Polycyclic aromatic hydrocarbons in soil and selected plants

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ABSTRACT

The influence of soil load with polycyclic aromatic hydrocarbons (PAHs) on their contents in selected plants was investigated. A set of experiments was realized in three years. The influence of extreme soil load with PAHs (soil contaminated by floods and sludge application) on their content in plants was observed in a pot trial. A laboratory column extract trial investigated PAHs transfer from the soil into soil solution in different conditions. The results showed that the transfer of PAHs into plants is influenced mainly by chemical characteristics of the substances (the number and position of aromatic nuclei); by soil characteristics (content and quality of soil organic matter) and by plant characteristics (plant species and plant bodies). The roots of tested plants were loaded with PAHs thanks to the transfer of less-nuclei compounds (2–3 nuclei) in soil solution into the roots and thanks to the binding of more nuclei compounds (4–6 nuclei) on organic substances in epidermis and primary bark of roots. These results were confirmed by a laboratory column trial.

Keywords: polycyclic aromatic hydrocarbons (PAHs); soil load; mobility; plant production

Polycyclic aromatic hydrocarbons (PAHs) are substances with of 2 and more aromatic nuclei and belong to the group of persistent organic pollutants (POPs). Attention is paid to this group of contaminants in many compartments of the environment, including soil. The persistence of different compounds of POPs and PAHs can differ according to the environment. Many substances decompose in soil in the span of a few years (naphtalene, anthracene) and some substances, such as benzo(ghi)perylene, are persistent (Starke et al. 1991) in spite of degradation processes in the soil environment (microbial activity, photodegradation, hydrolysis etc.).

Increased PAHs content in agricultural soil does not usually lead to acute intoxication of humans. On the other hand, health problems can be sometimes connected with symptoms of chronic intoxication. Carcinogenic, mutagenic, terratogenic effects, genotoxicity, an increased level of cholesterol in the blood or reproduction defects were observed after long-term POPs exposure and confirmed by toxicological experiments (Janošek et al. 2007).

In the Czech Republic, research on the most important POPs compounds in agricultural soils was begun in the early 1990s. The compounds in the 'Dutch List', including 13 PAHs compounds, were accepted and the proposal of the limit values of POPs in agricultural soils (background values) for the legislation purposes was prepared (Němeček et al. 1996).

The identification of the main inputs of PAHs into soils is the result of long-term observation of POPs in Czech agricultural soils (Podlešáková et al. 1998, Vácha et al. 2003). The main sources of increased soil load with PAHs are imission out-puts of industrial areas, emission out-puts from local furnaces in settlements, contaminated water in fluvial areas of some rivers, sludge and sediment application on agricultural soils.

The use of toxicity equivalents that maintain the toxicity of each compound seems to be a more progressive approach for the evaluation of the load in comparison with sum contents. The toxicity equivalents were derived from human toxicological studies focused on carcinogenic risk. The value of toxicity equivalent 1 is given to the most toxic compounds (benzo(a)pyrene from PAHs group). The total value is calculated as the sum of the products of equivalents and the contents of each compound. The resulting value is used as a sum of toxic equivalents in the case of PAHs (TEQ PAHs sum).

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Limit values of POPs (including PAHs) in agricultural soils are included in the directive of the Ministry of Environment of the Czech Republic (Directive No. 13/1994 Coll). These limit values were not derived from the values relevant for the soil in the Czech Republic and were based on the correction of external samples. As the suitability of these limit values for the evaluation of the load of Czech agricultural soils is problematic, an update of the directive was proposed. New limit values maintain the background values of POPs in Czech agricultural soils (Němeček et al. 1996) and are proposed as 'preventive limits' for legislative use. At present, the higher level of limit values, based on POPs transfer into plant production, is not available; their development is complicated by different level of persistence of individual compounds in soil and their decomposition and transformation into other forms. The microbial decomposition of PAHs in the soil seems to be one of the most important factors regarding degradation processes. Thiele and Brümmer (1999) defined the part of PAHs in the soil that could be decomposed by microbial activity, by using different chemical extract agents (a solution of acetone and toluene, tenzides). They compared the natural decline of PAHs in soil over a five-year field trial and the contents of PAHs in individual extract agents.

The observation of the transfer of PAHs into plants is complicated by other factors. Krauss et al. (2005) compared atmospheric and biological sources of plant loads by PAHs in a tropical area. Thiele and Brümmer (2002) confirmed the formation of PAHs in soil by plant material decomposition. They observed formation of 4–6 nuclei PAHs predominantly.

The behaviour and distribution of individual PAHs compounds of different molecular weights in the environment can differ markedly. Brandt et al. (2002) presented the information on low availability of compounds with a high value of K_{ow} partition coefficient (octanol/water) for plants. These compounds have high affinity to fat and the value of bioaccumulation in the animal tissues increases rapidly. The affinity to soil organic matter (K_{oc} partition coefficient) of 4–6 nuclei PAHs is two or three times higher than that of 2–3 nuclei PAHs (Holoubek 2005). It was investigated that the transfer of the chemicals with high hydrophobicity trough plant tissues occurs predominantly through pore water (Trapp et al. 2007) and the effect of 'growth dilution' influencing bioconcentration of PAHs in potatoes was described. Trapp (2002) presents a model for prediction of the transfer of PAHs from soil into plant tissues. He concluded that for the substances with low or medium lipophilicity (log K_{ow} < 2) there was no difference between developed dynamic model and equilibrium approach. For lipophilic substances equilibrium approach can be used only for prediction of their contents in the peel (tested on carrot), while for prediction of their contents in the core a dynamic (steady-state) flux model is more realistic. Mikeš et al. (2009) analysed the load of roots, bulbs and shoots of radish planted on contaminated soil by polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). They observed a decrease of bioaccumulation factors (BCF) of substances with increasing log K_{ow} value in the bulbs. Opposite root BCF values were constant and did not correlate with log K_{ow}. The authors confirmed the influence of chemicals concentrations in the air on the load of shoots of plants and the used prediction model of Trapp (2007) correlated with the measured values. Based on analytical data they concluded that soil resuspension and subsequent deposition of soil particles on leaf surfaces is dominant transport pathway and they also described entering of the compounds from the air into plant tissues of the shoot and even bulbs of radish. In spite of high affinity of lipophilic substances to soil organic matter some authors observed an increased load of plants by 4–6 nuclei PAHs after sludge application into soil in spite of a high content of soil organic matter (Oleszuk and Baran 2005). Trapp (2002) presents that the absorption of lipophilic organic substances to root is comparable with their absorption to soil organic carbon. At the same time the roots of plants can influenced the fate of PAHs in the soil. Rezek et al. (2009) used some plants (*Betula pendula*, *Morus rubra*, *Lolium perenne*) for bioremediation of contaminated soil by PAHs. The contents of fluoranthene and pyrene markedly decreased (to 50%) after 1 year of plant cultivation. They even observed successful degradation of benz(a)pyrene. On the contrary, benzo(ghi)perylene and indeno(cd)pyrene were very persistent in soil and practically undegradable. The potential of phytoremediation in soils loaded with PAHs was confirmed by Cheema et al. (2010) in the study focused on phenantrene and pyrene degradation by combined plant cultivation. The other ways to decrease PAHs in soil can be soil washing with some organic substances, like fatty acid methyl esters (Gong et al. 2010), vegetable oils (Yap et al. 2010) or products of mushroom metabolites (Li et al. 2010), for example.

Holoubek (2005) specifies the intake of PAHs by plants via the intake by roots from the soil so-

lution (depending on the plant water regime and the content of lipid compounds in the root), the absorption of PAHs on root surface, the absorption of volatilised PAHs (from the soil) on the shoot and the absorption of PAHs on leaves of plants (from imission fall-outs). Some PAHs could be synthesised in plant metabolisms.

The limit value for the content of benzo(a)pyrene in some foods was brought forward to the European Union legislation (Directive 208/2005/EC). The limit of benzo(a)pyrene in fats and oils for direct consumption is 2 μg/kg and the limit for children's food is 1 μg/kg. The regulation of the Czech Ministry of the Health, No. 305/2004 Coll., limits the values for nine PAHs compounds in edible oils with the maximum of 2 μg/kg for each individual compound.

MATERIAL AND METHODS

The study was performed on the basis of three experiments: pot trial, field plot trial and laboratory column experiments.

Pot trial. The trial was based in 2004 (October) and sieved for the first time in 2005 (April). The trial was finished in autumn 2007. The experimental soil contaminated by floods with increased PAHs contents was taken out on the basis of terrain monitoring as loaded variant L1 (Modal Fluvisol – FLm). Sludge from a wastewater factory was used as another source of increased PAHs contents. The mix of the sludge and the soil used in the control variant (Arenic Cambisol – CAa) in a mass rate 1:1 simulated the increased load of soil by PAHs after the sludge application (loaded variant L2). The soil used as the control variant (Arenic Cambisol – CAa) with PAHs contents under background values has comparable chemical and physical characteristics (pH, Cox, particle-size composition) as loaded variant 1. The sum of PAHs is comparable in the soil of L1 and L2 variants but TEF value is evidently higher in the soil of L2 variant with sludge application because of increased content of the substances with carcinogenic effect in used sludge. The characteristics of soils and PAHs contents are presented in Table 1. The contents of potentially risk elements in used sludge were under the limits of regulation of the Czech Ministry for Environment No. 504/2004 Coll. The same sludge was used in field trial in extremely increased dose and we had to fulfil the criteria for sludge contamination with limited contaminants at least. From this viewpoint the phytotoxicity due increased contents of risky elements in used sludge was eliminated. The pot trial was placed in a fenced area and was performed in four replications.

Mitscherlich pots filled with 6 kg of sieved soil were used. Soil acidity was treated on the value of 6.5 pH (dolomitic limestone, Ca and Mg in the form of $CaCO_3$ and $MgCO_3$) and basic fertilisation with N, P and K was realised. Soil samples for PAHs analyses were taken out after 20 days from the trial establishing. The pots were seeded with radish (*Raphanus sativus* var. *radicula*), Duo variety in 2005, with carrot (*Daucus carota*), Nantes 3 variety in 2006 and with parsley (*Petroselinum crispum*), Dobra variety in 2007. Only five plants were left in every pot after germination.

Seeded pots were periodically irrigated with a constant volume of drinking water (500 ml). The samples of soil and plants for PAHs analyses were taken out after the termination of the vegetation period (second week in October). Plant samples were analysed in the form of washed hypocotyles (radish 'bulbs') and washed and unwashed shoots. Plant washing was done with demineralised water in laboratory conditions. The samples of carrot and parsley were analysed in the form of washed roots separated into two parts – primary bark (external part) and central cylinder (inner part). Only roots of comparable size (carrot roots of cca 15 cm length and 2.5 cm thickness and parsley roots of cca 8 cm length and 1.5 cm thickness) were used for the analyses. The differences between the size of carrot and parsley roots were caused by lower yield of parsley plants.

Field plot trial. The field trial was located in the clean area of the Czech-Moravian Highlands (Jihlava district) and was based on a typical

Table 1. The characteristics of soils and PAHs contents

Soil	Variant	Particle-size class	Substrate	Σ PAHs	Σ TEF PAHs
				(mg/kg)	
Typic Fluvisol	L1	3	alluvial river sediments	21.05 ± 1.39	2.462 ± 0.20
Arenic Cambisol + sludge	L2	3	gneiss	24.59 ± 1.21	6.218 ± 0.51
Control – Arenic Cambisol	Co	2	terrace sands	0.454 ± 0.08	0.058 ± 0.02

Cambisol (CAm, acid variety, on gneiss). Sludge application (sludge identical with pot trial) in the dose of 25 kg/m of dry matter was used for increased PAHs load simulation in April 2005. The extent of individual plots was 2 × 2 m. The soil was seeded with mustard (*Sinapis alba*) in 2005 and 2006 and with carrot (*Daucus carota*) in 2007 in four replications. The samples of soil and plants were taken after the harvest (October). Plant samples were used in the form of washed and unwashed shoots in the first year and unwashed shoots in the second year of the trial. Only roots of carrot were used for PAHs analysis in the third year. The roots were separated into three parts, epidermis, primary bark and central cylinder.

Laboratory column experiment. The column experiment focused on the observation of the transfer of PAHs from the soil into the solution was realised in 2006. Plastic columns with an outlet in the bottom part were used. The bottom part was fitted with a 0.2 mm sieve. The columns were filled with 100 g of soil identical with the pot trial. The soil in the columns was saturated with demineralised water (the value of field capacity) and then was leached with 200 ml of solutions (in three repetitions) during 24 h in the following order:

demineralized H_2O

demineralized H_2O acidified by H_2SO_4 on the value pH 3

a 0.2% solution of the tenzide – $C_{12}H_{25}NaO_4S$ in demineralized H_2O

The use of a tenzide solution was derived from the work of Thiele and Brümmer (1999), which defined the mass fraction of PAHs extracted from the soil by a 0.2% tenzide solution as microbial degradable. The extractability by water and 0.2% and 4% tenzide solutions were compared in laboratory conditions. The extraction proceeded gradually and the weakest extract agent, the demineralized water, was firstly used. The extraction of the same sample by acidified water (pH 3) followed and the 0.2% tenzide solution extraction was the last step in this approach.

The obtained extract was centrifuged (2500 rpm) to separate the colloidal phase from the solution. Only 33% of PAHs contents flowed in an aqueous phase and the prevailing mass of PAHs was bound to the colloids (Holoubek 2005). The treated extract was analysed in a commercial accredited laboratory by standard methods of a PAHs analysis. Laboratory column trial was replicated three times and median values are used in the presentation.

Experimental data were processed by elementary statistics using the Excel programme (average, geomean, standard deviation, median, maximum, minimum, Pearson correlation coefficient).

PAHs analysis. The laboratory determination of the PAHs contents in the soils, plants and solution was realised in the accredited laboratory Aquatest JSC. The solid sample analysis comprises the exsiccation using waterless sulphate and the extraction procedure in the acetone solution. The raw extract is analysed without purifying. PAHs are determined by the high-performance liquid chromatography (HPLC) with fluorescence detection (mobile phase – acetonitrile/water). One instrument measured some PAHs portions during isocratic elution under invariable wavelength and the second plant detected the other PAHs portion on equal terms. Two detectors in series assemble the instrument configuration, and thus two different wavelengths are involved in the detection. Such procedure minimises the difficulties with the gradient elution and with the alteration of the wavelength setting during analysis by the division of unpurified samples.

The concentration levels of individual compounds, the sum values of the compounds (the PAHs sum), the sum value of 2–3 nuclei PAHs and of 4–6 nuclei PAHs were used for the assessment of the load of soils and plants. The sum of toxic equivalency factors for PAHs (the TEF PAHs sum) was involved as well to take into account various toxicological characteristics of individual PAHs compounds. The TEF PAHs sum is defined as the sum of the products of the concentration of each compound multiplied by the toxic equivalent value for carcinogenic compounds. The following compounds were used:

Benzo(a)pyrene and Dibenzo(a,h)anthracene – toxic equivalent value = 1

Benzo(a)anthracene, Benzo(b)fluoranthene and Indeno(1,2,3-cd)pyrene – toxic equivalent value = 0.1

Benzo(k)fluoranthene – toxic equivalent value = 0.01

The soil organic matter analysis. The selected characteristics of the content and quality of soil organic matter (SOM) were assessed in the central laboratory of the RISWC. The characteristics are defined below.

C_{org} – organic carbon indicative of the carbon content in primary SOM. It may be used for the humus content calculation ($1.72 \times C_{org}$). The determination procedure is based on the chromic acid oxidation of organic carbon under the sulphuric acid abundance and elevated temperature. Unexpended chromic acid is determined by the

iodometric method. The accredited method SOP 4/02 is the modification of the ISO 14235 norm. The assay of weakly and tightly bounded humus materials includes the determination of the humic acid carbon (C-HA), fulvic acid carbon (C-FA), humus matter carbon (C-FA + C-HA) and the assessment of the colour coefficient (Q4/6) indicating the humus quality. The determination procedure is based on the sample extraction using mixed solution of sodium diphosphate and sodium hydroxide, the carbon contents (C-FA, C-HA) are determined by titration and the coefficient Q4/6 results from the photometry.

C_{ws} – water-soluble carbon, indicating the quality of primary SOM (bioavailable carbon for soil microorganism). Laboratory determination consists in an hour sample extraction using 0.01M $CaCl_2$ solution (1:5 w/V) and in the determination of oxidizable carbon in the filtrate evaporation residue through the heating of filtrate with chromium sulphuric acid and the subsequent titration with Mohr's salt.

C_{hws} – hot water-soluble carbon, being similar in the assessment purpose to water-soluble carbon. After an hour soil sample boil in 0.01M $CaCl_2$ solution (1:5 w/V), the oxidizable carbon in filtrate evaporation residue is determined through the heating of filtrate with chromium sulphuric acid and the subsequent titration with Mohr's salt.

RESULTS AND DISCUSSION

The characteristics of content and quality of soil organic matter in the soil of the pot trial are presented in Table 2.

The highest values of C_{org} content were detected in the soil of L2 variant, the soil of L1 variant reached almost the same C_{org} value as the Co variant.

The content of hot water-soluble carbon (C_{hws}) indicated the content of an active micro degradable carbon fraction within the total organic matter. The highest value of C_{hws} was observed in the soil with sludge-L2 variant; control variant had a value ten times lower, while the lowest value was detected in the soil of L1 variant.

Similar ratios of values could be found in the case of water-soluble carbon (C_{ws}) that also indicated the quality of primary organic matter (Kolář et al. 2009). The highest total carbon content of humic substances (C-HS) was observed in the soil with sludge-L2 variant and the lowest in the soil of L1 variant. The quality of humic substances, according to the HA/FA ratio is comparable to that of the soil of L2 variant and control variant, where the predominance of humic acids was detected. A balanced humic and fulvic acid ratio was observed in the soil of L1 variant. The values of the Q4/6 coefficient confirm the lowest quality of humus substances in the soil of L1 variant, and the similar humus quality of the soils of the control variant and the variant with sludge – L2.

The PAHs total contents in soil were comparable between L1 variant and L2 variant, with concentrations moving from 20 000 μg/kg up to 30 000 μg/kg. Differences between the contents before and after the vegetation period were observed simultaneously. An increased PAHs content was detected in poor and sandy soil of L1 variant after the vegetation period, in comparison with that previously considered. This could be connected with PAHs syntheses by plant material decomposition in the soil (Thiele and Brümmer 2002, Holoubek 2005). On the contrary, the content of PAHs in the soil of L2 variant was lower after the vegetation period in the first year. The existence of soil heterogeneity could not be excluded in this case, because a perfect interfusion of the sludge with the soil is not realistic. A corresponding trend shows the values of TEF PAHs, indicating carcinogenic risk in the soils of the considered variants. The soil samples were taken only after the vegetation period in the following years and an increase of the sum of PAHs in L1 variant (more than 40 000 μg/kg) and a slight decrease in L2 variant (18 000 μg/

Table 2. The content and quality of soil organic matter, pot trial

Variant	C-HA	C-FA	HA:FA	C-HS	Q4/6	C_{org}	C_{ws}	C_{hws}
	(pyro) %			(pyro) %	(pyro)	(%)	(mg/kg)	
Typic Fluvisol	0.06	0.07	1:1	0.13	4.3	1.6	34	175
Arenic Cambisol + sludge	0.83	0.5	1:0.6	1.33	2.7	5.23	204	1944
Control – Arenic Cambisol	0.33	0.2	1:0.6	0.53	2.3	1.66	89	489

C-HA – humic acid carbon; C-FA – fulvic acid carbon; C-HS – humus substances carbon; Q4/6 – colour coefficient; C_{org} – organic carbon; C_{ws} – water soluble carbon; C_{hws} – hot water soluble carbon

Table 3. The median values of PAHs content in washed and unwashed radish shoot planted in pot trial

	PAHs (µg/kg of dry matter)												
	Fl	P	Ph	B(b)F	B(a)A	A	I(cd)P	B(a)P	B(k)F	B(ghi)P	Ch	N	D(ah)A	Sum PAHs
L1 unwashed	1000 ± 117.4	741 ± 29.32	644 ± 26.92	446 ± 16.44	402 ± 13.73	129 ± 17.18	232 ± 17.01	493 ± 34.62	231 ± 13.06	455 ± 23.28	403 ± 18.45	100 ± 10.7	68 ± 8.28	5344 ± 331.84
L2 unwashed	120 ± 45.95	60 ± 7.46	40 ± 7.87	130 ± 15.82	50 ± 10.46	< 10	90 ± 19.42	130 ± 15.05	80 ± 11.98	140 ± 20.36	90 ± 16.42	100 ± 17.84	< 10	< 1050
Control unwashed	90 ± 11.15	60 ± 9.33	70 ± 9.75	20 ± 8.67	20 ± 8.72	< 10	< 10	20 ± 8.66	< 10	20 ± 4.69	30 ± 12.11	100 ± 12.08	< 10	< 470
L1 washed	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 130
L2 washed	15 ± 2.45	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 135
Control washed	< 10	< 10	15 ± 2.87	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 135

L1 – Typic Fluvisol; L2 – Arenic Cambisol + sludge; Control – Arenic Cambisol

kg) were observed in the second year. The PAHs contents were comparable in the soil L1 variant between the second and third year and decreased slightly in the soil of L2 variant in the third year. Comparable values of TEF PAHs in these variants suggest the hypothesis of a more rapid decrease of less-nuclei and less carcinogenic PAHs substances and confirm the increased persistence of more-nuclei PAHs in the soil. The contents of PAHs in the soil of the control variant moved under their background values in Czech soils (1 000 µg/kg) in the intervals from 400 µg/kg up to 600 µg/kg even if a slight increase of their contents after the vegetation period was observed. This increase could relate with described effect of the influence of air load with PAHs on soil content in time (Mikeš et al. 2009).

Differences between PAHs contents in the plants were also observed. The increase of PAHs contents in unwashed radish shoots in L1 variant in comparison with L2 variant and especially with control variant was statistically significant at the 0.05 significance level. The contents of PAHs in the radish shoots decreased rapidly after washing the shoots and they are comparable in all variants of the trial. The increased PAHs contents in radish shoot in L1 variant – Typic Fluvisol in comparison with L2 variant – Arenic Cambisol + sludge relates with higher soil particles mobility in sandy soil very probably. The data are presented in Table 3. The results confirm the predominance of contamination on the surface of the plant shoots (dust articles in the air, surface contamination by soil particles during the irrigation) in comparison with the root intake. In spite of this finding the trend of a PAHs content increase in radish bulbs (hypocotyles) in the loaded variants (L2 variant < L1 variant) was observed. The values of TEF PAHs in radish roots showed similar trends, but on the other hand, PAHs were not analysed in the peel and inner part of radish bulbs and in the hair roots of radish separately where the differences in the accumulation of the substances depending on log K_{ow} value were described by some authors (Trapp 2002, Mikeš et al. 2009).

The load of unwashed radish shoots in L1 variant (493 µg/kg of dry matter) and L2 variant (130 µg/kg of dry matter) must be evaluated as extremely increased in comparison with the limit value of benzo(a)pyrene (2 µg/kg) in food for direct consumption (Directive 208/2005/EC). The contents of benzo(a)pyrene decreased under the detection limit (10 µg/kg of dry matter) after washing the shoots of all the variants. Increased contents of benzo(a)pyrene were also detected in radish bulbs in L1 variant (42 µg/kg of dry matter). The load of radish bulbs in L2 variant reached the value of 10 µg/kg of dry matter of benzo(a)pyrene and the content of the control variant moved under the detection limit. The decrease of PAHs contents in radish bulbs after washing supports the hypotheses of the influence of soil load with PAHs on their contents in radish bulbs, although Mikeš et al. (2009) confirmed a significant influence of air load on radish bulbs.

Differences between PAHs and TEF PAHs sum contents in primary bark and central cylinder of carrot (second year of the trial) are presented in

Figure 2. The increased contents of PAHs in the central cylinder in comparison with the primary bark were observed in the control variant and especially in L1 variant. On the contrary, the ratios of PAHs contents between the upper and inner parts of the carrot root were opposite in L2 variant. We lean to the hypothesis about the root intake of some PAHs substances and their transfer to the central cylinder of the carrot root in the case of poor and sandy organic matter of Karvina fluvisol (L1 variant) loaded with PAHs. Trapp (2002) describes in his model of POPs transfer into plant the input from soil and output to plant stems of the substances with log K_{ow} < 2 with transpiration stream where no relevant difference between dynamic model and equilibrium approach was observed, also less lipophilic substances have higher tendency to enter plant tissues via vascular system. In spite of the fact that minimal log K_{ow} value for PAHs is almost 4 (3.92 for acenaphtene) the observed trend follow these principles. The PAHs and TEF PAHs sums in the central cylinder of the carrot in L1 variant reached increased values in comparison with the control variant, whereas these values in the primary bark are comparable. Only slightly increased contents of PAHs were detected in the central cylinder of the carrot root in L2 variant, but rapidly increased PAHs contents were found in the primary bark of the carrot root in this variant in comparison with the control variant. It could be concluded that stronger bindings of PAHs on soil organic matter in the variant with sludge are impaired in the rhizosphere zone, while mobilised PAHs are consecutively bound onto organic substances in the external layer of the carrot root (lipids, plant pigments, growth regulators etc.). The intensive binding of PCBs on carotene in carrot roots is presented by Bobovnikova et al. (2000) for example.

On the basis of the comparison of 2–3 nuclei PAHs and 4–6 nuclei PAHs contents in carrot roots (Figure 1), an increased transfer by root intake seems to be evident in the case of less-nuclei PAHs.

Marginal transfer of 4–6 nuclei PAHs into the central cylinder of the carrot root via the primary bark could be hypothesised, considering their increased contents in the primary bark of the carrot roots in all the variants. Trapp (2002) analysed that the contents of benzo(a)pyrene in peel of carrot was up to 100 times higher in comparison with the core. These trends were confirmed by the use of parsley samples in the third year of pot trial. Nevertheless, PAHs contents were higher in parsley roots in comparison with carrot roots very probably thanks to lower parsley yield – smaller size of roots. The effect of 'growth dilution' is presented by Trapp et al. (2007) and is described also by Mikeš et al. (2008). It means an increase of root surface in contrary to root volume that could lead to the increase of PAHs intake. The problem with the limit values of benzo(a) pyrene in plant products for direct consume seems to be evident in this case (Figure 2). These results also show that not only carotene (Bobovnikova et al. 2000) plays the important role in the bindings of organic pollutants in the root of plants. Especially more nuclei PAHs with higher log K_{ow} value are close to the phase equilibrium with soil in the peel of roots and the adsorption of lipophilic compounds to root surfaces is similar to the adsorption to soil organic matter (Trapp 2002).

The results of the laboratory column experiment confirm findings coming from the pot trials. The sum contents of 2–3 nuclei PAHs and 4–6 nuclei PAHs in extracts (µg/l) are presented in Figure 3.

Intensive wash of 2–3 nuclei PAHs from the soil of L1 variant was predominantly observed after the first extraction step, while marginal wash of PAHs from the organic bindings in the soil of L2 variant

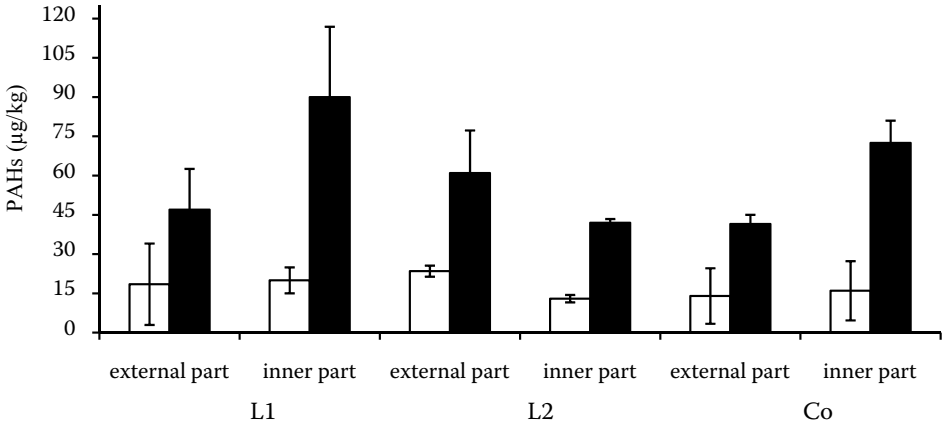

Figure 1. Comparison of 4–6 nuclei (white) and 2–3 nuclei (black) PAHs contents in carrot roots (µg/kg of DM)

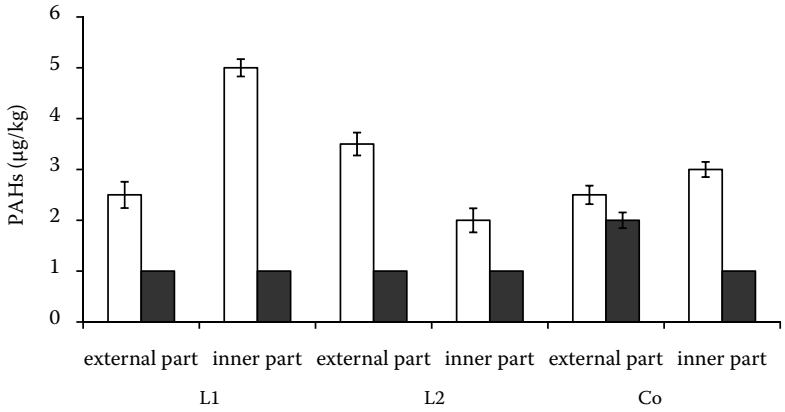

Figure 2. The contents od benzo(a)pyrene (white) and dibenzo(ah)anthracene (black) in parsley roots of pot trial (µg/kg of DM)

was detected. A decrease of PAHs content in the extract was observed after the second extraction step by the acidified water in L1 variant. A relatively low decrease was detected in the case of 4–6 nuclei PAHs thanks to their relatively low wash after the first extraction step related to their stronger binding with soil organic matter. The change of pH value of the solvent did not have a stronger influence on the extractability of PAHs from the soil with sludge (L2 variant). The use of the tenzide during the third extraction step led to the erosion of PAHs bindings on soil organic matter in the soil of L2 variant and massive wash of 2–3 nuclei > 4–6 nuclei PAHs from the soil to the extract was detected. The increased PAHs wash during the third extraction step was observed in L1 variant in comparison with the second extraction step, but this wash was significantly lower in comparison with the first extraction step. Simultaneously, the content of 4–6 nuclei PAHs was higher than the content of 2–3 nuclei PAHs in the third extraction and it could be concluded that lower weight PAHs with lower affinity to organic matter were washed predominantly during the first extraction step from the soil of L1 variant.

Other key factors could be derived from the comparison of the contents of individual compounds in the extracts (Figure 4).

The highest transfer of 2-nuclei naphthalene with a simple molecular structure from the soil into the liquid phase is visible. Its effect on the total PAHs content is the highest in the water extract from the soil of L2 variant and is the highest in soil organic matter and with the use of the weakest extraction agent. During the second extraction by acidified water, the proportion of naphthalene decreased at the expense of 2-nuclei fluorene with a more complexly structured molecule. The decrease of 3-nuclei phenanthrene at the expense of more-nuclei PAHs was also registered. It could be concluded that the second extraction by the acidified water slightly influenced the proportion of individual PAHs on the total PAHs content in the extract. The third extraction by the tenzide solutions strongly influenced this proportion, where the amount of more-nuclei PAHs increased.

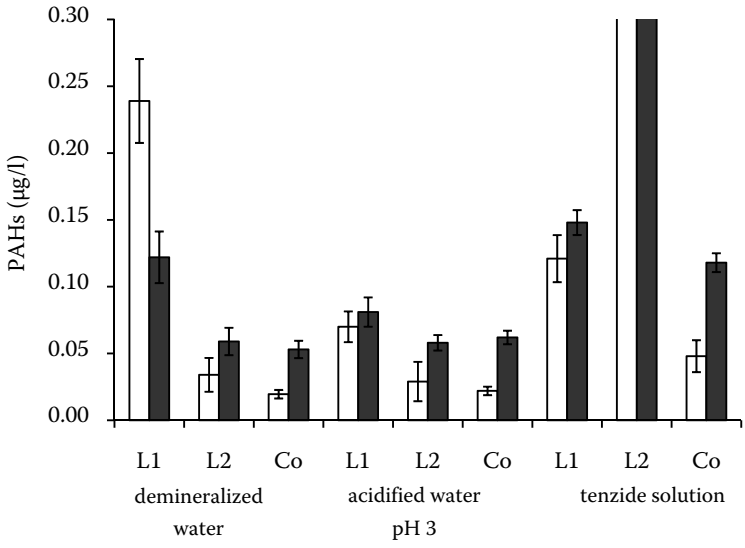

Figure 3. The sum contents of 4–6 nuclei (white) and 2–3 nuclei (black) PAHs in the extracts (µg/l)

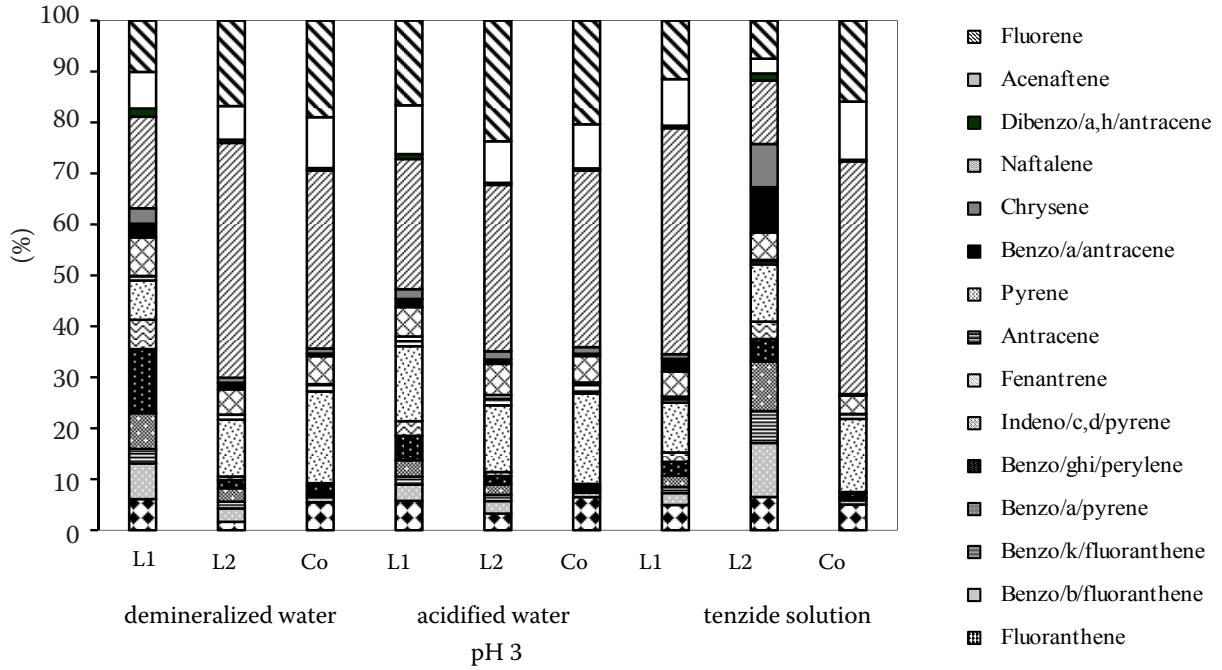

Figure 4. Comparison of the contents of individual PAHs compounds in the extract (%)

The mobilisation effect on PAHs by the tenzide solution from the soil with sludge (L2 variant) is comparable with the first extraction by L1 variant water, where the wash of non-bound PAHs from poor sandy soil was realised. The increased content of more-nuclei PAHs in the extract of L2 variant relates to the increased content in the sludge.

No verifiable changes of the proportion of individual PAHs during three extraction steps were observed in the case of the control variant (Figure 4) and low-nuclei PAHs were predominantly extracted. Cave et al. (2010) measured bioaccessible PAHs fraction in the soil (varied from 10–60%) and the multiple regression showed that the PAHs bioaccessible fraction could be explained using the PAHs compound, the soil type and the total PAHs to soil organic carbon content.

The results of field trial confirmed the trends of pot trial. The differences between loaded variant (sludge application) and control variant did not reached the level of pot trial because of lower dose of sludge in the soil of field trial. In spite of this fact, the dose of sludge was 50 times higher compared to the Czech regulation for sludge application on agricultural soils (Regulation of Ministry of Environment No. 504/2004 Sb). PAHs contents in shoots of mustard were comparable in loaded variant and control variant (550 μg/kg vs. 520 μg/kg of dry matter, respectively). The median values of sum of PAHs in carrot epidermis were 148 μg/kg on loaded variant with sludge compared to 75 μg/kg of dry matter in the control. PAHs contents in carrot primary bark were 105 g/kg vs. 60 μg/kg of dry matter and the PAHs contents in carrot central cylinder were 50 μg/kg vs. 55 μg/kg of dry matter, respectively.

On the basis of the above reported findings, it could be concluded that the increased load of the soil by PAHs influenced the root load of the tested plants. This load was caused by PAHs transfer (low-nuclei PAHs especially) into the plant vascular system by the soil solution or by PAHs binding (especially more-nuclei PAHs) to organic substances in the external root layer dependent on the soil properties. Different types of soil loads with PAHs, including sludge or sediment application, could cause the plant contamination with PAHs. It was confirmed by other authors (Oleszuk and Baran 2005, Zohair et al. 2005). Results of the pot trial led to conclusion that the plant shoots were predominantly loaded with over ground contamination. The similar trend was showed also in the field trial. The fulfilment of limit values of PAHs – especially of benzo(a)pyrene, in commodities for direct consumption could be problematic in the localities with increased PAHs load, with respect to the limit values in the Directive 208/2005/EC.

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