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Phytoremediation of freshwater crayfish (*Cherax quadricarinatus*) culture wastewater with spinach (*Ipomoea aquatica*) in aquaponic system

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Abstract. Aim of the study was to compare the ability of nutrient reduction of freshwater crayfish (*Cherax quadricarinatus,* family Parastacidae) wastewater cultured in aquaponic system using vegetable plant, water spinach (*Ipomoea aquatica*). Aquarium A - rearing freshwater crayfish without plant, and aquarium B - rearing freshwater crayfish with water spinach, were experimented for three weeks. Survival rates of crayfish were 85% (aquarium A), and 90% (aquarium B). Meanwhile mortality of crayfish was 15% and 10% at aquarium A and B, respectively. Aquaponic system applying water spinach can reduce freshwater crayfish culture wastewater particularly ammonia (NH_3) until 84.6%, and nitrate (NO_3) until 34.8%. Orthophosphate underwent reduction of 44.4% under spinach treatment. Relatively high ammonia concentration in control without plant treatment (aquarium A) affected the growth of freshwater crayfish. Hence, the survival rates of freshwater crayfish grown together with plant were higher than that without plant.

Key Words: ammonia, nitrate, survival rate, mortality rate.

Introduction. Aquaculture farms produce some waste resulting from unused pellet and feces. No more than 25% to 30% of the nitrogen and phosphorus applied to ponds in fertilizers and feeds is recovered in fish or shrimp at harvest (Boyd & Tucker 1998). Lin et al (2002) reported that nutrient concentrations in a fishpond increased as feed residue and fish excreta accumulated.

Disposal of aquaculture wastewater containing high organic matter resulting from unused pellet and feces can contaminate natural water bodies. High organic substances through decomposition process are converted into ammonia, nitrate, orthophosphate, etc. The main contaminants of the wastewater effluent are suspended solids, ammonium, organic nitrogen, and phosphorus (Piedrahita 2003; Sugiura et al 2006; Turcios & Papenbrock 2014). Aquatic pollution is mostly prevalent in developing countries where only a small proportion of the wastewaters are treated (Baruah et al 2006; Boyd 2003; Bunting 2004).

Pond effluents are potential sources of pollution in receiving waters, if untreated can cause sediment loading and eutrophication in receiving waters (Hall et al 2011; Tello et al 2010; Vaiphasa et al 2007). Nutrients released in the culture system can be converted into plant or other biomass, which can easily be removed and may often be a valuable by-product. The nutrient-assimilating photoautotrophic plants can be used to turn nutrient-rich effluents into profitable resources (Neori et al 2004). Effluents from aquaculture are characterized by increased nitrogen species (ammonia, nitrites, and nitrates), organic carbon, phosphates, suspended solids, and high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Boyd 2003). Significant issues can result in the release of nutrient rich effluents such as increased algal blooms, degradation of benthic communities, oxygen depletion, and overall degraded water quality (Boyd 2003).

In integrated intensive aquaculture systems, the waste load such as nitrates and phosphates can be reduced if the fish is cultured with other organisms, such as plants as biofilter, which can convert nutrient discharges into valuable products (Turcios & Papenbrock 2014). In addition, Buhmann & Papenbrock (2013) stated that the halophytic plants recycle the nutrients generated in a fish culture in terms of biomass production and maintain appropriate quality in the process water of the recirculating aquaculture system.

Phytoremediation, an emerging clean up technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants, including grasses, forbs, and woody species, to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water (Hegazy et al 2011; Mojiri 2012). Phytoremediation is on average tenfold cheaper than other physical, chemical or thermal remediation methods since it is performed in situ, is solar driven and can function with minimal maintenance once established (Hooda 2007).

Phytoremediation with aquatic plant through aquaponic system is an integrated system between aquaculture and hydroponic using recirculation system. The waste of this system can be absorbed by aquatic plant. In aquaponics, nutrient-rich effluent from fish tanks is used as nutrient source of hydroponic production beds for growing vegetables, herbs, and flowers. This negates the cost of a biofilter used for other recirculating aquaculture systems, and is more environmentally sustainable (Allsopp et al 2009; Diver 2006; Qin et al 2005).

Phytoremediation presents many advantages, as compared to other remediation techniques. It can be performed with minimal environmental disturbance. It is applicable to a broad range of contaminants, including many metals with limited alternative options, organic pollutants may be degraded to CO_2 and H_2O, removing environmental toxicity (Schwitzguebel 2000).

Furthermore, if plants with high transpiration rates are selected, they will be able to take organic micro pollutants up and distribute them in their tissues, where further metabolism will occur (Coleman et al 2002; Schröder & Collins 2002; Schröder et al 2005; Golan-Goldhirsh et al 2004). Some plant species such as *Eichhornia crassipes*, *Chara* spp., *Ipomoea aquatica*, *Lemna* sp., *Pistia stratiotes*, *Typha latifolia*, *Brassica juncea* and *Helianthus annuus* can be used for eliminating contaminant (Boyd 1970; Hazra et al 2011; Schröder et al 2007; Yapoga 2013).

Aim of the study was to compare the ability of nutrient reduction of freshwater crayfish (*Cherax quadricarinatus*) wastewater cultured in aquaponic system using water spinach (*Ipomoea aquatica*).

Material and Method. Treatments of rearing freshwater crayfish without plant (aquarium A), and freshwater crayfish and water spinach (aquarium B), were evaluated. Each unit consisted of rearing tank (80 x 40 x 40 cm), hydroponic ditch, and water container. As much as 100 liter water was used in each aquarium.

An aeration unit was installed in rearing tanks to provide oxygen. Water from rearing tanks (aquariums A and B) containing unused pellet and feces was transferred with pump to hydroponic tank for growing vegetable plants. Water from hydroponic tanks was accommodated in the container, before pumped back into rearing tank, being used again for aquaculture system. This cycle continued throughout experiment, without water replacement, called as Recirculating Aquaculture System (RAS) (Ackefors 1999; Oberdieck & Verreth 2009; Sandu et al 2008; Waller 2001). This RAS was combined with plant which is commonly called as aquaponic system. Research was conducted at Centre for Environmental Studies, Bogor Agricultural University, June-July 2013.

Freshwater crayfish and plant. Freshwater crayfish juvenile aging 2–3 months old, 4–5 cm length, originating from the hatchery of Aquaculture Diploma Program, were applied in the experiment. Each aquarium was stocked with 30 crayfishes. Crayfish was observed their growth (weight and length), survival rate, mortality rate, and relative growth. Growth of freshwater crayfishs were determined once a week. As much as 5 bunds of water spinach, 3 weeks old, originating from the farmer, who regularly supplies spinach to the market, located nearby campus, were planted on the water using styrofoam as a holder. Each bund was planted by spacing plants (20 x 20 cm) at aquaponic ditch. Plant growth was determined by measuring length of water spinach limb once a week.

Survival rate and mortality rate were caculated using the following formulas:

$$SR = \frac{Nt}{No} \times 100\% \qquad M = \frac{No - Nt}{No} \times 100\%$$

Where: SR = survival rate;
M = mortality rate;
No = number of freshwater crayfish at the onset of experiment;
Nt = number of freshwater crayfish at the end of experiment.

Relative growth rate was calculated using the formula:

$$RGR = \frac{\ln Xt - \ln Xo}{\Delta t}$$

Where: RGR = relative growth rate;
Xt = length of freshwater crayfish or plant at the end of experiment;
Xo = length of freshwater crayfish or plant at the onset of experiment;
Δt = observation period.

Water quality parameters. They were analyzed weekly and included: temperature, pH, dissolved oxygen, ammonia, nitrate, ortophosphate, and sulphide (APHA 2008). All experimental parameters were observed every week for three weeks. This research duration was considered sufficient, since spinach could be harvested after 6 weeks cultivation. Correlation among parameter was analyzed by Pearson correlation. Water quality alteration was calculated using the formula:

$$\% \text{ Reduction} = [(a-b)/a] \times 100\%$$

Where: a = control concentration of water quality parameter at time t;
b = treatment concentration of water quality parameter at time t.

Results and Discussion

Freshwater crayfish growth. Freshwater crayfishes were fed with shrimp pellets three times a day (morning, afternoon, and evening). Amount of feed was adjusted to the needs by weight percentage. In early rearing (week 0), crayfish length ranged 4.3–5.5 cm (aquarium A), and 3.7–4.5 cm (aquarium B). At week 3, crayfish length ranged 5.0–7.1 cm and 5.0–6.0 cm at aquariums A and B, respectively.

Similar to the length, crayfish weight also increased. Crayfish weight at week 0 ranged 1.93–4.14 g (aquarium A) and 1.25–2.5 g (aquarium B). At week 3, crayfish weight ranged 2.54–8.50 g and 2.71–4.75.1 g at aquariums A and B, respectively.

Survival rates of crayfish were 85% (aquarium A), and 90% (aquarium B). Meanwhile mortality of crayfish was 15% and 10% at aquariums A and B, respectively. Relative growth rate of crayfish at aquariums A and B was 0.006 and 0.006, respectively. The high mortality rate in aquarium A might be caused by water quality deterioration such as high ammonia concentration. High ammonia content at aquarium A correlated with high number of crayfish death (Pearson correlation coefficient -0.954).

Water spinach growth. At the early planting (week 0), length of water spinach ranged 0.4–0.7 cm. At the end of experiment, length of water spinach ranged 2.8–6.0 cm. Relative growth rate of spinach was 0.102 cm day^{-1}. This indicated that vegetable plant could grow in the aquaponic system without nutrient addition. The inorganic compounds in aquaculture systems comply to a large extent with the nutrient requirements of plants and algae (Turcios & Papenbrock 2014). The use of nutrient by plant and microorganism in this experiment was likely denoted by the reduction of ammonia and nitrate within the culture of crayfish together with water spinach, compared with that of control (crayfish only).

The concentration of contaminants to be removed should be from low to medium because excess concentration may inhibit plant growth (EPA 2000). The potential of recirculation at aquaculture system for plant cultivation is reported by Lewis et al (1978). The combination of fish and plant culture where the plants not only act as biofilter but also as food for humans such as vegetable, salad, etc. denotes that hormones and chemicals should not be applied within the system. Integration of algal and macrophyte cultures can also be optimized to increase wastewater treatment efficiency and profitability of the farms (Castine et al 2013).

The University of Virgin Island aquaponic RAS can produce one pound of lettuce (*Lactuca sativa*) using less than half the water of traditional farming techniques - around 7.6 gallons of water (Anonymous 2009).

Summary of crayfish and spinach growth performance in aquarium A and B at the end of experiment is presented in Table 1.

Table 1
Performance of freshwater crayfish and water spinach growth at the end of experiment

No	*Growth Indicator*	*Aquarium A*	*Aquarium B*	
		Freshwater crayfish	*Freshwater crayfish*	*Water spinach*
1	Length (cm)	5.0–7.1	5.0–6.0	2.8–6.0
2	Weigth (g)	2.54–8.50	2.71–7.51	-
3	Survival rate (%)	85	90	-
4	Mortality rate (%)	15	10	-
5	Relative growth rate (cm day^{-1})	0.006	0.006	0.102

It seems that no obvious different between growth indicator at aquarium A and aquarium B. Further experiment with much longer observation instead of 21 days needs to be pursued in order to represent the life cycle of water spinach. Treatment repicates have also to be designed to avoid artefactual sample. The reason of ending the experiment at 21 days is a consideration that this experiment was an initial step. Furthermore a comprehensive research with more variation of treatment should be conducted later on to reveal a better result and findings of growth performance of freshwater crayfish and water spinach under aquaponic recirculation system.

Water quality. Water temperature was relatively stable (27–29°C), suitable for freshwater crayfish growth requiring temperature range of 26–29°C. Acidity of water was normal (pH 6.4–7.5). Boyd & Tucker (1998) stated that optimum growth of fish occurs at pH 6–9 and death may occurs at pH 4 and 9.

Dissolved oxygen ranged 5.2–6.8 mg L^{-1}, in accordance with Boyd & Tucker (1998) statement that crustacea growth depends on dissolved oxygen levels > 5 mg L^{-1}. Increased ammonia (NH_3) occured at aquarium A from 0.06 mg L^{-1} (week 0) to 0.50 mg L^{-1} (week 2), 0.43 mg L^{-1} (week 3) due to the absence of plant as biofilter. Ammonia concentration at aquarium B was < 0.1 mg L^{-1} at week 2 and 3. In recirculating systems, level of ammonia should be kept < 0.5 mg L^{-1} (Phillips et al 1994). The presence of vegetable plants in aquaculture was able to keep ammonia level below its critical level.

Ammonia reduction of spinach treatment compared with control at week 3 attained 84.4%. Meanwhile nitrate reduction (34.8%) was much lower than ammonia reduction. However nitrate reduction was lower (23.3%) (Table 2). Another experiment by Effendi et al (2015a) using lettuce as phytoremediator found that ammonia reduction was slightly higher namely 91.5% at week 3 than that of spinach.

Table 2

Ammonia and nitrate reduction compared with control at the same sampling period

Treatment	*Observation period (week)*		
	1	*2*	*3*
Ammonia reduction (%)			
Aquarium B (crayfish and spinach)	47.6	84.6	84.4
Crayfish and lettuce (Effendi et al 2015a)	57.1	90.1	91.5
Nitrate reduction (%)			
Aquarium B (crayfish and spinach)	11.0	34.8	34.8
Crayfish and lettuce (Effendi et al 2015a)	18.5	23.3	23.3

A research of crayfish culture wastewater using spinach and bacteria by Effendi et al (2015b) denoted that reduction of ammonia, nitrate, and orthophosphate was 81%, 33%, and 89%, respectively after 4 week experiment also under aquaponic system.

After treatment of 120 days, 30.6% of total nitrogen and 18.2% of total phosphorus were removed by *I. aquatica* (Li & Li 2009). The aquatic plants were able to significantly reduce the pollution load of the aquaculture wastewater. The NH_4-N, NO_2-N, NO_3-N and PO_4-P reductions ranged 55.9-76.0%, 49.6-90.6%, 34.5-54.4%, and 64.5-76.8%, respectively (Snow & Ghaly 2008). The total reductions in NO_3–N, NO_2–N, and phosphate ranged 82.9-98.1%, 95.9-99.5%, and 54.5-93.6%, respectively (Ghaly et al 2005).

Treatment of domestic wastewater with lotus (*Nelumbo nucifera*) and hydrilla (*Hydrilla verticillata*) resulted in ammonia reduction of 80% and 87% respectively (Kanabkaew & Puetpaiboon 2004). Ammonium is one of the end products of protein metabolism (Walsh & Wright 1995). All these factors contribute to the high nitrogen residues in aquaculture water. In water, NH_3 (ammonia) and NH_4^+ (ammonium) are in equilibrium depending on the pH and the temperature (Timmons et al 2002).

Ammonia nitrogen in *Eichhornia crassipes* treatment of shrimp aquaculture wastewater decreased from 1.8-0.2 mg L^{-1} in 46 hours. Furthermore, percentage reductions ranged from 52.5 -100% and were in the order of NO_3-N > NO_2-N > TP > TAN > TN > TSS > RP > BOD_5 > COD (Nyanti et al 2010).

In the case of sweet flag (*Acorus calamus*) and common reed (*Phragmites communis*) the concentration of nitroglycerin was reduced by 50% after 3rd day. At the end of the experiment (day 21), decontamination efficiency reached 87 and 84% for sweet flag and common reed (Marecik et al 2013).

Although both NH_3 and NH_4^+ may be toxic to fish, unionized ammonia is the more toxic form attributable to the fact that it is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH_4^+ ions (Körner et al 2001). Ammonia-N is toxic to commercially cultured fish at concentrations above 1.5 mg L^{-1}. In most cases, the acceptable level of unionized ammonia in aquaculture systems is only 0.025 mg N L^{-1} (Neori et al 2004; Chen et al 2006). However, the toxicity threshold depends strongly on the species, size, fine solids, refractory organics, surface-active compounds, metals, and nitrate (Colt 2006).

In aquarium A, nitrate level increased from 0.05 to 0.11 mg L^{-1} (week 3), and aquarium B from 0.04 to 0.08 mg L^{-1} (week 3). Initial nitrate (0.56 ± 0.14 mg L^{-1}) from aquaculture wastewater decreased 21.4% to be 0.44 ± 0.14 mg L^{-1} after treatment with aquatic plant (Tavares & Boyd 2005).

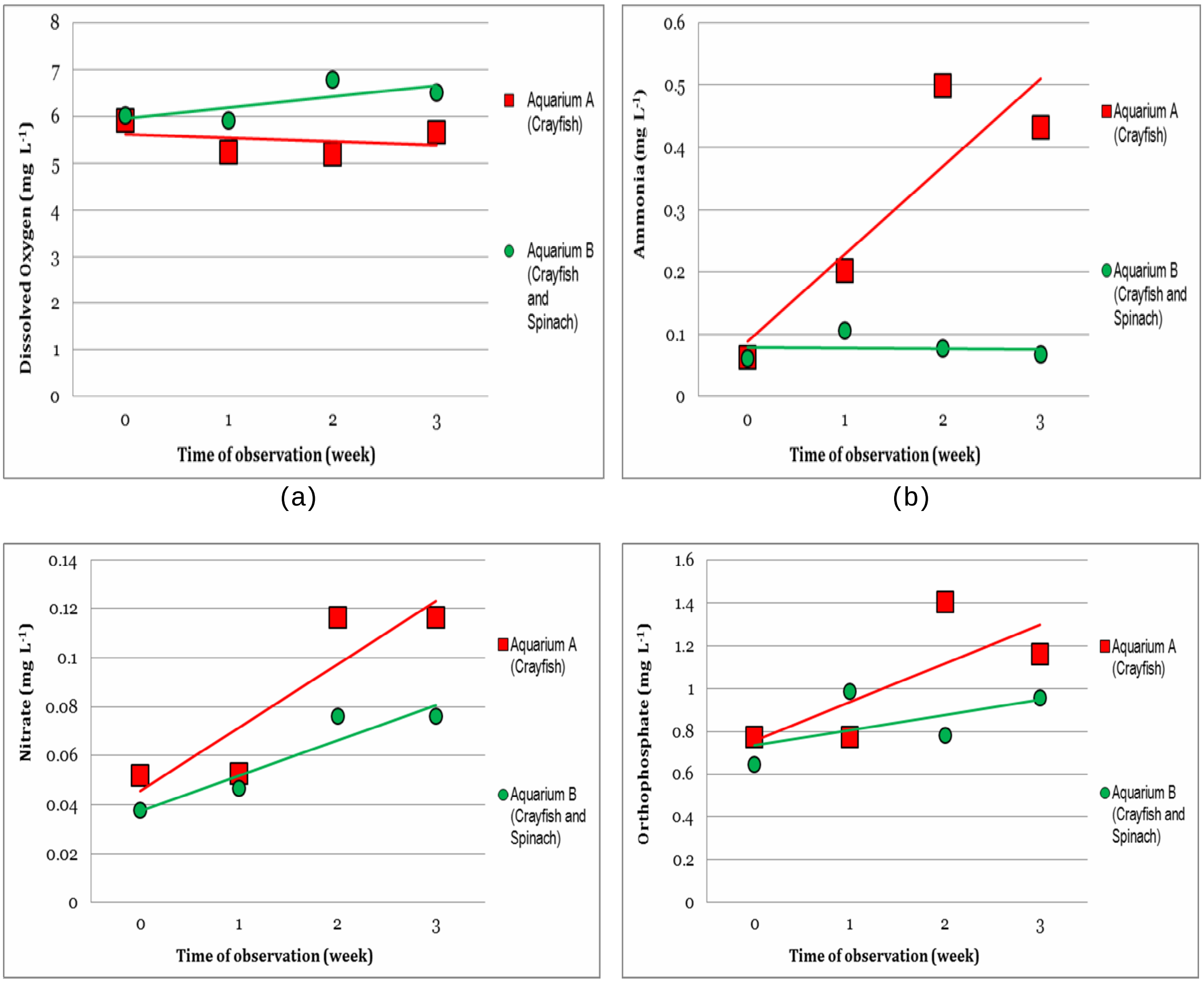
Orthophosphate (PO_4) increased within 4 weeks experiment. Orthophosphate at aquarium A increased from 0.77 mg L^{-1} (week 0) to 1.40 (week 2). At aquarium B also increased from 0.64 mg L^{-1} (week 0) to 0.78 mg L^{-1} (week 2). Orthophosphate reduction at week 2 occurred 44.4%.

The increase of nitrate and orthophosphate concentration is related to the input of crayfish feed (pellets), and decomposition of crayfish shell after moulting (Simbarashe et al 2011). Phosphorus is found in fish feeds and is broken down into a more useable form (phosphate) through decomposition (Miller & Semmens 2002).

Nitrate and phosphate concentration found in this experiment was in permissible limit for aquaculture namely 0 to 3.0 mg L^{-1} and 0.01 to 3.0 mg L^{-1}, respectively. The key mechanism of P removal in pond systems was uptake by plants (Tchobanoglous et al 2003).

The floating plants shield the water from sunlight and reduce the growth of algae. Systems of this kind have been effective in reducing BOD, nitrogen, metals and trace organics and in removing algae from lagoons and stabilization pond effluents. Supplementary aeration has been used with floating plant systems to increase treatment capacity and to maintain the aerobic conditions necessary (Dixit et al 2011).

During the 3-weeks observation period, sulphide concentration pattern was relatively stable. Sulphide concentration from week 0 to 3 was the same (< 0.002 mgL^{-1}). This condition denoted that the decomposition of organic waste resulting from feces and unused pellet was in aerobic state. This phenomenon was also supported by the presence of dissolved oxygen which was always > 5 mg L^{-1} throughout experiment. Water quality parameters during observation period are presented in Figure 1.



(a) (b)

(c) (d)

Figure 1. Alteration of water quality parameters (a) dissolved oxygen, (b) ammonia, (c) nitrate, (d) orthophosphate.

Conclusions. Survival rates of freshwater crayfish grown without plant (85%) and freshwater crayfish grown with spinach (90%) indicated that spinach might serve as the plant for freshwater crayfish wastewater quality phytoremediation. However further investigation with bigger scale and longer experiment duration must be pursued. Aquaponic system using spinach can reduced freshwater crayfish culture wastewater particularly ammonia (NH_3) until 84.6%, and nitrate (NO_3) until 34.8%. Orthophosphate underwent reduction of 44.4% under spinach treatment. Relatively high ammonia concentration in control without plant treatment affected the growth of freshwater crayfish. Hence, the survival rates of freshwater crayfish grown together with spinach were higher than that of freshwater crayfish cultured without plant. Aquaculture wastewater containing unused crayfish pellet and feces could support the growth of spinach at aquaponic system without nutrient addition. However optimum growth of spinach cultivated under this system needs further investigation.

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