POLYCYCLIC AROMATIC HYDROCARBONS CONTENT IN SHOOTS AND LEAVES OF WILLOW (*SALIX VIMINALIS*) CULTIVATED ON THE SEWAGE SLUDGE-AMENDED SOIL

PATRYK OLESZCZUK* and STANISŁAW BARAN
Institute of Soil Science and Environmental Management, University of Agriculture,
ul. Leszczyńskiego 7, 20-069 Lublin, Poland
*(*author for correspondence, e-mail: patol@agros.ar.lublin.pl; Tel: +48 (81) 534 35 48;*
Fax: +48 (81) 532 26 32)

(Received 7 March 2005; accepted 7 July 2005)

Abstract. The aim of the present study was to investigate the uptake of 16 PAHs by willow (*Salix viminalis*) from soil amended with contaminated sewage sludge. Uptake experiments were conducted on field plots using sludge applications of 0, 30, 75, 150, 300, and 600 Mg ha^{-1}. The total PAH content of control soil and sludge were 49.6 μg kg^{-1} and 5713 μg kg^{-1}, respectively. The concentrations for the 16 PAHs listed as priority pollutants were measured for soil and plant tissue samples obtained at 0.5, 1.5, 2.5, and 3.5 years. Soil total PAH content decreased significantly within the first half year, followed by minimal changes over the subsequent three years of treatment. PAH analysis was carried out on a HPLC-UV. Total PAH content in control plants was 3.6–7.3 μg kg^{-1} for shoots and 13–27 μg kg^{-1} for leaves. Treated plant tissue content was higher with shoots and leaves containing ~5.5–17.6 and 13.5–33.8 μg kg^{-1}, respectively. Plant total PAH content did not show a significant trend relative to controls with respect to time. However, uptake did increase in relation to an increased sludge application. Bioconcentration factors (BCF), adjusted by control values, were calculated for total PAH content. BCF values were highest for the initial sampling (6 months) and did not show a significant temporal relationship. BCF values did decrease with increasing sludge application. With respect to individual PAHs, elevated plant tissue concentrations were measured for "light" PAH (e.g. naphthalene, phenanthrene, acenaphthalene) with leaf BCF values correlated with solubility and organic partitioning coefficients.

Keywords: PAHs, sewage sludge, contaminants uptake, soil fertilization, plant contamination

Introduction

In recent years, concern about persistent organic pollutants (POPs) has considerably increased (Nadal *et al.*, 2004). Due to the toxic, mutagenic and carcinogenic properties of these compounds, much attention has been paid to those components which have direct contact with the human organism (water, food products). One of the most frequent "representatives" of POPs in the natural environment are polycyclic aromatic hydrocarbons (PAHs) (Baran *et al.*, 2004). These contaminants are characterized by high persistence in the environment, and due to their mutagenic and carcinogenic properties they can pose a risk to human health. The most frequently mentioned sources of PAHs in plants are (Topp *et al.*, 1986; Duarte-Davidson and

Water, Air, and Soil Pollution (2005) 168: 91–111

Jones, 1996): (1) their root uptake from soil solution, and subsequent translocation from roots to shoots in the transpiration stream; (2) absorption by roots or shoots of volatilized organics from the surrounding air; (3) uptake by external contamination of shoots by soil and dust followed by retention in the cuticle or penetration through it and uptake and transport in oil channels which are found in some oil-containing plants such as carrots.

There are divergent data in the literature on the possibility of PAH uptake by the roots and their transportation to the above-ground plant parts. Much information has been collected which indicates that many higher plants may not take up PAHs (Wild and Jones, 1992). It is assumed that this type of transportation is only possible in the case of 2–3 ring PAHs with low molecular weight, whereas 3–6 ring "heavier" PAHs show a tendency to sorb on the surface of root cells (Larsson and Sahlberg, 1981). The main accumulation pathway for such compounds is from the air to the leaf surface (Smith and Jones, 2004). It has been suggested that lipophilic organic pollutants including PAHs are strongly associated with the soil organic fraction, and are not expected to be susceptible to plant uptake and subsequent translocation (Wild and Jones, 1992; Gao and Zhu, 2004). Moreover, the uptake of organic pollutants from the soil by plants depends on the physical-chemical properties of these pollutants only, the plant species and the soil's properties.

Studies carried out so far (Kirchmann and Tengsved, 1991; Wild *et al.*, 1992; Harms, 1996; Oleszczuk and Baran, 2005a) on PAH uptake by plants from sewage sludge-amended soils concern mainly edible plants (carrots, corn, barley). There is little information on PAH uptake from sewage sludge-amended soils by other plants. Fast-growing plants commonly used in the short rotation coppice/forestry system (SRC/SFS) are a good example here. The ability of Salicaceae family trees, particularly the Populus Genus, in the removal of a large array of organic contaminants by absorption onto plant roots is well documented (Paterson and Schnoor, 1992; Schnoor *et al.*, 1995). Little is yet known about the impact of willow trees on the fate of organic pollutants in soils and sediments. However, the ability of poplar trees to remove organic contaminants is already documented (Schnoor *et al.*, 1995).

The genus Salix comprises about 450 species, which all are easily propagated, fast-growing, and tolerant of diverse soil conditions. The ability to propagate form cuttings, a high transpiration rates and potential production of energy biomass, makes it an attractive group of plants for restoration of contaminated land (Kuzovkina *et al.*, 2004). Willows can stabilize polluted substrates, recycle nutrients and accumulate pollutants in their tissues (Vervaeke *et al.*, 2003).

The aim of the present studies was to determine the content of 16 polycyclic aromatic hydrocarbons from the US EPA list (US EPA, 1985) in willow grown on soil fertilized with sewage sludge. The influence of the sewage sludge dose on the uptake of contaminants was studied together with the profile of individual PAHs in leaves and shoots of willow.

Materials and Methods

PLOT EXPERIMENT

The study block consisted of 6 plots (15 m^2 each) founded on light soils. Plots were allocated according to increasing doses of sludge as follows: soil without any fertilization – control; sewage sludge 30 Mg ha^{-1}, 75 Mg ha^{-1}, 150 Mg ha^{-1}, 300 Mg ha^{-1}, 600 Mg ha^{-1}. Fermented sewage sludge from a mechanical-biological sewage treatment plant was used. Sludge doses were calculated on dry mass basis. The sewage sludge was mixed with a surface soil layer up to a depth of 20 cm. Next, seedlings of willow were planted directly in each plot. The experiment was carried out for 42 months.

SAMPLE COLLECTION AND PREPARATION

Soil samples were collected for analysis after the introduction of sewage sludge, and then after 6, 18, 30 and 42 months. Surface (0–20 cm) soil and sewage sludge-amended samples were collected with a (5 cm i.d. × 60 cm) stainless steel corer. Six independent samples (replicates) were taken from each plot. The cores were placed into zip-lock bags. Shoots and leaves of willow were harvested 6, 18, 30 and 42 months after sewage sludge application. Samples of plants were collected at the beginning of October. Four plants were gathered from one plot. Whole plants were chosen and then transported to the laboratory where shoots were separated from leaves. Plant material was then homogenised, ground and air-dried prior to PAHs analyses. The soil samples were transported to the laboratory, air-dried in air-conditioned storage rooms (20–25 °C) for 2 d (in darkness), manually crushed and sieved (<2 mm) prior to chemical analyses. Properties of soil and sewage sludge are summarized in Table I. In Figure 1 are presented content of PAHs in control soil and sewage sludge.

ANALYSIS OF PAHS

PAHs were determined using the method of HPLC with UV detection after optimization of the analytical process with respect to the amount and type of solvent used and extraction time, by means of ultrasonic method (Oleszczuk and Baran, 2003) and optimization of the extract purification process by the solid phase extraction method (Oleszczuk and Baran, 2004). The samples were extracted in an ultrasonic bath (Sonic-3, Polsonic, Poland). The extract was purified by solid phase extraction using C_{18} Octadecyl columns (JT Baker-Mallinckrodt, Germany). A qualitative and quantitative analysis of PAHs was carried out on the liquid chromatograph with UV detection (Oleszczuk and Baran, 2003, 2004). All reported concentration values of PAHs are expressed on a dry-weight basis of soil, plants, sewage sludge and sewage

TABLE I

The physico-chemical properties of sewage sludge and soil used in the experiment

Properties		Soil	Sewage sludge
Soil texture (%)	1–0.1	86	–
	0.1–0.02	7	–
	<0.02	7	–
pH in KCl		5.8	6.4
CEC (mmol kg^{-1})		48.9	583.2
TEB (mmol kg^{-1})		71.2	607.7
BS (%)		68.7	96.0
Available forms (mg kg^{-1})	P	56	28
	K	87	84
	Mg	49	77
TOC (g kg^{-1})		12.1	277
N_t (g kg^{-1})		1.2	22.3
16 PAHs sum (μg kg^{-1})		49.6	5712

CEC – the cation exchange capacity. pH – reactivity in 1 M KCl. TEB – the total of the exchangeable bases. BS – the degree of the base saturation. Available forms P, K, Mg – available forms of phosphorus, potassium and magnesium respectively. TOC – the total organic carbon. N_t – the total nitrogen.

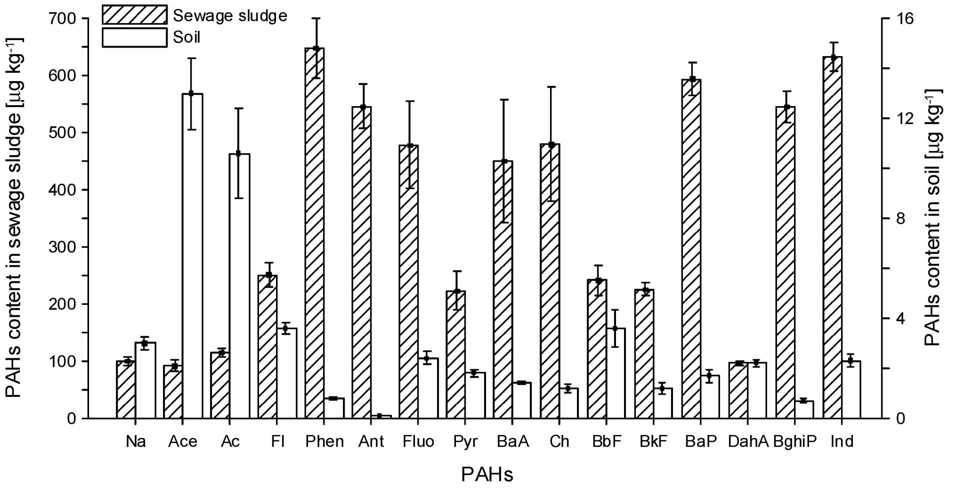

Figure 1. Content of individual PAHs in control soil and sewage sludge used in the experiment. Error bars represent the standard errors of the mean of 3 extractions.

sludge-amended soils determined by drying the samples for 24 h at 105 °C and are the average of triplicate extraction.

Recoveries for the total procedures (sample preparation, extraction and SPE) ranged between 81–90% (in soil and sewage sludge-amended soil) and 72–83%

(in plants and sewage sludge) for individual PAHs. Only in the case of naphthalene recoveries were in the range 50–60% (in all samples). Precision expressed as relative standard deviation (RSD) was below 21% (Oleszczuk and Baran, 2003; Oleszczuk and Baran, 2004) for investigated samples. The detection limits calculated with a signal to noise ratio of three (IUPAC criterion), for 20 μl loop injection, were less than 0.05 μg/mL or 0.5 ng for all PAHs.

DATA ANALYSIS

The relationships between BCF values and the properties of the PAHs (S, log K_{ow} and log K_{oc}) were determined by correlation analysis with Statistica 6.0. Significance was set at $^{*}P \leq 0.05$ or $^{**}P \leq 0.001$. Statistically significant differences between the results were evaluated on the basis of standard deviation determinations and analysis of variance method (ANOVA).

Bioconcentration factors (BCF) were calculated according to equation:

$$\text{BCF} = (\text{PAHs in plant})/(\text{PAHs in soil}) \tag{1}$$

In order to determine bioconcentration coefficient for the PAHs which originated from sewage sludges, PAH content was studied in the plants grown on the soil fertilized with sewage sludges and then reduced by the PAH content determined for the plants grown on the control soil according to the following equation:

$$\text{BCF}_{\text{a}} = (\text{PAH}_{\text{ps}} - \text{PAH}_{\text{pc}})/\text{PAH}_{\text{s}} \tag{2}$$

where BCF_{a} is bioconcentration factor after accounting for control values, PAH_{ps} is PAHs content in plant cultivated on sewage sludge-amended soil, PAH_{pc} is PAHs content in plant cultivated on un-amended soil, PAH_{s} is PAHs content in soil.

Results

PAHS IN SOIL

PAH transformations both in the control soil and in soil fertilized with sewage sludge have been presented in other studies (Baran and Oleszczuk, 2003; Oleszczuk and Baran, 2005b). The control soil was characterized by a very low content of polycyclic aromatic hydrocarbons (49.6 ± 5.5 μg kg^{-1}). Acenaphthylene and acenaphthene were predominant in this soil (Figure 1). In the sludge used in this experiment, the total PAHs content was 5713 μg kg^{-1} ($\pm$428 μg kg^{-1}) (Table I) and, as opposed to the soil, had a considerably high content of 3 and 6-ring PAHs.

The introduction of sludge into the soil resulted in an increase of the PAH content therein (Baran and Oleszczuk, 2003; Oleszczuk and Baran, 2005c). During the

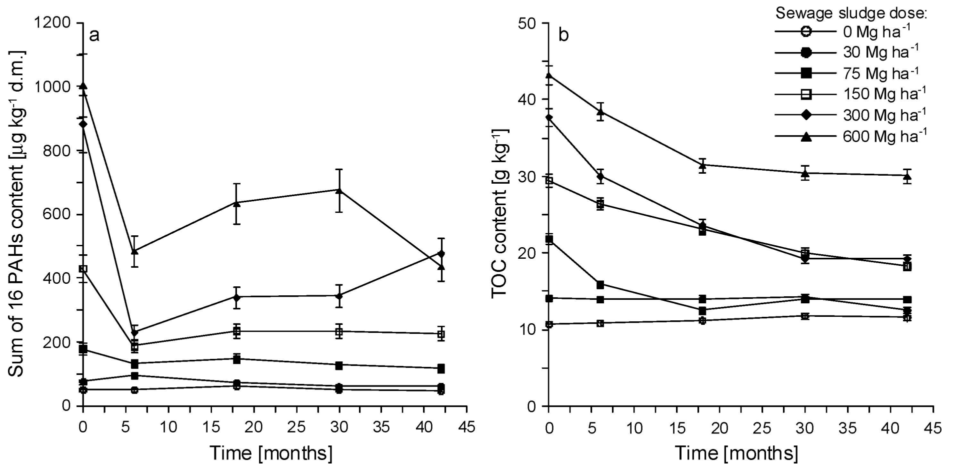

Figure 2. Changes of the (a) sum of 16 PAHs and (b) total organic carbon (TOC) content in control and sewage sludge-amended soil. Error bars represent the standard errors of the mean of 3 extractions.

whole study period, continuous variations in the content of the compounds studies were observed (Figure 2a). Analyzing changes in the content of the total PAH on the last study date as compared to their content at the beginning of the experiment, a statistically significant ($P \leq 0.05$) decrease in all experimental treatments was observed. A proportional relation between the intensity in the removal of the total PAH studied and an increase of the sewage sludge dose was noted in this case. In the experimental treatment with the highest dose of sludge, more than half (56.7%) of the content of the 16 PAHs degraded, and in the case of the remaining doses, i.e. 30, 75, 150 and 300 Mg ha^{-1}, the decrease in the total of their content was, respectively 20, 34, 48 and 46% (Oleszczuk and Baran, 2005b).

The removal range for individual PAHs depended on the sewage sludge dose. A relatively high losses of individual PAHs (>50%) were found in the experimental treatment with the highest sludge dose (600 Mg ha^{-1}) and, in the case of a few PAHs, in those soils with the sludge amounting to 150 Mg ha^{-1} and 300 Mg ha^{-1}. In the case of benzo[*a*]anthracene and dibenz[*ah*]anthracene, was a relation observed, in which the losses of these compounds increased with increasing sludge doses, as was also the case for the total of the 16 PAHs. In the case of the remaining PAHs, the range of their losses varied and clearly depended on the experimental treatment as well as the sewage sludge dose applied (Oleszczuk and Baran, 2005b).

THE CONTENT OF PAHS IN WILLOW TISSUES

Figure 3 presents levels of the total content of the PAHs in plants grown on control soil and on soil fertilized with sewage sludge. The PAH content during the study period ranged from 3.6 to 7.3 $\mu g\ kg^{-1}$ in the shoots and from 13.0 to 27.0 $\mu g\ kg^{-1}$

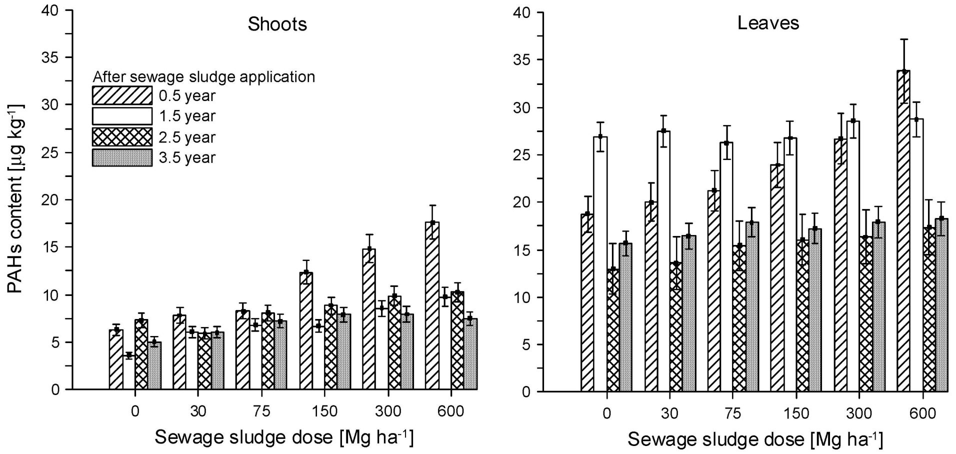

Figure 3. Changes of the sum of 16 PAHs content in willow shoots and leaves. Error bars represent the standard errors of the mean of 3 extractions.

in the willow leaves. Irrespective of the study date, naphthalene and acenaphthene were predominant in both plant parts studied and their contribution – depending on the year – ranged from 88% to 91% in the leaves, whereas in the shoots it was lower, i.e. from 45 to 86%. There were no mutagenic or carcinogenic 5- and 6-ring PAH present either in the leaves or the shoots (Tables II–V).

The content of polycyclic aromatic hydrocarbons in the shoots and leaves of willow grown in soil fertilized with sewage sludge was clearly related to the examination term (Figure 3). In 0.5 year after sludge application, when the willow seedlings were in the preliminary stage of growth, a gradual increase in the PAH content was noted with increasing sewage sludge doses. However, a statistically significant ($P \leq 0.05$) increase in relation to the control was noted only at a sludge dose of $\geq$150 Mg ha^{-1} in the case of the shoots and above 300 Mg ha^{-1} in the case of the willow leaves. In the consecutive year, i.e. 1.5 after the beginning of the experiment, the PAH content in the willow leaves was significantly higher than in the control which was already at the lowest sludge dose (30 Mg ha^{-1}), whereas in the case of the leaves, no statistically significant differences between the PAH content between plants grown on the control soil or on the soil fertilized with sewage sludge were observed.

No significant increase in the PAH content in the willow leaves in relation to the control, was observed before the 2.5 and 3.5 years from the beginning of the experiment (Figure 3). On the last two study dates, i.e. 2.5 and 3.5 years from sewage sludge application in the case of willow shoots, a more clear differentiation in the PAH content than in the case of the leaves of this plant was observed. A significant increase in the total content of the 16 PAHs in the shoots as compared to the control was noted when the sludge dose exceeded 300 Mg ha^{-1} (2.5 years of investigation) or 150 Mg ha^{-1} (3.5 years of investigation). As was observed in the

TABLE II

Polycyclic aromatic hydrocarbons content in willow six months after sewage sludge application (I term)

	Sewage sludge dose (Mg ha^{-1})											
	Shoots						Leaves					
PAHs*	0	30	75	150	300	600	0	30	75	150	300	600
Na	1.88 ± 13	2.05 ± 14	2.36 ± 11	4.26 ± 12[a]	4.38 ± 14[a]	4.87 ± 11[a]	9.39 ± 10	8.20 ± 12	9.44 ± 13	11.04 ± 11	12.52 ± 10[a]	15.48 ± 13[a]
Ace	n.d.	0.77 ± 13[a]	0.62 ± 12[a]	1.65 ± 14[a]	2.97 ± 11[a]	2.56 ± 13[a]	0.55 ± 12	1.35 ± 12[a]	1.28 ± 10[a]	1.29 ± 9[a]	1.93 ± 10[a]	2.74 ± 11[a]
Ac	2.31 ± 14	2.54 ± 10	2.43 ± 12	2.48 ± 13	2.45 ± 10	3.09 ± 10[a]	7.08 ± 10	7.62 ± 10	7.29 ± 9	7.44 ± 9	7.35 ± 9	9.27 ± 13[a]
Fl	0.18 ± 11	0.25 ± 11[a]	0.22 ± 11	0.25 ± 10[a]	0.38 ± 14[a]	0.22 ± 14[a]	n.d.	0.35 ± 8[a]	0.37 ± 13[a]	0.30 ± 12[a]	0.38 ± 11[a]	0.42 ± 14[a]
Phen	0.51 ± 10	0.11 ± 8[a]	0.11 ± 10[a]	0.18 ± 9[a]	0.19 ± 13[a]	0.30 ± 15[a]	0.29 ± 13	0.23 ± 11	0.24 ± 9	0.40 ± 13[a]	0.43 ± 12[a]	0.66 ± 10[a]
Ant	0.07 ± 12	0.03 ± 12[a]	0.03 ± 15[a]	0.14 ± 11[a]	0.11 ± 9[a]	0.17 ± 11[a]	n.d.	n.d.	0.09 ± 11[a]	0.13 ± 10[a]	0.12 ± 12[a]	0.19 ± 12[a]
Fluo	0.79 ± 12	0.83 ± 10	0.85 ± 14	1.10 ± 13[a]	1.27 ± 10[a]	1.12 ± 10[a]	0.50 ± 11	0.73 ± 10[a]	0.89 ± 10[a]	1.05 ± 8[a]	1.27 ± 9[a]	1.35 ± 10[a]
Pyr	0.56 ± 10	0.66 ± 11	0.52 ± 11	0.55 ± 12	0.71 ± 12	1.12 ± 12[a]	0.56 ± 12	0.81 ± 13[a]	0.64 ± 12	0.68 ± 12	0.88 ± 13[a]	1.38 ± 9[a]
B*a*A	n.d.	0.16 ± 13[a]	0.17 ± 13[a]	0.11 ± 15[a]	0.25 ± 12[a]	0.51 ± 13[a]	n.d.	n.d.	0.09 ± 14[a]	0.15 ± 12[a]	0.18 ± 14[a]	0.24 ± 13[a]
Ch	n.d.	0.11 ± 14[a]	0.16 ± 10[a]	0.14 ± 9[a]	0.36 ± 9[a]	0.57 ± 12[a]	0.40 ± 14	0.45 ± 9	0.42 ± 11	0.54 ± 9[a]	0.36 ± 13	0.59 ± 12[a]
B*b*F	n.d.	0.14 ± 10[a]	0.18 ± 12[a]	0.48 ± 11[a]	0.35 ± 11[a]	0.61 ± 14[a]	n.d.	0.10 ± 9[a]	0.10 ± 9[a]	0.18 ± 10[a]	0.23 ± 10[a]	0.16 ± 9[a]
B*k*F	n.d.	n.d.	0.18 ± 8[a]	0.11 ± 12[a]	0.20 ± 8[a]	0.36 ± 9[a]	n.d.	n.d.	n.d.	0.07 ± 13[a]	0.15 ± 10[a]	0.13 ± 10[a]
B*a*P	n.d.	0.12 ± 11[a]	0.18 ± 10[a]	0.30 ± 8[a]	0.37 ± 12[a]	0.59 ± 9[a]	n.d.	0.15 ± 12[a]	0.22 ± 14[a]	0.30 ± 9[a]	0.33 ± 12[a]	0.41 ± 12[a]
D*ah*A	n.d.	0.06 ± 12[a]	0.06 ± 12[a]	0.28 ± 13[a]	0.29 ± 10[a]	0.58 ±	n.d.	n.d.	0.07 ± 11[a]	0.07 ± 8[a]	0.12 ± 11[a]	0.09 ± 11[a]
B*ghi*P	n.d.	n.d.	0.11 ± 8[a]	0.12 ± 10[a]	0.28 ± 11[a]	0.45 ± 12[a]	n.d.	n.d.	0.08 ± 15[a]	0.11 ± 11[a]	0.18 ± 9[a]	0.25 ± 10[a]
Ind	n.d.	n.d.	0.06 ± 13[a]	0.20 ± 10[a]	0.27 ± 13[a]	0.48 ± 10[a]	n.d.	n.d.	0.06 ± 9[a]	0.20 ± 13[a]	0.27 ± 14[a]	0.48 ± 10[a]

* – Na – Naphthalene, Ace – Acenaphthalene, Ac – Acenaphtene, Fl – Fluorene, Phen – Phenanthrene, Ant – Anthracene, Fln – Fluoranthene, Pyr – Pyrene, B*a*A – Benz[*a*]anthracene, Ch – Chrysene, B*b*F – Benzo[*b*]fluoranthene, B*k*F – Benzo[*k*]fluoranthene, B*a*P – Benzo[*a*]pyrene, D*ah*A – Dibenz[*ah*]anthracene, B*ghi*P – Benzo[*ghi*]perylene, Ind – Indeno[1,2,3-*cd*]pyrene; ± – relative standard deviation error (for $n = 3$ extractions).

[a] – Statistically different in relation to plant cultivated on un-amended soil.

n.d. – not detected.

TABLE III

Polycyclic aromatic hydrocarbons content in willow 18 months after sewage sludge application (II term)

	Sewage sludge dose (Mg ha^{-1})											
	Shoots						Leaves					
PAHs*	0	30	75	150	300	600	0	30	75	150	300	600
Na	1.61 ± 13	1.58 ± 11	1.76 ± 14	1.99 ± 15	1.76 ± 13	1.98 ± 15	10.17 ± 14	11.97 ± 13	10.55 ± 13	11.00 ± 15	12.11 ± 12	11.95 ± 14
Ace	n.d.	1.20 ± 13^{a}	1.39 ± 12^{a}	0.81 ± 12^{a}	1.32 ± 10^{a}	1.49 ± 11^{a}	n.d.	1.49 ± 11^{a}	1.54 ± 14^{a}	0.90 ± 11^{a}	1.37 ± 13^{a}	1.44 ± 11^{a}
Ac	n.d.	1.04 ± 10^{a}	1.07 ± 12^{a}	1.08 ± 11^{a}	1.36 ± 13^{a}	1.49 ± 13^{a}	14.20 ± 13	11.30 ± 13	11.19 ± 11	12.23 ± 9	12.01 ± 14	11.99 ± 12
Fl	0.19 ± 11	0.15 ± 12	0.15 ± 10	0.25 ± 13	0.19 ± 10	0.18 ± 11	0.23 ± 10	0.18 ± 12	0.16 ± 12^{a}	0.23 ± 13	0.19 ± 10	0.24 ± 9
Phen	0.29 ± 12	0.26 ± 10	0.30 ± 15	0.30 ± 10	0.21 ± 10^{a}	0.19 ± 11^{a}	0.53 ± 13	0.43 ± 10	0.33 ± 10^{a}	0.27 ± 10^{a}	0.35 ± 11^{a}	0.36 ± 9^{a}
Ant	0.02 ± 14	0.01 ± 9	0.02 ± 11	0.03 ± 10	0.06 ± 12^{a}	0.06 ± 15^{a}	n.d.	n.d.	n.d.	n.d.	0.07 ± 8^{a}	0.08 ± 11^{a}
Fluo	0.69 ± 8	0.58 ± 9	0.66 ± 10	0.39 ± 9^{a}	0.71 ± 14	0.77 ± 10	0.45 ± 10	0.72 ± 11^{a}	0.73 ± 10^{a}	0.35 ± 12^{a}	0.58 ± 8^{a}	0.60 ± 8^{a}
Pyr	0.43 ± 11	0.43 ± 12	0.37 ± 12	0.35 ± 12	0.70 ± 12^{a}	0.87 ± 12^{a}	0.55 ± 10	0.33 ± 14^{a}	0.41 ± 12^{a}	0.32 ± 11^{a}	0.44 ± 12	0.44 ± 10
B*a*A	n.d.	0.06 ± 10^{a}	0.10 ± 13^{a}	0.18 ± 10^{a}	0.27 ± 12^{a}	0.32 ± 12^{a}	0.39 ± 12	0.38 ± 14	0.45 ± 11	0.38 ± 8	0.40 ± 12	0.43 ± 14
Ch	0.36 ± 9	0.37 ± 10	0.35 ± 11	0.41 ± 11	0.31 ± 10	0.31 ± 10	0.40 ± 11	0.46 ± 9	0.38 ± 13	0.38 ± 11	0.41 ± 9	0.45 ± 12
B*b*F	n.d.	0.09 ± 12^{a}	0.12 ± 9^{a}	0.30 ± 12^{a}	0.33 ± 10^{a}	0.44 ± 8^{a}	n.d.	0.08 ± 11^{a}	0.14 ± 10^{a}	0.19 ± 9^{a}	0.16 ± 13^{a}	0.20 ± 10^{a}
B*k*F	n.d.	0.06 ± 13^{a}	0.08 ± 9^{a}	0.15 ± 12^{a}	0.19 ± 13^{a}	0.28 ± 9^{a}	n.d.	n.d.	0.09 ± 12^{a}	0.14 ± 9^{a}	0.13 ± 10^{a}	0.16 ± 10^{a}
B*a*P	n.d.	0.09 ± 11^{a}	0.13 ± 10^{a}	0.08 ± 10^{a}	0.35 ± 11^{a}	0.41 ± 13^{a}	n.d.	0.11 ± 12^{a}	0.15 ± 12^{a}	0.15 ± 13^{a}	0.13 ± 11^{a}	0.16 ± 13^{a}
D*ah*A	n.d.	n.d.	0.10 ± 12^{a}	0.19 ± 8^{a}	0.26 ± 8^{a}	0.32 ± 12^{a}	n.d.	n.d.	n.d.	0.07 ± 11^{a}	0.07 ± 8^{a}	0.08 ± 9^{a}
B*ghi*P	n.d.	0.07 ± 14^{a}	0.11 ± 13^{a}	0.13 ± 10^{a}	0.32 ± 12^{a}	0.32 ± 10^{a}	n.d.	n.d.	0.05 ± 10^{a}	0.12 ± 10^{a}	0.11 ± 11^{a}	0.13 ± 10^{a}
Ind	n.d.	0.09 ± 10^{a}	0.11 ± 11^{a}	0.05 ± 11^{a}	0.24 ± 13^{a}	0.31 ± 11^{a}	n.d.	0.07 ± 10^{a}	0.12 ± 12^{a}	0.05 ± 13^{a}	0.06 ± 9^{a}	0.06 ± 12^{a}

* – descriptions of individual PAHs are presented in Table II; ± – relative standard deviation error (for $n = 3$ extractions).

[a] – Statistically different in relation to plant cultivated on un-amended soil.

n.d. – not detected.

TABLE IV

Polycyclic aromatic hydrocarbons content in willow 30 months after sewage sludge application (III term)

	Sewage sludge dose (Mg ha^{-1})											
	Shoots						Leaves					
PAHs*	0	30	75	150	300	600	0	30	75	150	300	600
Na	1.74 ± 15	1.77 ± 11	1.78 ± 15	2.01 ± 14	1.82 ± 14	2.38 ± 13	5.47 ± 12	4.99 ± 15	5.76 ± 14	5.51 ± 12	5.44 ± 15	5.90 ± 13
Ace	n.d.	0.63 ± 12^{a}	1.18 ± 11^{a}	1.44 ± 12^{a}	1.45 ± 10^{a}	1.08 ± 10^{a}	n.d.	0.71 ± 12^{a}	1.17 ± 12^{a}	1.79 ± 10^{a}	1.94 ± 13^{a}	1.84 ± 12^{a}
Ac	4.70 ± 14	2.61 ± 9^{a}	3.47 ± 13	3.54 ± 10	3.87 ± 13	3.44 ± 12^{a}	6.10 ± 11	5.93 ± 13	6.42 ± 13	6.32 ± 11	6.19 ± 10	6.18 ± 10
Fl	n.d.	0.06 ± 14^{a}	0.08 ± 11^{a}	0.08 ± 10^{a}	0.07 ± 12^{a}	0.09 ± 11^{a}	0.13 ± 12	0.07 ± 10^{a}	0.08 ± 11^{a}	0.09 ± 13^{a}	0.09 ± 10^{a}	0.11 ± 12
Phen	0.39 ± 14	0.27 ± 13^{a}	0.40 ± 10	0.36 ± 12	0.26 ± 9^{a}	0.29 ± 10^{a}	0.43 ± 11	0.31 ± 10^{a}	0.40 ± 13	0.45 ± 13	0.35 ± 13	0.43 ± 11
Ant	n.d.	n.d.	0.04 ± 12^{a}	0.04 ± 11^{a}	0.07 ± 13^{a}	0.14 ± 12^{a}	n.d.	n.d.	n.d.	0.05 ± 14^{a}	0.10 ± 11^{a}	0.18 ± 11^{a}
Fluo	n.d.	0.06 ± 9^{a}	0.16 ± 9^{a}	0.30 ± 13^{a}	0.45 ± 12^{a}	0.45 ± 13^{a}	0.50 ± 10	0.70 ± 12^{a}	0.66 ± 12^{a}	0.57 ± 10	0.61 ± 11	0.55 ± 9
Pyr	n.d.	0.06 ± 11^{a}	0.12 ± 11^{a}	0.18 ± 13^{a}	0.27 ± 14^{a}	0.23 ± 9^{a}	n.d.	0.06 ± 12^{a}	0.10 ± 9^{a}	0.13 ± 10^{a}	0.14 ± 12^{a}	0.28 ± 12^{a}
B*a*A	0.47 ± 12	0.30 ± 12^{a}	0.30 ± 10^{a}	0.22 ± 12^{a}	0.31 ± 12^{a}	0.44 ± 9	n.d.	0.33 ± 10^{a}	0.30 ± 9^{a}	0.28 ± 12^{a}	0.41 ± 10^{a}	0.54 ± 10^{a}
Ch	n.d.	n.d.	0.15 ± 13^{a}	0.12 ± 10^{a}	0.29 ± 12^{a}	0.38 ± 12^{a}	0.35 ± 10	0.35 ± 8	0.33 ± 11	0.41 ± 8	0.39 ± 10	0.46 ± 14
B*b*F	n.d.	0.05 ± 11^{a}	0.16 ± 10^{a}	0.23 ± 10^{a}	0.28 ± 9^{a}	0.40 ± 11^{a}	n.d.	0.06 ± 11^{a}	0.09 ± 10^{a}	0.13 ± 12^{a}	0.18 ± 14^{a}	0.17 ± 13^{a}
B*k*F	n.d.	n.d.	0.05 ± 13^{a}	0.12 ± 13^{a}	0.12 ± 13^{a}	0.21 ± 10^{a}	n.d.	n.d.	n.d.	n.d.	0.09 ± 12^{a}	0.08 ± 10^{a}
B*a*P	n.d.	0.05 ± 9^{a}	0.07 ± 11^{a}	0.06 ± 13^{a}	0.12 ± 10^{a}	0.12 ± 9^{a}	n.d.	0.06 ± 12^{a}	0.07 ± 13^{a}	0.07 ± 10^{a}	0.10 ± 10^{a}	0.15 ± 11^{a}
D*ah*A	n.d.	n.d.	n.d.	n.d.	0.12 ± 10^{a}	0.17 ± 9^{a}	n.d.	n.d.	n.d.	n.d.	0.08 ± 11^{a}	0.14 ± 11^{a}
B*ghi*P	n.d.	n.d.	0.06 ± 9^{a}	0.10 ± 10^{a}	0.22 ± 13^{a}	0.29 ± 12^{a}	n.d.	n.d.	n.d.	0.12 ± 11^{a}	0.14 ± 9^{a}	0.15 ± 9^{a}
Ind	n.d.	0.05 ± 11^{a}	0.06 ± 12^{a}	0.09 ± 14^{a}	0.16 ± 11^{a}	0.16 ± 10^{a}	n.d.	0.06 ± 12^{a}	0.06 ± 11^{a}	0.11 ± 9^{a}	0.14 ± 13^{a}	0.19 ± 12^{a}

[*] – descriptions of individual PAHs are presented in Table II; ± – relative standard deviation error (for $n = 3$ extractions).

[a] – Statistically different in relation to plant cultivated on un-amended soil.

n.d. – not detected.

TABLE V

Polycyclic aromatic hydrocarbons content in willow 30 months after sewage sludge application (IV term)

	Sewage sludge dose (Mg ha^{-1})											
	Shoots						Leaves					
PAHs*	0	30	75	150	300	600	0	30	75	150	300	600
Na	1.55 ± 14	1.77 ± 13	2.16 ± 11[a]	2.23 ± 13[a]	1.97 ± 14	1.72 ± 14	6.72 ± 13	7.46 ± 12	7.06 ± 14	7.03 ± 14	7.17 ± 13	7.27 ± 14
Ace	n.d.	0.63 ± 11[a]	1.04 ± 13[a]	1.27 ± 10[a]	1.27 ± 10[a]	0.95 ± 10[a]	n.d.	0.88 ± 11[a]	1.48 ± 10[a]	1.15 ± 12[a]	1.40 ± 10[a]	1.25 ± 11[a]
Ac	2.77 ± 12	2.61 ± 12	2.70 ± 10	2.76 ± 13	2.63 ± 13	2.69 ± 12	6.80 ± 11	6.62 ± 10	6.83 ± 13	6.53 ± 8	6.90 ± 10	6.55 ± 13
Fl	n.d.	0.06 ± 10[a]	0.07 ± 12[a]	0.09 ± 13[a]	0.09 ± 11[a]	0.07 ± 12[a]	0.06 ± 12	0.08 ± 13	0.10 ± 12[a]	0.08 ± 10[a]	0.10 ± 12[a]	0.09 ± 9[a]
Phen	0.29 ± 10	0.27 ± 10	0.31 ± 9	0.35 ± 12	0.33 ± 12	0.32 ± 9	0.35 ± 10	0.38 ± 9	0.45 ± 10[a]	0.42 ± 12	0.36 ± 10	0.42 ± 13
Ant	n.d.	n.d.	n.d.	n.d.	0.06 ± 9[a]	0.08 ± 11[a]	0.06 ± 10	n.d.	0.07 ± 11	n.d.	0.07 ± 11	0.11 ± 11[a]
Fluo	0.22 ± 13	0.23 ± 8	0.13 ± 9[a]	0.15 ± 11[a]	0.31 ± 10[a]	0.22 ± 10	0.59 ± 9	0.32 ± 9[a]	0.48 ± 10	0.55 ± 14	0.54 ± 13	0.59 ± 12
Pyr	n.d.	0.09 ± 11[a]	0.10 ± 12[a]	0.14 ± 9[a]	0.18 ± 13[a]	0.17 ± 10[a]	0.33 ± 12	0.23 ± 12[a]	0.29 ± 10	0.31 ± 10	0.31 ± 13	0.33 ± 10
B*a*A	0.19 ± 11	0.25 ± 12[a]	0.26 ± 13[a]	0.29 ± 8[a]	0.21 ± 12	0.25 ± 8[a]	0.41 ± 10	0.35 ± 12	0.37 ± 12	0.40 ± 13	0.33 ± 10	0.38 ± 10
Ch	n.d.	n.d.	0.13 ± 12[a]	0.12 ± 12[a]	0.20 ± 11[a]	0.22 ± 12[a]	0.36 ± 11	n.d.[a]	0.35 ± 12	0.31 ± 12	0.22 ± 12[a]	0.29 ± 11
B*b*F	n.d.	0.05 ± 10[a]	0.10 ± 10[a]	0.18 ± 11[a]	0.19 ± 10[a]	0.22 ± 12[a]	n.d.	0.07 ± 10[a]	0.15 ± 8[a]	0.16 ± 11[a]	0.21 ± 9[a]	0.29 ± 12[a]
B*k*F	n.d.	n.d.	0.06 ± 10[a]	0.09 ± 10[a]	0.08 ± 13[a]	0.10 ± 10	n.d.	n.d.	0.09 ± 8[a]	0.08 ± 10[a]	0.09 ± 11[a]	0.13 ± 9[a]
B*a*P	n.d.	0.05 ± 12[a]	0.08 ± 11[a]	0.10 ± 9[a]	0.08 ± 9[a]	0.11 ± 13[a]	n.d.	0.08 ± 11[a]	0.11 ± 11[a]	0.09 ± 10[a]	0.09 ± 12[a]	0.15 ± 9[a]
D*ah*A	n.d.	n.d.	n.d.	n.d.	0.08 ± 9[a]	0.08 ± 11[a]	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 13[a]
B*ghi*P	n.d.	n.d.	n.d.	0.08 ± 12[a]	0.15 ± 12[a]	0.16 ± 9[a]	n.d.	n.d.	n.d.	0.07 ± 12[a]	0.08 ± 10[a]	0.15 ± 10[a]
Ind	n.d.	n.d.	0.05 ± 12[a]	0.07 ± 10[a]	0.11 ± 11[a]	0.13 ± 12[a]	n.d.	n.d.	0.07 ± 9[a]	0.06 ± 11[a]	0.09 ± 12[a]	0.17 ± 12[a]

* – Descriptions of individual PAHs are presented in Table II; ± – relative standard deviation error (for $n = 3$ extractions).

[a] – Statistically different in relation to plant cultivated on un-amended soil.

n.d. – not detected.

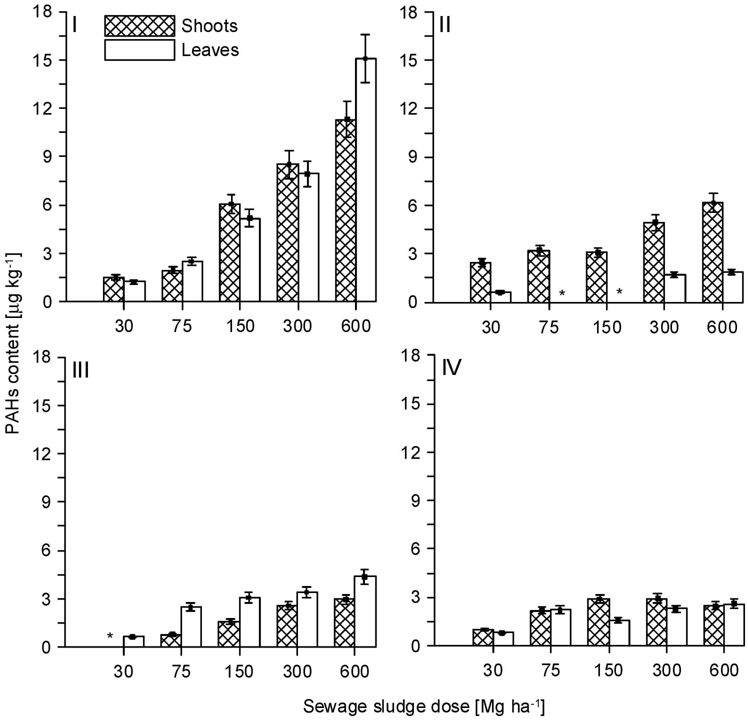

Figure 4. The sum of total PAHs content after accounting for control values. I, II, III and IV – 0.5, 1.5, 2.5 and 3.5 year after sewage sludge application. Error bars represent the standard errors of the mean of 3 extractions. * – a higher content of PAHs in the plants grown in the control soil than in the plants collected from the soil fertilised with sewage sludge was observed.

case of the plant grown in the control soil, a higher PAH content was observed in the willow leaves than in the shoots.

The total PAH content within willow shoots and leaves displayed a more pronounced trend after accounting for control content (Figure 4). Total PAH content among the tissues studied depended on the study term. In 1.5 and 2.5 years from the beginning of the experiment, significant differences in the PAH content between willow shoots and leaves were observed in the case of all sewage sludge doses applied.. After 1.5 years, a higher content of the total of PAHs in the willow shoots than in the leaves was noted. The PAH content in the willow leaves related to the sewage sludge dose ranged from 0.00 to 1.85 μg kg^{-1}, whereas in the shoots it ranged from 2.48 to 6.16 μg kg^{-1}. In 2.5 years from the sewage sludge application to the soil, a reversed tendency was observed. The PAH content in the shoots ranged from 0.00 to 2.97 μg kg^{-1}, and in the leaves it was higher and ranged from 0.65 to 4.36 μg kg^{-1}.The lowest differences between the tissues studied were observed in 0.5 and 3.5 year of investigation. Half year after sewage sludge application, only in the case of the highest sludge dose, were significant differences noted between the PAH content in the willow leaves and shoots. The PAH content in the willow leaves was 15.06 μg kg^{-1} whereas in the shoots 11.30 μg kg-1 in the case of the highest sludge dose. Tables II–V present individual PAHs content levels in willow grown on soils amended with sewage sludge. As in the case of plants grown on the control soil, 2- and 3-ring PAHs were predominant in the willow shoots and leaves. While analyzing the content of individual PAHs in the willow plant parts in relation to the sewage sludge dose applied, an increase in the PAH content with increasing sludge doses was observed irrespective of the study term in the case of most PAHs. The above phenomenon was more frequently found in the case of 4–6-ring PAHs than in the 2–3-ring forms of these compounds. Only 1.5 years after sewage sludge application to the soil, in the case of leaves and on the last experimental date in the case of shoots, PAH content did not differ irrespective of the sewage sludge dose applied.

PAH content after accounting for control values (data not presented) showed that in the case of willow shoots 0.5 and 2.5 year after sewage sludge application, a higher content of individual PAHs was observed in the willow shoots collected from the control plot than from the plots fertilised with sewage sludge. Half year after sewage sludge application, the above phenomenon concerned phenanthrene, whereas 2.5 years from the beginning of the investigation – it concerned acenaphthene regardless of sewage sludge dose. In the case of willow leaves, 1.5 years from the beginning of the experiment, a higher PAH content was observed in the willow leaves sampled from the control plot than in the samples collected from the soil fertilised with sewage sludge for more than half of the PAHs studied. Similar to the case of the shoots, it was true for phenanthrene irrespective of the sludge dose and also, additionally, for fluoranthene and pyrene. Irrespective of the plant tissue, sludge dose or experimental variant, the content of 5- and 6-ring PAHs in the plants grown on the soil fertilised with sludges was higher than in the control soil.

BIOCONCENTRATION FACTOR (BCF)

Values of the bioconcentration factor (BCF$_a$) calculated from Equation (2) for the total PAHs content varied, depending on the sludge dose, in a broad range (Figure 5). In the case of willow shoots, BCF$_a$ ranged from 0.004 to 0.037, whereas in the case of the leaves from 0.003 to 0.035. Higher values of the bioconcentration factor were more often noted in the willow shoots than in the leaves.

Both in the case of the willow shoots and leaves, the calculated factors of the bioconcentration depended on the study date as well as on the sewage sludge dose. The highest BCF$_a$ levels half year after sewage sludge application were noted in the experimental treatment with sewage sludge doses of 150, 300 and 600 Mg ha^{-1}, irrespective of the plant tissue. In the following terms (1.5 year after sewage sludge application) the reverse tendency was more frequently observed, in which an increase in the sludge dose resulted in a lowering of the BCF$_a$ level. Moreover, clearer differences between the willow shoots and leaves were observed (Figure 5).

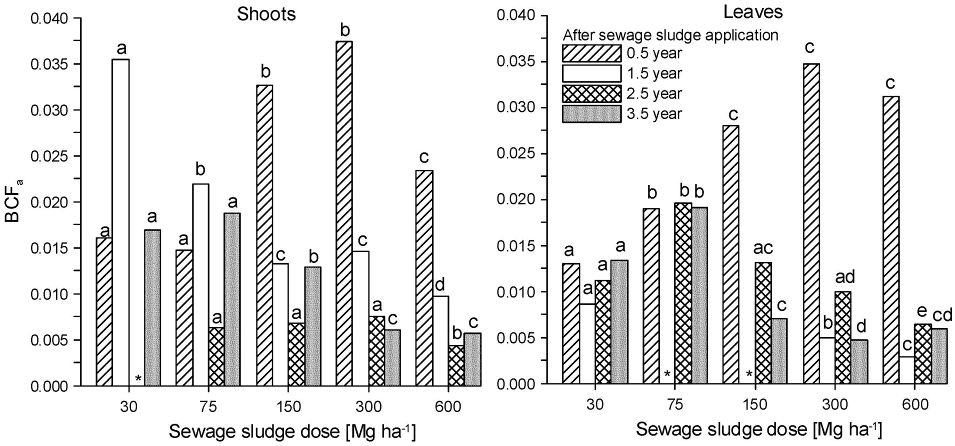

Figure 5. Bioconcentration factor of total PAHs in leaves and shoots of willow after accounting for control values. Values followed by the same letter (in relation to term of investigation and sewage sludge dose) are not significantly different (at 0.05). * – a higher content of PAHs in the plants grown in the control soil than in the plants collected from the soil fertilised with sewage sludge was observed.

Taking into consideration individual PAHs (data not presented), the highest BCF$_a$ values, irrespective of the planttissue, were observed 0.5 and 1.5 year after sewage sludge application. During this period high BCF$_a$ values were especially noticeable in the case of "light" PAHs i.e. naphthalene, acenaphthene and acenaphthylene. More pronounced differences between plant tissues were observed in relation to heavier PAHs. However, a decrease of the BCF$_a$ values with increasing sewage sludge doses was noted. The above phenomenon was more often noted in the willow leaves than in the shoots. In the last two study terms (2.5 and 3.5 year after sewage sludge application) in the case of willow shoots, a higher value of the bioconcentration factor than in the case of other doses was noted in the experimental treatment with a sludge dose of 75 Mg ha^{-1}.

Discussion

Concentrations in plants tended to increase with increasing PAH concentration in soils over the range of concentrations tested. Gao and Zhu (2004) claimed that both the uptake and accumulation of contaminants was correlated with their soil concentrations. This has also been confirmed by other authors (Fismes *et al.*, 2002). In the present study, a tendency in which an increase in the PAH content in the soil was accompanied by an increase of the PAH content in plants was also observed. A clear relationship was observed in the initial phase of the present experiment, i.e. 0.5 years after sewage sludge was introduced to the soil. On the consecutive dates, the relation in which an increased PAH content in the soil was accompanied by a similarly increased content of PAHs in willow was less and less frequent. A lowering of the relationships between the soil PAH content and the PAH content in plants can point to a strong binding of pollutants in the matrix, limiting their translocation in the soil, i.e. sequestration (Luthy *et al.*, 1997; Alexander, 2000) or bound-residue (Nieman *et al.*, 1999; Barraclough *et al.*, 2005). Aged soil contaminants are increasingly unavailable for uptake (Parrish *et al.*, 2000).

The results presented in this study do not provide proof that the pollutants determined are taken up by plants by the path: sewage sludge-amended soil – roots – shoots – leaves. However, they clearly show that there is a possibility of plant pollution by PAHs as a result of the application of sewage sludge. The above can be seen in the higher content of PAHs with low molecular weight in plants grown on soils fertilized with sludge than in plants grown in the control soil. Moreover, in the willow from the plots on which sewage sludge was applied, PAHs with a high molecular weight, which was unnoticed on the control plots, were found.

The most probable pathway for plant pollution by PAHs in the present experiment was the uptake of compounds that are volatilized from contaminated soil, particularly in the lower aerial parts of the plant (Duarte-Davidson and Jones, 1996; Trapp and Matthies, 1997). Wolters *et al.* (2002) stated that the volatilisation of fluoranthene from soil polluted with this compound, directly after application, ranged from 11% to 25% of the fluoranthene initially introduced and depended on air temperature and humidity. With time, the evaporation of xenobiotics clearly decreased and after 3 days it was below 2%. Volatilisation is directly related to the method by which the sewage sludge is applied to the soil (Cousins *et al.*, 1997). Also, contaminated soil particles can be transported directly to the plant surface by wind erosion or by splashing rain (Trapp and Matthies, 1997; Jones and Duarte-Davidson, 1997). Volatilization from sewage sludge-amended soil as a possible source of plant contamination by PAHs has also been confirmed by the relatively high content of light PAHs when compared to other compounds. Plant pollution by "heavy" PAHs, i.e. 4–6-ring, could be as a result of the second process mentioned above (wind erosion or splashing rain from sewage sludge-amended soil). There is little information on the behavior in soil of atmospherically derived particle associated semi-volatile organic compounds with their characteristically higher concentration (Smith and Jones, 2000). Studies by Guggenberger *et al.* (1996) showed 80% of the PAH burden to be in the silt (2–20 μm) and coarse clay (0.2–2 μm). Silt generally showed the highest concentrations of organic carbon related PAH concentrations, and there was an increase in the proportion of the high molecular weight PAHs as the size of the separates decreased.

The PAH content determined in the present study in plants grown on soils fertilized with sewage sludge was low (Tables II–V) when compared to data quoted by other authors. Data presented in the literature concerns mainly plant pollution by PAHs in the areas subjected to intense human influence (Voutsa and Samara, 1998;

Kipopoulou *et al.*, 1999; Nadal *et al.*, 2004; Tao *et al.*, 2004), near sources of PAH emission, e.g. large scale chemical fires (Meharg *et al.*, 1998) or near aluminium smelters (Larsson and Sahlberg, 1981). When analyzing PAH content in some selected plants grown on soils subjected to intense anthropopressure, Voutsa and Samara (1998) found that the PAH content ranged from 25 to 290 μg kg^{-1}. Similar results were obtained also by Camargo and Toledo (2003) for vegetables grown in Brazil. The content of 16 PAHs determined by Larsson and Sahlberg (1981) in lettuce grown in the vicinity of a highway ranged from 17 to 90 μg kg^{-1}. Kipopoulou *et al.* (1999) found PAH median values of 161 and 42 μg kg^{-1} in lettuces and cabbages, respectively, grown in the industrial area of Thessaloniki, Greece. Grova *et al.* (2000) reported a concentration of 51.8 μg kg^{-1} for 8 PAHs in rural grass samples, whereas Malawska and Wilkomirski (2001) found a concentration of 65.4 μg kg^{-1} for 14 PAHs in dandelion collected in the area of the railway junction. Relatively high PAH content levels were also noted in plants grown close to sewage treatment plants and in areas flooded by communal sewage (Jiries *et al.*, 2000; Tao *et al.*, 2004) as well as in the neighbourhood of petrochemical plants (Bakker, 2000).

PAH contents, observed by the present authors to be lower than the values mentioned earlier in their study, are related to the fact that the studies quoted concern plants grown in soils heavily polluted by the compounds studied. For example, the total of the 16 PAHs determined by Tao *et al.* (2004) in vegetables ranged from 500 to 1700 μg kg^{-1} while the PAH content in the soils was at a level of over 6000 μg kg^{-1}. Considerably higher values for the total of the 16 PAHs were also determined in vegetables by Fismes *et al.* (2002) (from 40 to 2600 μg kg^{-1}, depending on the plant part). However, the level of pollution in the soil in which these plants were grown ranged from 43 up to as much as 12000 mg kg^{-1}.

Even though there were certain differences between the results obtained by the present authors for the PAH content in plants and the data quoted in the literature, the percentage distribution of individual PAHs as well as the factor of bioconcentration are similar to the information quoted by other authors (Wild and Jones, 1992; Kipopoulou *et al.*, 1999; Fismes *et al.*, 2002; Gao and Zhu, 2004; Tao *et al.*, 2004). Almost all researchers point to the predominant contribution of 2 and 3-ring PAHs in plants despite the fact that in the soil in which these plants are grown, the above compounds are at a lower content level than the 5 and 6 rings PAHs with high molecular weight (Tao *et al.*, 2004). A high content of PAHs with low molecular weight is determined by the properties of these latter compounds, i.e. water solubility, volatility and bioavailability for plants (Wild and Jones, 1994), as opposed to 5 and 6 rings PAHs which are strongly bound to the soil particles and which, in turn, limits their transportation by the roots. The values of the bioconcentration factor for PAHs in plants observed by other researchers are within a very wide range, viz. from 0.001 to 0.4 for individual PAHs (Kipopoulou *et al.*, 1999; Fismes *et al.*, 2002; Gao and Zhu, 2004). The results obtained in this study are contained within the above given range.

Increase in BCF_a after the first 6 months and the associated decrease in sludge-amended soil total PAH content only during the first 6 months (Figure 2) it may point to the fact that the readily available PAH fraction was then taken up. The recent studies carried out by Ghosh *et al.* (2001, 2003) and Talley *et al.* (2002) showed that a particular fraction of the total PAH content can be desorbed within a relatively short period of time. A second fraction is more recalcitrant, requiring much longer to desorb and become bioavailable.

The lowering of the bioconcentration factor both in relation to the sewage sludge dose applied and time can be related, on one hand, to the biodegradation of these pollutants, and on the other hand, to the limiting of their bioavailability. An increase in the sewage sludge dose results also in an increase in the content of organic matter (Figure 2b) which, in accordance with generally accepted knowledge, is characterized by high affinity to hydrophobic organic pollutants such as PAHs (Sims and Overcash, 1983; Duarte-Davidson and Jones, 1996; Nieman *et al.*, 1999; Semple *et al.*, 2003). An increase in the organic matter content can limit the bioavailability of pollutants for plants to a considerable degree (Duarte-Davidson and Jones, 1996; Pignatello and Xing, 1996; Semple *et al.*, 2003). Bellin and O'Connor (1990), observed the negative relation between the content of organic carbon and the coefficient of the bioconcentration of pentachlorophenol in soil fertilized with sewage sludge. The highest BCF values determined in the present research study were characteristic of sewage sludge doses of 30, 75 or 150 Mg ha^{-1}. In almost all the cases of the PAHs determined, the lowest BCF values were observed in the experimental treatment with the highest sewage sludge doses (data not presented). This was especially true for the 5 and 6-rings PAHs which, as is well-known (Sims and Overcash, 1983), are characterized by the highest log K_{oc} values which indicate the affinity of hydrophobic pollutants to organic matter (Pignatello and Xing, 1996; Luthy *et al.*, 1997; Semple *et al.*, 2003).

While studying the relationships between the BCF values and the properties of the compounds in question which can significantly influence their uptake by plants, i.e. solubility in water (S), coefficient of partition log K_{oc} and log K_{ow}; statistically significant relations were most frequently observed in the case of S and, less frequently, in the case of log K_{oc}. The highest frequency of the occurrence of positive correlations, in the case of solubility in water, and the occurrence of negative correlations, in the case of log K_{oc} and log K_{ow}, was observed on the first study term and for willow leaves (Figure 6). As far as the shoots are concerned, the correlations described were neither very numerous nor very clear. Studies by other authors (Tolls and McLachlan, 1994; Kipopoulou *et al.*, 1999) showed similar relations. It is generally assumed that the solubility of organic compounds in water is decisive for their uptake by plants. Pollutants dissolved in water can be transported in the soil and then, through the roots, translocate to other planttissues. On the other hand, log K_{oc} and log K_{ow} show an affinity of the compound to organic matter which is a strong adsorbent limiting its movement in the soil environment. The values calculated, point to a clear regularity compatible with the rules stated above.

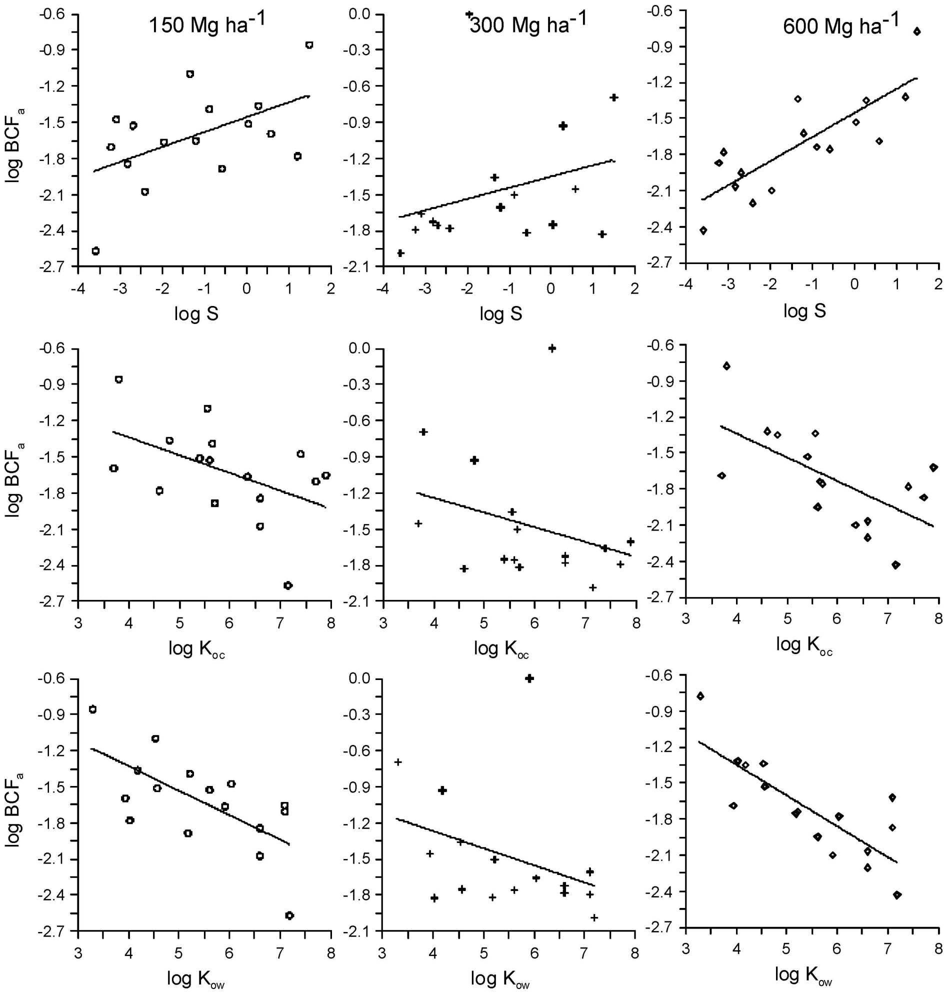

Figure 6. Logarithm of the soil-to-plant bioconcentration factor (BCF_a) for willow leaves versus the logarithms of solubility (S), organic carbon partition coefficient (log K_{oc}) and octanol-water partition coefficient (log K_{ow}).

Conclusion

Based on the results presented in this study, it is not possible to draw an unambiguous conclusion that a willow grown on a soil fertilized with sewage sludge takes up PAHs via the roots and translocates them to other plant parts. However, in the present study, the clear influence of the PAH content in the sewage sludge-amended soil on the content of these compounds in plants has been confirmed. The above statement has been confirmed by a higher content of 2–3 ring PAHs in plants grown on soil fertilized with sewage sludge as compared to those plants grown on control soil and also by the appearance of PAHs with high molecular weight which were not observed in plants grown on control soil. The content of the PAHs was at a low level in comparison to the PAH content in plants from areas subjected to moderate human influence. The PAH content in plants clearly depended on the sewage sludge dose applied and term of investigation which is especially visible in the evaluation of the bioconcentration of the total PAH in willow. BCF adjusted by control values were highest for the initial sampling (6 months) and did not show a significant temporal relationship. Increase in BCF after the first 6 months and the associated decrease in sludge-amended soil total PAH content only during the first 6 months it may point to the fact that the readily available PAH fraction was then taken up.

Acknowledgments

We appreciate the invaluable comments and suggestions given by the two anonymous reviewers. Financial support from the State Committee for Scientific Research (MNiI, Warsaw). Project no. P06H 062 20 is gratefully acknowledged. P. Oleszczuk received a granted by the Foundation for Polish Science and the Foundation Pro Scientia et Vita.

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