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Evaluation of a cost effective technique for treating aquaculture water discharge using *Lolium perenne* Lam as a biofilter

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Abstract

Wastewater stabilization ponds generate low cost by-products that are useful for agriculture. The utilization of these by-products for soil amendment and as a source of nutrients for plants requires a high level of sanitation and stabilization of the organic matter, to maintain acceptable levels of soil, water and air quality. In this study, two aquaculture wastewater treatment systems; recirculating system and a floating plant bed system were designed to improve the quality of irrigation water in local communities with low income. In both systems the grass species *Lolium perenne* Lam was used as a plant biofilter while vegetable specie *Amaranthus viridis* was used to evaluate the performance of the system and the suitability of the phyto-treated water for irrigation. It was found that the harmful material removal rate for recirculating system was 88.9% for TAN (total ammonia nitrogen), 90% for NO_2^--N, 64.8% for NO_3^--N while for floating plant bed system 82.7% for TAN, 82% for NO_2^--N and 60.5% for NO_3^--N. Comparative analysis of the efficiency of waste element removal between the two systems revealed that both systems performed well, however, plant growth was not robust for floating plant bed system while recirculating system is energy consuming.

Although both systems did not attain sufficient levels of TN (total nitrogen) and TP (total phosphorus) load reduction, the treatment with *L. perenne* remarkably improved the irrigation water quality. *A. viridis* plants irrigated with the phyto-treated discharge water had lesser concentrations of heavy metals in their tissues compared to those irrigated with untreated discharge. The control plants irrigated with untreated discharge were also found to be highly lignified with few stems and small leaves.

Key words: recirculating system; float system; plant filter; *Lolium perenne* Lam; *Amaranthus viridis*; Burundi

Introduction

The reuse of wastewater in agriculture and aquaculture is going to increase in the coming decades and thus need to strengthen technology options for wastewater management in rural and urban coastal areas.

Burundi's aquatic resources have contributed immensely to aquaculture development in the Lake Tanganyika that provides many options for sustainable economic development around trade and fisheries. However, the ignorance of realistic and holistic approach to environmental problems has led to the reduction of agricultural production and the disappearance of some indigenous plant and fish species in Burundi (PNUD/FAO, 1997).

In most aquaculture systems the primary motivating factor has been reuse of nutrients for food production rather than wastewater treatment. Neglecting the later has resulted in a key environmental concerns about aquaculture systems management of waste water discharged with high levels of nutrients and suspended solids into adjacent waterways (Burford *et al*., 2003). Aquaculture systems have two separate discharge components, solids and/or nutrients, which if left untreated, can have negative effects on receiving water bodies. Several reports have suggested that aquaculture discharges and other wastewater inputs should be limited to the assimilative capacity of the ecosystem, assuming that the system was assimilating the nutrients being discharged (Jones *et al*., 2001). Effluent compounds can be used as nutrients for plant production (McIntoshet and Fitzsimmons, 2003). Using aquaculture effluents as inputs for production of other farm products can improve overall facility sustainability (Summerfelt *et al*., 1999). However, Muchuweti *et al*. (2006) observed that untreated aquaculture discharge was capable of causing excessive accumulation of heavy metals in agricultural soils. This may not only result in environmental contamination, but lead to elevated heavy metal uptake by crops, which may affect food quality and safety. New technologies and improved management practices allow the aquaculture industry to be more sustainable and economically viable. Integrating aquaculture production into traditional agriculture may be one approach to reduce the organic load and provide an alternative use for the discharged water.

This article describes a model system for farm aquaculture water treatment using *Lolium perenne* Lam as plant

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biofilter for improving wastewater quality prior to use for irrigation and the removal of TAN (total ammonia nitrogen) from the discharge water.

We chose *L. perenne* because of its tolerance to a wide range of physiological stress conditions. Additionally, this grass has an extensive and deep root system that would help maintain the hydraulic conductivity and contribute to oxygen transport into the bed.

We used a nutrient film technique (NFT) for the grass culture. The grass was planted on hallowed plates using unwoven fabric as a growing medium. The dense grass roots acted as a filter by absorbing nutrients while trapping some solids.

Our major concern was nitrogen, particularly the removal of TAN from the discharge water. Our study had two objectives: (1) to assess a treatment system for reducing nutrients from aquaculture farm discharge; a system which is efficient, relatively inexpensive and which could be implemented in rural areas in developing countries with low income (e.g. Burundi); (2) to evaluate the potential contribution of the system using *Amaranthus viridis*.

1 Material and methods

The experiments were carried out in the key laboratory of environmental engineering and eco-agriculture of Zhejiang University, from September 2005 to late October 2006. Seedlings were cultured (5 g/plate) on plates with 30% of their bottom perforated, laid on four layers of moist unwoven fabrics for 60 d (Fig.1). Unwoven fabric was used as a growing medium. During the culture period, a commercial solution was used as nutrient solution. Each concrete trough consist of 40 plates arranged horizontally (Fig.2). The grass was clipped at 10 cm height before each series of experiments.

The first pilot experiment was designed to compare the efficiency of *L. perenne* to remove nutrients in recirculating and in the floating system.

In the recirculating system, six plates (60 d after sow: DAS) were placed on a bench elevated to a gentle slope (5%) that allows the flow of wastewater from a closed turtle pond regularly pumped from a large container (80 L). The flow rate was 128 ml/s. The system was equipped with a timer set at 15 s On and 5 min Off. This regime helped

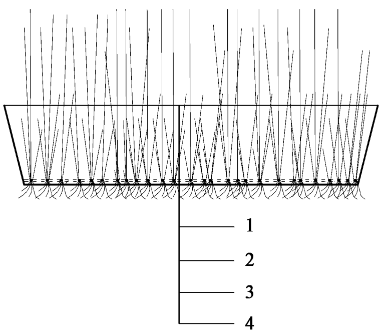

Fig. 1 Cross section of the grass-plate. (1) grass leaves and stems; (2) unwoven fabric layer(s); (3) hollowed plate bottom; (4) grass roots.

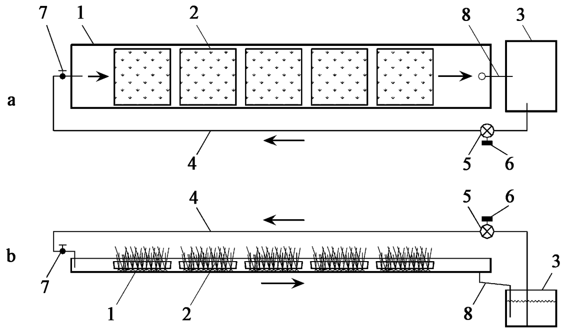

Fig. 2 Hydroponics botanical filter (a) top view (b) cross section view. (1) trough; (2) hydroponics grass-plate; (3) wastewater tank; (4) inlet pipe; (5) pump; (6) timer; (7) tap; (8) discharge pipe.

to maintain a high oxygen level in the root zone and as well allows assimilation of pollutants and back to the large container (Fig.3).

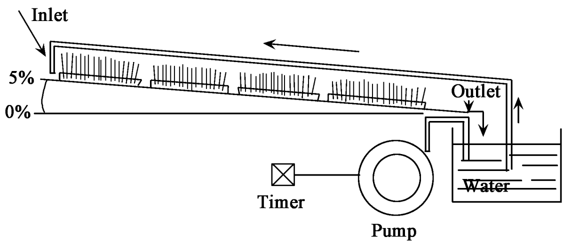

Fig. 3 Recirculating system.

The setting of the floating system consisted of a series of concrete trough compartments in which the same volume (80 L) of the turtle aquaculture farm discharge was put. Six grass plates were then made to float on the surface during the trial (Fig.4).

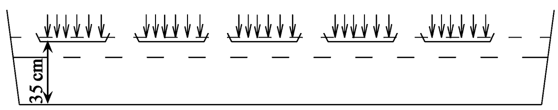

Fig. 4 Floating system.

Each experimental unit was replicated three times, the duration of each trial was 15 d, and the wastewater was replaced between trials.

Three replicate samples of wastewater at a depth of 10 cm from the surface of both systems were collected daily to quantify TAN, total nitrogen (TN), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), total phosphorus (TP) and chemical oxygen demand (COD). Except for TN and TP, all samples were first filtered with Wattman paper (GB/T 1914-93; ϕ12.5 cm) before analysis. Quantification of these 6 parameters allows the characterization of wastewater in each system. After the experimental period, the plant root and shoot lengths were measured, as well as the biomass and dry mass for each plate.

Laboratory analysis of TN was conducted by the Alkaline potassium persulfate digestion-UV spectrophotometric method (GB-11894); NO_3^--N was analyzed by spectrophotometric method with phenol disulfonic acid

(APHA, 1995), (UV-Vis spectrometer 752N); TAN was measured using Nessler's reagent colorimetric method (GB-7479-87); NO_2^--N was measured using the spectrophotometric method (GB-7493-87); TP was determined using ammonium molybdate spectrophotometric method (Spectrophotometer-Vis-723G) (GB-11893-89); COD was analyzed using the dichromate method (GB-11914-89). Measurements of temperature, electrical conductivity (Ec), dissolved oxygen (DO) and pH of the water samples were performed *in situ* during the sampling process, at a depth of 20 cm using the YSI multi-probe system (Model 556). NH_3-N-concentration as UIA (Unionized Ammonia) was calculated from the TAN using the following equilibrium equation (Erickson, 1985)

$$pK_a = 0.09108 + 2729.92/(273.2 + T) \quad (1)$$

$$UIA(\%) = 100(1 + 10^{(pK_a.pH)}) \quad (2)$$

The second set-up was designed to evaluate the suitability of the treated discharge water to irrigate *A. viridis*. This vegetable plant is widely consumed in Burundi and can adapt to low nutrient load at any season along the river banks or near fish pond discharge outlets.

The experiment was carried out in the greenhouse, in which environmental conditions were recorded but not controlled. Air temperature varied from 18–24°C during the course of the experiment. Seeds of *A. viridis* (0.5 g/pot) were sown in pots that were 15 cm deep and 27 cm in diameter containing silica sand on the top and gravel in the bottom. *A. viridis* was grown in the potting medium under the non-leaching regime. Three kinds of nutrient solution were used: the untreated wastewater, the treated wastewater and the commercial nutrient. There were 18–22 plants in each pot. The plants were watered manually four times daily (at 6:00, 8:00, 16:00 and 18:00 o'clock). Plant height and plant leaf area were recorded once every two weeks. Leaf area was determined using a portable area meter (Brand: Li-Cor, Model: Li-3000, Origin: USA). The trial was a factorial randomized block design with four replicates and three treatments. The data were analyzed statistically using the SAS system v 6.12 (1996), general linear model procedure.

At the end of the trial period, plants were cut off from the base; the roots were removed and washed. The stem, branches, leaves and roots were dried at 60°C for 72 h and then weighed to determine dry matter yield per pot. Biomass was determined before drying. Heavy metals (As, Cd, Cu, Pb, Zn, Hg) content of the plant tissue was assessed as follows: after harvest, tissue samples were immediately washed with detergent and rinsed with milli-Q water to remove adsorbed nutrients and solids. Roots were further washed with 0.06 mol/L $CaCl_2$ for 15 min and then rinsed with milli-Q water.

These tissues were dried in a forced draft oven at a temperature of 70°C and ground to powder using a ceramic pestle and mortar. Plant powder (50–100 mg) was digested with 2 ml of 70% nitric acid (HNO_3) at 150°C for 3 h, cooled and diluted to form a 2% HNO_3 final solution (prepared sample). Two milliliters of the prepared sample were analyzed according to standard methods for metals (Atomic absorption spectrophotometer ALPHA-4, CAMSPEC) (APHA, 1995).

2 Results and discussion

2.1 Aquatic environmental changes when using *L. perenne* as a biofilter

This experiment was carried out between mid-October and December 2005. Table 1 shows the fluctuations in environmental factors during the trial. Changes in wastewater temperature followed the same trend as the air temperature during winter varying from 13.54–1.49°C for the recirculating system and 11.74–4.13°C for the floating system. Water temperature fluctuates with the atmospheric temperature. The difference in temperature may also have resulted from the high specific heat capacity of water as well as from setting the two systems where one is static and the other is flowing. The slight increase in DO observed in the recirculating system could as well be attributed to the fact that the wastewater was regularly pumped out and flowing back to the container through the arranged grass plates. However, in both systems it is noted, the DO increased gradually from 9.2–13.1 mg/L and 8.3–12.4 mg/L for the recirculating and the floating systems respectively. The DO remained favorable for the culture of *L. perenne* in both systems during the course of the experiment. The Ec did not vary much but gradually decreased from 0.552–0.506 mS/cm^2 for the recirculating system, while it increased slightly from 0.57–0.632 mS/cm^2 for the floating system. Ec variation may have resulted from a combination of various biochemical processes, including,

Table 1 Aquatic environmental changes during the culture of *Lolium perenne* Lam for both recirculating and float systems

System	Parameter	Time (d)						
		1	3	6	8	10	13	15
Recirculating	Temperature (°C)	13.54 (0.07)	7.16 (0.056)	3.82 (0.08)	3.44 (0.04)	1.49 (0.06)	3.11 (0.19)	4.43 (0.2)
	Ec (mS/cm^2)	0.552 (0.002)	0.538 (0.006)	0.538 (0.01)	0.527 (0.008)	0.531 (0.014)	0.511 (0.012)	0.506 (0.014)
	pH	7.8 (0.02)	8.0 (0.03)	8.1 (0.07)	8.0 (0.0)	7.9 (0.01)	8.0 (0.0)	7.9 (0.05)
	DO (mg/L)	9.2 (1.2)	10.9 (1.0)	11.3 (0.3)	12.8 (1.2)	12.2 (0.0)	12.3 (0.4)	13.1 (0.1)
	COD (mg/L)	48.4 (1.7)	50.8 (1.7)	53.4 (2.8)	56.6 (0.8)	59.6 (2.8)	68 (0.6)	71 (1.4)
Floating	Temperature (°C)	11.74 (0.24)	8.03 (0.07)	5.66 (0.2)	5.12 (0.42)	4.13 (0.26)	5.01 (0.24)	6.08 (0.02)
	Ec (mS/cm^2)	0.57 (0.012)	0.61 (0.007)	0.62 (0.004)	0.619 (0.009)	0.624 (0.011)	0.629 (0.007)	0.632 (0.005)
	pH	7.6 (0.03)	7.5 (0.02)	7.7 (0.01)	8.0 (0.0)	8.0 (0.0)	8.0 (0.09)	8.0 (0.07)
	DO (mg/L)	8.3 (0.3)	10.2 (0.3)	10.7 (0.0)	11.8 (0.5)	11.8 (0.2)	11.6 (0.4)	12.4 (0.3)
	COD (mg/L)	48.6 (1.8)	55 (1.4)	74.8 (4.5)	82 (1.8)	85.4 (2.5)	97 (1.9)	99 (2.7)

* The values in parentheses represent the standard deviation of the means.

oxidation of organic compounds, accumulation of organic wastes, microbial activity and plant assimilation (Moore, 1989; Michaud, 1991). These processes are likely to differ between the recirculating system and the floating system. However, our results are not sufficient to determine the specific mechanisms involved in this fluctuation.

The COD increased steadily from 48.6 to 99 mg/L for the floating system and only from 48.4–71 mg/L for the recirculating system. The increase in COD may be a consequence of continuous settling of dead organic matter from plant debris. The more pronounced accumulation of organic compounds in the floating system than in the recirculating system may be as a result of the grass-plates arrangement with an intermittent flow for the recirculating system while the floating system consisted of static wastewater.

In addition, the rate of organic compounds mineralization and the fluctuation of organic nitrogen were observed to be different in the two systems (Fig.5h).

The slight increase in UIA, together with the increase in COD may be due to the organic matter accumulation as a result of wastewater stagnation. This observation is in agreement with the findings of Gross *et al*. (2003), who reported that bacteria decompose organic wastes releasing NH_3-N and PO_4^{3-}-P. The pH was not affected by the grass culture, remaining at 8 for both systems.

2.2 Comparative study of the recirculating and floating systems in nutrients removal efficiency

In aquaculture, TAN refers to NH_4^+ and NH_3. Our study focused on the trend of TAN during grass culture. For the recirculating system, TAN was 1.96 mg/L at day 1 and dropped to 0.581 mg/L after 3 d, and then to 0.217 at the day 15 (Fig.5a). The removal rate was 70.3% after 3 d, and 88.9% for the rest of the trial period. The trend was similar in the floating system with appreciable removal rates of 65.6% after 3 d, and 82.7% for the rest of the trial period. The reduction in the concentration of TAN may be due to the ability of *L. perenne* roots to assimilate NH_4^+. There was a reduction in UIA of 71.5% and 80.5% for the first three days and total removal rates of 93.3% and 73.1% at the end of the trial for the recirculating and the floating systems respectively (Fig.5b). Porello *et al*. (2003) earlier reported that in undissociated form, ammonia (NH_3) is one of the most toxic substances produced by intensive fish farms and should be removed from the discharge water. The results of Körner (2001) revealed that relative growth rates of *Lemna gibba* decreased linearly with increasing NH_3 concentrations up to a maximum level (8 mg/L), above which the duckweed died. Most aquatic organisms experience chronic toxicity at ammonia concentrations of 10–6 g/L and nitrite concentrations of 10–6 g/L (Lee *et al*., 2000). It is interesting to note that results obtained from our treatment system indicate that using *L. perenne* can be one of the ways to mitigate the toxicity effect of ammonia to plants and other living organisms.

In this study, the concentration of NO_2^--N gradually decreased from 30.5–3 μg/L for the recirculating system. The same decrease was observed in floating system where the concentration was reduced from 25–4.5 μg/L (Fig.5c). The NO_2^--N did not accumulate for the two systems, and since the plant does not assimilate NO_2^- the reduction of the nitrite might have resulted from the nitrification process. Qiao and Murray (1998) agreed with our observation when they reported that high levels of NO_2^- reduced the amount of nitrate uptake and increased the ammonium concentration in roots of soybean.

The concentration of NO_3^--N in wastewater decreased in both the recirculating and in the floating system but the decrease was more rapid for the recirculating system than for the floating system. The removal rate was 52.8% after 6 d and then 64.8% after 15 d for the recirculating system. It was about 44.3% after 6 d and then 60.5% after 15 d for the floating system (Fig.5d). Under natural conditions major sources of nitrogen for plants, are ammonium (NH_4^+) and nitrate (NO_3^-) (Olsson and Falkengren, 2000). In view

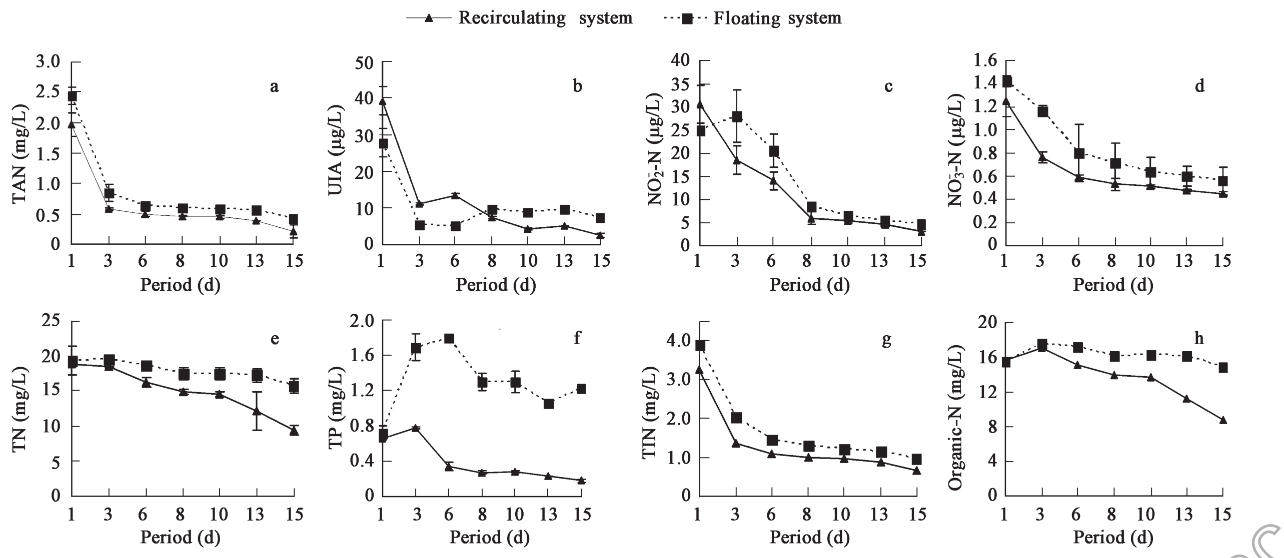

Fig. 5 Performance of *Lolium perenne* Lam in nutrients removal when used in a floating and a recirculating system. (a) TAN; (b) UIA; (c)NO_2^--N; (d) NO_3^--N; (e) TN; (f) TP; (g) TIN and (h) organic nitrogen fluctuation in different systems.

of the findings by Lee *et al*. (2000), who reported that nitrate is usually assimilated by plants in natural aquatic environments, we can assume therefore that the decrease in nitrate concentration is due to plant absorption. Contrary to ammonia and nitrite, nitrate is relatively non-toxic to aquatic organisms (van Rijn *et al*., 2006). Gross *et al*. (2003) also suggested that ammonia is oxidized by bacteria in biological filters to nitrite, which is also toxic, and to nitrate. Nitrate should be maintained below 100 mg/L, since high nitrates concentrations typically result in algal blooms, which over time can result in lowering of pH (Watson and Hill, 2006).

TN decreased gradually from 18.88 mg/L to 9.42 mg/L and 19.36 to 15.82 mg/L for the recirculating and the floating systems respectively (Fig.5e). The course of TP was different from that of TN. There was a progressive increase in TP from 0.72–1.22 mg/L for the floating system while at the same period; there was a gradual decrease from 0.66 to 0.208 mg/L for the recirculating system (Fig.5f). The fluctuations in TIN (total inorganic nitrogen) are shown in Fig.5g while those of organic nitrogen are presented in Fig.5h. The decrease in TN can only be attributed to plant assimilation of NH_4^+ and NO_3^-. *L. perenne* was not efficient in the removal of TN and TP. Discharge from the aquaculture pond is typically high in suspended solids and particulate organic nutrients. The elevated load in TN and TP resulted from the breakdown of organic matter from the plant debris to form the more stable end products of organic nitrogen and phosphate. Total solids were trapped in the root zone by the unwoven fabric in the recirculating system, though TN and TP decreased slightly. Meanwhile, in the floating system the solids could go through the plate and accumulate at the bottom of the trough. Ebeling *et al*. (2006) showed that the majority of the phosphorus discharged from intensive aquaculture systems (50%–85%) is contained in the filterable or settleable solids fraction. Phosphorus is often the limiting nutrient in natural ecosystems, and excessive algae blooms can occur if discharge concentrations exceed the absorption capacity of the receiving water body (Ebeling *et al*., 2006).

The recirculating system produced higher plant biomass and dry mass than the floating system. There was however, no significant difference in shoot length. The plants in the floating system developed longer roots than those from the recirculating system (Table 2).

Although both systems performed well in terms of nutrients removal, in the floating system plants were less robust than those in the recirculating system. The latter system is, however, energy consuming. The plants in the recirculating system developed thicker roots than those in the floating system, in which the roots were longer and thinner. We used the student's *t*-test to compare means. The differences (*t*-test) between the recirculating system and the floating system were highly significant for biomass (P = 0.005), dry mass (P = 0.0005) and root length (P < 0.0001). In summary, our report shows that *L. perenne* greatly contributed in improving wastewater treatment and may be utilized in remediation of aquaculture discharge.

In both recirculating and the floating systems, *L. perenne* showed a high performance by reducing the wastewater load in TAN, NO_2^--N and NO_3^--N. Nevertheless, the removal rate of TN and TP was low. The results indicate that the recirculating system was more efficient than the floating system, but this system bears a higher cost due to the consumption of energy for the pumping system.

Table 2 ***Lolium perenne*** **Lam growth after treatment (45 d after clipping)**

	Recirculating system	Float system
Plant biomass (g)	103.2 (5.25)**	82.54 (5.91)
Dry mass (g)	17.22 (1.03)**	13.18 (0.79)
Shoot length (cm)	17.92 (1.00)	18.56 (0.92)
Root length (cm)	7.86 (0.27)	17.9 (0.5)**

**The difference is highly significant; the values in parentheses represent the standard deviation of the means.

2.3 Performance of *L. perenne* in improving irrigation water

We used *A. viridis* to evaluate the efficiency of phytotreatment by *L. perenne* in improving water quality for irrigation. This trial was designed to assess the three sources of irrigation water on growth and development of *A. viridis*. Plant growth characteristics measured included plant height, leaf area index, biomass and dry mass.

Determination of leaf area was based on the five fully expanded leaves from each pot, taken five times during the growth period. Two weeks after sowing, there was no difference in leaf area between the three types of irrigation water; but along the duration of the trial, the plants irrigated with untreated wastewater developed the smallest leaves. The most expanded leaves were in pots irrigated with the commercial nutrient. However, the plants in pots irrigated with treated water also showed an appreciable leaf expansion (Table 3). Plants irrigated with untreated wastewater had the smallest and darkest leaves compared to those irrigated with treated effluent or commercial nutrient. *A. viridis* can therefore be used as an indicator of adverse environmental factors especially for aquaculture discharge.

In addition, we also observed that plants irrigated with untreated wastewater recorded the greatest height (Fig.6). The untreated wastewater enhanced plant growth. The plants irrigated with untreated effluent developed a very tough stem with a ligneous structure and the stem reached

Table 3 Effects of irrigation water quality on leaf expansion of ***Amaranthus viridis***

Time (wks)	Untreated wastewater	Phyto-treated water	CN
2	2.51 (0.23) a	2.63 (0.05) a	2.73 (0.05) a
4	4.73 (0.25) b	8.56 (0.4) a	9.06 (0.4) a
6	6 (0.25) b	11.06 (0.47) a	12.6 (1.1) a
8	6 (0.16) c	12.4 (0.64) b	16.0 (0.84) a
10	6.6 (0.2) c	13.2 (0.64) b	16.6 (1.1) a

A two-way analysis of variance (ANOVA) showed that there was a statistically significant difference (P < 0.001) among the means. The difference between treatments was highly significant. CN: commercial nutrient.

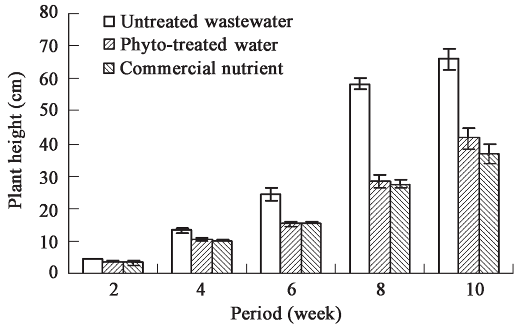

Fig. 6 Effects of irrigation water quality on *Amaranthus viridis* growth.

greater heights with fewer branches than those irrigated with the other kinds of nutrient solution. In contrast, plants irrigated with treated water or commercial nutrient solutions had a smooth stem, with many branches and the most expanded leaves. Periodic plant harvest is needed to prevent excessive vertical growth.

The irrigation water treated by *L. perenne* showed almost the same performance as the commercial nutrient in terms of plant growth. There was no need for addition of nutrients to sustain plant growth. Our results are in agreement with those of Montemurro *et al*. (2004), who reported that wastewater application increased growth parameters by 18.2% in 2001 and by 41.1% in 2002, suggesting the possible use of wastewater as a soil amendment for ryegrass production.

The plant biomass and dry mass were higher for the pots irrigated with untreated wastewater than those irrigated with treated effluent or commercial nutrient (Table 4).

Plants irrigated with untreated wastewater displayed the highest concentrations of heavy metals in their tissues, indicating that heavy metals were absorbed in the root zone and then translocated to the rest of the plant parts (stem, branches and leaves). On the other hand, the plants irrigated with the phytotreated wastewater showed less heavy metal concentrations in their tissues as compared to the untreated control (Table 5). This illustrates a dual function of *L. perenne*–the capacity to reduce the wastewater nutrient load and as the ability to alleviate the concentration of heavy metals in wastewater. The discharge of untreated wastewater may contaminate irrigation water, causing an accumulation of heavy metals in agricultural soils.

Table 4 Effects of irrigation water quality on biomass and dry mass of *Amaranthus viridis*

	Plant biomass	Dry mass
Untreated wastewater	345.13 (15.8) a	30.16 (2.63) a
Phyto-treated water	237.77 (23.5) b	14.63 (1.44) b
CN	241.6 (3.41) b	12.16 (0.7) b

Leaf area is expressed in cm^2; biomass and dry mass in grams; the values in parentheses represent the standard deviation of the means; different letters: significantly different at $p = 0.05$; CN: commercial nutrient.

3 Conclusions

The importance of integrating aquaculture and agriculture is clearly expressed throughout our study. *L. perenne* can be used in small scale farms in rural areas to treat aquaculture discharge with the purpose of improving the quality of irrigation water. However, its ability was not appreciable in terms of TN and TP removal. *L. perenne* has demonstrated potential to reduce nutrients load in aquaculture discharge water with an additional advantage of reducing the concentration of heavy metals, which accumulate in the plant systems and eventually in the human body. The system is reliable, inexpensive and easy to handle. Our studies also revealed the ability *A. viridis* to adapt to various environmental conditions, invariably making this unique plant growth features a bio-indicators of poor environmental conditions.

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Table 5 Concentrations of heavy metals in plant tissues of *Amaranthus viridis*

Metal	Untreated WW (mg/kg)	Treated WW (mg/kg)	Commercial nutrient (mg/kg)	Chinese standards for vegetables (mg/kg)
As	3.8 (0.32)	0.92 (0.021)	0.27 (0.013)	0.5
Cd	2.4 (0.19)	1.1 (0.042)	0.52 (0.036)	0.05
Cu	5.6 (0.81)	3.13 (0.033)	1.8 (0.086)	10
Pb	12.2 (1.21)	4.7 (0.014)	0.35 (0.032)	0.2
Zn	181 (12.2)	47.2 (3.3)	18.4 (1.85)	20
Hg	0.91 (0.04)	0.27 (0.03)	0.03 (0.008)	0.01

* The values in parentheses represent the standard deviation of the means; WW. wastewater.

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