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Removal of Eutrophying Nutrients (Nitrogen and Phosphorus) By Aquatic Macrophytes Biosorption System (AMBS) in Polluted River

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ABSTRACT
The present study assessed the efficiencies of Aquatic Macrophytes Biosorption System (AMBS) using Eichhornia crassipes (water hyacinth) and Najas tenuifolia var. pseudograminea (digman) as phytoremediators of eutrophied waters at the Niugan River, Cabuyao, Laguna. The following analyses were performed in three sampling sets and measured every 7th day for the period of one month: total Kjeldahl nitrogen (TKN), total phosphorus (TP), Lead (Pb), Cadmium (Cd), Dissolved oxygen (DO), Total Suspended Solid (TSS), pH, and temperature (TEMP). The results from the field experiment revealed that Pb uptake by N. tenuifolia var. pseudograminea was higher than E.crassipes (14.0 mg/g and 0.05 mg/g; respectively) after 28 days as an Aquatic Macrophytes Biosorption System (AMBS) component. In terms of eutrophying nutrients, E. crassipes accumulated more N and P (60.04 g/kg D.W. and 14 g/kg D.W.; respectively) than N. tenuifolia var. pseudograminea (40.01 g/kg D.W. and 12.20 g/kg D.W.; respectively) on the 28th day of the study. On the other hand, water quality was improved when the AMBS was introduced in the Niugan River resulting in the proliferation of fish species. The use of AMBS demonstrated its potential application in phytoremediation, showing reductions in Heavy Metals (Pb, Cd), TKN, TP and TSS as well as improvement of DO and pH throughout the duration of the study.
Keywords: *Phytoaccumulation, Eutrophying nutrients, Macrophytes, Water Quality*

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INTRODUCTION

Rapid urbanization and agricultural expansion are some of the leading causes of water pollution which is the major problem in many countries. Increasing use of fertilizer results in significant build-up of nutrients, such as nitrogen (N) and phosphorus (P), in the soils [1]. These nutrients are subject to loss to surface and ground water. Water quality is impaired and water availability is reduced because of accelerated eutrophication [2].

In the Philippines, the net effect of pollution is the deterioration of water quality that impact tremendously on biodiversity and overall productivity of these ecosystems including human health. Data from the World Bank [3] revealed that the range of impacts translates into environmental cost and economic losses estimated at PhP 67 Billion (US$1.3 billion) annually. These include PhP 3 billion for health, PhP 17 billion for fisheries production, and PhP 47 billion for tourism.

Phytoremediation in aquatic environment was also explored by Zafaralla *et al.* [4] by installing Aquatic Macrophyte Biosorption System (AMBS) across the Molawin Creek. Based on the results, it carries out initial steps towards a sustainable community-based restoration, protection and conservation program for this body of water. The data also showed improvement of water quality having acceptable BOD. The most remarkable effect of the AMBS was the proliferation of fingerlings in the creek.

Eichhornia crassipes Mart. Solms., commonly known as water hyacinth is a member of pickerelweed family (Pontederiaceae). It is a monocotyledonous, perennial, free floating (except when stranded in the mud) aquatic plant [5]. Since *E. crassipes* is an alien plant that is highly invasive to tropical regions, its application to remediate polluted water especially in constructed enclosures would help control the spread of the plant through periodic harvesting [6]. Wu and Raven [7] provided a key to the species of the genus Najas found from neotropical to tropical areas which was later used for the identification of *Najas tenuifolia var. pseudograminea* W. Koch, a native aquatic plant species of Laguna de Bay, Philippines [8]. The plant is a submerged aquatic plant with monoecious type of flower. Based on the extensive survey by Scopus (http://www.scopus.com), the largest abstract and citation database revealed that there is no work reported so far on using *N. tenuifolia* var. *pseudograminea* in phytoremediation of heavy metals. The study aims to evaluate the efficiency of *E. crassipes* and *N. tenuifolia* var*. pseudograminea* as components of Aquatic Macrophytes Biosorption System (AMBS) in the Niugan River, Cabuyao, Laguna. Specifically, this study: [1] examined the growth of the aquatic macrophytes utilized as AMBS in Niugan River [2] determined the uptake ability of eutrophying nutrients (Nitrogen and Phosphorus) by the AMBS; [3] examined the water quality improvements of the AMBS at the Niugan River ecosystem.

MATERIAL AND METHODS

Study Site

The site selected for the field experiment was the Niugan River located in Brgy. Niugan, Cabuyao, Laguna, with specific geographic coordinates 14^{0}15’57”N; 121^{0}7’34”E (Fig. 1). This was selected based on the following criteria: (a) evidence of industrial and domestic pollution (b) accessibility (c) river depth not more than 1.5 m deep; and (d) permission of the barangay captain. The study site had an average depth of 0.7 m. It is bounded to the east by Laguna de Bay, to the west by the Cavite, to the north by Sta. Rosa and on the south by Calamba. It is surrounded by varying types of land uses: agricultural, residential, commercial, and industrial (9). Among the most prominent industries surrounding the river basin are Nestlé Philippines Factory, Asia Brewery and Mina Oil Mill Corp.

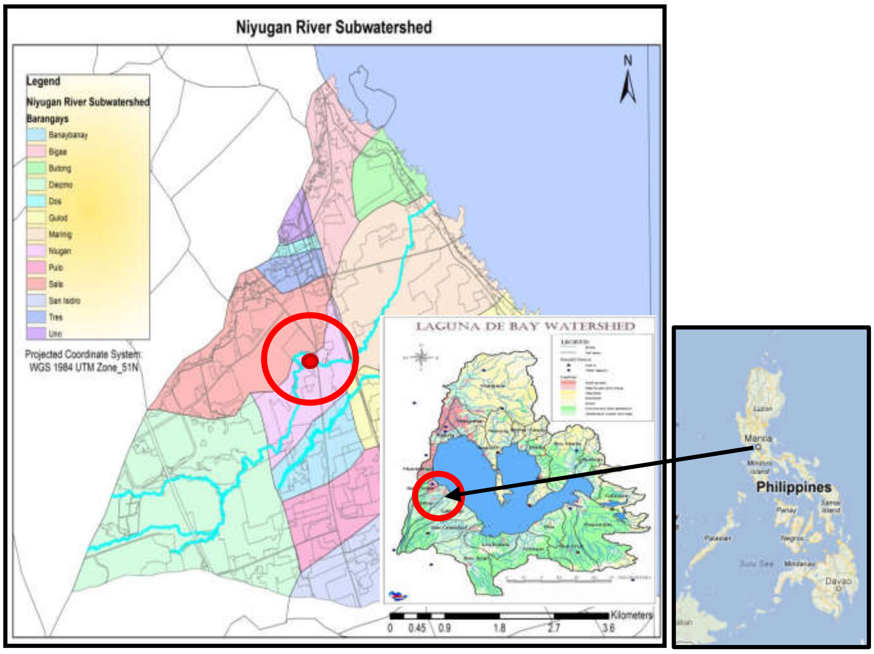


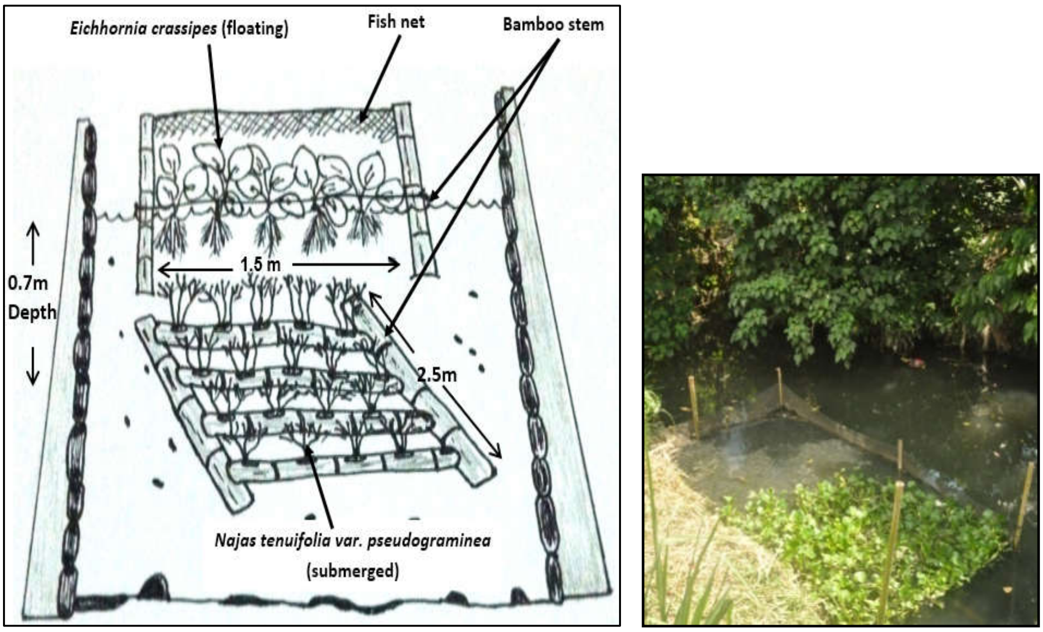
Figure 1. Location of field experimental set-up at Niyugan River (14^{0}15’57”N; 121^{0}7’34”E), Cabuyao City, Laguna.

Preparation and Installation of the Aquatic Macrophytes Biosorption System (AMBS)

The following materials were prepared for the establishment of the AMBS:

a) Bamboo pole measuring 2 m with 2-holes in each node.
b) Fish net (1.5m x 2.5m)
c) Aquatic macrophytes: *E. crassipes* (floating) and *N. pseudograminea* (submerged)

This study followed the concept of Aquatic Macrophytes Biofiltration System (AMBS) first introduced by Zafaralla et al. (4) in Molawin Creek, UPLB. Floating (*E.crassipes*) and submerged (*N. pseudograminea*) aquatic macrophytes were utilized in this study (Fig. 2). A 1.5m x 2.5m fishnet was used for *E. crassipes* and 8 bamboo stems (2.5 m) with soil was used as substrate for *N. pseudograminea*. *E. crassipes* floating pond was installed before the *N. pseudograminea* set-up.



A B

Figure 2. (A) Sketch of the field experimental set-up showing the modified Aquatic Macrophytes Biosorption System (AMBS) and the actual aerial view of the study (B).

Data Gathered

Water Quality Evaluation – The water quality of the study site was measured, and parameters such as dissolved oxygen, water temperature, and pH were determined on site. On the other hand, total suspended solids (TSS), heavy metal analysis were measured in various analytical laboratories of the University of the Philippines Los Baños (i.e. Institute of Chemistry, Institute of Plant Breeding and Institute of Biological Sciences) using the standard method by APHA [10].

The following data of AMBS site installation were collected:

1) **Measurement of Growth Parameters –** Weekly measurement of plant height and weight *in situ* using a ruler and weighing scale were done. Plant height of *N. tenuifolia* var*. pseudograminea* involved the above ground extending from the above-soil part to the apex of the main axis (Fig. 6). Plant weight of *E. crassipes* was taken from blot dried materials.
2) **Measurement of Eutrophying Nutrients –** TKN (Total Kjeldahl Nitrogen) and TP (Total Phosphorus) were determined from water and plant samples collected weekly by grab-sampling method. The three random grab samples of 1-L river water were taken around the AMBS.
3) **Measurement of Photosynthetic Pigments –** Fresh leaves were handpicked from both aquatic macrophytes and placed in a sealed plastic bag and transported in a styrofoam containing ice. In the laboratory, plants samples were rinsed in running tap water and blot-dried, shredded and placed in a mortar with pestle and then ground after adding liquid Nitrogen. A 250-mg ground samples was added with 10 ml of 90% acetone and decanted in a 10-ml test tube with screw cap for spectrophotometric determination of pigment content. Data were gathered weekly for a period of 28 days.

Statistical Analysis

Data were analyzed using a 3 factorial split-plot in CRD. There were three replicates used per analysis. Data were subjected to one way analysis of variance (ANOVA), using the SAS software version 9.1. All mean comparisons were tested using the Duncan' Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Growth and Survival of Aquatic Macrophytes

Floating (*E. crassipes*) and submerged (*N. tenuifolia* var*. pseudograminea*) aquatic macrophytes were utilized as AMBS (Acquatic Macrophytes Biofiltration System). The growth of the aquatic marophytes is shown in Fig. 49. Both of the plants exhibited increasing pattern of growth over the period of 28 days (Fig. 3A & B). The final height of *N. pseudograminea* has reached up to 320.19 cm which is more than double of the original height (150 cm) after 4 weeks in the field condition (Fig. 3B). In terms of percentage survival, the aquatic macrophytes used in this study have shown a 100% survival for the period of 28 days. This result was similarly observed by Souza *et al.* [11] which showed that Myriophyllum exhibited considerable growth throughout the study when utilized as phytoremediator of polluted water. Weekly examination of the growth (plant height and weight) of both macrophytes have showed high significant difference (*P<0.001*), indicating that both macrophtes adapted to the established AMBS environment in the Niugan river.

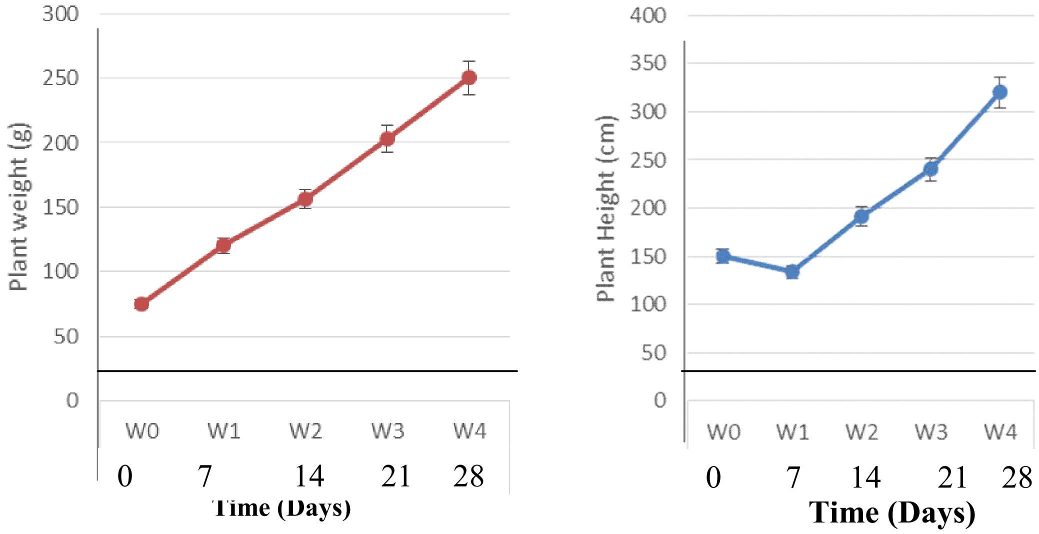


Figure 3. Plant weight of *E. crassipes* (A) and height of *N. tenuifolia* var. *pseudograminea* (B) utilized as AMBS component in the Niugan River for the period of 28 days under field condition.

Photosynthetic Pigments of the Aquatic Plants in AMBS

Photosynthetic pigment content from both of the aquatic macrophytes are presented in Figs. 8 A & B. It was revealed that the total Chl of *E. crassipes* and *Najas tenuifolia* var. *pseudograminea* growing in Niugan River were 2.4 mg/g and 1.9 mg/g respectively. Comparatively, the total Chl of the aquatic macrophytes *E. crassipes* and *N. tenuifolia* var. *pseudograminea* growing in their natural habitat in Bayog River are 2.3 mg/g and 2.1 mg/g respectively. Although the photosynthetic pigment of *N. tenuifolia* var. *pseudograminea* decreased slightly, there are no significant different detected on the photosynthetic pigment of the aquatic macrophytes. This result is also comparable to the total Chl of Hydrilla and rice which is 1.26 mg/g and 1.20 mg/g respectively (12) in its natural habitat. The decreasing amount of photosynthetic pigment of *N. tenuifolia* var. *pseudograminea* indicates that the plant was slightly stressed under the field condition in Niugan River.

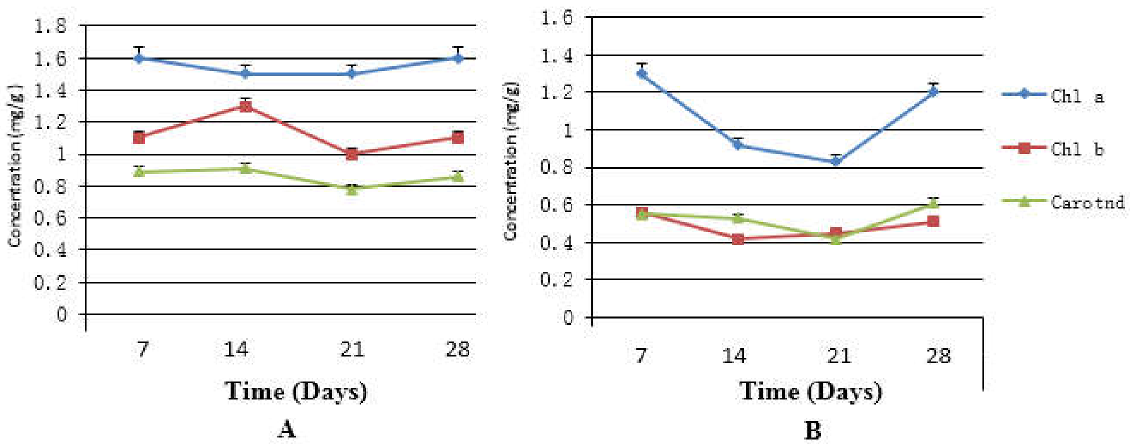


Figure 8. Photosynthetic pigment concentrations in the leaves of *E. crassipes* (A) and *N. tenuifolia* var. *pseudograminea* (B) utilized as AMBS component in the Niugan River for the period of 28 days under field condition.

Uptake of Eutrophying Nutrients (Nitrogen and Phosphorus) by Aquatic Macrophytes

Nitrogen (N) and Phosphorus (P) accumulation of *E. crassipes* were 32.83 g/kg N and 17.03 g/kg P respectively while *N. tenuifolia* var. *pseudograminea* yielded a concentration of 47.25 g/kg and 9.96g/kg respectively after 28 days under field condition (Figs. 10 and 11). The N and P uptake of the aquatic macrophytes used in this study was higher as compared to the uptake of eutrophying nutrients of other species of macrophytes, *Polygonium hydropiper* 13.23 g/kg N and 9.72 g/kg P [13]; *Pistia stratoites* 17.0 g/kg N and 3.0 g/kg P. The results have showed that *E. crassipes* accumulated more eutrophying nutrients than *N. tenuifolia* var. *pseudograminea*. The latter can tolerate high amount of eutrophying nutrients as previously described [14; 15; 16].

This finding was also similarly observed by Su *et al.* [17] on physiological responses of *Egeria densa* in which the high ammonium concentration reduced the amount of total chlorophyll, soluble proteins and carbohydrates. Phosphorus toxicity can vary in differently in plant varieties and cultivars. Research on other crops showed that excessive phosphorus does not create phosphorus toxicity but rather induces other micronutrient deficiencies. Potato plant can even tolerate as high as 200 mg/kg Phosphorus without even showing toxicity [18].

E. crassipes and *N. pseudograminea* can remove 89.83 mg/g N and 69.53 mg/g N, respectively. According to Brix [19], the uptake capacity of eutrophying nutrients by aquatic plants is in the order, floating plants (especially large-leaved species), followed by emergent species, and lastly, the submerged species.

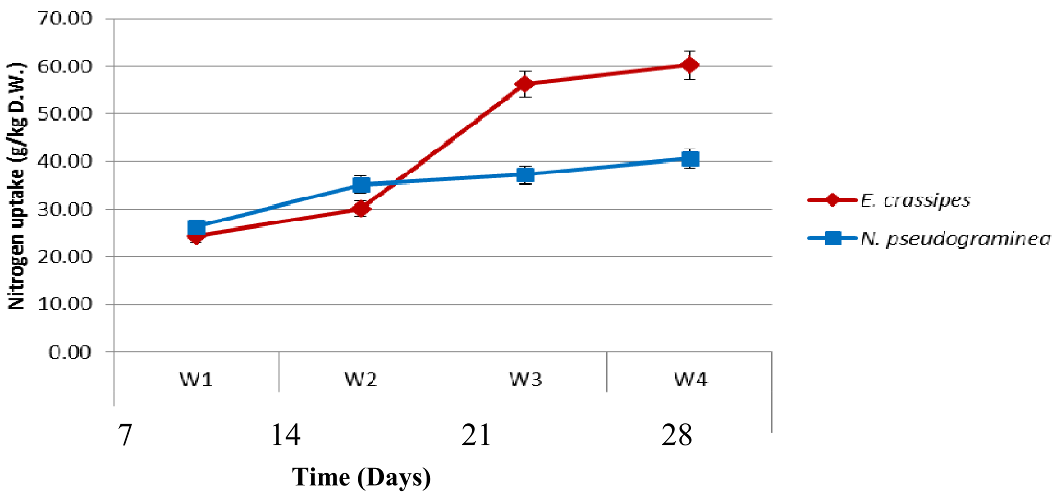


Figure 10. Nitrogen uptake of *E. crassipes* and *N. pseudograminea* utilized as AMBS component in the Niugan River for the period of 28 days under field condition

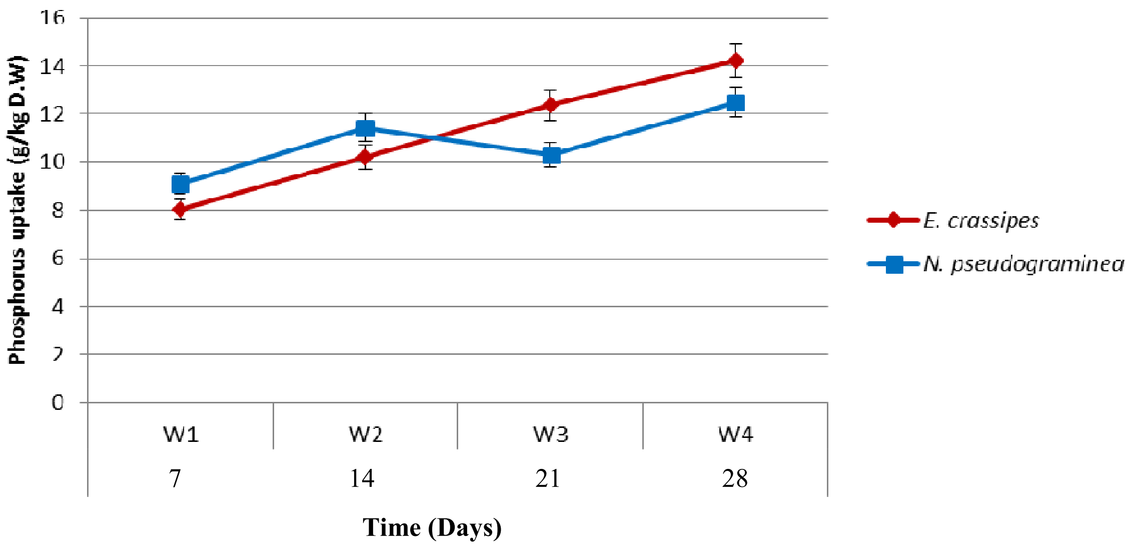


Figure 11. Phosporous ptake of *E. crassipes* and *N. pseudograminea* utilized as AMBS component in the Niugan River for the period of 28 days under field condition

Water Quality and the Aquatic Macrophytes

Physico-chemical analysis of Bayog River (source of the explants) qualified for Class B which for recreational and suitable for fishery production while Niugan River is classified under Class C (20). In terms of heavy metal (Pb and Cd) content in both rivers also passed the toxicity of the heavy metals with the amount of 0.038 and 0.011 mg/L respectively. The pH of Niyugan River was 7.03 while in Bayog River reach up to 6.8.

There is an improvement of DO and TSS by 22.8% and 14.6%, respectively, during the 21st day-operation of the AMBS in the field experiment (Table 1). This demonstrates that a combination of submerged and floating aquatic macropytes can improve the quality of water in a polluted river. Similarly, Souza et al. (21) revealed that Myriophyllum improved the water quality through removing excess BOD by 75.4% and COD by 67.4%. The result on the water quality parameter suggested that the application of Aquatic Macrophytes Biosorption System (AMBS) is effective in reducing pollutants of the eutrophic river. One of the concrete evidence of the improvement of water quality was the proliferation of many fish fingerlings around in the AMBS during the 21st day of the installation. This experiment have shown that the combined effect of floating (*E. crassipes*) and submerged (*N. tenuifolia* var. *pseudograminea*) aquatic macrophytes have reduced significant pollutants in the field experiment as well as the improvement of water quality in a eutrophic river as evidence by the proliferation of fish fingerlings population.

Table 1. Water quality of the inlet and outlet at the AMBS in Niyugan River.

PARAMETERS	0-DAY PERIOD	NO. OF DAYS							
		7th		14th		21st		28th	
		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
DO (mg/L)	4.21	4.28	4.75	4.58	4.93	4.38	5.18	5.81	6.66
pH	7.4	7.46	7.99	7.43	7.28	7.86	7.54	8.56	7.98
Temp(^{0}C)	18.31	18.36	18.16	18.23	18.13	18.11	18.64	18.68	18.23
TSS (mg/L)	80	91	72	102	83	97	82	96	81

CONCLUSION

The results obtained from the present study revealed that *E. crassipes* was found accumulate more N and P than *N. tenuifolia* var. *pseudograminea*. As a component of AMBS, *N. tenuifolia* var. *pseudograminea* attracts more faunal species thereby increasing biodiversity. On the other hand, water quality parameters (DO and TSS) have improved when the AMBS was introduced in the Niugan River. The AMBS particularly *N. tenuifolia* var. *pseudograminea* is also a potential aquaculture species by serving as a spawning and breeding ground for economically important fish. This consistent with the finding of Zafaralla et al. (2010) that AMBS enhances biodiversity species.

Apparently, the submerged nature of *Najas* in its habitat puts it in direct contact with the dissolved HM thus its tissue content is increased. As a component of AMBS, *N. tenuifolia* var. *pseudograminea* attracts more faunal species thereby increasing biodiversity. On the other hand, water quality parameters (DO and TSS) have improved when the AMBS was introduced in the Niugan River. The AMBS particularly *N. tenuifolia* var. *pseudograminea* is also a potential aquaculture species by serving as a spawning, breeding, foraging ground, shelter and honing ground of aquatic fauna for economically important fish.

Based on the results, this study also showed the potential use of *Najas tenuifolia* var. *pseudograminea* as a phytoremediation agent, aside from *E. crassipes*. These submerged aquatic macrophyte not only removed heavy metals but they also promoted and supported biodiversity of economically important aquaculture species. However, *N. tenuifolia* var. *pseudograminea* is not listed as an invasive species unlike its counterpart *E. crassipes.*

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CONFLICT OF INTEREST

The authors declares that is no conflict of interest in this study

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