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Submission date: 13-Apr-2023 01:23PM (UTC+0900)

Submission ID: 2063190279

File name: 2015_Phytoremediation_of_Vaname_Shrimp_AACL_Bioflux.pdf (155.36K)

Word count: 4546

Character count: 25021

Phytoremediation of vaname shrimp (*Litopenaeus vannamei*) wastewater using vetiver grass system (*Chrysopogon zizanioides*, L) in flow water surface-constructed wetland

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Abstract. One of the negative impacts of vaname shrimp (*Litopenaeus vannamei*) cultivation activities is the deterioration of water quality in rivers and coastal waters that functioned as a source of water for *L. vannamei* cultivation and other activities. This condition brings the need to treat the wastewater to restore the water quality. The study was focused on the evaluation of the ability of phytoremediation of vetiver grass (*Chrysopogon zizanioides*, L) in removing pollutants (NH₄⁺, NO₃⁻ and PO₄₃⁻) from the wastewater of *L. vannamei*. The results show that the vetiver grass can grow in mesohaline conditions and can perform phytoremediation for NH₄⁺, NO₃⁻, and PO₄₃⁻ pollutants. The planting treatment of emergent and hydroponic do not show significant difference in their ability of reducing pollutants. The growth of vetiver grass is determined more by the increasing age of the grass and is not affected by mesohaline's salinity.

Key Words: water quality, wastewater management, water pollutants, hydroponic, mesohaline.

Introduction. Indonesian aquaculture business is one of the most important sectors in the overall increasing fisheries production (MAF 2012). In 2013, aquaculture production contributed as much 68.54% of the total national fishery production (SI 2014). Annually, aquaculture has increased significantly, from 5.4% in 2007 to 23% in 2013 (MMF 2010; SI 2014).

Although increased aquaculture activities has positive impacts, it also has negative impacts: it can exceed the carrying capacity of the waters, it can disrupt the lives of aquatic organisms, deteriorates water quality, eutrophication, and deplete oxygen, which cause water pollution (Riani et al 2014; FAO 2011; Xin et al 2010; Boyd 2003; Naylor et al 2000). Poor water quality causes the spreading of various diseases of vanname shrimp. As a result, vanname shrimp (*Litopenaeus vannamei*) will fall into illness thus reducing productivity. As Riani (2015) stated, poor water quality lowers immunity and spread fish's diseases.

Aquaculture wastewater from shrimp cultivation activities cause increased levels of ammonia, nitrate, phosphorus and organic matter (Lanahan et al 2014; Mook et al 2012; Tilley et al 2002). Therefore, this situation must be properly managed to keep the shrimp cultivation sustainable. The development of aquaculture must be sustainable. In other words, aquaculture wastewater –before is discharged into the environment- should undergo some processing beforehand (Lin & Yang 2003). Thus, some strategies that can be taken is to treat the water source, recirculate the water and treating the wastewater. There have been several aquaculture wastewater treatment developed, such as adding beneficial microbes to help improving the water quality, applying biofilter systems, conventional wastewater treatment, recirculation, bioflok technology, bioremediation, bio-electrochemical, and some combinations of several methods (Mook et al 2012; Ray et

al 2010; Avnimelech & Kochba 2009; Schryver et al 2008; Crab et al 2007; Pan et al 2007; Avnimelech 2006; True et al 2004; Piedrahita 2003; Ridha & Cruz 2001; Dumas et al 1998; Twarowska et al 1997). However, these technologies have not been widely applied in Indonesia, especially by medium and small-scale fish farmers (traditional fish farmers). The arguments that can be mentioned is that the technology generally need specialized manpower and expertise, and it will significantly increase production costs and requires special maintenance, so the technology is difficult for fish farmers.

A cheap, easy, and effective technology in controlling aquaculture's wastewater is phytoremediation technology (Kadlec & Wallace 2009; Lananan et al 2014). Phytoremediation is the term that describes the ability of plants to detoxify, recover or purify the environment, reduce, transfer, stabilize or decrease pollutants, in both land and water (Ojoawo et al 2015; Dana 2014; Wang et al 2010; Willey 2007; Eapen & D'souza 2005; Schroder et al 2002). Phytoremediation is not only innovative but is also feasible, environmental-friendly, and economic (Wang et al 2010; Zhang et al 2007; Eapen & D'souza 2005; Ash & Truong 2003).

According to Truong & Hart (2001), removal of N and P elements from polluted waters through vegetation (phytoremediation) is the most effective, low cost, and environmentally friendly technique. Moreover, according to Ash & Truong (2003), the final phytoremediation product has several uses, including as animal feed and ingredients for organic farming.

The purpose of this study is to investigate the ability of phytoremediation of vetiver grass (*Chrysopogon zizanioides*, L) in removing pollutants (NH_4^+ , NO_3^- and PO_{43-}) derived from vannamei shrimp (*L. vannamei*) aquaculture wastewater. *C. zizanioides* is used in phytoremediation because it is able to live in very diverse and wide environmental conditions, is versatile to climatic conditions, habitat, and other environmental parameters (water quality) and water salinity. *C. zizanioides* also has a high reduction percent (high removal %) for pollutants N and P of organic water (wastewater), high removal percentage of COD, N, and P from the leachate originating from organic waste (Danh et al 2013; Truong et al 2008; Truong & Loch 2004; Vimala & Kataria 2004; Truong & Hart 2001).

Case studie of sustainable *L. vannamei* aquaculture with wastewater treatment by *C. zizanioides*, and its phytoremediation ability is presented as alternative aquaculture wastewater treatment solution.

Material and Method. The field research was conducted at the Center of Aquaculture Production Business service (BLUPPB) Karawang, in Dusun Sukajadi, North Pusakajaya Village, Cilebar District, Karawang County, Indonesia. Laboratory analysis was conducted at the Karawang BLUPPB Laboratory and Soil Laboratory of Faculty of Agriculture IPB Bogor, Indonesia.

Materials used for wetlands media are coarse sand (gravel), coral, and styrofoam. The used plants material *C. zizanioides*, planted on flow water surface-constructed wetland system (FWS-CWs), each consisting of 36 clumps (with total of 288 clumps).

This study was conducted with 2 treatments: planting the *C. zizanioides* using emergent (E) and hydroponics (H) technique. The size of FWS-CWS is 2 m x 1 m x 0.5 m, in form of vinyl plastic-lined tub, made from used banners using bamboo construction.

Wastewater used for this research is originated from *L. vannamei* cultivation activities with stocking density of 100 individuals/m². The shrimp (fries) stocking age is 10-day post larvae (PL-10).

The primary wastewater's pollutants originated from remains of foods and feces from the *L. vannamei* cultivation activities. Feed given during the study had a composition of 42% protein, 3% crude fiber, 6% fat, 12% moisture content, and 13% ash. The study was conducted over three months, and is set at the level of the stay time (t) or hydraulic retention time (HRT) within three days, with the average amount of debit of wastewater (aquaculture effluents) as much as 0.65 L/min or 233.33 L/day.

The determination of NO_3^- and NH_4^+ was performed using titrimetric methods and PO_{43-} was evaluated by the spectrophotometric method with a wavelength of 666 nm (APHA et al 1995).

The measurement of NO_3^- , NH_4^+ and PO_4^{3-} absorption capabilities of the plant were calculated by calculating the loading of wastewater of NO_3^- , NH_4^+ and PO_4^{3-} (LoW):

$$\text{LoW (mg/day)} = Q_{\text{wastewater (L/day)}} \times C_{\text{wastewater (mg/L)}} \quad (1)$$

Note: $Q_{\text{wastewater}}$ = debit wastewater in L/day;

$C_{\text{wastewater}}$ = Concentration of wastewater for NO_3^- , NH_4^+ and PO_4^{3-} in mg/L.

Furthermore, LoW was used to calculate vetiver uptake (VU):

$$\text{VU (mg/day)} = \text{LoW (mg/day)} \times \% \text{ vs} \quad (2)$$

Note: % vs = percentage of vetiver grass' absorption capabilities.

Regression and correlation analysis was conducted on the data from the experiment in order to determine whether the two treatments differ in reducing NO_3^- , NH_4^+ and PO_4^{3-} , then all of the data are processed further using MS-Excel software.

Results and Discussion

C. zizanioides performances in mesohaline conditions. Planted inside the FWS-CWs system, *C. zizanioides* phytoremediation capability toward wastewater from *L. vannamaei* cultivation is determined through whether the vetiver grass is able to grow (bud-growing and increasing height) and take pollutants in mesohaline condition (5-15‰) or not. *C. zizanioides* growth was measured by observing the growing number of clumps/buds and tall grass. Observations for *C. zizanioides* were conducted at the age of 14, 31, 41, 49, 69, 78, 89 and 95 days (observation started on 11 April 2013 and ended on July 1, 2013). During the research, the average weight of pollutants NH_4^+ , NO_3^- and PO_4^{3-} are 0.07, 5.54 and 2.94 g/day, respectively. *C. zizanioides* growth's performance is very good at high nutrient conditions (Figure 1a, 2a & Table 1).

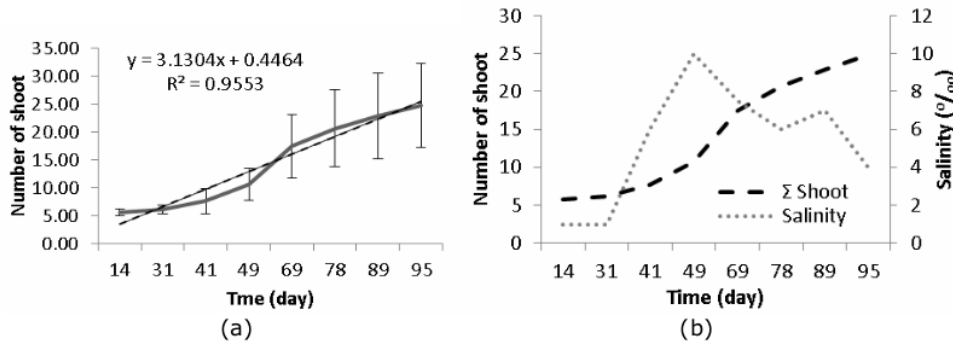


Figure 1. Pattern showing the increase in the number of shoots (a), and correlation between the number of shoots, salinity, and the age of *Chrysopogon zizanioides* (b).

Elimination rates by FWS-CWs and *Chrysopogon zizanioides* Tabel 1

| Specification | NH_4^+ | NO_3^- | PO_4^{3-} |
|-------------------------------------|-----------------|-----------------|--------------------|
| Influent (g/day) | 4.60 | 6.16 | 2.73 |
| Effluent (g/day) | 2.39 | 1.91 | 2.30 |
| Elimination by system (g/day) | 2.21 | 4.25 | 0.44 |
| Elimination by plant uptake (g/day) | 1.36 | 1.53 | 0.21 |
| Efficiency CWs (%) | 48 | 69 | 16 |
| Efficiency by plant uptake (%) | 62 | 36 | 49 |

Shoots growth. During the observation period, results showed the occurrence of growth as indicated by the growth of *C. zizanioides* shoots. Results of multiple regression and correlation analysis show that the growth of *C. zizanioides* is more determined by its age. Age determines growth, since p-value age = 0.000. Salinity does not significantly affect the growth (p-value = 0.275 for salinity). Results of multiple regression models for growth (total of shoots) = 0.279 age - 0.274 salinity - 0.241.

Age correlates strongly with the growing number of shoots with R = 0.979. Salinity does not correlate closely (the correlation between the growth *C. zizanioides* with mesohaline salinity level is very small, i.e. R = 0.355), or in other words, it does not affect the increase in the number of shoots. This means that the *C. zizanioides* is tolerant toward salinity mesohaline. Figure 1a shows the relationship between age and the increase in number of shoots. It appears that the number of shoots increases with age. This means that the increase in the number of shoots is strongly influenced by the age of *C. zizanioides* with a linear model: $Y = 3.13X + 0.45$ and $R^2 = 0.96$. Figure 1b is the observation results of the average number of *C. zizanioides* shoots between age 14 and 95 days. The increase in the average number of shoots per day ranges between 1-7 buds (average $2.73 \approx 3$). The increase the number of shoots follows the quadratic graph with the highest peak occurs at the age of 69 days of observation.

The increase in plant height. The increase in plant height during the study was measured as an indicator of growth. Growth of *C. zizanioides* plant's height was measured every ten days. The average height of the plants at the beginning is 20 cm. At the end of the study, *C. zizanioides* height reaches 89 cm. This means the grass' height was increased 4.5-fold over 3.2 months.

Results of multiple regression and correlation analysis to the increase in plant height show that *C. zizanioides* growth is more determined by its age. Age determines the height increment of plants, for it has the P-value = 0.028. Meanwhile, salinity does not significantly affect growth because it's P-value = 0.317. Multiple regression model obtained is the increase of plant height = 0.282 age + 0.945 salinity + 56.445. Age strongly correlates with the increase in grass vetiver's height with R = 0.849. Meanwhile, salinity does not correlate closely (the relationship between *C. zizanioides* height increment with mesohaline salinity levels, with R = 0.599). In other words, the salinity does not significant affect it. This means the *C. zizanioides* is still able to adapt to mesohaline salinity condition.

Figure 2a shows the relationship between age and height increment of the plants. It appears that the plant's height increases due to the increasing age of the plant. This means that an increase in plant height is strongly influenced by the age of *C. zizanioides* with orthogonal polynomial models of order three: $Y = 0.36X^3 - 5.68X^2 + 30.69X + 24.23$ and $R^2 = 0.97$.

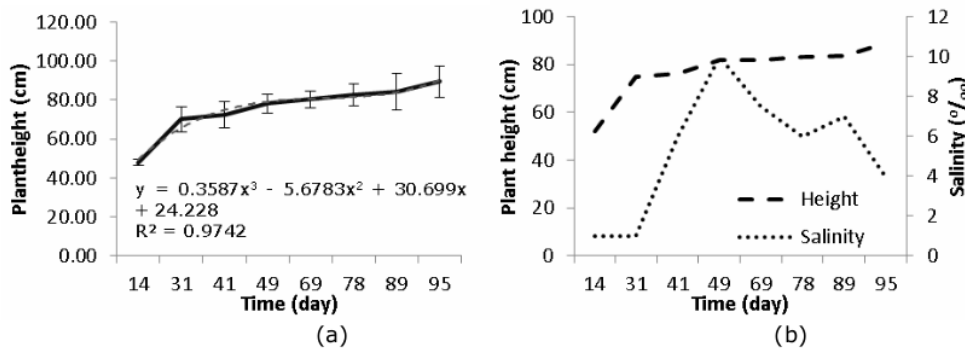


Figure 2. Pattern of vetiver grass' height increase (a). Relationship between *Chrysopogon zizanioides* height, salinity, and the age (b).

This pictorial analysis results revealed that the grass' age is one of the key factors in determining the growth of *C. zizanioides* in a constructed wetland system to reduce the organic waste from *L. vannahmei*. The polynomial relationship of order three shows that there are actually other biological, chemistry, and physics factors in work which has to be observed.

Figure 2b is an observation result of the *C. zizanioides* height increase from age 14 to 95 days. During those 95 days, plant's height increase reached 69 cm, making the average growth of *C. zizanioides* as much as 0.73 cm/day.

Pollutants uptake by vetiver grass (*C. zizanioides*). Statistical analysis show that the P-value > 0.05, which is 0.915 (NO_3^-), 0.879 (NH_4^+), and 0.861 (PO_4^{3-}), thus the two vetiver grass planting treatments of emergent and hydroponic for NH_4^+ , NO_3^- and PO_4^{3-} do not differ significantly. In other words, both the vetiver grass grown hydroponically and using emergent method have the same ability in taking these pollutants.

Plants perform a very important role in the FWS-CWs. They protect the water column, providing space for biofilm growth, and helping to recycle the nutrients and organic carbon (Kadlec & Wallace 2009; Lin et al 2002).

Pollutant uptake by vegetation is the main biotic process in this system (Lin et al 2002). Plants play a variety of roles in the CWs for wastewater treatment. The role includes the physical role of the plant itself in the form of sedimentation, erosion control, and providing a surface area for microbial growth (biofilm), thus increasing the reduction by the microbial process, including nitrification and denitrification. Vetiver grass has long roots that can reach 3.6 m length and the root system is very smooth with an average diameter of 0.5-1.0 mm (Truong et al 2008; Cheng et al 2003). In a plant that has many branches of fine roots, the surface roots are used by microorganisms as a place of growth (Tosepu 2012). Plants also have a metabolic role in the wastewater treatment with its ability to release O_2 into the rhizosphere and also to help the nitrification using the direct absorption of nutrients (Greenway & Woolley 2001; Brix 1997).

FWS-CWs and vetiver grass phytoremediation have good ability to remove nitrogen (NH_4^+ and NO_3^-) but not for phosphorus by the system (48%, 69% and 16%, Table 1), where the ability of phytoremediation vetiver grass in the removal of NH_4^+ and NO_3^- is 62% and 36% respectively but only 49% in PO_4^{3-} removal.

The dynamics of the pollutant (NH_4^+ , NO_3^- and PO_4^{3-}) give the performance capabilities of pollutant removal by the system and plant uptake (Figure 3).

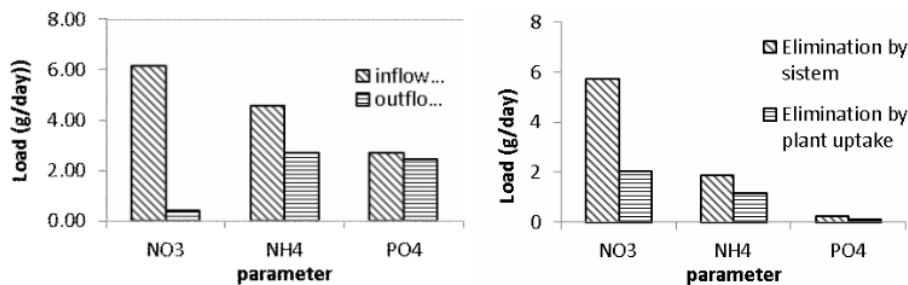


Figure 3. Comparison of the inflow and outflow (a). The rate of CWS elimination and *Chrysopogon zizanioides* (b).

Pollutant removal performance of NH_4^+ , NO_3^- and PO_4^{3-} , by an average uptake of *C. zizanioides* in both the root and in the leaves, respectively are 62%, 36% and 49%. This shows that the vetiver grass contributes to the removal of pollutants in the FWS-CWs system. Therefore, leveling up the uptake of those pollutants can be conducted by increasing the population of vetiver grass plants. According to Kennedy & Murphy (2004), the increase in plant density affects the decrease in the concentration of nitrogen.

The uptake amount of NH_4^+ by *C. zizanioides* is higher than NO_3^- because vetiver grass prefers nitrogen source from NH_4^+ more than from NO_3^- . According to Garnett et al

(2003), the different types of plants will have different preference of N source to be absorbed, depending on the resources available in the wetlands. According to Fang et al (2007), plants absorb nitrogen using roots and leaves. If both N sources are available, then plant tends to prefer to take NH_4^+ .

C. zizanioides becomes an option in removing nitrogen and phosphate pollutants in wastewater from *L. vannamei* cultivation. Results show that vetiver grass has a good tolerance to the mesohaline-salinity and nutrient-rich conditions. Danh et al (2013) wrote that *C. zizanioides* has the ability to take and increase the biodegradation of organic waste, thereby it has potential as phytoremediation plant. Moreover, it also has high biomass production, rapid growth rate, high survival rates, and the potential for accumulation.

Conclusions. *C. zizanioides* can grow and perform phytoremediation in mesohaline condition (salinity $<15^\circ/\text{oo}$). Phytoremediation using *C. zizanioides* can be applied to wastewater treatment from *L. vannamei* cultivation (aquaculture wastewater), primarily to the lower nitrogen concentrations in the ponds with mesohaline-salinity. *C. zizanioides* prefers nitrogen from NH_4^+ more than from NO_3^- . The advantage of this technology is that it is a green technology and environmentally friendly in treating wastewater and is a natural recycling method. The final product can be used, for example, as animal feed and ingredients for organic farming.

Acknowledgements. The authors would like to thank Karawang Aquaculture Bussines Development Centre for their support in this study, especially to Mr. Ir. Supriyadi, MSC and Mr. Ir. Warih Hardanu, MSc.

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Received: 28 July 2015. Accepted: 19 September 2015. Published online: 15 October 2015.

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How to cite this article:

Raharjo S., Suprihatin S., Indrasti N. S., Riani E., 2015 Phytoremediation of vaname shrimp (*Litopenaeus vannamei*) wastewater using vetiver grass system (*Chrysopogon zizanioides*, L) in flow water surface-constructed wetland. AACL Bioflux 8(5):796-804.

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