ECOLOGIA BALKANICA
2023, Vol. 15, Issue 1 June 2023 pp. 232-245

Are there plant biomarkers for microplastic pollution in soil? A review

Bogdan Nikolov[1], Slaveya Petrova[1,2*]

[1] - Department of Ecology and Environmental Conservation, Faculty of Biology, Plovdiv University, Plovdiv, BULGARIA
[2] - Department of Microbiology and Ecological Biotechnologies, Faculty of Plant Protection and Agroecology, Agricultural University, 12 Mendeleev Blvd, 4000, Plovdiv, BULGARIA
*Corresponding author: nikolov81bg@uni-plovdiv.bg

Abstract. The ubiquitous presence of microplastics (MPs) in the environment is due to their higher persistence and extensive use in in almost every sector of our society, including packaging, construction, medicine, automotive, electronics, etc. Nowadays they are becoming an emerging threat on the soil-plant system, especially in agricultural production. The main goal of the present review is to synthesize the studies in this field with the majority of literature spanning the last five years in order to highlight possible plant biomarkers for MPs pollution in soil. Data revealed that the effect of MPs on plants are predominantly negative, but many studies revealed no significant effects as well as some studies reveal at positive effect (stimulation) of some plant biomarkers. Different plant species demonstrated different responses to same MPs pollution, which could be explained by both biological specificities in plant structure and/or in plant physiology. Overall, there is a dire need to establish long-term studies for a better understanding of their fate and associated risks mechanisms in realistic environment scenarios for safe agricultural functions.

Key words: soil-plant system, phytotoxicity, soil pollution, plant response, biomarkers.

Introduction

Plastics are well known synthetic materials made up of polymers, representing long molecules around chains of carbons atoms, especially hydrogen, nitrogen, oxygen, and sulfur (Zainudin et al., 2020). Based on their size, plastics are separated on four group, namely macroplastics (>25 mm), mesoplastics (5–25 mm), microplastics (MPs) (0.1–5 mm), and nanoplastics (NPs) (<100 nm) (Alimi et al., 2018; Azeem et al., 2021).

The extensive use of plastics has resulted in an alarming rate of release of plastic waste into the environment (Geyer et al., 2017). Soil, especially arable soil, has become a major and permanent sink for plastic, coming mostly from anthropogenic activities such as manufacturing, wastewater treatment plants (WTPs), mulching by agricultural systems and so on (Chae & An, 2018; de Souza Machado et al., 2018; Geyer et al., 2017). Most plastic waste is ultimately landfilled and difficult to degrade, leading to the gradual accumulation of plastics in the environment (Wright and Kelly, 2017). MPs differ from other soil pollutants by their lack of background value under natural conditions (He et al., 2022). Once MPs enter the soil they are trapped into different organic-mineral complexes and remain there for a very long time (Fuller and Gautam, 2016).

Higher plants, an irreplaceable part of the terrestrial ecosystem, are inevitably exposed to microplastics in both air, water and soil. The ecological effects of MPs on plants are widely studied in hydroponic systems but there is a small number of papers concerning the processes in soil-plant system and the effect of MPs on plant organism. Furthermore, their presence in plant food destined for human consumption causes a potential risk for the human health, the harmful effects of which have not yet been assessed. So, the need of a more comprehensive and deeper understand-ding MPs behavior in soil-plant system, their bioaccumulation patterns and effects on higher plants arises.

In this context, the scope of the present review is to: i) summarize the most current progress in the recent five years; ii) investigate the potential toxic impact of MPs on plants, especially crop plants; iii) reveal possible plant biomarkers of soil MPs pollution. This review will improve the understanding of the environ-mental behavior of MPS in soil-plant system, the effects and influencing mechanisms of MPs on higher plants and provide a theoretical reference to better assess the ecological risk of MPs in agricultural land.

Material and methods

We performed a literature search to gain an understanding of what is the scientific know-ledge about the impact of soil MPs on plants as well as which plant biomarkers have been most studied. The initial identification of the scientific literature in the field was done by searching for articles in most popular databases – Scopus, Web of Science and Science Direct platforms. The keywords searched were "microplastics in soil", "microplastics in plants", "microplastics toxicity on plants" (first stage of the review process) (Fig. 1). Some additional keywords associated with "plant biomarkers" and "plants response to microplastics" were also used in order to refine the results achieved.

Second stage screening of the outputs included manual removal of duplicates, and after that by checking the title, abstract and conclusions. The focus was on plant-soil system interactions, so the publication that employed non-soil experiments were ignored. The year of publication was also checked as we wanted to analyze the most current progress in this field (2019-2023). Third stage screening of the outputs revealed 30 papers that were relevant from all 128 found at first stage (O'Riordan et al., 2021; Petrova & Nikolov, 2023).

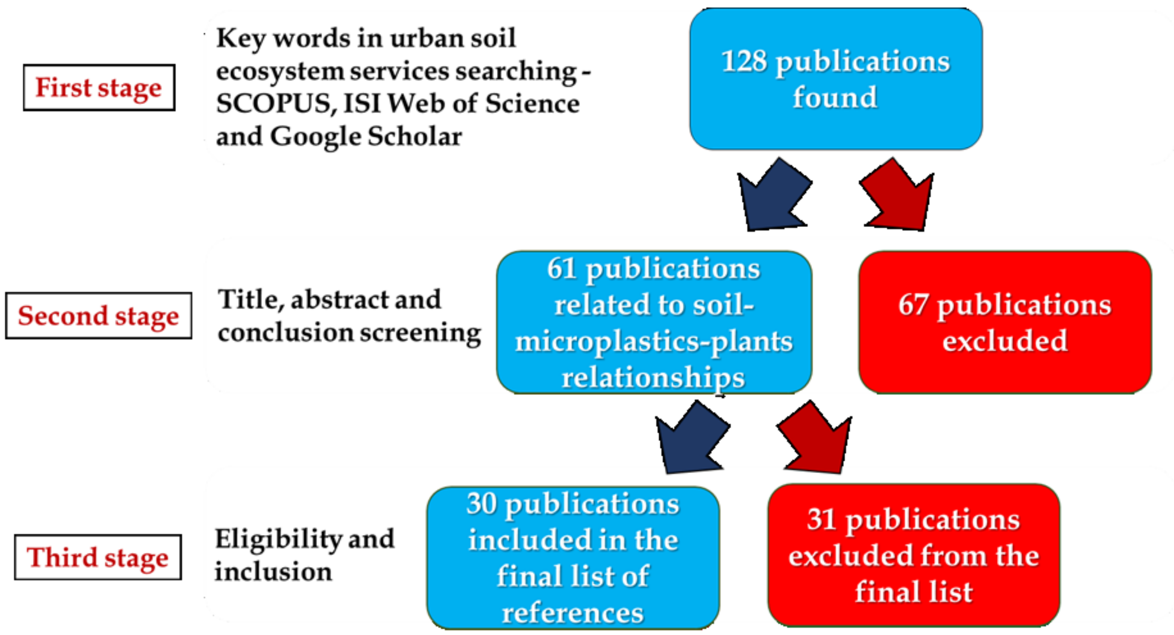

Fig. 1. Literature review process

The inclusion and exclusion criteria have been applied during the screening process (Table 1). From all 128 publications found at the first stage, only publications that addressed any context of soil-microplastics-plants relationships and were published in the period 2019-2023 were selected on the second stage – 61 passed and 67 excluded. At third stage, the thematic relevance was subjected to screening and total of 30 publications that addressed terrestrial plants response to MPs in soil were included into review analysis.

The literature review confirmed that the ecological effects of MPS on plants are widely studied in both aquatic or hydroponic systems but there is a small number of papers estimating the soil microplastics impact on plants (30 papers for the five-year period).

Table 1. List of criteria used in the three-step review process

Stage	Inclusion criteria	Exclusion criteria
1. Identification	Key words	no
2. Title, abstract and conclusion screening; publication year	Thematic relevance to the impact of microplastics in the soil to terrestrial plants Publication year in the range 2019-2023	Unrelated topic to soil-microplastics-plants interactions, abstract out of scope, no access, older than 2019
3. Eligibility and inclusion	Thematic relevance to the plants response (plant biomarkers) to MPs in soil	Content out of scope

Results and discussion

There are two major questions to be addressed within the soil-MPs-plants system: (a) plant uptake of MPs from soil and (b) ecological impact of absorbed MPs on plant's organism.

Uptake of soil MPs by terrestrial plants

Soil plastic pollution is a problem of a major concern but the problem with micro- and nanoplastics bioaccumulation by plants and their possible translocation through the trophic chains is still underestimated.

Generally, MPs have been considered to be difficult to uptake by plants due to their high molecular weight and/or large particle size (Ng et al., 2018; Zhang et al., 2022). Nevertheless, many experiments revealed that MPS can be uptake by the root system and transported to the shoot. Some studies have shown that the prevailing part of MPs accumulate on the root surface due to the large particle size and/or the strong hydrophobicity (Bosker et al., 2019; Taylor et al., 2020; Urbina et al., 2020). Sun et al. (2022) proved that the positively charged PS-NH_2 accumulate around the root zone but negatively charged PS-SO_3H can be translocated by root hairs to the mature zone. They supposed that different behavior of MPs is probably related to the effects of root exudates. Other authors revealed that the uptake of MPs by plants can be enhanced in the presence of heavy metals. Dong et al. (2021) reported that As in soil led to an increment of negatively charged MPs, so a greater plant uptake. They found also that As can deform or destroy the cell walls and thus allowing PS particles (<200 nm) to enter the cells of carrot.

There are many fractions of MPs which can get across plants' membranes and cell wall barriers as these processes are well documented using fluorescent microbeads (Li et al., 2021; Taylor et al., 2020). Taylor et al. (2020) revealed that MPs accumulate in wheat root cells although they cannot enter the plant. In a recent study Fajardo et al. (2022) used three types of MPs (white, blue and fluorescent blue) within a pot experiment with *Zea mays* seeds. They found that MPs not only accumulate in plat tissues but also act as pollutants carriers which poses a serious threat to both soil biota and terrestrial plants.

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. There is still a gap of knowledge in this field, probably due to the challenges like the inefficient extraction and quantification of MPS that are absorbed into plant tissues.

Plant biomarkers for MPs toxicity

Microplastics can affect plants both directly and indirectly. They can indirectly affect plant performance by changing the physicochemical properties or biological conditions of soil (Wang et al., 2022). For example, MPs in soil can significantly alter the soil pH, conductivity, and C:N ratio and thus they directly impact on abiotic composition in the soil-plant system (Qi et al., 2020a, Qi et al., 2020b) which might in further influence the plants development. The rhizosphere is regarded as the critical zone where various interacttions between soil, plants and microorganisms occur (Mendes et al., 2013). Other study has found that MPs also could impact on some of the fundamental physiological processes in plants by changing the rhizosphere microbial community's structure and metabolism (Ren et al., 2021). Consequently, it is obvious that soil MPs pollution not only interferes with soil nutrient cycling, but also potentially threatens the interspecific relationship and biodiversity of terrestrial ecosystems.

Direct effect of MPs is related to changes of various plant traits, mainly physiological (germination, growth, photosynthesis) and biochemical (metabolism, oxidative stress), but also genotoxic ones, as discussed below (Table 2). The effects of MPs were closely related to the species of plants and plastic type (chemical content) as well as to the MPs size and concentration in soil.

Nevertheless, we made an attempt to synthesize all recent knowledge in this field and to assess plants' response in order to evaluate the potential of some plant traits to be used as biomarkers for soil MPs pollution.

Seed germination

Plants' life cycle is quite dynamic as they constantly have to face changing environmental conditions. This permanent process of adaptation is particularly important for the seed germination, which, if occurring under unfavorable environmental conditions, can compromise the propagation of the species and crop yield (Golubinova et al., 2020; Longo et al., 2021). Seed germination is the first developmental process in the life cycle of vascular plants. This transition - from a dormant to a germinating seed – could be described as a crucial developmental switch in the plant ontogenesis. Subsequent transition from a germinating seed to an autotrophic organism is also very important step that requires a multilayered control due to its susceptibility to various factors. Seed germination and seedling growth are considered as multistep processes, involving both internal and external signals, aiming at provide a fine-tuning control network in plant (Longo et al., 2021). It is well-known that the process of transition from seed dormancy to seed germination is crucial also for the production of offspring, so it is often studied as an important ecological and commercial trait.

The effect of MPs on seed germination has been often reported to be negative especially in hydroponic conditions. Pehlivan & Gedik (2021) found that the germination potential of maize seeds was significantly reduced when exposed to 75-212 mm PVC particles in a soil culture media (0.02 g MPs/150 g soil), but Fajardo et al. (2022) revealed no significant effect on the germination of maize seeds exposed to PE microbeads (212-300 μm size, 0.1% w/w dose) in a pot experiment. An inhibition of seed germination of garden cress was reported by Pignattelli et al. (2020) which conducted a chronic toxicity experiment with natural soils and four type of MPs (0.125 mm) – PP, PE, PVC and PVC+PE mixture, at a concentration of 184 mg/kg. As Pflugmacher et al. (2020) supposed, the toxic leachates from plastic particles could be one of the possible factors for such inhibition. Other authors stated that MPs accumulate into seed coat, so they can suppress germination by blocking the surface pores and water uptake (Bosker et al., 2019).

Table 2. Effects of microplastics on plants

Plant species	Type of MPs	Size of MPs	Exposure concentration of MPs	Exposure time	MPs impact		Reference
					Biomarker	Effect	
Maize (*Zea mays*)	PE PLA	100-154 mm	0, 0.1%, 1% and 10% (w/w)	30 days	Growth	0/0 PE 0/- PLA	Wang et al. 2020
					Biomass	0/0 PE 0/- PLA	
					Chlorophyll	0/0 PE 0/- PLA	
Maize (*Zea mays*)	HDPE PLA		0, 0.1%, 1% and 10% (w/w)	30 days	Biomass	0/+ HDPE +/- PLA	Yang et al. 2021
Maize (*Zea mays*)	PE	212–300 µm	0.1% w/w	4 weeks	Seed germination	0	Fajardo et al. 2022
					Leaf length	-	
					Root length	-	
					CAT gene	0 root - leaf	
					GST gene	0	
					SOD gene root	+	
Maize (*Zea mays*)	PVC	75-150 mm 150-212 mm	0.02 g (w/w)/150 g soil		Germination	-	Pehlivan & Gedik, 2021
					Chlorophyll	-	
					genes POD1 and HSP1	+ HSP1 0 POD1	
					Relative water content	-	
					Membrane stability index	-	
					H_2O_2	+	
					quantum photosynthetic yield of photosystem-II Y(II)	0	
					max. electron transport rate (ETR)	-	
					max. quantum yield (FV/FM)	0	
					FV/F0	0	
Maize (*Zea mays*)	polyuretan - coated fertilizer (PCF)	3.79 ± 0.60 mm	0.01%, 0.1%, and 1% w/w		Plant height	0/+	Lian et al., 2021
					Shoot biomass	+	
					Root biomass	0	
					Pigments content	+	
					net photosynthetic rate	+	
					transpiration rate (Tr) and stomatal conductance (Gs)	0	
					intracellular CO_2 concentration	-	
					water use efficiency (WUE) and stomatal limitation values (Ls)	+	
Wheat (*Triticum aestivum* L.)	PVC and PE	125 µm	1%, 5%, 10%, and 20%	35 days	root-to-shoot ratio	0/+	Zang et al. 2020
					shoot biomass	0	
					Shoot and root biomass	-/+	
					harvested seed C:N ratio	0	

Wheat (*Triticum aestivum* L.)	LDPE	200–250 µm	0.5%, 1%, 2%, 5%, 8% (w/w)	15 days	Shoot and root length	- shoot + root	Liu et al. 2021
					Biomass	+/- shoot + root	
					chlorophyll	+/-	
					Root SOD, POD, CAT	+	
Wheat (*Triticum aestivum* L.)	LDPE and starch-based biodegradable plastic (Bio)	1 mm, 500 µm, 250 µm and 50 µm	1% (w/w) content	2 months 4 months 139 days	Plants height	0	Qi et al., 2018
					Number of tillers	0	
					Number of fruits	0	
					Shoot biomass	-	
					Root biomass	-	
					Root/shoot ratio	+	
					Leaf area	+	
					Number of leaves	0	
					Stem diameter	-/0	
					chlorophyll	0	
Wheat (*Triticum aestivum* L.)	PE	200 µm	0.2%	42 days	Growth/height	+	Guo et al., 2022
					biomass	-	
					Chlorophyll	+	
					Carotenoids	+	
					SOD	0	
					APX	-	
					POD	+	
Wheat (*Triticum aestivum* L.)	PE and PVC	125 µm	1% and 5%	54 days	Shoot biomass	+	Liu et al., 2022
					Root biomass	+ PVC 5%	
					Shoot-to-root ratio	-	
Soybean (*Glycine max*)	PS	100 nm, 1 µm, 10 µm and 100 µm	10 mg/kg	30 days	ROS, CAT, MDA, SOD and POD in roots	+	Xu et al., 2021
					ROS, MDA, SOD, Cat POD in stems	0	
					CAT and SOD in leaves	+	
					activity of soybean roots (KIT test)	-	
					genes SOD, CAT, POD	+	
Soybean (*Glycine max*)	PE PLA	20 and 50 µm from 20 to 60 µm	0.1% and 1%	49 days	Shoot length	+	Lian et al. 2022
					Shoot weight	0	
					Root length	-	
					Root weight	-	
					chlorophyll	0	
					SOD leaves	0	
					POD leaves	-	
					CAT leaves	+	
					Soluble protein	+	
					H_2O_2	+	
					31 metabolites in leaves	-/+	
Rice (*Oryza sativa*)	PS and PTFE	1–1 µm and 10–100 µm	0.25% and 0.5%		Biomass	-	Dong et al., 2022
					Rice root activity	-	
					H_2O_2 in grains	+	
					Enzyme activity (SSS and AGP) in grains	-	
					Starch (hemoglobin) contents in roots	-	

Rice (*Oryza sativa* L. II You. 900)	PS	<50 mm	50, 250, 500 mg/l	142 da	Shoot length	-	Wu et al., 2020
					Shoot biomass	-	
					CAT and ROS	+	
					POD, SOD, MDA	-	
					primary and secondary (metabolites	- Primary - Secondary + Cycloserine +D-asparagine	
Lettuce (*Lactuca sativa* L.)	PE	8.68–500 µm	0.1% 1% and 10%	45 days	Plant biomass	0/-	Wang et al., 2021
Lettuce (*Lactuca sativa* L.)	PE microfiber		0.1% and 0.2%	58 days	Chlorophyll	-	Zeb et al., 2022
					net photosynthetic rate	-	
					POD and CAT	+	
					SOD	0	
					MDA	-	
					46 leaf metabolites	- 17 metabolites	
					Shoot length	-	
					Root lenght	+	
Common bean (*Phaseolus vulgaris* L.)	LDPE and biodegra dable plastic (Bio-MPs)	250– 500 µm	1.0%, 1.5%, 2.0% and 2.5%	46 days	Shoot and root biomass LDPE	0	Meng et al., 2021
					Fruit biomass LDPE	0	
					Pod number LDPE	0	
					Leaf area LDPE	+	
					Chlorophyll LDPE	-	
					Root length LDPE	+	
					fine root surface area proportion LDPE	+	
					Shoot and root biomass Bio-MPs	-	
					Shoot-to-root-ratio Bio-MPs	-	
					Fruit biomass Bio-MPs	-	
					Leaf area Bio-MPs	-	
					Root length Bio-MPs	+	
					Chlorophyll Bio-MPs	+	
Mung bean (*Vigna radiata*)	shoe sole fragmen ts	< 1 mm 204 ± 131 µm, 57 ± 46 µm, 156 ± 144 µm, and 229 ± 108 µm	0, 0.1%, and 1% wt/wt	28 days	Shoot growth	- Sneaker + Slippers + Running shoes	Lee et al., 2022
					Root growth	+ Trekking + Sneakers	
					Polyphenolic compounds in leaves	+Flavonoids +Antocyanins - Nitrogen balance	
					Chlorophyll	-	
					Max. fluorescence (Fm), max. photoch. efficiency of photosystem II (QYmax), coefficient of photochemical quenching (qP)	- Sneakers	

Tomato (*Lycopersicon esculentum* Mill.)	PP, HDPE, LDPE and PET	0.31-2.11 mm	17.870 ± 2174, 27.821 ± 1357, 47.130 ± 3002 particles /kg dw of sludge	109 days	Biomass	+	Hernandez-Arenas et al., 2020
					Crop yield	-	
Rape (*Brassica napus* L.)	PE	293 μm	0.001%, 0.01% and 0.1%	60 days	MDA content	0/+	Jia et al. 2022
					SOD and POD activity	0/+	
					Soluble sugar content	-	
					Vit. C	-	
					Chlorophyll	0/-	
					Electrolytic leakage of root	-/+	
Sweet potato (*Ipomoea batatas* Lam.)	PE	5 μm	1% and 5%	15 days	Biomass	+	Shi et al. 2022
					Total protein		
					SOD, POD, GSH, MDA	+	
Garden cress (*Lepidium sativum*)	PP, PE, PVC, PE + PVC	0.125 mm	0.02% (w/w)	6 days 21 days	Seed germination	-	Pignattelli et al. 2020
					Plant height	-	
					Biomass	+	
					Leaf number	-	
					H_2O_2	+	
					Glutatione	-	
					Vit. C	-	
					Chlorophyll	+	
					Carotenoids	+	
					Aminolaevulinic acid	+	
					Proline	+	
Chinese cabbage (*Brassica chinensis* L.)	HDPE and general purpose PS	<25, 25–48, 48–150, and 150–850 μm	0.5, 5, 10, and 20 g MP kg^{-1}	30 days	Fresh weight	-	Yang et al., 2021
					Growth	-	
					Leaf soluble sugar concentration	+	
					Leaf concentra-tions of starch	-	
					Chlorophyll	-	
***Cucurbita pepo* L.**	HDPE, PVC, PP and PET	40–50 μm	0.02%, 0.1% and 0.2%,	28 days	Fresh biomass	-	Colzi et al., 2022
					Dry biomass	-	
					Chlorophyll	- PE -PVC	
					Fluorescence	- PE -PVC	
					Fv/Fm	- PE -PVC	
					Performance index (PIABS)	- PVC	
					NDVI	- PE -PVC	
					Water content	- PET	
					Leaf area	- PE -PVC	
					SLA	+ PE + PVC	
Spring onion *Allium fistulosum*	PA, HDPE, PET, PP, PS, PES fibers	15 μm - >800 μm		45 days	Root biomass	+ PES, PS	de Souza Machado et al., 2019
					Total root length	+	
					Root diameter	-	
					Total root area	+	
					Root tissue density	- PA + PES, PS	

Plant height

One of the most visible symptoms of MPs effect on plants is the altered plant growth varying from inhibition through indifferent up to stimulation. Maize plants showed decreased root and shoot length under PE contamination in the study of Fajardo et al. (2020) but maize plants in the experiment with polyuretan-coated fertilizer exerted no effect (Lian et al., 2021). Wheat cultivars shoot length was found to be inhibited by low-density polyethylene particles (200–250 μm) but the shoot length was stimulated in an acute experiment (15 days) (Liu et al., 2021). Other authors reported that PE MPs of 200 μm size and 0.2% concentration in soil exerted a stimulatory influence on wheat plants for a 42-days period (Guo et al., 2022). Same MPs have no effect on wheat growth in a long-term experiment conducted by Qi et al. (2018). When regarding rice plants, Wu et al. (2020) and Dong et al. (2022) revealed that PS MPs suppressed root and shoot elongation.

Another important parameter is the root-to-shoot ratio which is defined as the ratio of the amount of plant tissues that have supportive functions to the amount of those that have growth functions. Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy. So, this ratio is often analyzed in various plant studies. Zang et al. (2020) reported that the root-to-shoot ratio of wheat plants significantly increased under 10% and 20% MPs (PVC and PE) in the soil media. Same tendency was observed by Qi et al. (2018) under 1% of low-density polyethylene (LDPE) and starch-based biodegradable MPs. An opposite result has been reported by Liu et al. (2022) after their experiment with wheat plants and MPs (PVC and PE in concentrations of 1% and 5%).

Root traits

Apart from the impact on root length, MPs can negatively affect the root activity as reported for soybean (Xu et al., 2021) and for rice (Dong et al., 2022). Low density PE MPs (250– 500 μm) can enhance the fine root surface area proportion in common bean (Meng et al., 2021) as well as high density PE MPs (>800 μm) can promote the total root area in spring onion (de Souza Machado et al., 2019). Jia et al. (2022) proved that low doses of PE (293 μm) in soil decreased the electrolytic leakage of rape's roots while at dose of 0.1% (w/w) a stimulatory effect was pronounced.

Leaf traits

Plants often respond to unfavorable conditions by developing smaller or higher mass per leaf area (Reich et al., 1997). Colzi et al. (2022) reported that *Cucurbita pepo* plants developed smaller leaves with a reduced lamina when exposed to PE and PVC (40–50 μm) at 0.1-0.2% but an increase of specific leaf area was also observed. LDPE MPs could lead to bigger leaf area in wheat (Qi et al., 2018) and in common bean (Meng et al., 2021). Wheat cultivars with bigger leaf area due to the addition of 1% LPDE microplastics (50 μm-1 mm) in soil have been grown by Qi et al. (2018).

Biomass

Changes in plant growth, induced by the MPs in the soil, are inevitable related to some changes in plants' fresh and dry biomass. Tomato plants significantly enhanced their biomass when MPs (PP, HDPE, LDPE and PET) have been added to soil but crop yield decreased (Hernandez-Arenas et al., 2021). LDPE MPs did not affect the biomass of Common bean cultivars although an inhibition has been found under the biodegradable MPs toxicity (Meng et al., 2021). Furthermore, Pignatelli et al. (2020) found a reduction of plant height in garden cress but an increment of plant biomass when exposed to PP, PE and PVC MPs at 0.02% (w/w) for 21 days. A decrement of both fresh and dry biomass of *Cucurbita pepo* was observed under the influence of PE, PVC and PET at 0.1-0.2% for 28 days (Colzi et al., 2022). These findings confirm that plants response to MPs in a species-specific or cultivar-dependent manner.

Photosynthesis and transpiration

Photosynthetic parameters are considered as effective indicators of MPs induced stress in plants (Larue et al., 2021; Wang et al., 2022). The impact of MPs on the photosynthesis in maize was expressed by altering the photosynthetic rate (positively), transpiration rate (no effect), stomatal conductance (no effect), intercellular CO_2 concentration (negatively) (Lian et al., 2021). Other study on maize revealed that PVC MPs (75-220 mm, 0.02g/150 g soil) had no significant effect on quantum photosynthetic yield of photosystem-II Y(II), maximum quantum yield (FV/FM) and FV/F0, while on the maximum electron transport rate (ETR) they had a suppressive effect (Pehlivan & Gedik, 2021). The sneakers sole fragments decreased the maximum fluorescence (Fm), maximum photochemical efficiency of the photosystem II (QY max), and coefficient of photochemical quenching (qP) in mung bean leaves (Lee et al., 2022).

Chlorophyll content

Some studies have tracked the changes in chlorophyll content to assess the toxicity of MPs on plants, suggesting that MPs accumulated in leaves may alter both the pigments content and the pigments ratios. Pignatelli et al. (2020) found that PP, PE and PVC microplastics (0.125 mm size, 0.02% w/w) exhibited a positive effect on chlorophyll and carotenoids in garden cress leaves. Meng et al. (2021) reported that low density PE (250 µm-1000 µm) in soil decreased the chlorophyll content in common bean leaves while the same size and amount of biodegradable MPs had a stimulatory effect. Photosynthetic pigment in maize were not affected by PE and PLA microplastics (Wang et al., 2020), but they were significantly suppressed by PVC MPs (Pehlivan & Gedik, 2021) and significantly stimulated by the presence of polyuretan-coated fertilizer in the soil (Lian et al., 2021). Chlorophyll content of wheat was no changed or even stimulated by low doses of PE (up to 1% w/w), but it was significantly disturbed when concentration raised (Qi et al., 2018; Liu et al. 2021; Guo et al., 2022). 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.

Oxidative stress biomarkers

Oxidative stress is recognized as the most common ecotoxicity index in plants (Wang et al., 2022; Petrova et al., 2022; Petrova & Petkova, 2023). When this type of stress occurs, the level of reactive oxygen species (ROS) is enhanced and the antioxidant defense mechanisms are involved into plant response. Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) activities typically increase (Zhang et al., 2021) as well as the content of malondialdehyde (MDA) (Wu et al., 2020). Maize roots with increased SOD gene activity was observed in the presence of PE MPs, but the CAT gene and GST gene activity in root has not been influenced (Fajardo et al., 2022). Although the H_2O_2 in maize level raised due to the effect of PVC microparticles, the POD gene activity stayed the same while heat shock protein increased (Pehlivan & Gedik, 2021). Wheat roots with higher SOD, POD and CAT activities due to the low LDPE (0.5-8% w/w) in a 15-days treatment have been reported by Liu et al. (2021). Guo et al. (2022) revealed that after 42-days experiment with PE MPs the level of POD activity in wheat is higher. All enzymes activities (ROS, CAT, MDA, SOD and POD) both in roots and leaves of soybean plants treated by PS MPs significantly increased but in stems no change have been observed (Xu et al., 2021). Similar effect exerted PE MPs on sweet potato plants (Shi et al., 2022).

Metabolites changes

Metabolomics is an efficacy method for tracking the changes caused by toxicants on the metabolites concentrations and metabolic pathways in plant organism (Zhang et al., 2019; Wang et al., 2022). A series of changes in both primary and secondary metabolites have been described. In soybean exposed to PE and PLA microplastics (0.1% and 1%) for 49 days significantly changes were found in 7 out of 31 metabolites studied, indicating a strong type-dependent and dose-dependent effect (Lian et al., 2022). Wu et al. (2020) observed an inhibition of both primary (saccharides and aminoacids) and secondary (organic acids and fatty acids) metabolites in rice exposed to PS MPS for 142 days, with an exception of cycloserine and D-asparagine which have been enhanced. Polyester microfibers significantly reduced the content of 17 metabolites (from 46 studied) in lettuce (Zeb et al., 2022). The soluble sugar content and vitamin C content of rape was also negatively affected by PE (Jia et al., 2022). Pignatelli et al. (2020) found a higher level of aminolevulenic acid and proline in cress after 21 days of exposure to PVC.

Conclusions

As an overview of all above mentioned, some main conclusion could be made as follows:

1) The effects of MPs on plants are predominantly negative (toxic), but many studies revealed no significant effects as well as some studies reveal at positive effect (stimulation) of some plant biomarkers.

2) Different plant species demonstrated different responses to same MPs pollution, which could be explained by both biological specificities in plant structure and/or in plant physiology.

3) The size of MPs is an important factor – the smaller fractions of MPs had a more significant effect on plants.

4) The MPs concentration is also an important factor for plant growth and performance – some studies revealed a stimulatory effect in low doses of MPs presence in soil which transformed into an inhibition when MPs content increased.

5) Some enzymes activities and photosynthesis parameters could be evaluated as potential plant biomarkers for MPs pollution in soil although the lack of specificity in their reaction to different type of stress.

It should be pointed also, that currently the researchers' work on the effect of MPs on plants is limited to a few common plant species and short-time studies. So, some future research priorities should be dedicated to the long-term effects of MPs on a variety of plant species in both lab and filed conditions.

Acknowledgments

This research is supported by the Bulgarian Ministry of Education and Science under the National Program Young Scientists and Postdoctoral students – 2.

References

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., & Tufenkji, N. (2018). Microplastic and Nanoplastic in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. Sci. Total Environ., 52, 1704–1724. doi: 10.1021/acs.est.7b05559

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

Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., & Vijver, M.G. (2019). Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. Chemosphere 226, 774–781. doi: 10.1016/j.chemosphere.2019.03.163

Chae, Y., & An, Y.J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A review. Environ. Pollut., 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

de Souza Machado, A., Lau, C., Kloas, W., Bergmann, J., Bachelier, J., Faltin, E., Becker, R., Gorlich, A., & Rillig, M. (2019). Microplastics Can Change Soil Properties and Affect Plant Performance. Environ Sci Technol, 53(10), 6044-6052. doi: 10.1021/acs.est.9b01339.

Dong, Y., Gao, M., Qiu, W., & Song, Z. (2021). Uptake of microplastics by carrots in presence of as (III): combined toxic effects. J. Hazard Mater. 411, 125055. doi: 10.1016/j.jhazmat.2021.125055.

Dong, Y., Bao, Q., Gao, M., Qiu, W., & Song, Z. (2022). A novel mechanism study of microplastic and As co-contamination on indica rice (*Oryza sativa* L.). Journal of Hazardous Materials, 421, 126694. doi: 10.1016/j.jhazmat.2021.126694

Fajardo, C., Martin, C., Costa, G., Sanchez-Fortun, S., Rodrigez, C., de Lucas Burneo, J.J., Nande, M. Mengs, G., & Martin, M. (2022). Assessing the role of polyethylene microplastics as a vector for organic pollutants in soil: Ecotoxicological and molecular approaches. Chemosphere, 288, Part 1, 132460. doi: 10.1016/j.chemosphere.2021.132460

Fuller, S., & Gautam, A. (2016). A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780. doi: 10.1021/acs.es t.6b00816.

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

He, D.F., Luo, Y.M., Lu, S.B., Liu, M.T., Song, Y., & Lei, L.L. (2018). Microplastics in soils: analytical methods, pollution characteristics and ecological risks. TrAC, Trends Anal. Chem 109, 163–172. doi: 10.1016/j.trac.2018.10.006

He, S., Wei, Y., Yang, C., & He, Z. (2022). Interactions of microplastics and soil pollutants in soil-plant systems. Environmental Pollution, 315, 120357. doi: 10.1016/j.envpol.2022.120357

Golubinova, I., Nikolov, B., Petrova, S., Velcheva, I., Valcheva, E., & Marinov-Serafimov, P. (2020). Effect of Cycocel 750 SL on Germination and Initial Development of Some Sorghum Species. Ecologia balkanica, 12(1), 11-19

Guo, A., Pan, C., Su, X., Zhou, X., & Bao, Y. (2022). Combined effects of oxytetracycline and microplastic on wheat seedling growth and associated rhizosphere bacterial communities and soil metabolite profiles. Environmental Pollution, 302, 119046. doi: 10.1016/j.envpol.2022.119046

Hernandez-Arenas, R., Beltran-Sanahuja, A., Navarro-Quirant, P., & Sanz-Lazaro, C. (2021). The effect of sewage sludge containing microplastics on growth and fruit development of tomato plants. Environmental Pollution, 268, Part B, 115779. doi: 10.1016/j.envpol.2020.115779

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

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 Organisms. Small, 17, 2005834. doi: 10.1002/smll.202005834

Lee, T.-Y., Kim. L., Kim, D., An, S., & An, Y.-J. (2022). Microplastics from shoe sole fragments cause oxidative stress in a plant (*Vigna radiata*) and impair soil environment. Journal of Hazardous Materials, 429, 128306. doi: 10.1016/j.jhazmat.2022.128306

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

Lian, Y., Liu, W., Shi, R., Zeb, A., Wang, Q., Li, J., Zheng, Z., & Tang, J. (2022). Effects of polyethylene and polylactic acid microplastics on plant growth and bacterial community in the soil. Journal of Hazardous Materials, 435, 129057. doi: 10.1016/j.jhazmat.2022.129057

Liu, S., Wang, J., Zhu, J., Wang, J., Wang, H., & Zhan, X. (2021). The joint toxicity of polyethylene microplastic and phenanthrene to wheat seedlings. Chemosphere, 282, 130967. doi: 10.1016/j.chemosphere.2021.130967

Liu, Y., Xiao, M., Shahbaz, M., Hu, Z., Zhu, Z., Lu, S., Yu, Y., Yao, H., Chen, J., & Ge, T. (2022). Microplastics in soil can increase nutrient uptake by wheat. Journal of Hazardous Materials, 438, 129547. doi: 10.1016/j.jhazmat.2022.129547

Longo, C., Holness, S., De Angelis, V., Lepri, A., Occhigrossi, S., Ruta, V., & Vittorioso, P. (2021). From the Outside to the Inside: New Insights on the Main Factors That Guide Seed Dormancy and Germination.

Genes 2021, 12, 52. doi: 10.3390/genes12010052

Mendes, R., Garbeva, P., & Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev, 37(5), 634-63. doi: 10.1111/1574-6976.12028.

Meng, F., Yang, X., Riksen, M., Xu, M., & Geissen, V. (2021). Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. Science of The Total Environment, 755, Part 2, 142516. doi: 10.1016/j.scitotenv.2020.142516

Ng, E.L., Lwanga, E.H., Eldridge, S.M., Johnston, P., Hu, H.W., Geissen, V., & Chen, D.L. (2018). An overview of microplastic and nanoplastic pollution in agroecosystems. Sci. Total Environ. 627, 1377–1388. doi: 10.1016/j.jhazmat.2021.127750

O'Riordan, R., Davies, J., Stevens, C., Quinton, J., & Boyko, C. (2021). The ecosystem services of urban soils: A review. Geoderma, 395, 115076. doi: 10.1016/j.geoderma.2021.115076

Pehlivan, N., & Gedik, K. (2021). Particle size-dependent biomolecular footprints of interactive microplastics in maize. Environmental Pollution, 277, 116772. doi: 10.1016/j.envpol.2021.116772

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 2022, 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 2023, 14(4), 800. doi: 10.3390/f14040800

Petrova S., & Nikolov, B. (2023). Soil related ecosystem services in urban areas – a literature review. Ecologia balkanica, 15(1), 203-231.

Pflugmacher, S., Sulek, A., Mader, H., Heo, J., Noh, J.H., Penttinen, O.-P., Kim, Y., Kim, S., & Esterhuizen, M. (2020). The Influence of New and Artificial Aged Microplastic and Leachates on the Germination of *Lepidium sativum* L. Plants, 9, 339. doi: 10.3390/plants9030339

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

Qi, Y., Yang, X., Pelaez, A.M., Lwanga, E.H., Beriot, N., Gersten, H., Garbeva, P., & Geissen, V. (2018). Macro- and microplastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Science of The Total Environment, 645, 1048-1056. doi: 10.1016/j.scitotenv.2018.07.229

Qi, Y., Ossowicki, A., Yang, X., Lwanga, E.H., Dini-Andreote, F., Geissen, V., & Garbeva, P. (2020a). Effects of plastic mulch film residues on wheat rhizosphere and soil properties. Journal of Hazardous Materials, 387, 121711. doi: 10.1016/j.jhazmat.2019.121711

Qi, R., Jones, D.L., Li, Z., Liu, Q., & Yan, C. (2020b). Behavior of microplastics and plastic film residues in the soil environment: A critical review. Sci Total Environ, 703, 134722. doi: 10.1016/j.scitotenv.2019.134722.

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 volume 462, pages561–576. doi: 10.1007/s11104-021-04869-1

Shi, L., Hou, Y., Chen, Z., Bu, Y., Zhang, X., Shen, Z. & Chen, Y. (2022). Impact of polyethylene on soil physicochemical properties and characteristics of sweet potato growth and polyethylene absorption. Chemosphere, 302, 134734. doi: 10.1016/j.chemosphere.2022.134734

Sun, H., Lei, C., Yuan, Y., Xu, J., & Han, M. (2022). Nanoplastic impacts on the foliar uptake, metabolism and phytotoxicity of phthalate esters in corn (*Zea mays* L.) plants. Chemosphere, 304, 135309. doi: 10.1016/j.chemosphere.2022.135309

Taylor, S.E., Pearce, C.I., Sanguinet, K.A., Hu, D., Chrisler, W.B., Kim, Y.-M., Wang, Z.,

& Flury, M. (2020). Polystyrene nano- and microplastic accumulation at Arabidopsis and wheat root cap cells, but no evidence for uptake into roots. Environ. Sci. Nano 7, 1942–1953. doi: 10.1039/d0en00309c.

Urbina, M.A., Correa, F., Aburto, F., & Ferrio, J.P. (2020). Adsorption of polyethylene microbeads and physiological effects on hydroponic maize. Sci. Total Environ. 741, 140216. doi: 10.1016/j.scitotenv.2020.140216.

Wang, F., Zhang, X., Zhang, S., Zhang, S., & Sun, Y. (2020). Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere, 254, 126791. doi: 10.1016/j.chemosphere.2020.126791

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

Wu, X., Liu, Y., Yin, S., Xiao, K., Xiong, Q., Bian, S., Liang, S., Hou, H., Hu, J., & Yang, J. (2020). Metabolomics revealing the response of rice (*Oryza sativa* L.) exposed to polystyrene microplastics. Environ Pollut, 266 (Pt 1), 115159. doi: 10.1016/j.envpol.2020.115159

Xu, G., Liu, Y., & Yu, Y. (2021). Effects of polystyrene microplastics on uptake and toxicity of phenanthrene in soybean. Sci Total Environ, 783, 147016. doi: 10.1016/j.scitotenv.2021.147016

Zainudin, B.H., Wong, T.W., & Hamdan, H. (2020). Pectin as oral colon-specific nano- and microparticulate drug carriers. Polym. Sci. Innov. Appl. 2020, 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

Zeb, A., Liu, W., Meng, L., Lian, J., Wang, q., Lian, Y., Chen, C., & Wu, J. (2022). Effects of polyester microfibers (PMFs) and cadmium on lettuce (Lactuca sativa) and the rhizospheric microbial communities: A study involving physio-biochemical properties and metabolomic profiles. Journal of Hazardous Materials, 424, Part C, 127405. doi: 10.1016/j.jhazmat.2021.127405

Zhang, G.S., Zhang, F.X., & Li, X.T. (2019). Effects of polyester microfibers on soil physical properties: Perception from a field and a pot experiment. Science of The Total Environment, 670, 1-7. doi: 10.1016/j.scitotenv.2019.03.149

Zhang, S., Wang, J., Yan, P., Hao, X., Xu, B., Wang, W., & Aurangzeib, M. (2021). Non-biodegradable microplastics in soils: A brief review and challenge. Journal of Hazardous Materials, 409, 124525. doi: 10.1016/j.jhazmat.2020.124525

Zhang, Z., Cui, Q., Chen, L., Zhu, X., Zhao, S., Duan, C., Zhang, X., Song, D., & Fang, L. (2022). A critical review of microplastics in the soil-plant system: Distribution, uptake, phytotoxicity and prevention. Journal of Hazardous Materials, 424, Part D, 127750. doi: 10.1016/j.jhazmat.2021.127750

Received: 13.05.2023
Accepted: 03.06.2023