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RESEARCH ARTICLE

Proposal of invader *Pontederia crassipes* as a savior of micro and macro size plastic pollution

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ABSTRACT

This study is the first report evaluating the microplastic (MP) and macroplastics capture potential of *Pontederia crassipes*. Total of 3691 (508 microplastic and 3183 macroplastic) particles were extracted from the roots of 12 examined specimens. Mean macroplastic abundance in the roots was found as 265±44 macroplastic/specimen. Majority of the extracted macroplastics were fragment in shape, blue in color. Mean microplastic abundance was found as 42±23 MPs/specimen. Majority of the extracted microplastics were fragment in shape, blue in color and less than 500 µm in size. Results of this preliminary study showed that this species have significant ability to adsorb micro and macroplastics by the roots which makes them perfect employees for integrated floating systems.

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Introduction

Microplastics, defined as synthetic polymers less than 5 mm in size (Thompson et al., 2009), are persistent and widespread polluters in aquatic environments. Till the first detection of microplastics in rivers (Moore et al., 2005), only limited efforts were made to understand the distribution, transport, occurrence of microplastics in rivers compared to marine environments (Wagner et al., 2014; Li et al., 2018, Kılıç et al., 2022).

Rivers are more susceptible to any kind of pollution as they are usually surrounded by anthropogenic influences. They usually contain higher amounts of micro and macro size plastic particles and variety of chemicals that alter the biological and chemical dynamics of ecosystem (Scherer et al., 2018). So far, majority of the studies focused on effects of microplastics on microorganisms such as algae, daphnids, mussels, fish (Miloloža et al., 2021), and have been deficient in terms of aquatic plants (Kalčíková et al., 2020; Ceschin et al., 2023). Nevertheless, microplastic exposure might cause inhibition in

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photosynthesis (Yang et al., 2023), limitation in root growth (Ceschin et al., 2023), direct toxicity from contaminated surfaces of MPs (Mammo et al., 2020) in vascular aquatic plants. Besides, MPs can change soil characteristics in sediments (Wang et al., 2020), vary the turnover rate of microbial communities (Wang et al., 2020) and alter the nutrient cycle (Yu et al., 2022). These types of fluctuations in environmental conditions form an indirect impact of microplastics on aquatic plants (Lozano & Rillig, 2020).

Pontederia crassipes is known as the most invasive aquatic plant in freshwater environments (Villamagna & Murphy, 2010). Even though it is originally from Brazil, it is now distributed to the Africa, Asia, Australia, India and North America (Villamagna & Murphy, 2010, Virginia Invasive Species, 2024). It is more widespread in water bodies containing high nutrient concentration (Villamagna & Murphy, 2010). The studied environment, Orontes River, suffered heavily from agricultural runoff, industrial and domestic wastewater discharges (Kılıç & Yücel, 2019) making it an ideal habitat for *P. crassipes.* It was first observed in the eastern part of the Türkiye, Hatay-Altınözü near the border with Syria in August 2010 (Uremis et al., 2014). Since this plant can reproduce both sexually and vegetatively, it doubles its biomass every ten days under favorable conditions. Rapid spread of *P. crassipes* creates a potential problem for the river ecosystem (Uremis et al., 2014). On the other hand, field observations carried out in this study showed that *P. crassipes* is capable of adsorbing plastic debris in the surface water.

This study was undertaken to evaluate whether the invader *P. crassipes* can be solution to urgent plastic pollution in river systems.

Material and Methods

Sampling and Study Area

The specimens were collected from Orontes River, Hatay, Türkiye in November 2022. Randomly selected 12 specimens were collected from the riverbank where macroplastic density is high and water flow is low (Figure 1). Each specimen was collected using a plankton net with an adjustable mouth opening and a pore size of 65 µm.

Macroplastic Extraction

The collected specimens were wrapped in tin foil and transported to the laboratory. Randomly selected one secondary root was examined under microscope to identify the interaction between plastic debris and root structure. The remaining roots of the *P. crassipes* were washed three times with pre-filtered distilled water to wash off the macroplastic density in a glass beaker. After then, average diameter and root length were measured to estimate the root surface area ($Area = \pi \times average\ root\ diameter \times root\ length$).

Figure 1. Sampling site of examined *P. crassipes* specimens from Orontes River (coordinates: 36°15’17” N, 36°12’10” E)

The collected water represented the macroplastic density of each specimen was treated with 50 mL of hydrogen peroxide (30%) and placed on a hot plate at constant temperature of 60°C for 24 h to degrade organic content. Then, mixture was filtered through 5 mm steel sieves. Type and color (blue, black, red, white, yellow, green, brown) of extracted macroplastics were noted.

Microplastic Extraction

The primary roots of each specimen were separated from the main root system and placed in glass beakers. 20 mL of hydrogen peroxide (30%) per gram of roots was added to the beakers in order to remove organic material. Solution was kept on hotplate at 60°C for 24 h to complete degradation process. Next, saturated sodium chloride was added and stirred for 2 minutes to float microplastics in the mixture. Then, samples were allowed to settle for 24 h. Thereafter, the supernatant was filtered through 65 µm pore size mesh filters and filters were placed in glass petri dishes until they were dried. Finally, filters were examined under Olympus SZX7 microscope with an attached Olympus DP to determine accumulated microplastic in the roots. Estimated microplastics were categorized based on their size, shape (fiber, fragment or pellet) and color (blue, black, red, white, yellow, green, brown).

Plankton net which used in collection step was backwashed three times with pre-filtered distilled water and filtrate was processed for microplastic detection. Filtrate was treated with by 20 mL hydrogen peroxide (30%). Then floatation and microscope examination procedure was applied similar to the roots.

FTIR Analysis

Randomly selected 50 macroplastic particles were analyzed by Fourier transform infrared (FTIR) spectrometer. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with single reflection attenuated total reflectance (ATR) accessory. The spectrum range was 4000–400 cm^{-1} and a resolution of 4.0 cm^{-1} with 32 scans for each measurement. The polymer type identification was done by comparing absorbance spectra to reference libraries of SHIMADZU library.

Quality Control/Quality Assurance

In the laboratory, serious precautions are undertaken to prevent airborne contamination. First, the authorized personnel always wore cotton lab coats and nitrile gloves. Lab surfaces and laboratory materials (glass beakers, filtration unit, etc.) were rinsed with pre-filtered distilled water for three times before any analysis. Hydrogen peroxide and distilled water were filtered through GF/C filters before being used in the analysis. Mesh filters were checked with the presence of any MPs prior to use. All the used equipment including petri dishes and glass beakers were covered when they were not processing. Finally, at each step of the analysis (degradation, filtration, density separation, microscopic examination etc.) blank filters were replaced to detect contamination. Despite affords 0.3±0.5 MPs were detected in the blank filters which were subtracted from the dataset.

Statistical Analysis

In order to understand whether or not there is relationship between root surface area and microplastic or macroplastic abundance Pearson correlation analysis was used. Graphical representation was done using Florish program, online version.

Results

Results showed that *P. crassipes* have ability to capture microplastics and macro plastics present in the surface waters. Total of 508 microplastic particles were extracted from the roots of *P. crassipes* (Figure 2). Among them, 32% of them were fiber, 69% of them were fragment and 3% of them were pellet. Mean microplastic abundance was found as 42±23 MPs/specimen. Mean length of microplastics was found as 633±628 micrometer. Majority of the MPs were less than 500 µm. Detected MPs were commonly blue (30%), red (24%) and black (16%) in color, respectively (Figure 3).

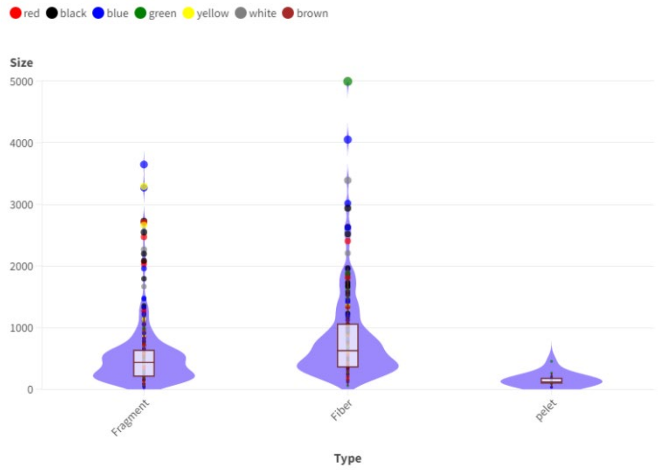

Figure 2. Type, size and color distribution of extracted microplastic from the roots of *Pontederia crassipes*

In terms of macroplastics, 3183 fragmented macroplastic particles were found. Mean macroplastic abundance in the roots was found as 265±44 macroplastic/specimen. Size of macroplastics were varied between 5000 µm and 10500 µm. Blue (22%), black (19%) and red (15%) colored particles form the majority of extracted macroplastics (Figure 3).

FTIR analysis showed that all of the examined macroplastics were high density polyethylene (HDPE).

Statistical analysis showed a strong correlation between root surface area and macroplastic abundance (r=0.07, $p<0.01$) (Figure 4). On the other hand, no correlation was observed in terms of microplastics ($p>0.05$) (Figure 5).

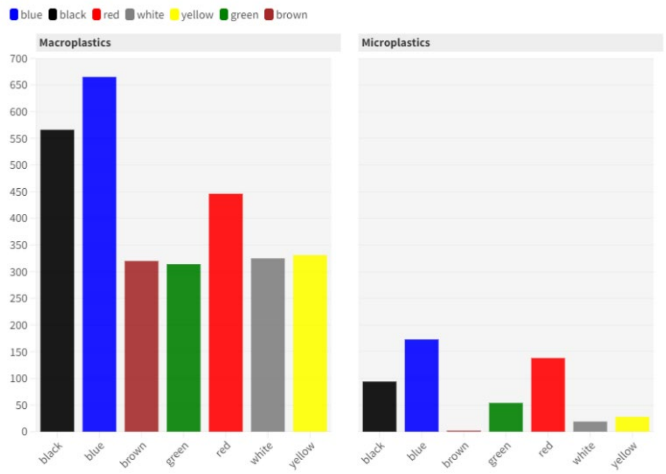

Figure 3. Color distribution of plastic particles extracted from roots of *Pontederia crassipes*

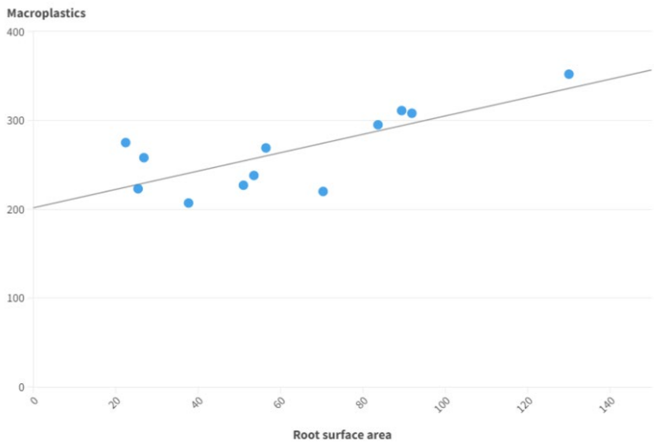

Figure 4. Relationship between root surface area of *Pontederia crassipes* and adsorbed macroplastics

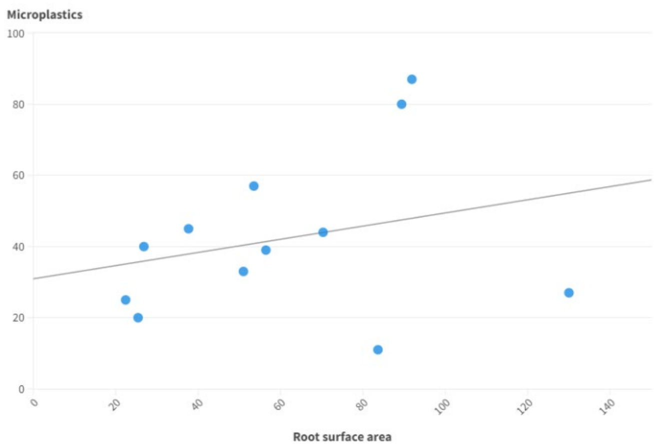

Figure 5. Relationship between root surface area of *Pontederia crassipes* and adsorbed microplastics

Discussion

Concerns regarding the spread of *P. crassipes* are mainly related to its dense malting, which leads to the intertwining of individual plants, reduces the phytoplankton community and reduces the concentration of dissolved oxygen concentrations beneath these malts (Villamagna & Murphy, 2010). On the other hand, previous studies have shown that they can harvest heavy metals in sediment (Greenfield et al., 2007), absorb organic contaminants (Zimmels et al., 2007), nitrogen, phosphate (Rommens et al., 2003) and heavy metals (Tiwari et al., 2007) in the surface water. These contradictory results lead to a debate on the ecological role, potential benefits and harms of *P. crassipes.*

Although this debate is still ongoing, previous studies have shown that *P. crassipes* can be successfully used for the treatment of urban wastewater (Zimmels et al., 2006; Kumari et al., 2014), heavy metals (Eid et al., 2021), ibuprofen and caffeine (de Oliveira et al., 2019). These recent advances have prompted us to think about the potential of *P. crassipes* for the removal of plastic particles in the surface waters. Similarly, Kalčíková et al. (2020) suggested that harvesting of duckweed species at predetermined intervals could reduce plastic litter.

The first characteristic that makes *P. crassipes* a perfect collector of plastic litter is its root structure. The roots are fibrous and unbranched (Penfound & Earle, 1948) and act like a plastic trap (Figure 6). As they tend to create a surface map, the plastic litter on the surface water can easily be caught by the roots. Moreover, the root length decreases when the nutrient concentration in the water increases (Rodríguez et al., 2012). Therefore, in regions where wastewater discharge is a systematic problem, they develop denser, more fibrous root structures that allow us to capture plastic litter discharged into water bodies. Correlation analysis showed that as the root surface area increases, number of adsorbed macroplastic particles increases (Figure 4). This mathematical relationship could be considered as an adsorption kinetics which in light 0f the macro debris harvesting potential of this species.

Figure 6. Microscopic view of macro plastics trapped in the roots of *P. crassipes*

Environmental parameters, especially surface flow rate, seems to be an important parameter for the collection of plastic litter. In this study, specimens were collected from the river bank where plastic litter was trapped (Figure 7) and the water flow was relatively low. *P. crassipes* formed a dense mat on the river surface (Figure 1). From an ecological engineering point of view, these conditions create a natural integrated floating system that allows plastic litter to be collected. For this reason, we are proposing creation of integrated floating systems by using these species to remove plastic litter from water. In a similar sense, this species was employed to remove heavy metals, organic contaminants, dissolved nutrients from freshwater environment (Rommens et al., 2003; Greenfield et al., 2007; Zimmels et al., 2007).

Figure 7. A) Picture of specimen collection point, B) Picture of collected *P. crassipes* specimen, C) Macroplastic debris remaining in the river, D) Picture of the roots of collected *P. crassipes* specimen

More importantly, floating aquatic plants seems not to be affected by micro and macro size plastics. Recent studies showed no impact on plant growth of duckweed species such as *Spirodela polyrhiza* (Dovidat et al., 2020), *Lemna minor* (Mateos-Cárdenas et al., 2019). Ceschin et al. (2023) reported that microplastics can only be adsorbed but not absorbed by the roots of *Lemma minuta*. On the other hand, a current study argued that nanoplastics less than 20 µm can only be absorbed by *P. crassipes*. Therefore, microplastics taken up by the root structure remain in the root structure until harvested, which is consistent with previous reports (Mateos-Cardenas et al., 2019; Dovidat et al., 2020; Kalčíková et al., 2020).

Small size MPs (<1000 µm) can be accidently consumed by aquatic organisms and could pose a significant threat in the food chain. In this study, the majority of microplastics ingested by the roots were smaller than 500 µm. Therefore, this plant appears to be effective in removing microplastics from the aquatic environment.

The macroplastics examined were all HDPE, which is an indicator that plastic has recently been discharged into the river. The results of this preliminary study showed that their complex root system has sufficient surface area (Yang et al., 2023) to capture discharged micro- and macroplastics in the water surface.

Conclusion

This study is the first report investigating the plastic capture potential of *P. crassipes*. The samples were collected from the Orontes River, which is known to have problems with water quality and plastic litter. A total of 3691 micro- and macroplastic particles were extracted from the roots of 12 examined specimens. This preliminary data indicates that *P. crassipes* is a good candidate for the micro and macro size plastic particle removal due to their ability to adsorb micro- and macroplastics through the roots. We proposed that this species can be used as employees for plastic litter removal. Future engineering studies needs to be carried out to understand the operating conditions for the design of floating wetland systems by employing *P. crassipes.*

Compliance With Ethical Standards

Authors' Contributions

EK: Conceptualization, Writing – original draft, Formal analysis, Data curation
NY: Conceptualization, Formal analysis, Data curation
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Not applicable.

Data Availability

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

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