*J. Aquat. Plant Manage.* 48: 116-120

# Efficiency of *Polygonum hydropiperoides* for Phytoremediation of Fish Pond Effluents Enriched with N and P

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## ABSTRACT

The present study assessed the nutrient depuration capacity of *Polygonum hydropiperoides* by cultivating plants hydroponically in water obtained from fish ponds. The experiment simulated varying degrees of nutrient enhancement by using pond water enriched with nitrogen (N) and phosphorus (P) at the following concentrations (in mg $L^{-1}$): 0-0 ($N_cP_c$), control group; 105-15 ($N_1P_1$); 155-30 ($N_2 P_2$); 185-45 ($N_3P_3$), and 205-60 ($N_4P_4$). The sources of N and P were urea and diammonium phosphate, respectively. Experiments were carried out using a completely randomized design, with six replicates and five levels of added N and P, and lasted for 40 d until plants reached maturity. Plant morphological and chemical variables were recorded, and depuration efficiency was measured based on the total shoot biomass and the nutrient content per treatment. The best performance was observed in the $N_1P_1$ treatment, achieving a water depuration rate of 74% for N and 81% for P. These results suggest promise for using emergent plants such as *Polygonum* grown in hydroponic culture to remove excess nutrients from aquaculture sites.


*Key words*: nitrogen, phosphorus, phytoremediation, swamp smartweed, water pollution.

## INTRODUCTION

Multiple uses of water during the past decades have seriously impacted its quality for humans and other organisms due to different sources of pollution including metals at toxic levels, harmful chemicals, sludge containing high amounts of nutrients, and other diffuse sources caused by inadequate soil management techniques. The trophic state index (IET) classifies Brazilian water quality using nutrient enrichment, especially N and P. According to nutrient input, the degree of eutrophication is classified as oligotrophic, mesotrophic, eutrophic, and hypereutrophic. The maximum value for mesotrophic classification of fresh waters is 0.1 mg $L^{-1}$ P (CONAMA 2005), and values that exceed this level are classified as eutrophic.

The Iraí reservoir, which serves 20% of the population of the city of Curitiba, Paraná State, Brazil (Lima et al. 2005), has been classified as hypereutrophic, based on chlorophyll *a* concentration (25 ug/L; Xavier et al. 2005). The eutrophication process is a consequence of domestic and industrial sewages in rivers that supply this reservoir. The first algal bloom was detected in 2001 when the toxic cyanobacteria, *Microcystis* sp. reached the density of 7,000,000 cells/mL (Carneiro et al. 2005). The excessive algal growth is associated with high levels of phosphorus in the water. Removal of this nutrient from water is one method to improve water quality (Bollmann and Andreoli 2005). To mitigate these problems, phytoremediation is becoming an innovative technology (Carvalho and Martin 2001) and an alternative to highly expensive procedures such as mechanical and chemical water treatments, particularly in the case of eutrophication (Kamal et al. 2004).

The reservoir water originates from several fish ponds in the surrounding area. These fish ponds have a diversified flora, especially emergent macrophytes, along their margins. Some species grow spontaneously in the water channels between the fish ponds and the reservoir, filtering organic matter in suspension. One of these species is *Polygonum hydropiperoides* Michx, a perennial rooted herb with adult plants reaching 90 cm in height. The genus *Polygonum* is distributed worldwide, from the tropics to polar regions (Melo 1999). In Brazil, *P. hydropiperoides* is found mostly in the south, southeast, and west-central regions (Kissmann and Groth 1997) where it occurs mainly in river channels, reservoirs, and lakes, especially along their margins. Natural propagation by seeds is as effective as vegetative sprouting (Lorenzi 2000).

This species occurs in both terrestrial and aquatic environments. In the later case, the sprouts follow the water level, maintaining floating leaves and emergent inflorescences. According to Baumeister and Ernst (1978), hydrophytes are able to exploit the subaquatic sediments as well as the water itself as nutritional sources. Rooted emergent hydrophytes are typically not limited by water availability, and hence high transpiration rates are possible (Pauliukonus and Schneider 2001). Nutrients in the aqueous solution can be readily absorbed either through diffusion or mass flow. Although transpiration rates are unknown for *P. hydropiperoides*, early

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Table 1. Experimental groups and their respective N and P concentration levels after supplementing the fish pond water.

| Treatment | Added Concentration (mg $L^{-1}$) N | P |
|---|---|---|
| *$N_cP_c$ | 0 | 0 |
| $N_1P_1$ | 105 | 15 |
| $N_2P_2$ | 155 | 30 |
| $N_3P_3$ | 185 | 45 |
| $N_4P_4$ | 205 | 60 |

*Unaltered pond water used as the control group.

Table 2. Values of electrical conductivity and pH of the different treatments of water from fish ponds.

| Treatment | Added Concentration (mg $L^{-1}$) N | P |
|---|---|---|
| $N_cP_c$ | 7.0 | 8.3 |
| $N_1P_1$ | 60.0 | 7.6 |
| $N_2P_2$ | 79.6 | 7.9 |
| $N_3P_3$ | 122.5 | 8.0 |
| $N_4P_4$ | 330.2 | 8.0 |

studies indicate this species has potential to remove excess nutrients from contaminated environments such as older aquaculture facilities.

This study tested the ability of natural populations of *P. hydropiperoides* to reduce nutrient loads from water flowing from aquaculture ponds. Although this is a rooted species, we investigated its growth while floating to test its capacity to increase absorption of nutrients.

## MATERIAL AND METHODS

The study took place in the Canguiri Experimental Research Station of the Universidade Federal do Paraná in the municipality of Pinhais, state of Paraná, Brazil (22°06'55"S; 47°45'15"W; Oliveira et al. 1998). According to Koeppen's classification, the climate of the region is humid mesothermic (*Cfb*), with the average temperature in the warmest month below 22 C (Fasolo et al. 2002).

The experiment was set up near experimental and commercial fish ponds in a greenhouse commonly used in horticulture to simulate critical eutrophication problems in aquaculture. The greenhouse was protected on its sides by a plastic frame to prevent the incursion of insects and other animals. Cultivations were conducted hydroponically on a platform 0.70 m above the ground. Each 5 L container received 3 sprouts and 4 L of pond water enriched with nitrogen (N) and phosphorus (P) in a completely randomized design with six repetitions and five treatments for a total of 30 containers (Table 1). Diammonium phosphate and urea were used as nutrient sources in several different concentrations (Table l). Although the nutrients already present in the fish pond water were not taken into account during the experimental design, their levels were measured at the beginning and end of each treatment. In addition, to avoid nutritional imbalances, complementation with a common fertilizer was provided with potassium (K) and iron (Fe) in the form of potassium chloride and Fe-EDTA, respectively; micronutrient concentrations were adapted from Machlis and Torrey nutrient solutions (Moore 1974). The final composition of this solution was 1 mg.$L^{-1}$ Fe, 0.7 mg.$L^{-1}$ Mn, 0.5 mg.$L^{-1}$ B, 0.05 mg.$L^{-1}$ Zn, 0.02 mg.$L^{-1}$ Cu and 0.01 mg.$L^{-1}$ Mo.

Calcium (Ca) and magnesium (Mg) were not provided because the liming treatment for fish farming provided sufficient amounts of these two elements. Variation in pH was not controlled during the experiments and remained as commonly encountered in the ponds (i.e., between 7.6 and 8.3). The *P. hydropiperoides* sprouts were obtained from the pond drainage channels and measured about 15 cm long. Each container received three cuttings. Aeration was provided constantly through a perforated hose connected to an air pump.

Table 3. Estimated export rate based on initial nutrient level in the culture solution and nutrient export by biomass harvesting of *P. hydropiperoides*, in eutrophicated water from fish ponds enriched with different levels of N and P, after 40 days cultivation.

| Treatments | Elements | Initial level (mg/4 L container) | Nutrient output via harvested biomass (mg) | End level (mg/4 L container) | Depuration rate (%)* |
|---|---|---|---|---|---|
| $N_c$ $P_c$ | P | 0.592 | 2.54 | 0.532 | 10 |
| | N | 44.36 | 28.79 | 22.16 | 50 |
| $N_1$ $P_1$ | P | 59.42 | 49.73 | 11.28 | 81 |
| | N | 421.40 | 236.82 | 110.88 | 74 |
| $N_2$ $P_2$ | P | 115.92 | 22.60 | 89.60 | 23 |
| | N | 621.00 | 132,43 | 510.08 | 18 |
| $N_3$ $P_3$ | P | 179.94 | 20,78 | 128.96 | 28 |
| | N | 742.96 | 163,25 | 676.44 | 09 |
| $N_4$ $P_4$ | P | 237.92 | 22,08 | 207.24 | 13 |
| | N | 831.68 | 137.67 | 742.96 | 11 |

*Depuration rate = (Initial level End level) × 100/Initial level.

TABLE 4. DRY SHOOT AND ROOT BIOMASS, ROOT LENGTH AND BIOMASS PRODUCTION OF *P. HYDROPIPEROIDES* IN DIFFERENT TREATMENTS.

| | Shoot Biomass | Root Biomass | Root Length | Biomass production |
|---|---|---|---|---|
| Treatment | g container[1] | | cm container[1] | g day[1].m[2] |
| $N_cP_c$ | 2.010 c | 0.39 b | 46.70 a | 0.63 |
| $N_1P_1$ | 7.575 a | 0.91 a | 20.97 b | 2.22 |
| $N_2P_2$ | 3.875 b | 0.63 b | 15.48 c | 1.16 |
| $N_3P_3$ | 4.236 b | 0.60 b | 14.27 cd | 1.27 |
| $N_4P_4$ | 3.980 b | 0.59 b | 12.90 d | 1.16 |

Values followed by the same letter represent homogeneous groups based on the Tukey Test at α = 0.05.

Water samples from each experimental container were obtained at the beginning (6 Aug 2004) and at the end (14 Sep 2004) of the experiment, when the plants reached maturity and began to bear flowers and fruits. Water loss by evapotranspiration, about 500 mL container[1] week[-1], was compensated by replenishing each container with water from the ponds with no additional fertilizer supplementation. Levels of N in the water were analyzed using the Kjeldahl procedure in concentrated sulfuric acid (APHA 1995) and determined through titration. Soluble P was analyzed calorimetrically (λ = 882 nm) with ascorbic acid according to Murphy and Rilley (1962) after being filtered through a 0.45 µm filter. Water conductivity and pH were measured directly in the sampling containers using an INOLAB-WTW pH meter and a SCHOTT conductivity meter. At the end of the experiment, plant material was separated into shoots (stalks, leaves, flowers, and seeds) and root biomass, and their lengths were measured. In the laboratory, all samples were washed with deionized water, dried at 60 C until weight stabilization, ground in a Wiley-type mill and stored in firmly closed flasks for chemical analysis. About 1 g of ground plant material (leaves, stalks, flowers, and seeds) was weighed in a porcelain crucible, incinerated at 500 C (Jones and Case 1990) for 3 h and, after cooling, the ash was dissolved in 10 mL HCl 3 mol L[-1] (Martins and Reissmann 2007), filtered through a "Blauband-SS" 593[3] filter, and the volume was completed to 100 mL in a volumetric flask. In the extract, P was measured using the yellow colorimetric method in a UV/VIS spectrometer at λ = 436 µm. Potassium and sodium (Na) were measured by emission in a flame photometer. Calcium, Mg, Fe, manganese (Mn), copper (Cu), and zinc (Zn) were measured by atomic absorption spectroscopy (Silva 1999). The depuration rate of the *Polygonum* plants was calculated based on the difference between initial and final nutrient concentration, and the depuration efficiency was calculated based on the accumulated biomass and its respective nutrient content. Analysis of variance was performed at $p \leq 0.05$ critical values, followed by a Tukey post-hoc test using the SANEST program from CNPF-EMBRAPA (Zonta and Machado 1985).

## RESULTS AND DISCUSSION

### Water Quality

The level of electrical conductivity of a water sample provides an indirect measurement of its eutrophication levels (water quality classification varies from 10-100 µS cm[-1] in clean water to 1000 µS cm[-1] in highly polluted conditions; (Brigante and Espindola 2003, CONAMA 2005). Average electrical conductivity levels in the present study (Table 2) elevated as the concentration of N and P increased among treatments, particularly in the case of $N_3P_3$ and $N_4P_4$ NA (Table 3). Initial and final levels of N and P are discussed later.

### Growth and Biomass Production

There were significant differences in shoot growth among treatments (Table 4). The highest biomass was recorded in the $N_1P_1$ treatment, followed by a homogeneous group composed of the treatments $N_2P_2$, $N_3P_3$, and $N_4P_4$. The poorest growth was observed in the control group ($N_cP_c$). A similar trend was observed with respect to root biomass, although in this case only $N_1P_1$ differed significantly from the other treatments. In contrast, root length showed an interesting pattern where $N_cP_c$ showed by far the greatest length, which was disproportionate to its biomass. Given that *P. hydropiperoides* has deep roots, one can speculate that root elongation without a corresponding increase in root biomass (Domingos et al. 2005) can represent an adaptive response to variation in nutrient availability. In this sense, and according to Barrat-Segretain (2001), the principles of biomass partitioning are influenced by environmental limitations, leading to special competitive abilities based on differences in growth, survival, and reproduction.

The smaller biomass observed in treatments considered to be above the optimum level ($N_1P_1$) is likely the result of increased stress associated with excess nutrient, as observed in

TABLE 5. CONTENT OF MACRONUTRIENTS, MICRONUTRIENTS, AND SODIUM DETERMINED IN *P. HYDROPIPEROIDES* SHOOTS.

| | N | P | K | Ca | Mg | Na | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | g kg[1] | | | | | mg kg[1] | | | | |
| $N_cP_c$ | 14.1 | 1.26 | 10.9 | 5.6 | 3.2 | 3.3 | 197 | 632 | 9.4 | 59.6 |
| $N_1P_1$ | 31.2 | 6.61 | 25.8 | 3.6 | 1.9 | 4.5 | 233 | 711 | 8.4 | 50.4 |
| $N_2P_2$ | 34.3 | 5.90 | 23.8 | 3.8 | 2.1 | 4.5 | 156 | 702 | 5.0 | 45.7 |
| $N_3P_3$ | 39.0 | 4.95 | 18.9 | 4.3 | 2.0 | 2.9 | 125 | 677 | 5.5 | 32.9 |
| $N_4P_4$ | 33.7 | 5.57 | 20.8 | 4.0 | 2.3 | 3.3 | 140 | 872 | 5.5 | 27.5 |

some studies on submerged macrophytes (Ni 2001). Plants subjected to nutrient concentrations below the observed optimum are also likely to experience stress and reduced biomass production.

## CHEMICAL COMPOSITION AND NUTRIENT REMOVAL

The chemical composition of *P. hydropiperoides* shoots were analyzed (Table 5). The effect of N and P supplementation is evidenced by the higher values in the treated samples in relation to the control. Nutrient content of shoots did not increase proportionately to N and P availability in treatments more extreme than $N_1P_1$. The same is observed for K, even though it was administered only once in each experimental treatment (except for $N_cP_c$). Given that biomass did not continue to increase with increasing nutrient levels, a concentration effect may explain this behavior. Nutrient concentration studies for *P. hydropiperoides* growing in environments with agricultural runoff inputs (Terry and Tanner 1986) showed similar results for N concentration with our control treatment. For P, some observations were higher than our data.

When compared to general micronutrient data from different species surveyed by Epstein and Bloom (2006), the present results suggest *P. hydropiperoides* can efficiently remove micronutrients, particularly Cu and Zn (Table 5). Furthermore, the high efficiency in micronutrient acquisition was apparent from the results of micronutrient levels during water analysis using flame atomic spectroscopy, which showed only traces of those nutrients despite their previous administration, as described earlier. Studies with this species carried out by Wang et al. (2003) reported its high capacity for Zn removal from soils contaminated with this metal. Note, however, that the decrease of Fe and Zn accumulation due to complexation with P increases its concentration in solution (Table 6)

Total content of elements in the shoots of *P. hydropiperoides* (Table 7) follow directly from the amounts of biomass produced and the element concentrations found in them. As a consequence of the superior growth performance (Table 3), $N_1P_1$ is by far the most effective treatment in accumulating the different nutrients available in solution.

Nutrient removal efficiency is directly related to growth and biomass production resulting in an estimated depuration rate of 81% and 74% for P and N, respectively, in the best treatment, $N_1P_1$ (Table 3). Root biomass seems not to be relevant in this case, given the high shoot/root biomass ratio on the best growth treatment (Table 4). In this sense, harvest can be restricted to the shoot. The higher rate of P removal in relation to N especially observed in the $N_1P_1$ treatment is a consequence of a prominent capacity of *P. hydropiperoides* to produce seeds that accumulate high levels of P, normally in the form of phytates. The performance of treatment $N_1P_1$ is also due to an appropriate nutrient balance for growth and seed production (Table 4).

Table 6. Micronutrients and sodium accumulation in shoots of *P. hydropiperoides* in each container (mg container[1]).

| Treatment | Fe | Mn | Cu | Zn | Na |
|---|---|---|---|---|---|
| $N_cP_c$ | 0.40 b | 1.27 c | 0.019 b | 0.122 b | 0.331 ab |
| $N_1P_1$ | 1.76 a | 5.34 a | 0.063 a | 0.381 a | 0.447 a |
| $N_2P_2$ | 0.60 b | 2.70 b | 0.019 b | 0.172 b | 0.440 a |
| $N_3P_3$ | 0.51 b | 2.81 b | 0.023 b | 0.134 b | 0.290 b |
| $N_4P_4$ | 0.54 b | 3.40 b | 0.021 b | 0.110 b | 0.330 ab |

Values followed by the same letter represent homogeneous groups based on the Tukey Test at $\alpha = 0.05$.

Table 7. Macronutrient accumulation in shoots of *P. hydropiperoides* in each container (mg container[1]).

| Treatment | N | P | K | Ca | Mg |
|---|---|---|---|---|---|
| $N_cP_c$ | 28.79 c | 2.54 c | 21.95 c | 1.12 c | 0.65 b |
| $N_1P_1$ | 236.82 a | 49.73 a | 194.75 a | 2.74 a | 1.46 a |
| $N_2P_2$ | 132.43 b | 22.60 b | 91.56 bc | 1.45 bc | 0.82 b |
| $N_3P_3$ | 163.25 b | 20.78 b | 79.94 b | 1.79 b | 0.84 b |
| $N_4P_4$ | 137.67 b | 22.08 b | 81.53 bc | 1.59 bc | 0.89 b |

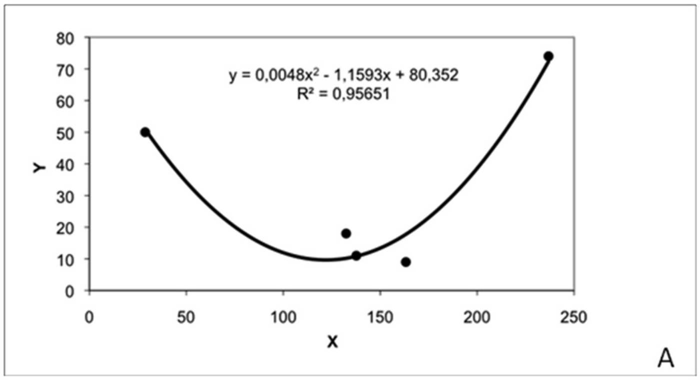
Values followed by the same letter represent homogeneous groups based on the Tukey Test at $\alpha = 0.05$.

Efficiency is given by high biomass production presenting high tissue nutrient contents. The accumulation of P in the $N_cP_c$ treatment is 2.54 mg, while the accumulation in the $N_1P_1$ treatment reaches 49.73 mg.,Concentration is not the only factor to be considered in water nutrient remediation, however; nutrient accumulation should be taken also in to account. Accumulation rate for $N_1P_1$ treatment was significantly higher than that observed in $N_cP_c$ (Table 7). Plants of $N_1P_1$ treatments accumulated 8.22 and 19.6 times more N and P, respectively, than plants of the $N_cP_c$ treatment. After these values, accumulation rates of nutrients became smaller due to reduction in both concentration (Table 5) and biomass (Table 4). Results from the present study, as well as those previously available (Wang et al. 2003), also corroborate the high capacity of *P. hydropiperoides* for the accumulation of Zn and therefore underscore its potential for phytoremediation purposes.

The higher water purification rate was obtained at the $N_1P_1$ treatment, showing the phytorremediation potential of *P. hydropiperoides.* This conclusion is the result of the higher biomass production (Table 4) and, consequently, of the higher exportation rate (output) of N and P through harvest. The relationship between water purification rate and nutrient export through harvesting was highly significant ($R^2 = 0.96$; $P < 0.001$) for both elements (Figure 1).

Based on the results of the present study, we can infer practical applications of *P. hydropiperoides* as a bioremediator. To reach the same efficiency obtained in the experiment in greenhouse during 40 d would require 750,000 sprouts in a fish pond of 1,000,000 L of water, an unreal possibility. Thus, a more feasible scenario should consider a lower purifying rate (25%), which is similar to purifying the root zone (20 cm) in the water column. For this volume (200,000 L), 37,500 plants, fixed with a fluctuating net, would be necessary.

Note that the removal of P and N by this method also implies a repetitive procedure over a long period and a large number of sprouts to increase its efficiency. In nature this species is restricted to fish pond shores and, thus, this method can be improved by using fluctuating nets to hydroponically grow the sprouts..



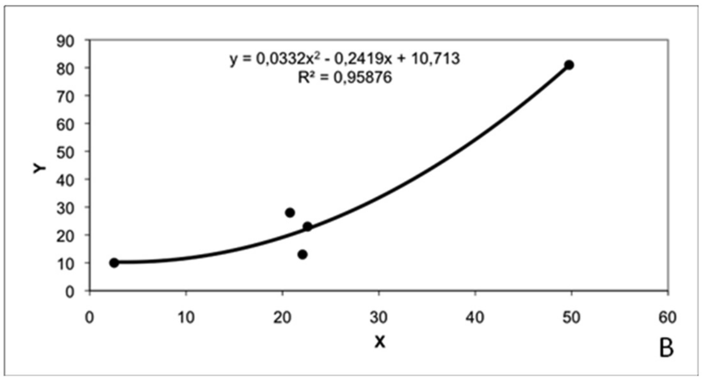




Figure 1. A. Relationship between nitrogen export by harvest (X) and water purification rate (%, Y). B. Relationship between phosphorus export by harvest (X) and water purification rate (%, Y).

## ACKNOWLEDGMENTS

We thank FINEP/CThidro (Financiadora de Estudos e Projetos) for financial support; Dr. Cleverson Andreoli and SANEPAR (Companhia de Saneamento do Paraná) for general coordenation support of the project.

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