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Nutrient uptake rate and removal efficiency of *Vetiveria zizanioides* in contaminated waters

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ABSTRACT: This research compares the performance of floating systems planted with Vetiveria zizanioides as a hydroponic approach for removing nutrients from two contaminated waters. For this purpose, two pilots with overall net volume of 60 litres were constructed and inoculated by secondary treated domestic wastewater (STDW) and irrigation water obtained from Minab reservoir (IWMR) in batch mode. Regarding the experimental results, the total nitrogen removal efficiency reaches more than 40 and 75%, in two and four days' detention time, respectively, while these figures are 75 and 85% for phosphorus. The comparative statistical analyses verify that the results reveal significant differences in nitrogen removal, its uptake and the shoots' dry weight. Conversely, phosphorus removal, its uptake and the roots' growth are not significantly different. The regression analysis shows that the nitrogen uptake is well correlated with the shoots' expansion rate as a matter of substrate type. The decay coefficient rates of nitrogen and phosphorus are calculated as 0.43 and 0.52 day^{-1}, respectively. It is then concluded that this system should be used for wastewater treatment rather than for surface water purification. However, it can be recommended as an environmental friendly approach for both, because of the high efficiency in nutrients' removal and the aeration capability.

Key words: Hydroponic, Minab reservoir, Phytoremediation, *Vetiveria zizanioides*, Wastewater treatment

INTRODUCTION

Eutrophication is introduced as a typical challenge in surface water quality management. This is mainly caused by algal blooms, overwhelmingly because of excess nutrients discharged. Point sources are required to construct and operate wastewater treatment plants with tertiary units (USEPA, 2007). However, large amounts of nutrients are discharged to the surface waters by non-point sources, such as agricultural activities (Kao et al., 2001). Yet, very few efficient, economical and operationally stable treatment units are used to control the nutrients discharged by rural areas. The collection and treatment mechanisms of these emission sources are not technologically identical, as the point sources are. Hence, in the literature, land-based treatment facilities, or phytoremediation techniques, such as constructed wetlands, are recommended in the form of decentralized systems (Kao et al., 2001; Minghui et al., 2011). However, due to some limitations (i.e., low removal efficiency, availability of sustainable macrophytes, or susceptibility to the environmental conditions), this has not developed in Iran.

Phytoremediation is defined as a collection of processes using a symbiotic consortium of living plants and bacteria to uptake, convert or stabilize contaminants

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with different mechanisms (Landmeyer, 2012). For example, constructed wetlands use a rhizoremediation process in which the attached growth bacteria cultured on the rhizomes reduce soluble nutrients and other pollutants (Ye and Li, 2009). The hydroponic systems can similarly be used as a cost-effective, user-friendly and efficient approach for decentralized pollution control (Keller et al., 2005; Oyama et al., 2005). These are small-scale floated plants provided with structural supports made of inert materials rather than being grown in soils (Haddad and Mizyed, 2011; Nonstrom et al., 2004). Consequently, they are useful for both surface waters and drainage canals (Azizur Rahman and Hasegawa, 2011; Chua et al., 2012) in which the plant/microbial uptake process is active for the remediation of non-point nutrients discharged (Jian-Hang et al., 2013). Yet, their efficiency relies on the hydraulic retention time and macrophyte types in use (Ghosh and Gopal, 2010; Haddad and Mizyed, 2011). For example, Ying et al. (2011) compared plants cultured in lakes for better application based on nutrient uptake capacity and growth conditions in China.

Recently, the soil-cultured application of *Vetiveria zizanioides* (abbreviated here as *Vetiver*) was studied to increase the shear stress resistance against flood, riverside erosion and landslides (Chomchalow, 2011). This study verified that the uprooting force is linearly dependent on the growth of leaves and roots (Mickovski et al., 2005). This plant was also recommended in wetlands, as a tertiary unit for domestic wastewater treatment (Boonsong and Chansiri, 2008). Previously, nutrients and sediments discharged by non-point sources and the upstream of surface waters were studied in full scale (Edelstein et al., 2009; Chua et al., 2012). However, *Vetiver* was identified as a relatively inefficient macrophyte in comparison with *Typha latifolia* for controlling nutrient transportation from the upstream of surface waters (Chua et al., 2012). Furthermore, the performance of *Vetiver*, as a tropical aquatic macrophyte, in inorganic nitrogen uptake, has been compared with three floating and emergent species by Jampeetong et al. (2012). Its application was recommended in subsurface constructed wetlands for better nutrient removal.

With respect to the literature, a comparison of the performance of floated *Vetiver* for treating nutrient-rich surface water or secondary treated domestic wastewater can be considered a novel study. This assessment focuses on interpreting the comparative results in nutrient uptake by *Vetiver* inoculated with these two substrates in the south of Iran in order to highlight the optimized application.

MATERIALS & METHODS

In order to perform the sampling at the same time, two similar rectangular pilots with an overall net volume of 60 litres were constructed to set the *Vetivers* floating on water. The total dimensions in length, width and height were 91, 17 and 52cm, respectively. These were equipped with sampling valves 30 cm in height from the bottom, and discharge valves beneath them. It should be noted that the whole plants were previously cultivated in gardening soil and watered in pots for 12 months. Then, in March, they were carefully taken out and placed in both pilots.

During the study, two pilots were discretely inoculated with two substrates:

1. the effluent of secondary treated domestic wastewater (STDW); and
2. water allocated for irrigation from the bottom of the Minab reservoir (IWMR).

This may assist in describing the performance of the *Vetiver* system for nutrient removal in different contaminated waters. The average values of influent parameters are shown in Table 1. It should be noted that the total nitrogen (TN) and phosphorus (TP) concentrations of substrates differed by less than 3 and 6%, respectively, while the ratio of TP to TN was also identical (Table 1). This ratio has critical effects on algal growth, and it is better that it remains identical for substrates. Otherwise, the comparative results may not be easily discussed. However, the average values of other compounds, like ammonia, nitrite, biochemical oxidation demand (BOD) and total suspended solids (TSS) were significantly different.

Minab reservoir (also termed the Esteghlal Dam) has been constructed on Minab River in the south of Iran (27º09’49” N latitude and 57º06’46” E longitude). This area has average and maximum temperatures of about 21ºC and 34ºC, respectively, with 53% moisture. The annual precipitation and evaporation are about 196.3 mm and 70.6 mm, respectively. Regarding the three-year water quality analysis, and based on trophic state indices (TSI) (Devi Prasad and Siddaraju, 2012), the lake is identified as eutrophic and hypereutrophic, with high algal blooms and extensive macrophyte problems. The TSI factors, calculated based on total phosphorous and the average of three main parameters (TP, chlorophyll-A and Secchi disc), are shown in Figure 1. It is noteworthy that TSI variations, particularly in winter and spring, may be due to rather high seasonal flows that wash pollutants from the upstream, and suspend the sediments in the reservoir.

Table 1. The quality specifications of substrates

Parameter	STDW	IWMR
DO (mg/L)	1.1 ± 0.9	2.5 ± 0.3
pH	7.7 ± 0.2	8.1 ± 0.1
TDS (mg/L)	457 ± 22	556 ± 16
TSS (mg/L)	13.6 ± 5.4	2.1 ± 0.3
EC (µs/cm)	736 ± 37	891 ± 29
BOD (mg/L)	13 ± 4	3 ± 1
NH_4^+ (mg/L)	0.53 ± 0.26	0.17 ± 0.03
NO_2^- (mg/L)	0.98 ± 0.1	0.04 ± 0.01
NO_3^- (mg/L)	46.8 ± 14.2	47 ± 3.7
TN (mg/L)	48.3 ± 15.5	47.2 ± 3.8
TP (mg/L)	2.9 ± 0.3	3.1 ± 0.1
TP/TN ratio	0.06 ± 0.013	0.07 ± 0.007

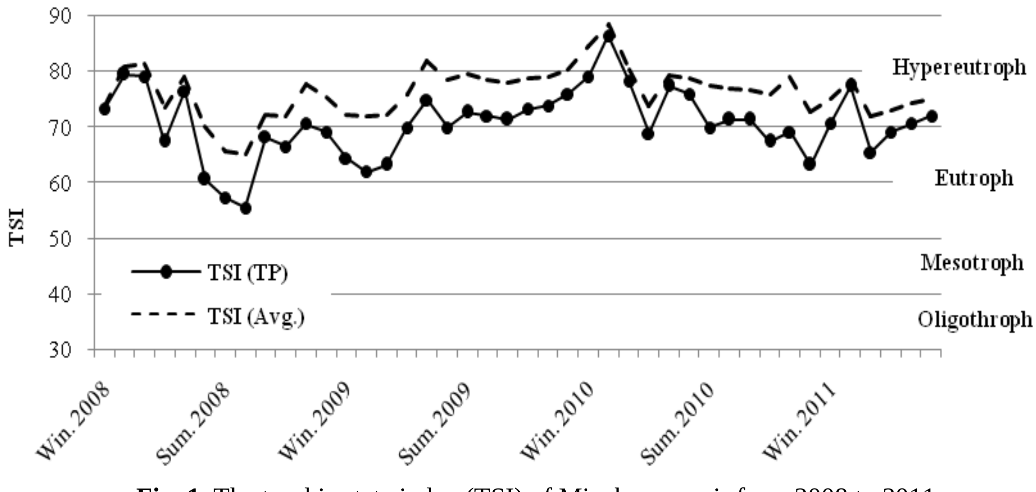

Fig. 1. The trophic state index (TSI) of Minab reservoir from 2008 to 2011

As plants were set in pilots, they were harvested and fed with substrates weekly in batch mode. The sampling was then carried out from the influent and through the pilots in four sequencing days, representing detention times of 24, 48, 72 and 96 hours. TN, TP and dissolved oxygen (DO) were analysed based on standard methods for the examination of water and wastewater (APHA, 2005). The dry weight and uptake rate of nitrogen and phosphorus restored to the roots and leaves were also measured based on standard practices (Jampeetong et al., 2012). For this purpose, at the beginning of each week, the contents of both pilots were discharged; they were washed and inoculated by fresh substrate. This continued for three months so that experiments were repeated 14 times (from March to June). The whole study was carried out in ambient temperature (22ºC to 33ºC). The data were then subjected to the analysis of variance (ANOVA) and Duncan test, using SPSS software to find out whether the differences observed were significant or not (Lu, 2009; Bianchi et al., 2010).

The time variations of nitrogen and phosphorus concentrations were estimated based on a first order reaction as per standard practices, as shown in Equation 1 (Chua et al., 2012). This may indicate the average nutrient uptake through phytoremediation.

$$\frac{C - C_t}{C_i - C_t} = e^{-kt} \quad (1)$$

in which C is the pollutant concentration (mg/L) at the sampling time t (day), C_t is the total concentration (mg/L), C_i is the initial concentration of substrate (mg/L), and k is the decay rate (day^{-1}).

RESULTS & DISCUSSION

Regarding the experimental results, it was observed that in both substrates, the system could remove soluble nutrients by more than 75% in four days' detention time. Actually, in this period, the total nitrogen removal efficiencies of pilots inoculated by STDW and IWMR were about 82 and 76%, respectively (Fig. 2). The ANOVA test determined the difference between the efficiencies of pilots as significant (P=0.001). However, phosphorus removal was calculated as 88% for both pilots without any significant difference (P=0.807). The same results were also observed in two days' detention time. The average TN removal efficiencies for STDW and IWMR were totally different, at 43 and 56%, respectively, while there were slightly significant (P=0.01) differences of TP efficiencies, which were 77 and 75%, respectively (Fig. 2). This means that the type of substrate is only effective on average nitrogen removal, and has little effect on phosphorus concentrations. This can probably be attributed to soluble phosphorus consumed by the aerobic bacteria consortium formed on rhizomes as an attached growth (Jian-Hang et al., 2013). It seems that the impacts of the substrate on system performance are more considerable in short detention times. In addition, it was observed that the variations in effluent quality (indicted here as standard deviations) for pilots inoculated by STDW were remarkably low. This shows a reliable performance by *Vetiver* in treating nutrients.

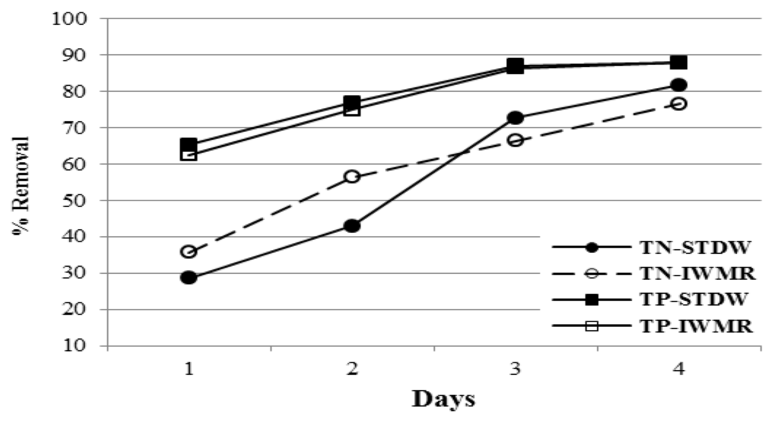

Fig. 2. Average nitrogen and phosphorus removal (%) in different HRTs

Consequently, the type of substrate may affect the performance of *Vetiver*, particularly with regard to TN removal. Similar results were achieved for *Water lettuce* and *Salvinia* (Lu, 2009). This may mainly be due to the fact that nutrient components are removed by different processes in phytoremediation. For example, ammonia, nitrite and organic nitrogen are initially oxidized to nitrate by rhizoremediation. Nitrate is then absorbed and extracted by plants. Therefore, the oxidation of the former may be completed in shorter detention times, while the extraction of the latter may take longer.

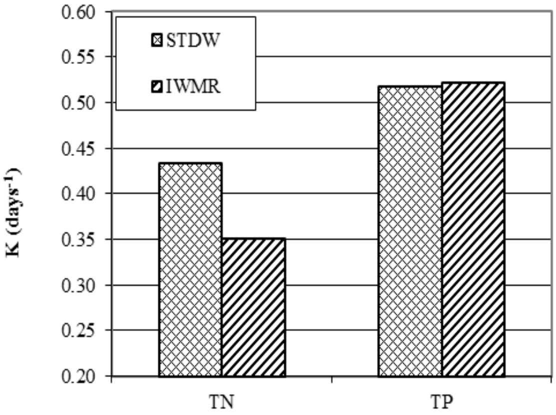

Fig. 3. Average nitrogen and phosphorus decay rates (day^{-1}) in different substrates

Based on equation 1, the total nitrogen decay rates (K_N) in STDW and IWMR were determined as 0.43 and 0.35 day^{-1}, respectively. This verifies that the nitrogen removal rate was much higher in more polluted waters. However, both values for total phosphorus decay rates (K_P) were relatively equal at around 0.52 day^{-1} (Fig. 3). It is noteworthy that the decay rates that were obtained are not far from the results of field studies in Singapore (Chua et al., 2012). These results may confirm the assumption that the *Vetiver* floating systems are able to inhibit algal blooms in surface waters.

Both substrates had low or even zero initial DO concentrations. STDW is the effluent of domestic wastewater treatment and scarcely exceeds 0.5 mg/L, while IWMR is, rather, an anoxic medium influenced by sediments and microbial consortia settled for long periods in Minab reservoir. In the experimental results, DO was increased to more than 7 mg/L for both systems. An increase in DO level by about 3 mg/L in the first 24 hours may prove the fact that plants have provided enough oxygen for the oxidation of organic components in rhizomes (Ye et al., 2012). This advantage in association with nutrient removal is introduced as an effective parameter for surface water purification or tertiary wastewater treatment. Recent studies have also implied these specifications (Chua et al., 2012; Krishnasamy et al., 2012).

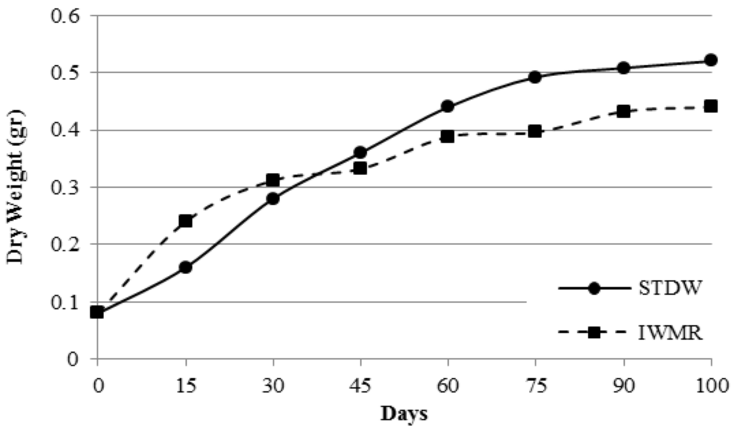

Fig. 4. Average dry weights of shoots during the study period, inoculated with different substrates

To verify the performance of *Vetiver* system in treating nutrients, the growth of leaves and roots was also compared. Based on statistical analysis, it can be concluded that growth of roots (P=0.077) in contrast to that of leaves (P=0.001) shows no significant difference between types of substrates. In other words, the leaves' growth may be influenced by the type of substrate, which was above about 23 cm in STDW. It displays a difference of about 0.08 gr for dry weight of leaves (Fig. 4). It is also implied that the leaves' growth remains continuous with STDW, while it shows a fluctuating trend with IWMR. This can be related to various nitrogen compounds that exist in STDW. In addition, it can be assumed that *Vetiver* is inclined to restore nutrients mainly in its leaves.

The uptake values of TN and TP in leaves and roots of five plants for two pilots are shown in Figure 5. It was observed that plants irrigated by STDW have more nutrient uptake in their shoots (P=0.001) compared to IWMR. This is less correlated in roots. Therefore, the type of substrate not only affects the leaves' growth, but also has an influence on their TN uptake rates. This can also be proved by regression analysis, in which a high linear correlation between TN uptake and growth rates was observed (R^2=0.86). Conversely, the TP uptake rates of leaves and roots irrigated by different substrates show no significant differences. The independency of TP uptake and its removal from the type of substrate is probably due to the fact that, on the first day, the soluble phosphate was reduced instantaneously as a matter of microbial uptake. Higher nutrient uptake for STDW may be verified, too, by the results of previous studies. For example, Jampeetong et al. (2012) observed that the tropical aquatic macrophytes, like *Vetiver zizanioides*, had higher growth rates and nitrogen uptake when inoculated by ammonia rather than nitrate.

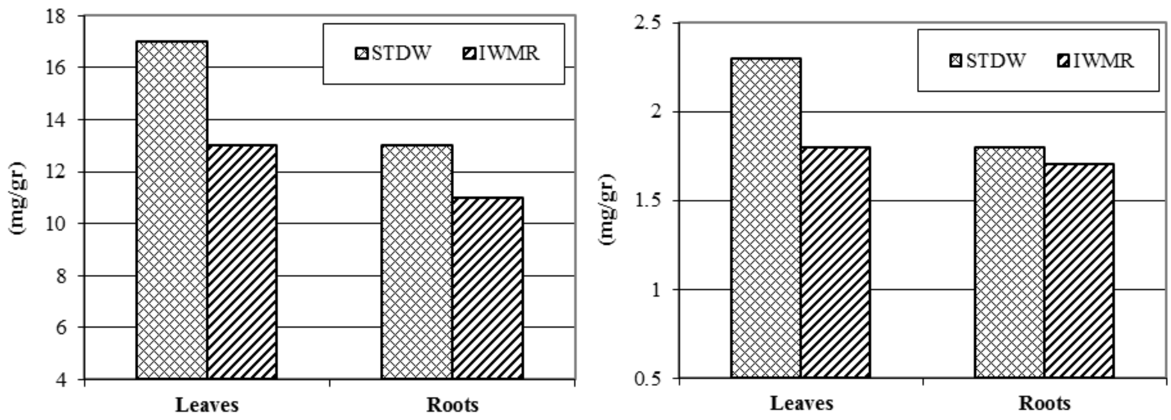

Fig. 5. The values of TN (left) and TP (right) absorbed into the leaves and roots at the end of study

It can be suggested that the *Vetiver* system in hydroponic conditions may be more effective as a hydroponic tertiary unit of wastewater treatment plants, than when used as a floating system for purifying surface water resources. As a result, a lower area (up to two days' detention time) would be required, while a higher removal efficiency of nutrients may be achieved through a combination of phytoextraction and rhizoremediation processes and very high oxidation rates. However, it can be recommended for both systems, especially because of its aeration capability. Consequently, it can also be introduced as an alternative solution for sustainable remediation of eutrophic conditions in surface waters.

CONCLUSION

This study aimed to assess and compare the performance of *Vetiveria zizanioides* in treating secondary treated domestic wastewater and contaminated surface water. Based on experimental results, it can be concluded that this method is capable of removing nutrients efficiently in two to four days' detention time. This approach can be recommended as an environmentally friendly solution for nutrient removal, and consequently for surface water quality improvement. The analyses of variance showed that the differences of system performance in TP removal and its uptake were not significant. Conversely, TN removal and its uptake were significantly different and correlated with the plant growth. This difference can be considered greater in shorter detention times. Consequently, the *Vetiver* system is mainly recommended as a tertiary wastewater treatment, either in hydroponic or subsurface constructed wetlands, rather than for floating on surface waters. It can also be applied as a treatment unit for non-point sources alongside rivers. These advantages would probably reduce the main causes of eutrophication and improve water quality.

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