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Environmental restoration effects of *Ranunculus sceleratus* L. in a eutrophic sewage system

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ABSTRACT

This study investigated the environmental restoration effects of *Ranunculus sceleratus* in a sewage system microcosm trial, including the removal of pollutants and algal inhibition. We compared the removal of pollutants by *R. sceleratus* in a eutrophic sewage system in the presence and the absence of algae. The rate of removal without algae was 16.2–20.5% of that with algae. $NH_4^+{-}N$ was removed most readily by *R. sceleratus*. The effects of *R. sceleratus* on the growth of *Microcystis aeruginos*a were also investigated in two allelopathic modes. The level of algal inhibition after the addition of an extract of *Ranunculus scleratus* was 57.1–78.9% greater than that in a co-culture test. To understand the role of allelopathy interference with algal development, we also determined the total flavonoid contents of plants, which ranged from 3.57 g to 20.19 g per plant. The cell density of *Microcystis aeruginosa* was negatively correlated with the total flavonoids in *R. sceleratus*, although aquatic macrophytes may contain other allelochemicals involved with algal inhibition in addition to flavonoid compounds. The environmental effects of *R. sceleratus* were significantly correlated with its growth stage (or water retention time), plant height, and biomass. This study suggests that *R. sceleratus* has potential for the low-effort and sustainable management of freshwaters, particularly the removal of nutrient pollutants and the reduction of excessive algal growth, which may be attributable to allelochemicals such as flavonoids. The *in situ* environmental restoration effects of *R. sceleratus* require further investigation at the ecosystem level.

1. Introduction

Ranunculus sceleratus, commonly known as cursed buttercup or celery-leaved buttercup, is a native of North America and Eurasia. In general, it grows in wet and moist habitats such as ponds and the banks of stream. *R. sceleratus* develops well with fertile soil, adequate water, and sufficient light. It branches vigorously, sometimes with a strong stem, and produces numerous seeds (Mei et al., 2012). In the floodplains of Dutch rivers, the pioneer species *R. sceleratus* is highly resistant to waterlogging and shallow floods. It can also ameliorate flooding stress because of its high root porosity and the capacity to increase its petiole elongation rate under water. *R. sceleratus* dies quickly if its leaf blades are unable to reach the water surface (He et al., 1999). Soo et al. (1999) found that polyamines were involved with the regulation of indoleacetic acid- and ethylene-induced growth, particularly in the later stages of petiole elongation in *Ranunculus* spp.

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R. sceleratus has been used widely on constructed wetlands because of its tolerance of flooding and the ability to accumulate and metabolize pollutants. In particular, it can adsorb many heavy metals from its surroundings. Thus, it has great potential in the field of phytoremediation. Hu et al. (2007) found that *R. sceleratus* accumulated Fe and Zn, where the roots had the highest capacity for Fe adsorption. *R. sceleratus* also performed well when used to treat different types of nutrient-polluted sewage. In some constructed wetlands dominated by *R. sceleratus*, it removed 74.1%, 73.4%, and 74.5% of the chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP), respectively (Wang et al., 2009). It also supports the growth of the microbial rhizosphere. Therefore, it has great value in artificial wetlands and for the biological monitoring of polluted environments. *R. sceleratus* is very common in China but there has been little development of its utilization for eutrophication remediation and harmful algae reduction. These functions probably rely on specific secondary metabolites. In the present study, therefore, *R. sceleratus* was cultured at different growth stages in growth aquaria containing eutrophic domestic sewage to test its effects on nutrient removal and algal inhibition. The flavonoid content was also determined. Correlation analyses were used to explore the potential environmental restoration effects of *R. sceleratus*. We aimed to determine the relationships between the pollutant removal effects, algal inhibition, and chemical composition. Our results provide a theoretical basis for the maintenance of clean freshwater by macrophytes.

2. Materials and methods

2.1. Plant culture in growth aquaria

Young *R. sceleratus* plants were collected from the banks of Huajin River in late February 2011. No specific permissions were required for the location and the field sampling did not involve endangered or protected species. The plants were cultured in growth aquaria according to the method of Zuo et al. (2011). The water bloom alga, *Microcystis aeruginosa*, was obtained from the Institute of Hydrobiology at the Chinese Academy of Sciences. Eutrophic domestic sewage was collected from the canteen at Anhui Normal University and was prepared by sedimentation, filtration, floating oil removal of the drained water, and sterilization with ultraviolet (UV) irradiation. The original values of the typical indicators, i.e., COD, NH_4^+−N, TN, TP, and pH, were 125–140 mg/L, 25.1–53.2 mg/L, 47.9–60.5 mg/L, 2.5–8.1 mg/L, and 6.9–7.3, respectively.

Ten similar *R. sceleratus* seedlings were transplanted into rectangular plastic aquaria and their aerial parts were kept above the sewage level, where the length, width, and height of the aquaria were 75, 50, and 50 cm, respectively. Three aquaria were treated as three replicates. Distilled water was supplemented to maintain a stable sewage volume of 15 L in each aquarium, thereby preventing water evaporation from reducing the water level. The transferred plants were acclimated to the sewage for a period of 7–15 days, before the experiment commenced and the relative indicators were measured. The overall trials lasted from early March until late September in 2011, which included the seedling stage (SE), jointing stage (JO), branching stage (BR), and flowering and fruiting stages (FF). The growth aquaria were placed on the balcony of the experimental buildings in the College of Environmental Sciences and Engineering. The sunlight level was adequate and the temperature varied with the weather. During each growth stage of *R. sceleratus* (without algae), the COD, NH_4^+−N, TN, and TP of the influent and effluent water were determined to calculate the pollutant removal rate. The plant height and fresh weight of *R. sceleratus* were also determined at each stage.

2.2. Pollutant removal and algal inhibition via the allelopathic potential of R. sceleratus

The allelopathic resistance of *R. sceleratus* to harmful algae was determined according to the method of Erhard and Gross (2006). Each of six growth aquaria was divided into two parts in the middle. Ten seedlings of *R. sceleratus* were placed in the left side and rooted in the sediment from a shallow eutrophic lake (Chaohu Lake, China), and *M. aeruginosa* was inoculated into the right side, based on the method of Zuo et al. (2011). The container was divided with transparent waterproof plastic, where a hole in the middle contained a 0.45 μm filter film that allowed water to pass through. The microcosm test was conducted under the light intensity of 70 μmol photons m^{-2} s^{-1}, a photoperiod of 12 h light/12 h dark with a temperature cycle of 25 °C light/20 °C dark in a temperature-conditioned green house. The treatment and the control each comprised three replicates. The control contained no plants. This test aimed to determine the *in vivo* allelopathic potential of *R. sceleratus* for inhibiting algal growth, while avoiding the effects of inter-specific competition. The algal density was recorded at the end of each of the four growth stages of *R. sceleratus*. The algal inhibition rate was then determined. The removal rates of pollutants, i.e., COD, NH_4^+−N, TN, and TP were calculated, and the plant height and fresh weight of *R. sceleratus* were also determined (with algae).

Another allelopathic mode was investigated using plant extracts. Plant samples were extracted using distilled water in the ratio of the dry weight of plant to water volume as 25:1 g ml^{-1}(Erhard and Gross, 2006). The aqueous extract was added to the growth aquaria instead of living plants. A constant ratio of plants to algae was maintained in all parallel trials. The other conditions were the same as those used in the *in vivo* trials. The methods used to determine the allelopathic potential of plant extracts was the same as that reported by Zhang et al. (2009). Samples of influent and effluent water were analyzed immediately, according to the method of Garnier et al. (2005). The COD, NH_4^+−N, TN, and TP were determined using the potassium dichromate oxidation method, Nessler's reagent colorimetric method, alkaline potassium persulfate UV spectrophotometry, and ammonium molybdate spectrophotometric method, respectively. All of the reagents used were analytical grade. The results were the averages of three replicates.