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Effect of polyethylene microplastics on physiological responses and vanadium accumulation in pakchoi (*Brassica rapa* subsp. *chinensis*) under vanadium stress

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Abstract

Vanadium (V) contamination poses potential risks to plant growth, while microplastics (MPs) may alter the behavior and bioavailability of heavy metals in soil. This study investigated the effects of polyethylene microplastics (PE) on the physiological responses and V accumulation of pakchoi (*Brassica rapa* subsp. *chinensis*) under V stress.

A pot experiment was conducted with four PE concentrations (0, 0.1, 1, and 5%) under 150 mg·kg⁻¹ V. Growth traits, photosynthetic characteristics, antioxidant enzyme activities, malondialdehyde (MDA), soluble protein, V accumulation, and soil physicochemical properties were determined.

V stress alone significantly inhibited pakchoi growth, reducing plant height, root length, and biomass by 60.26%, 70.11%, and 72.09%, respectively. It also decreased chlorophyll content and photosynthetic parameters, while increasing MDA content by 45.03%, indicating enhanced oxidative damage. Addition of 0.1% PE partially alleviated V toxicity by improving photosynthetic performance, reducing MDA content, and increasing superoxide dismutase (SOD) and peroxidase (POD) activities. However, the alleviating effect weakened at higher PE concentrations. PE addition also significantly decreased available V in soil and reduced V accumulation in pakchoi roots. These results indicate that low PE addition can mitigate V toxicity in pakchoi, likely by reducing V bioavailability in soil, whereas higher PE concentrations provide limited benefit.

Keywords Polyethylene microplastics Vanadium toxicity Pakchoi Soil-plant system Vanadium accumulation Antioxidant enzymes

1 Introduction

Vanadium (V) is a transition metal element widely present in the earth's crust and is released to the environment in large quantities during industrial production (such as steel smelting, petroleum catalysis), fossil fuel combustion, and mining (Chen et al. 2021; Yin et al. 2022). The dominant redox states of vanadium in environmental systems are V(IV) (e.g., VO²⁺) and V(V) (e.g., H₂VO₄⁻), with the latter being more soluble and bioavailable under aerobic conditions (Tambat et al. 2024). Vanadium (V) is a potentially toxic metal that can adversely affect plant growth and development.

(Hou et al. 2019). Vanadium can interfere with phosphate uptake, energy metabolism, and nucleic acid synthesis, thereby inhibiting root development and reducing plant biomass. (Wu et al. 2023). At the biochemical level, vanadium-induced reactive oxygen species (ROS) bursts, triggering membrane lipid peroxidation and, at the same time, destroying the photosynthetic mechanism, reducing the chlorophyll a/b content by 50-70% and reducing the net photosynthetic rate by 60-90%. At the molecular level, vanadium interferes with calcium signaling and hormone balance, inhibiting more than 80% of key enzyme activities (Hou et al. 2020). The study also found that vanadium can change the DNA methylation pattern and enrich the shoots transportation through the xylem, posing a threat to agricultural product safety.

Microplastics (particles < 5 mm) are an emerging environmental pollutant (Machado et al. 2018; Thompson et al. 2004). Their excellent properties, such as light weight, durability, and low cost, have been widely used in various fields, from daily necessities to industrial production materials, almost everywhere (Chae and An 2018; Horton et al. 2017). However, with the sharp increase in the use of plastic products, waste production has also increased daily, which has become a severe environmental challenge that has attracted much attention worldwide (Wang et al. 2022). Microplastics have extreme durability in the environment and are difficult to degrade naturally. They can enter the soil ecosystem continuously through various channels such as atmospheric sedimentation, surface runoff, and irrigation water (B. Zhao et al. 2024). In soil systems, microplastics can change soil structure and affect water migration (and microbiomes) (H. Yu et al. 2021); for plants, microplastics can affect growth and development through mechanical blockage and inhibit nutrient absorption. Microplastics may also pose risks to animals and human health (Lehmann et al. 2022; M. Zhao et al. 2023). Microplastics can be transmitted through the food chain and enriched in organisms, triggering inflammatory responses and metabolic disorders (K. Zhang et al. 2023). It is particularly noteworthy that the composite effect of microplastics and other pollutants often produces co-toxicity, such as polyethylene microplastics, which can increase the accumulation of cadmium in pakchoi by 40-60% (Li et al. 2024; Xiang et al. 2022). The coexistence of MPs and heavy metals in soil may pose additional risks to soil-plant systems (Liu et al. 2024; L. Yu et al. 2021). Based on the interaction mechanisms between MPs and heavy metals in the soil-plant system, the progression of their combined pollution can be categorized into three distinct stages: first, the adsorption of heavy metals by MPs from the soil matrix; second, the co-migration of MPs and adsorbed heavy metals within the soil environment (Zhang et al. 2019); and third, the translocation of both MPs and heavy metals toward plants.

Microplastics frequently act as carriers for heavy metals, thereby modulating the migration behavior of heavy metals in soil (Cui et al. 2022). The adsorption of heavy metals by MPs is primarily driven by electrostatic interactions between functional groups on the MP surface and heavy metal ions. Additionally, these surface functional groups can function as proton donors or acceptors, forming chemical bonds with heavy metals and consequently regulating the adsorption process. Yang et al. (2019) demonstrated that polar functional groups (-NHCO- and -COO-) on the surface of

polyamide (PA) microplastics exhibit a high propensity to form chemical bonds with Cu^{2+} , endowing PA with a significantly higher adsorption capacity compared to other types of MPs. The interaction mechanisms and migration patterns of MPs and V in soil are illustrated in Fig. 1.

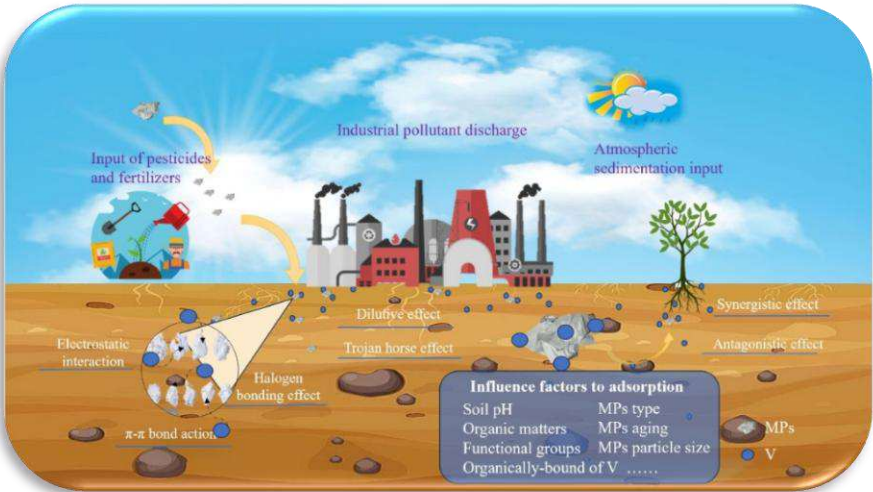


Fig. 1 The interaction and migration patterns of MPs and V in the soil

Pakchoi (*Brassica rapa* subsp. *chinensis*) is an important leafy vegetable crop with a short growth cycle and high nutritional value. (Men et al. 2024). Because of its relatively shallow root system and dense distribution, it is susceptible to various changes in the soil environment (Xie et al. 2023). Its edible parts (stems and leaves) are directly involved in the human food chain, and the accumulation of vanadium will pose potential risks to human health. Long-term intake of vanadium-enriched vegetables may interfere with human glucose and lipid metabolism, and damage the nervous and cardiovascular systems. When pakchoi grows in a soil environment polluted by V, its physiological and metabolic processes may be severely disturbed, its growth rate will slow down, and its quality will decrease. Microplastics in the soil may further indirectly affect the plant's absorption, transport, and accumulation process of heavy metals by changing the soil's physical structure, chemical properties, and microbial communities (Men et al. 2024). At the same time, it will also have more complex interactions with the physiological characteristics of the plant itself.

Although some progress has been made in the current research on microplastic and heavy metal pollution. However, limited information is available on how polyethylene microplastics affect the physiological responses and vanadium accumulation of pakchoi under V stress.. In-depth research on this area has crucial theoretical and practical significance for accurately assessing the ecological risks of microplastics and heavy metal composite pollution on vegetable growth, formulating scientific and reasonable soil pollution prevention and control strategies, ensuring the quality and safety of agricultural products, and maintaining the stability and balance of the ecosystem. Therefore, this study used a pot experiment to investigate the effects of polyethylene microplastics on pakchoi growth, photosynthesis, antioxidant responses, and V

accumulation under V stress. In addition, changes in soil physicochemical properties and available V were analyzed to better understand the interaction between PE and V in the soil–plant system.

2 Experimental materials and methods

2.1 Materials

The tested soil was collected from the topsoil (0~20 cm) of the Science and Education Park of Lanzhou City College, Anning District, Lanzhou City, Gansu Province, China (103°43'52"E, 36°6'48"N), and there is no history of agricultural film covering in the past 5 years and no visible or known plastic inputs. The soil type for testing is typical lime-calcic soil. The basic physical and chemical properties of the soil are as follows: pH is 7.42, organic matter content of 19.32 g/kg, total nitrogen (N) content of 1.25 g/kg, total phosphorus (P) content of 0.46 g/kg, total potassium (K) content of 1.25 g/kg, available V concentration of 5.32 mg/kg. The plant for testing is *Brassica rapa* subsp. *chinensis*, and the variety is "Suzhou Qing"; the microplastics in this test are polyethylene (PE), with a particle size range of 100 - 200 μm and purity greater than 99%, supplied by Huayu Co., Ltd. (Guangzhou, China); the vanadium (V) reagent for testing is ammonium metavanadate (NH_4VO_3), analytical pure reagent, purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2 Experimental design

The pots used in the experiment were clay pots with a diameter of 20 cm and a height of 15 cm. Each pot was filled with 2 kg of pretreated dry soil. The polyethylene microplastics were mixed with the soil using mass fractions of 0.1%, 1% and 5%, respectively. At the same time, V was added externally in the form of ammonium metavanadate solution to ensure that the ω (V) in the soil was $150 \text{ mg}\cdot\text{kg}^{-1}$, which is slightly higher than the vanadium screening value for agricultural land soil in China (The risk screening value for vanadium in agricultural land is $130 \text{ mg}\cdot\text{kg}^{-1}$ (GB 15618-2018)). There were five treatments in this experiment, which were CK(blank control, no PE and V addition), V stress alone [ω (V) was $150 \text{ mg}\cdot\text{kg}^{-1}$], V+0.1% PE [ω (PE) was $1.0 \text{ g}\cdot\text{kg}^{-1}$ + ω (V) was $150 \text{ mg}\cdot\text{kg}^{-1}$], V+1% PE [ω (PE) was $10 \text{ g}\cdot\text{kg}^{-1}$ + ω (V) was $150 \text{ mg}\cdot\text{kg}^{-1}$], and V+5% PE [ω (PE) was $50 \text{ g}\cdot\text{kg}^{-1}$ + ω (V) was $150 \text{ mg}\cdot\text{kg}^{-1}$], and each treatment was repeated 3 times, totaling 15 pots. Mix the soil well with the corresponding amount of V and PE evenly, then put it in a pot bowl. The samples were subjected to an 8-hour UVA-340 irradiation cycle with an irradiance of $0.76 \text{ W}/(\text{m}^2\cdot\text{nm})$ at a black panel temperature of 60°C , followed by a 4-hour dark condensation cycle at a black panel temperature of 50°C and 100% relative humidity. This 12-hour cycle was continuously repeated for a total aging period of 30 days. Before sowing, the pakchoi seeds were disinfected with 0.5% sodium hypochlorite solution for 10 min, then rinsed with distilled water and germinated in a 25°C constant temperature incubator for 24 h. 10 germinated seeds were sown in each pot. The seedlings were interspersed after emergence, and three seedlings with consistent growth were retained. During the experiment, the same growth conditions were maintained, the water was replenished by the constant weight method, and the harvest was obtained after 45 days.

2.3 Sample analysis and determination

Biomass determination: After 45 days of growing pakchoi, the plants were carefully removed from the soil, rinsed with distilled water, divided into the upper and lower parts, dried at 105°C for 30 min and then at 70°C to constant weight, and then dried at 70°C to a constant weight and weighed.

Photosynthetic performance determination: When pakchoi grows for 35 days, functional leaves that are fully expanded on the top of the plant were selected, and net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO₂ concentration (C_i) and transpiration rate (Tr) were determined using a portable photosynthesis instrument (LI-6400, LI-COR, USA). The soil and plant analyzer development value (SPAD) was measured using a chlorophyll meter (SPAD-502Plus, Japan). The chlorophyll a and chlorophyll b content was determined by the extraction method of ethanol-acetone mixed solution.

Determination of content of malondialdehyde (MDA) and soluble protein: Take 0.5 g of pakchoi leaves, add 5 mL of pre-cooled 5% trichloroacetic acid (TCA) solution, grind it into a homogenate, centrifuge it at 10,000 r/min for 10 min at 4°C, and then mix and react with 2 mL of 0.6% thiobarbituric acid (TBA). The MDA content was determined. The soluble protein content was determined by the Coomassie Bright Blue G-250 staining method, and a standard curve was prepared using bovine serum albumin as the standard protein.

Antioxidant enzyme system determination: Take 1 g of pakchoi leaves, add 5 mL of pre-cooled 50 mmol/L phosphate buffer (pH 7.8), contains 1% polyvinylpyrrolidone (PVP) to prevent the enzymatic degradation. Grind it into a homogenate in an ice bath, centrifuge it at 12,000 r/min for 20 min at 4°C, and take the supernatant to determine the antioxidant enzyme activity. The activity of superoxide dismutase (SOD) was determined by the actinic reduction method of nitrogen blue tetrazole (NBT) to inhibit the actinic reduction of NBT by 50% to 1 enzyme activity unit (U); the activity of peroxidase (POD) was determined by the guaiacol method, with a change of absorbance of 0.01 per minute as one enzyme activity unit (U); the activity of catalase (CAT) was determined by the ultraviolet absorption method, with a decomposition of 1 μ mol of H₂O₂ per minute as one enzyme activity unit (U).

Vanadium accumulation in pakchoi: The samples of the dried pakchoi above and underground areas were crushed and sieved at 0.15 mm. Weigh 0.5 g of the sample into a polytetrafluoroethylene digestion tank, add 5 mL of nitric acid and 2 mL of hydrofluoric acid, and digest it in a microwave digester. After the digestion solution was fixed, the V content was determined using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700x, United States).

Basic physical and chemical properties of soil: After the experiment, soil samples in the pot were collected, and soil pH, organic matter content, and available V content were determined. The soil pH was determined by glass electrode method (soil-water ratio 1:2.5); the organic matter content was determined by potassium dichromate

oxidation method; The bioavailable vanadium in soils was extracted using diethylenetriaminepentaacetic acid (DTPA) extraction solution following the standardized procedure., and the V content in the extract was measured by inductively coupled plasma mass spectrometer (ICP-MS).

2.4 Data analysis

One-way analysis of variance (ANOVA) was used to evaluate the effects of soil microplastics, cadmium contamination, and their interactions on all plant and soil properties variables. The correlation between plant and soil properties was tested using Spearman's correlation analysis, and the results were visualized using heat maps. Principal component analysis (PCA) was conducted using OriginPro 2021. All statistical analyses were performed using R software version 4.1.1.

$$V \text{ Transfer factor (TF)} = \text{Stem and leaf V content} / \text{Root V content} \quad (1)$$

$$\text{Root crown ratio} = \text{Dry weight}_{\text{root}} / \text{Dry weight}_{\text{shoot}} \quad (2)$$

$$\text{Root tolerance index (RTI)} = \text{Root dry weight}_{\text{treatment}} / \text{Root dry weight}_{\text{CK}} \times 100\% \quad (3)$$

$$\text{Stem and leaf tolerance index (STI)} = \text{Stem and leaf tolerance dry weight}_{\text{treatment}} / \text{Stem and leaf tolerance dry weight}_{\text{CK}} \times 100\% \quad (4)$$

3 Results

3.1 Effect of polyethylene on biomass and tolerance of pakchoi under V stress

It can be seen from Table 1 that the root length, plant height, root dry weight, and stem and leaf dry weight of pakchoi under V single stress were 70.11%, 60.26%, 72.09%, and 90.06%, respectively, compared with CK treatment. The single stress of V significantly inhibited the plant height and root length of pakchoi, significantly reducing the dry weight of the shoots and roots, and the toxic stress effect was significant. At the same time, the single V stress significantly increased the root crown ratio of pakchoi, which significantly reduced the shoot and root tolerance index. The root tolerance index (RTI) was lower than the stem and leaf tolerance index (STI), indicating that V had a greater inhibitory effect on the roots of pakchoi than the shoots. The roots were more sensitive to changes in V stress.

After adding polyethylene, the inhibition of V stress on the growth of pakchoi was alleviated to varying degrees. Among them, the plant height and root length of the V + 0.1% PE group recovered significantly, close to the level of the control group. The dry weight of the shoots and roots also increased, but there still was a certain gap between the control group. The growth parameters of pakchoi under V + 1% PE and V + 5% PE also increased to varying degrees, but their relieving effect on the growth of pakchoi under V stress was not noticeable. After adding polyethylene, the root crown ratio is reduced, indicating that polyethylene has a specific regulatory effect on the growth coordination of the shoots and roots of the pakchoi. From the tolerance index, it can be seen that the inhibition rates of V+0.1% PE treatment on the root and shoot of pakchoi were 70.17% and 51.16%, respectively, with the most apparent relief effect, which was

241 better than V+1% PE and V +5% PE treatment, indicating that polyethylene can
 242 improve the tolerance of pakchoi to V stress to a certain extent, but it is still far lower
 243 than that of the control group.

244 **Table 1** Effect of polyethylene on the biomass and tolerance index of pakchoi under
 245 vanadium stress

Treatment	Plant height/cm	Root length/cm	SDW/ g/plant	RDW/ g /plant	Root crown ratio	RTI/%	STI/%
CK	28.31±1.24 a	14.42±2.21 a	1.81±0.31 a	0.43±0.05 a	0.25±0.02 d	100.00±4.41 a	100.00±5.14 a
V	11.25±0.52 c	4.31±0.84 b	0.18±0.02 d	0.12±0.02 c	0.67±0.07 a	27.91±1.54 d	9.94±0.81 c
V+0.1%PE	26.36±3.35 a	15.62±2.04 a	0.54±0.05 b	0.21±0.01 b	0.39±0.05 c	48.84±2.63 b	29.83±1.52 b
V+1%PE	15.54±2.61 b	5.68±1.22 b	0.31±0.02 c	0.16±0.02 c	0.52±0.08 b	37.21±1.88 c	17.13±1.46 b
V+5%PE	14.28±2.05 b	6.31±0.54 b	0.27±0.03 c	0.15±0.02 c	0.56±0.12 b	34.88±1.62 c	14.92±1.92 bc

246 SDW- stem and leaf dry weight; RDW- root dry weight; STI-stem and leaf tolerance
 247 index; RTI-root tolerance index. Different lowercase letters in the same column indicate
 248 significant differences between different treatments ($P<0.05$), the same below.

249 3.2 Effect of polyethylene on photosynthetic properties and chlorophyll content of 250 pakchoi under V stress

251 Table 2 shows that the control group (CK) pakchoi is at a high level regarding
 252 photosynthetic performance and chlorophyll content. However, under vanadium (V)
 253 stress alone, P_n , G_s , C_i , and T_r were reduced by 72.48%, 62.35%, 64.52%, and 64.43%
 254 compared with CK, respectively. The photosynthesis of pakchoi leaves was
 255 significantly affected by V stress. Under vanadium (V) stress alone, the chlorophyll
 256 content also decreased significantly, and the SPAD decreased by 32.55% compared
 257 with CK, indicating that V stress had a serious inhibitory effect on the chlorophyll
 258 synthesis of pakchoi.

259 After adding different concentrations of polyethylene (PE) to soil V stress, the
 260 photosynthetic properties and chlorophyll content index of pakchoi have improved to
 261 varying degrees. Among them, the effect was relatively good when 0.1% PE was added,
 262 the net photosynthetic rate (P_n) increased to $15.83\pm2.35 \mu\text{mol} / \text{m}^2 \cdot \text{s}$, the stomatal
 263 conductance (G_s) returned to close to the level of the control group, and the chlorophyll
 264 content and SPAD value also increased significantly. However, as the polyethylene
 265 concentration increases to 1% and 5%, although the various indicators are still higher
 266 than the V stress group alone, but the increase is limited, and some indicators have even
 267 decreased, indicating that the appropriate amount of polyethylene can alleviate the
 268 inhibition of V stress on the photosynthetic properties and chlorophyll synthesis of
 269 pakchoi, while excessive concentrations will be ineffective.

270 **Table 2** Effect of polyethylene on the photosynthetic performance and chlorophyll
 271 content of pakchoi under vanadium stress

Treatmen	P_n /	G_s /	C_i /	T_r /	$\omega(\text{chlorop}$	$\omega(\text{chloro}$	SPAD
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t	$\mu\text{mol}/\text{m}^2\cdot\text{s}$	$\text{mol}/\text{m}^2\cdot\text{s}$	$\mu\text{mol}/\text{mol}$	$\text{mmol}/\text{m}^2\cdot\text{s}$	hyll a)/ mg/g	phyll b)/ mg/g	
CK	33.61±3.24 a	0.85±0.08 a	427.35±31.25 a	10.15±1.54 a	1.64±0.16 a	3.14±0.12 a	33.15±5.33 a
V	9.25±0.76 d	0.32±0.03 c	151.63±15.53 d	3.61±0.75 d	0.58±0.06 d	1.09±0.09 d	22.36±3.52 b
V+0.1%PE	15.83±2.35 b	0.71±0.06 a	325.51±21.31 b	8.44±0.62 b	1.23±0.09 b	2.61±0.08 b	31.81±3.61 a
V+1%PE	10.32±1.64 c	0.53±0.02 b	218.82±16.39 c	6.52±0.51 c	1.06±0.02 b	1.98±0.15 c	29.24±2.26 b
V+5%PE	6.41±0.83 e	0.48±0.05 b	154.25±13.25 d	4.01±0.36 d	0.81±0.08 c	1.55±0.03 e	24.32±3.96 b

272 P_n - net photosynthetic rate, G_s - stomatal conductance (Count in H_2O); C_i -
273 intercellular CO_2 concentration (Count in CO_2); Tr - transpiration rate (Count in H_2O);
274 SPAD- soil and plant analyzer development value

275 3.3 Effect of polyethylene on malondialdehyde and soluble protein in pakchoi 276 under V stress

277 It can be seen from Fig. 2(a) that the shoot and root MDA content of the pakchoi
278 in the CK was at a relatively low level. Under V stress alone, the MDA content of the
279 shoots increased by 45.03% compared with CK, indicating that the oxidative damage
280 was significantly exacerbated. After adding PE, the MDA content in the roots of the
281 V+0.1% PE treatment group decreased significantly, close to 1.41-fold higher than that
282 in CK, which was significantly improved compared with the vanadium stress group
283 alone. Although the MDA content at the root fluctuated between the treatment groups,
284 the change range was much smaller than that of the shoots, indicating that polyethylene
285 had a more outstanding effect on alleviating the oxidative damage caused by vanadium
286 stress in the shoots.

287 In contrast to MDA, V stress significantly reduced the soluble protein content in
288 the shoots and roots of pakchoi ($P<0.05$), and compared with CK, it decreased by 38.36%
289 and 32.05%, respectively. After the addition of PE, the content of soluble protein in the
290 shoots in pakchoi rebounded in different concentrations of treatment groups, but was
291 still lower than that of the control group; the content of soluble protein at the root did
292 not change significantly after the addition of PE, indicating that PE can partially
293 alleviate the negative impact of V stress on the synthesis of soluble protein in shoots of
294 pakchoi (Fig. 2(b)). Polyethylene microplastics can alleviate the toxic effects of V stress
295 by promoting osmotic regulation substance accumulation of pakchoi, and the effect is
296 better when a small amount of PE is added. This shows that the low soil contents of PE
297 and V have a specific antagonistic impact, but it still cannot completely eliminate the
298 toxic damage caused by V stress on pakchoi.

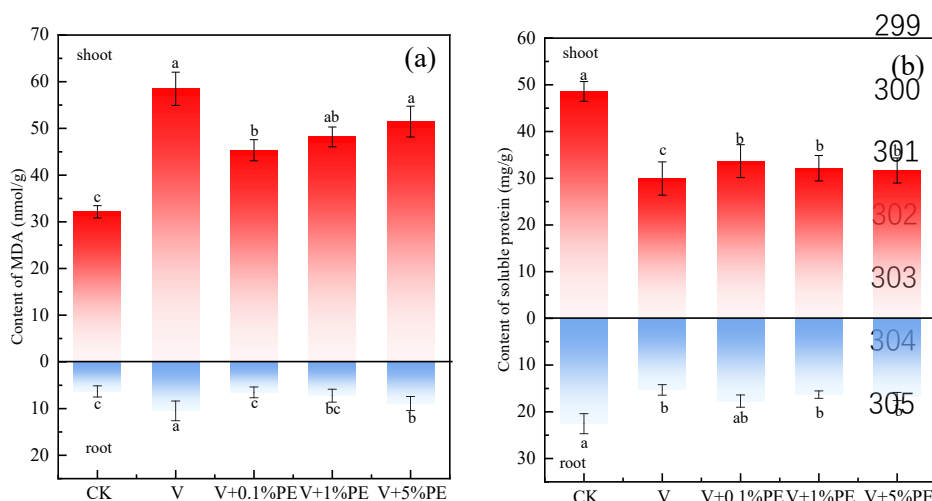
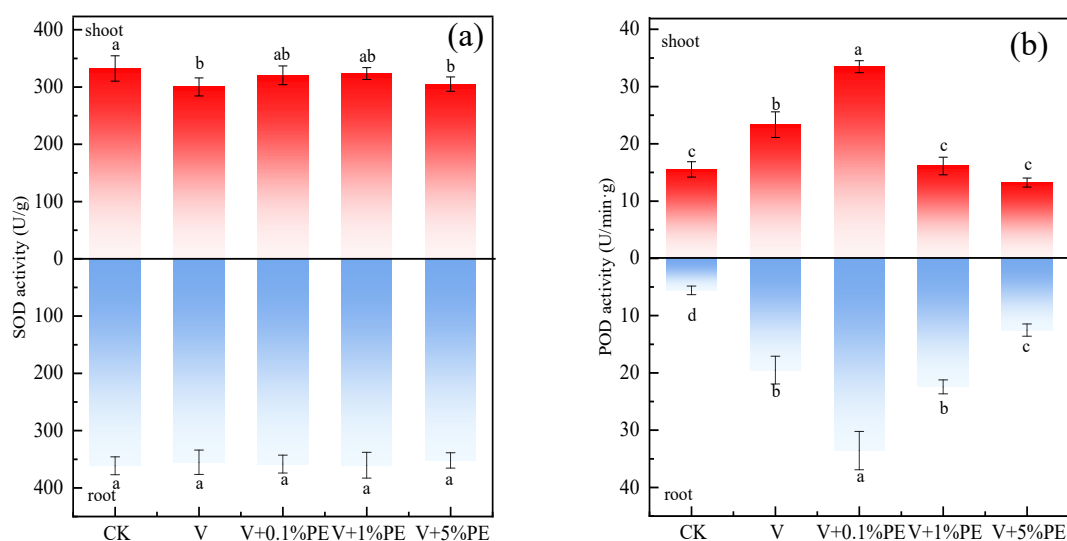


Fig. 2 Effect of polyethylene on the content of MDA **(a)** and soluble protein **(b)** of pakchoi under vanadium stress. MDA- Malondialdehyde. Different lowercase letters represent significant difference among each treatment ($n = 3$, $P < 0.05$), the same below.

3.4 Effect of polyethylene microplastics on the antioxidant enzyme system of pakchoi under V stress

Studies have shown that SOD and POD can coordinate the removal of excess ROS induced by environmental stress induced plant cells (Ghuge et al. 2023). From Fig. 3(a), it can be seen that compared with CK, the SOD in the shoots and roots of pakchoi under V single stress was reduced by 9.71% and 1.69%, respectively. In contrast, the addition of PE under V stress caused the SOD activity in the shoots and roots of pakchoi under V +1% PE treatment to reach the highest value. Under V single stress, the POD activity of the shoots and roots of pakchoi was significantly higher than that of the CK, which was increased by 33.48% and 71.37%, respectively (Fig. 3(b)). Under V stress, with the increase of PE addition, the POD in the shoots and roots of pakchoi tended to increase first and then decrease, and the highest value both appeared in the V+0.1% PE treatment group.

325



326

327 **Fig. 3** Effect of polyethylene on the antioxidant enzyme activities of pakchoi under
 328 vanadium stress. (a) SOD activity; (b) POD activity. SOD- Superoxide Dismutase;
 329 POD- Peroxidase.

330 **3.5 Effect of polyethylene microplastics on V accumulation and transport of** 331 **pakchoi under V stress**

332 In the control group (CK), the vanadium content at the roots of pakchoi was
 333 extremely low, and almost impossible to detect in the shoots. Under V stress alone, the
 334 vanadium content at the root and shootss increased significantly, which was 96.55%
 335 and 91.52% higher than the CK, and the V content in the roots was 6.66 times that in
 336 the shoots, indicating that most of the V absorbed by pakchoi accumulated in the root
 337 system (Fig. 4). After the addition of polyethylene (PE), the vanadium content at the
 338 root decreased significantly, with the V + 0.1% PE treatment group being the most
 339 significant. At the same time, the V translocation factor (TF) of each PE-added
 340 treatment did not change much, which shows that polyethylene can effectively reduce
 341 the vanadium content of pakchoi roots and reduce the accumulation of V in the plant
 342 body, but has little impact on the transport capacity of V from the root to the shoots.

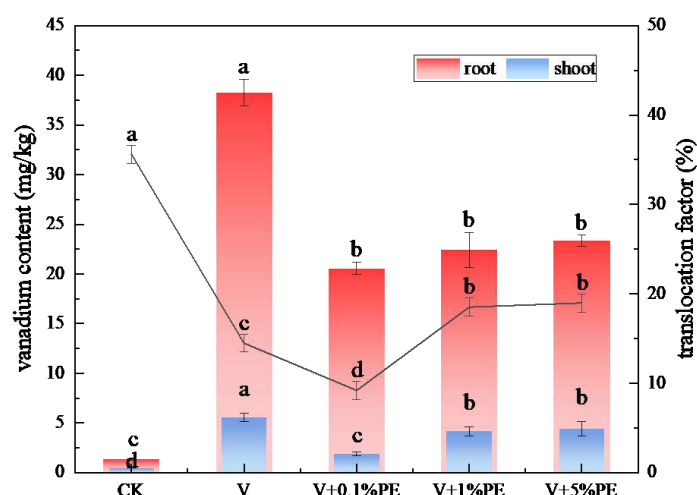


Fig. 4 Effect of polyethylene on the vanadium content and translocation factor of pakchoi under vanadium stress

3.6 Effects of polyethylene on basic physical and chemical properties of soil under V stress and correlation analysis

From Fig. 5, it can be seen that under vanadium (V) stress alone, the available V content in soil increased sharply. After the addition of polyethylene (PE), the available V content in the V + 0.1% PE, V + 1% PE, and V + 5% PE treatment groups was significantly reduced, which was 44.30%, 50.31%, and 43.13% lower than that in the V stress treatment group alone. Exogenous addition of PE under V stress caused the soil organic matter content to decrease first and then increase. It can be seen from this that the soil organic matter content was the lowest under 0.1% PE treatment, which was 1.11 g/kg and 0.83 g/kg lower than CK and vanadium (V) stress treatment alone, respectively, but the difference did not reach a significant level. In comparison, the soil organic matter content was significantly higher than the 0.1% PE treatment under V +1% PE and V +5% PE treatment, which indicated that the exogenous addition of high concentrations of polyethylene could promote the accumulation of soil organic matter. The pH value of the soil was increased to a certain extent compared with CK treatment under single V stress, and the PE was added to show a trend of different degrees of decrease. However, the differences between the treatments did not reach a significant level.

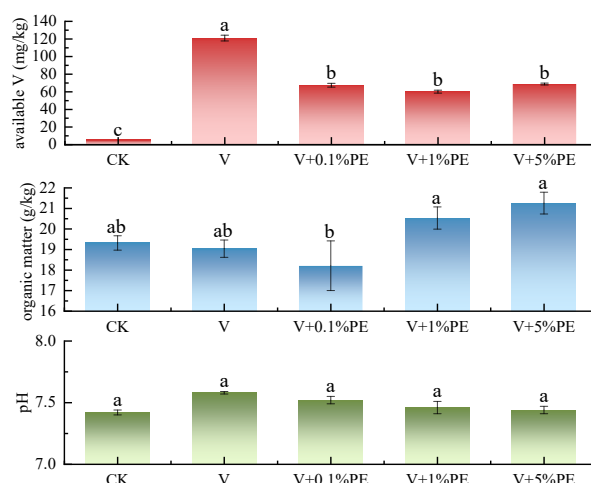
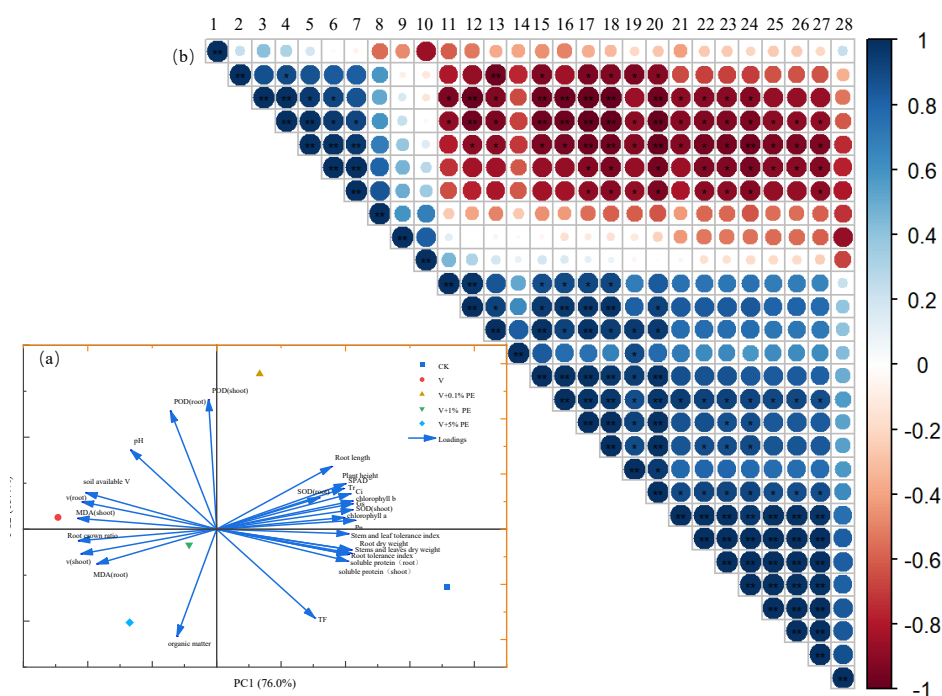


Fig. 5 Effect of polyethylene on the physical and chemical properties of soil under vanadium stress

The response of pakchoi growth, soil properties, available V concentration, V migration and physiological parameters in the shoots and roots of pakchoi induced by polyethylene under vanadium stress was evaluated using principal component analysis (PCA). There was a strong positive correlation between V treatment and antioxidant enzyme systems in the shoots and roots. Conversely, the V+0.1% PE treatments had a significant positive impact on the photosynthetic performance, chlorophyll content and growth parameters in both the shoots and roots of pakchoi (Fig. 6a). The correlation analysis results show that (Fig. 6b), the plant height, G_s , C_i , Tr , ω (chlorophyll a), and ω (chlorophyll b) of pakchoi were significantly negatively correlated with the V content in the above ground ($P<0.01$). At the same time, the Root length, Stem and leaf tolerance index, P_n , and SPAD were significantly negatively correlated with the V content in the above ground ($P<0.05$). The Root crown ratio of pakchoi was very significantly positively correlated with the V content in the above ground ($P<0.01$). The available V content in the soil was significantly negatively correlated with the V content in the underground ($P<0.01$), and the root dry weight, root tolerance index, G_s , ω (chlorophyll a), ω (chlorophyll b), and soluble protein (root) were significantly negatively correlated with the V accumulation in the underground ($P<0.05$).



1. organic matter, 2. MDA content in the root, 3. V content in the shoots, 4. root crown ratio, 5. MDA content in the stem and leaf, 6. V content in the roots, 7. soil available V content, 8. soil pH, 9. POD content in the root, 10. POD content in the stem and leaf, 11. root length, 12. plant height, 13. SPAD, 14. SOD content in the root, 15. transpiration rate, 16. intercellular CO₂ concentration, 17. chlorophyll b, 18. stomatal conductance, 19. SOD content in the stem and leaf, 20. chlorophyll a, 21. net photosynthetic rate, 22. stem and leaf tolerance index, 23. root dry weight, 24. soluble protein content in the root, 25. root tolerance index, 26. Stems and leaves dry weight, 27. soluble protein content in the stem and leaf, 28. TF

Fig. 6 Principal component analysis (PCA) about each index change of pakchoi induced by polyethylene under vanadium stress (a). Correlation analysis of each index change of pakchoi induced by polyethylene under vanadium stress (b). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.7 Structural characteristics of polyethylene microplastics

Fig. 7(a) displays the scanning electron microscopy (SEM) image of the polyethylene (PE) microplastics. As observed in the micrograph, the pristine PE particles possess a macroscopically smooth surface, yet they exhibit a certain degree of physical roughness, localized depressions, and uneven micro-structures. These morphological features provide potential sites for the mechanical interception and physical adsorption of heavy metals in the soil environment. The Fourier transform infrared (FTIR) spectrum of the pristine PE microplastics is presented in Fig. 7(b). Two highly intense absorption peaks located at 2915.60 cm⁻¹ and 2847.02 cm⁻¹ are attributed to the asymmetric and symmetric stretching vibrations of the methylene (-CH₂) groups, respectively. Additionally, the absorption peak at 1468.01 cm⁻¹ corresponds to the bending vibration of the -CH₂ groups, while the band at 717.63 cm⁻¹ is assigned to the rocking vibration

of the $-CH_2$ groups within the long aliphatic carbon chains. After one month of artificial aging, the aged PE were recovered from the soil and analyzed using FTIR (indicated by the red line of Fig. 7(b)). A broad and strong absorption peak corresponding to the hydroxyl ($-OH$) stretching vibration appeared at 3400.21 cm^{-1} , which is one of the most prominent characteristics of aged PE. This indicates that under the combined effects of artificial accelerated aging and the soil environment, the PE carbon chains underwent oxidative cleavage, resulting in the generation of abundant alcoholic hydroxyls, hydroperoxides, or carboxylic acids. Furthermore, the emergence of this peak suggests a significant increase in the surface hydrophilicity of the aged PE, potentially leading to the adsorption of more environmental moisture. Additionally, the strong peak near 1718 cm^{-1} clearly indicates the substantial formation of ketone, aldehyde, or carboxylic acid functional groups. Under the intervention of UV radiation and heat, the C-H bonds in the PE molecular chains were broken and combined with free oxygen, forming a dense carbonyl network. These newly generated functional groups can serve as highly active adsorption sites, binding heavy metal vanadium (V) ions in the soil through physicochemical processes such as surface complexation, electrostatic attraction, or ion exchange.

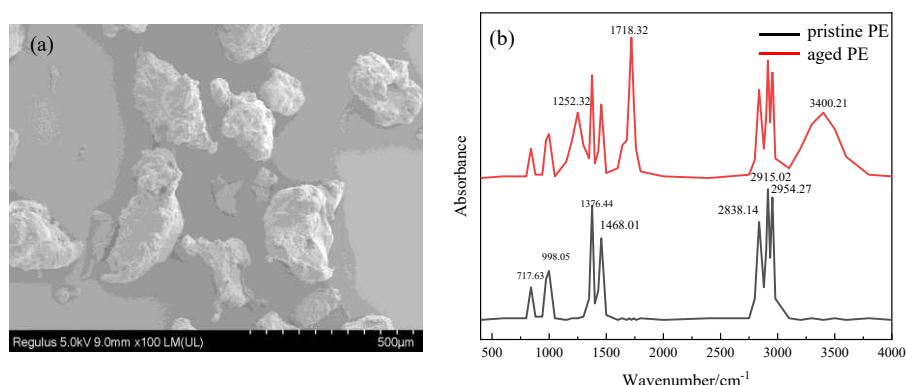


Fig. 7 SEM images (a) and FTIR spectrum (b) of pristine and aged polyethylene microplastics recovered from soil

4 Discussion

4.1 Effect of polyethylene on soil physical and chemical properties and growth of pakchoi under V stress

The results of this study demonstrated that the addition of polyethylene (PE) microplastics significantly reduced the concentration of vanadium (V) in the roots of pakchoi seedlings compared to the V-only treatment (Section 3.5). This decrease in V bioaccumulation was closely correlated with a marked reduction of DTPA-extractable V in the soil (Section 3.6). We propose that the PE microplastics may act as an external sink within the soil matrix, actively sequestering V ions and restricting their mobility, thereby mitigating the initial exposure of pakchoi roots to high levels of toxic V species. Crucially, after undergoing a one-month aging process in the soil environment, the surface chemistry of the PE microplastics was fundamentally altered (Mateos-Cárdenas et al. 2021). While SEM analysis (Fig. 7(a)) revealed physical roughness, localized

depressions, and micro-structures that provide sites for the physical interception of V complexes, may contribute to V immobilization was chemical adsorption. The natural aging process induced oxidative degradation on the microplastic surfaces, leading to the generation of abundant oxygen-containing functional groups(Adeleye et al. 2024; Bian et al. 2024). These newly formed oxygen-rich sites may significantly enhanced the surface polarity and hydrophilicity of the PE particles, allowing them to bind with V ions through specific chemical interactions, including surface complexation, electrostatic attraction, and ion exchange(An et al. 2024). Therefore, alongside mechanical trapping, adsorption and surface interactions could represent one possible mechanism responsible for reducing the concentration of bioavailable vanadium in soil pore water. This potent immobilization alleviated metal-induced oxidative stress, leading to a significant recovery in vital plant growth parameters, including biomass accumulation and photosynthetic rate.

Interestingly, the alleviating effect of the aged PE exhibited a distinct dosage-dependent biphasic trend. While the 0.1% and 1% PE treatments significantly improved plant growth by effectively utilizing chemical adsorption to reduce V uptake, the highest concentration (5% PE) resulted in diminishing returns in growth recovery, despite causing a further reduction in soil available V. This paradoxical phenomenon suggests that at excessively high concentrations, the intrinsic physical deterioration of the soil microenvironment caused by abundant microplastics—such as altered soil bulk density, reduced water-holding capacity, and mechanical interference with root-to-soil contact—begins to outweigh the chemical benefits of V immobilization(Ingraffia et al. 2022). The physical hindrance posed by abundant PE particles likely imposed secondary environmental stress on the pakchoi seedlings, partially offsetting the physiological benefits gained from reduced V toxicity(Chakraborty et al. 2025; Hui et al. 2024).

Unlike numerous previous ecotoxicological studies where microplastics were reported to enhance heavy metal toxicity via the well-documented "Trojan horse" effect, our findings present a contrasting scenario. In the context of V-contaminated agricultural soil, the aged PE particles primarily serve to immobilize V externally through strong chemical complexation and physical interception, reducing its overall bioavailability to the plant. While the present study highlights the dominant role of chemical adsorption driven by oxygen-containing functional groups, future research should incorporate high-resolution spectroscopic techniques (such as XPS or in situ synchrotron radiation) to precisely elucidate the micro-interfacial chemical coordination bonds between the aged microplastics and V.

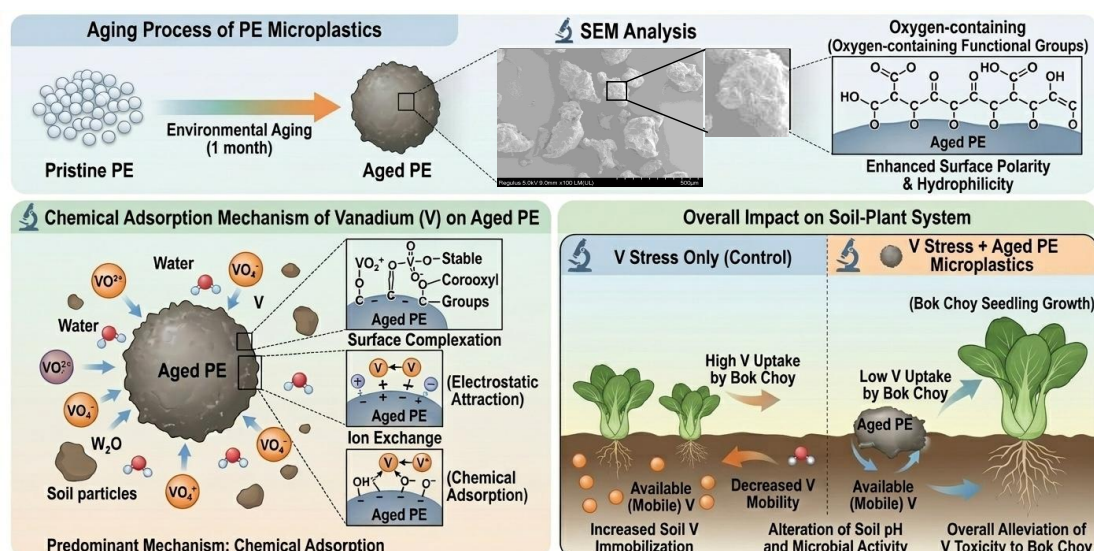


Fig. 8 Mechanism of aged PE microplastics on soil properties and pakchoi growth under V stress

4.2 Effect of polyethylene on physiological and biochemical and V accumulation of pakchoi under V stress

Photosynthesis is the core link of plant energy metabolism and is extremely sensitive to heavy metal stress. Several studies have shown that V stress significantly inhibits plant photosynthetic performance. For example, Yuan et al. found that $50 \text{ mg} \cdot \text{L}^{-1}$ V treatment reduced the net photosynthetic rate (P_n) of rice leaves by 68.3%, which is consistent with the result of a 72.5% decrease in P_n of pakchoi under $150 \text{ mg} \cdot \text{kg}^{-1}$ V stress in this study. In this study, the photosynthetic parameters and chlorophyll content of pakchoi were significantly reduced under V single stress. This may be related to the disruption of chloroplast structure and pigment synthesis under V stress. Metal ions replace Fe^{2+} , Zn^{2+} , and Mg^{2+} in the chlorophyll structure, resulting in loss of chlorophyll function and denaturation of chlorophyll enzymes, which ultimately promotes the degradation of chlorophyll, shows that the exogenous addition of a certain amount of PE can alleviate the photosynthetic inhibition of V stress on pakchoi, but its relieving effect depends on the amount of PE added. The impact of microplastics on plant photosynthesis is restricted by various factors such as plant species, environmental conditions, type, concentration, and particle size of microplastics (Jiang et al. 2024; Yang and Gao 2022).

At the same time, high concentration V stress can lead to nutritional imbalance in pakchoi, restriction of the electron transfer system, peroxidation of cell membrane lipids, and cell metabolism disorders, disrupting its normal growth and metabolism. The MDA content is usually used to determine the peroxidation level and damage to the cell membrane. Imtiaz et al. showed that V stress can damage the activity of the cell membrane of chickpea seedlings and reduce the soluble protein content of seedlings (Imtiaz et al. 2015). In this study, the MDA content in the upper and lower parts of pakchoi under single V stress was significantly increased, and the soluble protein

content was significantly reduced; however, the addition of exogenous PE will reduce the MDA content to a certain extent, and improve the soluble protein content. The effect varies with the change of PE addition, and the relief effect of low-level PE is relatively more obvious, but the toxicity of V stress is still not completely eliminated.

Pakchoi has evolved various defense strategies to enhance the tolerance to V, and the antioxidant enzyme system is a vital link (Imtiaz et al. 2018). The effect of V stress on the antioxidant enzyme activity of pakchoi varies by enzyme. Under V single stress, POD activity in the shoot and roots part of pakchoi is significantly increased, and SOD activity is slightly reduced. When PE is added under V stress, the SOD activity of the shoots will increase within a certain range. The SOD activity of the shoots and roots will be the highest when 1% PE is treated; the POD activity will increase and then decrease, while the 0.1% PE treatment group will be the highest. These results suggest that an appropriate amount of PE may enhance antioxidant defense and thereby alleviate V-induced oxidative stress. In this study, the correlation analysis showed that both the shoot and root soluble proteins of pakchoi were negatively or very negatively correlated with the V content in pakchoi. In contrast, the MDA content of pakchoi was significantly positively correlated with the V content of pakchoi, which also confirmed the toxic and damaging effect of high-content V stress on pakchoi. Regarding V accumulation, the content of V in the pakchoi tissue is manifested as the V accumulation followed the pattern roots > shoots. V is chelated or complexed in the root cell wall. The compartmentalization of V within root cell vacuoles reduces the transport of V from the root system to the shoots (Aihemaiti et al. 2020; Tian et al. 2015). Under V stress, the V content of the roots of pakchoi increased significantly, and the shoots also increased. After adding PE, the V content of the roots and shoots of the pakchoi are both reduced, and the decrease is more obvious with the increase of PE within a certain range. The effect is weakened when the concentration is too high, indicating that PE can inhibit the absorption and accumulation of V of the pakchoi to reduce toxicity, and the effect is related to the added amount.

5 Conclusion

Low PE addition (0.1%) partially alleviated V toxicity in pakchoi by improving photosynthetic performance, enhancing antioxidant defense, and reducing V accumulation in roots. PE also decreased available V in soil, suggesting that reduced V bioavailability was an important mechanism underlying the mitigation effect. However, higher PE concentrations provided limited benefit, indicating a dose-dependent trade-off between V immobilization and potential physical stress caused by excessive microplastic addition.

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Data Availability Statement The original contributions presented in this study are included in this article. Further inquiries can be directed to the corresponding author.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Competing Interests The authors declare no competing interests.

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