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## Tracing and trapping micro- and nanoplastics: Untapped mitigation potential of aquatic plants?

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### ABSTRACT

Micro- and nanoplastics are emerging concerns due to their environmental ubiquity and currently largely unknown ecological impacts. Leveraging on a recently developed method using europium-doped polystyrene particles (PS-Eu), our present work aimed to accurately trace the uptake and transport of micro- and nanoplastics in aquatic plants and shed insights into the potential of different aquatic plants for trapping and removal of plastics from water environment. Seedlings of *Vallisneria spiralis* Makino (submerged plant), *Iris tectorum* Maxim (emergent plant), and *Eichhornia crassipes* Solms (floating plant) were exposed to 100 nm and 2 μm PS-Eu in freshwater (5 μg/mL) or sediments (5 μg/g) for 8 weeks. Fluorescence imaging clearly evidenced that PS-Eu mainly accumulated in the intercellular space and were transported from roots to leaves via the apoplastic path and vascular bundle. Mass spectrum analysis demonstrated that up to 6250 μg/g nanoplastics were trapped in aquatic plants (mainly in roots) with a bioconcentration factor of 306.5, depending on exposure routes and plant species. Owing to their excellent capture capability and high tolerance to plastic exposures, floating plants like *E. crassipes* are promising for immobilizing and removing fine plastics from the water environment.

### 1. Introduction

Plastics are a true hallmark of the Anthropocene (Zalasiewicz et al., 2016). Over the past few decades, the global production of plastic polymers has increased exponentially to meet the growing demands for human use (Maity and Pramanick, 2020). Although the benefits of plastics are far-reaching, mismanagement and improper disposal of plastic waste are escalating plastic pollution worldwide. It is estimated that only 9% of the 9 billion metric tons of plastic ever produced has been recycled and that most plastic ends its life in landfills and the natural environment (Geyer et al., 2017). Complex sources, such as sewage discharge, surface runoff, atmospheric fallout, and direct waste disposal, contribute to the occurrence of plastics in aquatic environments (Dris et al., 2016; Mintenig et al., 2020; Murphy et al., 2016; Wu

et al., 2022). By 2030, even with ambitious efforts to reduce and manage plastic waste globally, up to 53 million metric tons per year will enter the water environment (including fresh and saltwater) (Borrelle et al., 2020). Rivers and lakes carry plastic waste from deep inland to the sea, making them major contributors to ocean pollution (UNEP, 2018).

Plastics undergo uncontrolled deterioration and fragmentation into microplastics (<5 mm) and nanoplastics (<1 μm) by physicochemical processes in the natural environment (da Costa et al., 2016). Although the actual concentrations of nanoplastics in aquatic environments are still unknown due to sampling and analytical limitations, it is generally believed that the environmental concentration of microplastics could be as high as parts per million (Cai et al., 2021; Fischer and Scholz-Bottcher, 2019). These plastic particles can be transported with and carry a panoply of hazardous chemicals and pathogens to diverse

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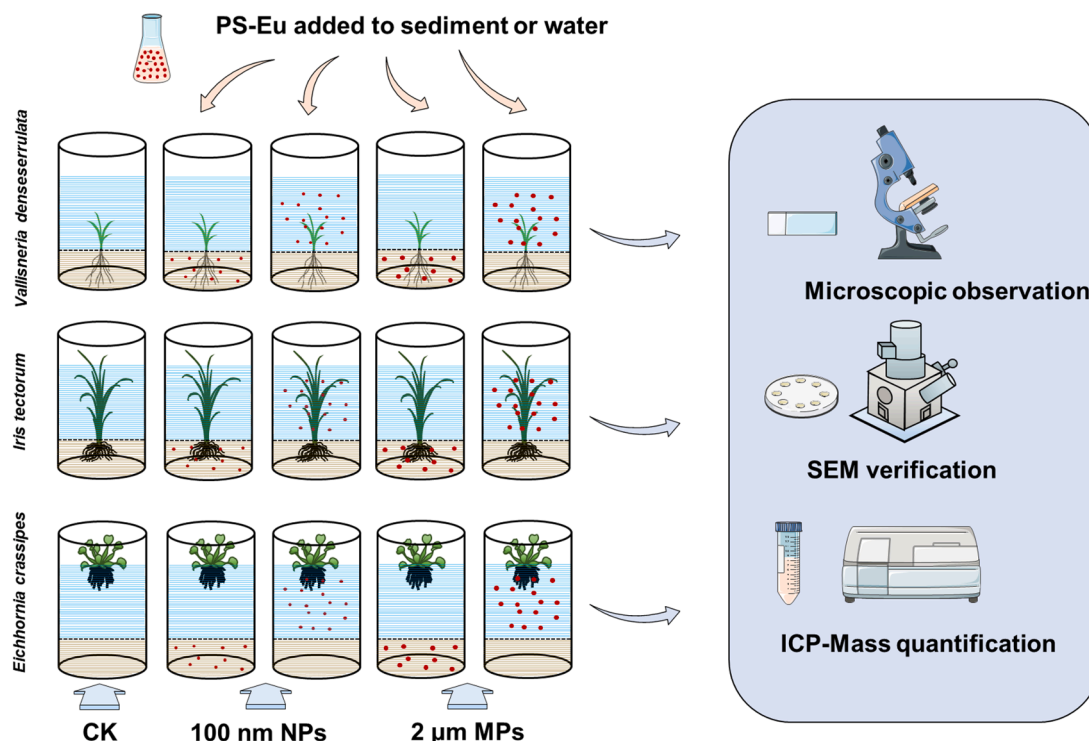


Fig. 1. Experimental design of the present study.

aquatic environments, potentially affecting aquatic organisms and consequently threatening ecosystem functioning and services (Alimi et al., 2018; Arias-Andres et al., 2018; Yang et al., 2020). Increasing reports on the ingestion of plastic particles by aquatic animals and plants in organismal guts, tissues, and food webs, so far, mainly focusing on invertebrates or small animals (Granek et al., 2020). Given their troubles in the food chain, small plastic particles have been cited as an emerging issue of environmental concern (Eerkes-Medrano et al., 2015).

There remain still significant knowledge gaps in understanding uptake and ecological effects of micro- and nanoplastics in aquatic plants. Several studies have demonstrated the potential of aquatic plants to trap and filter plastics (Cesarini and Scalici, 2022; Helcoski et al., 2020). Penny grass (*Hydrocotyle vulgaris*) was shown to accumulate nanoscale polymer dots in stems and blades (Li et al., 2020a). Physical adsorption of polystyrene microplastics by edible seaweed (*Fucus vesiculosus*) was confirmed, suggesting the microplastic-trapping ability of seaweed (Sundbæk et al., 2018). The previous study has also confirmed that fluorescent nanoplastics could be absorbed by the freshwater plant (*Ceratopteris pteridoides*) and affect its growth and development (Yuan et al., 2019). However, quantitative analyses of plastic particles, particularly in the submicrometre range, are still challenging due to analytical difficulties in extracting and measuring carbon-based polymers in plant samples. Compared to fluorescence-based methods, metal-doped plastic particles provide a promising and quantitative tool to assess the biological fate of micro- and nanoplastics at trace levels (Abdolahpur Monikh et al., 2022; Facchetti et al., 2020; Mitrano et al., 2019). Meanwhile, the high photostability and lower power density requirements of the lanthanide family (e.g. europium), make these background-free persistent fluorophores ideal tools for imaging purposes (Crawford et al., 2015; Luo et al., 2022).

In this study, leveraging europium-doped polystyrene (PS-Eu), we aim to quantify the uptake and transport of micro- and nanoplastics in three typical freshwater plants representing submerged, emergent, and floating plants, i.e., *Vallisneria denseserrulata*, *Iris tectorum*, and *Eichhornia crassipes*. We hypothesize that the growth habitat and root structures of different aquatic plant species affect their uptake and

translocation of micro- and nanoplastics. To simulate different environmental conditions, *V. denseserrulata* and *I. tectorum* were exposed to the overlying water or sediment contaminated by PS-Eu, while *E. crassipes* were only exposed to the water contaminated by PS-Eu (Fig. 1). After 8 weeks of chronic exposure, the distribution and translocation of PS-Eu in plant tissues were verified by confocal laser scanning microscopy and scanning electron microscope imaging. The uptake of PS-Eu particles into various plants was accurately determined by inductively coupled plasma mass spectrometry. Meanwhile, the growth responses and bioconcentration factors of various aquatic plants to micro- and nanoplastic under different exposure routes were evaluated. The results provide the first quantitative assessment on absorption, accumulation, and transport of micro- and nanoplastics in different types of freshwater plants after chronic exposure, showing the potential of hyper-accumulator aquatic plants for the phytoremediation of fine plastics in natural waters.

## 2. Material and methods

### 2.1. Collection and preculture of aquatic plants

Seedlings of *V. denseserrulata*, *I. tectorum*, and *E. crassipes* were collected from the Wuhan Botanical Garden of the Chinese Academy of Sciences (30°32'54"N, 114°25'39"E) in May 2022. The three species represent different types of aquatic plants, i.e., submerged, emergent, and floating plants, which are facing different exposure environments to micro- and nanoplastics. All seedlings were cleaned with deionized water and acclimated to well-controlled experimental conditions (light intensity of 5000lx, the day-night interval of 12 h: 12 h, and temperature of 25 ± 2 °C) in a plant culture room for two weeks. Nitrile gloves and a cotton lab gown were worn during field sampling and laboratory operation for minimizing anthropogenic plastic contamination. To provide adequate nutrition sources, the sediment used to cultivate the plants was collected *in situ* where the seedlings grew whereas the culture medium was based on a 10% Hoagland solution (Coolaber, Beijing, China; Table S1).

## 2.2. Characterization of PS-Eu particles

Polystyrene was used as a model plastic in this study because it is one of the most frequently observed plastic types in freshwater environments (Yuan et al., 2022). The PS-Eu particles were custom synthesized by Shanghai Huge Biotechnology Co., Ltd, China. The PS-Eu particles with nominal sizes of 100 nm and 2  $\mu\text{m}$  were prepared via a combined swelling-diffusion technique as described in our previous study (Luo et al., 2022). Free Eu that was not incorporated into the polystyrene particles was removed by dialysis at a molecular weight cut-off of 3000 Da. The spherical particles were supplied in deionized water as mono-dispersed suspensions and stored at 4  $^{\circ}\text{C}$  in the dark before use. The Eu leaching from the PS-Eu particles in the 10% Hoagland solution was monitored to be less than 0.5% after 8 weeks using a centrifugal ultra-filtration technique (Luo et al., 2022). The morphology of the PS-Eu particles was characterized by scanning electron microscopy (Quanta250, FEI, Hillsboro, US), and their actual particle sizes were shown to be  $101.1 \pm 1.2$  nm and  $19.7 \pm 1.5$   $\mu\text{m}$ , respectively (Fig. S1a, b). The fluorescence lifetime imaging of the PS-Eu particles was conducted by a time-resolved fluorescence microscope (MicroTime200, PicoQuant, Berlin, Germany) (Fig. S1c). The fluorescence spectrometer analysis of the PS-Eu particles was performed with a fluorescence lifetime spectrometer (FluoTime300, PicoQuant, Berlin, Germany) (Fig. S1d).

## 2.3. Exposure of plants to PS-Eu particles

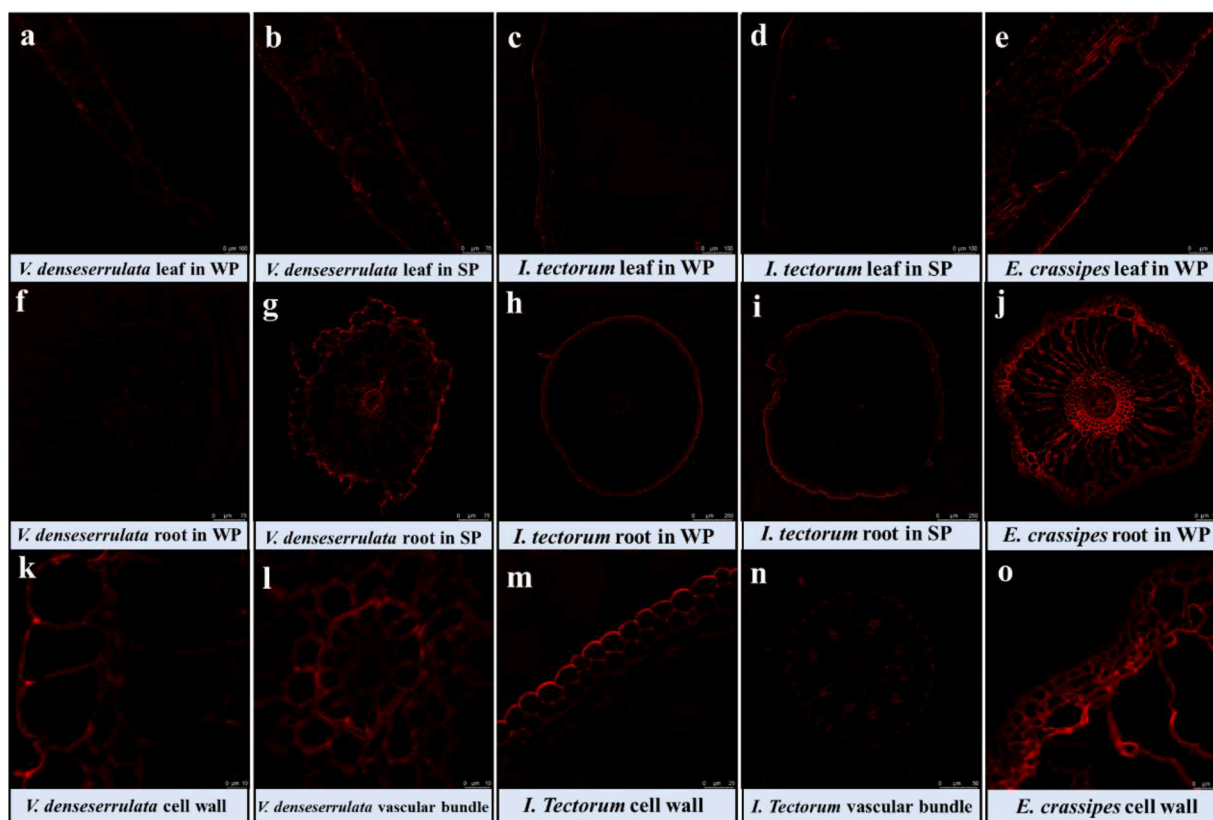
The sediment was first filtered with a 50  $\mu\text{m}$  stainless steel mesh to remove gravel and residual leaves and then stirred and homogenized with a steel mixer for 20 min. To prepare PS-Eu-contaminated aquatic microcosms, PS-Eu particles were either added to the homogenized sediment (5  $\mu\text{g/g}$  wet weight; referred to as SP microcosm) before

adding the overlying water (10% Hoagland solution) or added directly to the overlying water (5  $\mu\text{g/mL}$  PS-Eu particles; referred to as WP microcosm). The concentration used has been proven to be effective in observing the distribution of PS-Eu in plants (Luo et al., 2022). Each microcosm contained 250 g of sediment, 250 mL of water, and one seedling (Fig. 1). The control group of each plant species was set as a microcosm without any PS-Eu particles. There were nine replicated microcosms for each treatment group. To avoid the technical plastic contamination, all containers and apparatus were washed thoroughly with filtered tap water before use. The environmental parameters of the culture room were the same as the preculture conditions described above (i.e., 5000lx, 12 h: 12 h day-night interval, and  $25 \pm 2$   $^{\circ}\text{C}$ ). The prolonged exposure lasted for 8 weeks, and the overlying water was replenished twice a week during the entire exposure period.

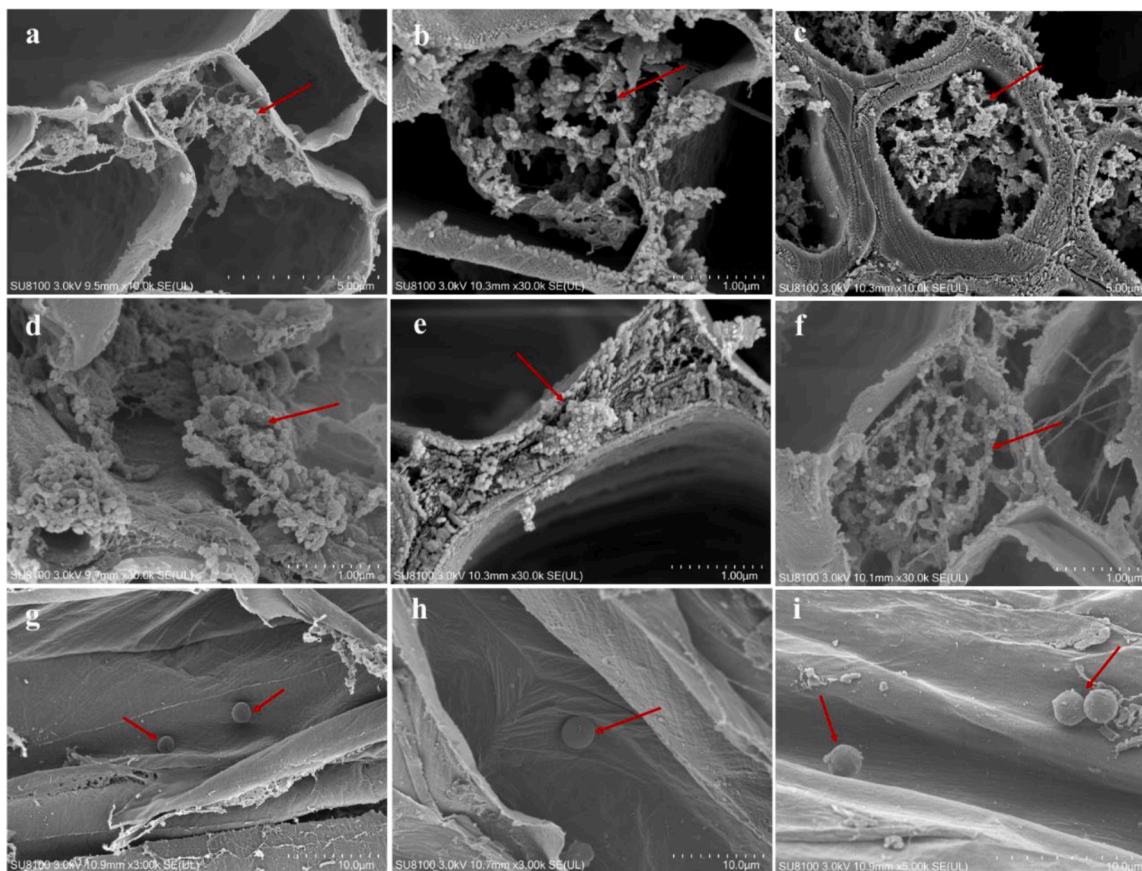
## 2.4. Visualization of PS-Eu particles in aquatic plants

After 8 weeks of exposure, plants were gently removed from the microcosms and carefully washed with distilled water. Before fluorescence imaging, the roots and leaves of fresh plants were separated by a scalpel and embedded in 4% agarose. The roots (in the mature zone) and leaves (with the primary vein) were sectioned into 40- and 100  $\mu\text{m}$ -thick slices, respectively, using a vibrating microtome (VT1000S, Leica, Wetzlar, Germany). Subsequently, these slices were placed on glass slides and covered with a coverslip after adding a drop of phosphate buffer solution. The PS-Eu particles in roots and leaves were visualized with a confocal laser scanning microscopy (TCS SP8, Leica, Wetzlar, Germany) under an excitation of 405 nm and an exposure time of 100 ms.

To verify the presence of PS-Eu particles in plant tissues, the samples were sectioned into small pieces and freeze-dried with a freezing vacuum dryer (FreeZone 4.5 L, Labconco, Kansas, US). The samples were



**Fig. 2.** Fluorescent images of the PS-Eu particle distribution in different aquatic plants in WP and SP microcosms. Cross section of leaves (a–e); cross section of roots (f–j); cell wall (k, m, and o) and vascular bundle (i and n) of roots. Seedlings were exposed to 5  $\mu\text{g/mL}$  or 5  $\mu\text{g/g}$  PS-Eu particles for 8 weeks. WP, PS-Eu added to water; SP, PS-Eu added to sediment.



**Fig. 3.** SEM images of PS-Eu particle localization in different aquatic plants. Nanoplastic localization in the roots of *V. denseserrulata* (a), *I. tectorum* (b), and *E. crassipes* (c). Nanoplastic localization in the leaves of *V. denseserrulata* (d), *I. tectorum* (e), and *E. crassipes* (f). Microplastic localization in the roots of *V. denseserrulata* (g), *I. tectorum* (h), and *E. crassipes* (i). Seedlings were exposed to 5  $\mu\text{g}/\text{mL}$  or 5  $\mu\text{g}/\text{g}$  PS-Eu particles for 8 weeks.

affixed to double-sided conductive carbon adhesive and coated with gold for 60 s in an ion beam sputter coater (MC1000, Hitachi, Tokyo, Japan). Then the root and leaf samples were examined with a scanning electron microscope (SU8100, Hitachi, Tokyo, Japan) at an electron accelerating voltage of 3.0 kV. Three random samples were examined for each treatment group.

### 2.5. Quantification of PS-Eu particles

The PS-Eu-exposed plant samples were cleaned by ultrasonication to remove adsorbed particles. Then, 5 mL  $\text{HNO}_3$  and 1 mL  $\text{H}_2\text{O}_2$  were added to 0.25 g tissue and the sample was digested completely with a microwave digestion system (ETHOS ONE, Milestone, Milan, Italian) at 180  $^\circ\text{C}$  for 6 h. To determine the biotic and abiotic exchange of PS-Eu particles between water and sediment during the experiment, 5 mL  $\text{HNO}_3$  and 1 mL  $\text{H}_2\text{O}_2$  were added to 0.25 g sediment (or 0.25 mL water). The concentration of Eu in the samples was analyzed with an inductively coupled plasma-mass spectrometry (7500i, Agilent, Santa Clara, US). The quantification of the PS-Eu concentrations in samples was performed based on the measured concentrations of Eu using the linear equations obtained by exogenous PS-Eu particles after subtracting the background value from the control group (Fig. S3). The ratio of PS-Eu concentration in water and sediment was calculated to estimate the sedimentation and dispersion of PS-Eu in water and sediment. The bioconcentration and translocation of PS-Eu particles in aquatic plants were quantified by the bioconcentration factors (BCFs) and the translocation factors (TLFs), respectively. The bioaccumulation factors (BCFs) were defined as the ratio of the average concentration of PS-Eu particles in the plants to the corresponding concentration in the media. The translocation factors (TLFs) were defined as the ratio of the

calculated concentration of PS-Eu particles in the leaves to the corresponding concentration in the roots.

### 2.6. Assessment of the biological effects

At the end of the exposure, the seedlings were gently removed from the microcosms and thoroughly rinsed with deionized water. Subsequently, the plant heights of seedlings in all microcosms were recorded and the fresh weights of root and shoot tissues were measured immediately. For oxidative stress assays, 0.1 g plant tissues were homogenized in 1 mL of distilled water. The samples were centrifuged at 3000 r.p.m. for 5 min to remove the residues. The catalase activity was measured using kits (Librui Biotechnology Co., Ltd, Wuhan, China). It was analyzed following the manufacturer's instructions with a UV-visible spectrophotometer (UV-1800, Thermofisher, Waltham, US).

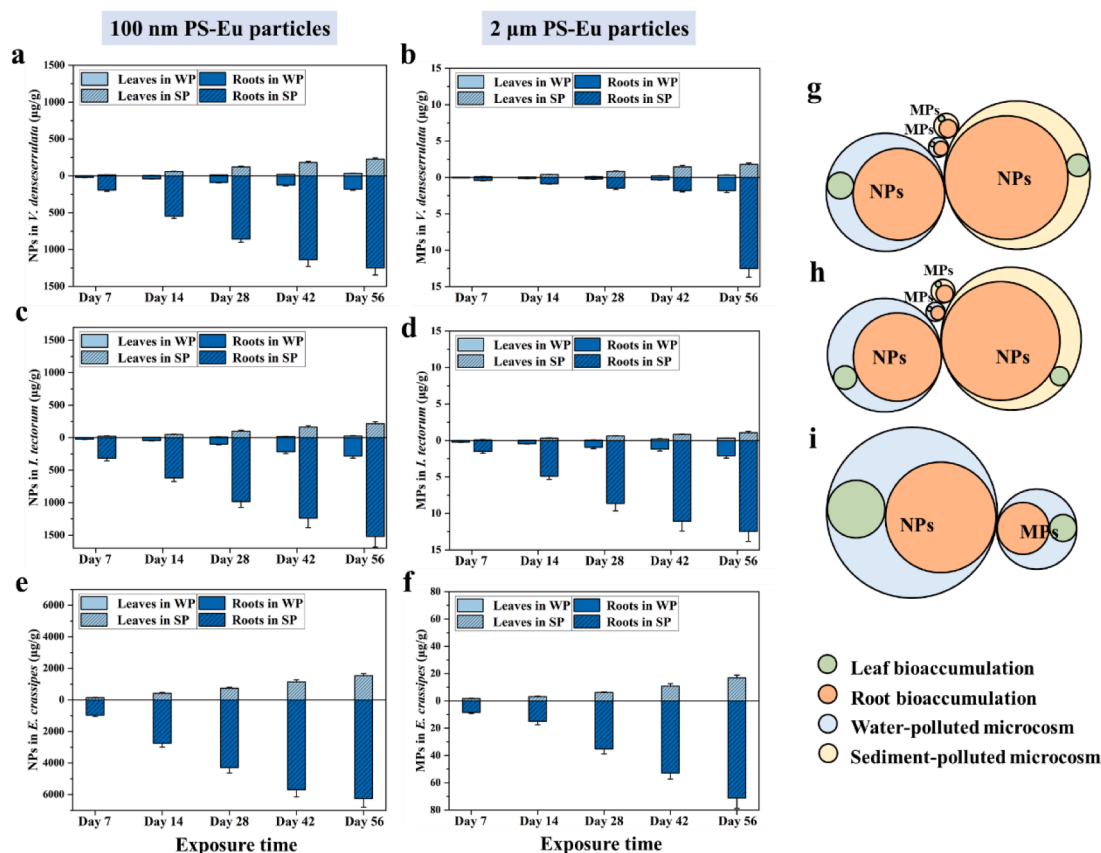
### 2.7. Statistical analysis

All values were expressed as mean  $\pm$  standard errors ( $n = 9$ ). Data in this study were evaluated by one-way analysis of variance followed by Tukey's test. Statistical analysis was performed using the software package IBM SPSS Statistics (v.16.0). The difference was considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. Uptake and distribution of PS-Eu particles in aquatic plants

After exposure to PS-Eu particles for 8 weeks, the presence of PS-Eu particles in the roots and leaves of the exposed aquatic plants was



**Fig. 4.** Concentrations of PS-Eu particles in plant tissues. Concentrations of nanoplastics and microplastics in the roots and leaves of *V. denseserrulata* (a,b), *I. tectorum* (c,d), and *E. crassipes* (e,f). Circular packing showing the bioaccumulation of micro- and nanoplastics in *V. denseserrulata* (g), *I. tectorum* (h), and *E. crassipes* (i) under different exposure routes. All the concentrations were calculated with dry weight. Seedlings were exposed to 5 µg/mL or 5 µg/g PS-Eu particles for 8 weeks. The concentration of Eu determined by ICP-MS was converted to the concentration of PS-Eu particles using the linear equation obtained using exogenous PS-Eu particles after subtracting the background Eu value from the control. WP, PS-Eu added to water; SP, PS-Eu added to sediment.

observed under UV light excitation. In contrast to the control group, wherein no fluorescence was exhibited (Fig. S2), red fluorescence was observed in the roots and leaves of the plants exposed to PS-Eu particles (Fig. 2). The red fluorescence in the roots and leaves of *E. crassipes* was stronger than that in *V. denseserrulata* and *I. tectorum*. In *V. denseserrulata* and *I. tectorum*, the red fluorescence in roots and leaves was stronger when PS-Eu particles were added to sediment than when PS-Eu were directly added to water. The red fluorescence in the roots of the same plant was generally stronger than the fluorescence in the leaves. Specifically, PS-Eu particles were mainly located in the intercellular space of the epidermis and the vascular bundle of the roots of *I. tectorum*, and small amounts of PS-Eu particles were distributed in the leaf margin and vein of *I. tectorum*. Moreover, strong red fluorescence was detected in the root cracks of *V. denseserrulata* and reached vascular tissues through apoplastic channels. Similar results were obtained for *E. crassipes*. Unlike 100 nm PS-Eu particles, 2 µm PS-Eu particles were only observed at the edge of the plant roots and leaves, indicating limited uptake by the aquatic plants.

The uptake of PS-Eu particles by the tested aquatic plants was further verified by scanning electron microscopy (SEM) imaging. The SEM images confirmed that accumulation of the 100 nm PS-Eu particles was mostly confined to the cytoderm gap and vascular bundle of roots, and some particles were observed in the leaf vein (Fig. 3a-f). The presence of 2 µm PS-Eu particles was only observed in the roots after longitudinal cutting (Fig. 3g-i).

### 3.2. Concentrations of PS-Eu particles in roots and leaves

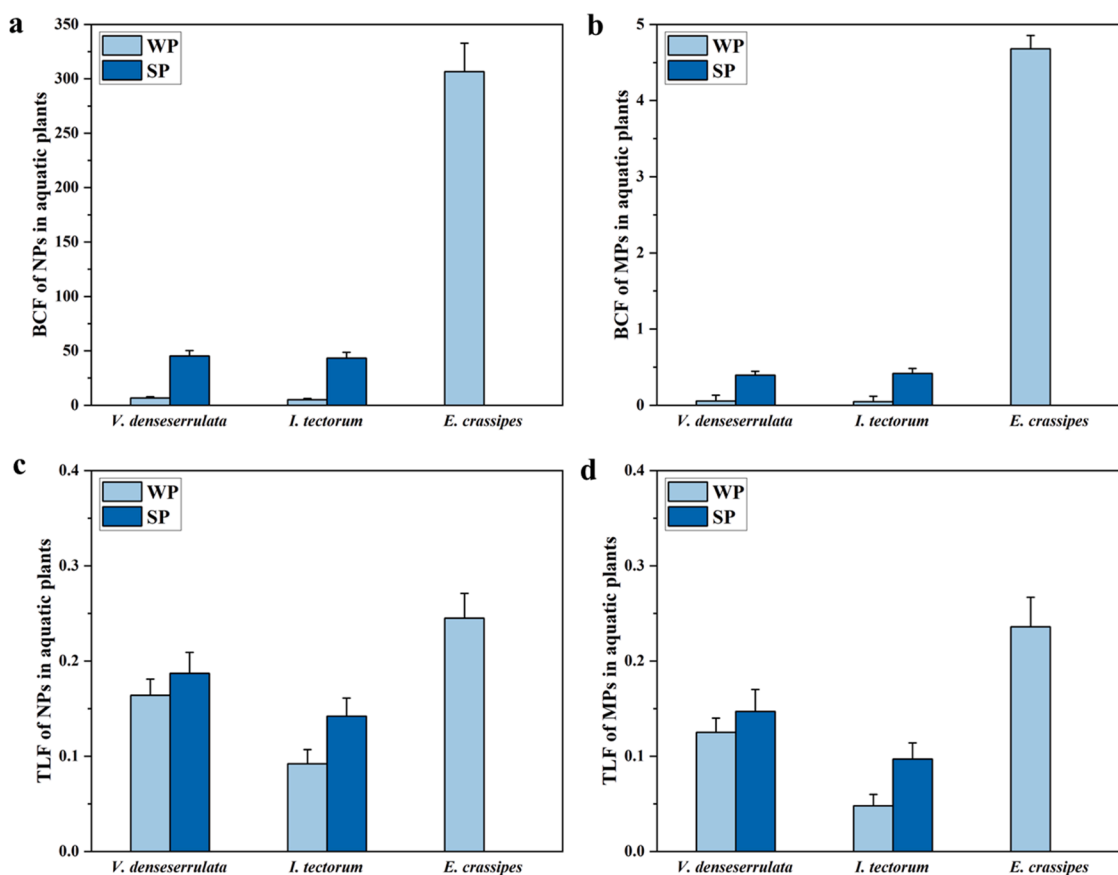
Serial dilutions of the 100 nm and 2 µm PS-Eu particles were

prepared for ICP-MS analysis to quantify Eu concentrations as a function of their mass. The Eu concentration increased linearly with increasing calculated PS-Eu particle mass (Fig. S3). The Eu in the 100 nm and 2 µm PS-Eu particles were estimated to be 2.0 and 1.5 wt%, with loads of  $4.4 \times 10^4$  and  $2.1 \times 10^9$  Eu chelates for each 100 nm and 2 µm PS-Eu particle, respectively. The loading relationships were used to determine the mass and quantity of PS-Eu particles in leaf and root samples of different aquatic plants.

No detectable Eu was found in the tissues of the control plants. The concentration of PS-Eu particles in aquatic plants increased gradually with exposure time (Fig. 4). After 8 weeks of exposure, the concentration of nanoplastics in aquatic plants reached 216.4~1532.5 µg/g and 1247.6~6250 µg/g in leaves and roots, respectively, which were significantly higher than those of microplastics (1.2~16.9 µg/g and 12.1~71.4 µg/g in leaves and roots, respectively; Table S2). For submerged plant *V. denseserrulata* and emergent plant *I. tectorum*, sediment-polluted treatments lead to higher concentrations of PS-Eu particles in plant tissues (216.4~1519.7 µg/g) than those of water-polluted treatments (25.98~282.2 µg/g) (Fig. 4a-d). While in the water-polluted treatments, the concentrations of PS-Eu particles in floating plant *E. crassipes* (16.9~71.4 µg/g nanoplastics and 1532.5~6250 µg/g microplastics) were much higher than that of submerged plants and emergent plants (Fig. 4e,f).

### 3.3. Enrichment and translocation of aquatic plants to PS-Eu particles

After 8 weeks of exposure to 5 µg/mL PS-Eu particles in water, the average bioconcentration factors (BCFs) of nanoplastics in *V. denseserrulata*, *I. tectorum*, and *E. crassipes* were 6.59, 5.23, and



**Fig. 5.** Enrichment of PS-Eu particles in aquatic plants. Bioconcentration factors (BCFs) of nanoplastics (a) and microplastics (b) in different aquatic plants. Translocation factors (TLFs) of nanoplastics (c) and microplastics (d) in different aquatic plants. WP, PS-Eu added to water; SP, PS-Eu added to sediment.

306.51, respectively (Fig. 5a). When PS-Eu particles were added to sediment, the calculated BCFs reached 45.3 and 43.3 for *V. denseserrulata* and *I. tectorum*, respectively (Table S3). The BCFs of microplastics were several orders of magnitude lower than those of nanoplastics (Fig. 5b). The translocation factors (TLFs) of PS-Eu particles ranged from 0.048 to 0.245 (Fig. 5c,d). Among them, the TLFs of PS-Eu particles in *E. crassipes* (0.236~0.245) were higher than those in *V. denseserrulata* and *I. tectorum* (0.048~0.187).

At the end of the exposure, higher bioaccumulation was found in the water-polluted microcosm than in the sediment-polluted microcosm (Fig. 5e-g). Most of the PS-Eu particles (78.24~91.56%) accumulated in the roots of plants, while fewer particles (8.44~21.76%) accumulated in the plant leaves (Fig. S4). The concentration of PS-Eu particles was also measured in the overlaying water in the microcosms, where it decreased by 11.9%, 13.8%, and 32.7% for the *V. denseserrulata*, *I. tectorum*, and *E. crassipes* after 8 weeks of exposure to 5  $\mu\text{g}/\text{mL}$  PS-Eu particles, respectively (Fig. S5).

### 3.4. Biological effects after exposure

The chronic exposures to PS-Eu particles (5  $\mu\text{g}/\text{mL}$  in water or 5  $\mu\text{g}/\text{g}$  in sediment) did not show any significant effect on the primary growth factors of aquatic plants, including height and biomass (Table S4). Moreover, there were no significant differences in catalase activities of aquatic plants exposed to PS-Eu particles compared to the controls (Table S4), indicating a relatively high tolerance of aquatic plants in response to the exposure of PS-Eu particles.

## 4. Discussion

To our knowledge, this is the first exploration to trace and quantify

the absorption of micro- and nanoplastics by aquatic plants using metal-doped PS particles. PS is the most-used model polymer in laboratory experiments and represents one of the most common plastics found in freshwater environments (Yuan et al., 2022). Due to the current analytical challenges, our understanding of the environmental and biological fates of nanoplastics remains rather limited. Mitrano et al. (2019) developed a method to synthesize nanoplastics doped with metals, which was successfully applied to investigate the fate of nanoplastics in a wastewater treatment plant. Lanthanide chelates are distinctly advantageous, as they have long luminescence lifetimes, large Stokes shifts, sharp emission profiles, and visible-light excitation wavelengths (Weissman, 1942). Our recent study has demonstrated labeling plastic particles with lanthanide chelates as an effective way to trace the uptake and translocation of submicrometre plastics in crop plants (Luo et al., 2022). Furthermore, the Eu element doping allows the indirect quantification of submicrometre plastics in plant and animal tissues by ICP-MS, even at low concentrations. Together with previous studies, the present research shows the high advantages and utility of Eu-doped plastic particles to investigate the biological fate and behavior of submicrometre plastics in aquatic plants.

Nanoplastics effectively accumulated in the roots of aquatic plants and were transported to leaves in the present study (Fig. 6). The uptake of nanoplastics was also observed by crop plants in our previous study in lettuce (Li et al., 2020b). In the root tip, PS-Eu particles were mostly adsorbed on border cells and arranged along the root surface. A large amount of red fluorescence was attached to the root surface and the intercellular space. These results suggest that PS-Eu particles might be absorbed by the root in the maturation region and internalized into the stele via the apoplastic pathway (Sun et al., 2020). Consistent with the results of confocal imaging, the presence of PS-Eu particles was further confirmed in root and shoot tissues by electron microscopy imaging,

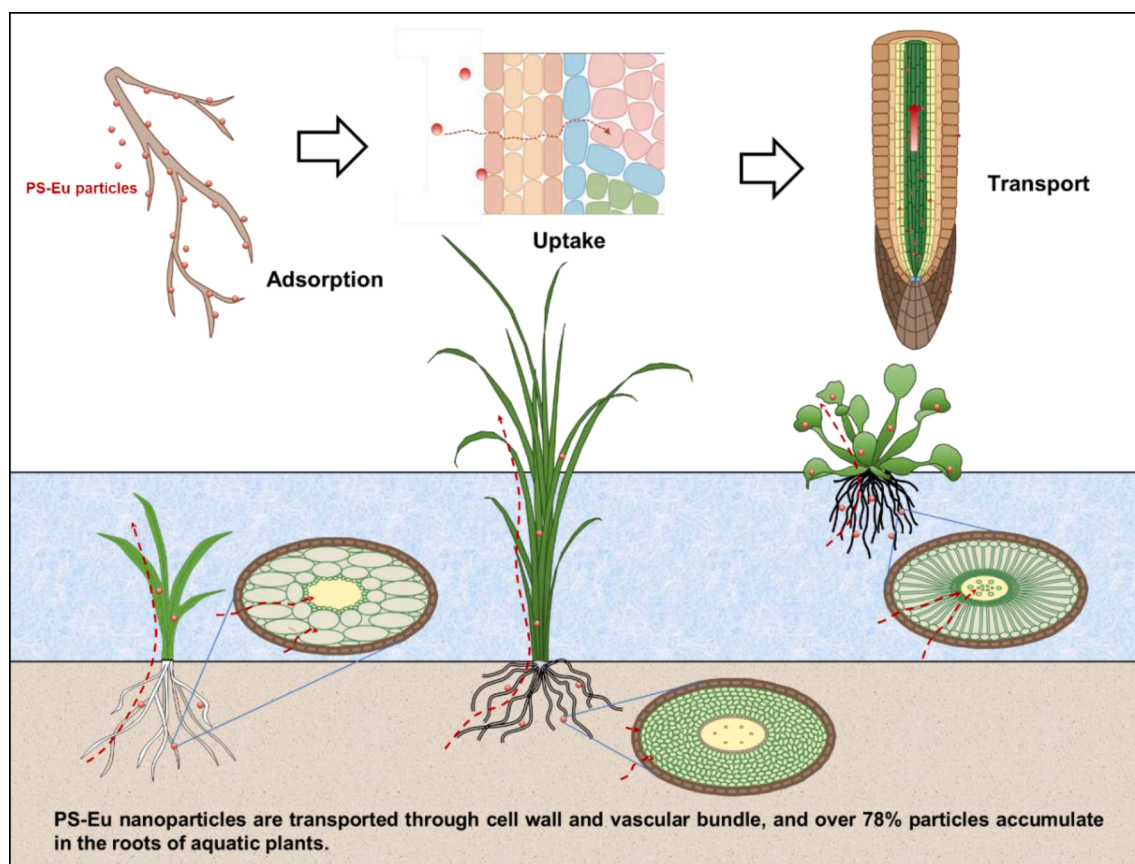


Fig. 6. Graphic showing the uptake and transport of PS-Eu particles by aquatic plants.

mainly in epidermal cells and the catheter of the xylem as well as in the leaf vein. Comparing different types of aquatic plants (i.e., submerged, emergent, and floating plants), we found higher translocation and bioaccumulation of nanoplastics in floating plants, which could be explained by the role of transpiration and different root structures.

Particulate matter must cross a series of chemical and physiological barriers of plants before uptake and translocation, which control the size exclusion limits (SELs) (Eichert et al., 2008). The root structures of different aquatic plant species may affect their uptake and translocation of micro- and nanoplastics. Compared with other plants, *E. crassipes* have a more concise apoplastic path, which may lead to easier transportation of plastic particles. It is generally accepted that relatively large nanoparticles (> 100 nm) can be taken up by plants despite the very small SELs (< 20 nm) of plant roots (Li et al., 2020b; Lian et al., 2020; Sun et al., 2021). One possible reason is that PS beads may compress and deform when being internalized because they are less stiff than the plant cell walls (Li et al., 2020b). This intrinsic property is essential for the migration of plastic particles within plant tissues, enabling them to reach the root central cylinder and the vascular tissues and resulting in their upward movement to the aerial parts of the plant.

The bioaccumulation of micro- and nanoplastics by aquatic plants is much higher than what was observed in terrestrial plants (Luo et al., 2022). In particular, the BCF of nanoplastics in *E. crassipes* reached 306.5 after 8 weeks of exposure, which is hundreds of times higher than those in crop plants (1.8–2.9). Previous studies have investigated the bioaccumulation of micro- and nanoplastics by various aquatic organisms, including invertebrates (Kuehr et al., 2022), zooplankton (Rist et al., 2017), zoobenthos (Redondo-Hasselerharm et al., 2021), shellfish (Al-Sid-Cheikh et al., 2018), as well as with fish species (Kim et al., 2021). Although it is difficult to compare the bioaccumulation in different organisms, floating plants like *E. crassipes* show a higher ‘plastic particle trapping’ potential than submersed or emersed plants

under similar exposure concentrations of plastic particles. The developed root system of *E. crassipes* has a large contact area with water, and its rhizosphere microorganisms and secretions might accelerate the aggregation and adsorption of plastic particles on the roots.

Compared with *E. crassipes*, *V. denseserrulata* and *I. tectorum* showed a lower uptake efficiency of plastic particles, yet bioaccumulation of nanoplastics was evident. In particular, the BCF values of these two plant species in the plastics contaminated-sediment microcosms were higher than those in the water-plastics contaminated-water microcosms, which is different from what has been observed for terrestrial plants in our previous study (Luo et al., 2022). The bioaccumulation of plastic particles by roots of *I. tectorum* was higher than in the case of *V. denseserrulata*, whereas the opposite was found for the leaves. It should be noted that this study cannot rule out the absorption of nanoplastics by submerged leaves. Zhao et al. (2017) indicated that CuO nanoparticles can be taken up by both roots and submerged leaves of *E. crassipes*. Aquatic plants might internalize more plastic particles than terrestrial plants in a more efficient way as both roots and leaves of aquatic plants can interact with plastic particles. Also, aquatic plants have more flexible cell wall structure and less developed vascular tissue, making them easier to transfer plastic particles than terrestrial plants.

There is currently no information on the environmental concentrations of nanoplastics but the environmental water concentrations of submicrometre plastics is expected to be in the range of a few to several hundred  $\mu\text{g L}^{-1}$  (Al-Sid-Cheikh et al., 2018). Therefore, the concentrations of micro- and nano-plastics used in this study (5  $\mu\text{g/mL}$  in water and 5  $\mu\text{g/g}$  in sediment) may be close to or slightly higher than their actual concentration in the water environments. The exposure concentrations of micro- and nanoplastics have no observable negative impact on all aquatic plants during the chronic exposure period. The effective bioaccumulation and enrichment of plastic particles by aquatic plants, as well as their high tolerance to exposure to plastic particles, make



aquatic plants useful for potential phytoremediation of plastic pollution in aquatic environments. In fact, floating plants like *E. crassipes* have been used to eliminate many other pollutants in water, such as heavy metals (Mishra and Tripathi, 2008), organic pollutants (De Laet et al., 2019), and antibiotics (Yan et al., 2020). Therefore, the high tolerance of *E. crassipes* to micro- and nanoplastics makes the species a good candidate for plastic remediation, while removing multiple pollutants from the water environment.

## 5. Conclusions

The PS-Eu particles are used for accurately quantifying the bioaccumulation of micro- and nanoplastics in different species of aquatic plants. Micro- and nanoplastics mainly accumulated in the intercellular space of aquatic plants and were transported from roots to leaves via the apoplastic path and vascular bundle. *E. crassipes* has the highest absorption capacity of plastic particles among the three plants investigated. A large amount of submicrometre plastic particles accumulated in the roots of aquatic plants, which have a far higher enrichment performance than known information in terrestrial plants. Prolonged exposure to relatively high concentrations of plastic particles has no significant adverse impact on the growth of aquatic plants, suggesting a low health risk of plastic particles at currently predicted environmental concentrations. The unique advantages of aquatic plants in absorbing nanoplastics and high tolerance to nanoplastic exposure show their great potential for the phytoremediation of nanoplastics in freshwater environments. The use of metal-doped plastic particles in plant uptake experiments also enables reliable screening for hyper-accumulator plants highly efficient for application in mitigation of environmental plastics pollution.

## 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.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2023.120249](https://doi.org/10.1016/j.watres.2023.120249).

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