



## Original Research

## Phytoremediation of microplastics by water hyacinth

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## ARTICLE INFO

## Article history:

Received 7 October 2024

Received in revised form

6 February 2025

Accepted 6 February 2025

## Keywords:

Plastic pollution

Root caps

*Eichhornia crassipes*

Adsorption

Bioremediation

Aquatic environment

## ABSTRACT

Microplastics have emerged as pervasive environmental pollutants, posing significant risks to both terrestrial and aquatic ecosystems worldwide. Current remediation strategies—including physical, chemical, and microbial methods—are inadequate for large-scale, in situ removal of microplastics, highlighting the urgent need for alternative solutions. Phytoremediation, an eco-friendly and cost-effective technology, holds promise in addressing these challenges, though its application to microplastic pollution remains underexplored. Here we show the capacity of *Eichhornia crassipes* (water hyacinth), a fast-growing, floating aquatic plant, to remove microplastics from contaminated water. Our results show that within 48 h, water hyacinth achieved removal efficiencies of 55.3 %, 69.1 %, and 68.8 % for 0.5, 1, and 2  $\mu\text{m}$  polystyrene particles, respectively, with root adsorption identified as the primary mechanism. Fluorescence microscopy revealed that the extremely large and abundant root caps, featuring a total surface area exceeding 150,000  $\text{mm}^2$  per plant, serve as the principal sites for the entrapment of microplastics. Furthermore, a unique “vascular ring” structure within the stem prevents the translocation of microplastics to aerial tissues, safeguarding leaves for potential downstream applications. This study offers the first microstructural insight into the mechanisms underpinning water hyacinth's exceptional microplastic adsorption capacity and resilience, providing a promising framework for developing phytoremediation strategies to mitigate microplastic pollution in aquatic ecosystems.

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## 1. Introduction

In recent years, microplastics (MPs) pollution has raised widespread global concern. Land, the primary site for the use and disposal of plastic products, is heavily contaminated with MPs [1–3], which tend to accumulate in rivers, lakes, and wetlands following precipitation events and surface runoff [4–10]. Polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) are the most prevalent and frequently detected MPs in freshwater ecosystems [11,12]. Elevated concentrations of MPs have been detected in urban lakes and in rivers that traverse populated regions [13]. For instance, a concentration of 12,611 items  $\text{m}^{-3}$  of MPs was recorded in the surface water of the Three Gorges in China [14]. In the Yellow River, MP concentrations ranged from 5358 to 654,000 items  $\text{m}^{-3}$  [15], while in Lake Michigan, sediment samples revealed an

abundance of MPs as high as 6229 items  $\text{kg}^{-1}$  [16]. Additionally, in Poyang Lake, China's largest freshwater lake, the MP content reached up to 34,000 items  $\text{m}^{-3}$  [17].

Numerous studies have shown that MPs and their further degradation products, nanoplastics (NPs), can be detrimental to aquatic plants and animals. For example, exposure of floating duckweed (*Lemna minor*) to PET-MPs over ten generations has resulted in disrupted chloroplast distribution and inhibited photosynthesis [18]. Similarly, research on the microalga (*Scenedesmus obliquus*) demonstrated that 2  $\mu\text{m}$  PS-MPs blocked light transmission and impacted photosynthesis, while 0.1  $\mu\text{m}$  PS-NPs adhered to the microalga's surface, compromising cell wall integrity [19]. PE-MPs have been found to cause oxidative damage to cladocerans (*Daphnia magna*), reducing mobility and heightened vulnerability to predation by damselfly larvae [20]. Furthermore, PE-MPs were shown to inflict physical damage to the gills of shrimp (*Litopenaeus vannamei*), which subsequently impaired oxygen uptake and increased the risk of infection [21]. A major concern is the potential for MPs to accumulate in aquatic organisms, ultimately

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entering the food chain through larger shellfish and fish [22], thus posing a grave threat to human food safety. Given these risks, urgent research is needed to develop technologies for efficiently removing MPs from freshwater and wetland ecosystems.

Various methods have been developed to remove MPs from water bodies [23], including membrane filtration, the use of synthetic adsorbents, coagulation-flocculation-sedimentation (CFS), and electrocoagulation (EC). However, these conventional remediation methods often present disadvantages, such as high energy consumption, elevated costs, the potential for secondary pollution, and limited applicability. For instance, membrane filtration is less effective at removing MPs smaller than 3–5  $\mu\text{m}$  [24,25], while CFS and EC are primarily suited for wastewater treatment facilities [26]. In contrast, phytoremediation techniques offer numerous advantages, such as low cost, high efficiency, improved biosafety, and the non-introduction of secondary pollutants, making them more suitable for the remediation of natural water bodies [27]. Nevertheless, research on the application of phytoremediation to remove MPs is still in its infancy. Therefore, the identification of excellent remediation plants and the investigation of their mechanisms for trapping MPs are critical tasks at this stage.

Water hyacinth (*Eichhornia crassipes*), a large floating plant of the Pontederiaceae family, reproduces rapidly through stolons and can double its biomass every 7–10 days under optimal conditions [28,29]. Recent studies have highlighted its potential application in the remediation of MP/NP pollutants. Yang et al. [30] demonstrated that water hyacinth can remove up to 66.4 % of 20 nm PS-NPs and 44.7 % of 200 nm PS-NPs from water. Furthermore, the absorption of PS particles by the plant roots was found to account for only a small portion of the total removal. Yuan et al. [31] found that the uptake of 100 nm PS-NPs and 2  $\mu\text{m}$  PS-MPs by water hyacinth was 6250 and 143  $\mu\text{g g}^{-1}$ , respectively, which was significantly higher than the uptake observed in *Vallisneria spiralis* and *Iris tectorum*. These two studies primarily concentrated on nano-sized particles, specifically examining the uptake of NPs and their impact on plants. However, two critical perspectives warrant further investigation. First, although water hyacinth is virtually incapable of absorbing micron-sized plastics, the adsorption ability of MPs on its roots remains poorly understood—mechanism which may play a critical role in the removal of MPs. The capacity of the roots to capture MPs, the nature of the adsorption sites, and the trapping mechanisms in this process have yet to be elucidated. Second, water hyacinth does not exhibit significant growth inhibition when exposed to NPs/MPs, indicating that it may possess effective defense mechanisms. Nonetheless, the transport, blockage, and tolerance of MPs within the plant remain largely unexplored.

Alarmingly, a single microplastic particle in the environment can fragment into more than  $10^{14}$  NPs [32,33]. Therefore, employing phytoremediation to eliminate substantial quantities of MPs in natural aquatic environments before they degrade into NPs offers an efficient and economical strategy. We propose that the interaction of water hyacinth with MPs differs from its interaction with NPs. It is likely that water hyacinth can capture a substantial amount of MPs and exhibit good tolerance due to its unique biological microstructure. In the present study, we used PS-MPs, one of the primary components of plastic pollution in natural water, to assess exposure over a period of 1–30 days, with the aims of (1) comparing the removal efficiency and tolerance of water hyacinth to various sizes of PS-MPs, (2) analyzing the removal mechanism of its roots in terms of both the absorption and adsorption of MPs, and (3) in particular, revealing the mechanism of water hyacinth's strong capture ability of and resilience to MPs from the new perspective of its unique anatomy and microstructures. The results will help deepen the understanding of the mechanisms through

which aquatic plants trap MPs and thus promote the application of phytoremediation technology in managing water bodies polluted with MPs.

## 2. Materials and methods

### 2.1. Preparation of PS-MPs

Three sizes of monodisperse PS microspheres (purchased from Jiangsu Zhichuan Technology Co., Ltd., Jiangsu, China), with nominal diameters of 0.5, 1, and 2  $\mu\text{m}$ , were used, denoted as PS-MPs<sub>0.5 $\mu\text{m}$</sub> , PS-MPs<sub>1 $\mu\text{m}$</sub> , and PS-MPs<sub>2 $\mu\text{m}$</sub> . The actual sizes of these microspheres were examined by microscopy, as shown in Fig. S1 (Supplementary Material). Water hyacinth roots were pre-tested under a fluorescence microscope and were found to display some green autofluorescence. To mitigate the potential interference of this autofluorescence, all PS microspheres were fluorescently labeled with rhodamine 6G in an orange-red color (Ex-Max = 525 nm, Em-Max = 580 nm). Additionally, the zeta potential of the PS-MPs in water was measured using a Zetasizer (Malvern Instruments, UK) and is listed in Table S1 (Supplementary Material). Prior to use, the suspension of PS microspheres was enclosed in a dialysis bag for 48 h following the method outlined by Zhou et al. [34]. The outer distilled water of the dialysis bag was replaced every 12 h to eliminate any potentially dissolved substances in the suspension.

### 2.2. Preparation of water hyacinth plants and PS-MPs exposure treatment

Water hyacinth plants were collected from a river in Jiading District, Shanghai, China, and cultivated in a greenhouse under controlled conditions. The plants were grown in half-strength Hoagland's nutrient solution under a light intensity of 6000 lx for 12 h light and 12 h dark, with temperatures at 30/26 °C (day/night). To ensure consistency, all plants used in this study were propagated from the same mother plant. For the PS-MPs exposure, water hyacinth plants of uniform size were placed in glass culture bottles with a 1-L nutrient solution, with two plants per bottle and their roots fully submerged. PS-MPs of 0.5, 1, and 2  $\mu\text{m}$  were added to achieve a final concentration of 50  $\text{mg L}^{-1}$ . The 50  $\text{mg L}^{-1}$  does not represent the average concentration of MPs in natural waters, as surveys of MPs in aquatic environments have typically used the unit of items  $\text{L}^{-1}$  or items  $\text{m}^{-3}$ . However, this concentration has been commonly used in previous studies [35–37] and may approximate the localized “hot spot” in heavily polluted waters [38]. A nutrient solution without PS-MPs was used as the control (CK). Prior to exposure, the nutrient solution underwent sonication for 5 min to prevent particle aggregation. Each treatment with PS-MPs and the CK group contained seven replicates, and the nutrient solution was changed every seven days.

### 2.3. Analysis of plant physiological indicators

To measure the effects of PS-MPs on plant growth, a total exposure time of 14 days was utilized. Plant fresh weight was measured at the beginning and end of the exposure to calculate the growth rate of biomass in  $\text{g day}^{-1}$ . Additionally, leaf weight, chlorophyll content, root weight, root length, root diameter, number of adventitious roots per plant, and number of lateral roots were recorded. Root diameter was determined by the cross-sections of adventitious roots under a microscope.

Chlorophyll concentration was assessed following the method outlined by Senthilkumar et al. [39]. Fully expanded leaves (0.5 g) were ground in 10 mL of 96 % ethanol on ice, and the homogenate

was stored at 4 °C for 24 h. Following centrifugation at 10,000 g for 10 min, the green supernatant was collected, and the absorbance (OD) was measured at 665 and 649 nm. Chlorophyll content was calculated as follows:

$$\text{Chlorophyll } a \text{ (mg kg}^{-1}\text{)} = (13.95 \times OD_{665} - 6.88 \times OD_{649}) \times V/W \quad (1)$$

$$\text{Chlorophyll } b \text{ (mg kg}^{-1}\text{)} = (24.96 \times OD_{649} - 7.32 \times OD_{665}) \times V/W \quad (2)$$

$$\text{Total chlorophyll (mg kg}^{-1}\text{)} = \text{Chlorophyll } a + \text{Chlorophyll } b \quad (3)$$

where  $V$  (mL) is the volume of 96 % ethanol used for extraction, and  $W$  (g) is the fresh weight of the extracted leaves.

#### 2.4. Oxidative stress analysis

The hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) levels, as well as the activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) in the leaves and roots of each treatment group, were assessed using commercially available kits from Grace Biotechnology (<http://www.geruisi-bio.com>). These measurements were conducted according to the manufacturer's instructions.

#### 2.5. Quantification of the roots' removal rate, adsorption capacity, and absorption capacity of MPs

The removal rate, adsorption capacity, and absorption capacity of PS-MPs from the nutrient solution by water hyacinth roots were analyzed using a fluorescence spectrophotometer (F96pro, Lengguang Technology, China) according to the method presented in previous studies [24,30], with modifications. Briefly, the fluorescence intensity (FI) of the initial nutrient solution (PS-MPs added at  $50 \text{ mg L}^{-1}$ ) was measured ( $\text{Ex} = 465 \text{ nm}$ ,  $\text{Em} = 580 \text{ nm}$ ), and it was confirmed that the FI did not decrease significantly throughout the experiment. A standard curve was then plotted, and the FI of the nutrient solution was measured after 1, 2, 3, and 5 days of exposure. The removal rate was calculated based on fluorescence attenuation. To analyze the adsorption and absorption of PS-MPs by the roots, samples were collected after two days of exposure. The roots were submerged in distilled water with 0.1 % (v/v) Tween 20, followed by three ultrasonic washings (2 min each) to fully elute the PS-MPs adsorbed on the root surface. The FI of the eluate was then measured. Next, the cleaned roots were weighed and homogenized in distilled water (containing 0.1 % Tween 20). The homogenate tissues were filtered through a 600-mesh sieve, and the residue was ultrasonically cleaned by adding distilled water (containing 0.1 % Tween 20) and then filtered. After six repetitions, the filtrates (homogenates) were mixed and concentrated in a freeze dryer and the FI was then determined. Final FI was obtained by deducting the background FI values of the non-exposed samples. For the stem samples, the FI of their homogenates was determined using the same procedure. The adsorption and absorption of PS-MPs were evaluated according to the FI and the respective volumes of the eluate and homogenate. These measurements were expressed as  $\mu\text{g g}^{-1}$ , signifying the weight of PS-MPs that could be adsorbed or absorbed per gram of plant tissue (fresh weight).

#### 2.6. Observation of the fluorescently labeled PS-MPs in plants

Three sizes (0.5, 1, and 2  $\mu\text{m}$ ) of PS-MP microspheres were initially examined under a fluorescence microscope ( $\text{Ex} = 525 \text{ nm}$ ,  $\text{Em} = 590\text{--}680 \text{ nm}$ ) to ensure the visualization of individual MP particles and their distribution in plant tissues (Supplementary

Material Fig. S2).

To observe the entry of PS-MPs into the roots, water hyacinth was collected after two days of exposure and rinsed sequentially with tap water and distilled water. The adventitious roots were sliced into consecutive sections of 200  $\mu\text{m}$  thickness using a vibrating slicer (HS-1205, ZEEDO). Sections that bore lateral roots were selected. To observe the adsorption of PS-MPs, the lateral roots were separated from the adventitious roots. Some of the lateral roots with root caps were stained with berberine-aniline blue, according to Brundrett et al. [40], to highlight the epidermal cells of the root body. The others were carefully stripped off their caps using pointed forceps. After 30 days of exposure, the stems and petioles were sliced to 100  $\mu\text{m}$  thickness to observe the translocation of PS-MPs from the root to the aerial part. All these samples were examined in bright-field and red fluorescence channels (some were also examined in yellow and blue fluorescence channels).

#### 2.7. Data analysis

All measurements and observations were based on samples from at least three biological replicates. Data were collated and graphed using Microsoft Excel 2021 and Origin 9.0 software, and one-way ANOVA was performed using SPSS v21 software.

### 3. Results

#### 3.1. Water hyacinth plants grew well under $50 \text{ mg L}^{-1}$ PS-MPs exposure

After a 14-day exposure period, all sizes of PS-MPs (0.5, 1, and 2  $\mu\text{m}$ ) showed no significant inhibitory effects on the plants (Fig. 1). The growth rate, leaf weight, and chlorophyll content of the water hyacinths were not reduced. In terms of roots, PS-MPs of 0.5  $\mu\text{m}$  caused a slight reduction in the length of the adventitious roots, while PS-MPs of 1 and 2  $\mu\text{m}$  had no significant impact on root weight, length, diameter, number of adventitious roots, and number of lateral roots.

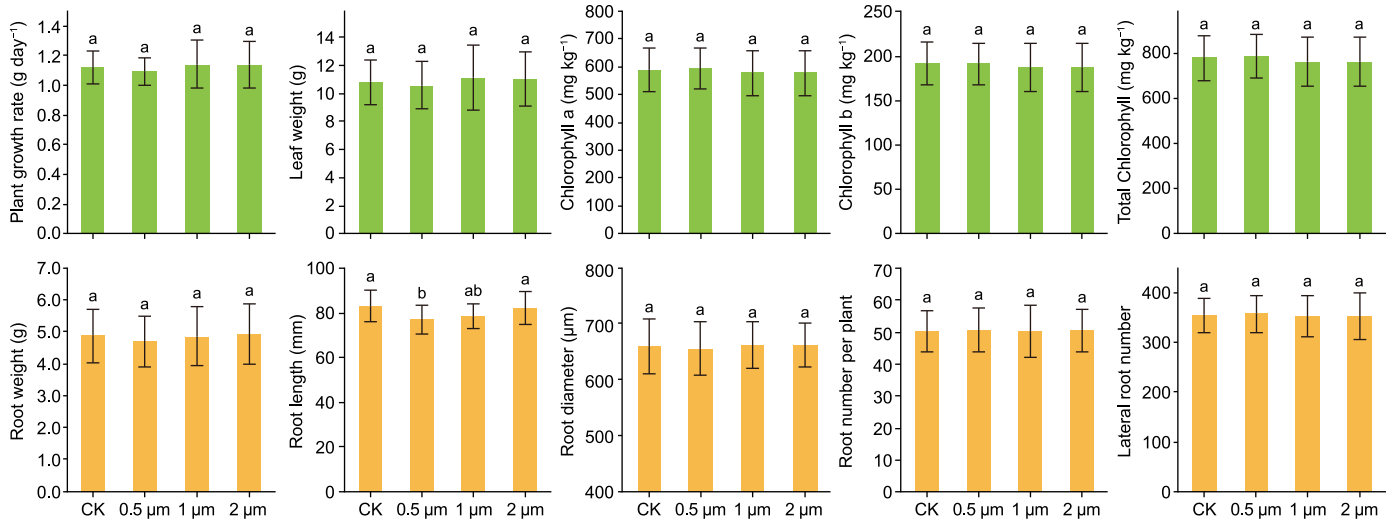
#### 3.2. PS-MPs activate antioxidant systems in roots

The activities of the three antioxidant enzymes, POD, SOD, and CAT, showed immense differences between the leaves and roots (Fig. 2). The roots exhibited notably high POD activity, which was approximately 10 times higher than that of the leaves (Fig. 2a). Conversely, the SOD and CAT activities in the roots were only about 20 % and 7 % of those in the leaves, respectively (Fig. 2b and c). The exposure to PS-MPs had minimal impact on the POD, SOD, and CAT activities in the leaves but resulted in a slight elevation of the POD activities in the roots. Specifically, 0.5, 1, and 2  $\mu\text{m}$  PS-MPs induced a 16.6 %, 11.3 %, and 10.1 % rise in POD activity in the roots, respectively. Furthermore, negligible effects on the production of  $\text{H}_2\text{O}_2$  were observed (Fig. 2d).

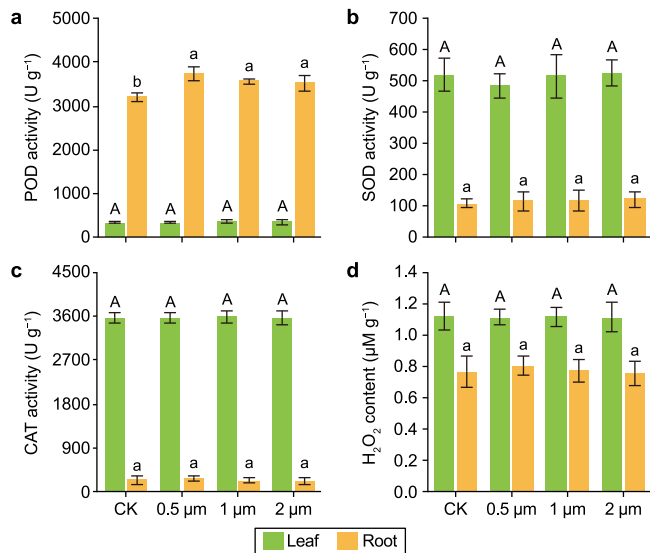
#### 3.3. Water hyacinth roots were effective in adsorbing and removing PS-MPs

The quantitative assay demonstrated that water hyacinth roots could remove over 55 % of PS-MPs from the nutrient solution within the initial two days (48 h) of exposure. Following five days of exposure, the removal rates for the three sizes of PS-MPs (0.5, 1, and 2  $\mu\text{m}$ ) were recorded at 62.8 %, 78.3 %, and 76.8 %, respectively (Fig. 3a).

To elucidate the specific mechanism of PS-MPs removal by water hyacinth, the amounts of adsorption (Fig. 3b) and absorption



**Fig. 1.** Effects of polystyrene microplastics (PS-MPs) on physiological parameters of water hyacinth after 14 days of exposure. Significant differences between the effects of three sizes of PS-MPs are indicated by letters “a” and “b,” and the same letter indicates no significant difference ( $p > 0.05$ ) based on least significant difference (LSD) test, one-way ANOVA.

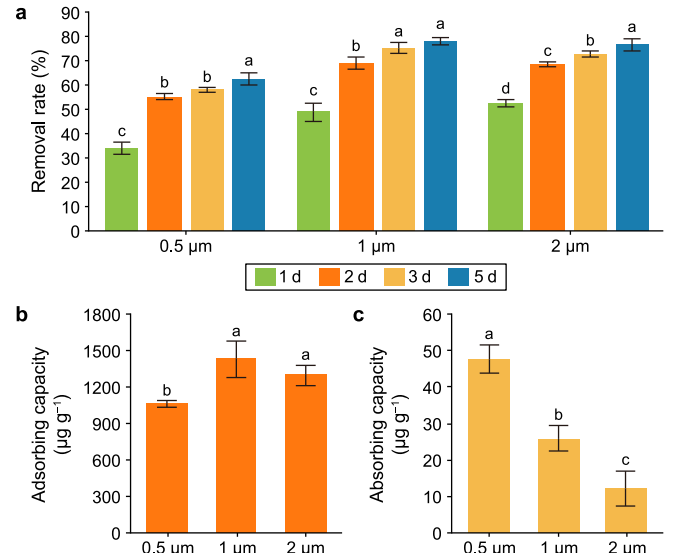


**Fig. 2.** Effects of PS-MPs on peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) activities and hydrogen peroxide ( $H_2O_2$ ) content of water hyacinth leaves and roots after two days of exposure. Significant differences between the effects of different sizes of PS-MPs are indicated by uppercase (for leaf) or lowercase letters (for root), and the same letter indicates no significant difference ( $p > 0.05$ ), LSD test, one-way ANOVA.

(Fig. 3c) were evaluated separately after two days of exposure. The roots exhibited a notable adsorption capacity for all three sizes of PS-MPs (0.5, 1, and 2  $\mu m$ ), with the amounts reaching 1,060, 1,426, and 1294  $\mu g g^{-1}$  (fresh weight), respectively. However, the absorption amount was far lower than adsorption, measuring only 48, 26, and 12  $\mu g g^{-1}$  (fresh weight), respectively.

### 3.4. PS-MPs were mainly captured by root caps

Despite lacking root hair, water hyacinth expanded its root surface by forming numerous lateral roots (Fig. 4a). Each lateral root was characterized by a huge cap at its front end, measuring over 1 mm in length, which was five times larger than the lateral root

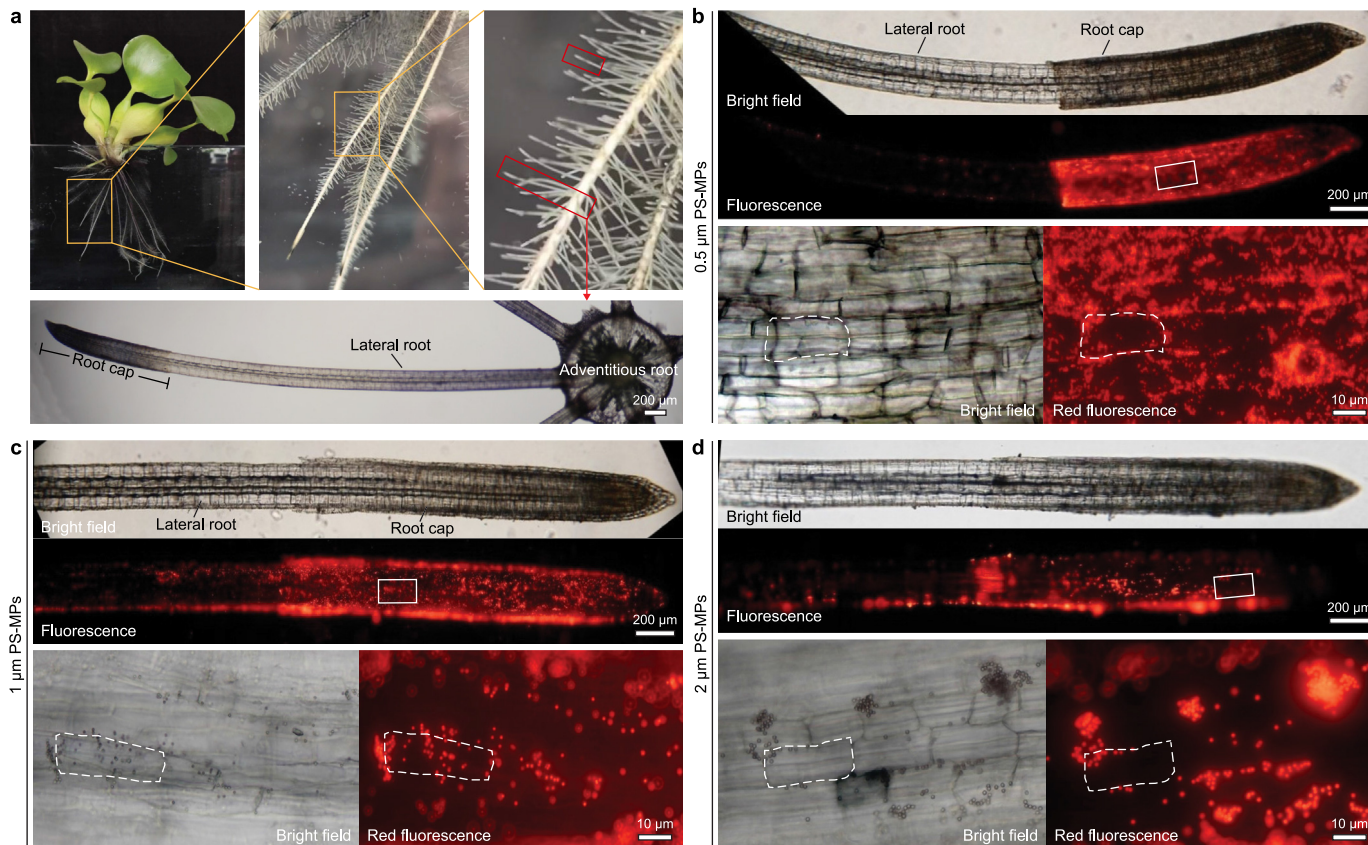


**Fig. 3.** Removal rate (a), adsorption capacity (b), and absorption capacity (c) of water hyacinth roots on different sizes of PS-MPs. The exposure time in panels b and c was two days. The different lowercase letters indicate significant differences between series days within each group in panel a, and significant differences among treatment groups in panels b and c, as determined by one-way ANOVA (LSD test,  $p < 0.05$ ).

diameter (Fig. 4a and 5). The total surface area of the caps on each adventitious root was calculated to be as high as 289  $mm^2$  (Fig. 5c and d), which was almost 400 times larger than that of rice (0.72  $mm^2$ ).

PS-MPs (0.5, 1, and 2  $\mu m$ ) were predominantly adsorbed on the root caps (Fig. 4b–d, and 5a,b). Notably, the amount of PS-MPs adsorbed on the caps exceeded that on the root bodies by more than 50 times (Fig. 5e). Upon removal of the caps, no fluorescence of PS-MPs was detected at the anterior end of the lateral root (Fig. 5b). Furthermore, the cap cells were much smaller than the root epidermal cells (Fig. 5a), and the PS-MP particles were predominantly clustered at the boundaries of the cap cells, appearing to be trapped within the cap cell interstices (Fig. 4b–d).





**Fig. 4.** Lateral root micro-structure (a) and adsorption of 0.5 μm (b), 1 μm (c), and 2 μm (d) PS-MPs on root caps after two days of exposure. The lower panels in b–d are magnified views of the white boxed areas in the upper panels, respectively. Note that the front end of each lateral root is covered with a huge cap in a. PS-MP particles were mainly adsorbed on the caps (red fluorescence channels in b, c, and d) and preferentially trapped in borders (white dashed lines) of the cap cells.

### 3.5. PS-MPs invaded through the root cracks

The cracks formed on the adventitious root, where the lateral roots emerged and appeared in a V-shape in the transverse section, extending from the lateral root surface to the stele (Fig. 6b and c). PS-MPs were observed to enter along the lateral root epidermis (Fig. 6a–c). The PS-MPs<sub>0.5μm</sub> were able to invade the cracks more effectively compared to the PS-MPs<sub>1μm</sub> and PS-MPs<sub>2μm</sub>. The larger size of PS-MPs<sub>1μm</sub> and PS-MPs<sub>2μm</sub> hindered their entry, causing them to be predominantly blocked at the crack surface (Fig. 6b and c). This aligns with the quantitative analysis shown in Fig. 3b.

### 3.6. Transfer of PS-MPs was blocked by stem “vascular ring”

The presence of PS-MPs in the stems and leaves was analyzed after 5, 15, and 30 days of exposure. No fluorescence from PS-MPs was detected in the leaves (Supplementary Material Fig. S3). Some PS-MPs were found in the roots, but the levels were much lower than in the roots. For instance, the content of PS-MPs<sub>0.5μm</sub> in the stems after 5 days of exposure was 1.83 μg g<sup>-1</sup> (fresh weight) (Fig. 7a), representing only 3.84 % and 0.17 % of the absorption and adsorption in the roots (see Fig. 3b and c), respectively. Furthermore, the content of PS-MPs<sub>0.5μm</sub> in the stem was higher than that of PS-MPs<sub>1μm</sub> and PS-MPs<sub>2μm</sub>, with a gradual increase during the exposure period.

Subsequently, an examination of the stems using a stereo microscope revealed a “vascular ring” structure. The tissues outside the “ring” connected to the adventitious roots, while the tissues inside were linked to the shoot apical meristem (SAM) and the

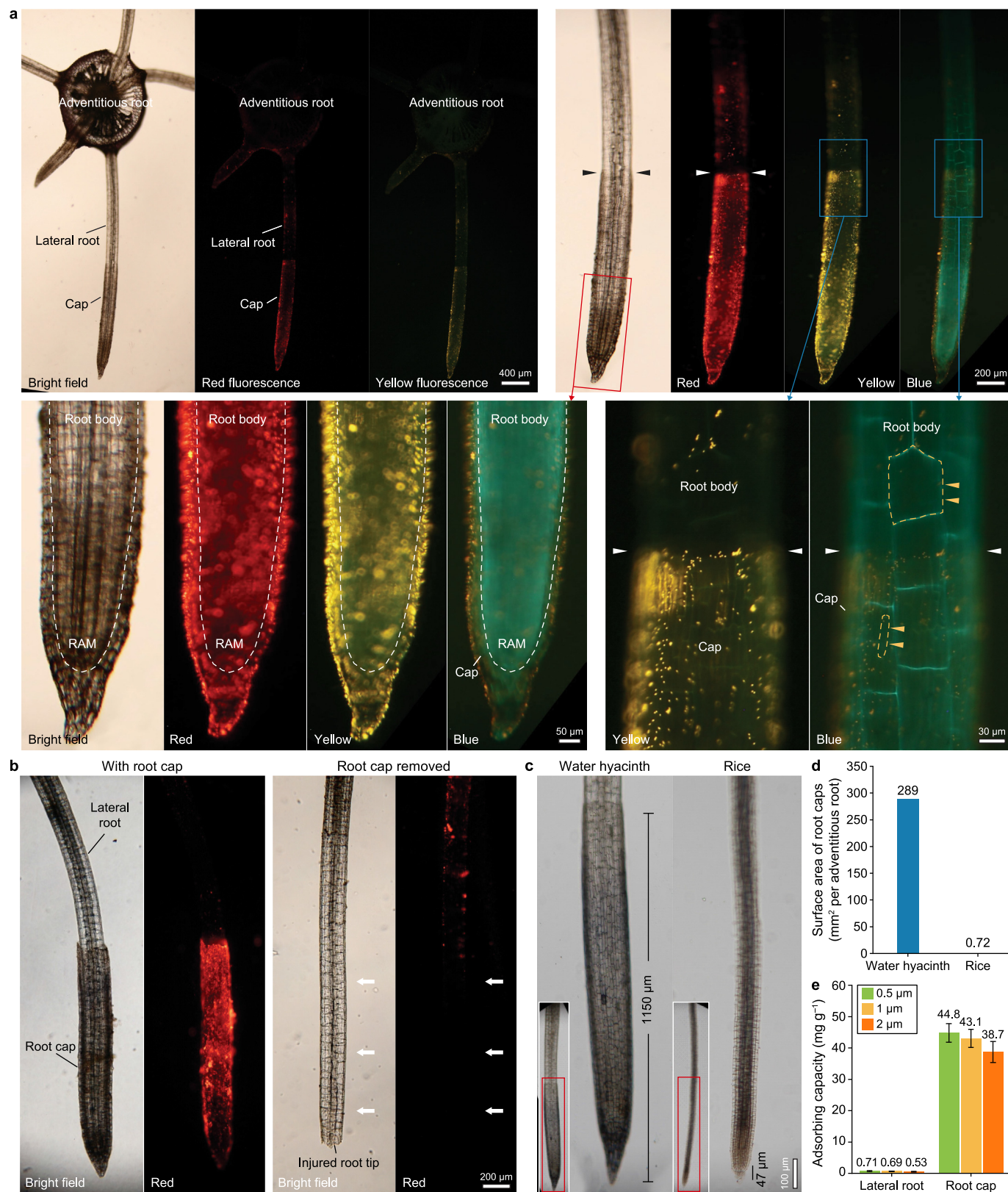
young leaves (Fig. 7b). After a 30-day exposure period, the PS-MPs entered the stems through the vascular bundles of the root but were obstructed totally out of the vascular ring (Fig. 7c and d). As a result, PS-MPs that entered the stem could not reach the leaf and delicate SAM, and no PS-MP particles were detected in the petioles (Supplementary Material Fig. S4).

## 4. Discussion

### 4.1. Water hyacinth is highly tolerant to PS-MPs

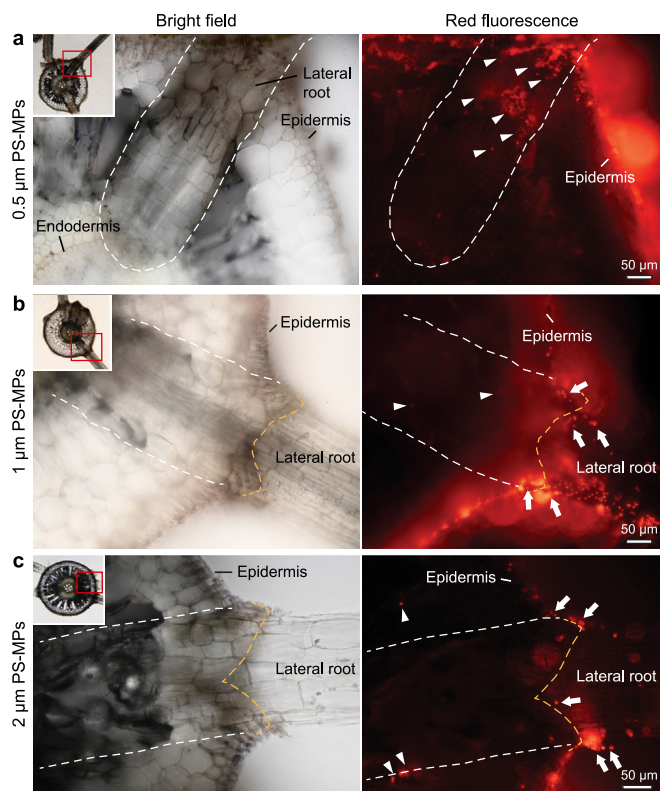
Previous studies have demonstrated that exposure to 50 mg L<sup>-1</sup> of MPs is sufficient to induce significant biological injury and growth inhibition in plants. For instance, root exposure to 50 mg L<sup>-1</sup> of PS-MPs led to a nearly 50 % reduction in the biomass of the aerial portion of cucumber plants, accompanied by a two-to three-fold increase in H<sub>2</sub>O<sub>2</sub> content and POD activity in the leaves [41]. After 15 days of exposure to 50 mg L<sup>-1</sup> of PP-MPs and PE-MPs, floating duckweed exhibited about 50 % and 35 % reductions in root length, respectively, along with an approximate 30 % decrease in growth rate [42]. Additionally, exposure to 3 mg L<sup>-1</sup> of PS-MPs and PVC-MPs resulted in a 67 % and 125 % increase in H<sub>2</sub>O<sub>2</sub> content in rice plants, respectively, and a reduction in the photosynthetic rate by 32 % and 44 %, respectively [43]. Similar injury phenomena were also observed in lettuce [44] and wheat [45].

In this study, we demonstrated that the biomass of water hyacinth exposed to 50 mg L<sup>-1</sup> of PS-MPs was unaffected, and several physiological indices of its leaves and roots did not significantly differ from those of the CK plants. This indicates that water



**Fig. 5.** Trapping (a), blocking (b), and adsorption capacity (e) of PS-MPs by water hyacinth root caps, and comparison of root cap size between water hyacinth and rice (c, d). The red and yellow fluorescence channels in panel a show the distribution of PS-MPs<sub>1 $\mu\text{m}$</sub> , and the blue fluorescence channel shows the root epidermis (stained with berberine-aniline blue). White and black arrowheads in panel a indicate the end of the root cap, white dashed lines mark the region of the root body, and yellow dashed lines and yellow arrowheads mark one epidermal cell (larger) and one root cap cell (smaller). Also, note that the red fluorescent signal of PS-MPs<sub>0.5 $\mu\text{m}$</sub>  disappeared (white arrows) after the root cap was stripped in panel b. Red boxes within the small graphs in panel c indicate the enlarged parts. The adsorption capacity was calculated as the fresh weight of the lateral roots or root caps in panel e.





**Fig. 6.** Accumulation and invasion of PS-MPs of 0.5  $\mu\text{m}$  (a), 1  $\mu\text{m}$  (b), and 2  $\mu\text{m}$  (c) at the root cracks after two days of exposure. The left and right columns show bright-field and red fluorescence channel photographs of the same sections, respectively. Red boxes within the vignettes in panels a, b, and c indicate the enlarged areas. White dashed lines mark the boundaries of lateral roots and yellow dashed lines in panels b and c mark root cracks. White arrowheads indicate PS-MP particles that enter the root interior, and white arrows in panels b and c indicate PS-MP particles blocked by the cracks.

hyacinth possesses considerable resilience to MPs. Previous research has established that water hyacinth is highly tolerant and capable of accumulating a wide range of heavy metals and organic pollutants. For instance, it exhibited growth rates that exceeded those of the control in a 1  $\text{mg L}^{-1}$  cadmium (Cd) environment and was able to tolerate toxicity levels of up to 10  $\text{mg L}^{-1}$  Cd [46]. Fan et al. [47] found that water hyacinth could grow normally under 10  $\text{mg L}^{-1}$  ciprofloxacin treatment, while the growth of *Vallisneria denserrulata* was severely impeded. However, most existing studies have primarily focused on the enrichment and/or removal capacity of water hyacinth in relation to pollutants, leaving the underlying tolerance mechanisms largely unexplored. One notable finding of the present study is that the POD activity in the roots of water hyacinth was nearly tenfold higher than that in the leaves, regardless of whether the plants were subjected to PS-MPs or CK treatment (Fig. 2a). This substantial difference is uncommon among plants, as studies on rice [48], wheat [49], and maize [50] have shown that the POD activity in the roots of all these species is comparable to that in their aboveground parts (leaves).

Environmental stress leads to the accumulation of reactive oxygen species (ROS) in plants, which in turn activates the synthesis of antioxidant enzymes [51]. The high POD activity in the roots of water hyacinth likely serves as a buffer for the detoxification of ROS. We hypothesized that tissue-specific, exceptionally high constitutive POD activity in the roots of water hyacinth (Fig. 2a) enables the plant to adapt to a harsh aquatic environment. Following injury induced by PS-MPs, the inherently elevated levels of POD in the

roots are sufficient to swiftly eliminate ROS, thereby preventing further damage to the aerial part (leaf). This can also elucidate why no great increase in antioxidant enzyme activity or  $\text{H}_2\text{O}_2$  content was detected (Fig. 2).

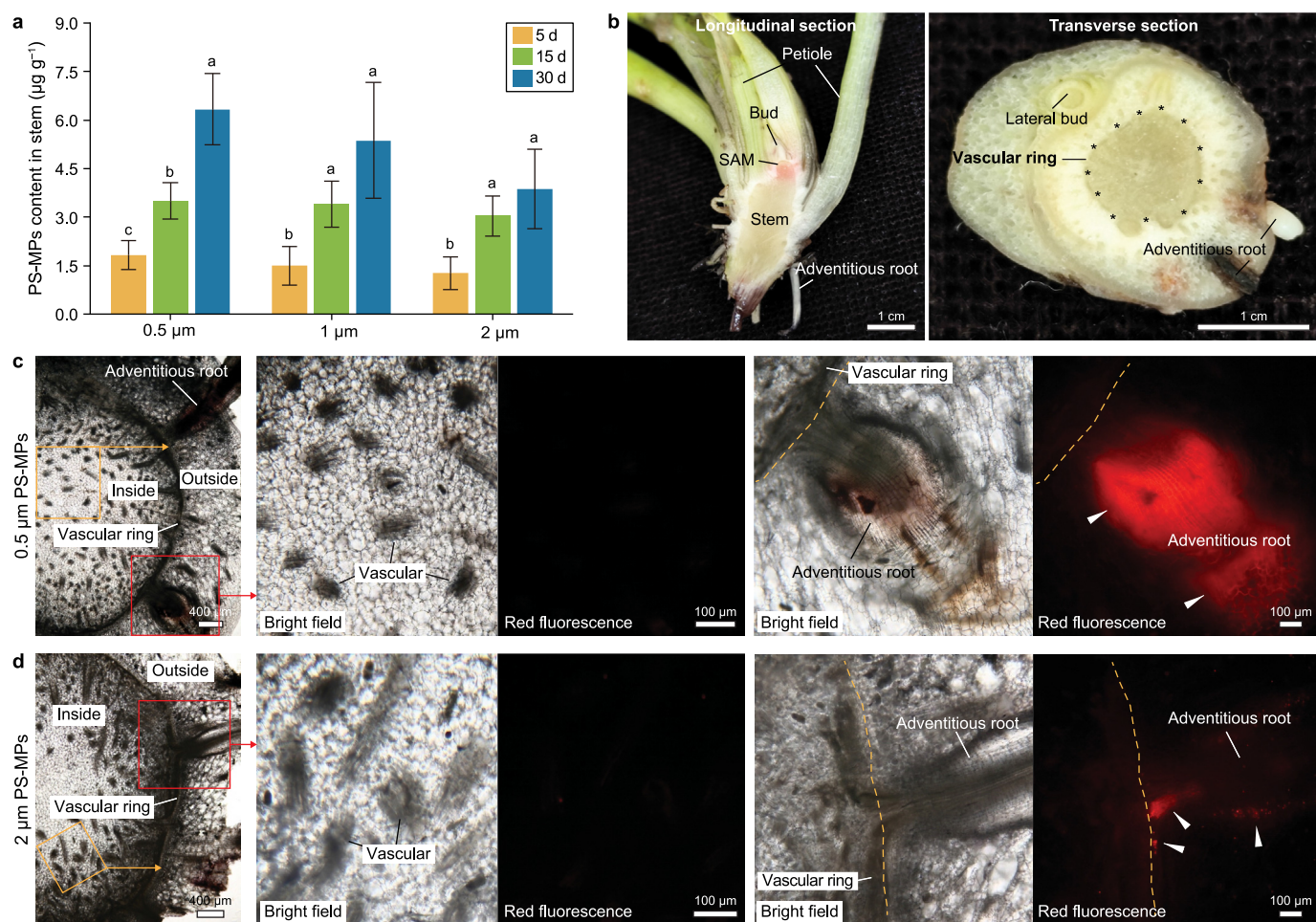
#### 4.2. Adsorption of PS-MPs by root caps is crucial for the removal of MPs

Previous studies have documented the adsorption of NPs/MPs by various plant species, including nori [52], duckweed [53], water spinach [54], and *Egeria densa* [55]. However, these reports provided only qualitative descriptions, highlighting the lack of quantitative evaluation of the plants' removal efficiency, adsorption capacity, and absorption capacity concerning MPs. In this study, it was observed that water hyacinth can develop extremely dense lateral roots (Fig. 4a), showing a robust ability to remove MPs in water (Fig. 3). The removal rates for 0.5, 1, and 2  $\mu\text{m}$  PS-MPs (at 50  $\text{mg L}^{-1}$ ) were found to be 55.3 %, 69.1 %, and 68.8 %, respectively, within 48 h (Fig. 3a). Moreover, the adsorption of roots on 0.5, 1, and 2  $\mu\text{m}$  PS-MPs was 22.3, 54.8, and 105 times that of absorption, respectively (Fig. 3b and c), underscoring the critical role of adsorption in the removal process. Further microscopy analyses revealed the preferential capture of PS-MPs by the extensive root caps of water hyacinth (Figs. 4 and 5).

The root cap is a specialized organ that serves the dual purpose of safeguarding delicate stem cells in the root tip and facilitating the reception and transmission of environmental signals to the developing root [56]. While the cap cells of the terrestrial plant are continuously generated, the overall small size of the organ remains constant [57]. Senescent cap cells naturally shed or dissolve, ensuring that the root cap consistently covers only the most apical region of the root [57,58].

However, water hyacinth has remarkably large root caps, with the surface area of a single cap approximately 100 times that of rice (refer to Fig. 5c and d). Together with the fact that each daughter plant can generate over 20,000 lateral roots, the total cap surface of one water hyacinth plant in the natural environment can reach up to 150,000–180,000  $\text{mm}^2$ . The huge cap not only covers the root apical meristematic tissue (RAM) but also extends to the elongation zone, protecting these tissues from harmful substances. This was confirmed by observing that after removing the cap, the front end of the root remained free from contamination by PS-MPs (Fig. 5b). Large root caps likely evolved over time to adapt to harsh aquatic environments [59]. They serve the dual purpose of shielding young root tips from aquatic organisms and microbial damage while unexpectedly capturing substantial amounts of MPs. Previous studies have observed a tendency for NPs/MPs to attach to the relatively small root caps of rice [35], *Arabidopsis*, and wheat [60], which may partially align with the adsorption mechanism of water hyacinth regarding MPs. However, the small and easily dislodged root caps of mesophytes [61,62] make them unsuitable for capturing MPs. In farmland, MPs tend to bind tightly to soil particles [63,64], indicating the limited effectiveness and feasibility of using mesophytic plants for MPs removal. On the contrary, the root caps of water hyacinth, with their characteristics of large number, huge size, and enormous surface area, endow it with a great ability to adsorb and remove MPs. Thus, floating plants, such as water hyacinth, present a more optimistic solution for addressing the pollution of MPs in aquatic environments.

Furthermore, this study encompassed the addition of other types of MPs — including PET, PE, PVC, and PP — into the nutrient solution. These MPs were also observed to be significantly adsorbed onto the root caps of water hyacinth (see Fig. S5, Supplementary Material), indicating that the plant can capture a wide range of MPs. Aquatic ecosystems often become contaminated with multiple



**Fig. 7.** Content of PS-MPs (a), anatomy (b), and distribution of 0.5  $\mu\text{m}$  (c) and 2  $\mu\text{m}$  (d) PS-MPs in the stems of water hyacinth. The significant differences in the content of PS-MPs in panel a between series days of exposure within each treatment are marked by the letters “a,” “b,” and “c” ( $p < 0.05$ , LSD test, one-way ANOVA). The exposure time in panels c and d was 30 days. The middle and right panels in c and d are the magnified views of the yellow and red boxed areas in the left panels, respectively. Note that the fluorescence of PS-MPs appears only in the vascular tissues where the root is attached to the stem (white arrowheads in panels c and d), indicating that PS-MPs were blocked outside the “vascular ring” (yellow dashed lines in panels c and d).

hazardous substances, including MPs, heavy metals, and organic pollutants [65,66]. Water hyacinth has shown remarkable tolerance and removal capabilities in relation to these pollutants [67–70], highlighting its potential application in managing complexly polluted water bodies.

#### 4.3. Root cracks are the main site of PS-MPs invasion

It has been demonstrated that MPs/NPs can infiltrate plants via apoplastic transport [27,71], root apices [35], or root cracks [72]. However, the representation of these invasion pathways differs among studies, which may be attributed to variations in plant species and the types of MPs/NPs employed. This study revealed that PS-MPs were unable to breach the epidermis of water hyacinth root (Fig. 6), indicating a low likelihood of entering the root stele via the apoplastic pathway. Despite the weak cell walls at the root tips, the presence of huge caps shielded them from PS-MPs contact (Fig. 5a and b). Consequently, the root apices did not emerge as prominent sites for PS-MPs invasion. Through the serial sections of adventitious roots, this study pinpointed root cracks as the precise location of MPs invasion (Fig. 6). The growth of lateral roots broke through the epidermis of the adventitious root, generating numerous cracks (Fig. 6, left column). The specific distribution of

PS-MP particles between these cracks and the middle column (Fig. 6, right column) further demonstrates that cracks are the main pathway for the uptake of MPs. This crack-entry mechanism mirrors the phenomenon observed in lettuce and wheat roots [72].

A further finding of this study indicates that the valid size for PS-MPs to penetrate water hyacinth's root crack is  $\leq 0.5 \mu\text{m}$  (Fig. 6a). PS-MPs larger than 1  $\mu\text{m}$  were mostly blocked from entering the cracks (Fig. 6b and c). Additionally, the uptake of PS-MPs in three different sizes (0.5, 1, and 2  $\mu\text{m}$ ) by the roots was minimal, with only 48, 26, and 12  $\mu\text{g g}^{-1}$  (fresh weight), respectively (Fig. 3c). Thus, it can be concluded that water hyacinth root achieves rapid removal of PS-MPs primarily by adsorption.

It is worth noting that not all plant roots are capable of absorbing MP or NP particles. For instance, 80 nm PS-NPs adhere only to the root surface of water spinach, without entering the vascular bundles [54]. On the other hand, 1  $\mu\text{m}$  PS-MPs attach to the surface of *Arabidopsis* and wheat roots but do not penetrate the root interior [60]. This phenomenon may be attributed to the limited number of lateral roots and cracks in these plants. Therefore, more comparative studies are needed to understand the variations in the resistance of different plant roots to NPs/MPs invasion and the underlying mechanisms.



#### 4.4. The stem “vascular ring” prevents PS-MPs from entering the leaves

Several studies have shown that PS-MPs can move from roots to aboveground tissues. For instance, 1  $\mu\text{m}$  PS-MPs have been observed to penetrate rice roots and reach the stalks' vascular bundles [35], while 0.2  $\mu\text{m}$  PS particles can enter pea roots and reach fruit pods and seeds [73]. This raises concerns about the potential NPs/MPs contamination of food and vegetable crops. The findings of this study revealed that following an extended 30-day exposure period, only a minimal amount of PS-MPs was detected in water hyacinth stems (Fig. 7), with no presence of PS-MPs in the leaves (Supplementary Materials Figs. S3–S4). This indicates the existence of a defense mechanism in the stem that hinders the further transport of PS-MPs. Microscopic examinations of the stems revealed the presence of distinct vascular ring structures (Fig. 7b). This vascular ring is densely organized and blocks all MP particles transported from the root (Fig. 7c and d). Actually, PS-MPs remain confined at the root-stem junction and do not enter the stem, thereby safeguarding the aerial portion from MPs contamination. This effective protective mechanism for aerial tissues also ensures that water hyacinth can sustain leaf function, photosynthesis, and rapid biomass growth, even under 50  $\text{mg L}^{-1}$  PS-MPs exposure (Fig. 1).

Water hyacinth grows rapidly, and its leaves are rich in cellulose, starch, and protein, making it a potential source of sustainable energy and clean fuel [74,75]. Numerous studies have demonstrated the feasibility of using water hyacinth in biofertilizer and biofuel production [76–78]. Additionally, biochar derived from water hyacinth can aid in  $\text{CO}_2$  sequestration [79,80]. Furthermore, our study revealed that water hyacinth plants used for MPs removal had partially uncontaminated leaves, which can be effectively utilized to offset the costs of treating MP-polluted water, while the relevant techniques require further in-depth exploration.

## 5. Conclusion

The contamination of MPs in aquatic environments is on the rise, posing significant risks to both ecosystems and human health. Phytoremediation offers a cost-effective solution for the removal of MPs while minimizing the risk of secondary pollution. Viewed through the lens of microstructure, we first clarified the dual mechanisms by which water hyacinth effectively captures MPs and is resilient to them. This capture is facilitated by the large, highly absorptive root cap, which is absent in terrestrial plants and enables water hyacinth to remove MPs from the water effectively. For the small number of MPs that invade the root, water hyacinth employs effective resilience reactions. First, it mitigates oxidative damage through inherently high POD activity in its root. Second, it prevents the transfer of MPs to aerial parts via a dense “vascular ring” in the stem, ensuring the normal functioning of the leaves and supporting rapid plant growth. Our study demonstrates that water hyacinth is a promising remediation plant, highlighting the potential of phytoremediation techniques in addressing the issue of MPs pollution.

### CRedit authorship contribution statement

**Jingjing Yin:** Writing – original draft, Investigation, Formal analysis, Conceptualization, Methodology, Software. **Tongshan Zhu:** Writing – original draft, Data curation, Formal analysis. **Xiaozun Li:** Resources, Investigation, Project administration. **Fayuan Wang:** Writing – review & editing, Data curation, Resources. **Guoxin Xu:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization, Writing – original draft.

### Declaration of competing interest

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.

### Acknowledgments

This research was supported by the Key Research and Development Project of Shandong Province (2022LZGC024), and the Natural Science Foundation of Shandong Province (ZR2021MC178).

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ese.2025.100540>.

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