

**Fast and reliable determination of phthalic acid esters in soil and lettuce samples based  
on QuEChERS GC-MS/MS**

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## Abstract

Phthalates are commonly used as plasticizers, and solvents in industry and households. We propose an application of the QuEChERS method for the determination of six PAEs in the soil and lettuce (roots and leaves) by GC-MS/MS. The QuEChERS method validation procedure was performed and good linearity ( $>0.997$ ), recovery (97.2-99.1%), very low detection limits (0.09-0.43 ng/g), and satisfactory inter- and intraday precision ( $\sim 4\%$ ) were obtained confirming that QuEChERS GC-MS/MS applied for PAEs determination in the environmental samples is a cheap and environmentally friendly method. In general, the higher the number of carbon atoms in PAEs, the higher the percentage noted in the lettuce roots. At higher PAEs concentration (60 ng/g) the main bis(2-ethylhexyl) phthalate (DEHP) sink were roots whereas at lower concentrations (30 ng/g) most of DEHP was noted in lettuce leaves implying that the fate of PAEs was governed not by the chemical structure of PAEs but rather partitioning ( $\log K_{ow}$ ).

## Keywords

Phthalates; Phthalic Acid Esters; QuEChERS; food contamination; lettuce

## 1. Introduction

Vegetables are essential for human health. They contain a lot of vitamins, antioxidants, minerals, and dietary fiber (Tankiewicz & Berg, 2022). Additionally, they are low in fat, soluble sugars, and calories (da Costa et al., 2023). World Health Organization (WHO) recommends daily intake of fruits and vegetables greater than 400 g/capita day, with minimal vegetable consumption of 240 g/capita day (WHO/FAO, 2003) (Dong et al., 2022). Such daily consumption may decrease the risk of various cancers, cardiovascular diseases, and all-cause mortality. A vegetable-rich diet is also climate-friendly and favors the Sustainable Development Goals of the United Nations (Dong et al., 2022). Despite the many benefits of consuming

vegetables, they may be a source of harmful substances, especially pesticide residues. They also contain many other dangerous substances which they take up from a contaminated environment. Therefore, it is important to determine their content before they are put on sale or consumed.

Phthalates (PAEs) are phthalic acid esters commonly used as plasticizers in the polymer industry to promote the transparency, strength, plasticity, and durability of materials (Giuliani et al., 2020). They are also used as solvents in the cosmetic industry and pesticides (Y. Zhang et al., 2021). Dimethyl phthalate (DMP) and diethyl phthalate (DEP) are mainly used as solvents in cosmetics whereas dibutyl phthalate (DBP) and bis(2-ethylhexyl) phthalate (DEHP) are the main plasticizers in plastic industry (Edwards et al., 2022). Since PAEs are present in various products and they are not chemically bound to the polymer (Hou et al., 2021) they may be easily washed out to the environment a water, soil, and air pollutants (Tuan Tran et al., 2022). Phthalates are taken up by plants from the environment and introduced into the food chain and the main route of human exposure to them is contaminated food (Coltro et al., 2023; Sun et al., 2015). Phthalates may cause many diseases like allergies and asthma and more serious ones like cancer (Cheng et al., 2021). Phthalates also affect children's development (Ghosh & Sahu, 2022). It is important to monitor their content in food (N. Chen et al., 2017).

Many methods of PAEs extraction are described in the literature. One of the most common are Soxhlet extraction and ultrasonically assisted extraction. But these methods have some disadvantages. They require several different steps like extraction in an ultrasonic bath and centrifugation or repeating the entire procedure twice (C. Li et al., 2016; Wei et al., 2020) being time-consuming. Other procedures (C. Li et al., 2016; J. Wang et al., 2015; Wei et al., 2020; H.-M. Zhao et al., 2015) required additional sample purification after extraction using packaged columns. Known methods also required large volumes of organic solvents. Various PAEs extraction methods described in the literature are summarized in Table 1.

Table 1. PAEs' extraction methods presented in the literature.

Extraction method	Solvents	Sample weight [g]	Extraction time	analyzed compounds	References
Soxhlet	Methanol 100 mL n-hexane 10 mL	2	45 min	DMP DEP DBP DEHP	(Sablayrolles et al., 2005)
Contact	Acetone 60 mL Hexane 160 mL	1	8 h	DBP DEHP	(Müller & Kördel, 1993)
Contact	Acetone 10 mL Hexane 15 mL	3	all night	DMP DEP DBP DEHP BBP DNOP	(J. Wang et al., 2015)
Contact ultrasound-assisted	Acetone 10 mL Hexane 15 mL	2	2x 21 min	DMP DEP DBP DiBP DEHP BBP DNOP Bis (2-methoxyethyl) phthalate Diamyl phthalate Di-n-hexyl phthalate Bis(2-n-butoxyethyl) phthalate Dicyclohexyl phthalate	(C. Li et al., 2016)
Contact ultrasound-assisted	Hexane 10 mL Dichloromethane 10 mL	1	2x 65 min	DMP DEP DBP DEHP BBP DNOP	(Wei et al., 2020)
Contact ultrasound-assisted	Acetonitrile 10 mL	0,5	40 min	DBP DiBP Di(2-ethyl-hexyl) phthalate	(F. Zhao et al., 2022)

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Soxhlet	Acetone 50 mL Dichloromethane 50 mL (Hexane 50 mL dichloromethane 50 mL for pre-extraction)	20 g	24 h + 24 h for pre-extraction of cellulose extraction thimble	DBP Di(2-ethyl-hexyl) phthalate	(H.-M. Zhao et al., 2015)
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77 Sample preparation for analysis is very important for reliable results. One of the  
78 methods that are Quick, Easy, Cheap, Effective, Rugged, and Safe is called QuEChERS  
79 (González-Curbelo et al., 2022). This method was presented in 2002 by Anastassiades, Lehotay,  
80 Stajnbaher, and Schenck (Anastassiades et al., 2003). Originally QuEChERS method was  
81 developed for the extraction of pesticide residues from fruits and vegetables (González-Curbelo  
82 et al., 2015, 2022). But now it is used for the analysis of antibiotics, hormones, mycotoxins,  
83 polycyclic aromatic hydrocarbons (Kim et al., 2019), lipids, antioxidants and food additives,  
84 endocrine disruptors, and surfactants (González-Curbelo et al., 2015) in food and  
85 environmental matrices including soil, sediments, and water (González-Curbelo et al., 2015;  
86 Kim et al., 2019). QuEChERS method combines extraction using acetonitrile and dispersive  
87 solid-phase extraction (d-SPE) to purify the extract from compounds from the matrix (e.g.,  
88 organic acids, certain polar pigments and sugars) (González-Curbelo et al., 2015; S. Wang et  
89 al., 2020). The main advantage of the method is that only acetonitrile (ACN) is required as a  
90 solvent to extract, anhydrous magnesium sulfate to salt out, sodium chloride to remove the  
91 moisture, and N-propyl ethylenediamine (PSA) as dispersive solid-phase extraction agent (C.  
92 Zhang et al., 2019). Additionally, the amount of these reagents is low. For 10 g of sample  
93 original QuEChERS method required 10 mL of ACN, 4 g of  $\text{MgSO}_4$ , 1 g of NaCl, and 25 mg  
94 of PSA (González-Curbelo et al., 2022) making QuEChERS a cheap and environmentally  
95 friendly method due to the small volumes of organic solvents and generating much less wastes  
96 than other extraction methods. It also meets the principles of green chemistry (González-  
97 Curbelo et al., 2022). Another advantage of the QuEChERS method is that it allows the analysis  
98 of many compounds, it may be easily modified and successfully used for the extraction of  
99 various substances from different matrices. QuEChERS method also has no time-consuming  
100 steps so it allows many analyses in a shorter time (Kim et al., 2019). Samples prepared using  
101 the QuEChERS method may be analyzed by gas chromatography (GC) and high-performance

liquid chromatography (HPLC) coupled with mass spectrometry (MS) which increases the applicability of this method (Kim et al., 2019).

In literature, one modification of the QuEChERS method to extract phthalates is described (González-Curbelo et al., 2015). For a sample of 10 g of jelly 10 mL of ACN as a solvent, 4 g of MgSO<sub>4</sub>, and 1 g of NaCl were used. In the d-SPE step, they used as sorbents 25 mg of PSA and 200 mg of MgSO<sub>4</sub>. LC-MS/MS was used as an analytical technique. The authors obtained recovery equal to 84-104% and a limit of detection (LOD) equal to 0.09–3.68 µg/L (González-Curbelo et al., 2015). Thus the objective of the presented studies was verification of the applicability of QuEChERS in i) the determination of the PAEs in the samples of soil, ii) the determination of PAEs in lettuce (*Lactuca sativa*), iii) estimation of the fate of PAEs in the soil-vegetable system.

## 2. Materials and methods

### 2.1. Chemicals

The certified reference standards of phthalates: dimethyl phthalate, diethyl phthalate, dibutyl phthalate, benzyl butyl phthalate, bis(2-ethylhexyl) phthalate and di-n-octyl phthalate (Sigma-Aldrich, Poland) (Table S1), and deuterated phthalates (internal standards): bis(2-ethylhexyl)phthalate-3,4,5,6-d<sub>4</sub> (DEHP-3,4,5,6-d<sub>4</sub>), dimethyl phthalate-3,4,5,6-d<sub>4</sub>, diethyl phthalate-3,4,5,6-d<sub>4</sub> and dibutyl phthalate-3,4,5,6-d<sub>4</sub> were acquired through Sigma-Aldrich (St. Louis, MO, USA). They were all kept under stable conditions at -20 °C. A standard mixture was prepared from individual stock standard solutions to obtain the concentration at 1 µg/mL in hexane. All other working solutions for determining calibration curves, recoveries, limits of detection (LODs), and limits of quantification (LOQs) were prepared by diluting the standard mixture in acetonitrile. Acetonitrile and methanol (LCMS grade) were purchased from Merck (Darmstadt, Germany); hexane and ethyl acetate (HPLC grade) from POCH S.A. (Gliwice, Poland); anhydrous magnesium sulfate (99.5 % powder; MgSO<sub>4</sub>) and sodium chloride from

Merck (Warszawa, Poland). For the QuEChERS process, Septra C18-E (50  $\mu$ m, 65 Å), and Septra PSA sorbents were acquired from Phenomenex (Torrance, CA, USA).

## **2.2. Pot experiment**

The fate of PAEs in soil-vegetable systems was established using agricultural acidic brown soil developed from deep loess from Podborcze, Poland (50°41'47.5"N 22°50'41.3" E) composed of silt, sand and clay particles. The soil was characterized by: pH=7,59, TC 13.573 mg/g, IC 0.00584 mg/g, TOC 13.567 mg/g, total dissolved carbon 33.33 mg/L, dissolved inorganic carbon 11,61 mg/L and dissolved organic carbon 21.72 mg/L. The topsoil samples (0-20 cm) were air-dried, sieved (<2 mm), homogenized, and divided into four groups (340 g each). Control (uncultivated soil), soil spiked with 1 mL of PAEs mixture containing 1  $\mu$ g/mL of each tested compound were transferred into 500 mL glass containers (12.5 cm diameter, 6.3 cm height), and tap water was added to reach 65% of the water-holding capacity of the soil. The 10 lettuce seeds (*Lactuca sativa* L.) were placed in each container (except for two controls). The samples were exposed to regular light/dark cycles (12:12 h) in Conviron GEN100 (temperature 22°C/18°C day/night, humidity 65%). After cultivation, the plants were carefully removed from the soil, and cut to separate the roots from the leaves. The subparts were firstly frozen in the freezer (-20°C, Liebherr) and lyophilized in Alpha 1-2 LDplus (Christ). The same procedure was conducted for the samples treated with 60 ng/g of PAEs mixture. Each experiment was performed with three replicates.

## **2.3. QuEChERS optimization**

The QuEChERS technique was first used to test pesticides in plant material (Wilkowska & Biziuk, 2011). To estimate the concentration of the analytes in the complex matrix two-step extraction procedure is required. The first step involves liquid-liquid extraction (LLE) or solid-liquid extraction (SLE) assisted with various types of organic extractants (most often acetonitrile, ethyl acetate, hexane, and others) with extraction salts – NaCl (sample salting



effect) and anhydrous  $\text{MgSO}_4$  (exothermic effect during water binding and increase of analyte extraction rate) (González-Curbelo et al., 2022). The second step involves extract purification using a dispersive solid-phase extraction (d-SPE) process in which different types of sorbents are used, usually PSA and C18 or their mixture (Rudel & Perovich, 2009).

To optimize the QuEChERS procedure, the influence of the following factors was examined:

- the amount of the dry sample (using 300, 400, 500, 600 and 700 mg)
- the type and quantity of the extractant (using 300, 400, 500, 600, and 700  $\mu\text{L}$  of acetonitrile, ethyl acetate, and hexane)
- the amount of extraction salts – a mixture of NaCl and  $\text{MgSO}_4$  1:3 (using 300, 400, 500, 600, and 700 mg)
- the type and amount of sorbent – C18, PSA, and their mixture 1:1 (using 5, 10, 15, 20, and 25 mg)

During the optimization process, it was possible to determine the optimal QuEChERS conditions for the determination of PAEs both in the soil and vegetable samples.

#### **2.4.GC-MS/MS analysis**

For qualification and quantification of the QuEChERS extracts, a gas chromatograph with a tandem mass spectrometer detector (GC–MS/MS TQ8040; Shimadzu) equipped with a ZB5-MSi fused-silica capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness; Phenomenex) was used. Helium (grade 5.0) as carrier gas and argon (grade 5.0) as collision gas were used. Column flow was 1 mL/min, and 1  $\mu\text{L}$  of the sample was injected by an AOC-20i+s type autosampler (Shimadzu). The injector was working at the temperature of 320 °C; the ion source temperature was 220 °C.

For qualitative purposes, the SCAN mode (full scan monitoring the mass range) with a range of 40-550  $m/z$  was employed and for quantitative analyses, the MRM mode (multiple reaction monitoring) was used. In both cases, electron ionization (EI) at 70 V was employed.

## 2.5. Method validation and statistical analysis

For the quantitative analysis of phthalates, the internal standard calibration method was employed. The specificity of the method was evaluated by analyzing soil, leaf, and root blank samples. The samples were tested in the absence of individual phthalates or internal standards. The presented method of phthalates determination in environmental samples was validated in terms of the limit of detection (LOD), the limit of quantification (LOQ), linearity, specificity, precision, and accuracy.

The linearity of the assay was calculated by the least square method and expressed as a coefficient of determination ( $R^2$ ). Calibration plots were prepared using the blank samples spiked with individual analytes at concentration levels equal to 0.25, 0.5, 1, 2.5, 5, 10, and 30 ng/g and a fixed concentration of the internal standard. Each solution was prepared in triplicate. The signal-to-noise ratios of LOD and LOQ were 3 and 10, respectively. The QuEChERS recovery was investigated using blank samples spiked with an individual analyte and internal standard at five different concentration levels (0.5, 1, 2.5, 10, 30 and 50 ng/mL). Based on the weighed analyte a calculated 100% of the recovery of the analyte response after sample workup in comparison to the initial solution was applied.

## 3. Results and discussion

### 3.1. QuEChERS optimization

Based on the analytes' chemical structures (Figure S1) and their fragmentation pathways the MRM transitions were estimated. For quantitation analysis of individual phthalates, the best conditions for our instrumentation were optimized. The MRM analysis was optimized in terms

of MRM transitions and optimal collision energies of the examined compounds. The selection of the optimal MRM transitions and the collision energy for quantitation purposes was made based on the conducted experiments, the results of which are shown in Fig. S2 and Table 2. For each compound three MRM transitions of the highest intensity were selected for further experiments:

- 194 > 163 (CE = 5 V), 194 > 133 (CE = 23 V) and 163 > 133 (CE = 8 V) for dimethyl phthalate;
- 222 > 177 (CE = 5 V), 222 > 149 (CE = 14 V) and 177 > 149 (CE = 8 V) for diethyl phthalate;
- 278 > 223 (CE = 5 V), 278 > 205 (CE = 5 V) and 223 > 149 (CE = 11 V) for dibutyl phthalate;
- 312 > 206 (CE = 5 V), 312 > 149 (CE = 11 V) and 149 > 91 (CE = 17 V) for benzyl butyl phthalate;
- 390 > 167 (CE = 8 V), 390 > 149 (CE = 20 V) and 167 > 149 (CE = 5 V) for bis(2-ethylhexyl) phthalate;
- 390 > 279 (CE = 5 V), 390 > 149 (CE = 14 V) and 279 > 149 (CE = 8 V) for di-n-octyl phthalate.

The MRM transitions and optimal collision energies of the examined compounds are collected in Table 2. The exemplary SCAN (see Fig. S3a) and MRM chromatogram (see Fig. S3b) of a soil sample spiked with DMP, DEP, DBP, BBP, DEHP, DNOP (30 ng/g) after QuEChERS sample preparation procedure are presented in Fig. 1. As results from the obtained data, the applied GC-MS/MS conditions are acceptable for both the qualitative and quantitative analysis of mentioned compounds.

Table 2. MRM transitions and collision voltages of analyzed phthalates for gas chromatography-tandem mass spectrometry (GC-MS/MS)

Compound	Retention time [min]	Qualitative MRM transition [mass > product mass]	Quantitative MRM transition [mass > product mass]	Collision voltage [V]
DMP	14.7	194>163 194>133 163>133	163> 133	8
DEP	16.8	222>179 222>149 179>149	179 > 149	8
DBP	21.7	278>223 278>205 223>149	223 > 149	11
BBP	26.2	312>206 312>149 149>91	149 > 91	17
DEHP	28.1	390>167 390>149 167>149	167 > 149	5
DNOP	29.8	390>279 390>149 279>149	279 > 149	8

*MRM* - multiple reaction monitoring

Appropriate selection of process conditions allows optimal extraction of sample components with very high efficiency from complex matrices, given that each factor crucially influenced the recoveries of the examined analytes. During the research, optimization of the phthalates extraction process from soil samples and plant fragments was carried out. Optimization of the extraction process (first step of procedure QuEChERS) was performed using acetonitrile, ethyl acetate, and hexane as solvents. The best results were obtained when acetonitrile was used for this purpose. It should be mentioned here that the use of inorganic extraction salts, the amount of which was also subject to optimization, has a huge impact on the extraction process.

The QuEChERS procedure also requires the final purification of the interferences from the extract (the second step of the QuEChERS procedure - step d-SPE). Optimization of this

stage was carried out with the use of C18, PSA, and their mixture as a purifying sorbent. The best results were obtained using a mixture of C18 and PSA. The applied optimal QuEChERS conditions are presented in Fig. 1.

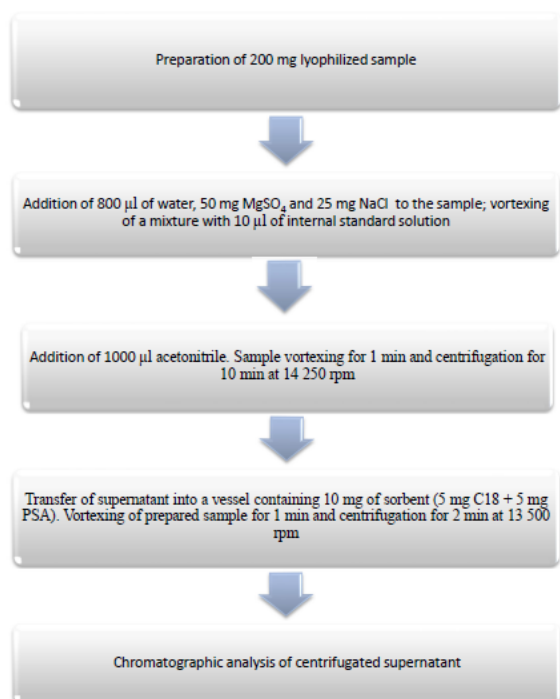


Fig 1. The optimal sample preparation procedure for the soil and the lettuce sample (root and leaves).

To estimate the analytical utility of the described method, its validation procedure was performed. The results of the validation experiments are gathered in Table 3, and they show that the method is characterized by good linearity, very low detection limits as well and satisfactory inter- and intraday precision for all the examined phthalates.

252 Table 3. Results of validation for the applied method.

Tested parameter	DMP	DEP	DBP	BBP	DEHP	DNOP
Linearity ( $R^2$ )	0.9971	0.9991	0.9994	0.9989	0.9974	0.9969
Intraday precision (% RSD)	3.97	4.05	4.11	3.99	4.69	4.22
Interday precision (%RSD)	4.11	4.46	4.33	4.74	5.18	4.99
Intraday accuracy (%)	99.07	98.38	99.49	97.47	97.77	98.03
Interday accuracy (%)	98.41	97.99	101.12	98.55	102.41	101.93
LOD ( $\mu\text{g/kg}$ )	0.11	0.21	0.09	0.37	0.43	0.41
LOQ ( $\mu\text{g/kg}$ )	0.36	0.69	0.30	1.23	1.43	1.36
Recovery	97.41	98.08	99.12	98.44	97.19	97.37
Matrix effect	No significant differences were found between the slopes. The results led to the conclusion that the presented method was not subjected to any matrix effect					
Selectivity	No peaks of the examined analytes and/or their significant interferences on MRM chromatograms confirm the high selectivity of the described method					

253  $R^2$  coefficient of determination, *RSD* relative standard deviation, *LOD* limit of detection, *LOQ* limit of quantification

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### 3.2. Analysis of the fate of PAEs in soil-vegetable system

PAEs leached from plastics in the environment may be present in the dissolved phase, adsorbed to soil (Liu et al., 2023) or sediments (Y. Chen et al., 2023), complexed with organic matter, transported with water or degraded by microbes (Ramanayaka et al., 2023). The presence of PAEs in the cultivated soil may affect soil productivity (microorganisms abundance and characteristics) and plant growth. The results of the fate of selected PAEs in the soil and soil under cultivation of the vegetables are presented in Figure 2. In the control soil (blank) the presence of PAEs was below LOD stressing that the matrix effect of PAEs was not important. In the literature in the estuarine soil the concentration range of  $\Sigma 6$ PAEs (the sum of all six PAEs) was 0.709–9.565 mg/kg, with an average of  $3.258 \pm 2.031$  mg/kg (Liu et al., 2023), DEHP and DBP in agricultural soil constituted up to 2.2–25.2 mg/kg (Ramanayaka et al., 2023) whereas in paddy soils - from 0.17 to 1.97 mg/kg (with DBP and DEHP accounting for 81% of the total PAEs) (Y. Li et al., 2023). The other sink of PAEs was sediments. It was established that 4.67 ng/g to 274.0 ng/g of  $\Sigma 16$ PAEs was noted in Yangtze River sediments, again with predominant DEHP, DBP, and DIBP (>90 % of  $\Sigma$ PAEs in the sediments) (Y. Chen et al., 2023) implying that residential activities have stronger impacts than industrial activities on PAE distributions (Lu et al., 2023) and PAEs such as DEHP revealed tendency to accumulate in sediments and living there demersal fish (Ai et al., 2023) causing the inflammation and tissue damage in zebra fish (Lyu et al., 2023). Calculated Risk Quotients (RQs) for DEHP present in wastewater were above 1, indicating a high risk to biota, whereas LMW-PAEs posed medium risks (RQ 0.1 – 1) (Ngeno et al., 2023). When considering mussels, Risk Quotient values were below 1, so the consumption of Galician raft mussels did not pose a risk to human health (Carro et al., 2023). Thus, there is a need to establish the fate of PAEs in soil-vegetable system to avoid risk for humans.

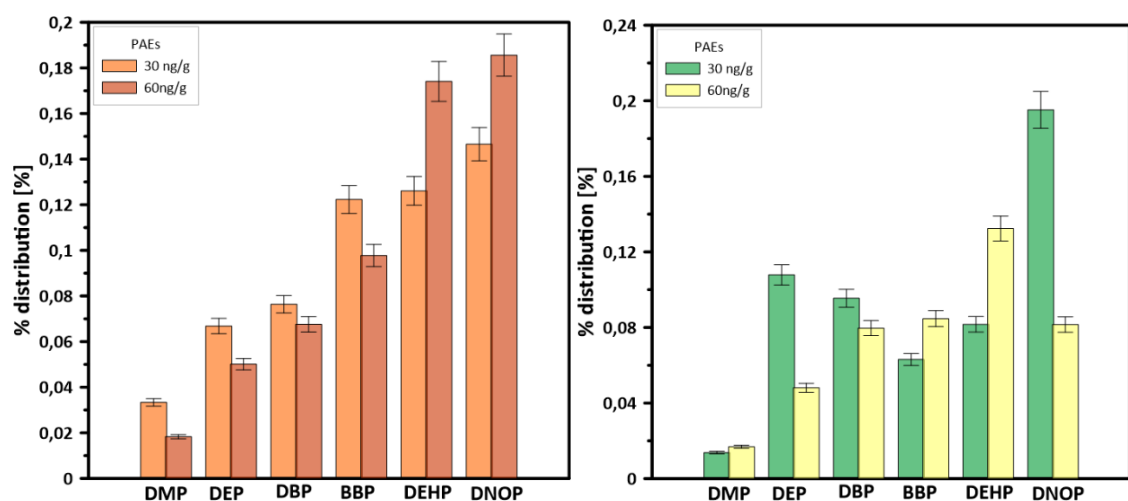
The data from our studies indicate that the fate of PAEs was dependent on the target molecule (Fig. 2). In general, DMP was mostly immobilized in the soil sample and did not tend to migrate into lettuce (below 5% of the initial value in the roots and 2% in leaves). The other PAEs revealed different behavior but in general, the lowest concentration in the roots was noted for DMP and DEP, DBP (low-molecular-weight PAEs; below 8% of the initial value), whereas PAEs such as BBP, DEHP, and DNOP (high-molecular-weight PAEs) were noted in roots at higher level (12-16% of initial concentration) implying that the higher molecular mass, higher number of carbon atoms in the molecule, the greater tendency to accumulate in the lettuce roots. It can be observed that although the number of PAEs noted in roots or leaves was below 6 ng/g, the highest affinity to roots was noted in the case of DMP, BBP, and DEHP, whereas to the leaves for DEP, DBP, DNOP.

Lots of attention in the literature is paid to DEHP, as it is considered a possible carcinogenic agent to humans with high rates of bioaccumulation (Ai et al., 2023). DEHP is widely noted in the environmental matrices due to its high leachate from polymers. It was observed that DEHP is immobilized in the soil due to weak physical bonding with soil organic matter (the hydrophobic and  $\pi$ — $\pi$  bonds (Ramanayaka et al., 2023) or via preferred strong but reversible chemisorption to soil mineral fraction when organic matter is absent implying its easier migration into soil water and thus increased availability in soil organisms such as earthworms and plants (Ramanayaka et al., 2023). Thus DEHP decomposition in the environment can be correlated with the soil organic carbon nitrogen rather than soil organic carbon and CEC (cation exchange capacity) of the soil (thus soil characteristics) and its microbial activity (Liu et al., 2023). It was observed that even a slight change in the environmental conditions may result in the desorption of PAEs from soil minerals (Ramanayaka et al., 2023). The transport of DEHP in the food chain (algae-crustacean-fish: *Chlorella pyrenoidosa*, *Daphnia magna*, and



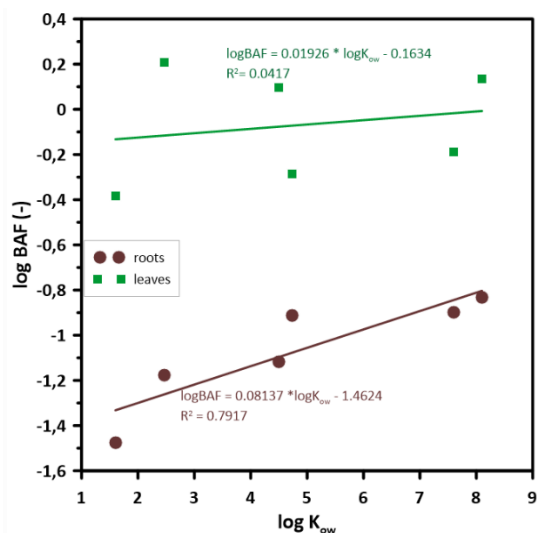
*Micropterus salmoides*) was noted (Liao et al., 2023) stressing the necessary monitoring of PAEs in different matrices (Mohammadi et al., 2023).

An increase in PAEs concentration usually did not result in increased root accumulation (Fig. 2a). Lower molecular mass PAEs were noted at lower concentrations in the roots showing that there is a mechanism for PAEs compensation in the roots. The most used DEHP, however, revealed the tendency to accumulate in the roots. Similar results were observed by Sun et al. (Sun et al., 2015), where the DBP accumulation in lettuce roots was noted, in contrast to DEHP behavior in our studies, that was also present mainly in the lettuce roots. However, the differences may arise from the fact that in the studies of Sun et al. the vegetables were grown in hydroponic nutrient solution. Thus it indicates that the bioavailability of PAEs to plant is affected by nutrient presence. At higher PAEs concentrations the distribution in lettuce was also changed (Fig 2). DEP and DNOP were showing the other tendencies. In the case of DEP at lower PAEs concentrations higher fraction was noted in the leaves (11%) than in roots (7.5%), whereas an increased amount resulted in a uniform distribution of about 7% of the initial value. DNOP at lower concentrations revealed a higher tendency to accumulate in leaves (20% of initial concentration) than roots (15%) whereas at doubled initial concentration, roots were the main DNOP sink (20% vs. 8% in the roots) suggesting that higher concentrations do not favor the migration in the plant. It was observed that the fate of PAEs is connected both with their concentration and soil characteristics. In long-term greenhouse cultivation accumulation of DEHP in soil was noted whereas DnBP concentration in prolonged time was lowered (N. Chen et al., 2017).



a

b



c

Fig. 2. Fate of PAES in soil-lettuce system a) roots, b) leaves, c) The relationship between the mean logarithmic bioaccumulation factors (log BAFs) and the octanol–water partition coefficients (log K<sub>ow</sub>) of the phthalate esters.

Due to its low water solubility and high octanol-water partition coefficient, it has a strong affinity and adsorption for solid particles and organisms. Bioaccumulation factor (BAF) was used to estimate the hazard of PAEs in lettuce. BAF is defined as the ratio of the concentration

of a chemical in an organism to its concentration in the matrix, here soil, which was calculated according to the following equation:

$$BAF = C_{biota} / C_{soil},$$

where  $C_{biota}$  is the concentration of PAEs in the biota ( $\mu\text{g/kg}$ ) and  $C_{soil}$  is the PAEs concentration in the soil ( $\mu\text{g/kg}$ ) (Ai et al., 2023). The bioaccumulation was combined with two main parameters:  $\log K_{ow}$  and molar mass of PAEs and the results were presented in Fig. 2c. Firstly,  $\log BAF$  increased with increasing lipophilicity of the PAEs (Ai et al., 2023). It was noted that a strong positive correlation ( $R^2 = 0.7917$ ,  $p < 0.1$ ) was observed between  $\log BAF$  and  $\log K_{ow}$  or molar mass ( $R^2 = 0.9050$ ,  $p < 0.02$ ) in lettuce roots. Such dependence is not noted in the case of leaves. These results verified that PAEs with higher molecular weights can be absorbed by lettuce roots more efficiently than those with lower molecular weights.

A lipid–water partitioning is used for the description of bioaccumulation in food and  $\log K_{ow}$  higher than 4 indicates increased bioaccumulation in organisms. The bioaccumulation in roots may be especially dangerous when these parts are consumed as dietary intake is the dominant exposure route (over 90% of the total phthalate intake) in humans (Ai et al., 2023).

It was observed that highly hydrophobic phthalates like DEHP are salted out to a minor degree in freshwater and a higher degree in seawater (Henkel et al., 2023) changing their bioavailability. However, at low levels of PAEs pollution increased bacterial richness and promoted carbon and nitrogen transformation in paddy soils were noted (Y. Li et al., 2023) implying that careful monitoring of PAEs in the soil-vegetable system should be provided.

#### 4. Conclusions

PAEs are widely produced and emitted into the environment. Their environmental fate is connected with several processes such as hydrolysis, photo- and microbial degradation, whereas the biodegradation and hydrolysis of PAEs in the environment are very slow revealing a potential hazard to organisms living in the soil. PAEs released into the soil may be transported

or even accumulated in the vegetables (e.g., DEHP) stressing the necessity to monitor their concentration. The results indicate that QuEChERS GC-MS/MS is a viable method of PAEs determination both in soil and in the lettuce samples with high linearity ( $>0.997$ ), recovery (97.2-99.1%), and very low detection limits (even 90 ng/kg). Although some PAEs revealed the tendency to accumulate in the lettuce roots, however, mostly used DEHP and DNOP were noted mainly in the lettuce leaves revealing greater hazards to humans.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### **Author Credit Statement**

A. Sokołowski Investigation; Writing - original draft; M. P. Dybowski Formal analysis; Investigation; Methodology; Validation; Visualization, Writing - original draft; P. Oleszczuk Supervision; Y. Gao Supervision; B. Czech: Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Visualization; Writing - original draft; and Writing - review & editing.

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