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THE EFFECTS OF DI-N-OCTYL PHTHALATE (DNOP) ON *SINAPIS ALBA* IN A MICROCOSM EXPERIMENT

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Phthalates, such as di-n-octyl phthalate (DnOP), are micropollutants released from microplastics, entering the environment mainly through biowaste collected and stored in plastic bags. When compost becomes contaminated, these compounds may be transferred into agricultural systems, potentially reducing crop yield and quality as well as posing risks to food safety. This study examined DnOP leaching from PVC under different conditions and its effects on white mustard (*Sinapis alba*) in a microcosm experiment with the aim of evaluating agricultural risks. Leaching experiments showed that DnOP release was most pronounced in water, reaching 60.43 µg/L by Month 6. In contrast, release into soil was minimal with only 14.41 µg/L detected, suggesting stronger retention. The 'Simple' samples displayed consistent and significant increases over time with concentrations of up to 45.25 µg/L measured. Plant experiments revealed that DnOP inhibited germination and early growth in a concentration-dependent manner. By Day 12, control plants grew to a height of 5.5 cm, while those exposed to 200 mg/L DnOP were only 3 cm tall. By Day 30, plants that were exposed were shorter due to a reduction in growth rate and visible stress, although flowering was observed, indicating an altered growth strategy under chemical pressure. Importantly, no distinct morphological abnormalities were detected, even at the highest concentrations. Chemical analysis confirmed a dose-dependent accumulation of DnOP in plant tissues. Uptake was particularly significant between 100 and 150 mg/L, resulting in a 13.8% increase, while the rise between 150 and 200 mg/L was smaller (+6.1%). Plants exposed to higher concentrations contained nearly double the DnOP levels of their control counterparts, highlighting the significance of the growth medium and high exposure scenarios. The findings demonstrate that substantial amounts of DnOP can accumulate in white mustard. Further studies are needed to clarify its impact on plant nutrient metabolism, particularly sulfur-related compounds, as yield, nutritional quality and agricultural sustainability might be under threat.

Keywords: biowaste, phthalate, agricultural sustainability, *Sinapis alba*, microplastic

1. Introduction

With the widespread use of plastics, research into their environmental risks has also come to the forefront. In addition to microplastics, increasing attention has been paid to the leaching of plasticizers (phthalates) as well as their associated environmental hazards and effects on ecosystems. The extensive application of polyethylene terephthalate (PET) poses significant environmental challenges as its resistance to biological degradation leads to the fragmentation of discarded products into microplastics, which infiltrate water resources, soils and the food chain [1]. This is particularly concerning since microplastics are widely detectable in bodies of surface water, occurring in diverse forms and compositions, moreover, can also be ingested by fish as well as birds [2]. Such microplastics may contain endocrine-disrupting compounds and other harmful substances, such as bisphenols and phthalates, which severely affect the hormonal balance of living organisms [3]. Furthermore, these microplastics can also be consumed by humans through drinking water, air or food, where they may accumulate in the liver, spleen, lungs and other organs, thereby potentially compromising human health [4].

Based on the measurements of Tóth et al. [5] conducted on the B-fraction of mixed municipal solid waste, it was established that the plastic content of the >4 and 2–4 cm fractions was 40 and 25%, respectively. In contrast, the polyvinyl chloride (PVC) content did not decrease proportionally with the reduction in particle size. While the proportion of PVC in the >4 cm B-fraction was minimal, it reached 10.86% in the 2–4 cm fraction. Compared with other size fractions, the 2–4 cm

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range contained the highest amount of PVC-based plastics and therefore should be studied further. Regarding organic matter, the >4 cm fraction contained 5%, whereas the 2–4 cm B-fraction contained 10% [6]. These results may also be influenced by seasonal variability in waste composition.

During the separate collection of municipal green waste, it is frequently observed that a considerable amount of plastic film is introduced into composting facilities. Prior to waste processing, these plastics begin to fragment under environmental influences such as temperature fluctuations, ultraviolet radiation and moisture. As a result, green waste subjected to windrow composting already contains large amounts of microplastics [7]. During aerobic stabilization, the internal temperature of the windrow can reach up to 70 °C, which promotes the leaching of plasticizers from microplastics. Consequently, these substances can be incorporated into the compost material that is later applied for soil improvement in agricultural fields and residential gardens [8].

In addition to the challenges posed by inadequate waste management, it is also important to assess the biological risks associated with plastic-containing materials that agriculture faces directly. In this context, discarded waste is not of concern but rather tools and materials intentionally used to support agricultural practices. Such materials include drainage systems, mulching films, geogrids and pesticides, many of which contain the plasticizer Di-n-octyl phthalate (DnOP). As a widely used additive, DnOP remains a source of considerable environmental concern due to its potential release, particularly from PVC-based products [9].

The effects of DnOP on plants have been extensively studied. DnOP, as a phthalate compound, can act as an endocrine disruptor, influencing plant growth processes and reproductive cycles, which in the long term may affect ecosystem stability [10]. Beyond its morphological effects on plants, it is also crucial to investigate the potential impacts of DnOP on nutrient uptake and biochemical pathways [11]. Should DnOP negatively affect nutrient assimilation, it would not only reduce crop yield but also compromise the overall quality of agricultural products.

The central focus of the experiments presented in the current research is the evaluation of the effects of DnOP on white mustard (*Sinapis alba*), a plant species widely used in agriculture and applied here as a test organism. The key questions of this study are as follows: Can DnOP adversely influence the growth intensity and morphological traits of this species of plant? Is this species of plant capable of taking up DnOP from the growth medium? If so, to what extent can the plasticizer present in the substrate accumulate in it? In the present study, first, the leaching dynamics of DnOP from PVC samples under varying environmental conditions were investigated in order to determine potential exposure levels. Following this, the response of white mustard was evaluated across a range of DnOP concentrations with a particular emphasis on the assessment of morphological alterations and growth-related parameters.

2. Materials and methods

2.1. Materials

Green waste compost

Green waste compost (GWC) with a moisture content of approximately 30% was collected from the composting facility at the University of Pannonia (Hungary). The feedstock material consisted of green waste generated on the university campus, e.g. grass clippings, leaves and pruning residues, moreover, its composition and origins are comparable to material typically used in industrial-scale composting plants. Therefore, the compost applied in this study can be considered representative of standard GWC used in agricultural and environmental applications.

Commercial garden soil (GS)

During the experiment, standard potting soil with low peat content was used, which is widely accessible, basically black in color and has a greasy texture. The purpose of selecting this material was to rule out the presence of DnOP in the starting material and guarantee a suitable growing medium for the plants during the experiment with the indicators necessary for ideal plant development.

Di-n-octyl phthalate

DnOP is a phthalate ester produced by the esterification of phthalic acid with n-octanol. Its molecular formula is $C_{24}H_{38}O_4$ with a molar mass of 390.56 g/mol and is a colorless or pale yellow oily liquid, practically insoluble in water but miscible with many organic solvents. Due to its high boiling point (~384 °C) and low volatility, it is primarily used as a plasticizer for PVC and other polymers, providing flexibility and durability. The compound is chemically stable and degrades slowly under environmental conditions, thereby persists in soils and sediments. DnOP with a purity of 99.0% was used in the experiment. The PVC used in the leaching test was shrink wrap suitable for packaging beverage bottles.

2.2. Measurement methods

X-ray fluorescence analysis

Elemental analysis of garden soil samples was performed using a handheld SPECTRO xSORT COMBI XHH03 X-ray fluorescence spectrometer equipped with a docking station. Measurements were conducted under the SPECTRO environmental fundamental parameter calibration mode, which allows for matrix-independent quantification of major and trace elements. Prior to taking measurements, soil samples were oven-dried at 105 °C to constant weight, homogenized and sieved to <2 mm in order to reduce heterogeneity as well as ensure comparability. After each series of measurements was taken, both calibrated concentration values and net count data were plotted as cross-plots to assess consistency and linearity of response. The coefficient of determination

(R^2) was typically >0.9 with the lowest acceptable value being 0.8, indicating robust analytical performance. Replicate measurements (n ≥ 3 per sample) were taken and their average values regarded as the final concentrations.

Orthophosphate (PO_4^{3-}) determination

Orthophosphate (reactive phosphate) was measured according to the Hungarian Standard MSZ EN 1189:1998 [12]. In this method, dissolved orthophosphate reacts with ammonium molybdate under acidic conditions (L-ascorbic acid) to form a phosphomolybdate complex which is subsequently reduced typically using stannous chloride or ascorbic acid to yield a blue-colored molybdenum complex ("molybdenum blue"). The intensity of the color is directly proportional to the concentration of orthophosphate in the sample. Absorbance was measured spectrophotometrically at a wavelength of 880 nm using deionized water as a blank. Calibration curves were prepared from a series of potassium dihydrogen phosphate (KH_2PO_4) standard solutions. The method is applicable within a concentration range of approximately 0.05–5.0 mg/L PO_4^{3-} with a limit of detection (LOD) around 0.01 mg/L.

pH determination

A portable pH meter (Hanna Instruments, Model HI 83141) equipped with a combined glass electrode was used to measure the pH, which was calibrated daily prior to taking measurements using standard buffer solutions of pH 4.01, 7.00 and 10.01 at laboratory temperature in line with the manufacturer's instructions.

Soil samples were prepared by mixing a subsample with deionized water in a soil-to-water ratio of 1.0:2.5 (w/v), which was homogenized and allowed to equilibrate for 30 minutes before taking measurements. Each measurement was triplicated and the average values reported as the final pH.

Moisture content determination

The moisture content (M) of the soil samples was measured using an OHAUS MB 25 moisture analyzer. 10 g of representative soil subsample was placed into the instrument before being heated at 105 °C to constant weight. Instrument performance was checked by routine verification with reference mass samples to ensure accuracy and reproducibility.

Maximum water holding capacity determination

The maximum water holding capacity (WHC) of the soil samples was determined according to the OECD Guideline 222 (2004) [13]. Air-dried soil was saturated with deionized water, allowed to drain freely for 2 hours and then weighed. WHC was calculated gravimetrically as the percentage of water retained relative to the dry soil mass. Measurements were triplicated and their average values reported.

GC-MS measurements

Gas chromatographic measurements were recorded by a GCMS-QP2010 SE Single Quadrupole GC-MS system equipped with a ZB-5ms column. The oven temperature was initially kept constant at 60 °C for 1 min before being increased to 310 °C at a rate of 10 °C/min and maintained at this final temperature for 5 mins. The instrument was operated in splitless mode with helium as the carrier gas at a constant flow rate of 1 mL/min.

During sample extraction, hexane as well as acetone were added to the samples and the extraction process accelerated using an ultrasonic bath. The filtrate was subsequently concentrated using a rotary evaporator at 50 °C.

Preparation of the DnOP solution

For the soil incubation experiment, DnOP was applied at three different concentrations (100, 150 and 200 mg/L). Stock solutions were prepared separately for each concentration. The required amount of DnOP was first dissolved in a small volume of analytical grade ethanol to facilitate solubilization since its solubility in water is very low. Having completely dissolved, the solutions were diluted with distilled water up to the target final volumes.

2.3. Microcosm experiment

Stones and other larger particles as contaminants were removed by sieving from the green waste compost and garden soil. The green waste compost and garden soil were homogenized before a homogeneous mixture containing equal amounts of garden soil and green waste compost was made as a control soil (Control). The designated volume of DnOP solution was poured onto the 300 g of homogenized soil in each pot before being carefully aerated and thoroughly mixed to ensure the phthalate was evenly distributed throughout the substrate and allow close contact with the root zone during plant growth. No more DnOP was added during the experimental period. The plants were only regularly watered with distilled water according to their needs. The residual ethanol from the DnOP stock solutions was allowed to evaporate completely before plant seeding to prevent any phytotoxic effects from the solvent itself. White mustard (*Sinapis alba*) served as test plant. After the evaporation period, 4 *S. alba* seeds were planted in each growth test pots (3 replicates for each DnOP concentration) which were incubated in POL-EKO KK 1200 Smart PRO climatic chambers with a steam humidifier as shown in *Figure 1*. The setting parameters in order to enhance plant growth were the following: 70% humidity with 100% light intensity (- 16992 LUX -) at 22 °C over 16 hours. Following this, the light intensity was set to 0% (representing nighttime) at 20 °C for 8 hours. Every day, the growth rate as well as mortality of the plants were monitored and they were irrigated with tap water.

Table 1: General initial properties of the test soils

Sample	pH	OP mg/kg	M %	WHC %
GWC	8.1	21.5	31.4	144
GS	7.4	40.1	72.6	158

2.4. Leaching experiment

The leaching conditions (temperature, UV radiation) of plastics containing plasticizers were investigated in 1, 3 and 6-month-long experiments. This involved exposing PVC shrink wrap to various environmental matrices to quantify the release rates of DnOP, simulating real-world conditions. 2×2 cm plastic (PVC) pieces containing DnOP were investigated in an ageing chamber in 1, 3 and 6-month-long experiments under the following conditions over 3 replicates (*Figure 2*):

- 'Simple': without water and soil, UV and 35 °C;
- 'Water': in water, UV and 35 °C;
- 'Soil': in soil moistened with water, UV and 35 °C.

The DnOP concentration in the leachate was subsequently analyzed using gas chromatography-mass spectrometry to determine the extent of plasticizer release under different environmental conditions.

Figure 1: Microcosm experiment - from left to right: Control, DnOP 100, DnOP 150, DnOP 200

Figure 2: Leaching experiment - from left to right: 'Simple', 'Water', 'Soil'

3. Results and discussion

3.1. Green waste compost and garden soil

The soil samples were characterized for various physicochemical properties, including pH, electrical conductivity, organic matter content and nutrient levels, to establish the baseline conditions for the experiment. These initial analyses are essential for understanding the intrinsic fertility and potential buffering capacities of the different soil matrices, which can influence the bioavailability and mobility of DnOP.

Green waste compost

The pH of the compost was 8.1, remaining below the critical threshold of 9.0, thereby indicating that the compost can be considered chemically stable and does not exert inhibitory effects on the target plant group. The organic phosphate content was measured at 21.5 mg/kg, therefore, is a potentially bioavailable nutrient source for plants. The initial moisture content was 31.4%, while the water holding capacity (WHC) reached 144%, both parameters being favorable for sustaining adequate soil–plant water interactions during the experiment.

Elemental analysis revealed a substantial calcium concentration, indicating enrichment in calcium carbonate, with quartz likely present as the dominant mineral phase. Such a composition may enhance the buffering capacity and provide structural stability to the substrate. Regarding trace elements, none of the measured concentrations fell within ranges known to exert inhibitory effects on plant growth, thereby confirming that the compost matrix represents a suitable medium for evaluating DnOP exposure under controlled experimental conditions.

Potting soil

The pH of the potting soil was 7.4 as shown in *Table 1*, that is, close to neutral, which represents optimal conditions for the proper growth of most plant species. The orthophosphate (OP) level was measured to be 40.1 mg/kg, approximately twice as high as that observed in the previously characterized compost. This elevated value is likely attributable to microbial activity, providing an increased source of bioavailable phosphorus for plants. The initial moisture content (M) was 72.6% (*Table 1*), which deviates from the commonly reported value of ~60%. This difference can be explained by the period between packaging and use, during which microbial activity does not cease. As a result of decomposition processes, both CO_2 and H_2O are released. The water holding capacity (WHC) was found to be nearly identical to that of the compost used (*Table 1*). In terms of the inorganic elemental composition, the soil contained higher amounts of calcium, iron, magnesium and aluminum, consistent with the characteristics of carbonate-rich soils (*Table 2*). Based on the present analyses, however, it could not be determined whether the detected sulfur content is bound to mineral phases or associated with the organic fraction.

Table 2: Elemental composition of the green waste compost and garden soil according to XRF at 700 °C

Element		GWC	GS
P	%	1.29	0.67
S	%	1.38	9.80
K	%	2.84	1.18
Ca	%	19.49	30.03
Mg	%	0.30	0.53
Al	%	0.01	0.29
Fe	%	1.99	6.54
Si	%	12.52	14.60
Cu	mg/kg	74.93	51.80
Zn	mg/kg	292.17	144.93
Pb	mg/kg	35.73	81.30
Ti	mg/kg	2354.67	3794.33
Ni	mg/kg	48.23	142.40

3.2. Results of the leaching experiment

The concentrations of DnOP in three different sample types ('Simple', 'Water' and 'Soil') measured after 1, 3 and 6 months are presented in *Figure 3*. A general trend of increasing DnOP concentrations over the 6-month-long period was observed in most sample types, indicating continued leaching or accumulation of the plasticizer. The concentration in the 'Simple' sample consistently and significantly increased over time, nearly doubling from Month 1 (13.99 µg/L) to Month 3 (29.22 µg/L) and then continued to rise substantially to 45.25 µg/L by Month 6. This suggests a steady release or presence of DnOP in this sample type.

The 'Water' sample consistently exhibited the highest concentrations of DnOP throughout the study, starting at 44.50 µg/L in Month 1, steadily increasing to 45.44 µg/L by Month 3 and reaching its peak at 60.43 µg/L by Month 6. This might indicate that water acts as an effective medium for the leaching and accumulation of DnOP. The 'Soil' sample generally yielded the lowest concentrations compared to the other two sample types. Interestingly, its concentration slightly decreased from Month 1 (10.94 µg/L) to Month 3 (10.71 µg/L) before increasing to 14.41 µg/L by Month 6. Although an increase was observed by Month 6, its overall concentration remained significantly lower than those in the 'Water' sample, possibly suggesting different leaching dynamics or retention capabilities of DnOP within the soil matrix.

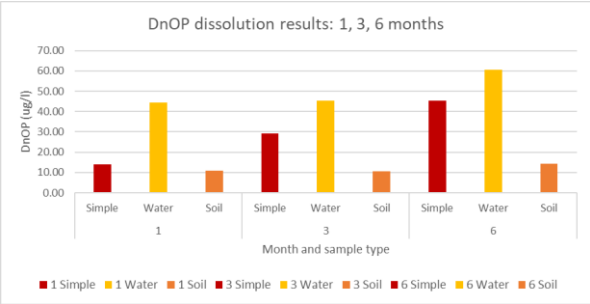

Figure 3: Results of the leaching of DnOP experiment after 1, 3 & 6 months

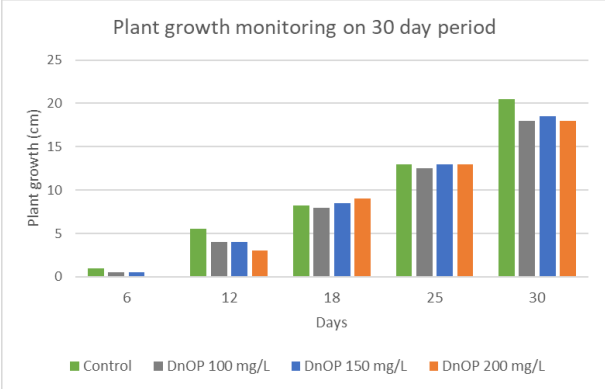

Figure 4: Results regarding plant growth intensity

3.3. Results of the Microcosm experiments

The soil substitute medium was homogeneous and its moisture content adequate to start the plant experiment. Based on the growth intensity results presented in *Figure 4*, it can be concluded that germination started later due to the effect of DnOP. By Day 12, the control was 5.5 cm tall, while the samples containing DnOP concentrations of 100 as well as 150 mg/L were 4 cm tall and the one with 200 mg/L of DnOP was the shortest at 3 cm in height. This indicates an inhibitory effect of DnOP on early plant growth with higher concentrations showing a more pronounced effect. By the 30th day of the experiment, the stress effect of DnOP could be observed. Even though the height of the plants was slightly reduced compared to the control samples, signs of flowering were visible. This phenomenon resulted from a stress response to DnOP exposure, decreasing the growth intensity of the plants but accelerating the vegetation process, namely flowering required for reproduction. Even at the highest applied concentration of DnOP, no morphological alterations could be detected in the plants.

3.4. Results regarding DnOP content

The uptake of DnOP by the plants exhibited a clear dose-dependent trend, although the response was not strictly linear as seen in *Figure 5*. Between 100 and 150 mg/L, a pronounced increase in DnOP accumulation of 13.8% was observed, indicating active absorption of the available phthalate within this concentration range. In contrast, the increment between 150 and 200 mg/L was notably smaller at +6.1%, suggesting the partial saturation of uptake mechanisms or regulatory constraints limiting further accumulation at higher concentrations. Compared to the control, plants exposed to concentrations of 150–200 mg/L contained approximately double the DnOP content, highlighting the significant impact of medium-to-high exposure levels on plant phthalate burden. These findings underscore the nonlinear dynamics of DnOP uptake and the potential for increased bioaccumulation when exposed to elevated environmental concentrations.

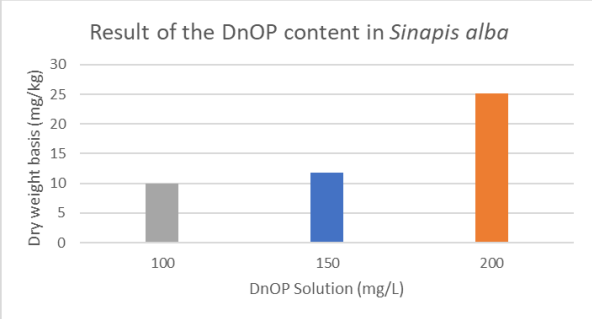

Figure 5: Results regarding the DnOP content in *Sinapis alba*

4. Conclusions

Phthalic acid esters are ubiquitous environmental pollutants with di-n-octyl phthalate being a prominent member frequently detected in soil ecosystems. Its widespread presence necessitates a thorough investigation into potential ecological impacts, particularly on biomass such as white mustard (*Sinapis alba*), a plant commonly used in phytoremediation studies due to its robust growth and metal accumulation capabilities [14]. While the remediation potential of white mustard is well-documented, the influence of emerging contaminants like di-n-octyl phthalate on its growth and broader soil health remains less understood [15].

Based on the outcomes of the present study, it can be established that white mustard (*Sinapis alba*), applied here as a test plant, is able to accumulate considerable amounts of the phthalate ester DnOP, which is widely used as a plasticizer in PVC products. This observation raises important questions regarding the potential physiological and ecological consequences of DnOP exposure in agricultural systems. For a more comprehensive assessment, future investigations should not only quantify the extent of DnOP uptake but also address its possible role in interfering with nutrient dynamics, especially in relation to macro- and micronutrient availability as well as assimilation.

Of particular concern is the potential impact of DnOP on sulfur uptake, as sulfur-containing glucosinolates are directly responsible for the pungency of mustard and constitute key secondary metabolites with both ecological and nutritional relevance. Any disruption in sulfur assimilation, as well as in the uptake of other essential elements, could therefore result in significant alterations to plant growth performance, yield stability and the overall nutritional quality of the harvested biomass. Such changes may have cascading effects not only on crop productivity but also on the broader agro-economic context, potentially influencing market value, consumer acceptability and food security. Consequently, systematic and multidisciplinary studies are warranted to elucidate the mechanistic pathways through which DnOP interacts with plant physiology, soil chemistry and agroecosystem sustainability.

SYMBOLS

B-fraction	fraction smaller than 8 cm of municipal solid waste
DnOP	di-n-octyl phthalate
GS	garden soil
GWC	garden waste compost
M	moisture
PET	polyethylene terephthalate
pH	
PVC	polyvinyl chloride
OP	orthophosphate
WHC	maximum water holding capacity
XRF	X-ray fluorescence spectrometer

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