Phytoremediation of Nutrient-Controlled Water using Duckweed and Water Fern

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Abstract

Phytoremediation is a way to use plants to remove nitrogen and phosphorus from water bodies to reduce negative impacts of eutrophication. This study used duckweed, water fern, and their combination to analyze nutrient removal from water environments with varying levels of these two elements. For these environments, most of the N concentrations were within the range o

2006). The organisms that perform this decomposition deplete oxygen from the water column, which can alter the community composition and structure of the ecosystem can change. Eventually, only certain species tolerant of anoxic conditions are able to survive (Frey *et al.* 2006). The growth of the plant material itself can be harmful to a pond ecosystem, as certain plants begin to dominate and block sunlight from penetrating to greater depths (Frey *et al*. 2006). Eutrophication can cause unpleasant smells and water colors and in some cases, a coating of foam on the surface of the water (Frey *et al.* 2006). Additionally, when added nitrogen is converted to ammonia, it is toxic to fish (El-Bestawy *et al.* 2005). Decreases in fish populations can have mild to disastrous effects on local fishing economies (Hunt *et al*. 2006). Increased rates of eutrophication can cause food web alterations (Qin 2009), hinder establishment of communities that would normally be parts of succession in that location or cause loss of biodiversity. Furthermore, in high enough concentrations, both nitrogen and phosphorus can be toxic to humans. Nitrates can be converted to nitrites and combine with hemoglobin in the blood, depleting oxygen levels and causing “blue-baby syndrome” in infants, and can be transformed into cancer-causing nitrosamines inside the human body (Frey *et al.* 2006, El-Bestawy *et al.* 2005).

Eutrophied water bodies may become no longer useful as water sources for humans and other organisms. In light of population pressures (El-Bestawy *et al*. 2005) reversing the ecosystem changes that we have caused is desirable. One approach is to pump water from a lake and treat it off-site with physicochemical methods. However, this can be expensive or environmentally harmful (Frey *et al.* 2006).

Bioremediation is an alternative that is less expensive and makes use of natural processes. It is defined as the use of any living organisms to degrade waste (Litchfield 2005). In the broadest sense, the process of bioremediation has been occurring since human beings have disposed of their trash and relied on natural systems to convert it to organic matter (Litchfield 2005). In more recent times, the process has been used in more intentional ways. During the late nineteenth century, wastewater treatments plants were developed, and along with them the first intentional application of biological processes to treat waste and wastewater (Litchfield 2005).

During the 1990s, phytoremediation became an established technique to clean polluted sites. (Litchfield 2005). Plants have a diverse range of applications in remediating polluted sites, with a capacity to hyperaccumulate metals and take up large quantities of organic “pollutants” such as nitrogen and phosphorus that they not only accept but require for their biological processes. Harvesting plants used in remediation efforts removes the nutrients contained in the biomass from the water body, decreasing the concentration of those nutrients in the ecosystem.

Duckweed (*Lemna minor*) and water fern (*Azolla sp.*) have been used successfully in phytoremediation applications. Duckweed, in particular, is commonly used in the United States to phytoremediate municipal, industrial and septic waste (Iqbal 1999). Many small-scale phytoremediation efforts are found in other locations and can be non-mechanized. For example, in one village in Bangladesh, duckweed, cultivated on raw sewage, is fed to fish (Iqbal 1999). For our study in phytoremediation, we chose to use both duckweed and water fern based on their growth patterns, nutrient uptake rates and the fact that they are native to the study region. Due to space constraints, we were interested in plants that grow primarily outward rather than upward. Duckweed, a prolific aquatic plant, has a life cycle of several weeks; an individual frond may produce ten generations of progeny over a period of ten days to several weeks (Skillicorn *et al.* 1993). It has been shown to double in mass every two days (Skillicorn *et al.* 1993) and can remove 75% of total phoshospqu2.1.5 Td   [(pr)h15 Td   [(ki)-2(l)5(n(n)-101%)3(ot2(ha)4(t)]TJ   2   o fcc [(e)6(

Water fern is common in many parts of the world and is used as a fertilizer and livestock feed. It has a unique potential for remediation because of its association with nitrogen-fixing cyanobacteria called *Anabaena azollae* Strasb.(Forni *et al.* 2001). Its fast growth rate is also amenable to phytoremediation applications; it can produce approximately 18 kg/m^2/yr of plant material (Sela *et al*. 1989).

The use of plants for nutrient uptake is especially valuable because following site remediation, it is possible to identify practical and value-added uses for the plant material. These could include conversion of plant biomass to energy, animal feed, or further breakdown of the material by using fungi. In particular, five duckweed species have been shown to be a valuable additive for animal fodder because of its high protein and low fiber and lignin contents (Vermaat and Hanif 1998). Between fifteen and forty percent of its dry weight is protein (Cheng *et al*. 2002; Alaerts *et al*. 1995).

One site that could benefit from nutrient removal is located in the Saratoga Lake watershed in Saratoga County, New York. Most water bodies in the watershed are not severely impacted by the effects of cultural eutrophication. The region is monitored by several water resource protection organizations, a fact which contributes to the relative health of the major water bodies. However, one powe

Methods

Collection of Water Sample from Field Site

We collected a water sample at the field site near the Skidmore horse stables (henceforth referred to as the Stables site) in February 2010 and stored it in a Nalgene container. The sample was filtered through 0.7 micron Milipore filters and tested

Root length

In each container we measured plant root length after 24 days. To do this we stirred the contents and randomly selected 20 individuals, measuring with calipers the longest root from each individual. For the combination treatment, however, we measured 10 duckweed individuals and 10 water fern individuals. (We hereafter refer to these two groups as duckweed in combination and water fern in combination.) Some individuals selected did not have any measureable roots, and we did not include them in the data analysis. In these instances, the sample size is less than 20 or 10. We compared the root lengths for each of four groups (duckweed alone, duckweed in combination, water fern alone, and water fern in combination). Different species of plants, or plants grown in the different treatments, could have different maximum growth potentials. Therefore, we normalized each average root length by the highest average root length in each of the four groups to directly compare the plants of different species and subjected to different treatments.

pH

We used Accumet Basic AB15 pH meters to determine pH of water in each of the containers after 29 days.

Results

Water nitrogen concentration decreased compared to the control (the corresponding water environment without plants) in all duckweed and combination treatment water environments except DI water. The amount of N remaining in each water environment, for each treatment group, is shown in Figure 1. This amount is expressed as the ratio of the N remaining in each treatment water environment, to the N remaining in the corresponding control water environment. For example, for duckweed grown in the 75% environment, the ratio was calculated by dividing the amount of N remaining in that container by the amount remaining in the 75% control container. This normalization by the initial amount of N in each water environment allows for direct comparison of these N values. The ratio is less than one which, on a logarithmic scale, translates to a negative value. Therefore its change in N is shown as negative on the graph.

In the water fern and combination treatments, the DI water environment shows increased final N concentration compared to the control. Higher percent N removal occurred when plants were in lower initial water concentrations, as illustrated by the smaller bars on the graph at higher initial concentrations. The these(i)-2(s)-5(he 1f)3(i)-2(na)4(1.19)3(e)44tW

that plants can absorb in that time.

Water fern showed smaller decreases in N water concentration, with a maximum decrease of 26%. This lesser removal of N can probably be attributed to its ability to fix N from the atmosphere, thus reducing its N demand from the water (Forni *et al*. 2001). Furthermore, it could be that water fern suffered from P deficiency. Several water fern individuals in almost all containers showed a red color. This is a symptom of, a

This would allow N removal to occur with no associated plant growth, which, in turn, could explain the lack of P removal by plants.

The combination of decreases in water N with little change in P results in an altered N:P ratio in the water. The ratio of nitrogen to phosphorus in a water body has implications for ecosystems and organisms (Bulgakov and Levich). Duckweed and combination treatments showed a decrease in N:P ratio (Figure 3). Duckweed preferentially took up N, thereby reducing the N concentration. The N:P reduction for the combination was intermediate between the reductions for duckweed and water fern.

The shift in N:P ratio in the presence of plants has significance with regards to the characteristics of a water body’s ecosystem. Unaltered environments, such as a water body that has not been impacted by humans, have typical N:P ratios, which change with anthropogenic pollutant inputs. If we see plants shifting the N:P ratio of their environment in the direction of the “natural” value, we see them changing that environment to resemble more closely its pre-altered condition. In our study, the N:P molar ratio for the duckweed treatments were reduced (almost consistently, with the exception of the 100% water environment) from upwards of 140:1 to less than 35:1. The latter ratio falls in the range of river water (Sterner and Elser 2002). General leafy plant biomass N:P ratios cluster around 6:1 to 18:1 (Downing and McCauley 1992). It has been determined that there the N:P ratios of marine plant life are consistent globally, and that this N:P ratio coincides with that of many marine water environments. The similarity and commonality of this ratio s(mona)8( )-106hmpuEifhad otio

Vermaat and Hanif 1998), supporting the explanation of toxicity. Root lengths measured in the present study decreased significantly in the elevated concentrations (Figure 4). Additionally, final pH levels clustered around and below 4, levels which can be toxic to plants directly. The pH range at which duckweed growth is not inhibited is between 5 and 8 (Caicedo *et al*. 2000). Water fern can survive between pH values of 3.5 and 10 but has optimum growth at pH values between 4.5 and 7. One potential explanation for low pH levels in elevated nutrient environments, with or without plants added, is the significant additions of NH_4Cl and KH_2PO_4. These cause dissociation of H+ ions, which causes decreased pH values.

useful not only for the Stables site but also as a model for phytoremediation efforts of other water bodies with similar nutrient regimes.

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Tables and Figures

Table 1. Comparison of Expected and Observed Final N Concentrations in Combination

Figure 1. Change in N Represented by the Ratio of Final N in the Remediated Water to the Corresponding Final N in the Control, in Each Water Environment

Figure 2. Change in P Represented by the Ratio of Final P in the Remediated Water to the Corresponding Final P in the Control, in Each Water Environment

Figure 3. Depression of Average N:P Ratios

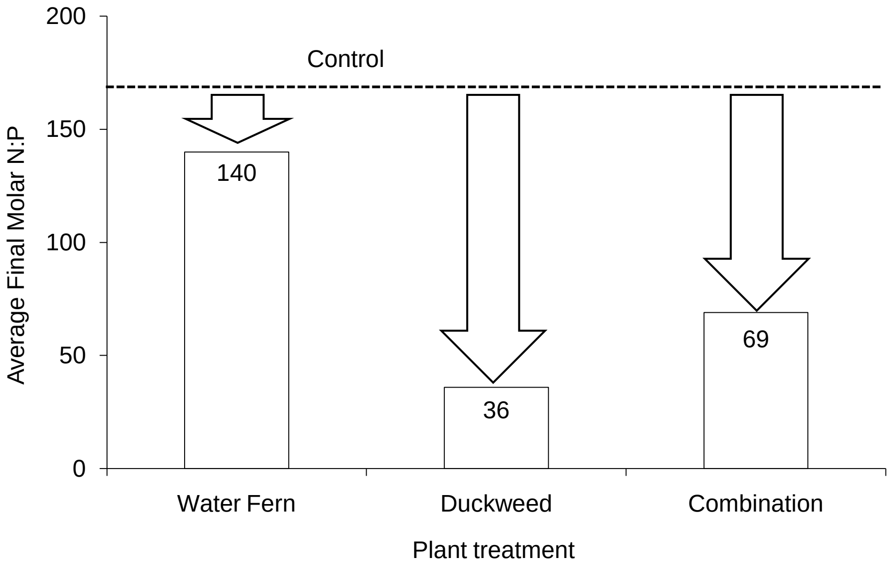

Figure 4. Average Length of Longest Root

Figure 5. Standardized Maximum Root Length