Original Research Article

Influence of microplastics on seedling growth of blackgram under different soil types

.

ABSTRACT

The prevalence of microplastics in soil has recently attracted substantial interest as they pose a major threat to agricultural system. A pot experiment was conducted to evaluate the effect of polyethylene microplastics (PE-MPs) on seedling development of blackgram (*Vigna mungo L*.) in two different types of soil during April, 2022 under controlled conditions in the Department of Environmental Sciences, Tamil Nadu Agricultural University, India. The treatments included four different concentrations of PE-MPs (0.25, 0.50, 0.75 and 1.00%) along with a control which had no microplastics. All the treatments were replicated thrice. In loamy soil type, germination rate declined from 87.1±0.57 (control) to 73.1±0.33 (1.00% PE-MPs) and in clay loam soil, from 83.3±3.08 (control) to 79.8±0.03 (1.00% PE-MPs). Similarly root and shoot characteristics also showed distinct reduction along with increasing microplastics concentrations. Hence, the results of the study reveal that soil type has influence over the magnitude of variations in blackgram growth parameters in the presence of polyethylene microplastics.

Keywords**:** *Blackgram, Growth indices, Microplastics, Soil type*

1. INTRODUCTION

Soil is one of the factors that form the basis for all the living organisms on the planet. After the advent of green revolution and modernization, the quality of soil has been constantly deteriorating due to release of various forms of pollutants into the soil. Microplastics (MPs) (both primary and secondary) are newly emerging contaminants whose abundance is found to be increasing in both terrestrial and aquatic ecosystem. Often people are realizing that soil, in addition to the aquatic environment, is a significant sink for microplastics. Hence, the microplastics impact on the soil ecology has been an area of mass scientific interest in recent years [1, 2]. Change of any nature in the properties of the soil, whether positive or negative would in turn influence crop growth. Hence, the indiscriminate release of microplastics into the soil is an alarming situation that needs to be addressed. In modern agriculture, polyethylene (PE) film is widely employed and thus forms a significant source of MPs in soil. The mulch films disintegrate into microplastics due to sunlight, water, chemical and biological degradation [3] and gradually accumulate in the field [4, 5]. In addition to mulch film fragments, MPs might also reach soil through the application of compost [6, 7], organic fertilisers [8, 9], sewage sludge [10-12], and waste water irrigation [12, 13].

Microplastics have been found to directly modify soil bulk density and water-holding capacity [14, 15], which in turn interfere with the stability of soil aggregates and biological properties of soil. Furthermore, microplastics may modify the dominant bacterial phyla in the soil and enzymes linked to the carbon, nitrogen, and phosphorus cycle, which might have a consequence on nutrients cycles in the soil [15-17].

Microplastics cause a variety of harmful effects in plants, including (i) preventing plant nutrients from being absorbed and transported by blocking cell wall pores or cell connections [18-21]; (ii) reducing or delaying seed germination by preventing water absorption; (iii) altering root and shoot growth [19-21]; (iv) interfering with the balance of plant chlorophyll a/b ratios [22]. The presence of microplastics around the plant roots is more likely to promote phytotoxicity of other soil contaminants. The influence of microplastics on soil-plant system varies based on the shape, size, type and concentration of microplastics [20, 23-25] and soil type [17, 26]. Yet, the studies focusing on the impacts of microplastics on plant system in different soil types is scarce. Hence, the objective of this study is to analyze the influence of soil type in the presence of polyethylene microplastics (PE-MPs) on the growth of blackgram seedlings. The study was assessed based on the hypothesis that microplastics exhibit impacts at different magnitudes on seedling growth when present in different soil types.

2. MATERIAL AND METHODS

2.1. COLLECTION AND CHARACTERIZATION OF EXPERIMENTAL SOIL

The experimental soils (loamy and clay loam) were collected from farms of Tamil Nadu Agricultural University, India (11º 01’ 2.28” N, 76º 56’ 13.2” E and 426.6 MSL; 11º 00’ 24.804” N, 76º 56’ 9.816” E and 426.6 MSL). The soil was shade dried, sieved to 2 mm to get rid of plant residues, large rocks and gravel

and characterized as per the standard procedure [27, 28].

2.2. Characterization of microplastics and collection of blackgram seeds

The polyethylene microplastics (PE-MPs) powder used in the study was obtained from a local polymer recycling industry named Arunachal Polymer Industries (11.07469 ºN, 76.91207 ºE), Tamil Nadu, India. The size, shape and elemental composition as characterized by Scanning Electron microscope with EDAX (Quanta 250 (FEI, Netherlands)) were 6 to 600 µm, irregular particles with 90.88% Carbon, 9.09% Oxygen, 0.01% phosphorus and potassium.

To reduce microbiological contamination, the microplastics were microwaved at 0.8 KW for 3 minutes [24] and their sterility was assessed by nutrient agar and potato dextrose media at different dilutions. Microplastics were added immediately to processed farm soil at five different concentrations (T1- 0, T_2 - 0.25, T_3- 0.5, T_4- 0.75 and T_5- 1.00% on w/w basis). The concentrations were fixed based on the previous studies that quantified microplastics in different soils [8]. Mixing of microplastics with soil was done by stirring with ethanol sterilized metal rod in a metal container. The Blackgram (CO 6) variety was obtained from the National Pulse Research Centre, Vamban, India.

2.3. Experimental methodology

The test soils (400g) with different microplastics concentrations were transferred to clean containers. Three replications were maintained for each treatment (2 soil types and 5 concentrations). The containers were arranged randomly inside glass house at 50% humidity and at 12h/12h day and night hours. The containers were covered with aluminum foil and incubated for 2 weeks at 30-35º C for stabilization [24]. During incubation, the water saturation was maintained at 50-60% water holding capacity on weight basis of each soil sample.

At the end of stabilization period, 5 uniformly sized and healthy seeds of CO 6 blackgram variety were sown in each treatment. The seedlings were maintained for duration of 15 days after sowing (DAS) during April, 2022 and watered regularly (at the rate of 50mL per day) to provide sufficient moisture. To assess the influence of soil types in the presence of PE-MPs on blackgram seedling growth, the critical parameters like germination rate, germination time, root length, shoot length, root to shoot ratio, vigour index, leaf length and breadth, and chlorophyll content were recorded at appropriate stages.

2.4. Statistical analysis

The data recorded for each treatment was processed and represented as mean ± SD. The statistical design fixed was Factorial Completely randomized design (FCRD) and the data was tested using R Studio 4.1.3, to test the significance of variations among the treatments (T), Soil type (S) and their interactions (T x S). Furthermore, Graphs were drawn using OriginPro 2021.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the initial soil

The physicochemical properties of the soils were characterized as per the standard procedures and the results obtained were given in Table 1. The soil types are found to be Loamy and Clay loam soil with a water holding capacity of 34 and 41%.

Table 1. Initial characteristics of the experimental soils

Parameters	Loamy soil	Clay loam soil
Water holding capacity (%)	34.00	41.00
Bulk density (g/cc)	1.08	1.60
Porosity (%)	41.78	49.50
Soil pH	8.68	8.47
Soil EC (dSm^{-1})	0.34	0.39
Soil Organic Carbon (%)	0.32	0.45
Available Nitrogen (kg ha^{-1})	267.00	240.00
Available Phosphorus (kg/ha)	25.00	20.00
Available Potassium (kg/ha^{-1})	323.00	341.00
Texture -		
Clay (%)	23.60	47.30
Silt (%)	40.50	8.70
Sand (%)	30.70	37.20

3.2. Germination rate and time taken for shoot emergence

The soil type in the presence of PE- MPs had a significant influence on germination rate. In clay loam soil, the germination rate ranged from 83.3 (T_1- control (0% PE-MPs)) to 79.8% (T_5 – 1.00% PE-MPs); while in loamy soil it varied between 87.1 (T_1- control (0% PE-MPs)) and 73.1% (T5 - 1.00% PE-MPs). Irrespective of the soil type, application of 1.00% PE-MPs significantly recorded the least germination rate. On comparing the soil type, the germination rate was significantly lower in loamy soil compared to clay loam (Table 2). The decrease in germination rate could be due to physical or chemical interference of microplastics with seed imbibition or water uptake [20]. In previous studies, microplastics were reported to reduce germination rate in cress [20, 29, 30], rye grass [31], soybean [25] and lettuce [22].

The time taken for shoot emergence was significantly affected by different concentrations of microplastics in both of the soils. In loamy soil, the shoot emerged 4 DAS in T_1 (Control - 0% PE-MPs) and after 5 DAS in T_5 (1.00% PE-MPs); while in clay loam soil shoot emergence was recorded 3 and 5 DAS in T_1 and T_5 respectively. The delay was more pronounced in clay loam than in loamy soil (Table 2).

3.3. Growth characteristics

The soil type with presence of different concentrations of PE-MPs exhibited significant influence on root length. Compared to T_1 (control- 0% PE-MPs), an 18 and 24% reduction was observed in T_5 (1.00% PE-MPs) in loamy and clay loam soil respectively. In loamy soil, the root length varied from 17.4 cm in T_1 to 14.2 cm in T_5; while in clay loam soil, the root length of seedlings declined from 19.2 cm in T1 to 12.8 cm in T_5 (Table 2). Similar results regarding altered root length were reported in cress [20, 30], *Plantago lanceolata* [32], wheat [19], maize [33], rye grass [31], broad bean [34], and carrot [23]. On comparing root lengths of seedlings from both the soils, the decrease in root length was higher in clay loam soil.

Similarly, there were significant differences in shoot length between two soil types with PE-MPs. Similar to germination rate, shoot length of blackgram was affected the most in loamy soil and in both of the soils T_5 recorded the lowest shoot length 14.21 (loam) and 14.74 cm (clay loam). The shoot length declined by a 35 and 23% in T_5 (1.00% PE-MPs) from control (T_1) in loamy and clay loam soil respectively. The observations on shoot length from this study are similar to the findings of Yang et al. 2021, who reported reduction in shoot biomass by 28-50% in maize after application of a high-dose of polylactic acid (PLA) microplastics [33].

The ratio of root to shoot (R/S ratio) varied significantly between two soil types in the presence of PE-MPs. In loamy soil, R/S ratio was observed to gradually increase from control (T_1 – 0% PE-MPs) to T_5 (1.00% PE-MPs) from 0.79 to 1.01. Whereas, in clay loam soil the R/S ratio decreased constantly from 0.99 in T_1 to 0.87 in T_5.

In comparison with control, the overall vigour index of blackgram seedlings declined by 39 and 31% in the treatment with the highest microplastics concentration (T_5-1.00% PE-MPs) in loamy and clay loam soil respectively (Table 2). It was observed that the vigour index gradually decreased as the concentration of microplastics in the soil increased. In maize [8], duckweed [35], plantain [32], broad bean [34] and cress [29], microplastics were found to reduce biomass.

Table 2. Effect of different concentrations of PE-MPs on blackgram seedling parameters (15 DAS) in two loamy and clay loam soil

Type of soil	Treatment	Germination Rate (%)	Time taken for shoot emergence (DAS)	Root length (cm)	Shoot length (cm)	Root to shoot ratio	Vigour index (VI)
Loamy	T_1	87.1±0.57[a]	4±0.05[e]	17.4±0.06[b]	21.9±0.66[a]	0.79±0.03[e]	3430±77[a]
	T_2	80.9±0.02[bc]	4±0.16[f]	16.5±0.18[c]	18.7±0.73[c]	0.88±0.01[cd]	2856±59[d]
	T_3	79.5±0.86[c]	4±0.10[g]	16.5±0.54[c]	17.5±0.16[d]	0.94±0.01[b]	2709±70[e]
	T_4	73.3±0.53[d]	5±0.13[a]	14.5±0.01[e]	14.6±0.61[e]	0.99±0.04[a]	2138±56[g]
	T_5	73.1±0.33[d]	5±0.12[b]	14.2±0.53[e]	14.2±0.40[e]	1.01±0.01[a]	2080±81[g]
Clay loam	T_1	83.3±3.08[b]	3±0.05[j]	19.2±0.40[a]	19.2±0.54[b]	0.99±0.03[a]	3202±98[b]
	T_2	81.7±3.09[bc]	4±0.17[h]	17.6±0.40[b]	19.1±0.07[b]	0.92±0.02[c]	2997±87[c]
	T_3	80.4±2.97[bc]	4±0.13[i]	15.5±0.63[d]	18.1±0.75[d]	0.85±0.04[d]	2699±91[e]
	T_4	80.0±1.66[bc]	5±0.11[c]	13.1±0.01[f]	18.6±0.15[c]	0.70±0.02[f]	2540±76[f]
	T_5	79.8±0.03[bc]	5±0.21[d]	12.8±0.44[f]	14.7±0.57[e]	0.87±0.03[c]	2200±59[g]

3.4. Leaf parameters and chlorophyll content

The length of leaf in seedlings was significantly affected in both the soils in the presence of PE-MPs. A 6 and 9% reduction in 1.00% PE-MPs from control was observed in loam and clay loam soil respectively. In contrast, no significant difference in leaf breadth was observed among soil types in the presence of PE-MPs. As previously observed in other parameters, overall changes in the leaf length and leaf breadth were more pronounced in clay loam in the presence of microplastics (Fig. 1). Similarly, a reduction in leaf development was reported in lettuce due to microplastics by [36].

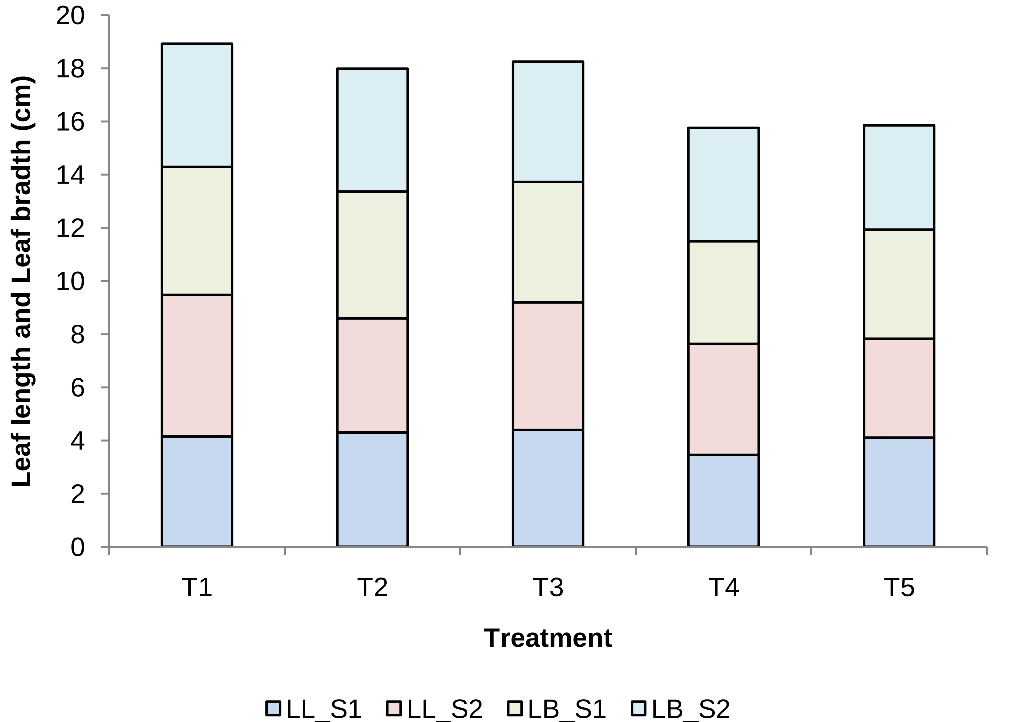

Figure 1. Effect of PE-MPs on Leaf length and leaf breadth of blackgram seedlings in loamy and clay loam soil (LL – leaf length, LB – leaf breadth, S1 – loamy soil, S2 – clay loam soil)

In this study, chlorophyll a content among different treatments did not vary significantly, however, chlorophyll b was observed to decline with increasing concentrations of microplastics in blackgram seedlings grown in both the soil types. The total chlorophyll content was also affected as a result of changes in the chlorophyll b (Fig. 2). Similarly, in Chinese cabbage, polystyrene and HDPE [22] and in maize, PE-MPs were reported to reduce chlorophyll content in leaf [8].

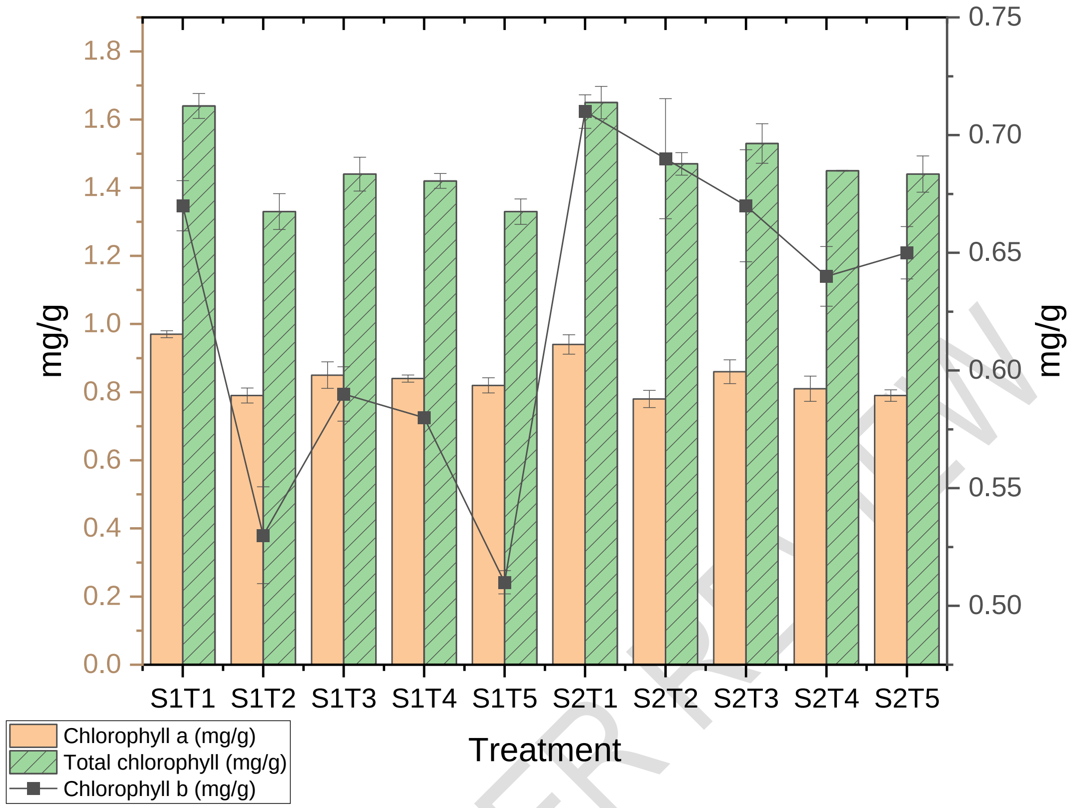

Figure 2. Effect of PE-MPs on Chlorophyll a, b and total chlorophyll content in blackgram seedlings grown with loamy and clay loam soil (S1 – loamy soil, S2- clay loam)

The changes observed in seedling growth parameters could be due to changes in physical, chemical and biological properties caused by the microplastics in soil. For example, microplastics are reported to favour a certain group of microbes over others [16, 17] which would undoubtedly disturb soil enzyme activity and in turn plant nutrient uptake dynamics.

4. CONCLUSION

The effect of microplastics on plants varies based on type, shape, size and their interaction with various parameters surrounding the plant. So it is necessary to study how each polymer with varying properties behave in different soil-plant systems. Through this study, the behavior of polyethylene microplastics of size <600 µm (larger microplastics) in two different soil types on blackgram seedling growth under controlled conditions was analysed. The results showed that the effect on blackgram varied between the soil types. Hence, further studies on how polyethylene microplastics could influence plant growth in natural settings are necessary to fully establish their potential impacts on soil-plant system.

REFERENCES

1. Zhao S, Zhang Z, Chen L, Cui Q, Cui Y, Song D, et al. Review on migration, transformation and ecological impacts of microplastics in soil. Appl. Soil Ecol. 2022;176:104486.

2. Ding L, Huang D, Ouyang Z, Guo X. The effects of microplastics on soil ecosystem: A review. Curr. Opin. Environ. Sci. Health. 2022:100344.
3. Zhang K, Hamidian AH, Tubić A, Zhang Y, Fang JK, Wu C, et al. Understanding plastic degradation and microplastic formation in the environment: A review. Environ. Pollut. 2021;274:116554.
4. van Schothorst B, Beriot N, Huerta Lwanga E, Geissen V. Sources of light density microplastic related to two agricultural practices: the use of compost and plastic mulch. Environments. 2021;8(4):36.
5. Zhang Z, Peng W, Duan C, Zhu X, Wu H, Zhang X, et al. Microplastics pollution from different plastic mulching years accentuate soil microbial nutrient limitations. Gondwana Res. 2021.
6. Vithanage M, Ramanayaka S, Hasinthara S, Navaratne A. Compost as a carrier for microplastics and plastic-bound toxic metals into agroecosystems. Curr. Opin. Environ. Sci. Health. 2021;24:100297.
7. El Hayany B, Rumpel C, Hafidi M, El Fels L. Occurrence, analysis of microplastics in sewage sludge and their fate during composting: A literature review. J. Environ. Manage. 2022;317:115364.
8. Lian J, Liu W, Meng L, Wu J, Zeb A, Cheng L, et al. Effects of microplastics derived from polymer-coated fertilizer on maize growth, rhizosphere, and soil properties. J. Clean. Prod. 2021;318:128571.
9. Zhang S, Li Y, Chen X, Jiang X, Li J, Yang L, et al. Occurrence and distribution of microplastics in organic fertilizers in China. Sci. Total Environ. 2022:157061.
10. Nizzetto L, Futter M, Langaas S. Are agricultural soils dumps for microplastics of urban origin? : ACS Publications; 2016.
11. Mahon AM, O'Connell B, Healy MG, O'Connor I, Officer R, Nash R, et al. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. 2017;51(2):810-8.
12. He D, Luo Y, Lu S, Liu M, Song Y, Lei L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. Trends Anal. Chem. 2018;109:163-72.
13. Turan NB, Erkan HS, Engin GO. Microplastics in wastewater treatment plants: Occurrence, fate and identification. Process Saf. Environ. Prot. 2021;146:77-84.
14. de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. Glob. change biol. 2018;24(4):1405-16.
15. Zhang M, Zhao Y, Qin X, Jia W, Chai L, Huang M, et al. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. Sci. Total Environ. 2019;688:470-8.
16. Rong L, Zhao L, Zhao L, Cheng Z, Yao Y, Yuan C, et al. LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci. Total Environ. 2021;773:145640.
17. Yan Y, Chen Z, Zhu F, Zhu C, Wang C, Gu C. Effect of polyvinyl chloride microplastics on bacterial community and nutrient status in two agricultural soils. Bull. Environ. Contam. Toxicol. 2021;107(4):602-9.
18. Ma Y, Huang A, Cao S, Sun F, Wang L, Guo H, et al. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ. Pollut. 2016;219:166-73.

19. Qi Y, Yang X, Pelaez AM, Lwanga EH, Beriot N, Gertsen H, et al. Macro-and micro-plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci. Total Environ. 2018;645:1048-56.
20. Bosker T, Bouwman LJ, Brun NR, Behrens P, Vijver MG. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere. 2019;226:774-81.
21. Yu H, Peng J, Cao X, Wang Y, Zhang Z, Xu Y, et al. Effects of microplastics and glyphosate on growth rate, morphological plasticity, photosynthesis, and oxidative stress in the aquatic species Salvinia cucullata. Environ. Pollut. 2021;279:116900.
22. Ren X, Tang J, Wang L, Liu Q. Microplastics in soil-plant system: effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues. Plant Soil. 2021;462(1):561-76.
23. Lozano YM, Lehnert T, Linck LT, Lehmann A, Rillig MC. Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. Front. Plant Sci. 2021;12:616645.
24. de Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, et al. Microplastics can change soil properties and affect plant performance. Environ. Sci. Technol. 2019;53(10):6044-52.
25. Wang L, Liu Y, Kaur M, Yao Z, Chen T, Xu M. Phytotoxic Effects of Polyethylene Microplastics on the Growth of Food Crops Soybean (Glycine max) and Mung Bean (Vigna radiata). Int. J. Environ. Res. Public Health. 2021;18(20):10629.
26. Wang F, Wang Q, Adams CA, Sun Y, Zhang S. Effects of microplastics on soil properties: current knowledge and future perspectives. J. Hazard. Mater. 2022;424:127531.
27. Jackson ML. Soil chemical analysis: advanced course: UW-Madison Libraries parallel press; 2005.
28. Pansu M, Gautheyrou J. Handbook of soil analysis: mineralogical, organic and inorganic methods: Springer Science & Business Media; 2007.
29. Pignattelli S, Broccoli A, Piccardo M, Felline S, Terlizzi A, Renzi M. Short-term physiological and biometrical responses of Lepidium sativum seedlings exposed to PET-made microplastics and acid rain. Ecotoxicol. Environ. Saf. 2021;208:111718.
30. Pignattelli S, Broccoli A, Piccardo M, Terlizzi A, Renzi M. Effects of polyethylene terephthalate (PET) microplastics and acid rain on physiology and growth of Lepidium sativum. Environ. Pollut. 2021;282:116997.
31. Boots B, Russell CW, Green DS. Effects of microplastics in soil ecosystems: above and below ground. Environ. Sci. Technol. 2019;53(19):11496-506.
32. van Kleunen M, Brumer A, Gutbrod L, Zhang Z. A microplastic used as infill material in artificial sport turfs reduces plant growth. Plants, people, planet. 2020;2(2):157-66.
33. Yang W, Cheng P, Adams CA, Zhang S, Sun Y, Yu H, et al. Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with ZnO nanoparticles. Soil Biol. Biochem. 2021;155:108179.
34. Jiang X, Chen H, Liao Y, Ye Z, Li M, Klobučar G. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environ. Pollut. 2019;250:831-8.
35. Mateos-Cárdenas A, Scott DT, Seitmaganbetova G, van Pelt Frank N, AK JM. Polyethylene microplastics adhere to Lemna minor (L.), yet have no effects on plant

growth or feeding by Gammarus duebeni (Lillj.). Sci. Total Environ. 2019;689:413-21.
36. Li Z, Li Q, Li R, Zhao Y, Geng J, Wang G. Physiological responses of lettuce (Lactuca sativa L.) to microplastic pollution. Environ. Sci. Pollut. Res. 2020;27(24):30306-14.