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Effect of co-presence of cadmium or procymidone with microplastic films in soil on lettuce growth

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Abstract

Agricultural environment is often contaminated with various chemicals (e.g., pesticides, heavy metals) and microplastics due to the uses of plastic products. The effects of chemical contaminants or microplastics on terrestrial environment have been extensively studied, but the studies on the co-presence of chemical contaminants and microplastics are relatively limited. This study was set to investigate the effect of co-presence of microplastics (i.e., low-density polyethylene (LDPE) and polyvinyl chloride (PVC) microplastic films) and chemical contaminants (i.e., cadmium (Cd) and procymidone (PCM)) in soil on the lettuce growth and Cd and PCM uptake by lettuce using pot tests. The lettuce leaf lengths were not affected by the presence of only Cd or PCM, but the rates of change in the lettuce leaf number were adversely affected by the presence of PCM. The presence of only LDPE or PVC in soil at the concentrations used in this study did not have significant impacts on the lettuce growth. But the co-presence of Cd and LDPE and the co-presence of PCM and PVC resulted in the negligible increases in the lettuce leaf length and leaf number with time, although the lettuce growths were statistically similar in the Cd- or PCM-contaminated soils regardless of the presence of microplastics. The results suggest that the adverse effects of Cd or PCM can be intensified by the co-presence of microplastics, and the effects can be different depending on the types of microplastics. The promoted adverse effects of chemical contaminants in the co-presence of microplastics can be supported by the tendency of the increased absorption of Cd or PCM by lettuce in the co-presence of microplastics. Overall, this study shows the need for management of both chemical contaminants and microplastics that may reside in the agricultural environment.

Keywords Microplastics, Cadmium, Procymidone, Lettuce

Introduction

The increasing use of plastic products has resulted in the contamination of environment with plastics with <5 mm in size, which are known as microplastics [1, 2]. Microplastics of various characteristics (e.g., sizes, shapes, types) can impose threats to organisms in different environmental media [2–4]. In particular, many studies have explored the sorption characteristics of microplastics suggesting the role of microplastics as a carrier of various chemical contaminants including heavy metals and antibiotics [5–7].

Agricultural environment is prone to contamination by various chemicals such as pesticides, heavy metals, and

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antibiotics. The residual chemicals in the agricultural soil can be absorbed by crops and may lead to the human health risk [8, 9]. Procymidone (PCM) is one of the dicarboximide fungicides that has been widely used to control gray mold rot and stem rot [10]. Cadmium (Cd) can be introduced to the agricultural soil through various activities such as fertilizer uses and nearby industrial activities (e.g., mining, metal processing) [11]. The residual pesticides in soil can be absorbed by crops and human consumption of these exposed crops can lead to the presence of pesticides and their metabolites in the excretion [12, 13]. Also, the absorption of Cd by crops may impose toxic effects on crops inhibiting the growth and the absorbed Cd can end up in human by crop consumption [14].

The effects of microplastics in soil on terrestrial organisms (e.g., plants, earthworms, microorganisms) have been reported in previous studies [1–3]. For example, microplastics can inhibit the growth of plants such as lettuce [3, 15]. However, most previous studies used certain microplastic shapes such as beads or fragments, and the studies dealing with more than one type of microplastics in order to compare the effect of microplastic types are still limited [2, 3]. Thus, there is a need to study the effect of microplastics having various shapes and types on terrestrial organisms. Also, the presence of microplastics can change the toxic effects of chemical contaminants in environmental media, and the studies looking at the effects of co-presence of microplastics and chemical contaminants are increasing [16, 17]. For example, the bioavailability of Cd was affected by the co-presence of microplastics and this resulted in the greater accumulation of Cd in earthworms [17] and lettuce [18]. On the other hand, the reduced uptake of dibutyl phthalate by lettuce in the co-presence of microplastics has been reported [19]. The changes in the availability of chemicals in soil by the co-presence of microplastics can be partly explained by the interference of microplastics with the chemical sorption on soil [16]. Most of the previous studies also used microplastic fragments prepared by grinding commercially sold particles or beads, but there are other shapes of microplastics such as fibers and films in soil environment and the effect of different shapes of microplastics needs to be explored.

Although recent studies started to investigate the effect of microplastics on the fate of co-existing chemical contaminants, available information is limited. In particular, microplastics of different types (e.g., polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS)) can have different sizes and shapes (e.g., spherical particles, irregularly-shaped particles, fibers, films), and the interaction between microplastics and chemical contaminants can be different depending on the characteristics of microplastics [15]. Low-density polyethylene (LDPE) and PVC

plastic products such as mulching films and pesticide containers are widely used in the agricultural environment, and microplastics generated from the use of the plastic products can reside in agricultural soil. Therefore, this study was set to investigate the effect of co-presence of microplastic films (i.e., LDPE and PVC microplastic films) and chemical contaminants (i.e., Cd and PCM) in soil on the lettuce growth and Cd and PCM uptake by lettuce.

Materials and methods

Preparation of microplastic films

Polyvinyl chloride plastic film (Hwashin Ind. Co., Incheon, South Korea) and LDPE plastic film (Hanjung Chemical, Geumsan, South Korea) were purchased and then they were manually cut into small pieces of <5 mm (length) × <5 mm (width). The films were analyzed by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR; IRTracer-100, Shimadzu, Japan) and the spectrum data (in the range of 4000–400 cm^{-1} at the resolution of 4 cm^{-1}) can be found in the previous study [6].

Soil preparation

Air-dried and sieved (<2 mm) soil samples were spiked to prepare the Cd-contaminated soil samples and PCM-contaminated soil samples. The soil pH, total organic carbon, and cation exchange capacity were 4.7, 5.9%, and 13.8 cmol kg^{-1} , respectively. The background Cd concentration of the soil sample was 11 mg kg^{-1} , while PCM was not detected (i.e., the limit of detection = 1.25 ng). Procymidone (analytical standard ($\geq 98.0\%$), Sigma-Aldrich, USA) and $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ (ACS reagent ($\geq 99\%$), Sigma-Aldrich, USA) were used to contaminate the soil samples. The soil samples were soaked in Cd- or PCM-containing water and the water was dried to contaminate the soil samples with Cd or PCM. The initial concentrations of Cd and PCM of the Cd- and PCM-contaminated soil samples were 103 and 2.7 mg kg^{-1} , respectively. In order to study the effect of the presence of microplastics on the absorption of Cd or PCM by the lettuce grown in the contaminated soil samples, both the contaminated soil samples were mixed with either PVC (0.54% w/w) or LDPE (0.57% w/w).

Pot tests

Pot tests were carried out using the Cd- or PCM-contaminated soil samples with and without microplastic films. The soil samples without microplastic films were used as the controls. Each pot was filled with 200 g of the soil samples and one lettuce (*Lactuca sativa*) seedling grown for one month was placed in the pot. The lettuce seedlings of similar sizes were used in the pot tests. Triplicate

samples were prepared for each condition. The lettuce seedlings were exposed to either Cd or PCM in the presence or absence of microplastics for 25 d. During the exposure period, the temperature and the relative humidity were maintained at $24.6 \pm 0.5^\circ\text{C}$ and $66.6 \pm 7.6\%$, respectively, and the pots were exposed to the light cycle of 16 h light and 8 h dark. The growth of lettuce (e.g., number of leaves, shoot height) was monitored during the test period, and the lettuces were harvested at the end of the test to extract the absorbed Cd or PCM.

Analytical methods

The Cd concentrations in the soil and lettuce samples were extracted by following the EPA 3052 method [20] and measured using inductively coupled plasma-mass spectroscopy (ICP-MS) (Agilent 7800 ICP-MS, Agilent Scientific Instruments, USA). In order to extract PCM from the soil and lettuce samples, the samples were mixed with acetone, and then the liquid and solid parts were separated by filtration. The filtered extracts were placed in a separatory funnel containing deionized water, saturated sodium chloride solution, and dichloromethane to carry out liquid-liquid partition, and the PCM containing part was concentrated. The concentrated extracts were purified using the solid-phase extraction (SPE) for analysis using high-performance liquid chromatography with diode-array detection (HPLC-DAD) (Agilent

1100 series, Agilent Scientific Instruments, USA). The HPLC was equipped with the Kinetex® EVO C18 column ($250 \times 4.6 \text{ mm} \times 5 \mu\text{m}$). The PCM concentration was determined at 220 nm using acetonitrile and water as the mobile phase solutions. The flow rate of the eluent was 1 mL min^{-1} and the injection volume was $5 \mu\text{L}$.

Statistical analysis

The difference between the samples were determined by using the one-way analysis of variance (one-way ANOVA) followed by the Tukey test as a post-hoc test. Linear regression was carried out to estimate the change rates of lettuce leaf lengths and number of leaves with time. The statistical analyses were done using the GraphPad Prism software (v8.0) (GraphPad Software, USA).

Results and discussion

Effect of Cd, PCM, or microplastics in soil on lettuce growth

Figure 1 shows the effect of Cd or PCM contamination on the lettuce growth. The length of the lettuce leaves and lettuce leaf number were increased significantly with time after an initial stagnation period (p -value < 0.05) regardless of the presence of Cd or PCM (Fig. 1a, b and Table 1). The lettuce length and leaf number change rates estimated by fitting a linear line to the increasing parts were higher for the control and Cd-contaminated soil samples than the PCM-contaminated

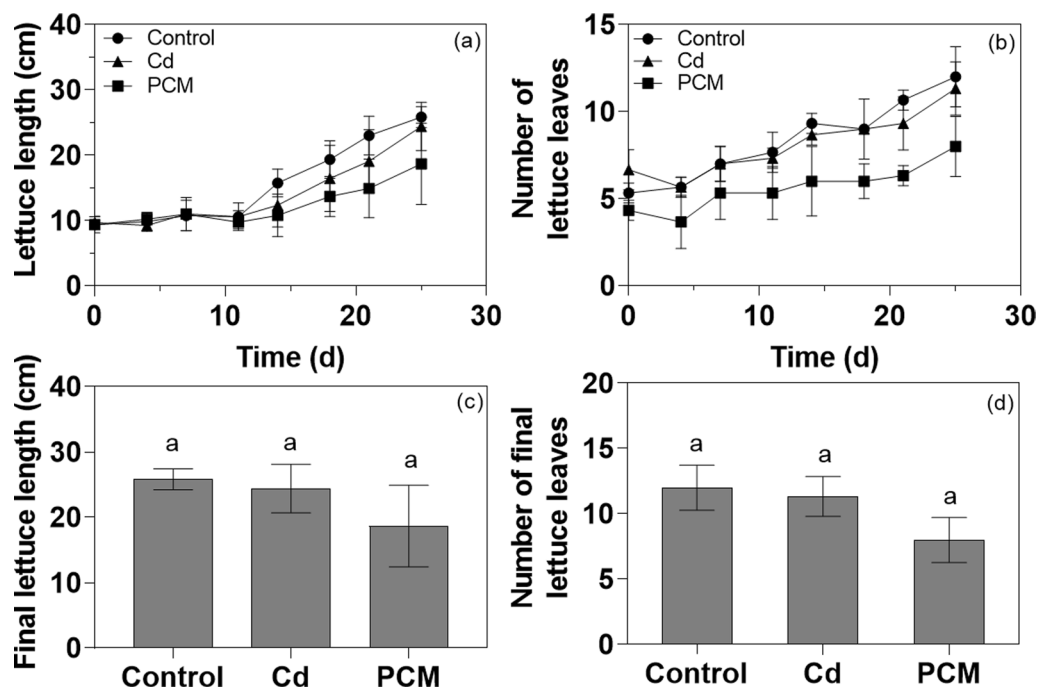


Fig. 1 Effect of cadmium (Cd) or procymidone (PCM) contamination on the **a** length of lettuce leaves per lettuce seedling during the 25 d-growth period, **b** number of lettuce leaves per lettuce seedling during the 25 d-growth period, **c** final length of lettuce seedling after 25 d, and **d** final number of lettuce leaves after 25 d. Control: soil without Cd or PCM contamination, *Cd* Cd-contaminated soil, *PCM* PCM-contaminated soil

Table 1 Changes in the lettuce length and leaf number with time in the soils contaminated with cadmium (Cd), procymidone (PCM), polyvinyl chloride (PVC) microplastics, or low-density polyethylene (LDPE) microplastics after 25 d-growth period

Soil samples	Lettuce length		Lettuce leaf number	
	Change rate* (cm d ⁻¹)	p-value	Change rate* (d ⁻¹)	p-value
Control soil	1.074	<0.0001	0.2828	0.0007
Cd-contaminated soil	0.986	0.0001	0.2481	0.0004
PCM-contaminated soil	0.633	0.0046	0.1629	0.0371
PVC-contaminated soil	0.960	0.0091	0.2562	0.0592
LDPE-contaminated soil	1.126	<0.0001	0.2459	0.0020

*The change rates were estimated by linear regression of the lettuce length or the number of lettuce leaves after an initial stagnation period

soil samples (Table 1). However, the lettuce lengths of the controls were not statistically different from that of the Cd-contaminated soil (p -value=0.7585) or PCM-contaminated soil (p -value=0.2647) during the 25 d-growth period (Fig. 1a and Table 1). Also, the final lengths of the lettuce leaves were statistically similar regardless of the presence of contaminants (i.e., Cd, PCM) (p -value>0.05), although the average value was lower in the PCM-contaminated soil (Fig. 1c). Similarly, the lettuce leaf number was statistically similar in the Cd-contaminated soil and the control soil (p -value=0.7791), but it was significantly lower in the PCM-contaminated soil than in the control soil during the 25 d-growth period (p -value=0.0060) (Fig. 1b and Table 1). However, the final number of lettuce leaves were statistically similar regardless of the presence of PCM, although the average value was lower in the PCM-contaminated soil (Fig. 1d).

Overall, the results show that the presence of contaminants did not affect the lettuce leaf lengths or number after the 25 d-growth period (Fig. 1), but the change rates in the lettuce length and leaf number were adversely affected by the PCM contamination (Table 1). Similarly, previous studies reported varying effects of Cd contamination on the plant growth [12, 21–23]. One study showed that the heights of sassafras seedlings at different concentrations of Cd (i.e., 0, 5, 20, 50, 100 mg kg⁻¹) were statistically similar [23]. In another study, the stem length and number of leaf of sorghum cultivars were decreased with increasing Cd concentration [22]. The studies on the effect of PCM in soil on plant growth are limited, while the toxic effects on PCM in water have been studied using aquatic plants (i.e., *Lemna minor* and algae) [24].

Figure 2 shows the effect of soil contamination with microplastics (i.e., microplastics in the control soils without Cd or PCM contamination) on the lettuce growth. The lettuce length was increased significantly with time after an initial stagnation period (Fig. 2a and Table 1). The change rates of the lettuce length were similar in the soils with and without microplastics (Table 1). This suggests that the lettuce lengths were not significantly affected by the contamination of PVC (p -value=0.8128) or LDPE (p -value=0.9723) during the 25 d-growth period (Fig. 2a and Table 1). Also, the final lengths of the lettuce leaves were statistically similar regardless of the presence of microplastics (i.e., PVC, LDPE) (p -value>0.05) (Fig. 2c). The lettuce leaf number was changed significantly with time for the control and LDPE-contaminated soils, but the changes were insignificant for the PVC-contaminated soil (p -value=0.0592) (Fig. 2b and Table 1). This suggests adverse effects of PVC on the lettuce growth; however, the lettuce leaf numbers of the controls were statistically similar to that of the PVC-contaminated soil (p -value=0.0799) or LDPE-contaminated soil (p -value=0.4377) (Fig. 2b and Table 1). The final lettuce leaf numbers were also statistically similar regardless of the presence of microplastics (i.e., PVC, LDPE) (p -value>0.05) (Fig. 2d). Overall, the soil contamination with microplastics at the concentrations used in this study did not have significant impacts on the lettuce growth. However, previous studies reported varying degrees of effects of microplastics in soil on plant growth [3]. For example, the increase in the microplastic concentrations from 0 to 3% resulted in the decreasing number of lettuce leaves and lettuce lengths [15]. In this previous study, LDPE fragments, PVC fragments, and PVC fibers at 0–3% were used to study the effect of microplastics; however, LDPE (0.57%) and PVC (0.54%) films at lower concentrations were used in this study. Such differences can explain the different effects observed in different studies since different microplastic shapes and concentrations can have different effects on the lettuce growth. Based on the observed results, it can be expected that the lettuce growth is likely to be adversely affected if higher microplastic concentrations were used.

Combined effect of Cd or PCM and microplastics in soil on lettuce growth

Figure 3 shows the combined effect of microplastics and Cd or PCM on the lettuce growth. In the Cd-contaminated soils, the lettuce length was significantly increased with time after the initial stagnation period, except for the Cd-contaminated soil with LDPE (Fig. 3a and Table 2). But the lettuce lengths were statistically similar regardless of the presence of microplastic films during the 25 d-growth period (p -value>0.05), and the final lettuce

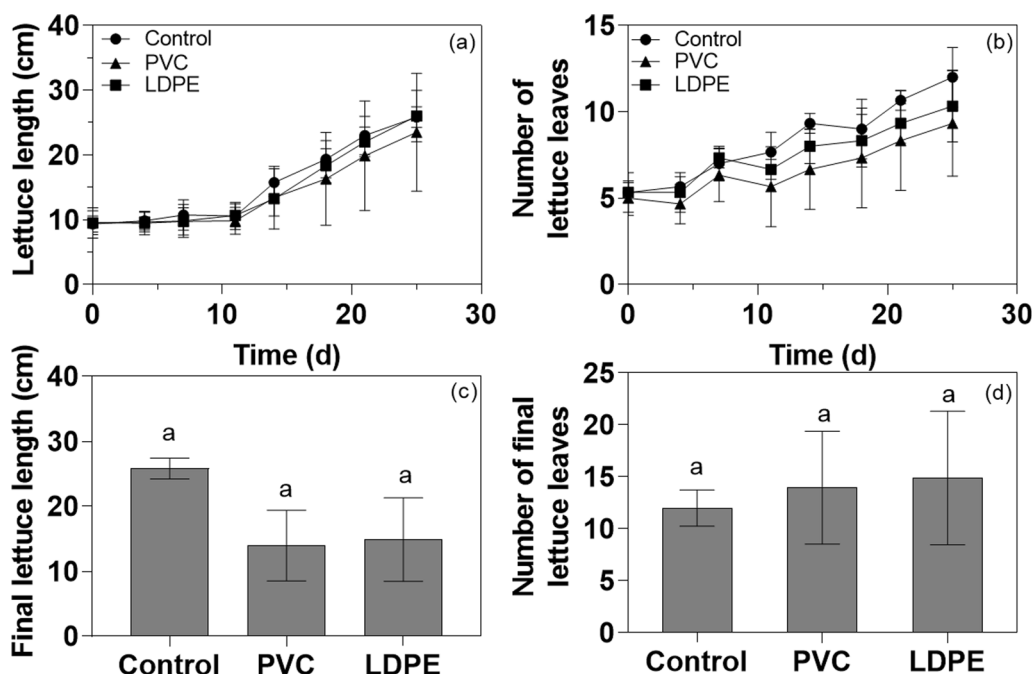


Fig. 2 Effect of microplastics in the control soils (i.e., uncontaminated soil) on the **a** length of lettuce leaves per lettuce seedling during the 25 d-growth period, **b** number of lettuce leaves per lettuce seedling during the 25 d-growth period, **c** final length of lettuce seedling after 25 d, and **d** final number of lettuce leaves after 25 d. Control: soil without microplastic contamination, *PVC* control soil with polyvinyl chloride (PVC) contamination, *LDPE* control soil with low-density polyethylene (LDPE) contamination

lengths were also statistically similar in the Cd-contaminated soils regardless of the presence of microplastics (p -value > 0.05) (Fig. 3a). The changes in the number of lettuce leaves with time in the Cd-contaminated soils with microplastics were negligible (p -value > 0.05), while the number of leaves was significantly increased with time in the Cd-contaminated soil without microplastics (p -value = 0.0004) (Fig. 3b and Table 2). During the 25 d-growth period, the lettuce leaf numbers were statistically similar in the presence and absence of PVC films (p -value = 0.2677), but it was significantly lower in the presence of LDPE films (p -value = 0.0011) (Fig. 3b). However, the final lettuce leaf numbers were statistically similar (p -value > 0.05) (Fig. 3b). The negligible increases in the lettuce length and number of leaves with time in the co-presence of Cd and LDPE suggest that the adverse effects of Cd were promoted in the presence of LDPE. This is partly because LDPE films can adsorb more Cd than PVC films [6]. The desorbed Cd from the Cd-contaminated soil can sorb on LDPE or PVC films increasing the contact time between Cd in soil environment and lettuce. Since LDPE films can sorb more Cd, a greater amount of Cd is likely to last in soil environment and exhibit greater adverse effects on the lettuce growth. It may be expected that the longer growth period may lead to the significant difference in the lettuce growth in the

presence of LDPE. With the PCM-contaminated soils, the leaf length was increased with time after the initial stagnation time regardless of the presence of microplastics, except for the PCM-contaminated soil with PVC (Fig. 3c and Table 2). But the lettuce lengths did not show statistically significant differences regardless of the presence of microplastic films during the 25 d-growth period (p -value > 0.05) (Fig. 3c). The leaf number did not change with time in the presence of PVC or LDPE in the PCM-contaminated soil, while it was increased with time in the PCM-contaminated soil without microplastic films (Fig. 3d and Table 2). The lettuce leaf numbers in the presence of only PCM were statistically different from that in the co-presence of PCM and PVC films, but were similar to that in the co-presence of LDPE films during the 25 d-growth period (p -value > 0.05) (Fig. 3d). But the final numbers of the lettuce leaves were statistically similar regardless of the presence of PVC or LDPE (Fig. 3d). Unlike the results observed with the Cd-contaminated soils, the co-presence of PCM and PVC tends to promote the adverse effects of PCM on the lettuce growth, while the co-presence of PCM and LDPE did not. Previous study showed that PVC films have a greater PCM sorption potential than LDPE films [25]. Thus, it is more likely that more PCM lasts for longer in soil environment in the co-presence of PVC films since the desorbed PCM

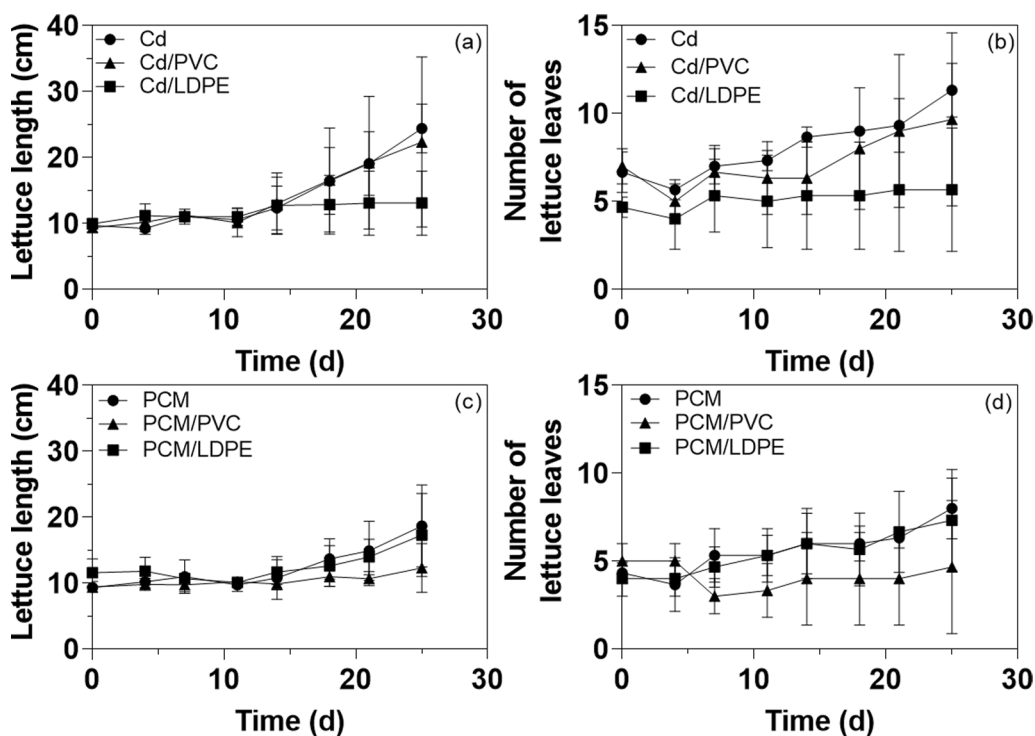


Fig. 3 Effect of microplastics in the cadmium (Cd)-contaminated soils on the **a** length of lettuce leaves and **b** number of lettuce leaves and effect of microplastics in the procymidone (PCM)-contaminated soils on the **c** length of lettuce leaves and **d** number of lettuce leaves per lettuce seedling during the 25 d-growth period. *Cd* Cd-contaminated soil, *Cd/PVC* Cd-contaminated soil with polyvinyl chloride (PVC) microplastic films, *Cd/LDPE* Cd-contaminated soil with low-density polyethylene (LDPE) microplastic films, *PCM* PCM-contaminated soil, *PCM/PVC* PCM-contaminated soil with PVC microplastic films, *PCM/LDPE* PCM-contaminated soil with LDPE microplastic films

Table 2 Changes in the lettuce length and leaf number with time in soils contaminated with cadmium (Cd) and microplastics or procymidone (PCM) and microplastics after 25 d-growth period

Soil samples	Lettuce length		Lettuce leaf number	
	Change rate** (cm d ⁻¹)	p-value	Change rate** (d ⁻¹)	p-value
Cd-contaminated soil	0.9864	0.0001	0.2481	0.0004
Cd-contaminated soil with PVC	0.8781	0.0408	0.2676	0.1274
Cd-contaminated soil with LDPE	0.1244	0.5326	0.0467	0.7527
PCM-contaminated soil	0.6328	0.0046	0.1629	0.0371
PCM-contaminated soil with PVC	0.1469	0.1299	0.0760	0.5563
PCM-contaminated soil with LDPE	0.4785	0.0113	0.1319	0.2012

PVC polyvinyl chloride, *LDPE* low-density polyethylene

**The change rates were estimated by linear regression of the lettuce length or the number of lettuce leaves after an initial stagnation period

from the PCM-contaminated soil can sorb on PVC films. With longer growth periods, the difference between the effects of the PCM with PVC and the effects of the PCM with LDPE may be greater. Different types of microplastics can have different effects on the lettuce growth [15]. For example, the number of lettuce leaves was significantly lower in the presence of LDPE or PVC fragments than in the controls (i.e., 0% microplastics), but

the shoot length was significantly lower in the presence of LDPE fragment, but not in the presence of PVC fragment [15]. Similarly, the effect of co-presence of chemical contaminant and microplastics can depend on the types of microplastics. For instance, the co-presence of Cd and high-density polyethylene did not affect the shoot biomass of maize plants, while the co-presence of Cd and PS led to the reduction in the shoot biomass [26]. Also, the

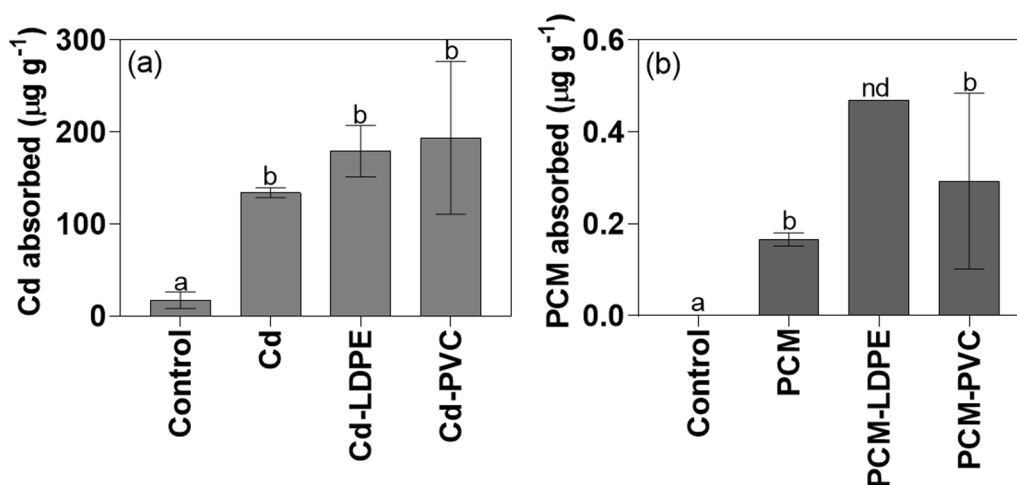


Fig. 4 Absorption of **a** cadmium (Cd) and **b** procymidone (PCM) by lettuce. Control: control soil without any contamination and microplastics, *Cd* Cd-contaminated soil, *Cd-LDPE* Cd-contaminated soil with low-density polyethylene (LDPE), *Cd-PVC* Cd-contaminated soil with polyvinyl chloride (PVC), *PCM* PCM-contaminated soil, *PCM-LDPE* PCM-contaminated soil with LDPE, *PCM-PVC* PCM-contaminated soil with PVC. Single sample was analyzed for the PCM-LDPE sample. nd: The statistical differences cannot be determined due to the presence of single sample in the group

co-presence of Cd and polylactic acid (PLA) resulted in the higher bioavailability of Cd than the co-presence of Cd and PE [27].

Effect of microplastics on the absorption of Cd and PCM by lettuce

Figure 4 shows the absorbed amount of Cd and PCM by the lettuce grown in different soil samples. The Cd absorbed by the lettuce in the control soils was $17 \mu\text{g g}^{-1}$, on average, and it was increased significantly (i.e., about eight times) in the Cd-contaminated soil (Fig. 4a). Similarly, the PCM absorbed by the lettuce in the PCM-contaminated soil was significantly higher than that in the control soil (i.e., negligible PCM absorption) (Fig. 4b). The co-presence of Cd or PCM and microplastics increased the average absorbed amount of Cd or PCM; however, they were not statistically different (Fig. 4). The tendency of increased Cd or PCM absorption by lettuce in the co-presence of LDPE or PVC can be supported by previous studies that reported increased chemical contaminant availability in the presence of microplastics [18, 26, 28]. For example, the bioavailable Cd concentrations in soil and the absorbed Cd amounts by lettuce were significantly higher in the co-presence of PE [18]. Also, the co-presence of Cd and PE promoted the Cd absorption by plants more strongly than the co-presence of Cd and other microplastics (i.e., PS, PVC) [28]. However, in this study, the Cd absorption by lettuce were similar regardless of the types of microplastics (Fig. 4a). On the other hand, some studies reported decreased absorption of chemicals by plants in the co-presence of microplastics.

For example, the absorption of dibutyl phthalate by lettuce was decreased in the co-presence of PS, and this was attributed to the sorption of dibutyl phthalate on microplastics [16, 19]. This indicates that the effect of co-presence of microplastic on the availability of chemical contaminants can vary depending on the characteristics of chemical contaminants and microplastics as well as their interaction (e.g., sorption). The increasing Cd or PCM absorption tendency in the co-presence of microplastics (Fig. 4) can also support the negligible increases in the lettuce length and number of lettuce leaves in the co-presence of microplastics (Fig. 3).

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Author contributions

EHJ: Conceptualization, Data curation; Methodology; Formal analysis; Investigation; Writing—Original Draft; Writing—Review & Editing; Visualization; Supervision; Project administration; Funding acquisition; JWY: Visualization, Writing—Original Draft; WJJ: Methodology, Formal analysis, Visualization, Writing—Original Draft; MMH: Visualization, Writing—Original Draft.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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