PALYGORSKITE REDUCING CD ACCUMULATION IN LETTUCE (*LACTURA SATIVA* L.) AND REMEDIATING SOIL CONTAMINATED WITH BOTH CADMIUM AND MICROPLASTICS

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Abstract. Microplastics (MPs) increase the available state of cadmium (Cd) in soil, which could seriously threaten the safe production of lettuce (*Lactura sativa* L.). Palygorskite (PAL) can potentially improve MPs-Cd co-contaminated soil, but the mechanism is unclear. PAL (2%, 4%) and MPs (0.1%, 1%) were mixed into soils with different levels of Cd pollution. After 30 days, the bioavailability of Cd and soil physical and chemical properties were determined. The dry weight of lettuce and the concentration of Cd in the shoot and root parts of lettuce were determined. The results showed that MPs hurt the growth of lettuce and increased the Cd accumulation in plant. The content of available Cd in soil was also increased. PAL could increase the lettuce biomass of the shoot and root part by 2.46%-40.64% and 2.75%-36.25%, respectively. Soil bioavailable Cd decreased by 14.15%-53.85%. Cd content in the shoot and root part of lettuce decreased by 6.12%-27.29% and 2.36%-44.90%, respectively. Regression path analysis (RPA) showed that PAL reduced the accumulation of Cd in plants and promoted the growth of lettuce by changing the properties of MPs-Cd co-contaminated soil. This study provides a new perspective for the remediation of MP-Cd co-contaminated soil with natural clay minerals.
Keywords: *palygorskite, cadmium, polyethylene, lettuce, co-contaminated soil*

Introduction

Microplastics (MPs) refer to plastic-like pollutants with a particle size of less than 5 mm, including the primary microplastics used as raw materials for industrial production and secondary microplastics formed by physical, chemical, and biological weathering and cracking (Yu et al., 2022; Zhang et al., 2021). As a global environmental problem, the pollution of microplastics in marine environments has attracted widespread attention (Chang et al., 2022; Rillig et al., 2020; Wang et al., 2020). In recent years, studies have shown that soil microplastic pollution is more common than that in the sea, and its abundance may be 4 to 23 times higher than that of the ocean (Khalid et al., 2021; Xu, 2020; Zhou et al., 2019). It is estimated that about 6.3-43.0×10^4 tons and 4.4-30×10^4 tons of microplastics are imported into farmland in Europe and North America annually (Futter et al., 2016). MPs in the soil can change the physical and chemical properties of soil, interfere with the normal growth and development of plants, and pose environmental and ecological risks to the soil-plant system. It can change the water cycle in soil. When the content of MPs reaches a certain value, it will obstruct soil moisture infiltration and increase the probability of soil erosion (Baile et al., 2020; Feng et al., 2021). MPs and their additives can also affect the pH of the soil (Bandow et al., 2017; Yang et al., 2019), such as high-density polyethylene significantly reduces the pH of the soil, while PVC filled with calcium carbonate leads to an increase in soil pH. Moreover, MPs significantly impact soil

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material circulation and enzyme activity, resulting in reduced soil fertility (Cao et al., 2017). Microplastics not only affect the development of plant roots but are also easily absorbed and enriched by plant roots, accumulating in edible parts and leading to transfer, enrichment, and health risks through the food chain (Rillig et al., 2019). In addition, microplastics have a large specific surface area and easily adsorb other pollutants in the soil, thus forming compound pollution (Wu et al., 2022).

Soil heavy metal pollution is one of the main problems faced by soil environmental security (Liu et al., 2020; Qin et al., 2021). Human activities such as the discharge of industrial “three wastes”, the improper use of chemical fertilizers and pesticides, and the emission of municipal solid waste have led to increasing serious soil heavy metal pollution (Li et al., 2014). Heavy metals in soil are not easily decomposed by microorganisms, but they can be readily absorbed by plants and concentrated in plant tissues, thereby disrupting normal cell activity and affecting the growth and development process of plants (Xiang et al., 2024). Notably, areas polluted with microplastics often coincide with those polluted with heavy metals. The combined pollution of microplastics adsorbing heavy metals will bring new risks to the soil-plant ecosystem, posing more significant environmental and ecological security challenges (Liu et al., 2024; Yu et al., 2022). Based on the interaction between MPs and heavy metals in the soil-plant system, the process of combined pollution by microplastics and heavy metals can be divided into three stages: firstly, microplastics adsorbing heavy metals from soils (Mei et al., 2020; Wu et al., 2018); secondly, the migration of microplastics and heavy metals together in soils (Zhang et al., 2019); thirdly, the migration of MPs and heavy metals towards plants (Li et al., 2021; Yu et al., 2020). Microplastics are often used as carriers to adsorb heavy metals, affecting the migration of heavy metals in soil (Cui et al., 2022). In this process, microplastics have an adsorption effect on heavy metals, mainly through electrostatic attraction between functional groups on the surface of microplastics and heavy metals. In addition, functional groups on the surface of microplastics can also act as proton donors or acceptors to chemically bond with heavy metals, thus affecting adsorption (Qi et al., 2020). Yang et al. (2019) found that polar functional groups (-NHCO- and -COO-) on the surface of polyamide (PA) are more likely to form chemical bonds with Cu^{2+}, making their adsorption capacity much higher than other types of MPs. The interaction and migration patterns of MPs and Cd in the soil are shown in *Figure 1*.

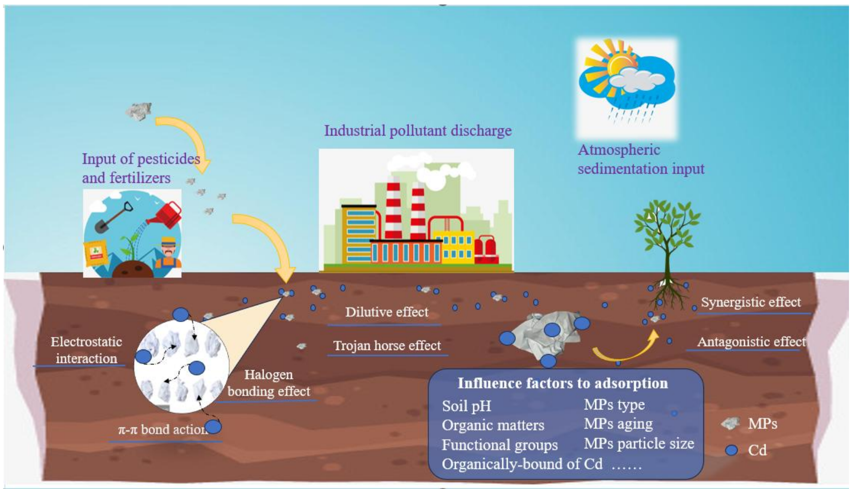

Figure 1. *The interaction and migration patterns of MPs and Cd in the soil*

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Palygorskite (PAL), also known as attapulgite, is a natural nano-sized hydrated magnesium aluminosilicate clay mineral. The theoretical chemical formula is $Mg_5Si_8O_{20}(OH)_2(OH_2)_4 \cdot 4H_2O$ (Guggenheim et al., 2011). As a kind of water-rich aluminosilicate clay mineral, palygorskite has good adsorption capacity and ion exchange capacity due to its rich rod-like crystal structure and a large amount of negative charge on its surface (Han et al., 2014; Li et al., 2019). Liang et al. (2017) studied the stabilization effect of palygorskite on Cd^{2+} in paddy soil. The results showed that palygorskite could increase the pH value of soil and significantly reduce the concentration of biologically available Cd in soil, resulting in a significant decrease in the concentration of Cd in rice. The adsorption mechanism is complexation and surface precipitation. Han et al. (2014) studied the adsorption stability and passivation mechanism of palygorskite for Cd in soil. The results show that the adsorption of palygorskite to Cd is less affected by environmental conditions such as pH, temperature, and background electrolyte and shows good stability in the passivation process. The characterization results show that the adsorption mechanism of palygorskite to Cd in the soil is the surface precipitation of $CdCO_3$ and the surface complexation with the hydroxyl group. Therefore, it has the potential to be used in the passivation and remediation of heavy metals in farmland soil.

In this study, a natural clay mineral—palygorskite, was added to microplastic-heavy metal-contaminated farmland soil to establish a new soil resource remediation mechanism. The main purposes are as follows: i) the action mechanism of PAL on microplastic-heavy metal contaminated soil was discussed by measuring the physical and chemical properties of soil (pH, CEC, DOC, MBC, and MBN), the content of Cd in soil, the growth status of lettuce (*Lactura sativa* L.) and the absorption concentration of Cd; ii) Regression path analysis (RPA) was used to reveal the effect mechanism of palygorskite in lettuce under the condition of microplastic storage.

This work fills the gap in the research on the influence of PAL on the available form of HMs in the presence of MP. The results will increase our understanding of the risk of heavy metal and microplastic pollution in farmland soil and provide a theoretical basis for preventing and controlling soil microplastic-heavy metal compound pollution.

Materials and methods

Experimental materials

The tested plant selected in this study is lettuce, which is widely growing in China and purchased from the Gansu Academy of Agricultural Sciences (Lanzhou, China). The seeds were cleaned before use, sterilized with 1%NaClO for 10 min, and then soaked in ultra-pure water for 24 h.

The palygorskite used in this study is produced in Banqiao Town, Linze County, Gansu Province, China, and purchased from Gansu Hanxing Environmental Protection Technology Co., Ltd (Lanzhou, China). The pH of palygorskite was 8.25 ± 0.20, the content of organic matter was $5.55 \pm 0.3\%$, the content of cation exchange capacity (CEC) was 18.35 ± 2.54 cmol/kg, the content of available potassium was 21.54 ± 3.15 mg/kg, and the content of Cd was 0.21 ± 0.01 mg/kg. The contents of available nitrogen and available phosphorus were not detected.

Polyethylene (PE) is one of the most common plastics in daily life and agricultural production, purchased from Dongguan Zhangmu Huachuang Plastic Raw Material Co.,

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Ltd (Dongguan, China). The particle size of PE microplastics is 50-200 um, and the density is 0.853-0.904 g/cm^3. The PE microplastics were soaked in 5% nitric acid for 24 h to remove surface impurities, then washed to neutral with deionized water and dried at low temperatures.

The soil used in this experiment was collected from Anning Village, Lanzhou City (36°09’E, 103°69’N, Gansu Province, China). The soil type was brown soil. Collect 0~20 cm topsoil, remove plant roots, sand, and other sundries, air-dry, grind, sift through 10 mesh, and set aside. The pH of the soil was 7.48 ± 0.30, DOC content was 2.88 ± 0.15 mg/kg, the content of CEC was 22.35 ± 2.14 cmol/kg, the content of available potassium was 28.54 ± 3.61 mg/kg, the content of total nitrogen content is 0.92 ± 0.25 mg/kg, and the content of Cd was 0.36 ± 0.04 mg/kg.

Experimental design

First, the soil contaminated by Cd was prepared. The Cd concentration of collected soil is 0.36 ± 0.04 mg/kg, which is lower than the risk control value of Cd concentration (0.60 mg/kg) (China State Administration of Market Supervision and Administration, Ministry of Ecology and Environment, 2018). This concentration is called Cd-L. Adding $Cd(NO_3)_2 \cdot 4H_2O$ to the soil nd setting the concentration of Cd at 2.5 and 5.0 mg/kg, which was called Cd-M and Cd-H, respectively. The collected soil was packed into two wooden containers containing 60 kg of soil. 0.15 L and 0.30 L solutions of 1 g Cd^{2+} were dissolved in 18 L distilled water and added to two containers. Balance them for 30 days, maintain 30% moisture content during this period, and stir every three days. The Cd concentration of each soil sample was measured at the end of equilibrium, and the soil was digested with a mixed acid solution of HNO_3:HF:H_2O_2 (5:2:1). Then, the content of Cd was determined by ICP-OES. The physical and chemical properties of the three soils were shown in *Table 1*.

Table 1. *The physical and chemical properties of the three soils (means ± std, n = 3)*

	Soil 1 (Cd-L)	**Soil 2 (Cd-M)**	**Soil 3 (Cd-H)**
pH	7.48 ± 0.30	7.46 ± 0.10	7.41 ± 0.05
CEC (cmol/kg)	22.35 ± 2.14	22.15 ± 1.65	21.73 ± 2.47
DOC (mg/kg)	2.88 ± 0.15	2.60 ± 0.07	2.74 ± 0.21
MBN (mg/kg)	26.71 ± 2.18	23.58 ± 1.69	21.98 ± 1.25
MBC (mg/kg)	203.52 ± 5.21	200.15 ± 6.61	147.79 ± 4.46
Total-Cd (mg/kg)	0.36 ± 0.04	2.53 ± 0.05	5.32 ± 0.02

After that, MPs and PAL were mixed into Cd-L, Cd-M, and Cd-H soils, respectively. Ceramic flowerpots with a diameter of 21.5 cm and a height of 15 cm were selected, and each pot was filled with 2 kg of soil. The amounts of MPs added was 0, 0.1% and 1% (w/w), and the amount of PAL added was 0, 2% and 4% (w/w). Before mixing PAL, add MPs with Cd-contaminated soil and balance them for a week. There were nine treatments of Cd concentration in each group, which were blank (0 MPs + 0 PAL), 0.1%MPs, 1%MPs, 2%PAL, 4%PAL, 0.1%MPs + 2%PAL, 0.1%MPs + 4%PAL, 1%MP + 2%PAL and 1%MP + 4%PAL. A total of 27 treatments were carried out in the soils with three kinds of Cd concentrations, and three groups of repeats were set for each group, which was 81 pots.

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Ten lettuce seeds in each basin were sowed after the soil was prepared ready and maintained at 70% field capacity. After the emergence of the seedlings, five seedlings with excellent growth were selected, while the other seedlings were pulled out and discarded. The position of the flowerpot was changed every week to ensure the randomness of the growing environment. The flowerpot was cultivated under 25 ± 2°C, 8 h of natural light, and 1 h of ventilation per day. After 30 days, the plants were harvested, Cd accumulation of lettuce was measured by ICP-OES after digested with a mixed acid solution of HNO_3 and $HClO_4$ (2:1) (Jia et al., 2022). The biomass of lettuce was also determined.

Chemical analysis of samples

The pH value of the soil sample was determined with a pH electrode (Leici pH-3C, Shanghai), with a soil-liquid ratio of 2.5:1 (w/w). The sample CEC was determined by the barium chloride-sulfuric acid forced exchange method. Available nitrogen was determined by alkaline diffusion method, available phosphorus was determined by sodium bicarbonate extraction-molybdenum-antimony resistance colorimetry, and available potassium was determined by ammonium acetate extraction-flame photometer. DOC was determined by a total organic carbon analyzer (Jena Mull 3100 TOC, Germany). Before determination, soil DOC was extracted with water (1:5, soil-water ratio). Diethylenetriaminepentaacetic acid (DTPA) (soil-liquid ratio 1:5) was used to extract available Cd from the soil, and its content was determined by ICP-OES (Agilent 5800 ICP-OES, USA). Soil microbial biomass carbon content (MBC) and soil microbial biomass nitrogen content (MBN) were fumigated with $CHCl_3$, then extracted with 0.5 mol/L K_2SO_4 solution, and determined by TOC analyzer (Qikun, CDLC-800M, Shanghai).

Statistical analyses

One-way analysis of variance (ANOVA) (SPSS, 2019) was used to process the data at the level of $P < 0.05$ for statistical analysis. Duncan test was used to compare the differences among different MP, PAL, and Cd content treatment groups. Pearson correlation analysis was used to analyze the correlation between plant Cd content and soil parameters. Regression path analysis (RPA) was used to analyze the relationship between available Cd, Cd concentration in plants, and soil physicochemical properties. The graphics were drawn by Origin 2021 software.

Results

Plant biomass

The biomass of lettuce's shoot and root parts under different treatments were displayed in *Figure 2*, and the figure notes explained capital and lowercase letters on the bar chart. The results showed that when MPs and PAL were not added, the dry weight of the shoot and root parts of lettuce decreased significantly ($P < 0.05$) with the increase of heavy metal concentration, and heavy metal had an obvious inhibiting effect on plant growth. Adding MPs intensified the pollutants' effect on lettuce growth, with shoot and root biomass most affected at higher MP concentrations. In the experiment, when cabbage plants were exposed to different levels of Cadmium (Cd-L, Cd-M, and Cd-H) without PAL addition groups, the shoot biomass of the plants treated with 1% MPs was

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lower than that of the plants in the 0 MP treatment. The shoot biomass of plants in the Cd-L, Cd-M, and Cd-H groups was 2.48 g/pot, 2.27 g/pot, and 1.54 g/pot, respectively, which represented a decline of 7.12%, 2.58%, and 10.99% compared to the biomass of the plants in the 0 MP treatment. The roots part of lettuce showed the same trend. In the Cd-L, Cd-M, and Cd-H groups, the dry weight of roots treated with 1% MPs was decreased than that of the blank at 7.55%, 8.60%, and 7.58%, respectively. Adding PAL ($P < 0.05$) increased the biomass of shoots and roots of lettuce significantly and weakened the inhibitory effects of MPs and Cd on plant growth. For example, in the Cd-L and 1% MPs concentration group, the biomass of shoots was 1.79 and 2.17 g/pot under 2% and 4% PAL addition, respectively, which improved by 16.23% and 40.91% compared to 0 PAL (1.54 g/pot) treatment. The promoting effect of 4%PAL treatment on the growth of lettuce was significantly higher ($P < 0.05$) than that of 2% PAL addition.

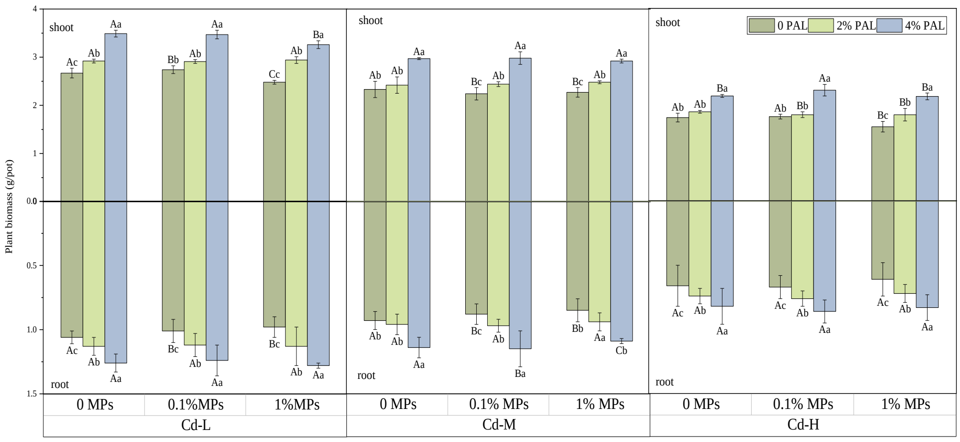

***Figure 2.** Effect of PAL on the lettuce biomass grown in soil with both MPs and Cd*

Cd concentration in lettuce

Figure 3 depicts the variations in Cd accumulation in the shoots and roots of lettuce. A one-way ANOVA was performed to compare Cd concentrations in shoots and roots for different MP treatments under the same PAL dosage and cadmium level (capital letters in *Fig. 3* represent the results). The results showed that under the same concentrations of cadmium and PAL, 1% MP treatment significantly increased Cd accumulation in lettuce ($P < 0.05$). In the CD-H treatment without PAL, the influence of 1% MP treatment on the accumulation of Cd in shoots and roots was the greatest (3.94 and 3.01 mg/kg, respectively), which was 1.15 and 1.06 times that of 0 MP treatment, respectively. Cd content of shoots and roots under 0.1% MP treatment was 3.63 mg/kg and 2.96 mg/kg, lower than that under 1% MP treatment but higher than that under 0 MP treatment by 5.83% and 3.86%, respectively. The same trend was observed in the Cd-L and Cd-M treatment groups.

A one-way ANOVA analysis was conducted on the Cd concentrations of lettuce treated with 0%, 2%, and 4% PAL at the same MP and Cd levels (lowercase letters in *Fig. 3* represent the results). The findings indicate that the application of PAL significantly ($P < 0.05$) reduced the accumulation of Cd in plants. In the CD-H treatment group without MPs, the accumulation of Cd in the shoot part of 2% and 4%

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PAL treatment was 3.22 and 2.59 mg/kg, respectively, which was 6.12% and 24.49% lower than that in 0 PAL (3.43 mg/kg) treatment. The accumulation of root parts was 2.75 and 2.19 mg/kg, which was 3.5% and 23.16% lower than in 0 PAL (2.85 mg/kg) treatment. In the CD-H soil containing 1% MPs, the shoot Cd accumulation under 2% and 4% PAL treatment was 3.50 and 3.40 mg/kg, respectively, which was 11.17% and 13.71% lower than that under 0 PAL (3.94 mg/kg) treatment, respectively. The accumulation of root parts was 2.86 and 2.67 mg/kg, which was lower than 4.98% and 11.30% that of 0 PAL (3.01 mg/kg) treatment. Adding PAL can significantly reduce the Cd accumulation in lettuce regardless of the presence of MPs in the soil, and the effect increases with the amount of PAL added.

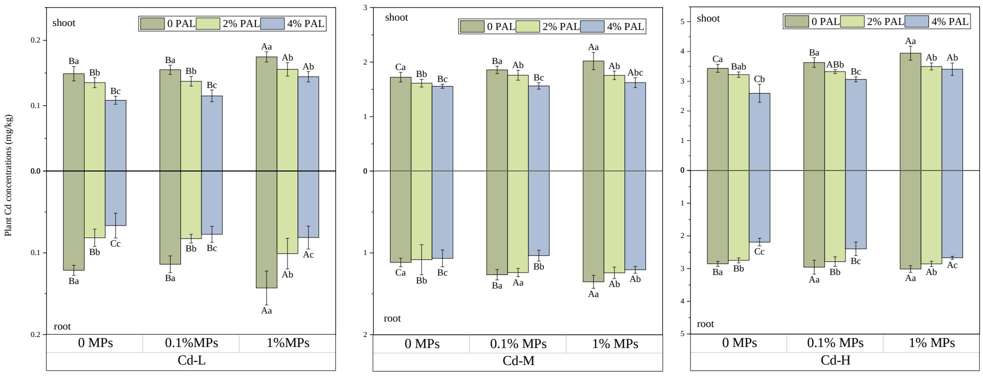

Figure 3. *Effect of PAL on the Cd concentrations in lettuce grown in soil with both MPs and Cd*

DTPA-extractable Cd in soil

Figure 4 illustrated the PAL application on the DTPA-extractable Cd content in 27 treated soils with MPs and/or Cd. Under the same concentration of MPs and Cd treatment, the content of bioavailable Cd was analyzed by one-way ANOVA with different dosages of PAL (lowercase letters in *Fig. 4*). The results showed that PAL could significantly reduce the content of soil bioavailable Cd with or without MPs. For example, in the Cd-M treatment group, without the addition of MPs, the extractable Cd concentrations of DTPA treated with 2% and 4% PAL were 4.68 and 4.52 μg/L, respectively, which were 19.89% and 21.66% lower than those treated with 0 PAL (5.77 μg/L). Similarly, in the Cd-H treatment group, DTPA-Cd concentrations were reduced by 14.15% and 16.06% in 2% and 4% PAL treatment (7.22 and 7.06 μg/L), respectively, compared to the control group (0 PAL treatment). When the same concentration of Cd and MPs was used, 4% PAL treatment was found to be more effective than 2% PAL treatment in reducing bioavailable Cd.

Additionally, under the same PAL dosage and Cd contamination degree, a one-way ANOVA was performed for bioavailable Cd in the MPs treatment groups with different concentrations (capital letters in *Fig. 4*). It was observed that, at the same PAL dosage and Cd contamination levels, the increase in DTPA-Cd contents was found to be significantly higher in the group treated with 1% MPs compared to that of the group treated with 0.1% MPs ($P < 0.05$). For example, in the Cd-M and 4% PAL treatment group, DTPA-Cd concentrations were increased by 56.85% and 23.01% in 1% (7.09 μg/L) and 0.1% (5.56 μg/L) MPs compared to the 0 MPs treatment (4.52 μg/L).

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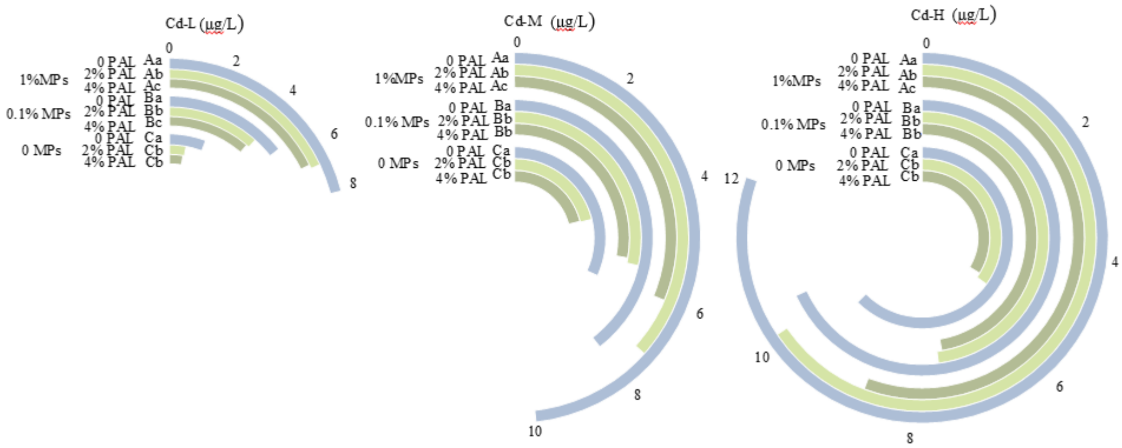

***Figure 4.** Effect of PAL application on the content of DTPA-extractable Cd in soil with both MPs and Cd*

Soil physicochemical properties

Table 2 showed the physical and chemical properties of soil under different treatment groups. In the absence of PAL, the presence of MPs reduced the soil pH and CEC and increased DOC significantly. Moreover, the difference was more significant with the increase of microplastic content. For example, when Cd pollution was at Cd-M level, pH, CEC, and DOC in the treatment group without PAL and 1% MPs concentration were 7.13, 19.37 cmol/kg, and 2.78 mg/kg, respectively. Compared with the 0 MPs treatment group, pH and CEC decreased by 4.42% and 12.55%, respectively, and DOC increased by 6.47%. In addition, when PAL was not added to the soil, MPs increased soil MBC and MBN, but the difference was insignificant. For example, in the 0 PAL and Cd-H treatment groups, MBC and MBN were 24.21 mg/kg and 161.32 mg/kg, respectively, under 0.1%MPs treatment, which increased by 10.15% and 9.14% compared with the 0 MPs treatment group.

Soil pH, CEC, DOC, MBC, and MBN increased significantly after PAL was added to MPs-Cd co-polluted soil. For example, in the Cd-M and 0 MPs treatment groups, pH (7.72), CEC (27.52 cmol/kg), DOC (2.85 mg/kg), MBC (281.63 mg/kg) and MBN (30.58 mg/kg) increased by 3.49%, 24.24%, 9.62%, 29.69% and 40.71% respectively compared with the 0 PAL treatment group under the 4% PAL treatment group. In the Cd-M and 1% MPs treatment groups, the pH, CEC, DOC, MBC, and MBN of the 2% PAL treatment group were increased by 3.37%, 10.89%, 1.44%, 13.80%, and 28.26%, respectively. 2% and 4% PAL treatments have different effects on soil physical and chemical properties, and the existence of PAL limits the effects of MPs on soil physical and chemical properties. The results indicated that the increase in the amount of PAL has a more significant effect on plant growth.

Relationships between the soil properties and the accumulation of Cd

The regression path analysis (RPA) was conducted to determine the relationship between the concentration of Cd in the shoot or root parts of lettuce and various soil physicochemical properties such as DTPA-Cd, pH, DOC, CEC, MBC, and MBN. The study revealed that pH, DOC, DTPA-Cd, MBN, and MBC were the critical factors influencing the concentration of Cd in the shoot and root parts (*Fig. 5*). The

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concentration of Cd in the shoot and root part of lettuce was found to be negatively correlated with soil pH, MBC and MBN, but positively correlated with DOC and DTPA-Cd. The CEC of the soil did not significantly affect the Cd concentration in the shoot and root part of the lettuce. In addition, there was no significant difference in the direct effects of MBN and DOC on Cd accumulation in plants. The amount of CEC may indirectly affect the shoot and root Cd concentration by promoting the influence of soil pH, DOC, MBC, MBN, and other soil variables, and the indirect contribution values were 0.64, 0.57, 0.51, and 0.47, respectively. These results indicate that adding PAL changes the shoot and root Cd concentrations of lettuce by affecting soil properties and directly or indirectly affects the mechanism of soil property change, in which soil pH, DOC, Cd bioavailability, MBC, and MBN play a central role.

Table 2. *Effect of PAL application on the soil physicochemical properties*

Cd levels	Index	0 MPs			0.1% MPs			1% MPs		
		0 PAL	2% PAL	4% PAL	0 PAL	2% PAL	4% PAL	0 PAL	2% PAL	4% PAL
Cd-L	pH	Ac	Ab	Ba	Bc	Bb	Ba	Cc	Cb	Aa
	CEC (cmol/kg)	Ac	Bb	Ba	Bc	Bb	Ba	Cc	Ab	Aa
	DOC (mg/kg)	Cc	Cb	Ba	Bc	ABb	Aa	Ac	Aab	Aa
	MBN (mg/kg)	Ac	Bb	ABa	Ac	Bb	Aa	Ac	Ab	Aa
	MBC (mg/kg)	Ac	Cb	Ba	Ac	Bb	Aa	Ac	Bb	Aa
Cd-M	pH	Ac	Ab	Aa	Bc	Ab	Ba	Cc	Bb	Ca
	CEC (cmol/kg)	Ac	Bb	Ba	Bc	Bb	Ba	Cc	Ab	Aa
	DOC (mg/kg)	Cc	Cb	Ba	Bc	ABb	Aa	Ac	Aab	Aa
	MBN (mg/kg)	Ac	Bb	Ca	Ac	Bb	Ba	Ac	Ab	Aa
	MBC (mg/kg)	Ac	Cb	Ca	Ac	Bb	Aa	Ac	Ab	Aa
Cd-H	pH	Ac	Ab	Ba	Bc	Ab	Aa	Cc	Bb	Aa
	CEC (cmol/kg)	Ac	Ab	Ba	Bc	Ab	Ca	Cc	Bb	Aa
	DOC (mg/kg)	Bb	Bb	Aa	Ab	Aa	Aa	Ab	Aab	Aa
	MBN (mg/kg)	Bc	Cb	Ca	ABc	Bb	Ba	Ac	Ab	Aa
	MBC (mg/kg)	Ac	Cb	Ca	Ac	Bb	Ba	Ac	Ab	Aa

2.60 3.16 7.13 7.85 18.95 44.39 147.79 374.36

The uppercase letters in the table represent the differences under different MP analyzed by A one-way ANOVA in the same PAL and Cd processing group. The lowercase letters in the table represent the differences under different PAL analyzed by A one-way ANOVA in the same MPs and Cd processing groups

Discussion

Effect of PAL on the Cd bioavailability and lettuce growth in MP-and Cd contaminated soil

Cd has high toxicity, concealment, and difficult degradation after entering the farmland, which will negatively affect the soil environment, plant growth, and human health. After planting plants in Cd-contaminated soil, the increase of reactive oxygen species (ROS) in plants will lead to the change of enzyme specificity, the destruction of cell function, and the damage of cell membrane and DNA structure, resulting in the

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decrease of plant growth rate, nutritional imbalance and inhibition of photosynthesis (Fajardo et al., 2020; Song et al., 2023). Parmar et al. (2013) found that Cd interferes with a series of physiological processes of plants, such as photosynthesis, water relationship, ion metabolism, and mineral uptake, and interferes with the production of aminolevulinic acid dehydratase, reduces the photosynthetic rate of plants and hinders the synthesis of chlorophyll. The toxicity of Cd in soil depends more on its form in soil. When it is in the active state, it is more toxic to plants (Xu et al., 2021).

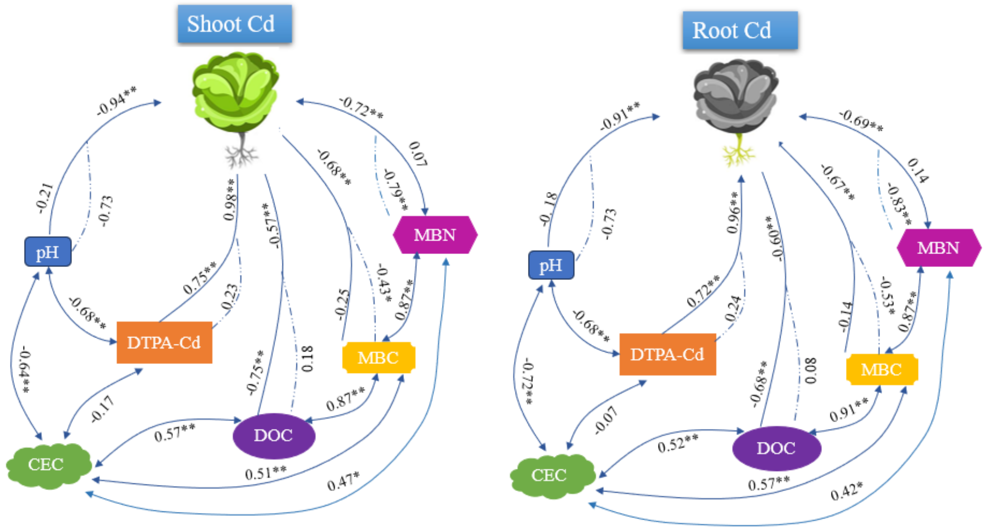

Figure 5. *Relationship between soil physical and chemical properties and Cd uptake by lettuce. The solid lines with arrowheads, solid lines, and dotted lines represent the comprehensive, direct, and indirect effects on the concentration of Cd in different parts, respectively*

Microplastics can enrich heavy metals locally, which is not conducive to solute transport in heavy metal-contaminated soil and aggravates local soil pollution (Li et al., 2024). The existence of MPs will compete with the metal cations in the soil, thus increasing the mobility and toxicity of Cd (Liu et al., 2024). This study showed that the existence of MPs did increase the DTPA-extracted Cd in soil and the accumulation of Cd in the root and root parts of lettuce (*Figs. 3* and *4*). The decrease in the biomass of lettuce also showed the inhibitory effect of MPs-Cd compound polluted soil on crop growth (*Fig. 2*). This finding was consistent with Wang et al. (2021), who found that when MP exists in Cd-contaminated soil, the bioavailability of Cd would be increased, while plant height and root length would be inhibited. It should be noted that this study does not consider the effects of PAL on different varieties of *Lactuca sativa* and can be used as a future research direction.

Clay minerals (such as PAL) are called natural scavengers of heavy metal pollutants in the soil environment. There are a large number of free and associated functional groups (- OH, ≡ Si-OH, Al-O, Si-Al, and hydrogen bonds) on the surface of PAL, which can stabilize heavy metals in soil by complexation, cation exchange, or electrostatic interaction, and the migration ability and bioavailability of heavy metals are reduced (Tao et al., 2021). The addition of PAL promoted the growth of lettuce; on one hand, the bioavailability of Cd in soil decreased significantly, and the toxicity to plants decreased after adding PAL. On the other hand, the surface of PAL is rich in silanols and other trace elements necessary for plant growth, such as Ca^{2+} and Mg^{2+},

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which promote the development of crops with the increase of palygorskite content. The results showed that the addition of PAL did reduce the content of DTPA-extracted Cd in soil and Cd accumulation in the shoot and root parts of lettuce (*Figs. 3* and *4*).

The soil repaired by PAL has a significantly reduced effective content of heavy metals, which can reduce the risk of being absorbed by crops, thereby ensuring the quality and safety of agricultural products and reducing the possibility that agricultural products cannot enter the market or pose a threat to human health due to excessive heavy metals. In addition, it can also reduce the accumulation and loss of pollutants in farmland and reduce the pollution pressure of agriculture on the surrounding environment, which is conducive to realizing the sustainable development of agriculture.

Effect of PAL on the alteration in the properties of MP-and Cd contaminated soil

Soil physical and chemical properties are important factors that can directly or indirectly affect the bioavailability and stability of heavy metals in soil, which can affect the release and fixation of pollutants (Jia et al., 2022). For example, neutral or acidic media can increase the solubility of heavy metals in the environment. At the same time, higher pH is more likely to form ion forms with lower solubility and more substantial stability. This study showed that the pH value and CEC of soil contaminated by MP and Cd could be increased by adding PAL (*Table 1*). This may have been because metal-organic complexes, metal hydroxides, and carbonates are more easily formed in a high pH environment. Besides, with the increase of soil pH, more negatively charged adsorption sites will be produced on soil organic-inorganic colloids and clay particles, which promote electrostatic adsorption, surface complexation, and cationic metal precipitation to reduce the migration and bioavailability of heavy metals in soil. In addition, when clay minerals have ion exchange or adsorption with HM, $Na^{+,}$ and K^{+} can be released, thus increasing soil CEC. What is noteworthy is that MP can indirectly increase the bioavailability of Cd by reducing soil pH and increasing water content, therefore inhibiting the growth of plants (Feng et al., 2021). In this paper, the results showed that the presence of MP decreased the soil pH value (*Table 1*), which indirectly increased the accumulation of DTPA-extracted Cd in soil and Cd content in the shoot and root of lettuce. RPA further showed that PAL affected the accumulation of Cd in lettuce by directly affecting soil pH, DTPA-extracted Cd, DOC, CEC, MBN, and MBC (*Fig. 5*). Therefore, in MPs-Cd compound polluted soil, PAL can indirectly alleviate the direct effect of Cd on lettuce and promote plant growth by changing soil properties.

Soil moisture, nutrients, and trace elements directly affect crop yield and quality (Liu et al., 2023). When the content of DOC is higher, it shows that the content of nutrients that can be absorbed by plants is higher, which will increase the yield of the crop. MP itself can be used as a carbon source to increase the content of DOC in the soil. Combining clay minerals (such as PAL) with soil humus can increase the stability of aggregates, maintain soil structure and organic matter content, and provide a favorable environment for the survival of microorganisms (Song et al., 2023). The coexistence of MP and PAL in soil can contribute to the cycle and transformation of carbon in soil and provide favorable nutrients (carbon source) for crop growth. The increase of DOC content in the soil further confirmed the increase of MBC and MBN (*Table 1*). In addition, adding exogenous substances reduced the soil bulk density and increased soil porosity to a certain extent. Higher porosity increases soil air content, resulting in a decrease in organic acid production and an increase in soil pH.

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Therefore, PAL can alleviate the effect of MP on the decrease of soil pH and CEC by increasing soil pH and CEC. In addition, PAL works with MP to promote DOC, MBC, and MBN to provide nutrition and a stable environment for lettuce growth.

Conclusions

The effects of PAL on the growth and Cd accumulation of lettuce in Cd-MP co-contaminated soil were studied in this research. The existence of MPs changed the physical and chemical properties of Cd-contaminated soil, promoted plant absorption of Cd, and inhibited the growth of lettuce. However, the application of PAL to polluted soil could effectively improve the soil properties, reduce the content of bioavailable Cd in soil, reduce the enrichment of Cd by plants, and promote the growth of lettuce. RPA results showed that PAL affected the Cd content of lettuce by changing soil properties, in which soil pH, DTPA-Cd, DOC, MBN, and MBC played a key role. In general, the negative effects of microplastics and heavy metals on soil properties and plant growth could be alleviated by applying PAL in co-polluted soil. This study provides a new perspective for the remediation of MP-Cd co-contaminated soil with natural clay minerals.

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REFERENCES

[1] Cao, D., Wang, X., Luo, X., Liu, G., Zheng, H. (2017): Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. – IOP Conference Series: Earth and Environmental Science 61: 012148.

[2] Chang, X., Fang, Y., Wang, Y., Wang, F., Shang, L., Zhong, R. (2022): Microplastic pollution in soils, plants, and animals: a review of distributions, effects and potential mechanisms. – Science of the Total Environment 850: 157857.

[3] Cui, Y., Zhang, Q., Liu, P., Zhang, Y. (2022): Effects of polyethylene and heavy metal cadmium on the growth and development of Brassica chinensis var. chinensis. – Water Air and Soil Pollution 233: 426.

[4] Fajardo, C., Sanchez-Fortun, S., Costa, G., Nande, M., Botias, P., Garcia-Cantalejo, J., Mengs, G., Martin, M. (2020): Evaluation of nanoremediation strategy in a Pb, Zn and Cd contaminated soil. – Science of the Total Environment 706: 136041.

[5] Feng, X., Wang, Q., Sun, Y., Zhang, S., Wang, F. (2021): Microplastics change soil properties, heavy metal availability and bacterial community in a Pb-Zn-contaminated soil. – Journal of Hazard Material 424: 127364.

[6] Futter, M., Langaas, S., Nizzetto, L. (2016): Are agricultural soils dumps for microplastics of urban origin? – Environmental Science & Technology 50: 10777-10779.

[7] Han, J., Xu, Y., Liang, X., Xu, Y. (2014): Sorption stability and mechanism exploration of palygorskite as immobilization agent for Cd in polluted soil. – Water Air and Soil Pollution 225: 2160-2173.

[8] Jia, H., Wu, D., Yu, Y., Han, S., Sun, L., Li, M. (2022): Impact of microplastics on bioaccumulation of heavy metals in rape (Brassica napus L.). – Chemosphere 288: 132576.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_735748

[9] Khalid, N., Aqeel, M., Noman, A., Khan, S. M., Akhter, N. (2021): Interactions and effects of microplastics with heavy metals in aquatic and terrestrial environments. – Environmental Pollution 290: 118104.

[10] Li, M., Liu, Y., Xu, G., Wang, Y., Yu, Y. (2021): Impacts of polyethylene microplastics on bioavailability and toxicity of metals in soil. – Science of the Total Environment 760: 144037.

[11] Li, Q., Chang, J., Li, L., Lin, X., Li, Y. (2024): Soil amendments alter cadmium distribution and bacterial community structure in paddy soils. – Science of the Total Environment 924: 171399.

[12] Li, S., Lei, N., Chen, J. (2019): Differential effects of natural palygorskite and its modified Form on alleviation of heavy metals toxicity to tomato grown in soil contaminated with Cd, Pb and Zn. – Polish Journal of Environmental Studies 28: 4519-4528.

[13] Li, Z., Ma, Z., Kuijp, T., Yuan, Z., Huang, L. (2014): A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. – Science of the Total Environment 468-469: 843-853.

[14] Liang, X., Qin, X., Huang, Q., Huang, R., Yin, X., Cai, Y., Wang, L., Sun, Y., Xu, Y. (2017): Remediation mechanisms of mercapto-grafted palygorskite for cadmium pollutant in paddy soil. – Environment Science and Pollution Research 24: 23783-23793.

[15] Liu, B., Zhao, S., Qiu, T., Cui, Q., Yang, Y., Li, L., Chen, J., Huang, M., Zhan, A., Fang, L. (2024): Interaction of microplastics with heavy metals in soil: mechanisms, influencing factors and biological effects. – Science of the Total Environment 918: 170281.

[16] Liu, J., Yin, M., Xiao, T., Zhang, C., Tsang, D., Bao, Z., Zhou, Y., Chen, Y., Luo, X., Yuan, W. (2020): Thallium isotopic fractionation in industrial process of pyrite smelting and environmental implications. – Journal of Hazard Material 384: 121378-121378.

[17] Liu, Y., Cui, W., Li, W., Xu, S., Sun, Y., Xu, G., Wang, F. (2023): Effects of microplastics on cadmium accumulation by rice and arbuscular mycorrhizal fungal communities in cadmium-contaminated soil. – Journal of Hazard Material 442: 130102.

[18] Mei, W., Chen, G., Bao, J., Song, M., Li, Y., Luo, C. (2020): Interactions between microplastics and organic compounds in aquatic environments: a mini review. – Science of the Total Environment 736: 139472.

[19] Parmar, P., Kumari, N., Sharma, V. (2013): Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. – Botanical Studies 54: 45.

[20] Qi, Y., Ossowicki, A., Yang, X., Lwanga, E. H., Dini-Andreote, F., Geissen, V., Garbeva, P. (2020): Effects of plastic mulch film residues on wheat rhizosphere and soil properties. – Journal of Hazard Material 387: 121711.

[21] Qin, G., Niu, Z., Yu, J., Li, Z., Ma, J., Xiang, P. (2021): Soil heavy metal pollution and food safety in China: effects, sources and removing technology. – Chemosphere 267: 129205.

[22] Rillig, M. C., Lehmann, A. (2020): Microplastic in terrestrial ecosystems. – Science 368: 1430-1431.

[23] Rillig, M. C., Anika, L., Abel, S., Gaowen, Y. (2019): Microplastic effects on plants. – New Phytologist 223: 1066-1070.

[24] Song, X., Jin, J., Li, H., Wang, F., Liu, J., Wang, X., Huang, X., Chai, C., Song, N., Zong, H. (2023): Kaolinite reduced Cd accumulation in peanut and remediate soil contaminated with both microplastics and cadmium. – Ecotoxicology and Environmental Safety 266: 115580.

[25] Stephen, G., Krekeler, M. (2011): The structures and microtextures of the palygorskite–sepiolite group minerals. – Developments in Clay Science 3: 3-32.

[26] Tao, L., Mi, X., Ren, H., Tong, Y., Wang, Y., Ren, J. (2021): Stabilization of heavy metals in mining soil using palygorskite loaded by nanoscale zero-valent iron. – International Journal of Environmental Science and Technology 19: 6789-6802.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_735748

[27] Wang, F., Wang, X., Song, N. (2021): Polyethylene microplastics increase cadmium uptake in lettuce (*Lactuca sativa* L.) by altering the soil microenvironment. – Science of the Total Environment 784: 147133.
[28] Wang, J., Coffin, S., Schlenk, D., Gan, J. (2020): Accumulation of HOCs via precontaminated microplastics bye earthworm Eisenia fetida in soil. – Environmental Science and Technology 54: 11220-11229.
[29] Wu, C., Tanaka, K., Tani, Y., Bi, X., Liu, J., Yu Q (2022): Effect of particle size on the colonization of biofilms and the potential of biofilm-covered microplastics as metal carriers. – Science of the Total Environment 821: 153265.
[30] Wu, P., Cai, Z., Jin, H., Tang, Y. (2018): Adsorption mechanisms of five bisphenol analogues on PVC microplastics. – Science of the Total Environment 650: 671-678.
[31] Xiang, Y., Rillig, M. C., Penuelas, J., Sardans, J., Liu, Y., Yao, B., Li, Y. (2024): Global responses of soil carbon dynamics to microplastic exposure: a data synthesis of laboratory studies. – Environmental Science and Technology 58: 5821-5831.
[32] Xu, B. L., Liu, F., Cryder, Z., Huang, D., Lu, Z. J., He, Y., Wang, H. Z., Lu, Z. M., Brookes, P. C., Tang, C. X., Gan, J., Xu, J. M. (2020): Microplastics in the soil environment: occurrence, risks, interactions and fate—a review. – Critical Reviews in Environmental Science and Technology 50: 4772.
[33] Xu, D., Fu, R., Liu, H., Guo, X. (2021): Current knowledge from heavy metal pollution in Chinese smelter contaminated soils, health risk implications and associated remediation progress in recent decades: a critical review. – Journal of Cleaner Production 286: 124989.
[34] Yang, J., Cang, L., Sun, Q., Dong, G., Ata-Ul-Karim, S. T., Zhou, D. (2019): Effects of soil environmental factors and UV aging on Cu2 + adsorption on microplastics. – Environmental Science and Pollution Research 26: 23027-23036.
[35] Yu, H., Hou, J., Dang, Q., Cui, D., Xi, B., Tan, W. (2020): Decrease in bioavailability of soil heavy metals caused by the presence of microplastics varies across aggregate levels. – Journal of Hazard Material 395: 122690.
[36] Yu, H., Zhang, Y., Tan, W., Zhang, Z. (2022): Microplastics as an emerging environmental pollutant in agricultural soils: effects on ecosystems and human health. – Frontiers in Environmental Science 10: 855292.
[37] Zhang, K., Hamidian, A. H., Tubi, A., Zhang, Y., Lam, P. K. S. (2021): Understanding plastic degradation and microplastic formation in the environment: a review. – Environmental Pollution 274: 116554.
[38] Zhang, S., Han, B., Sun, Y., Wang, F. (2019): Microplastics influence the adsorption and desorption characteristics of Cd in an agricultural soil. – Journal of Hazard Material 388: 121775.
[39] Zhou, Y., Liu, X., Wang, J. (2019): Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. – Science of the Total Environment 694: 133798.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_735748