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Bioaccumulation of microplastics in Pisum sativum L. - a pot experiment

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Abstract. Microplastic (MP, 1 μm-5 mm size) pollution of the environment is a global problem with a significant risk to ecosystems and human health. The aim of the present study is to assess the potential of pea plants (*Pisum sativum* L.) to assimilate MPs from soil and to translocate them into aboveground organs (stem and leaves) as well as to investigate the effects of MPs on plants. Bioaccumulation of MPs from the three studied fractions (1 μm, 5 μm, 100 μm) into all plant organs was established. They exerted a suppressive effect on the biosynthesis of photosynthetic pigments, and hence on the intensity of photosynthesis.

Key words: microplastic pollution, soil, plant uptake, translocation, pea, bioassay.

Introduction

Microplastics (MPs) are well known plastic particles within the range of 1 μm – 5 mm (Nikolov and Petrova, 2023; Zainudin et al., 2020). They become one of the most serious global problem because of their ubiquitous distribution and persistent presence in the environment (Zang et al., 2020). Many studies have shown their occurrence and impacts in aquatic environments (Horton et al., 2017; Larou et al., 2021), suggesting that terrestrial ecosystems are probably receiving much more plastic waste that the oceans (Horton et al., 2017; Ebere et al., 2019).

MPs can enter soil via multiple routes turning it, especially arable soil, into a permanent sink for plastic, coming mostly from various anthropogenic activities – wastewater treatment plants, manufacturing, mulching by agricultural systems and so on (Chae & An, 2018; de Souza Machado et al., 2018; Geyer et al., 2017). The presence of MPs in soil could change soil properties (Chae & An, 2018; de Souza Machado et al., 2018) which could be beneficial or detrimental for plants (Colzi et al., 2022; Nikolov & Petrova, 2023). Some studies have shown the possibility of plants to accumulate nano- and microplastics from polluted soil revealing at species-specific effects on their development and metabolism (Azeem et al., 2021; Wang et al., 2022). Given in mind that plants are primary producers in a food chain and are consumed by human, the bioaccumulation of MPs poses a potential risk for human health (Wright & Kelly, 2017).

Pea (*Pisum sativum* L.) is well-known test species in ecotoxicological studies (Kim et al., 2022) as well as an important source of protein and aminoacids for humans (Iqbal et al., 2006). Accordingly, in this study we aimed to assess the potential of pea to assimilate MPs from soil and to translocate them to the aboveground organs (stem and leaves) as well as to investigate the effects of MPs on plants. To the best of our knowledge, similar study regarding the impact of MPs on pea via exposure in soil media was made only once before (Kim et al., 2022).

Materials and Methods

Test seeds, soil and MPs materials

Pea seeds were obtained from a comercial seed producer GBM Commerce LTD (Lactofol, Bulgaria). Pea seed germination was pre-tested and 100% germination was demonstrated (20 germinated out of 20 seeds planted).

Standardized soil (substrate) was used for sowing and propagation, purchased from a certified manufacturer (Agro CS, Czech Republic). Substrate characteristics are as follows: total N content 50-300 mg/l; P content 80-300 mg/l; K content 80-400 mg/l; pH = 5-6.5; Soil organic matter - min 35%.

Polystyrene microplastic spheres (PS MPs) of three sizes have been used, colored with a different fluorescent pigment as follows: 1 μm - yellow; 5 μm - red; 100 μm – blue. The MPs were purchased from Sigma Aldrich (Belgium), and have all the necessary quality certificates.

Bioassay design

Bioassay was carried out in pots with a volume of 0.1 liter. Each pot was filled up to 2/3 of the volume with standardized soil to which one of the studied fractions of MPs was added at a concentration of 0.01% v/w. Two pea seeds were placed in each pot and a 1.5 cm layer of soil was added on top. Controls were made by a similar manner without adding MPs. All experimental variants were of a 4 replicates.

Pots were placed in a greenhouse under controlled conditions - air temperature in the range of 22-28°C±3°C and relative air humidity in the range of 45-65%±10%, for a period of 21 days (07-28 April 2023).

Measurement of endpoints

Shoot and root lengths and biomass (analytical balance KERN, AMB-BA-def-1636, Germany) were determined at the end of exposure (21-th day). Intensity of photosynthesis and transpiration, as well as the photosynthetic pigments content in plants, were measured following standard procedures (Petrova, 2020; Petrova et al., 2022). Photosynthesis intensity, transpiration and stomatal conductance measurements were performed with a Q-box CO650 portable photosynthetic system (Quibit Systems Inc., Canada). They were performed on three fully developed, undamaged leaves of the same physiological age, with at least 30 values taken for each individual leaf.

To confirm the uptake of MPs, the harvested plants were dissected and analysed using a Axio Scope.A1 fluorescence microscope (Carl Zeiss). Pictures were taken and processed with a digital camera adapted to the microscope – ProgRes CapturePro.

Statistical analysis

Descriptive statistical analysis (min, max, mean, median, mode, SD) was applied and the relationships between the individual parameters of pea plants and MPs were tested using Spearman rank correlation coefficients. All analyzes were performed using SPSS 20.0 software (IBM SPSS Statistics, Armonk, NY, USA).

Results and Discussion

The penetration of MPs from the three studied fractions (1 μm, 5 μm, 100 μm) into all plant organs was established (Fig. 1). Overall, the highest number of microplastic particles in one field of view was observed in plants treated with the finest fraction (1 μm), and higher levels of MPs were found in the belowground biomass (central and lateral roots) compared to aboveground biomass (stem and leaves).

To date, most studies addressed the presence of MPs in the roots and leaves of plants exposed to MPs (either in soil or Hoagland solution), whereas relatively few studies have observed MPs in plant stems. Kim et al. (2022) proved the translocation of MPs from the roots to the stem of pea plants via soil media. Dong et al. (2021) investigated the impact of polystyrene microspheres on red lettuce development and nutritional quality, where they also found bioaccumulation of the MPs in the root system. Furthermore, this study revealed that MPs effects on plant physiology were mainly expressed as a decrease in the chlorophyll content, the intensity of photosynthesis and transpiration, as well as a decrease in soluble sugars and proteins. As He et al. (2018) stated, terrestrial plants have a plenty of specific structures and mechanisms which may promote the uptake of MPs from soil, i.e. root, xylem, cytoplasm and vacuoles, transpiration, water and lipid fractions, potential of tonoplast, of plasma membrane, etc. By this way, the bioaccumulated MPs in plants could be transferred and even biomagnified in the food chains, threatening human health.

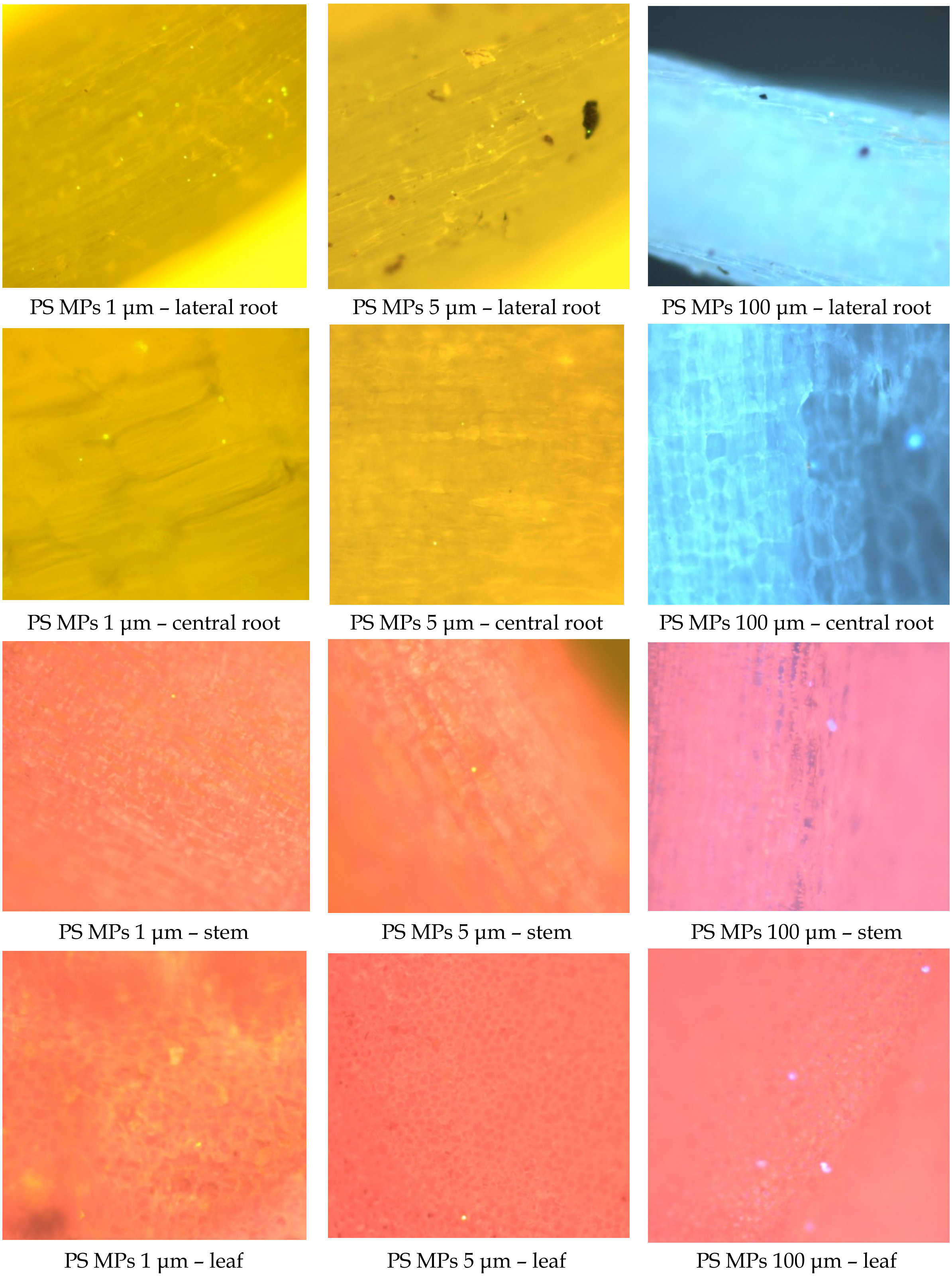

Fig. 1. Bioaccumulation of polystyrene microplastics (PS MPs) - luminous spheres accumulated in plant tissues.

In our study, the shoot length of pea plants varied from 7.20 cm (in the control) to 13.00 cm (5 µm MPs) (Fig. 2). Significant differences were found in the experimental variants with 5 µm and 100 µm MPs when comparing to the control ($p<0.05$). The root length of pea plants varied from 5.30 cm (in the control) to 15.60 cm (100 µm MPs) (Fig. 2), being reliable in the experimental variants with 5 µm and 100 µm MPs ($p<0.05$).

The shoot biomass varied from 0.59 g (in the control) to 1.64 g (5 µm MPs), and reliable differences compared to the control were also found for 5 µm and 100 µm MPs ($p<0.05$). The root biomass ranged from 0.48 g (5 µm MPs) to 1.31 g (5 µm MPs), with no significant differences from the control (Fig. 3).

Photosynthesis is one of the fundamental and at the same time one of the most sensitive processes in the plant organism, which is why it is often used as an early indication of stress conditions (Petrova & Petkova, 2023; Pignatelli et al., 2020). The content of photosynthetic pigments and the ratios between different functional groups of pigments also have a similar meaning (Ren et al., 2021).

In the course of the present study, it was found that MPs had a proven suppressive effect on the biosynthesis of chlorophyll and carotenoids ($p<0.05$), but this did not affect the ratios between them (Table 1). MPs exposure significantly reduced the amount of chlorophyll in pea plants, which was most pronounced in the 5µm fraction ($p<0.05$). Regarding carotenoids, a significant inhibitory effect was also observed, except for the 5µm fraction, where the effect was stimulatory (Table 1).

Data on the intensity of photosynthesis, transpiration and stomatal conductance are presented in Table 2. A statistically significant suppressive effect on the photosynthetic process was observed in the pea plants treated with 5 µm and 100 µm MPs ($p<0.05$), while a stimulatory effect was observed in the 1 µm MPs experiment, although no significant differrence from control was demonstrated. Regarding the intensity of transpiration and the conductivity of the stomatal apparatus, higher values were recorded in all test plants compared to those measured in the control ($p<0.05$). This can be taken as a confirmation that the toxic effect is not related to the stomata, that is, they are not the route by which the stress factor enters the plants.

The presence of MPs in plant tissues and organs can be associated with the observed disturbances in the metabolism, biosynthesis of key substances and the functioning of the plant organism. They, in turn, have an impact on the growth and development of the pea plants observed during the bioassay. Similar experiments need to be carried out in laboratory and field conditions to trace the overall manifesttation of the effects during the complete vegetative development cycle.

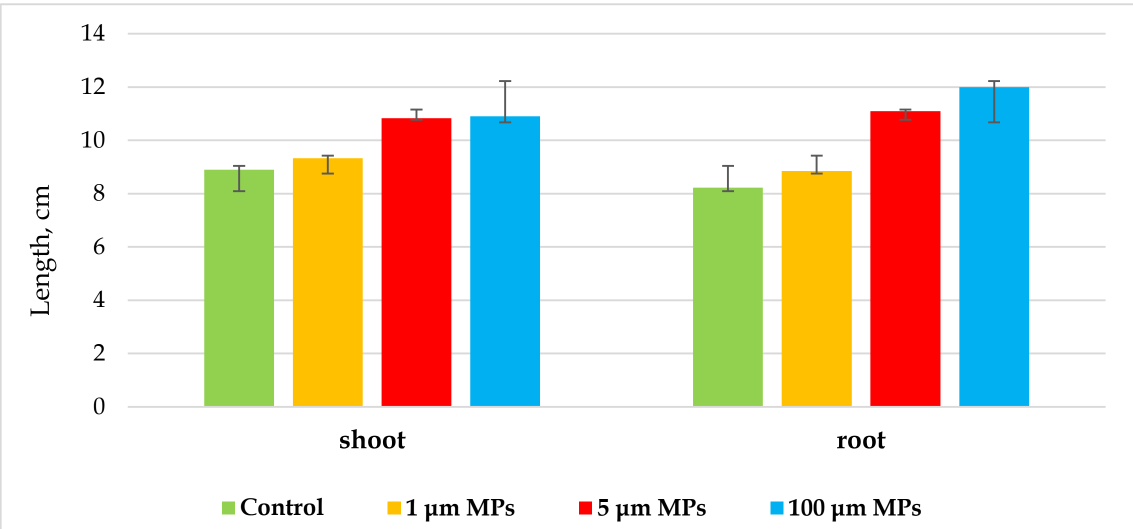

Fig. 2. Shoot and root length of pea plants after 21 days of MPs exposure

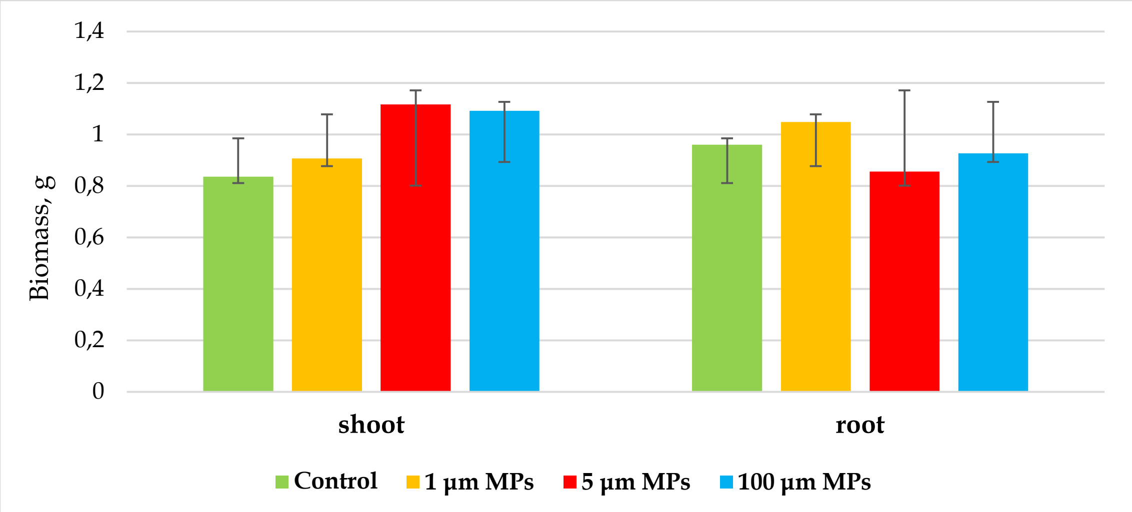

Fig. 3. Shoot and root biomass of pea plants after 21 days of MPs exposure

Table 1. Content of photosynthetic pigments in leaves of pea plants after 21 days of MPs exposure.

Experimental variant	Concentration, mg g^{-1}				Ratio	
	chl a	chl b	chl a+b	car	chl a/b	chl a+b/car
Control	12.76	7.19	19.95	5.50	1.77	3.63
1 µm MPs	4.97	2.93	7.90	1.96	1.69	4.04
5 µm MPs	3.76	2.19	5.95	1.54	1.71	3.87
100 µm MPs	4.25	2.44	6.69	1.74	1.74	3.84

Table 2. Photosynthesis, transpiration and stomatal conductance of pea plants after 21 days of MPs exposure.

Experimental variant	Intensity of photosynthesis, mol m^{-2} s^{-1}	Intensity of transpiration, mol m^{-2} s^{-1}	Stomatal conductance, mol m^{-2} s^{-1}
Control	5.580	1.92	50.23
1 µm MPs	5.851	2.12	56.11
5 µm MPs	2.182	2.37	63.55
100 µm MPs	3.945	2.76	78.16

Similar research with cucumber as a test species and polystyrene nanoplastic granules (100 nm, 300 nm, 500 nm, 700 nm fractions) was conducted by Li et al. (2021). They found a reliable decrease in the length and biomass of the underground and aboveground parts of the treated plants only at the 300 nm fraction, where they concluded that the size of the plastic particles has a decisive importance for the degree of manifesttation of the impact. This is also confirmed by the observed changes in the intensity of photosynthesis and the decrease in the amount of photosynthetic pigments depending on the fraction of nanoplastics.

Pignatelli et al. (2020) also conducted a similar series of bioassays to determine the impact of different types of MPs on cress over a 7-day period and over a 21-day period. Their results correlate with ours both in terms of the lack of effect on germination and the stimulatory effect on the growth and accumulation of biomass in the aerial part of the plants.

Same effect of PE MPs was reported by Jia et al. (2022) for chlorophyll in rape and by Colzi et al. (2022) for *Cucurbita pepo*. Recent studies have shown that MPs alter also the chl a/chl b ratio of plants, and thus reducing the effectiveness of photosynthesis.

Conclusions

Although the effect of MPs on seed germination has been often reported to be negative, in the present bioassay with polystyrene MPs they did not affect the germination of pea seeds. The penetration and bioaccumulation of 1 µm, 5 µm, 100 µm MPs in all plant organs - roots, stem, leaves, has been observed. A reliable suppression of the growth and biomass synthesis of pea plants has been found, and it was most strongly manifested in the 5 µm and 100 µm fractions. MPs had also a suppressive effect on the biosynthesis of photosynthetic pigments, and hence on the intensity of photosynthesis, while at the same time a stimulating effect on the transpiration process is taken into account.

The present study demonstrates that MPs from soil are rapidly absorbed by plants through the root system, transported to and accumulated in even the most distant aerial organs. Thus, they have a significant impact on plants, which in the aboveground organs manifests itself as suppression of one of the fundamental processes – photosynthesis, responsible for the synthesis of the necessary compounds for their existence. Reduced bioproductivity probably results in growth stimulation to increase photosynthetic area to compensate for reduced photosynthetic activity. The size of the particles is an important factor determining their ability to bioaccumulate, on the one hand, and the degree of impact on plant life processes, on the other. Future comprehensive studies are needed to more fully reveal the impact of MPs on plants and their ability to travel through food chains to humans.

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References

Azeem, I., Adeel, M., Ahmad, M.A., Shakoor, N., Jiangcuo, G.D., Azeem, K., Ishfaq, M., Shakoor, A., Ayaz, M., & Xu, M. (2021). Uptake and Accumulation of Nano/Microplastics in Plants: A Critical Review. *Nanomaterials*, 11, 2935. doi: 10.3390/nano11112935

Chae, Y., & An, Y.J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A review. *Environmental Pollution*, 240, 387–395. doi: 10.1016/j.envpol.2018.05.008

Colzi, I., Renna, L., Bianchi, E., Castellani, M.B., Coppi, A., Pignatelli, S., Loppi, S., & Gonnelli, C. (2022). Impact of microplastics on growth, photosynthesis and essential elements in *Cucurbita pepo* L. *Journal of Hazardous Materials*, 423, Part B, 127238. doi: 10.1016/j.jhazmat.2021.127238

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., & Rillig, M.C. (2018). Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Chang. Biol.*, 24, 1405–1416. doi: 10.1111/gcb.14020

Dong, Y., Song, Z., Liu, Y., & Gao, M. (2021). Polystyrene particles combined with di-butyl phthalate cause significant decrease in photosynthesis and red lettuce quality. *Environmental Pollution*, 278 (2021) 116871. doi: 10.1016/j.envpol.2021.116871

Ebere, E., Wirnkor, V., & Ngozi, V. (2019). Uptake of microplastics by plant: a reason to worry or to be happy? *World Scientific News*, 131, 256-267

Geyer, R., Jambeck, J.R., & Law, K.L. (2017). Production, use, and fate of all plastics ever made. *Sci. Adv.*, 3, e1700782. doi: 10.1126/sciadv.1700782

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, 586, 127-141. doi: 10.1016/j.scitotenv.2017.01.190.

Jia, H., Wu, D., Han, Y., Sun, L., & Li, M. (2022). Impact of microplastics on bioaccumulation of heavy metals in rape (*Brassica napus* L.). *Chemosphere*, 288, Part 2, 132576. doi: 10.1016/j.chemosphere.2021.132576

Kim, D., An, S., Kim, L., Byeon, Y.M., Lee, J., Choi, M.-J., & An, Y-J. (2022). Translocation and chronic effects of microplastics on pea plants (*Pisum sativum*) in copper-contaminated soil, *Journal of Hazardous Materials*, 436, 29194. doi: 10.1016/j.jhazmat.2022.129194.

Larue, C., Sarret, G., Castillo-Michel, H., & Pradas del Real, A.E. (2021). A Critical Review on the Impacts of Nanoplastics and Microplastics on Aquatic and Terrestrial Photosynthetic Orga-

nisms. *Small*, 17, 2005834.
doi: 10.1002/smll.202005834

Li, Z., Li, Q., Li, R., Zhou, J., & Wang, G. (2021). The distribution and impact of polystyrene nanoplastics on cucumber plants. *Environmental Science and Pollution Research*, 28, 16042–16053. doi: 10.1007/s11356-020-11702-2

Nikolov, B., & Petrova, S. (2023). Are there plant biomarkers for microplastic pollution in soil? A review. *Ecologia Balkanica*, 15(1), 232-245.

Petrova, S. (2020). Efficiency of *Pinus nigra* J.F. Arnold in removing pollutants from urban environment (Plovdiv, Bulgaria). *Environmental Science and Pollution Research*, 27(31), 39490-39506. doi: 10.1007/s11356-020-09975-8

Petrova, S., Velcheva, I., Nikolov, B., Vasileva, T., & Bivolarski, V. (2022). Antioxidant Responses and Adaptation Mechanisms of *Tilia tomentosa* Moench, *Fraxinus excelsior* L. and *Pinus nigra* J. F. Arnold towards Urban Air Pollution. *Forests*, 13, 1689, 1-16.
doi: 10.3390/f13101689

Petrova, S., & Petkova, M. (2023). Plant Traits of *Tilia tomentosa* Moench, *Fraxinus excelsior* L., and *Pinus nigra* J.F.Arnold as a Proxy of Urbanization. *Forests*, 14(4), 800.
doi: 10.3390/f14040800

Pignatelli, S., Broccoli, A., & Renzi, M. (2020). Physiological responses of garden cress (*L. sativum*) to different types of microplastics. *Science of the Total Environment*, 727, 138609.
doi: 10.1016/j.scitotenv.2020.138609

Ren, X., Tang, J., Wang, L., & Liu, Q. (2021). Microplastics in soil-plant system: effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues. *Plant and Soil*, 462, 561–576. doi: 10.1007/s11104-021-04869-1

Wang, F., Feng, X., Liu, Y., Adams, C.A., Sun, Y., & Zhang, S. (2022). Micro(nano) plastics and terrestrial plants: Up-to-date knowledge on uptake, translocation, and phytotoxicity. *Resources, Conservation and Recycling*, 185, 106503. doi: 10.1016/j.resconrec.2022.106503

Wright, S., & Kelly, F. (2017). Plastic and Human Health: A Micro Issue? *Environmental Science & Technology*, 51 (12), 6634-6647. doi: 10.1021/acs.est.7b00423

Zainudin, B.H., Wong, T.W., & Hamdan, H. (2020). Pectin as oral colon-specific nano-and microparticulate drug carriers. *Polym. Sci. Innov. Appl.*, 257–286. doi: 10.1016/B978-0-12-816808-0.00008-1

Zang, H., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., & Jones, D.L. (2020). Microplastics in the agroecosystem: Are they an emerging threat to the plant-soil system? *Soil Biology and Biochemistry*, 148, 107926. doi: 10.1016/j.soilbio.2020.107926

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