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DISTRIBUTION OF PHTHALIC ACID ESTERS IN AGRICULTURAL PLANTS AND SOIL

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Abstract

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The study observed the occurrence of di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in the soil and agricultural crops (*Triticum aestivum, Brassica napus, Zea mays*) and their distribution to the individual parts. For the experiment were selected 4 locations in central Moravia. At two locations (L1, L2) winter wheat (*Triticum aestivum*) was grown, at the third location (L3) winter oilseed rape *(Brassica napus)*, and at the fourth location (L4) flint corn *(Zea mays)*. The soil samples (n = 72) and whole plant samples (n = 78) were collected during the vegetation. The aboveground and underground parts of plants, ears, siliques and seeds were analyzed separately.

The values of DBP in soil at all areas ranged from 0.28 to 1.59 mg.kg^{-1} of dry matter and DEHP < 0.03 to 0.73 mg.kg^{-1} of dry matter. The soil at the L4 site, which was the most fertilized with organic fertilizers, especially manure, was detected significantly ($P < 0.05$) to show the highest values of both the PAEs (phthalic acid esters) observed (DBP_{L4} 1.59 ± 0.07 mg.kg^{-1} of dry matter, $DEHP_{L4}$ 0.73 ± 0.18 mg.kg^{-1} of dry matter).

Average concentrations measured in underground parts of monitored crops ranged from 1.68 to 14.26 mg.kg^{-1} of dry matter for DBP, and 0.12 to 10.34 mg.kg^{-1} of dry matter for DEHP. Values detected in aboveground parts were 0.03 to 8.84 mg.kg^{-1} of dry matter for DBP, and 0.25 to 4.59 mg.kg^{-1} of dry matter for DEHP. Average values of DBP in final products ranged from 0.05 to 0.83 mg.kg^{-1} of dry matter, and < 0.06 to 0.98 mg.kg^{-1} of dry matter for DEHP.

phthalic acid esters, di-n-butyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP)

Phthalic acid esters (PAEs) have long been used as plasticizers of polymer materials. These materials gain flexible features, improve adhesion and solubility. They are most often added to paints, varnishes, adhesives, sealants, coatings, floor coverings, wire insulation, or dialysis equipment, and blood transfusion bags.

In order to produce the above mentioned products, most used are the esters of di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP). In 2007, the EU produced about 10,000 tons of DBP and 341,000 tons of DEHP, while in the last decade the production has dropped by about one-third (ECHA, 2009).

Phthalates are released to the environment during their production and processing of plasticizer, particularly through wastewater. However, a greater degree of contamination of the environment is caused by their release from the goods or landfill leachate. The contamination of agricultural soils can occur through fallout from the air, oil leakages from farm machinery or frequent fertilization with organic fertilizers (Vikelsoe *et al.*, 2002; Cai *et al.*, 2006).

A plant receives nutrients, including toxic substances, particularly through the root system as well as above-ground green parts of plants. For some types of crops, a greater accumulation of phthalates in stems was demonstrated (Yin *et al.*, 2003). Species specificity in the sensitivity of crops to the income of phthalic acid esters is an important factor (Dueck *et al.*, 2003; Yin *et al.*, 2003; Kato *et al.*, 1980).

Incidence of di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in agricultural crops and their distribution in various parts of plants were observed in this study. The emphasis was placed on the transport of phthalates in plant crops which were further processed for food production or from feeding livestock. During the growing period, plants and soil were collected from four locations in central Moravia..

MATERIAL AND METHODS

Investigations were carried out at 4 locations in central Moravia selected on the basis of preliminary analyses of phthalates in soil. At two locations (L1, L2) winter wheat (*Triticum aestivum*) was grown, at the third location (L3) winter oilseed rape *(Brassica napus)*, and at the fourth location (L4) flint corn *(Zea mays)*. It the L1 and L2 locations in April, June and July (I–III. sampling) soil samples (n = 18) from arable layer by a probe rod and plant samples (n = 18) were collected. At the L3 site soil samples (n = 18) and plant samples (n = 24) were also collected in April, May, June and July (period I.–IV.). Samples of flint corn plants (n = 18) and soil samples (n = 18) from the site of L4 were obtained in June, September and November (period I.–III.). The sampled plants were properly cleaned, aboveground and underground biomasses were separated and lyophilization was implemented. The aboveground and underground parts of plants, ears, siliques and seeds were analyzed separately.

Lyophilized samples were extracted three times through a mixture of *n*-hexane:acetone (1:1). Solvent was evaporated on a rotary evaporator and dried with nitrogen from united extracts. For samples of winter oilseed rape, from the obtained extract lipid fraction was separated using gel permeation chromatography Bio Beads S-X3 column. As a mobile phase a mixture of dichloromethane:cyclohexane (1:1) was applied. For the purification of samples, hydrated sulfuric acid was used. Sample purified in this way was dissolved in 1ml of acetonitrile and analyzed by HPLC in harmony with protocols by Jarošová *et al*. (1999).

Detection and quantification was performed by HPLC analysis with a Cogent e-Column C18 (grain 5 µm, length 150 mm, mobile phase of acetonitrile:water 99:1) and UV detection at a wavelength of 224 nm. Chromatograms obtained were evaluated on a five-point calibration curve in the software of Agilent Chemstation for LC and LC / MS systems.

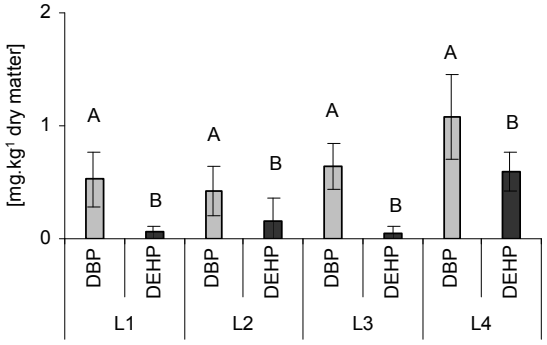
For statistical evaluation the software of Statistica 9.0 was used. The results were evaluated by Student's distribution methods.

RESULTS

During analyses concentrations of DBP and DEHP in the analyzed samples of soil and plant parts were detected (Table I.–III).

The values of DBP in soil at all sites ranged from 0.28 to 1.59 mg.kg^{-1} of dry matter and DEHP < 0.03 to 0.73 mg.kg^{-1} of dry matter. Concentrations below the detection limit were detected in soil at sites L1–L3 only at the first sampling (Tab. I).

The average values for the whole growing season (Fig. 1) show obvious significant difference ($P < 0.05$) between the observed concentrations of DBP (e.g. DBP_{L1} 0.53 ± 0.24 mg.kg^{-1} of dry matter) and DEHP ($DEHP_{L1}$ 0.06 ± 0.06 mg.kg^{-1} of dry matter) in soil at individual locations. In harmony with the hypothesis, higher ability of soil particles to adsorb of DEHP than DBP was confirmed ($P < 0.05$) for all sites (Fig. 1) in the experiment.

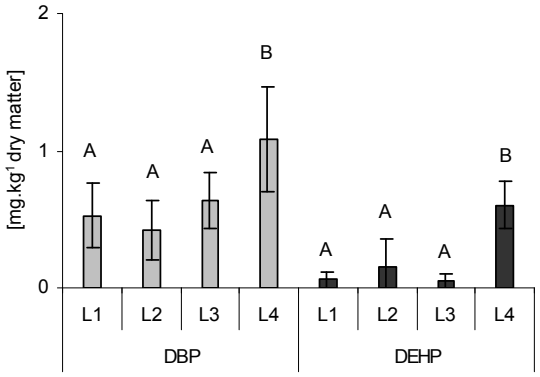


1: *Average concentrations of DBP and DEHP [mg.kg^{-1} of dry matter] in the soil at locations L1–L4 during the vegetation (A, B – average values labeled by another letter within the same factor differ significantly from each other at a confidence level of P < 0.05; factor: location)*

I: *Average concentrations (± standard deviation) of DBP and DEHP [mg.kg^{-1} of dry matter] in the soil at all selected locations L1–L4 from depth of arable profile (0–30 cm)*

	sampling I.		sampling II.		sampling III.	
Location	**DBP**	**DEHP**	**DBP**	**DEHP**	**DBP**	**DEHP**
L1	0.46 ± 0.39	< 0.03	0.64 ± 0.03	0.10 ± 0.01	0.51 ± 0.04	0.11 ± 0.04
L2	0.55 ± 0.29	< 0.03	0.37 ± 0.00	0.09 ± 0.01	0.28 ± 0.02	0.45 ± 0.05
L3	0.84 ± 0.10	< 0.03	0.45 ± 0.00	0.10 ± 0.00	0.53 ± 0.08	0.09 ± 0.02
L4	0.76 ± 0.16	0.4 3± 0.09	1.03 ± 0.03	0.70 ± 0.07	1.59 ± 0.07	0.73 ± 0.18

The detection limit of PAE was determined 0.03 mg.kg^{-1}
L1–L3: sampling I. = April, sampling II. = June sampling, III. = July; L4: sampling I. = June, sampling II. = September, sampling III. = November



2: *Comparison of the observed concentrations of DBP and DEHP [mg.kg^{-1} of dry matter] in the soil between sites (A, B – average values labeled by another letter within the same factor differ significantly from each other at a confidence level of P < 0.05; factor: concentration of DBP, concentration of DEHP)*

In our experiment, the resulting concentration of DBP, or DEHP did not differ significantly between locations L1, L2 and L3 ($P > 0.05$, Fig. 2). Only between these sites and the L4 site in all cases was a significant difference ($P < 0.05$). The soil at the L4 site, which was the most fertilized with organic fertilizers, especially manure, was detected significantly ($P < 0.05$) to show the highest values of both the PAE observed (Table I, DBP_{L4} 1.59 ± 0.07 mg.kg^{-1} of dry matter, $DEHP_{L4}$ 0.73 ± 0.18 mg.kg^{-1} of dry matter). One of the major sources of phthalates in agricultural soils are organic fertilizers.

Average concentrations measured in underground parts of monitored crops ranged from 1.68 to 14.26 mg.kg^{-1} of dry matter for DBP, and 0.12 to 10.34 mg.kg^{-1} of dry matter for DEHP (Table II, III). Values below the detection limit were found in winter oilseed rape (L3) in the final sampling for both the underground and aboveground parts. Values detected in aboveground parts ranged from 0.03 to 8.84 mg.kg^{-1} of dry matter for DBP, and 0.25 to 4.59 mg.kg^{-1} of dry matter for DEHP.

II: *I: Average concentrations (± standard deviation) of DBP [mg.kg^{-1} of dry matter] in the underground (U) and aboveground (A) parts of plants (**Triticum aestivum**, **Brassica napus, Zea mays**) during the vegetation in 2009*

DBP	A	U	A	U	A	U
	sampling I.		sampling II.		sampling III.	
Triticum aestivum (L1)	7.38 ± 1.50	13.47 ± 1.48	0.79 ± 0.11	9.03 ± 1.23	0.78 ± 0.07	6.04 ± 0.30
Triticum aestivum (L2)	8.84 ± 0.21	14.26 ± 0.11	2.17 ± 0.58	12.38 ±0.60	0.68 ± 0.07	7.52 ± 0.11
Brassica napus (L3)	3.50 ± 0.15	1.77 ± 0.09	2.71 ± 1.44	4.02 ± 0.19	1.70 ± 0.66	3.56 ± 0.21
Zea mays (L4)	0.03 ± 0.04	1.68 ± 0.23	0.34 ± 0.06	2.39 ± 0.35	0.94 ± 0.33	10.78 ± 0.56

The detection limit of PAE was determined 0.03 mg.kg^{-1}
L1–L2: sampling I. = April, sampling II. = June, sampling III. = July; L3: sampling I. = April, sampling II. = May, sampling III. = June; L4: sampling I. = June, sampling II. = September, sampling III. = November

III: *II: Average concentrations (± standard deviation) of DEHP [mg.kg^{-1} of dry matter] in the underground (U) and aboveground (A) parts of plants (**Triticum aestivum**, **Brassica napus, Zea mays**) during the vegetation in 2009*

DEHP	A	U	A	U	A	U
	sampling I.		sampling II.		sampling III.	
Triticum aestivum (L1)	0.53 ± 0.09	1.37 ± 0.49	0.74 ± 0.12	1.11 ± 0.26	2.38 ± 0.06	2.61 ± 0.06
Triticum aestivum (L2)	2.27 ± 0.84	0.68 ± 0.11	0.48 ± 0.06	6.22 ± 0.25	2.36 ± 0.21	7.38 ± 0.23
Brassica napus (L3)	0.42 ± 0.25	0.12 ± 0.02	0.59 ± 0.23	0.39 ± 0.16	0.25 ± 0.10	0.61 ± 0.02
Zea mays (L4)	2.37 ± 0.28	4.78 ± 0.94	4.59 ± 0.55	10.34 ± 0.01	1.84 ± 0.21	3.18 ± 0.35

The detection limit of PAE was determined 0.03 mg.kg^{-1}
L1–L2: sampling I. = April, sampling II. = June, sampling III. = July; L3: sampling I. = April, sampling II. = May, sampling III. = June; L4: sampling I. = June, sampling II. = September, sampling III. = November

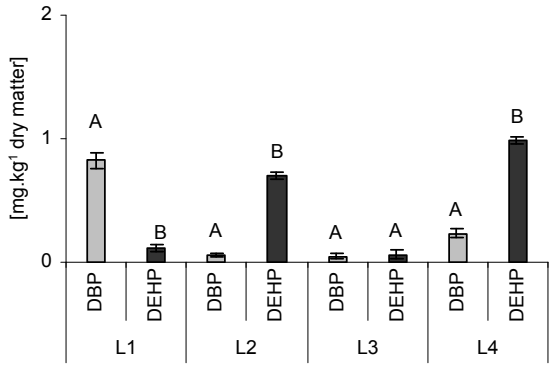
IV: *III: Average concentrations (± standard deviation) of DBP and DEHP [mg.kg^{-1} of dry matter] in the ears, siliques, grain and seeds of the monitored crops (**Triticum aestivum**, **Brassica napus, Zea mays**) in 2009*

	DBP	DEHP	DBP	DEHP
Triticum aestivum (L1)	0.42 ± 0.03^{a}	0.79 ± 0.10^{a}	0.83 ± 0.07^{b}	0.11 ± 0.03^{b}
Triticum aestivum (L2)	2.13 ± 0.25^{a}	0.41 ± 0.09^{a}	0.06 ± 0.01^{b}	0.70 ± 0.03^{b}
Brassica napus (L3)	1.53 ± 0.42^{c}	0.70 ± 0.04^{c}	0.05 ± 0.02^{d}	0.06 ± 0.04^{d}
Zea mays (L4)	0.03 ± 0.01^{e}	0.55 ± 0.15^{e}	0.23 ± 0.03^{f}	0.98 ± 0.03^{f}

The detection limit of PAE was determined 0.03 mg.kg^{-1}.
^{a} ears, sampling II. = April; ^{b} grain, sampling III. = July; ^{c} green siliques, sampling III. = June; ^{d} seeds, sampling IV. = July; ^{e} milky ripeness, sampling II. = September; ^{f} full ripeness, sampling III. = November

Mean values found in the ears, siliques and finished products of crops are given in Tab. IV. Average values of DBP in final products ranged from 0,05 to 0.83 mg.kg^{-1} of dry matter, and 0.06 to 0.98 mg.kg^{-1} of dry matter for DEHP.

For all crops, except for rape seed, grain content of DBP and DEHP differed significantly ($P < 0.05$) (L3, Fig. 3).



3: *Average concentrations of DBP and DEHP [mg.kg^{-1} of dry matter] in the grains of individual crops (A, B – average values labeled by another letter within differ significantly from each other at a confidence level of P < 0.05*

The highest content of DBP in grain was detected in winter wheat (L1) 0.83 ± 0.07 mg.kg^{-1} of dry matter and DEHP in flint corn (L4) 0.98 ± 0.03 mg.kg^{-1} of dry matter. The lowest concentrations of PAE were detected in rape seed 0.05 ± 0.02 mg.kg^{-1} of dry matter for DBP, or 0.06 ± 0.04 mg.kg^{-1} of dry matter for DEHP.

DISCUSSION

Adsorption ability of phthalic acid esters to bind to soil particles varies depending on their physical and chemical properties (Wang *et al.*, 1998; Staples, 1997). On the basis of different solubility in aqueous environment (DBP 11.2 mg.l^{-1}; DEHP 0.003 mg.l$^{-1)}$) leads to different sorption to soil particles and different leaching into the soil solution, and thus to the intake of a plant (Trapp and McFarlane, 1994). In their study, Peijnenburg and Struijs (2006) observed the effect of temperature on the solubility of the PAE, while DEHP was less soluble in spring (average temperature of 8 °C), DBP dissolved at least at higher autumn temperatures (average temperature 12 °C). These results are consistent with our findings, where the measured values of DEHP at all sites during the spring season (sampling I., Tab. I) were below the detection limit.

At all sites higher proportion of DBP than DEHP were detected on the average for the whole growing season. In their study, Xu and Wang (2008) compared the occurrence of phthalates in arable soils from two different areas of eastern China and in both cases higher proportion of DBP was confirmed. By contrast, in a study by Vikelskøe *et al.* (2002) higher proportion of DEHP (16 µg.kg^{-1} of dry weight) was observed in the uncultivated soil than DBP (2.1 µg.kg^{-1} of dry weight).

The measured values (Tab. I) detected in soil samples were collected by a probe rod from arable layer of 0–30 cm depth. The study by Vikelsøe *et al.* (2002) found differences in the proportion of monitored contaminants depending on the depth of the soil profile. In this study, the highest concentration of DBP were discovered in top layers (0–20 cm), whereas DEHP was detected in deeper layers (30–40 cm) of arable profile, which may therefore be another factor influencing the proportion of PAE in cultivated crops. Depending on the concentration of these contaminants in a particular depth and the depth of rootage of cultivated crops, differences in individual crops can be measured.

Xu and Wang (2008) compared the values of DBP and DEHP in soil between differently cultivated locations. In his study he found lower levels of DBP (4.27 mg.kg^{-1} of dry matter) and DEHP (1.51 mg.kg^{-1} of dry matter) in uncultivated land, and on the other hand, he detected higher DBP (15.46 mg.kg^{-1} of dry matter) and DEHP (4.61 mg.kg^{-1} of dry matter) in area cultivated by organic agriculture,which is heavily fertilized with organic fertilizers. In our experiment, the L4 site was intensively fertilized with organic fertilizers, which mainly reflected the higher incidence of DEHP (e.g. sampling III.: $L1_{DEHP}$ 0.11 mg.kg^{-1}, $L4_{DEHP}$ 073 mg.kg^{-1}; Tab. I).

Several studies have also confirmed the dependence of resulting concentration of DBP and DEHP in crops on the initial concentration of PAE in the soil (Yin *et al.*, 2003; Sablayrolles *et al.*, 2005; Zeng *et al.*, 2006). When using sewage waste as a fertilizer, a significant increase in the content of phthalates in soil occurs in comparison to the normal organic fertilizer (Xu and Wang, 2008; Cai *et al.*, 2008). For this reason, the EU set a limit for DEHP content in the waste sludge used as a fertilizer to 100 mg.kg^{-1} of dry matter (Aparicio *et al.*, 2009).

Many authors have observed interspecies differences in both plant intake (Kato *et al.*, 1980, Cai *et al.*, 2006) as well as in effects on physiological functions of plants (Aranda, *et al.*, 1989). Dueck *et al.* (2003) observed different responses of plants (*Phaseolus vulgaris, Brassica chinensis, Trifolium repens*) to an increased incidence of DBP in the air. The most common symptoms included chlorosis, leaf distortion and reduced production of biomass.

Study of Mo *et al.* (2009) the measured values for 11 crops grown on nine differently located farms where in all cases a higher incidence of DEHP than DBP was detected. In our study, these results were found only in flint corn grain (L4), where the detected values of DBP were 0.23 ± 0.03 mg.kg^{-1} and DEHP 0.98 ± 0.03 mg.kg^{-1} (Tab. IV).

SUMMARY

The study examined the transport of phthalates (PAEs) di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) from soil to agricultural crops (*Triticum aestivum, Brassica napus, Zea mays*) and their distribution among aboveground and underground parts, and any accumulation in the final crop product.

The values of DBP in soil at all areas ranged from 0.28 to 1.59 mg.kg^{-1} of dry matter and DEHP < 0.03 to 0.73 mg.kg^{-1} of dry matter. The soil at the L4 site, which was the most fertilized with organic fertilizers, especially manure, was detected significantly ($P < 0.05$) to show the highest values of both the PAEs observed (DBP_{L4} 1.59 ± 0.07 mg.kg^{-1} of dry matter, $DEHP_{L4}$ 0.73 ± 0.18 mg.kg^{-1} of dry matter).

Average concentrations measured in underground parts of monitored crops ranged from 1.68 to 14.26 mg.kg^{-1} of dry matter for DBP, and 0.12 to 10.34 mg.kg^{-1} of dry matter for DEHP. Values detected in aboveground parts were 0.03 to 8.84 mg.kg^{-1} of dry matter for DBP, and 0.25 to 4.59 mg.kg^{-1} of dry matter for DEHP. Average values of DBP in final products ranged from 0.05 to 0.83 mg.kg^{-1} of dry matter, and < 0.06 to 0.98 mg.kg^{-1} of dry matter for DEHP. For all the monitored crops, the amount of DBP in both underground and aboveground parts of plants was lower than that of DEHP, but the final product showed just the opposite results.

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