PLANT UPTAKE OF POLYBROMINATED DIPHENYL ETHERS FROM CONTAMINATED SEWAGE SLUDGE

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in many countries of the world for more than thirty years. They are lipophilic with bioacumulative properties. These compounds are structurally similar to polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dichloro-diphenyl-trichloroethane (DDT), therefore their chemical properties, persistence and distribution in the environment are very similar [1,2]. Despite their low acute toxicity the lower brominated congeners operate as endocrine disruptors, carcinogens and/or neurodevelopment toxicants. The debromination of decabromodiphenylether (BDE 209) to more toxic lower brominated congeners was also reported [3, 4, 5, 6].

Phytoremediation is technology that utilizes green plants and also the associated rhizosphere microorganisms to remove, contain or transform environmental contaminants that are located in soils, sediments, water and also in the air. This in situ method is very popular because it is more cost-effective method than alternative mechanical or chemical methods of removing hazardous compounds from soils, sediments, water and air. Besides, this technology is aesthetically pleasing because the contaminated substrate stays on site and it is planted. But it can last longer than other technologies and it exists possibility of food chain contamination [7, 8, 9]. It is known that plants can withstand relatively high concentration of organic xenobiotics and may stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates and enzymes [10].

The objective of this study was to evaluate PBDEs plant uptake from sewage sludge contaminated with PBDEs. We have chosen *Nicotiana tabacum* and *Solanum nigrum* as the model plants for their known ability to accumulate PCBs, that are structurally similar to PBDEs.

Material and methods

Sewage sludge and control substrate

On the basis of chemical analysis we have chosen sewage sludge sampled in Hradec Králové (it contained all monitored congeners of PBDEs - ∑BDE: c = 700,8 ng/g (2,4,4´-triBDE (BDE 28); 3,4,4´-BDE (BDE 37); 2,2´,4,4´-tetraBDE(BDE 47); 2,2´,4,5´-tetraBDE (BDE 49); 2,3´,4,4´-tetraBDE (BDE 66); 2,2´,3,4,4´-pentaBDE (BDE 85); 2,2´,4,4´,5-pentaBDE (BDE 99); 2,2´,4,4´,6-pentaBDE (BDE 100); 2,2´,4,4´,5,5´-hexaBDE (BDE 153); 2,2,4´,4,5´,6´-hexaBDE (BDE 154); 2,2´,4,4´,5´,6-BDE (BDE 183)); BDE 209 (deca-BDE): c = 2973,1 ng/g). As the control substrate was used substrate for garden plants (AGRO CS a.s., Česká Skalice, CZ)

Planting

The aluminium foil was used to line plastic pots (V = 200 ml) to prevent sorption of PBDEs from sewage sludge on plastic pots. Three different treatments consisting of 5 replicate pots were established. The first 5 replicates were left unplanted, the remaining were planted either with tobacco or with nightshade. Pots were maintained at laboratory temperature and were regularly watered for 3 months. Dry leaves were removed from the plant and stored at -20°C till the end of experiment.

Harvesting and storage of plants and substrates

Plants were harvested after 3 months of growth. They were gently removed from the substrate and separated into roots, stalks, leaves and fruits fractions. Dry fractions from 5 pots were used to calculate average weight of roots, shoots, fruits and then PBDEs were extracted for GC/MS-NCI analysis. The substrates of each treatment were mixed thoroughly and stored at 4°C before microbial analysis. These substrates were also used to ecotoxicological analysis, PBDEs extraction and GC/MS-NCI analysis.

Plant growth

Plant growth was determined on the basis dry weight. The dry biomass of the plant fractions was measured gravimetrically after drying at 50°C for 48-72 h. The plants were also photographed after 1, 2 and 3 months since the beginning of the experiment.

Chemical analysis of the substrates and plant fractions

Chemicals were obtained from the folowing sources: Deca-BDE (99,5%) from Dr. Ehrenstorfer GmbH; following set of standard solutions containing PBDE congeners (concentration 50 µg/ml in nonane): 2,4,4´-triBDE (BDE 28); 3,4,4´-BDE (BDE 37); 2,2´,4,4´-tetraBDE(BDE 47); 2,2´,4,5´-tetraBDE (BDE 49); 2,3´,4,4´-tetraBDE (BDE 66); 2,2´,3,4,4´-pentaBDE (BDE 85); 2,2´,4,4´,5-pentaBDE (BDE 99); 2,2´,4,4´,6-pentaBDE (BDE 100); 2,2´,4,4´,5,5´-hexaBDE (BDE 153); 2,2,4´,4,5´,6´-hexaBDE (BDE 154); 2,2´,4,4´,5´,6-BDE (BDE 183) and deca-BDE (BDE 209) were obtained from Cambridge Isotope Laboratories (CIL, Andover, USA). The organic solvents (cyclohexane, dichloromethane, hexane, ethylacetate and isooctane) declared for "organic trace analyses" grade were all supplied by Merck (Darmstadt, Germany).

Extraction of PBDEs was performed by dichloromethane (sewage sludge) and by mixture hexane: dichloromethane (1:1, v/v, plants) in Soxhlet apparatus. The crude extract was carefully evaporated and then sample was dissolved in solvent mixture cyclohexane-ethylacetate (1:1, v/v) that was used as a mobile phase in gel permeation chromatography (GPC) for separation of interfering co-extracts. Agilent 6890 (Agilent, USA) gas chromatograph equipped with a single quadrupole mass analyser Agilent 5975 XL operated in negative chemical ionization mode (GC/MS-NCI).

Microbial analysis

Enumeration of bacteria in the substrates was carried out by culturing onto Plate Count Agar (PCA; Oxoid). 10 g of the substrate was shaken in the Erlenmeyer flask with 90 ml 1% $Na_4P_2O_7$ (Sigma-Aldrich GmbH) and glass beads (at 28°C for 2 h, 130 RPM). The dilution series were prepared in physiological solution. 100 µl of the sample was plated onto PCA and incubated at 28°C. Each sample of the dilution series was analyzed in 3 replicates. Bacterial counts were made after 72 h.

Ecotoxicological analysis of the substrates

A measurement of the ecotoxicity was performed using 3 bioassays:

- Inhibition of the root growth using the seeds of the lettuce (*Lactuca sativa* L. var. capitata L., SAFÍR; SEMO s.r.o.), direct contact test, modified version ISO 11269-1, 1993
- Inhibition of the root growth using the seeds of the lettuce (*Lactuca sativa* L. var. capitata L., SAFÍR; SEMO s.r.o.), test using water extracts, modified version of guideline of Ministry of the Environment of the Czech Republic (339/1997)
- Bioluminescence assay using bacteria *Vibrio fischeri*, ČSN EN ISO 11348-2

These tests were performed with dry substrates before and after phytoremediation.

Results and discussion

Plant growth measured by plant weight

The plant growth is influenced by many factors. In our experiment the difference was the substrate quality. The plant growth was monitored by measuring the plant weight (g) (Table 1). The dry fractions from 5 pots were used to calculate average weight of roots, shoots and fruits (in case of nightshade). At the end of the experiment the plant shoot weight was higher when the plants were cultivated in the sewage sludge (in case of tobacco more than 2 times). These plants were also taller.

Accumulation of PBDEs in plant tissues
Our results demonstrate that plants are able to take up and accumulate PBDEs. Table 2 shows accumulation and translocation of PBDEs in roots, stalks, leaves, eventually fruits of the nightshade and tobacco. (Tobacco didn't have fruits.) From these results is obvious that the nightshade is able to absorb higher amount of PBDEs than tobacco (ng/g dry weight).

Microbial analysis
Figure 1 shows the total colony forming units in 1 g dry sewage sludge after phytoremediation. There are no significant differences between the treatments.

Ecotoxicological analysis
Figure 2 shows the root growth inhibition of lettuce (using contact test and using water extract test) and inhibition of bioluminescence of photobacteria *Vibrio fisheri*. The growth inhibition and light inhibition decreased rapidly in planted as well as in unplanted sewage sludge.
Due to the reason that the sewage sludge contained other toxic chemicals (heavy metals, polycyclic aromatic hydrocarbons, PCBs, etc.) it is possible that they were eluted from the sewage sludge by regular watering.

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Table 1: Plant growth in control substrate and sewage sludge sampled in Hradec Králové

Plant dry wt (g)						
Substrate	**Nightshade**			**Tobacco**		
	Root	**Shoot**	**Fruit**	**Root**	**Shoot**	**Fruit**
Control substrate	0.26	2.48	2.12	0.32	2.80	-
Sewage sludge Hradec Králové	0.11	3.14	1.70	0.34	6.40	-

Table 2: PBDEs accumulation in roots, stalks, leaves and fruits of nightshade and tobacco from sewage sludge Hradec Králové

Uptake and dislocation of PBDEs in plant tissues (ng/g dry wt)					
Substrate	**Plant fraction**	**Nightshade**		**Tobacco**	
		∑BDE	BDE 209	∑BDE	BDE 209
Sewage sludge Hradec Králové	Root	25.87	28.17	49.3	0
	Stalk	8.12	0.80	2.8	16.1
	Leaves	3.61	0	3.0	0
	Fruit	5.37	1.21	-	-

Figure 1: Total colony forming units (cfu/g) in the unplanted and planted sewage sludge that contains PBDEs

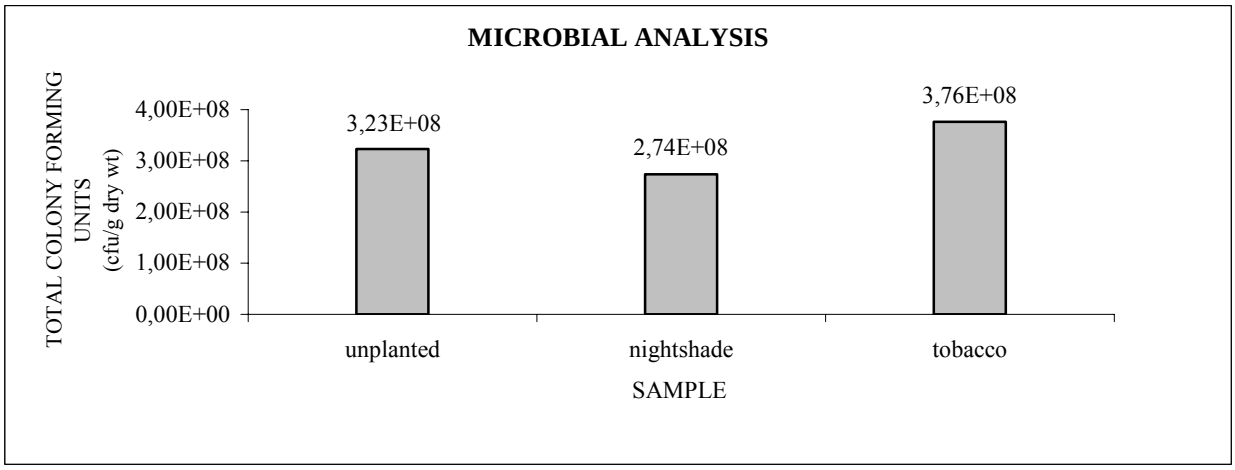

Figure 2: Change of the sewage sludge ecotoxicity after the experiment using three different tests