiles of seven indicator congeners in root vegetables (carrot, parsley, and red beet) in dependence on soil characteristics was investigated. There were no visible differences among any of the studied vegetable plants, neither in root nor in aboveground biomass between contaminated and control plants. These results confirmed our previous findings that soil spiked with 0.7 mg PCB_7 kg^{-1} (PCB_7 – the sum of seven indicator congeners) does not lead to significant biomass reduction or to toxicity symptoms [23]. However, the yield of plants grown on Fluvisol (F) was restricted 1.5-1.6-fold in carrot, 3.3-4.9-fold in parsley, and even 20.1-25.2-fold in red beet compared to Chernozem (CH). Soil F had a poor fertility and high sand content, leading to an insufficient water regime. These factors probably subsequently contributed to the fungi development and plant stress resulting in restriction of plant growth.

Soil samples taken before planting and after harvest revealed a significant decrease of PCB_7 concentration. Initial concentration in soil CH and F measured three days after spiking was 643 μg kg^{-1} and 616 μg kg^{-1}, respectively. There were no statistically significant differences in PCB_7 concentration after planting in terms of soil type. The losses in PCB_7 contents in soil CH and F were about 40.6% and 35.3% for carrot, 39.5% and 38.2% for parsley, and 47.2% and 47.5% for red beet compared to the spiked amount (Fig. 1). Immediately after spiking, the distribution of individual PCB congeners was steady while at the end of the experiment the concentration dropped with a decreasing number of Cl atoms in biphenyl (Fig. 1), which is in accordance with our previous results [24]. In general, highly chlorinated congeners have a greater affinity for soil organic matter and are thus more persistent in the environment than lower ones that are more likely to be degraded or have a stronger tendency to volatilize [25, 26].

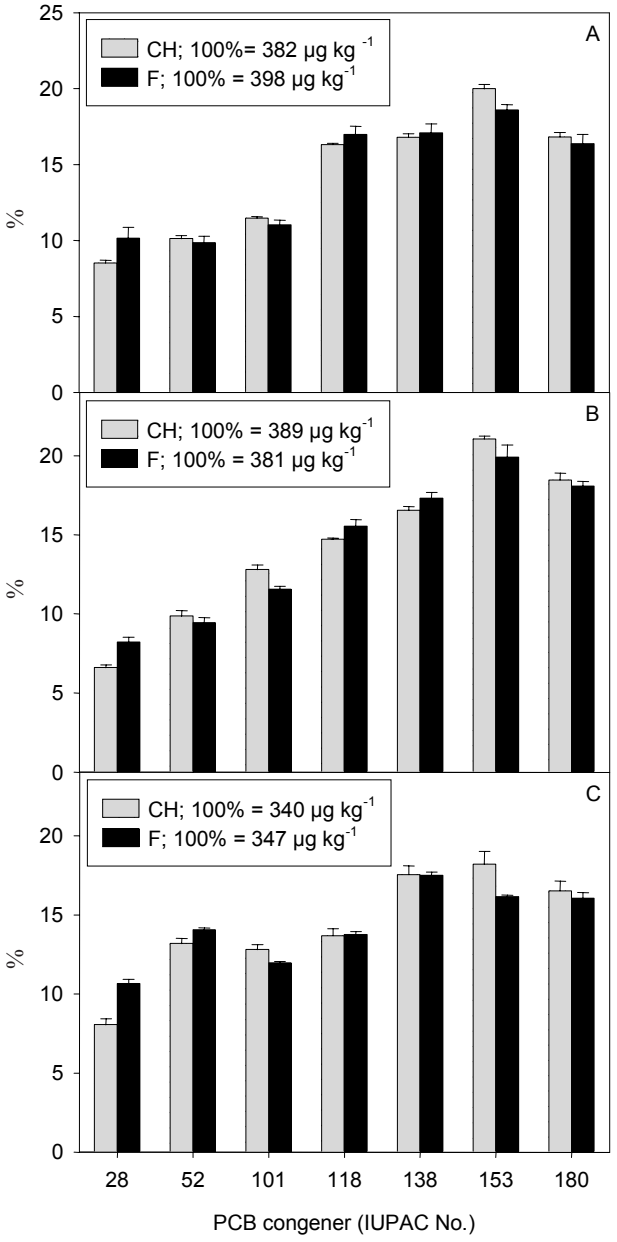

Fig. 1. Relative concentrations of individual PCB congeners (100%=PCB_7) in soil samples after harvest of (A) carrot, (B) parsley, and (C) red beet.

Concentrations of PCB_7 in all screened plant tissues was greater in the roots than in the shoots with a higher PCB_7 content in vegetables grown on soil F (Table 1). These findings correspond to our previous results [23, 24] and showed that soil physico-chemical characteristics plays a dominant role in the level of organic pollutant accumulation in plant biomass. The highest root concentration was found in parsley, followed by carrot and red beet. However, the difference between parsley and carrot was not statistically significant. Several authors indicated that lipid and carotene contents in plant tissues are main factors determining the retention of liphophilic compounds and their accumulation [27-29]. According to Kopec [30], parsley, carrot, and red beet have lipid and carotene contents of about 6.005, 3.035, and 1.002 g kg^{-1}, respectively. However, it should also be noted that lipid quality or other plant components such as lignin, suberin, or waxes may also absorb organic chemicals; they might thus be further responsible for the differences in uptake of liphophilic chemicals [13, 31].

It was suggested that octanol-water partition coefficient (K_{ow}) can predict the interaction between plants and organic chemicals [32]. According to Hawker and Connell [33], the log K_{ow} of these seven indicator congeners ranges between 5.67 and 7.36. Many works demonstrated that hydrophobic chemicals (log K_{ow}>3.5) are not easily transported within plants [32, 34]. In contrast, some studies have reported that uptake of chemicals with a log K_{ow}>5 and their translocation to aboveground biomass is possible [14, 19]. Our results proved that transport of PCB from soil to aboveground biomass of root vegetables is a possible route for shoot contamination [9, 17, 19, 23, 24]. In our study it was confirmed by the fact that PCB_7 content in the control plants was bellow the detection limit and thus secondary contamination by the deposition of PCB volatilized from soil was unlikely. It is evident that compound accumulation is a complex result of chemical and biological processes and K_{ow} itself is not sufficient to predict biological behaviour [35].

However, as mentioned before, all treatments indicated several times lower PCB_7 content in shoot biomass compared to root biomass, which is well documented by the translocation factor (TRF) values – calculated as the ratio of PCB_7 concentration in shoot to PCB_7 concentration in roots ($[PCB_{shoot}]/[PCB_{root}]$) (Table 1). All screened species had a higher TRF at soil CH, indicating that the higher mobility of PCBs in the soil and higher concentration in roots do not necessarily increase the mobility of PCBs within the plant. A study made by Zeeb et al. [19] investigated the phytoremediation potential of squash, sedge, grasses, and legumes grown on three different weathered soils contaminated with Aroclor 1260. Most of the plants grown on soils with contamination levels ranging from 90 to 4,200 mg kg^{-1} showed a poor translocation of PCB to the shoots (TRF<0.05).

Table 1. Total PCB_7 concentration, translocation factor (TRF), and total PCB_7 amount taken up by roots and shoots of the studied plants.

Plant species	Soil Type	μg PCB_7 kg^{-1}		TRF	PCB_7 extracted (μg)*	
		Roots	Shoots		Roots	Shoots
Carrot	CH	$1,257^{aA}$	250^{aA}	0.24^{aA}	65.6^{aA}	2.67^{aA}
	F	$1,859^{bA}$	215^{aA}	0.11^{bA}	68.8^{aA}	2.06^{bA}
Parsley	CH	$1,312^{aA}$	120^{aB}	0.09^{aA}	37.9^{aB}	2.91^{aA}
	F	$2,154^{bB}$	114^{aB}	0.06^{aB}	19.6^{bB}	0.91^{bB}
Red beet	CH	319^{aB}	133^{aB}	0.44^{aC}	14.5^{aC}	1.84^{aB}
	F	$1,245^{bC}$	279^{bC}	0.22^{bC}	2.87^{bB}	1.36^{bC}

*per pot; data marked with the same letter did not significantly differ at α=0.05; lower case – relationship between soil types within each plant species; capital letter – relationship among plant species within each soil.

TRF is not sufficient to express total PCB uptake, and their further translocation by plants because biomass yields of individual plants have to be taken into account. Therefore, plant dry weights and PCB concentrations were converted to total PCB amount determined in roots and shoots (PCB extracted, Table 1). Roots accounted for 89-97% from the total extracted amount, whereas the above-ground biomass was only about 3-11%. By contrast, in the study performed by Zeeb et al. [19], up to 98% of PCB was present in shoots, despite the fact that most calculated TRF values were bellow ours. While the PCB root concentrations of all samples were 1.5-3.9-fold higher on soil F than on soil CH, total PCB amount extracted on that soil was lower (parsley and red beet) or comparable (carrot plants). This was the result of the above mentioned reduced yields of plants grown on soil F. The results indicate that due to both high root uptake and biomass production, carrot is more effective in PCB extraction from soil than other screened plants.

The concentration of PCB_7 in carrot root was 1.4-fold higher compared to our previous results [24]. While concentrations of higher chlorinated isomers (mainly of those with seven, six or five chlorine atoms – No. 180, 153, 138, 118, and 101) were comparable, the concentrations of lower ones (PCB 28 and PCB 52) were much lower in the previous study [24]. The difference was most probably caused by changing sample processing. Our present method using the sample evaporation to near dryness reduced the loss of the most volatile congeners in comparison with the former procedure using sample evaporation to total dryness [36-38].

Congener profiles displayed as a portion of total PCB_7 contents in carrot, parsley, and red beet are presented in Fig. 2. Concentrations tended to decrease from the lowest to the highest chlorinated isomers in the present study. The steepest decrease was found in carrot plants grown on soil F. Muntean et al. [39] investigated long-term contamination by persistent organic pollutants in food samples and found that carrots, onions, and potatoes tended to be contaminated only with the lowest chlorinated isomers (PCB 28 and PCB 52). Similarly, Bobovnikova et al. [40] stated that the mass fraction of lower chlorinated biphenyls with three and four Cl atoms was 85% for carrot roots. However, it is difficult to compare these results with our findings since their used soils were predominantly contaminated with low chlorinated biphenyls (Aroclor 1242) [40], or a congener profile was not determined at all [39].

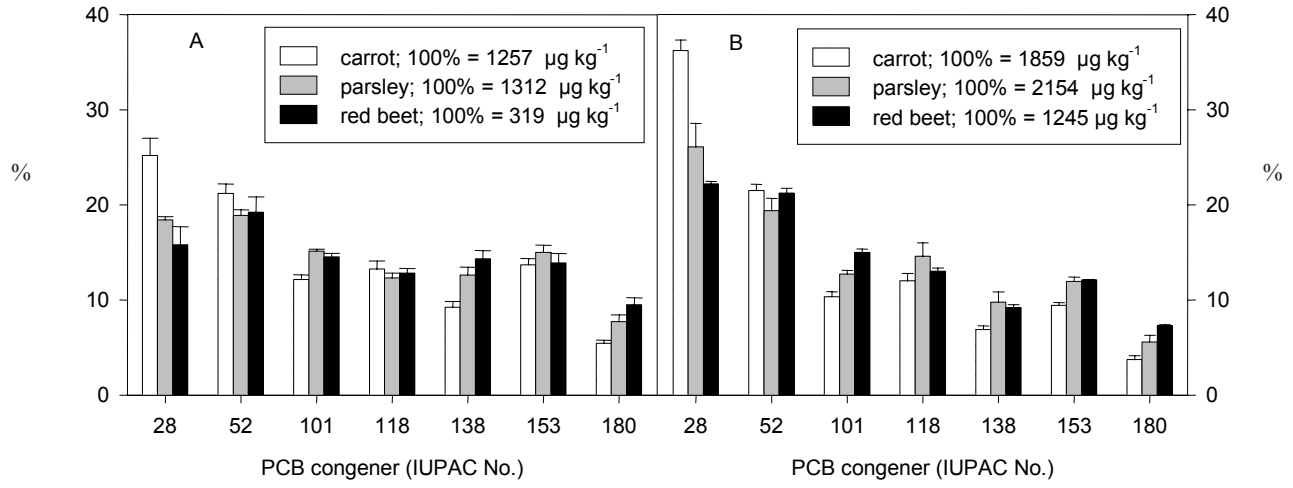

Fig. 2. Distribution of individual PCB congeners (100%=PCB_7) in roots of tested plants grown on (A) Chernozem and (B) Fluvisol.

Table 2. PCB_7 distribution between peel, cortex, and core of studied plant roots grown on different soils.

Plant	Soil type	Root distribution of PCB_7 (%)		
		Peel	Cortex	Core
Carrot	CH	93.0	5.8	1.2
	F	93.6	5.9	0.5
Parsley	CH	88.8	10.1	1.1
	F	83.4	13.3	3.3
Red beet	CH	75.3	-	24.7

Lower chlorinated congeners also contributed significantly to total shoot concentration in carrot plants, whereas in parsley and red beet all congeners were distributed more or less uniformly (data not shown). In many studies investigating PCB uptake, lower chlorinated congeners were more abundant in plant shoots when the soils were contaminated with a technical PCB mixture of lower (Aroclor 1254, Aroclor 1242) or highly chlorinated PCB (Aroclor 1260) [9-11, 19]. Lower chlorinated PCB congeners are more water soluble and thus the translocation to the shoots through the transpiration flow is easier [19]. In contrast, Bush et al. [41] found in chamber experiments that more volatile and less lipophilic 2-chlorobiphenyl and 2,2'-dichlorobiphenyl were released from plant leaves of purple loosestrife. This finding may explain the relatively even isomer distribution without the prevalence of lower chlorinated biphenyls in parsley and red beet shoots. Several researchers have stated that important plant characteristics may influence pollutant concentrations in the shoots, resulting from root uptake and translocation, including the content of lipophilic solids and the plant transpiration flow rate [31, 32, 42].

Our study investigated the horizontal distribution of PCB_7 as well as their congener profile in each root compartment. Table 2 summarizes the percentage ratio of PCB_7 in individual root compartments. Since the red beet grown on soil F had a low biomass yield, the PCB distribution was calculated only for red beet planted on soil CH. The measured concentrations showed that the majority of PCBs were accumulated in peels of all screened plant without any soil type dependency. Our results confirm the findings of previous studies that very lipophilic chemicals diffuse into plant tissue very slowly, so that they are likely to remain in the peel of root vegetables [8, 9, 28, 43]. Waliszewski et al. [44] demonstrated that the peel of carrot root accumulated

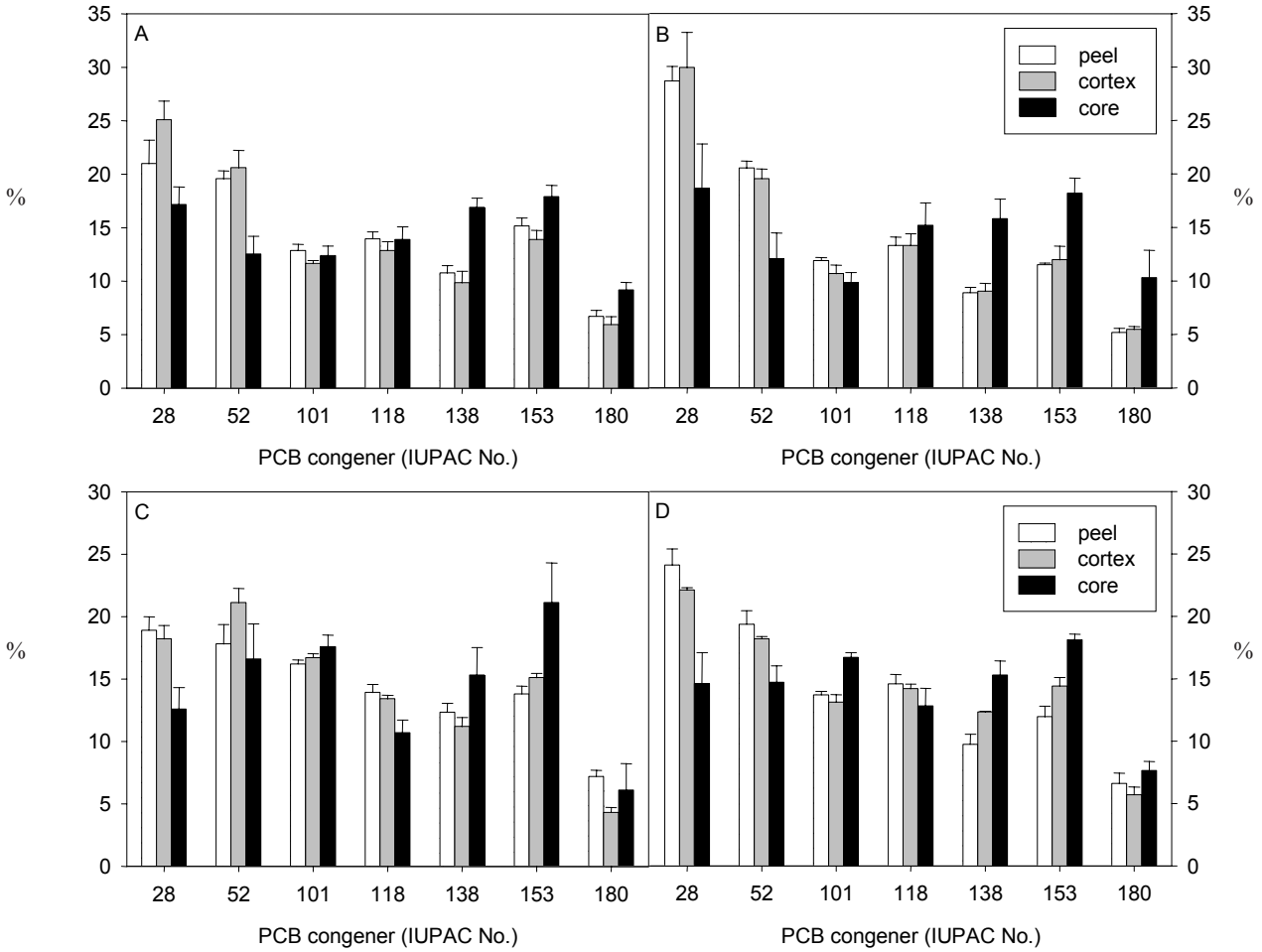

Fig. 3. PCB congener distribution (%) between root compartments of (A) carrot – soil CH, (B) carrot – soil F, (C) parsley – soil CH, and (D) parsley – soil F.

3-7 times more organochlorine pesticides than the root core. Trapp [8] found even 100 times higher concentrations of liphophilic compounds (benzo(a)pyrene, PCB, chlorobenzens) in carrot peels than in the core. Kipopoulou et al. [29] ascribed this to the fact that the peels have higher lipid contents than the core, thus the transfer from the root peel to the core of the root appeared to be minimal. Decreasing lipid gradient concentration from outer to inner parts of carrots was found by Rowston [45], which is a possible explanation for higher PCB concentration in peels.

Concerning the individual congener distribution in root compartments, peel of red beet and surface layers (peel, cortex) of carrot and parsley contaminated more lower chlorinated biphenyls, while the congener distribution in the core of all screened plants was not specific (Fig. 3). Lower molecular weight chemicals, that are more bioavailable in the soil and more likely to be transferred and sorbed onto peel tissue, may also be more able to move into the core and further to the shoots [29]. Another explanation is that plants produce a specific enzyme that might degrade higher PCB congeners to lower ones that can be stored in lipophilic root solids like lipids in membranes and cell walls of surface layers [46, 47]. Interestingly, congeners 138 and 153 with six Cl atoms were relatively more abundant. It is possible that the symmetric structure of congener 153 allows it to more easily be transported further. Congener 138 has two adjacent unsubstituted carbons that might provide an active site for root exudates and thus enable the transport of such hydrophilic complex through the plant more easily. However, these solubilizing effects have only been demonstrated so far for soil contaminated with dioxins [48-50].

Conclusions

1. The PCB_7 concentration in all screened vegetables was higher in the roots than in the shoots, whereas concentration in roots increased in the following order: red beet<carrot<parsley. Our results proved that uptake of PCB from soils and translocation to aboveground biomass of root vegetable is a possible route to shoot contamination.
2. A decrease in the uptake of individual congeners into vegetables with increasing rate of chlorination was observed.
3. While the root PCB_7 concentrations of all plants were several fold higher in the Fluvisol than in the Chernozem, their total PCB concentrations extracted from Fluvisol were lower (parsley and red beet) or comparable (carrot plants). This is the result of reduced yield of plants grown on Fluvisol, indicating that fertility and physico-chemical properties of soils influence PCB extraction.
4. Most of the PCBs in all vegetables grown on the contaminated soils were associated with the peel without any soil type dependency. Therefore, peeling might be a feasible method to reduce PCB levels in vegetables grown on contaminated soils.
5. Surface layers (peel, cortex) contained more lower chlorinated biphenyls, whereas the congener distribution in the core of all screened plants was almost homogenous with tendency to accumulate hexachlorinated biphenyls.

Acknowledgements

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