Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [10] September 2018 : 17-23

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Phytoaccumulation of Heavy Metals (Pb & Cd) by *Najas tenuifolia* var. *pseudograminea* and *Eichhornia crassipes* in Polluted River

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ABSTRACT
Phytoaccumulation of heavy metals (Lead and Cadmium) in the polluted river of Niugan, Cabuyao, Laguna, Philippines was studied using the modified Aquatic Macrophytes Biosorption System (AMBS) by utilizing Najas tenuifolia var. pseudograminea and Eichhornia crassipes as phytoremediators. The following analyses were performed in three sampling sets and measured every 7th day for the period of one month: Lead (Pb), Cadmium (Cd), Dissolved oxygen (DO), Total Suspended Solid (TSS), pH, and temperature. The results revealed that Pb uptake by N. tenuifolia var. pseudograminea was significantly higher (P<0.001) than E.crassipes (14.0 mg/g and 0.05 mg/g; respectively) after 28 days. However in the case of the Cd uptake, E. crassipes, have significantly (P<0.001) accumulated high amount of Cd than N. tenuifolia var. pseudograminea during the 28 days period of the experiment. On the other hand, the water quality parameter DO and TSS was improved by 22.8% and 14.6% respectively. One of the concrete evidence of water quality improvement was the proliferation of fish fingerlings around the AMBS during the 21st day of its installation. Our findings suggest that by introducing AMBS in the polluted river, it did not only removed pollutants but also serve as a foraging and spawning ground of economically important fish.
Keywords: *Phytoaccumulation, Heavy Metals, Lead, Macrophytes,*

Received 12.03.2018 Revised 30.05.2018 Accepted 10.08. 2018

INTRODUCTION

Preservation of environmental quality is one of the major concerns in our society. Pollution of the water bodies is only one of the many resources that are getting polluted – soil, land and air [1]. Due to its topography or being catchment the area in the watershed, aquatic ecosystems are directly or indirectly end destinations of anthropogenic substances as well as from natural systems which often present high pollutant concentrations that may be detrimental for organisms therein.

Unlike other pollutants, heavy metals are difficult to degrade, but can accumulate throughout the food chain, producing potential human health risks and ecological disturbances. Their presence in water is due to discharges from residential dwellings, groundwater infiltration and industrial discharges. The discharge of wastewater containing high concentrations of heavy metals to receiving water bodies has serious adverse environmental effects [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.

Aquatic macrophytes have great potential for the phytoremediation of water contaminated with heavy metals [4, 5, 6]. They are utilized in the removal of eutrophic storm water [7], copper [8], nitrogen and phosphorus [9, 10]. Aquatic Macrophyte Biosorption System (AMBS) a phytoremediation technology developed by Zafaralla *et al*. [11] which involve the process of rhizofiltration was installed across the

Molawin Creek, University of the Philippines Los Baños. Their data revealed the improvement of water quality having acceptable BOD and the most remarkable effect of the AMBS was the proliferation of fingerlings of *Oreochromis niloticus* (Tilapia) 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 [12]. 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 [13]. Wu and Raven [14] 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 [15]. 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 heavy metals (lead and cadmium) by the AMBS; [3] examined the water quality improvements of the AMBS at the Niugan River ecosystem.

MATERIAL AND METHODS

Study Site

The study was conducted at 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 (16). Among the most prominent industries surrounding the river basin are Nestlé Philippines Factory, Asia Brewery and Mina Oil Mill Corp.

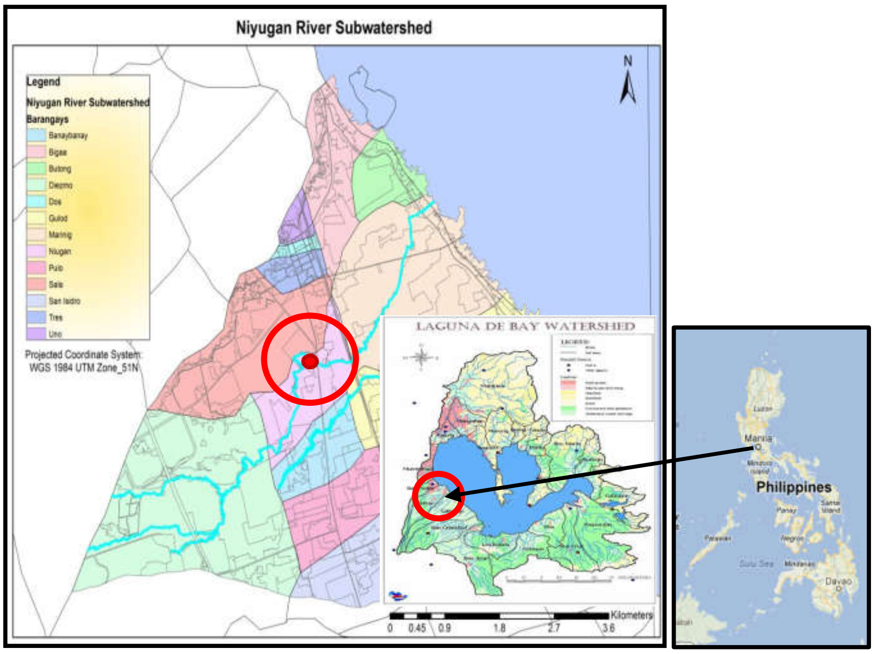


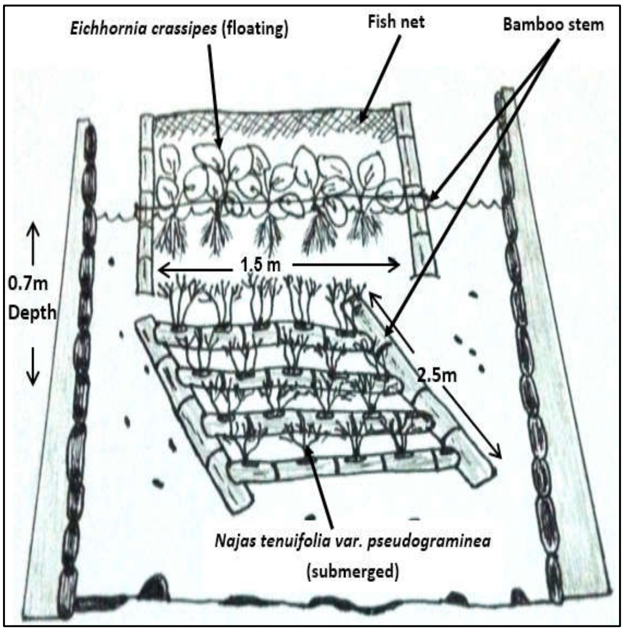
Figure 1. Location of field experimental set-up at Niyugan River (14^{0}15’57”N; 121^{0}7’34”E), Cabuyao City, Laguna.

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. [11] 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 [17].

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 Heavy Metal Uptake –** Plant materials were taken weekly by pulling out from the AMBS (one whole plant in 3 replicates). These were brought to the laboratory and washed thoroughly under running tap water. After blot-drying these were oven-dried at 70°C for 72 hrs. After drying the replicate plants were pooled and ground. From the ground material 1 g was measured out and submitted for analysis of Pb and Cd.

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.* [18] 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.

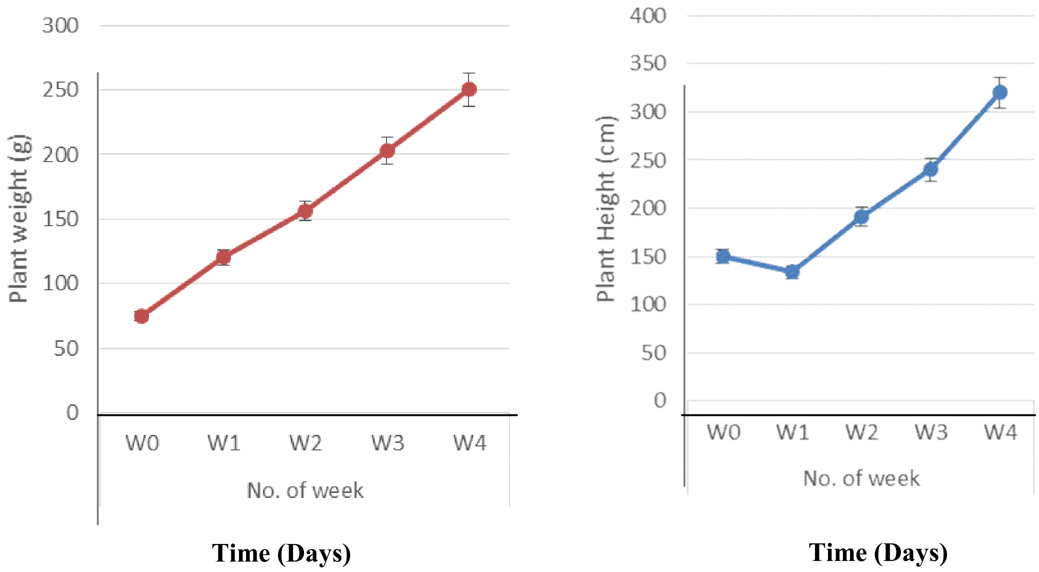


Figure 3. Plant weight of *E. crassipes* (A) and height of *N. tenuifolia* var. *pseudograminea* (B) utilized as AMBS component in the Niugan River for the period of 28 days under field condition.

Uptake of Heavy Metals by Aquatic Macrophytes

Results of the heavy metal analysis in the water of Niugan River revealed that Lead (Pb) and Cadmium (Cd) content was 0.04 and 0.01 mg/L respectively. This amount was also comparable to the previous study on the water analysis of the river conducted by Baltazar, [19] which yield 0.05 mg/L Pb and 0.01 mg/L Cd. Uptake of heavy metal (Pb and Cd) by *E. crassipes* and *N. tenuifolia* var. *pseudograminea* are presented in Fig. 4 (A and B). The result showed that *N. tenuifolia* var. *pseudograminea* have significantly (*P<0.001*) accumulated high amount of Pb as compared to *E. crassipes* (Fig. 4A). Penga *et al*. [20] also demonstrate the effectiveness of the two species of submerged macrophyte, Potamogeton in the bioaccumulation of heavy metals (Cd and Pb) in industrial waste water.

However in the case of Cd uptake, *E. crassipes* have significantly (*P<0.001*) accumulated high amount of Cd than *N. tenuifolia* var. *pseudograminea* during the 28 days period of the experiment. This result corroborated with the study of Mishra et al. [21] which revealed that *E. crassipes* is the most efficient for the removal of cadmium followed by *P. stra*tiotes and *S. polyrrhiza*. Interestingly, both aquatic macrophytes were able to bioaccumulate significant high amount of HM than in their environment during the 28 days study period.

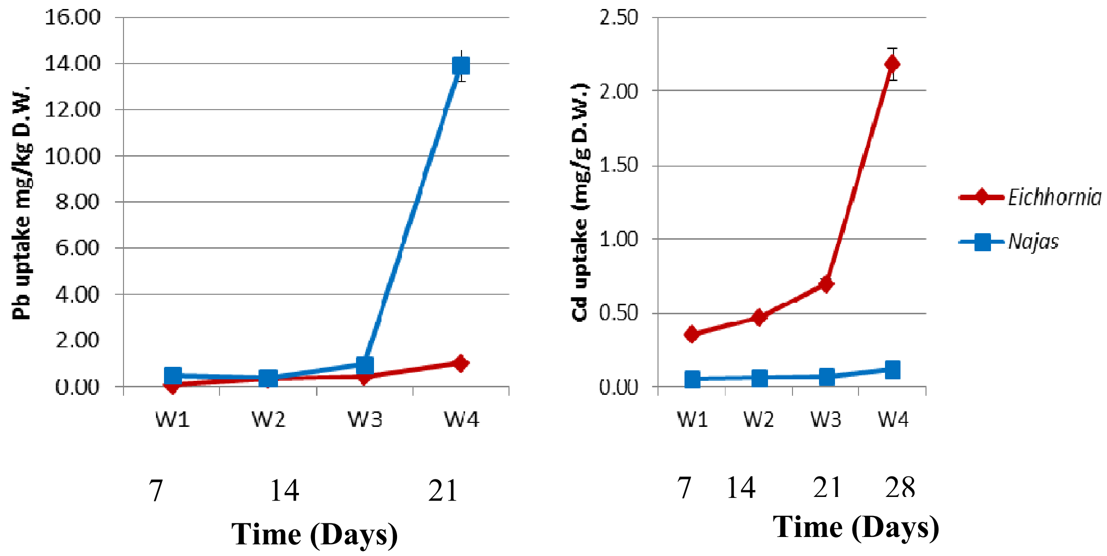


Figure 4. Weekly concentrations of Pb and Cd in the whole plant of *E. crassipes* (A) and *N. tenuifolia* var. *pseudograminea* (B) utilized as AMBS component in the Niugan River for the period of 28 days under field condition.

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 [22]. 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. (18) 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 field experiment based on the relative concentration of the two heavy metals (Pb and Cd), indicated that both aquatic macrophytes used in this study has a potential in restoration of polluted aquatic ecosystem. In terms of Pb (lead) uptake, *N. tenuifolia* var. *pseudograminea* had a higher HM content than *E.crassipes* (14.0 mg/g and 0.05 mg/g, respectively) after 28 days of growth as AMBS component. 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.*

ACKNOWLEGEMENT

The authors would like to acknowledge the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development of the Department of Science and Technology (DOST) - for funding the research study.

CONFLICT OF INTEREST

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

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CITATION OF THIS ARTICLE

Lituanas, CRM., Cadiz, N.M. Phytoaccumulation of Heavy Metals (Pb & Cd) by *Najas tenuifolia* var. *pseudograminea* and *Eichhornia crassipes* in Polluted River. Env. Pharmacol. Life Sci., Vol 7 [10] September 2018. 17-23