Analysis of phthalic acid esters in agricultural soils

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Abstract The aim of the study was monitoring of phthalic acid esters in agricultural soils of the Czech Republic over the period of 6 years, namely dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). Monitoring took place in twelve regions of the Czech Republic. Soil samples were taken evenly from the top and bottom soil horizons using a zig-zag pattern. Soil samples were taken from arable land, permanent grassland, and hop field. Lyophilisation of the samples was done by ultrasound-assisted extraction using a mixture acetone-hexane (1:1). Phthalic acid esters were analysed using high-performance liquid chromatography with UV detection. Subsequently, the results were statistically compared by analysing the principal components (PCA) to determine the effect of individual factors on the content of phthalic acid esters in agricultural soil. Factors such as precipitation, distance from a pollution source, amount of pesticides, and amount of artificial and organic fertilizers were taken into account. If we compare the concentrations established in this study with the limits set out in the Methodological Instruction of the Ministry of the Environment of the Czech Republic based on RSLs (Regional Screening Levels) issued by the USEPA (United States Environmental Protection Agency), none of these values were exceeded.

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Introduction

Agricultural soil has an impact not only on quality but also on the health safety of food of plant and animal origin. It is the primary environment in which both of these food groups are created. From this point of view, it is very important to know the content of all substances in the soil that can adversely affect soil quality and ultimately endanger human health, which in this case also means knowing the content of phthalic acid esters in agricultural soils of the Czech Republic.

The two main sources of phthalates in the soil are the atmospheric deposition and fertilization. Atmospheric deposition is a problem especially in areas with extensive industrial activity, but at the same time, it is also the reason for the occurrence of lower concentrations of phthalic acid esters in uncultivated soils. As for the fertilization, the type, dose, and concentration of phthalates in fertilizers are of course decisive (Vikelsøe et al. 2002). Therefore, monitoring the presence and concentration of phthalic acid esters in agricultural soils is highly important.

Material and methods

Material

Samples of agricultural soils were collected in cooperation with the Central Institute for Supervising and Testing in Agriculture in Brno as part of their annual monitoring of other organic pollutants. The monitoring of phthalic acid esters in this study has been going on from 2011 to 2017. The approach of selecting a grid of non-uniform areas with a proportional representation of the main soil types and species and individual cultivated agricultural crops was chosen. Soil samples were collected in 12 regions of the Czech Republic on 40 places of basic monitoring observation areas: Central Bohemia (7 locations), South Bohemia (2 locations), Plzeň (4 locations), Karlovy Vary (1 location), Ústí nad Labem (4 locations), Liberec (1 location), Pardubice (2 locations), Vysočina (4 locations), Zlín (4 locations), South Moravia (1 location), Olomouc (2 locations), and Moravian-Silesian region (8 locations).

Sampling

Approximately 0.75 kg of soil was taken. This quantity was homogenized by hand directly in the field by mixing on a suitable pad. When homogenized, a coarse-grained skeleton was removed. The labelled samples were transported in refrigeration boxes and then stored at − 18 °C until handed over to the laboratory.

In the first years, samples were taken during the autumn months, later in the spring months. In 2017, samples were collected both in the spring and autumn of this year.

Chemicals and devices

Alpha 1–2 LD Plus laboratory freeze dryer (Christ, Germany) was used to lyophilise the samples. Extraction of samples was performed under ultrasound guidance by PS 10000 ultrasonic cleaner (Notus-Powersonic, Slovakia). A mixture of acetone/hexane p.a. (Penta, Czech Republic) was used for extraction. The extracts were evaporated on a vacuum rotary evaporator RV 05-ST (JK IKA-Werke, Germany) and dried under industrial grade nitrogen 4.0 (SIAD, Czech Republic).Purification of the sample was carried out using concentrated sulphuric acid p.a. (Penta, Czech Republic);GFL orbital shaker 3005 (GFL,

Germany) and Universal 32R centrifuge (Hettich, Germany) were used. Analysis was performed on HP 1100 liquid chromatograph (Agilent Technologies, USA) on Zorbax Eclipse XDB-C8 column, 150×4.6 mm, 5 μm (Agilent Technologies, USA). Standards of DEHP (Fluka Analytical, Germany) and DBP (Supelco Analytical, USA) were used. Acetonitrile gradient grade (J. T. Baker, the Netherlands) was used as the mobile phase.

Sample preparation

Sample preparation included lyophilisation, extraction, and purification. Each sample frozen at 0.75 kg was thawed and about 10 g of soil was subsequently duplicitously collected from each of them. This amount was weighed into aluminium plates, which had been rinsed three times with hexane as well as all the other utensils and containers used. The samples were then frozen and subsequently lyophilised for 18 h. After lyophilisation, samples were extracted with acetone:hexane mixture (1:1) using ultrasound, 3 times for 5 min. Combined extracts were filtered, evaporated on a vacuum rotary evaporator, and dried under nitrogen. The samples were then transferred with hexane to the vials, followed by purification with sulphuric acid (Thurén and Södergren 1987). Purified samples were dried under nitrogen and supplemented with acetonitrile with a standard addition of DBP and DEHP (39 μg/ml) to 1 ml volume.

HPLC analysis

Analysis of the content of phthalic acid esters was made by high-performance liquid chromatography with UV detection at a wavelength of 224 nm. All samples were injected twice. Sample injection volume was 10 μl. A Zorbax Eclipse C8 column was used, and acetonitrile was used as the mobile phase. The results were evaluated with a calibration curve using Agilent ChemStation software for LC and LC/MS systems. The calibration curve was generated in the range of concentrations 1 to 112 μg/ml for DBP and DEHP, and the correlation coefficient for both DBP and DEHP was 0.9999. Limit of detection for DBP and DEHP was 0.11 μg/ml and 0.05 μg/ml, respectively.

Statistical evaluation

Statistical evaluation of analysed samples was performed by SAS software (SAS Institute 2018) where phthalate content between seasons, years, soil types,

locality, and types of fertilisers used were compared by Turkey's test. Principal component analysis (PCA) on correlation matrix with the Varimax rotation was used to find the relationship among phthalate content and several environmental and anthropogenic variables by XLSTAT-Pro software (Addinsoft 2006). Environmental and anthropogenic variables were given by Central Institute for Supervising and Testing in Agriculture in Brno as dosage of organic and synthetic fertilizers, precipitation, pesticides dosage, and distance from known pollution source. Organic and synthetic fertilizers were further divided by the amount of element added (N, P, K, Mg, Ca, S). The dosage of fertilizers, precipitation, and pesticide dosage were summed for a period of 6 months before sampling.

Results and discussion

Table 1 shows the average content of phthalic acid esters in comparison of the season and the year.

As can be seen from the table, there is a statistically significant difference between spring and autumn and between 2011 and 2013. The study by Zhang et al. (2015) also showed a significant difference in the concentration of phthalic acid esters in agricultural soil between different seasons (spring, summer, autumn, and winter). The concentrations of phthalic acid esters differed significantly from spring to autumn. The highest average phthalate concentration was found in summer.

The differences in phthalate content between localities and fertiliser types were examined and it was found that no statistically significant difference were established (data not shown). Those results are different than the conclusions given by Zorníková et al. (2011) who found the highest concentrations of phthalic acid esters in soils at the place with the highest amount of organic fertilisers added and especially manure. This implies the influence of added fertiliser type and amount on phthalic acid esters concentration in the soil. The effect of fertiliser was assessed by Vikelsøe et al. (2002) too. Concentration of phthalic acid esters in samples from areas fertilised by organic fertilisers, synthetic fertilisers, and low amounts of sewage sludge was small and comparable. The concentrations of the pollutant were just slightly different from concentrations measured in preserved area without agricultural use which was taken as a reference sample. The increased amount of phthalic acid esters was found in soils fertilised with high amounts of sewage sludge.

Table 2 shows a comparison of phthalate content in different soil types. It was found that there is a significant difference of phthalate content between fluvisol and other soil types. Fluvisol had a higher concentration of phthalates possible because of higher organic matter content as it was found by Xu et al. (2008).

To explore the possible relationships between phthalate content and environmental and anthropogenic variables, three separate analyses of principal components were performed. Each principal component (PC) represents an independent cause of variation; thus, variables near each other are positively correlated, variables separated by 90° are independent, and variables separated by 180° are negatively correlated. All PCs are linear combinations of variables, but a variable far from the origin that lies on a PC is predominant in defining this PC.

The first principal component analysis (PCA_1) (Fig. 1) was run on the whole data set having values of variables for the year and season. The first two principal components accounted for 44.14% of the total variance, wherein 27.88% of the total variance was explained by the first principal component (PC1), and 16.26% by the second principal component (PC2). PC1 was mainly explained by variables regarding the dosage of organic fertilizers with a contribution of 97.09%. Phthalate content mostly contributed to the PC2 characterization (71.52%), while precipitation (11.98%) and pesticide dosage (8.06%) were minor contributors. It was observed that the precipitation and pesticide dosage were close to the soil phthalate content, indicating that the phthalate content was higher when precipitation and pesticide dosage were higher. Similar was observed in the study by Zeng et al. (2010) who found that the phthalates with a higher molecular weight showed a slight increase in atmospheric deposition in the rainy season compared with the drought periods. Seasonal variations of atmospheric phthalate deposition were demonstrated, with deposition affected by seasonal changes in meteorological parameters. Organic fertilizer dosage was located on PC1 axis at around 90° to the phthalate content, indicating that these variables were independent of each other. Other variables like synthetic fertilizer dosage and pollution distance are closer to the plane origin, thus being weakly correlated to the both PCs and the soil phthalate content. These findings are somewhat different than the results reported by Hongjun et al. (2013) who identified the impact of agricultural activity and the proximity of industry to the content of

Table 1 Effect of the year and the season on the phthalate content (mean ± standard error mg/kg)

Phthalates	Season[1]		Year[1]		
	Spring	Autumn	2011	2013	2017
DBP	0.17 ± 0.05^b	0.39 ± 0.03^a	0.47 ± 0.05	0.47 ± 0.03	0.39 ± 0.04
DEHP	0.20 ± 0.05^b	0.38 ± 0.02^a	0.48 ± 0.06^a	0.27 ± 0.04^b	$0.38 \pm 0.02^{a,b}$
Total	0.37 ± 0.08^b	0.77 ± 0.05^a	0.95 ± 0.09	0.74 ± 0.05	0.77 ± 0.05

[1] Means with a common superscript are not significantly different

Indices [a] and [b] indicate whether there is a statically significant difference between the samples at the significance level 0.05

phthalates in soil. Therefore, we can conclude that precipitation and pesticides had the strongest relationship with phthalates content, while other variables like distance from pollution or the addition of synthetic and organic fertilizers had weak relationship with phthalates.

Additional principal component analyses were applied for better understanding of relationships between variables during 3 years (PCA_2) and during two seasons within 1 year (PCA_3). The first two principal components of PCA_2 accounted for 42.63% of the total variance as shown in Fig. 2. The most positively correlated with PC1 were the variables of organic fertilizers which contributed 97.32% to PC1 characterization. Again, there was a strong positive correlation between the phthalate content and PC2 and having a contribution of 86.48%. Pesticides were also positively correlated to PC2 but had a low contribution (4.79%). Other variables had negligible contribution to the characterization of both PCs. The main pattern of relationships between soil phthalate content and other variables, which was

already found in the PCA_1, was observed in this case, too. However, pesticides were more far from the phthalate content, and precipitation had no correlation to PCs or phthalate contents. This could lead to a conclusion that on a year basis, none of the used variables had any meaningful relationship with phthalate content. This could mean that the soil phthalate content is more correlated or even affected by other factors, as it was presented by Rhind et al. (2013).

Third PCA was run on the values of variables related to the season and the phthalate content, as it is shown on Fig. 3 as the plot of the variables in the plane defined by the first two PCs obtained which accounted to 54.58% of the total variance, 35.75% and 18.83% by first and second PC, respectively. It was observed that the variation among samples at the PC1 is mainly described by the organic fertilizers correlated positively and contributing 89.25% to the PC characterization. Synthetic fertilizers based on nitrogen content negatively correlated with PC1 but with low contribution (2.85%). The second principal component was mainly positively related to the phthalate content (49.23% contribution), precipitation (11.91% contribution), and pesticides (3.62% contribution). Synthetic fertilizers based on Mg, Ca, and N content had negative correlation with PC2 contributing 32.80% to the PC2 characterization. It was observed that the soil phthalate content appeared to be positively correlated to precipitation and pesticides, and negatively related to the addition of synthetic fertilizers based on Mg, Ca, and N. Therefore, more precipitation and pesticide addition led to the increase of soil phthalate content, while higher addition of synthetic fertilizers had the opposite relationship. Research by Wu et al. (2015) also shown that the adsorption of phthalates to the soil decreases with increasing pH, to which also the presence of alkaline minerals, such as magnesium or calcium, in the soil may contribute.

Table 2 Effect of the soil type on the phthalate content (mean ± standard error mg/kg)

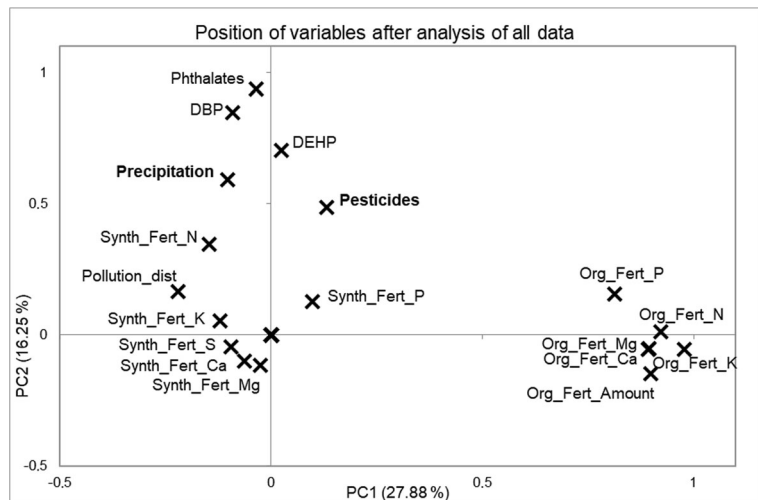| Soil type	Phthalates[1]		
	DBP	DEHP	Total
Chernozem (black soil)	0.28	$0.39^{a,b}$	$0.67^{a,b}$
Fluvisol	0.46	0.54^a	0.99^a
Gleysol	0.23	0.15^b	0.38^b
Cambisol (brown soil)	0.36	0.31^b	0.67^b
Cambisol	0.35	0.27^b	0.62^b
Luvisol	0.47	0.22^b	$0.69^{a,b}$
(Pseudo)gleysol	0.37	$0.33^{a,b}$	$0.70^{a,b}$

[1] Means with a common superscript are not significantly different within column

Indices [a] and [b] indicate whether there is a statically significant difference between the samples at the significance level 0.05

Fig. 1 Principal component analysis of the whole data set as a combination of the year and season

Projection of the observations in the area defined by the first two PCs in PCA_1 and PCA_2 analyses did not give a satisfactory or clear separation of groups and therefore are not shown. There are several reasons which could imply poor separation: small individual differences among observations in many variables, having an effect of outliers in the data set, or the used variables were not explanatory enough for the observations. However, an obvious group separation was found in PCA_3 analysis on PC2 axis as shown in Fig. 4. Circles which represented spring observations were located mostly in the lower quadrants, while all triangles representing autumn observations were in the upper quadrants. Two distinctive outliers were seen, one close to the positive part of PC1 axis, and one close to negative part of the PC2 axis, both shown as filled out marks.

Therefore, it is possible that the outliers affected the PCA performance. Nevertheless, this could mean that environmental and anthropogenic variables could change substantially without changes in the soil phthalate content.

As reported previously in Fig. 3, the PC2 axis from PCA_3 analysis was mostly defined by the phthalate content, precipitation, and pesticides, indicating that those variables were predominant for observation positions. In the study by Rhind et al. (2013), no consistent effects of soil, vegetation type, carbon content, soil pH, altitude, or distance from centres of settlement on the concentrations of contaminants were found. The concentrations of phthalates in this study were highly variable, depending on the factors affecting each other, where a clear source of contamination cannot be

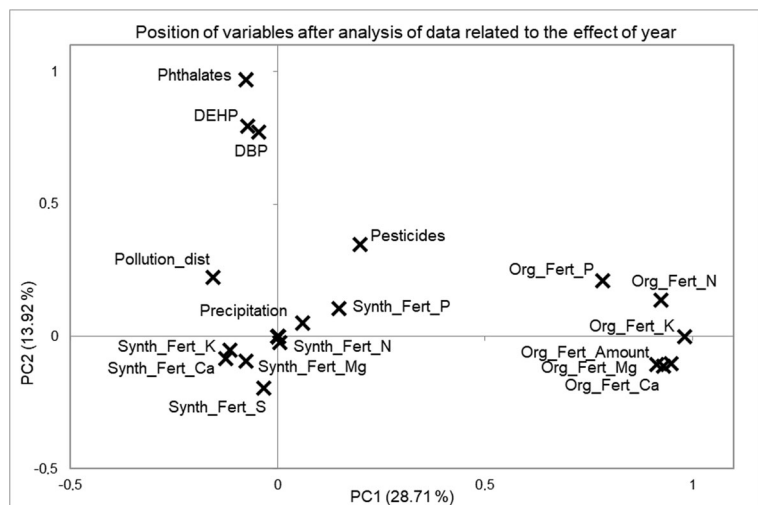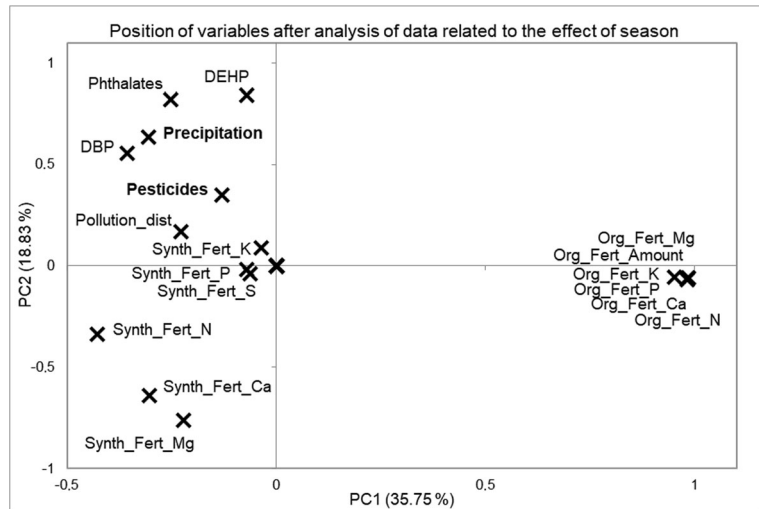**Fig. 2** Principal component analysis of the data set related to the effect of year

Fig. 3 Principal component
analysis of the data set related to
the effect of season

identified, but there may be a connection with wet atmospheric deposition and organic carbon content in the soil. However, the absence of consistent statistically significant differences in concentrations associated with the different carbon content, pH value, soil, vegetation, altitude, or distance from major pollution sources does not make it possible to establish a single factor determining the amount of pollutants, because too many interacting factors are involved. This is seen in total variances explained by PCA analyses which were not high, giving only 44.14, 42.63, and 54.58% explanation for PCA_1, PCA_2, and PCA_3, respectively. Therefore, it could be stated that other factors have an underlying relationship with the soil phthalate content, and more research on that is needed.

Conclusion

Samples were obtained from four types of land: arable land, permanent grassland, hop field, and uncultivated areas. Monitoring was carried out in 12 regions of the Czech Republic: Central Bohemia, South Bohemia, Plzeň, Karlovy Vary, Ústí nad Labem, Liberec, Pardubice, Vysočina, South Moravia, Olomouc, Zlín, and Moravian-Silesian region. If we compare the concentrations established in this study with the limits set out in the Methodological Instruction of the Ministry of the Environment of the Czech Republic (2013) based on RSLs (Regional Screening Levels) issued by the USEPA (United States Environmental Protection Agency), none of these values were exceeded. The limit for

Fig. 4 Projection of the
observations in the plane defined
by the first two principal
components referring to the effect
of season: circle, spring; triangle,
autumn
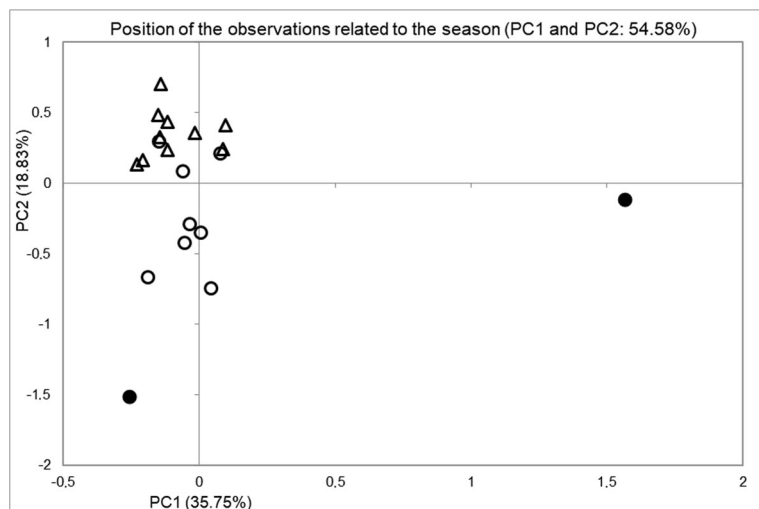
industrially used fields and other fields is 160 mg/kg and 39 mg/kg for DEHP and 82,000 mg/kg and 6300 mg/kg for DBP respectively.

When comparing the seasons, a statistically significant difference between the period of sampling during spring and autumn was found at a significance level $\alpha = 0.05$. When the individual years 2011, 2013 and 2017 are compared, a statistically significant difference was also found at the significance level $\alpha = 0.05$.

The probable cause for increased DBP and DEHP content in some locations could be intensive farming activity associated with the use of higher pesticide doses. Another reason may be greater rainfall in the area where the content of phthalic acid esters is transmitted by atmospheric deposition, both in the dry way and above all the wet way. As indicated by the principal component analysis, the atmospheric deposition is also likely to be responsible for the observed concentrations of DBP and DEHP on non-cultivated land. Insufficient explanation of the effects of factors by PCA analysis can be explained by the interaction of many factors that affect the content of phthalates in one or the opposite direction, and therefore a higher influence of any single factor cannot be determined.

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