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Phytoremediation of Phosphorous and Ammonia with *Eichhornia crassipes* and *Azolla pinnata* in Waste Waters *from Estero de San Miguel* Mendiola Manila Philippines

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Abstract. Wastewater treatment and removal of pollutants by phytoremediation, remain a major concern of the 21th century. This study is focused on the phytoremediation study in Philippine setting, specifically in one of the tributaries of Pasig river-the Estero de San Miguel. It determined the pH, Ammonia and Phosphorous before, 7th and 14th day of phytoremediation with the use of *A. pinnata and E. crassipes*. Twelve improvised water ponds/troughs, 3 ponds per treatment were used for 14 days. T- control (only wastewater), T1 for *A. pinnata* + wastewater, T2 for *E. crassipes* + wastewater and T3 for *A. pinnata E. crassipes* + wastewater. Potential hydrogen, Ammonia-N mg/L, phosphorous mg/L were analyzed before, on the 7th and 14th day of the study. Data gathered was analyzed using ANOVA and Fisher Least Significant Difference test as post hoc test. Result revealed that T1 (*A. pinnata*) lowered the pH and ammonia-N (mg/L) of wastewaters from Estero de San Miguel. T3 (combination of *A. pinnata + E. crassipes)* has significantly lowered the Phosphorous level of the wastewaters. Thus both aquatic macrophytes can be used as phytoremediation agents in the said Estero.

1 Introduction

Urban industrialization is always coupled by environmental pollution particularly bodies of water, turning previously biocompatible lakes and rivers and rivulletes. There are many methods of removing pollutants from wastewater but phytoremediation is gaining popular. It has been observed that phytoremediation of wastewater using the floating plant system is a predominant method which is economic to construct, requires little maintenance and increase the biodiversity [1]. Phytoremediation strategies, based on the abilities of aquatic plants to recycle nutrients, offer an attractive solution for the bioremediation of water pollution, and represents one of the most globally researched issues. The subsequent application of the biomass from the remediation for the production of fuels and petrochemicals offers an ecologically friendly and cost-effective solution for water pollution problems and production of value-added products [2].Phosphorous in wastewaters came from decomposition of organic matter from human waste and from detergents and soaps which is one of the many pollutants in wastewaters. Phosphorous in the form of phosphate plays an important role in metabolic activities of the plant such as; photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement. Ammonia on the other hand, gives wastewater a pungent odour due to action taken by most bacteria in its metabolism and is mostly preferred by pond plants than nitrate. Aquatic plants can use ammonia directly. Together with sugars aquatic plants can metabolize even in dark reaction.

Estero de San Miguel is located in Manila, Philippines. It is one of the 47 major tributaries of Pasig river, and has a sub-cathcment area of 259261.2 square meters [3]. A study on the analyses of physicochemical parameters of the esteros – turbidity, temperature, salinity, EC, pH, DO, BOD, COD,TKN, TP, chl-a, and oil and grease-showed the *estero* waters fall under Class D water quality criterion. TKN and TP values indicated hypertrophic conditions AT all stations. Low chl-a values were indicative of stressful conditions to the primary producers. Nineteen phytoplankton genera - Cyanophyta (3 spp), Chlorophyta (9 spp) and Bacillariophyta (7 spp) thrived in the esteros [4].

Azolla pinnata with a preferred common name, mosquito fern, under the domain: Eukaryota, Kingdom:

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Plantae, Phylum: Pteridophyta, Class: Filicopsida, Family: Azollaceae; is small aquatic plants. It is 1.5-2.5 cm long, with a +/- straight main axis with pinnately arranged side branches, progressively longer towards the base, thus roughly triangular in shape; the basal branches themselves becoming pinnate and eventually fragmenting as the main axis decomposes to form new plants. Roots have fine lateral rootlets, giving a feathery appearance in the water. Leaves minute, 1-2 mm long, overlapping in two ranks, upper lobe green, brownish green or reddish, lower lobe translucent brown; minute, short, pillae, +/- cylindrical unicellular hairs often present on the upper lobes. When fertile, round sporocarps 1-1.5 mm wide can be seen on the underside at the bases of the side branches. The leaves often have a maroon-red tinge and the water can appear to be covered by red velvet from the distance. *A. pinnata* is often applied to rice paddies as a nitrogen fertilizer and weed suppressant [5]. *Azolla pinnata* has many uses. It can be used as bio-fertlizer, feed for poultry and fish [6].The plant is an aquatic fern of stagnant water. It can spread rapidly and has the ability to survive on moist soil in and around rivers, ditches, and ponds; it also occurs in Papyrus swamps, on sandbanks, in sluggish rivers and in neglected paddy fields. The occurrence of a vigorously growing population of azolla in a farm dam generally indicates high nutrient levels in the water. Azolla can survive within a water pH range of 3.5 to 10, but optimum growth occurs in the pH range of 4.5 to 7 and temperature range of 18^{o}C to 26^{o}C. [7].. Under optimum conditions, azolla's growth spreads across the dam surface until it covers the surface of the water in a dense cover. Azolla can double its leaf area in seven days if conditions of high nutrient levels and water temperatures persist [8].\Azolla does not thrive under adverse conditions: extreme cold or heat. But it can be preserved even under such adverse conditions in very slow moving water bodies such as streams, canals, sewage channels, small ponds and tanks and unused wells. They are known as inoculum banks. The optimum temperature for azolla ranges between 15-35 ^{o} C [9].

Water hyacinth (*Eichhornia crassipes*), is a native of Brazil and is introduced to and is naturalized in many tropical countries like Philippines [10]. All along the Pasig River in the Philippines, water hyacinths can be found almost year-round; they sometimes cover a complete section of the river so that no water is visible. Obviously, it has acclimated very favorably, but no one cares about controlling it and using its merits for the environment [11].Water hyacinth can be recommended as costless, efficient and friendly environmental process for waste water treatment [12]. The presence of phosphorus and nitrogen in excess amounts could lead to the eutrophication of water sources, which may also create environmental conditions that favour the growth of toxin-producing cyanobacteria. Chronic exposure to some of such toxins produced by these organisms can cause a host of other diseases [13]. Objective of the study. This study determined the chemical characteristic (pH, Phosphorous and ammonia-N) of wastewaters from Estero de San Miguel, Mendiola Manila Philippines before, 7th and 14th day use of *azolla pinnata* and *Eichhornia crassipes* as phytoremediation agents.

2 Methodology

A. Materials

Plant materials used in this study were *A. pinnata* and *E. crassipes* which were collected from Azolla Farm and water pond in La Union, Pangasinan, Philippines (figure 1)

Fig. 1. Samples of A. *pinnata* (left) and *E. crassipes* (right).

Other materials used are; measuring glass, plastic strainer, weighing scale, Whatman paper no. 1, digital pH meter, 12 improvised plastic containers as water troughs/ ponds. (see figures 2-a, 2-b, and 2-c).

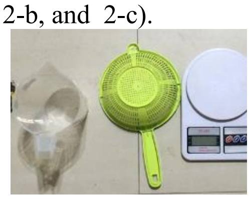


Fig. 2-a. measuring glass, plastic strainer and weighing scale.

Fig. 2-b. Whatmann paper no. 1, digital pH. 2-c. Improvised plastic trough/ pond.

B.*Methods*

A study on phytoremediation of wastewaters [14] served as a guide in the conduct of this research. Wastewaters was collected from Estero de San Miguel Mendiola Manila Philippines and brought to the experimental site in plastic containers.

The aquatic plants selected for phytoremediation were *A pinnata* and *E. crassipes*. It was collected freshly from natural pond at Azolla Farm in La Union, Pangasinan Philippines. These plants were cleaned properly to remove dirt and dust under tap water and stabilize in laboratory conditions for 2-3 days to normalize their growth. Complete randomized design with four treatments and three samples was used to conduct the

experiment as shown in the experimental set-up (figure 3).
The laboratory conditions were maintained uniform throughout the experimental period. This study is limited to 3 main chemical characteristics of wastewater samples (pre and post treatments) such as pH, phosphorous and ammonia. These characteristics were determined by using standard methods, such as digital pH meter for its potential hydrogen. The Phosphorous and ammonia content were determined by American Public Health Association (**APHA**), the American Water Works Association (**AWWA**) methods. The data were analyzed statistically by using ANOVA and Fisher Least Significant Difference test as post hoc test.

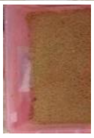
Sample Number	Treatment Number			
	- (waste water)	1 (*A. pinnata* + wastewater)	2 (*E crassipes* + wastewater)	3 (*A. pinnata* + *E crassipes* + wastewater)
1				
2				
3				

Fig.3. Experimental lay-out

E. crassipes about 8-10 inches long and *A. pinnata* were used as experimental plants The weight of *Azolla pinnata* was recorded after keeping them on a filter paper to remove excess water. An initial analysis of main pH, phosphorous mg/l and ammonia-N mg/l was done. Newly collected wastewater samples were delivered to Water Analysis laboratory in clean tighten glass containers (before and 7^{th} and 14^{th} day of experimentation), for the determination of phosphorous and ammonia content. Plants were contained in plastic ponds/troughs having capacity of 33 liters wastewater following the treatments as follows; T-: control no aquatic plant.T1:400 grams of *A. pinnata* in wastewater from Estero de San Miguel; T2: 20 pieces *E. crassipes* in wastewater from Estero de San Miguel; and T3: 200 grams *A. pinnata* + 10 *E. crassipes* in wastewater from Estero de San Miguel

3 Results

3.1 Initial Potential Hydrogen (pH) of wastewater per treatment per sample

Table 1 posited the initial potential hydrogen of waste water samples per treatment. Since wastewaters came from one source, (*Estero de San Miguel* in Mendiola Manila Philippines), the pH of waste waters, before the study is 6.9. The pH of waste water samples are below the neutral pH of 7 which indicate that aquatic plants used can thrive in the water. *Azolla* can survive within a pH range of 3.5 to 10 [15]. *E. crasippes* (water hyacinth) plants survived in a pH range of 4.0 to 8.0. Both alkaline pH (above 8.0) and highly acidic pH (below 4.0) had inhibitory effect on the growth of plants [16].Potential hydrogen (pH) varies depending on the geology of the river catchment, on river flow, and on wastewater discharges but is generally in the range 6 – 9 [17].

3.2 Initial Phosphorous (mg/L) of wastewater per treatment per sample

In freshwater, phosphorous the natural background levels of total phosphorus are generally less than 0.03 mg/L. The natural levels of phosphate usually range from 0.005 to 0.05 mg/ [18]. **Table 2** shows that the initial phosphorous level of wastewaters, are beyond the level of freshwaters (1 mg/l) thus, wastewater is euthrophic. Euthrophic water source has 0.03-0.1 mg/l of total phosphorous [19].
Phosphorus (P) is the most critical and limiting input for Azolla rice cultivation Azolla absorbs P from the floodwater and makes it available to the plant [20].

Table 1. Initial potential hydrogen (pH) per treatment per sample

Sample	Treatment			
	-	1	2	3
1	6.9	6.9	6.9	6.9
2	6.9	6.9	6.9	6.9
3	6.9	6.9	6.9	6.9
Total	20.7	20.7	20.7	20.7
Mean	6.9	6.9	6.9	6.9

Table 2. Initial phosphorous content per treatment per sample

Sample	Treatment			
	-	1	2	3
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
Total	3	3	3	3
Mean	1	1	1	1

3.3 Initial Ammonia (mg./L) of wastewater per treastment per sample

Table 3 displays the initial Ammonia content of wasters which is 8 mg/l. Natural (unpolluted) waters contain relatively small amounts of ammonia, usually less than 0.02 mg/l as N [21]. With the above initial ammonia and phosphorous content of the wastewaters from Estero de San Miguel, it shows that it can favor the growth of *Azolla pinnata* and *E. crassipes*

The levels of available nitrogen and phosphorous are the most important factors limiting in growth. The half-saturation co-efficients for water hyacinths grown under constant conditions have been found to be from 0.05-1 mg/l for total nitrogen and from 0.02-0.1 mg/l for phosphates. Growth quickly tails off below the lower limits [22].

Table 3. Initial ammonia (mg/L) content per treatment per sample

Sample	Treatment			
	-	1	2	3
1	8	8	8	8
2	8	8	8	8
3	8	8	8	8
Total	24	24	24	24
Mean	8	8	8	8

3.4 Mean Potential hydrogen of wastewaters, before, 7th and 14th day of experimentation

Table 4 shows the mean pH from initial, 7th and 14th day of experimentation. Treatment 1 had the highest mean pH (7.36), followed by T3 (7.13), T2 (7.01) and T- (6.9) respectively. The result revealed that the ponds/troughs with *A. pinnata* had increased the pH from 6.9 to 7.36 which is nearest to neutral. Analysis of Variance (ANOVA) demonstrate that there is significant difference (p value is lower than 5% level of significance) among the treatment means in 14 days of experiment in terms of pH. Fisher Least significant difference calculated that significant difference exist between T1vs.T2 and as indicated by different superscripts in the mentioned treatment.

The result indicates that *A. pinnata* can increase the pH of wastewaters from Estero de San Miguel. The pH after phytoremediation using *A. pinnata* is 7.36. Potential hydrogen rises with photosynthetic activities of azolla plant. A study on phytoremediation using *E. crassipes* reported that there was no much change for pH. The pH value was found to be between 6 and 8 [23].

Table 4. Mean potential hydrogen, before, 7th and 14th day of experimentation

Days	Treatment			
	-	1	2	3
initial	6.9	6.9	6.9	6.9
7th	6.9	7.3	7	7.1
14th	6.9	7.9	7.3	7.4
Total	20.7	22.1	21.2	21.4
Mean	6.9^{acd}	7.36^{b}	7.01^{c}	7.13^{d}

ANOVA

SV	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.461	2	0.230	5.36*	0.04	5.14
Columns	0.336	3	0.112	2.60	0.14	4.75
Error	0.258	6	0.043			
Total	1.056	11				

Legend: * significant at 5% level of significance

3.5 Mean phosphorous level (mg/L) of wastewater before, 7th and 14th day of experimentation

The mean phosphorous in mg/L of treatments is displayed in **table 5**. Lowest phosphorus level was obtained in T3 (.31), followed by T1 (.783), T2 (.83) and T- remained 1. ANOVA showed significant difference and Fisher LSD revealed significant difference in pairs of T- vs T3, T1 vs. T3 and T2 vs T3 as shown in the difference of superscripts in treatment means. The result further implies that a combination of *A. pinnata ad E. crassipes* in wastewaters significantly lowered the Phosphorous level of wastewaters in Estero de San Miguel Mendiola Manila Philippines. Azolla plants showed substantial P-removal efficiency from P-eutrophicated solutions. Several studies showed that both macrophytes can lower the phosphorous level in wastewaters. Water hyacinth showed its ability to survive in high concentration of nutrients. Significant removals of ammonia and phosphorus, respectively was obtained using the water hyacinth plants. Use of water hyacinths can help reduce eutrophication effects in receiving streams and also improve its water quality [24]. The Aquatic fern Azolla was used to treat waste water. Because of the capability of N fixation by Azolla, the efficient removal of phosphorus would be expected even after N is consumed [25].

Table 5. Mean phosphorous (mg/L) level, before, 7th and 14th day of experimentation

Days	Treatment			
	-	1	2	3
initial	1	1	1	1
7th	1	0.96	0.99	0.62
14th	1	0.39	0.5	0.32
Total	3	2.35	2.49	0.94
Mean	1^{ab}	$.783^{ad}$	$.83^{bd}$	$.31^{c}$

ANOVA

SV	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.433	2	0.216	6.803*	0.028	5.14
Columns	0.189	3	0.063	1.982	0.22	4.75
Error	0.191	6	0.031			

3.6 Mean ammonia-N Level (mg/L) of wastewater before, 7th and 14th day of experimentation

Table 6 reveals the mean ammonia-N level (mg/L) on the initial, 7th and 14th day of experimentation. Treatment 1 had the lowest mean of 5.03 mg/L, followed by 2 (5.32), T3 (5.75) and T- (8) respectively. The result revealed that the ponds/troughs with *A. pinnata* had lowered the ammonia-N (mg/l) from 8 to 5.03. Analysis of Variance (ANOVA) demonstrate that there is significant difference (p value is lower than 5% level of significance) among the treatment means on the 7th and 14th day of experimentation .Fisher Least significant difference calculated that significant difference exist between T- vs.T1 and T- vs. T2 as indicated by different superscripts in the mentioned treatment.
The result indicates that *A. pinnata* can lower the ammonia-N (mg/L) of wastewaters from Estero de San Miguel, in 14 days of experimentation much faster than *E. crassipes* and the combination of *E. crassipes* and *A. pinnata*. Azolla can grow well in effluents from wastewater stabilization ponds despite the high ammonium content of the medium [26]

Table 6. Mean ammonia-N (mg/L) content, before, 7th and 14th day of experimentation

Days	Treatment			
	-	1	2	3
initial	8	8	8	8
7th	8	4.85	5.23	5.97
14th	8	2.26	2.74	3.28
Total	24	15.11	15.97	17.25
Mean	8^{a}	5.03^{bde}	5.32^{cdf}	5.75^{aef}

ANOVA

S.V	SS	df	MS	F	P-value	F crit
Rows	30.89	2	15.44	8.66*	0.017	5.14
Columns	16.33	3	5.44	3.05	0.11	4.75
Error	10.69	6	1.78			
Total	57.92	11				

Legend: * significant at 5% level of significance

4 Conclusion

Eutrophication of wastewaters can be minimized by the used of indigenous plants like *A. pinnata* and *E. crassipes*. Result of this study implied that *A. pinnata* increased the pH of wastewaters from Estero de San Miguel and lowered the ammonia (mg/L) content in 14 days. The combination of *A. pinnata* and *E. crassipes* lowered the phosphorous (mg/L) content of wastewater. Thus, both aquatic macrophytes can be used as potential phytoremediation agents. Based from the result of this study several phytoremediation studies and projects can be proposed in order to control ammonia and phosphorous level in wastewaters. Since this study was conducted on improvised waterpond, a study on-pond can be done to determine the growth response of the plants in lotic environment. Proper spacing, and growth management of both aquatic plants, in a given area to avoid O2 depletion in wastewaters is also recommended. A study on the use of both aquatic plants as organic fertilizer in other aquatic plants can be undertaken.

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