Phytoremediation: An Innovative Wastewater Treatment Method For Removal of Microplastic Particles

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Abstract

Microplastic contamination of marine and freshwater bodies is an increasingly pressing concern, with dangerous implications for the health of aquatic ecosystems and human communities. Although conventional wastewater treatment processes capture most large plastic particles, they have high energy and maintenance requirements and are ineffective at filtering microplastics [1]. Reports indicate that 94% of drinking water in the US is contaminated with microplastics [2]. Phytoremediation is an emerging method of removing pollutants such as heavy metals from the environment via accumulation in plants, but has never been investigated in the context of microplastics [3]. This project investigated the potential of phytoremediation by the common duckweed (*Lemna minor*) as an inexpensive and sustainable wastewater treatment method (WWTM) for the removal of microplastics. *L. minor* was exposed to microplastic concentrations ranging from 5 to 500 mg L^{-1} for 168 h. Growth endpoints were assessed, revealing no significant toxicity at tested levels. Overall, *L. minor* was able to successfully remove over 65% of microplastic particles from wastewater at concentrations of 5 and 50 mg L^{-1} and over 12% of particles at 500 mg L^{-1}. The vast majority of recovered particles were absorbed onto the surface roots and fronds of *L. minor*, with no evidence of accumulation inside the plant. These results demonstrate the potential of *L. minor* as a phytoremediator for microplastic pollution and support further research of its implementation in wastewater treatment processes.

Objectives

Study objectives were twofold:

1. Assess degree of toxicity of microplastics towards *L. minor* to determine the organism's fitness for phytoremediation of microplastics – if exposure to microplastics produces too much stress or tissue damage towards the organism, then it may not be very effective at absorption of microplastics.
2. Assess ability of *L. minor to* absorb microplastics: after exposure, colonies of *L. minor* will be harvested and examined for microplastic recovery, i.e. the proportion of microplastics it removed from the suspension, to test its viability for integration into wastewater treatment methods

Materials & Methods

1 Cultivation of L. minor

Common duckweed (*L. minor*) was obtained from a commercial source (Carolina Biological) and cultured in Steinburg growth medium at 25°C with a 16h light/8h dark photoperiod under a fluorescent lamp.

2 Microplastic Preparation & Characterization

Green fluorescent polyethylene (PE) microplastic spheres of diameter of 27–32 µm and peak fluorescence at 515 nm were obtained as hydrophobic, dry powder from a commercial source (Cospheric LLC). PE microplastics were transferred to distilled water containing 0.01% v/v Tween-20.

3 Spectrophotometer Construction

A homemade spectrophotometer was constructed using a black cardboard box containing a webcam covered by DVD-derived diffraction grating (Fig. 1). To analyze samples, a light source was shined through a 1 mm slit and analyzed with Spectragryph software.

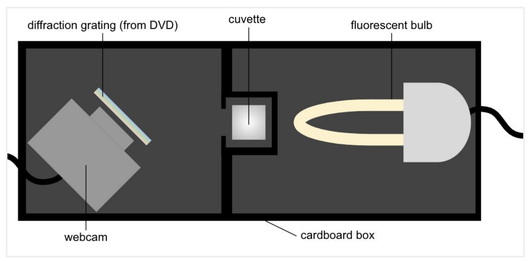

Fig. 1. Top down diagram of homemade spectrophotometer

4 Experimental Setup

L. minor colonies were randomly assigned to 300 mL Erlenmeyer flasks containing 100 mL of fresh growth medium and cultured under the conditions described in section 1. *L. minor* colonies were exposed to microplastic concentrations of approximately 5, 50, and 500 mg L^{-1} (n = 5 replicates) over 168 h. Colloidal stability was confirmed with dynamic light scattering (DLS).

5 Growth Assay

Microplastic toxicity towards *L. minor* was assessed by measuring growth endpoints for frond area and root length. Growth inhibition was calculated using the equation $\%I_r = \frac{\mu_c - \mu_t}{\mu_c}(100)$ ere $\%I_r$ is percent growth inhibition relative to control, μ_c is sample mean for the control, and μ_t is sample mean for the treatment.

6 Microplastic Recovery

At 0, 24, 48, 96, or 168 h, a set of colonies from each level of microplastic exposure (n = 5 replicates) were carefully harvested and washed three times to remove microplastics adhering to their surfaces. To assess accumulation of microplastics inside *L. minor*, washed samples were dissolved in 5.8% sodium hypochlorite for 24 h and filtered through a 25 µm nylon mesh (Fig. 2). Microplastics collected from washing and dissolution were resuspended in Tween 20 solution and sonicated. Aliquots were measured with the spectrophotometer. Absorbance values at 515 nm were used to approximate concentrations using the Beer-Lambert Law to indirectly calculate microplastic recovery.

7 Data Analysis

A Kruskal-Wallis test was used to analyze collected data, which was considered statistically significant if $p < 0.05$. A Tukey post hoc test was used to analyze statistically significant results.

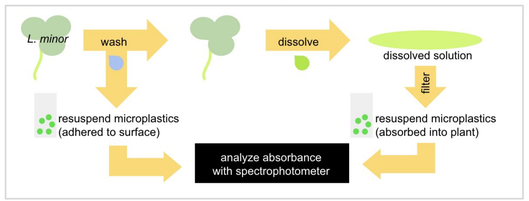

Fig. 5. Microplastic recovery procedure

Results & Interpretation – Figures & Tables

Concentration (mg • L^{-1})	Fronds			Roots	
	Largest frond (mm^2)	Avg. frond area (mm^2)	Quantity (number)	Longest root (cm)	Avg. root length (cm)
Control	15.22	12.33	98.46	3.50	3.26
5	16.34	13.42	105.94	3.56	3.27
50	14.96	12.52	112.87*	3.27	2.73*
500	15.85	11.12	80.67*	3.661	3.01

Table 1. Effects of microplastics on growth rate of *L. minor* after 168 h are shown. Results that were statistically significant relative to control were marked with * ($p < 0.05$).

Concentration (mg • L^{-1})	Fronds			Roots	
	Largest frond (mm^2)	Avg. frond area (mm^2)	Quantity (number)	Longest root (cm)	Avg. root length (cm)
Control	0.00	0.00	0.00	0.00	0.00
5	-7.33	-8.84	-7.60	9.99	-0.23
50	1.73	-1.58	-14.63*	7.79	16.31*
500	-4.15	9.83	18.07*	-3.13	7.71

Table 2. Effects of microplastics on growth rate of *L. minor* after 168 h expressed as percent growth inhibition relative to the control ($\%I_r$). Results that were statistically significant relative to control were marked with * ($p < 0.05$).

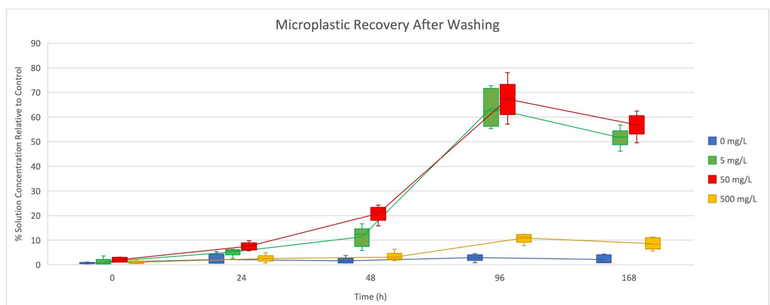

Fig. 2. Microplastics recovered after washing, as determined indirectly by relative concentration, are shown as a function of time in a box plot graph (n = 5 replicates).

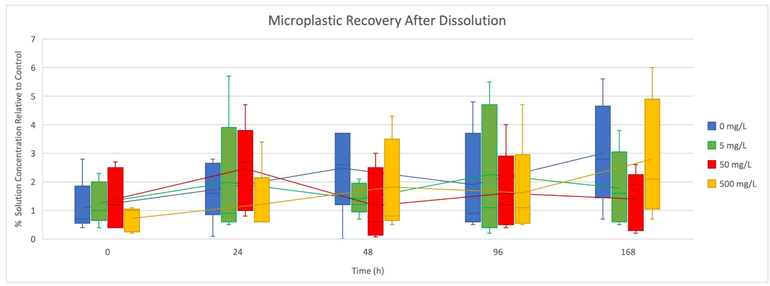

Fig. 3. Microplastics recovered after dissolution, as determined indirectly by relative concentration, are shown as a function of time in a box plot graph (n = 5 replicates).

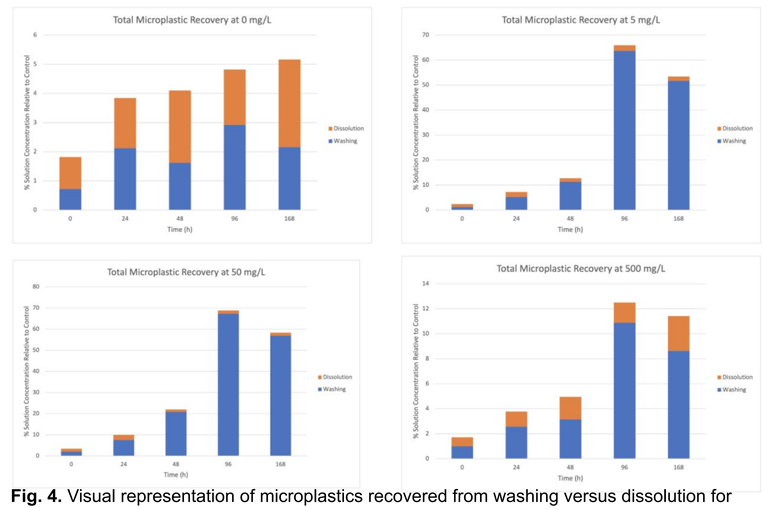

Fig. 4. Visual representation of microplastics recovered from washing versus dissolution for each experimental treatment, as well as the total recovery rate of microplastics.

Results & Interpretation – Discussion

- Microplastics absorb to the surface of *L. minor*, but did not accumulate inside the plant
  - At the peak period of 96 h, washing microplastics absorbed to the surface of *L. minor* recovered 68.8% of microplastics from a concentration of 5 mg L^{-1}, 65.9% of microplastics from a concentration of 50 mg L^{-1}, and 12.5% of microplastics from a concentration of 500 mg L^{-1}
  - Results from washing (Fig. 2) were statistically significant at concentrations of 5 and 50 mg L^{-1} from 24 h to 168 h ($p < 0.01$) and at 500 mg L^{-1} from 48 h to 168 h ($p < 0.01$)
  - None of the microplastic recovery from dissolution (Fig. 3) was statistically significant relative to the control ($p > 0.05$), suggesting that none of the microplastics were internalized
  - Even if microplastics were absorbed, Fig. 5 suggests that the vast majority of microplastics were removed via absorption to plant surfaces
- Evidence for MP toxicity towards *L. minor* was viewed with skepticism
  - Although variations in growth inhibition were observed, most were deemed not statistically significant, and those that were had p-values close to 0.05
  - Results also did not follow a dose-dependent trend: in fact, for 50 mg L^{-1}, measurements indicated that the quantity of fronds increased by 14.63%
- Microplastic recovery by L. minor relative to the control was significantly lower at high concentrations of 500 mg L-1 compared to those at medium and low doses
  - The logarithmic exposure gradient means that even if a less proportion of particles were removed, the total amount of particles removed was still higher than the previous concentration as long as recovery rate is > 10%
  - Suggests that capacity of *L. minor* to accumulate microplastics follows a logarithmic trend and may approach an upper limit at high enough concentrations
- For statistically significant measurements, microplastic removal by L. minor increased over 0–96 h, but decreased between 96–168 h
  - Suggests that particle aggregation or colony growth may have impeded the adsorption of particles in the later periods of the experiment
- Nonzero microplastic recovery in control groups suggests some degree of uncertainty in terms of measurements conducted with the homemade spectrophotometer
  - Statistical significance relative to the control still suggests that results are valid

Conclusion & Applications to Biotech

The results of this experiment support phytoremediation as an inexpensive, effective, and innovative extraction process for the removal of small microplastic particles from wastewater.

1. PE microplastics did not inhibit root and frond growth of *L. minor* over the experimental period and observed concentrations, supporting its use as a sustainable microplastic bioremediator.
2. PE microplastics accumulated on root and frond surfaces of *L. minor* as determined by thorough washing, but were not internalized by the plant, as suggested by the lack of statistical significance in the results of the dissolution assay.
3. *L. minor* was able to remove over 60% of microplastic particles of diameter 27–32 µm at concentrations of 5 and 50 mg L^{-1} and over 10% at concentrations of 500 mg L^{-1}. Current WWTMs are only able to effectively remove particles up to 300 µm in size [1].
4. Higher eukaryotes are an innovative already to bioremediatory microorganisms, which have hard-to-control growth rates and may facilitate unwanted gene transfer if leaked into the environment [4]. This experiment tested phytoremediation as a novel alternative to current bioremediatory techniques.
5. Harvesting *L. minor* colonies is a simple, inexpensive, and effective method for removal of PE microplastics. Results of this experiment support further exploration of macrophytes for their implementation in WWTMs for microplastic extraction

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