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Phytoremediation Potential of Vetiver Grass (*Chrysopogon* sp.) System for Improving the Water Quality of Aquaculture Ponds along the Marilao and Meycauayan River in Bulacan, Philippines

ABSTRACT

The Marilao and Meycauayan Rivers are known to be polluted with heavy metals and organic matter due to different anthropogenic and industrial activities along the river system. Many aquaculture ponds are situated along the river system and obtain water from the river. In order to address this problem, phytoremediation or the use of plants was tested as a low-cost remediation system to reduce the pollution on the ponds. The vetiver grass was utilized because of its unique features and its ability to accumulate heavy metals. A vetiver pontoon was established on fishponds located at Brgy. Nagbalon, Marilao and Brgy. Liputan, Meycauayan. The vetiver roots and leaves were analyzed for heavy metal content. There is an accumulation of toxic heavy metals such as lead, chromium, manganese and copper in the roots and leaves. Manganese had the highest accumulated metal by the vetiver grass. It was observed that there is a significant difference of heavy metal absorption of Pb, Zn, Mn and Cr through time. The vetiver grass favored accumulating heavy metals in the roots based on the translocation factor (TF). Vetiver grass can potentially improve some water quality parameters such as lowering levels of ammonia, BOD and COD and absorb heavy metals such as Pb, Zn, Mn and Cr which are harmful to fish. The vetiver grass is a low-cost phytoremediation technology with a high potential impact in cleaning up the water in ponds.

Key words: *Phytoremediation, vetiver grass, heavy metals, water quality*

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INTRODUCTION

The Marilao and Meycauayan River which is part of the Marilao-Meycauayan-Obando River System (MMORS) is known to be polluted with heavy metals and organic matter due to anthropogenic and industrial activities along the river system. Heavy metal and organic pollution are severe on the river system and has caused environmental resource degradation and posed numerous public health problems. The waters on the aquaculture ponds of Marilao and Meycauayan Bulacan came from the river system. The likelihood that the aquaculture ponds along the river system is polluted with heavy metals and organic matter is very high. In order to address this problem, remediation strategies using plants could be employed. Phytoremediation is a technology that employs plants and their associated microbes to remove pollutants from contaminated soils and waters. It takes advantage of the natural plant processes-physical, chemical and biological interactions occurring between plants and the environmental media. (*Nagendran et al. 2006; Odjegba and Fasidi,\ 2007*). Phytoremediation works best at sites with low to medium amounts of pollution (*USEPA 2002*). The vetiver grass was utilized for phytoremediation because of its unique features and its ability to accumulate heavy metal and improve water quality. The vetiver grass was first used in the 1990s and recognized for having a "super absorbent" characteristics suitable for the treatment of wastewater and leachate generated from landfill in Queensland (*Ash, R. and Truong P. 2004*). Vetiver grass has high ability for pollutant removal in terms of organic or inorganic materials from the environment (*Suelee A.T. 2016*). Vetiver grass has been used for a long time in land conservation by means of soil and water by World Bank (*Darajeh et al. 2014*), but its advantages of being cheap, effective and easy for water and soil conservation, particularly in wastewater treatment, only emerged in the 1980s (*Danh et al. 2009; Truong. 2000*), due to its extraordinary and outstanding physiological and morphological characteristics. The use of vetiver grass to address water quality problems are always used in wastewater treatment facility. Using vetiver grass in aquaculture ponds to reduce heavy metal pollution was very unusual application.

The study evaluated the performance of vetiver grass as phytoremediation species in improving the water quality of ponds in Brgy. Nagbalon, Marilao and Brgy. Liputan, Meycauayan, Bulacan. This also aimed to determine the accumulation potential of heavy metals of the vetiver grass.

MATERIALS AND METHODS

Study area and treatment ponds

Fishponds supplied with water from Marilao and Meycauayan River of the MMORS were chosen and is said to be contaminated with different heavy metals and is heavily polluted with organic and inorganic matter. The first sampling area was located at Brgy. Nagbalon, Marilao, Bulacan while the other is at Brgy. Liputan Meycauayan Bulacan. Two ponds on each site were utilized in this study: one pond was installed with a phytoremediation set-up and the other as control pond (**Table 1**).

Set up of the vetiver grass pontoons

The vetiver grasses (*Chrysopogon* sp.) were obtained from Vetiver Farms Philippines. The 1 x 1 m^2 vetiver grass pontoon consisted of 10 or more young vetiver grasses that were balled-out. A total of 10 bamboo pontoons were spread out on the whole pond.

One pond on each study site served as the phytoremediation pond where the vetiver grass pontoons were installed. A control pond on each study site was established to serve as a comparison with the treatment pond.

Data collection and analysis

In situ and ex situ procedures were followed in the determination of the levels of water quality parameters. Those analyzed in situ were dissolved oxygen (DO), pH, temperature, and salinity. Ex situ determination were done to the following parameters: Ammonia (NH_3), Phosphate (PO_4), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD).

A baseline water quality monitoring was done before installing the vetiver grasses to determine the current condition of the ponds. A daily monitoring of dissolved oxygen (DO), temperature, pH, salinity was done. Ammonia (NH_3) and phosphates (PO_4) were analyzed weekly. Monthly monitoring of parameters includes chemical oxygen demand (COD) and biochemical oxygen demand (BOD). In situ parameters were determined on the four random sampling points of each pond. This was done around 5:30 to 8:30 in the morning and 13:00 to 16:00 in the afternoon. For the ex situ parameters such as ammonia and phosphates, it was done on a weekly basis. Water samples were collected from the four random sampling points on each pond and pooled in two 1L polyethylene bottles. The remaining parameters such as the Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) were analyzed on a monthly basis. The data collection was done in a span of two months.

Analysis of covariance (one-way ANOVA) was done in order to in order to determine if there are significant changes on the water quality parameters and heavy metal absorption of vetiver grass through time using the SPSS Statistical software.

Heavy metal accumulation of vetiver grass

Samples of vetiver grass were collected for heavy metal analysis. The roots and leaf were separated and analyzed for heavy metal using Niton X-ray Fluoresence (XRF) Spectrophotometer at CASL Laboratory,

Table 1. Geographic coordinates, physical characteristics and treatments of the ponds.

Pond (study site)	Treatment	Geographic Coordinates		Size (m^2)	Depth (m)
		Longtitude	Latitude		
Nagbalon Pond	Phytoremediation	120°56’46.03”E	14°44’53.02”N	670	1.0
Control Pond 1	No treatment	120°56’42.95”E	14°44’52.74”N	300	1.0
Liputan Pond	Phytoremediation	120°56’32.77”E	14°44’17.56”N	350	1.5
Control Pond 2	No treatment	120°56’32.00”E	14°44’18.02”N	300	1.5

BIOTECH-UPLB. The collected roots and leaves were pooled for each pond respectively and analyzed. Root and leaf samples were air dried and ground into fine powder using a mortar and pestle. Analysis of heavy metals was done before installing the plants, after a month and two months of thriving in the ponds. The translocation factor (TF) is the ratio of plant ability to extract heavy metal from root to shoot. A value of TF< 1 means that most of heavy metal accumulates in the root. The translocation factor is computed by the formula below (*Zhang et.al. 2014*):

$$TF = \frac{Metal\ concentration\ in\ plant\ shoot\ (mg\ kg^{-1})}{Metal\ concentration\ in\ plant\ root\ (mg\ kg^{-1})}$$

RESULTS AND DICUSSION

Baseline physico-chemical water quality parameters of ponds

Baseline characterization of the physico-chemical parameters of ponds were done in order to determine the initial condition of the ponds. The dissolved oxygen values of ponds at Nagbalon site were below the recommended level of 5.0 ppm (**Table 2**). The water temperature was relatively low on both sites. There was an exceedance of pH level at the Liputan site. Other parameters such as ammonia, phosphates, BOD and COD exceeded the recommended limit. BOD measures the amount of oxygen consumed by microorganisms in decomposing organic matter in water which directly affects the DO level (*APHA 1992*). The higher the BOD level, the more rapidly oxygen is depleted in water which is evident in the collected data.

Monitoring of the physico-chemcal water quality parameters of ponds

Pond monitoring was done to determine the impacts of phytoremediation on the different physico-chemical parameters. In general, the DO levels for both sites are usually below the recommended limit (5.0 ppm) in the morning monitoring and at supersaturated level in the afternoon (**Figures 1** and **2**). In non-aerated ponds, DO is generally through the photosynthesis of phytoplankton depending on the amount of light available. Oxygen is also incorporated into the water from the air, especially when the wind blows on the surface of the water causing water movement and mixing. The typical diurnal pattern of dissolved oxygen is very low at dawn (around 5:00 am) and at the highest peak during dusk (around 12:00 pm). This is primarily because oxygen has been used up at night until morning in the process of respiration while carbon dioxide has been released. At daytime, DO levels start to increase as photosynthesis takes place and oxygen is produced. During the summer months, the stratification of pond affects the level of DO. There is high dissolved oxygen on the upper layer, where the warmer temperature and good phytoplankton growth indicates good water quality while on the lower layer, there is very low DO and poor phytoplankton growth which indicates poor water quality (*Crochet D.W.*). The daily temperature and pH levels are within the recommended limit based on the Department of Environment and Natural Resources (DENR) Administrative Order No. 2016-08 (DAO 16-08). There was a significant difference on the DO and temperature levels in the morning and afternoon monitoring for both ponds ($p<0.05$). The salinity levels of ponds were relatively high for both sites. It ranges from 15–29 ppt for all ponds. The water source which is the river system had relatively high salinity. The ammonia levels in the ponds had reductions for both sites (**Figure 3**). Ammonia levels at the Liputan site had significant difference ($p<0.05$) through time. The ammonia levels at Liputan site had a decreasing trend after the installation of the phytoremediation setup. For phosphates levels, only the

Table 2. Baseline physico-chemical water quality parameters of ponds in the study.

Pond Name	DO (PPM)	Temperature (°C)	PH	Salinity (PPT)	Ammonia (PPM)	Phosphates (PPM)	BOD (PPM)	COD (PPM)
Nagbalon Site								
Phytoremediation Pond	3.58±0.18	23.95±0.14	7.81±0.19	15.00±0.87	5.99±0.54	0.87±0.21	23.00	189.46
Control Pond 1	4.69±0.98	24.18±0.09	8.05± 0.27	15.75±1.16	0.43±0.33	0.68±0.11	29.00	148.80
Liputan Site								
Phytoremediation Pond	5.77±0.86	25.77±0.13	8.61±0.09	18.00±2.12	5.79±0.19	0.65±0.17	12.00	243.54
Control Pond 2	6.53±2.34	26.47±0.04	8.62±0.27	15.33±0.91	4.28±0.28	4.13±0.49	6.00	228.63
Recommended Level	5.0 ppm (DAO 16-08)	25 - 31°C (DAO 16-08)	6.5 – 8.5 (DAO 16-08)	25 ppt (*Garg et.al. 2003*)	0.05 ppm (DAO 16-08)	0.5 ppm (DAO 16-08)	7 ppm (DAO 16-08)	100 ppm (DAO 16-08)

control pond in Liputan had reduced level and no observed reductions on the treated ponds (**Figure 4)**. Vetiver grass is good in removing nitrogen and phosphorus as compared to other grasses. Based on a study, it was able to reduce total N and P of a polluted river by 71% and 98% respectively after 4 weeks (*Danh, L.T. et.al. 2012*).

There was a reduction of BOD level after the 30-day culture but increased towards the 60-day culture for all ponds except for the Phytoremediation pond in

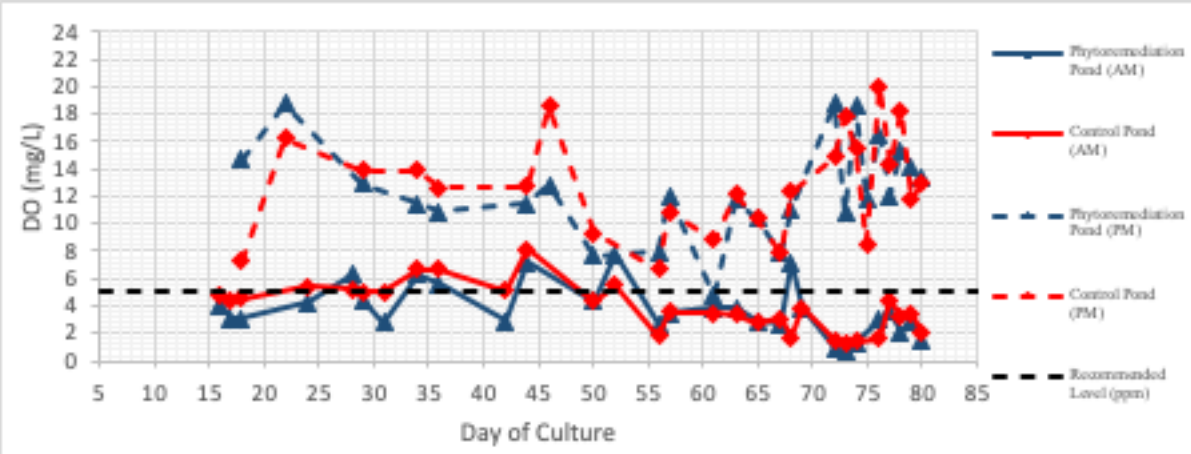

Figure 1. Daily dissolved oxygen level (ppm) of ponds at Nagbalon site during the morning and afternoon monitoring.

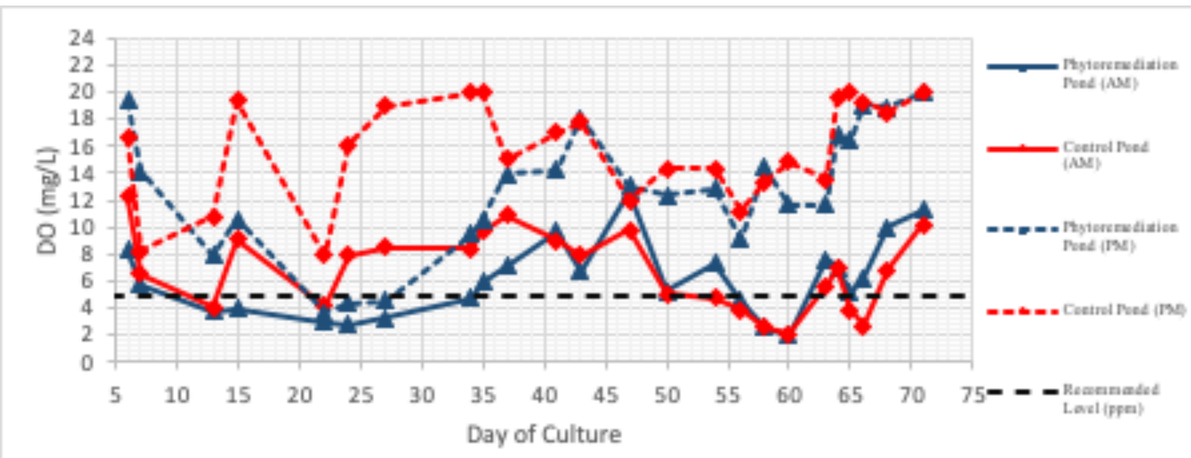

Figure 2. Daily dissolved oxygen level (ppm) of ponds at Liputan site during the morning and afternoon monitoring.

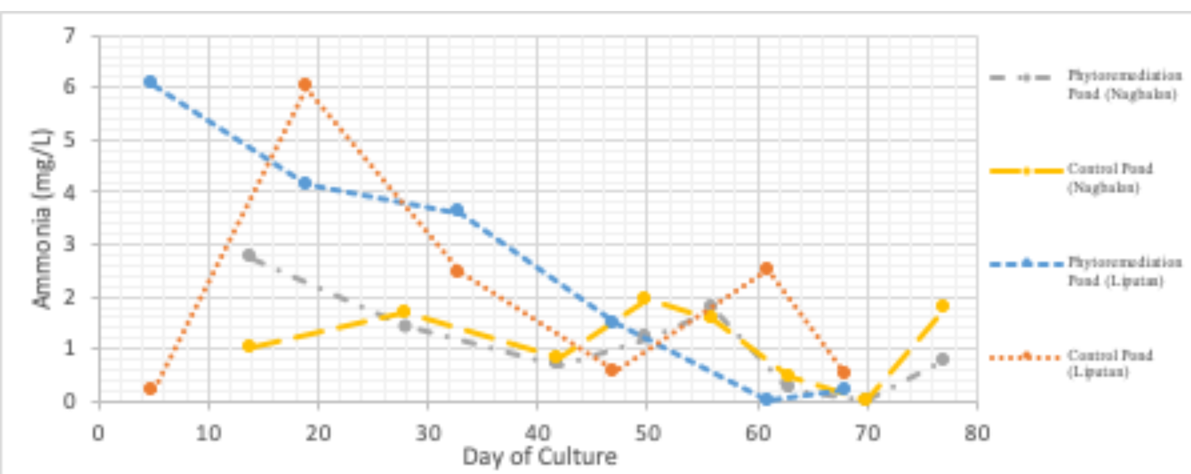

Figure 3. Ammonia level (ppm) monitoring of treatment and control ponds for both sites.

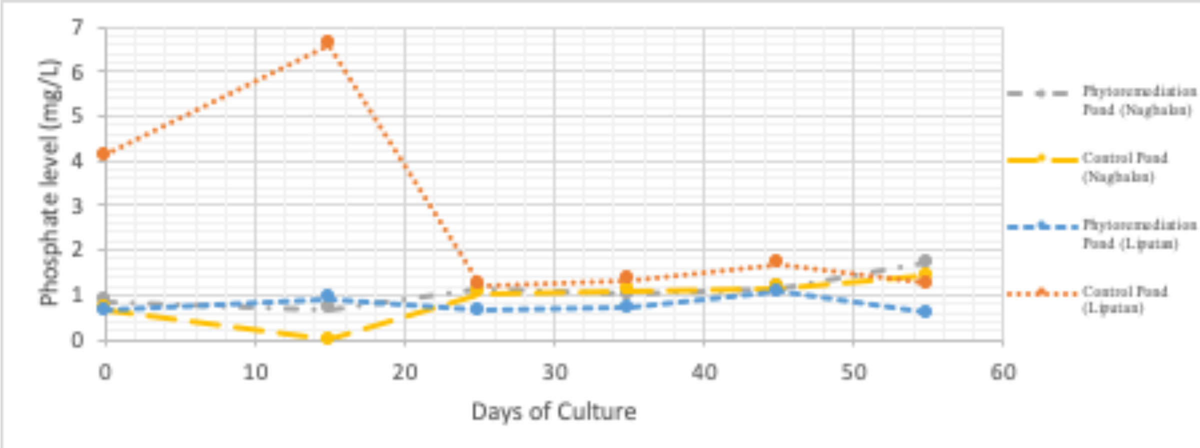

Figure 4. Phosphate level (ppm) monitoring of treatment and control ponds for both sites.

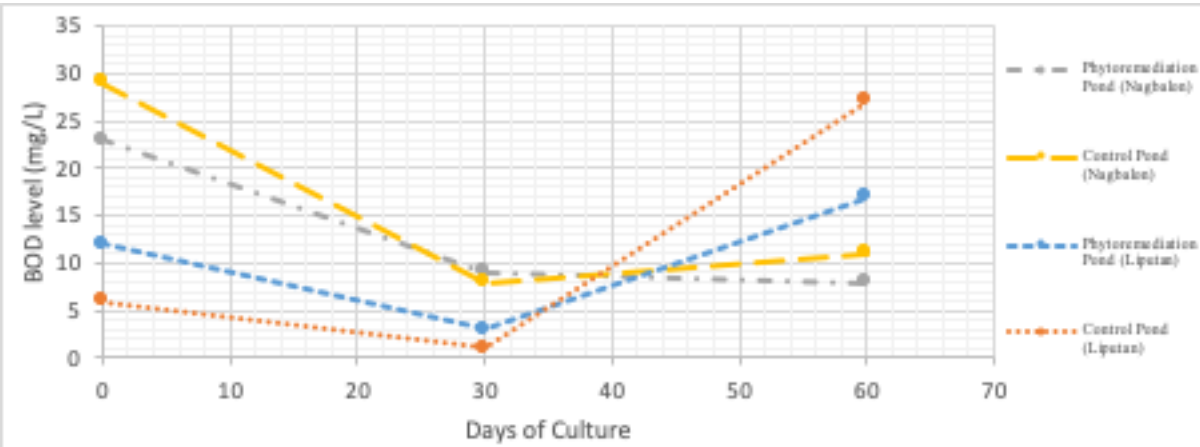

Figure 5. Biochemical oxygen demand (BOD) level (ppm) monitoring of treatment and control ponds for both sites.

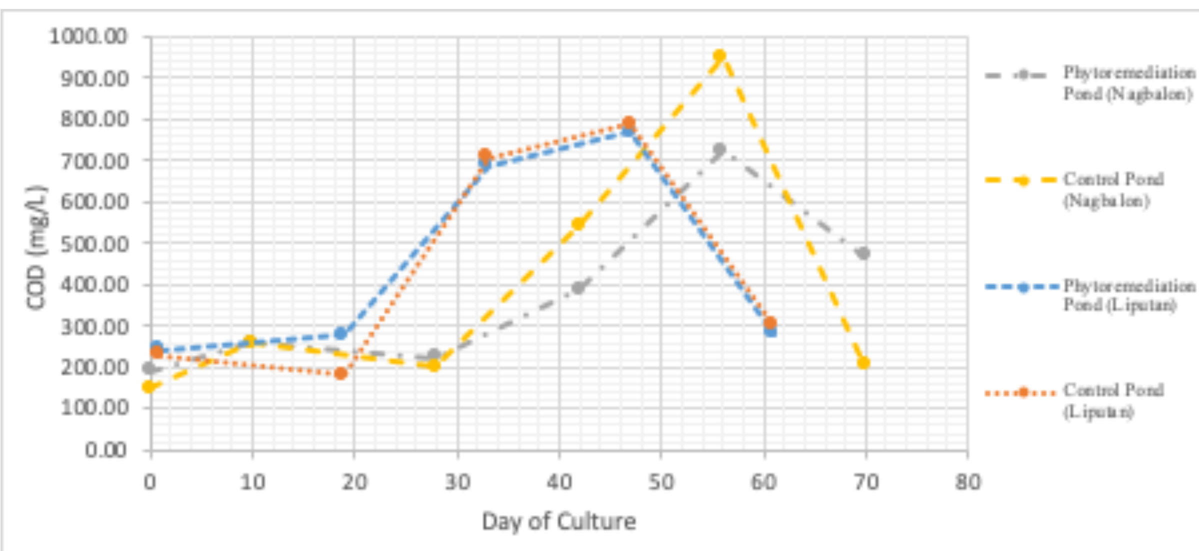

Figure 6. Chemical oxygen demand (COD) level (ppm) monitoring of treatment and control ponds for both sites.

Nagbalon site. The COD levels of the phytoremediation at Nagbalon site were relatively low compared to the control pond after the two months culture. In the Liputan site, there were relatively minor difference on the COD levels for both ponds throughout the culture period. There is no significant difference observed in the BOD and COD level for both ponds. Based on the study by *Darajeh N. et.al*. (*2014*), vetiver grass with welldeveloped root and shoots were able to reduce BOD and COD in water. Vetiver grass has great potential in dissolved nutrient uptake and other organic elements such as nitrogen, phosphorus, BOD and COD (*Truong 2000*).

Heavy metal accumulation of vetiver grass

The young vetiver grass had zinc, copper and manganese present in roots and leaves (except for chromium in leaves). Lead, arsenic, mercury, and cadmium were not detected on both roots and leaf. According to the study of *Danh, L.T. et.al*. (*2012*), vetiver grass can tolerate high concentrations of individual and combination of heavy metals. It has a high tolerance to wide range of heavy metals.

A river quality monitoring of the Marilao and Meycauayan River was conducted in 2008 and found out that Arsenic, Cadmium, Copper, Chromium, Lead, Manganese and Zinc exceeded the limit set by the DENR. The water in aquaculture ponds coming from these two rivers would probably be contaminated with heavy metals. After a month, results showed that vetiver grass accumulated lead in the roots with 16.92 ppm for the Nagbalon site. The accumulation in the leaves was below the detection limit of the XRF which is 13 ppm. There was an increase in the zinc content in the roots and leaves of vetiver grass after a month. There was an increase in the absorption of manganese in vetiver after one month of culture in the ponds of Nagbalon and Liputan site. Chromium was also absorbed by the vetiver grass. Arsenic, mercury and cadmium were below the limit of detection of the XRF analyzer. At the Liputan site, the vetiver grass recorded lead accumulation of 20.71 ppm in roots and 14.40 ppm in leaves. There was an increase in the absorption of zinc and manganese by vetiver as compared with baseline data. Arsenic (11 ppm), mercury (10 ppm), cadmium (12 ppm) and chromium (85 ppm) were below the limit of detection. After two months of culture, several vetiver grass died and were removed in the pond. The remaining existing vetiver grass were harvested and analyzed for heavy metals. During the second month of culture, zinc, copper and manganese were observed on the roots and leaves for both sites. It was noted that there was high accumulation of manganese. In general, most of the heavy metals for both sites were accumulated in the roots rather than the shoots. It was also noted that lead was not detected during the baseline test but the vetiver grass accumulated lead after one and two months of culture. Vetiver grass roots take up heavy metals such as lead through rhizofiltration process which is the adsorption and precipitation onto the roots (*Tangahu B. et.al. 2011*). Based on the study of *Roongtanakiat et. al.* (*2007*), vetiver grass installed in industrial wastewater had the following degree of heavy metal accumulation: Fe>Mn>Zn>Cu>Pb and it is more concentrated in the roots rather than the leaves. This is relatively the same as the result of the study wherein Manganese had the highest accumulated heavy metal.

In terms of the translocation factor (TF) of the first month sampling at Nagbalon site, the chromium had the highest TF of 1.02 and manganese had the lowest with 0.59. For the Liputan site, manganese had the highest TF with 1.38 while lead had the lowest with 0.70. After two months of culture, copper had a translocation factor of 1.05 which was the highest and manganese with only 0.15 for the Nagbalon site. The heavy metal zinc had the highest TF of 1.44 and manganese had the lowest with 0.42 for the Liputan site. A translocation factor (TF) greater than 1 is a feature of an accumulator (*Agunbiade, et.al. 2009 and Zhang et.al. 2014*). A TF > 1 indicates the greater translocation of metals from roots to the shoot part of the plant. A TF lower than 1 indicates that vetiver prefers to accumulate heavy metal in the root more than in the shoot (*Aksorn E. and Chitsomboon B. 2013*). Based on the results, most of the TF are below 1 which shows that vetiver grass prefers accumulating heavy metals in the roots rather than the leaves. An important finding is that since not much heavy metal are translocated into the leaves, the leaves can be used for grazing or mulch (*Anjum 2013; and Truong 2000*).

Based on the statistical analysis (One-way ANOVA), it shows that there is a significant difference ($P<0.05$) of the heavy metal content of vetiver grass through time. It shows that heavy metal content (Pb, Zn, Mn and Cr) significantly increased from the baseline assessment. This proves that vetiver grass can absorb heavy metals and store it in the roots and leaf.

Nutrient availability is an important factor for a successful phytoremediation (*Hutchinson et.al 2001*). The plants absorb contaminants through the root systems and store them in the root biomass and transport them to the stem or leaves. Based on the study of *Truong* (*1999*), the distribution of heavy metals in vetiver plant can be into three groups: (i) very little of arsenic, cadmium, chromium and mercury absorbed, were translocated to the shoots; (ii) moderate proportion of copper, lead, nickel and selenium were translocated (16-33%); and (iii) zinc was almost distributed between shoot and roots. Based on the research study, very little amount of arsenic, cadmium, chromium and mercury were absorbed and translocated to the leaves. Zinc were both distributed on the leaves and roots for both sites of the study. Other

Table 3. Heavy metal content (ppm) of vetiver grass used in the research study.

Vetiver Part	Baseline					After one month					After two months				
	Pb*	Zn*	Cu	Mn*	Cr*	Pb*	Zn*	Cu	Mn*	Cr*	Pb*	Zn*	Cu	Mn*	Cr*
Nagbalon Site															
Vetiver roots	ND	58.82	77.52	277.31	74.03	16.92	101.46	75.97	3002.51	125.59	< LOD	117.12	69.17	775.64	35.59
Vetiver leaf	ND	37.98	53.04	381.36	ND	<LOD	65.79	< LOD	1780.16	128.43	< LOD	57.09	72.67	114.37	< LOD
Translocation Factor (TF)	-	0.65	0.68	1.37	-	-	0.65	-	0.59	1.02	-	0.49	1.05	0.15	-
Liputan Site															
Vetiver roots	ND	58.82	77.52	277.31	74.03	20.71	145.03	52.05	3240.00	< LOD	< LOD	53.26	42.97	422.19	< LOD
Vetiver leaf	ND	37.98	53.04	381.36	ND	14.40	141.81	46.76	4471.74	< LOD	< LOD	76.54	< LOD	176.42	< LOD
Translocation Factor (TF)	-	0.65	0.68	1.37	-	0.70	0.98	0.90	1.38	-	-	1.44	0.81	0.42	-

researchers concluded that vetiver roots accumulate higher heavy metal concentrations than in the shoot (*Yang, et.al. 2003; Roongtanakiat et.al. 2007 and Singh et.al. 2007*). Mature vetiver grass cannot concentrate more heavy metals in the shoots. The shoot heavy metal concentrations decreased as the plants age, possibly due to dilution effect of increasing biomass, while the root heavy metals concentration increased (*Roongtanakiat and Chairoj 2001*).

There is a high potential that vetiver grass could reduce organic matter and heavy metal content of the aquaculture ponds. It is relatively low in terms of the cost to produce but effective when used. Another positive impact of the vetiver pontoons is that it served as shade for fishes especially during noontime when there is intense heat. In some way, it reduces the stress to the fishes and help them survive the high temperature. In India, vetiver grass is known as “desert coolers” have been used since ancient time due to its cooling effect and pleasant aromatic air (*Greenfield, J., n.d. and Lavinia 2003*).

CONCLUSION AND RECOMMENDATION

The vetiver grass system has a high potential in cleaning up the water coming from the river system. The results had shown that there was accumulation of heavy metals on roots and leaves. Toxic heavy metals such as lead, chromium, manganese and copper were absorbed by the vetiver grass. Manganese was accumulated the highest for both culture months. The vetiver grass preferred accumulating heavy metals in the roots based on the computed translocation factor for both sites. Based on statistical analysis, it shows that there is a significant difference of heavy metal content (Pb, Zn, Mn and Cr) from the baseline assessment. It proves to show that the vetiver grass is somehow effective in absorbing heavy metals in water. Vetiver grass could potentially improve some water quality parameters such as ammonia, BOD and COD. Another positive impact of vetiver grass is that its roots has cooling effect to reduce the stress of fishes during summer months. The vetiver grass system is a relatively low-cost technology with a high potential positive impact on cleaning up pollution in water.

It is recommended that another study be done over a longer span of time to improve the design and effectiveness of the vetiver grass system. Increasing the area of the pontoon vetiver grass would be recommended. Plant cultivation of the vetiver grass must be done in soil before installing it to the pontoons so that the roots will grow faster and longer. It is also suggested that mature vetiver grass with long roots must be utilized so that it could accumulate more heavy metals.

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