DOI: 10.11598/btb.2023.30.2.1038

REMOVAL EFFICIENCY OF NITROGEN, PHOSPHORUS AND HEAVY METALS ASSOCIATED WITH SWINE WASTEWATER USING AQUATIC MACROPHYTES

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Received 23 March 2018 /Revised February 26, 2023 /Accepted 3 March 2023

ABSTRACT

Wastes from breeding farms have globally increased greenhouse gases and caused a serious pollution to aquatic environments. Biogas treatment polymer bags could significantly reduce organic compounds; however, they could not effectively treat other pollutants in animal wastewater. The objective of this study was to assess removal efficiency of salinity and pollutants associated with pig wastewater using aquatic macrophytes. Four macrophytes namely *Acrotichum aureum, Eleocharis dulcis, Typha domengensis,* and *Limnophyton obtusifolium* and a soil control without vegetation were randomly assigned into fifteen mesocosms (1.2 x 0.7 x 0.6m) with 3 replicates for each treatment. Pig wastewater was filled continuously into input chambers of mesocosms in every three day with 5 liters. Water samples were collected from output chambers with 60 and 120 days after treatment while soil and vegetation samples were collected at the beginning and the end of the experiment. The results showed that *E. dulcis, T. domengensis*, and *L. obtusifolium* were dominant in removal of N, P, Cu and Zn and suspended solids as well; *E. dulcis* and *T. domengensis* significantly increased the dissolved oxygen; whereas the treatment of *L. obtusifolium* species showed the best efficiency in salt-ion removal. Pollutants of N, P, Cu and Zn tend to accumulate more in the macrophyte roots than in their leaves. Accumulation of N, P, Cu and Zn in the *L. obtusifolium*'s biomass is the highest compared with other treatments. From findings, it is suggested that a combination of three aquatic macrophytes including *E. dulcis, T. domengensis*, and *L. obtusifolium* could establish a constructed wetland system to directly treat pollutants of livestock wastewaters

Keywords: Aquatic macrophytes, Nitrogen, Phosphorus, Salinity, Swine wastewater treatment

INTRODUCTION

Rapid growth of poultry and livestock sectors have caused seriously adverse effects on environmental quality and natural ecosystems and threatened human health (Mawdsley *et al.* 1995, Honda *et al.* 2000, Nguyen 2010). Wastes from poultry and livestock activities annually contribute to a large number of greenhouse gases which result in the global warming (FAO 2014). Throughout the Vietnam, there are more than 10 million different households and farms with approximately 27 million pigs that consume annually about 18 million tons of industrial feed (Nguyen 2009). The livestock sector discharges about 73 million tons of wastes per year, in which wastes of pig farms count for 33.4% (Xuan 2009). This could not only reducing the quality of products but also increasing pollutants and heavy metals in wastes. Major pollutants associating with the swine wastes include organic matters, nitrogen, phosphorus, copper, and zinc and other trace metals (Cang *et al.* 2004, Truong 2010, Dang *et al.* 2014).

According to the surveyed results about status of waste treatment of pig farms from eight different regions throughout Vietnam, Nguyen (2010) showed that 74% of pig farms have applied biogas plants to treat pig wastes while about 26% of the households and farms have been directly discharged the wastes into the environment. Although treatment polymer bags have effectively been removed organic carbon but they could not reduce pollution of nitrogen (N), phosphorus (P) and other trace metals. Nguyen & Pham (2012) who evaluated the treatment effectiveness of pig wastewater removal by biogas treatment polymer bags at a

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level of households showed that biogas plants could reduce up to 85% COD (Chemical Oxygen Demand) and 76% BOD_5 (Biological Oxygen Demand in five days) but only 12% and 7% for total nitrogen (TN) and total phosphorus (TP). Other study which assessed the wastewater treatment effectiveness by biogas plants at 12 pig farms in the provinces of Red river delta in the Northern part of Vietnam, Vu *et al.* (2008) pointed out that biogas reduced 65-70% COD, 75-81% BOD_5, and 10-28% TN. Meanwhile, biogas treatment polymer bags did not impact on reducing heavy metals in pig wastewater.

In recent decades, the use of constructed wetlands to treat different polluted sources has achieved a very high efficiency (Vymazal 2011, Shelef *et al.* 2013). Four major factors determining a successful application of constructed wetlands include type of the aquatic plants, retention time of wastewater staying on the treatment system, the depth of the mud, and the depth of water column (USEPA 2000). Cattail and *Eleocharis* species are two species of aquatic plants that have a wide distribution throughout the world and have a high performance in pollutant treatment (Marois *et al.* 2015, Mitsch *et al.* 2015). Particularly, *Eleocharis* species is recently used to remove N and P because of its high treatment efficiency in a short time (Pham 2013). Suhendrayatna *et al.* (2012) used cattails (*Typha sp*) and reed species (*Phragmites communis)* to treat municipal wastewater and showed that cattailspecies were more resilience to the wastewater and removed more BOD, COD and TSS (Total Suspended Solid) higher reed species. In the other research, Lam & Ngo (2013) pointed out that cattail species was capable of absorbing 0,17g N and 0,09g P/m^2/day in wastewater treatment of intensive catfish ponds with closed recirculation, whereas absortion ability of heavy metals was 456 - 1549 mg/kg (Fe) and 1.2 to 7.6 mg/kg (Pb) (Anning *et al.* 2013).

The main goal of this research was to identify some species of aquatic plants that peform a high efficiency in handling salinity and majority pollutants in pig wastewater. To achieve the research objectives, a series of examinations were conducted : (i) assessing capability to remove BOD, COD, N, P, Cu, Zn and salinity in pig wastewater between treatments, (ii) determining the ability to absorb and accumulate N, P, Cu and Zn in soil and biomass of plant species. Research results will provide a scientific basis for a suitable choice of aquatic plant species in handling organic pollutants, salinity and heavy metals for wastewater in general, particularly pig wastewater.

MATERIALS AND METHODS

Experimental Design

Four plant species consisting of *Typha domengensis, Eleocharis dulcis, Acrostichum aureum, Limnophyton obtusifolium* and one control without plant were assigned a completely random into 15 composite barrels with 1.2m (length) x 0.6m (width) x 0.7m (depth) with 3 replications for each treatment (Figure 1). A layer of soil was placed at the bottom of each barrel with a depth of 30cm. Plants were grown with an initial density of 6 plants/experimental barrels, exception to the control. Water level in each treatment was gradually raised to plant height after plantation, and water column-maintained depth of 30 cm from the mud surface by an effluent system with PVC Φ-34 pipes. The composite barrels were buried into the soil with a depth of 30 cm equal to the soil depth inside each barrel. Raw wastewater collected from pig farms was contained in a wastewater tank and filled into the barrels with frequency of every 3 days and 5 liters per time at an inlet zone of each treatment through the influent wastewater system as described in Figure 1. Based on the volume of water column, the surface area of barrel and the amount of wastewater entered (Adelaja *et al.* 2014; Dong *et al.* 2022), a retention time of wastewater stayed in each treatment system was estimated approximately 14 days.

Determination of Soil and Water Temperature

In order to examine if temperature affects efficacy of pollutant removal, HOBOWare device was used to measure temperature in water column and soil of treatments during experimental time. Fifteen HOBOWare gauges (Figure 2a) were deployed into the middle of soil layer and water column within each treatment and established to automatically recorded temperature in every 30 minutes. The gauges were mounted on PVC Φ-27 pipes and fasten by

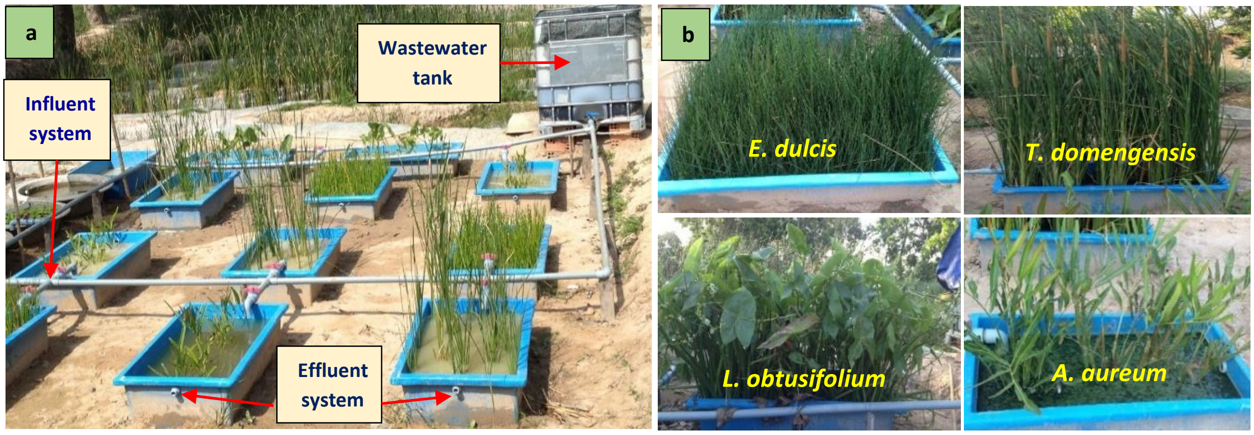

Figure 1 a) Experimental design with a treatment system; b) plant growth after 3-months handling

plastic tubes. Every two gauges were arranged on the same PVC pipe and aligned in the middle of water column and soil layer of each treatment.

Measurement of Salinity, DO, and pH

To measure salinity among experimental treatments, electrical conductivity (EC) was measured, and then be converted EC to salt concentration (Rhoades *et al.* 1999, NRW Service Centre 2007). Dissolved oxygen (DO), EC, and pH in effluent were measured at the outlet zone of each effluent system in every 2 weeks from 9:00 am to 10:00 am using YSI650 MDS machine (Figure 2b). YSI's readers were deployed into water column with a depth of 10cm at the outlet zone to measure DO, EC and pH. The readers were set up stabilizing in 60 seconds before recording and displaying results. To ensure QA/QC, the machine was calibrated in one hour before conducting the measurement and checked right after measurement by following the user manual's standard operating procedures (YSI Incorporated 2009).

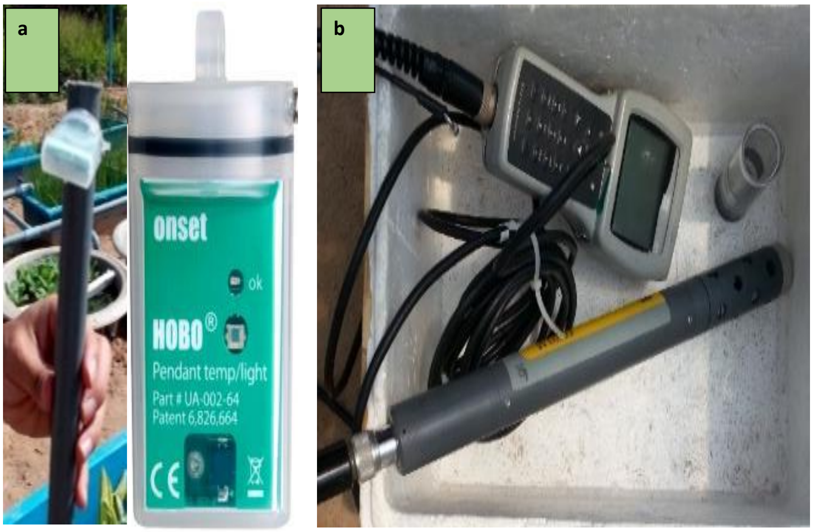

Figure 2 a) HoboWare; b) YSI650 MDS machine

Collection and Analysis of Effluent Samples

To evaluate effectiveness of pollutant removal among experimental treatments, samples of effluent water were collected after two and four months. Collection of effluent samples was conducted at the outlet zone of effluent system in the morning by using a mini pump and collected in one day before putting the wastewater. Effluent water was directly pumped into a 2-liter plastic bottle and kept inside an ice cooler before sending to NLU's Research Institute of Biotechnology and Environment on the same collection day for analyzing BOD_5, COD, N, P, Zn, and Cu. Methods applied to analyze for these components are presented in Table 1.

Table 1 Methods used to analyze samples

Sample type	Criteria	Analytical methods
Water	BOD_5	TCVN 6001:2008
	COD	SWEWW 5220C:2012
	TN	TCVN 6638:2008
	TP	TCVN 6020:2008
	Cu^{2+}, Zn^{2+}	ACIAR-AAS 007-2007
Soil	TN	TCVN 6498 – 1999
	TP	TCVN 8940 – 2011
	Cu, Zn	ACIAR-AAS 019-2007
Plant	TN	10TCN 451 – 2001
	TP	10TCN 453 – 2001
	Cu, Zn	ACIAR-AAS 019- 2007

Accumulation of Pollutants in Soil and Plant

Soil and vegetation samples were collected in one day before wastewater was initially filled into barrels corresponded with one month after planting, and at the end of experiment to determine contents of N, P, Cu and Zn in soil and plant biomass. Soil samples were taken at a depth of 5cm from soil surface by a soil core. In each treatment barrel, soil was randomly collected at three different positions, mixed them together, then placed it into a ziplock bag and kept it in an ice cooler before sending to laboratory for analysis. Similarly to soil sampling, plant sample was collected from three plants in three different locations of each barrel. The sample was distinctly divided into leaves and roots. These samples were cut into 2-cm pieces, arranged neatly in paper bags, and dried in oven at 65^oC for 7 days before sending for analysis. Analytical methods of N, P, Cu, and Zn applied for plant and soil are presented in Table 1.

An increasing accumulative rate of pollutants in biomass computed by the following equation:

$$H\ (\%) = 100 \times (C_{b\text{-final}} - C_{b\text{-initial}})/C_{b\text{-final}}$$

where:

$C_{b\text{-initial}}$ = concentration of pollutants in plant biomass (mg/kg) collected in one day before putting wastewater

$C_{b\text{-final}}$ = concentration of pollutants in plant biomass (mg/kg) collected in one day before putting wastewater at the end of the experiment.

N:P ratio = TN/TP, where concentrations of N and P accumulated in the plant biomass (mg/kg).

Statistics and ANOVA Analysis Method

This one-factor experiment was completely randomized factor with three replications for each treatment. Software of Minitab 17 was used for ANOVA analysis to evaluate significantly differences among treatments, and for Tukey's multiple comparision method ($\alpha = 0.05$). Charts and Figures were created by using the software of Sigma Plot 12.

RESULTS AND DISCUSSION

Concentrations of Influent Pollutants

Table 2 shows that concentrations of untreated pollutants in raw pig wastewater were very high. BOD_5 and COD were 2687.0 and 31516.0 mg O_2/L, whereas concentrations of total nitrogen (TN), total phosphorus (TP), Zn^{2+} and Cu^{2+} were 2207.0; 1335.0; 23.4; 15.4 mg/L respectively. Especially the pig wastewater contained a high electrical conductivity (EC) of 8.3 mS/cm, but a very low DO content of 1.8 mg O_2/L. Concentrations of these pollutants were much higher than those of previous studies (Chang & Liu 2002, Chung *et al.* 2004, Nguyen & Pham 2012). The reason for that was because wastewater samples were taken from tunnels of pigsty which directly received waste from pig manures and pigsty washing, and have not been decomposed with a condensed concentration. Based on concentration of the untreated pollutants in the raw wastewater, loading rate established for each treatment, and retention time of wastewater in each treatment, initial influent concentrations of COD, BOD_5, TN, TP, Zn and Cu were 806.1; 9455.0; 662.2; 400.6; 7.0; and 4.6 mg/L respectively.

Table 2 Concentration of influent pollutants

Parameter	Unit	Raw wastewater	Initial average influent*
pH	-	7.2	-
EC	mS/cm	8.3	-
DO	mg/l	1.8	-
BOD_5	mg/l	2687.0	806.1
COD	mg/l	31516.0	9455.0
TN	mg/l	2207.0	662.2
TP	mg/l	1335.0	400.6
Zn^{2+}	mg/l	23.4	7.0
Cu^{2+}	mg/l	15.4	4.6

* *Concentration estimated by basing on the raw wastewater concentration and retention time of the wastewater*

Temperature Variation in Soil and Water Column

Temperature is an important ecological factor and directly affects respiration of microorganisms in a treatment system. Variation of daily average temperature in soil and water among treatments were showed in Figure 3. In general, the temperature in soil and water fluctuated from 24 – 31°C. In the first stage of development, there was a temperature difference. There was also a temperature difference among treatments in soil due to young plants, but no difference of those in water. In the final growth period, there was no different temperatures among the treatments in both soil and water. Temperatures of *T. domengenis* and *L. obtusifolium* showed lower than those of other treatments. Variations of temperatures among treatments could impact on removal efficiency of pollutants (Ab-Halim *et al.* 2016; Alisawi, 2020; Shatat and Al-Najar, 2011).

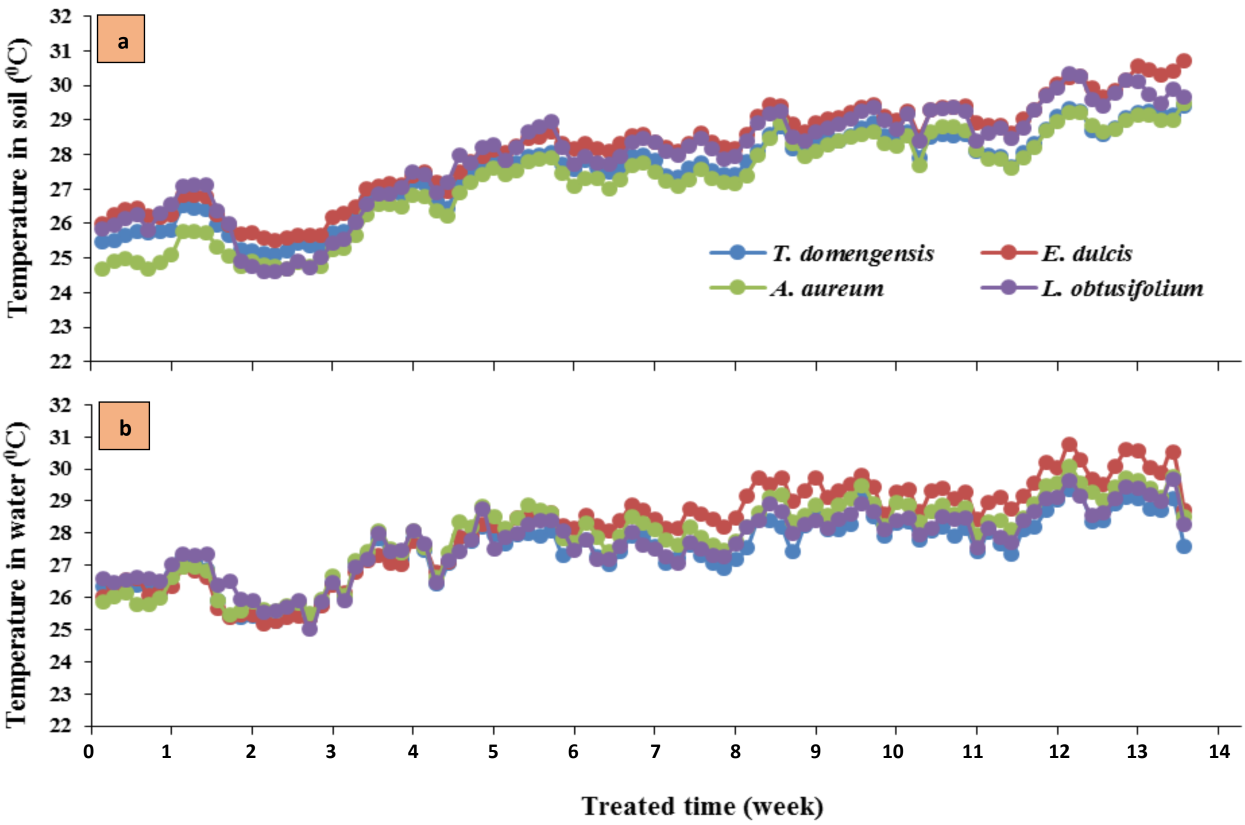

Figure 3 Temperature fluctuation of treatments following the treated time: a) soil; b) water

DO, EC and Removal Efficacy of Salt

Dissolved oxygen (DO) in water is dependent on photosynthesis processes of aquatic plants and a microbial respiration in water column and soil. In the respiration, microorganisms consume a large amount of DO to decompose organic compounds. An amount of DO used for the microbial respiration are often supplemented by photosynthesis of aquatic vegetation. The low level of dissolved oxygen in water is not only a sign of contamination and is but also an important factor in determining water quality and pollution (Bozorg-Haddad, 2021; Kulkarni, 2016). In freshwater ecosystems, most of aquatic animals require amount of dissolved oxygen higher than or equal to 3 mg O_2/L for survival. For example, fish cannot survive for long in water with dissolved oxygen less than 5 mg/L (Bozorg-Haddad, 2021). Table 2 shows that the pig wastewater contained a large amount of organic compounds with a low DO of 1.8 mgO_2/L. Althought DO concentrations of each treatment fluctuated in over the experimental periods, two treatments of *T. domengenis* and *E. dulcis* generally improved the DO (Figure 4). DO concentration of these two treatments was generally ranged from 5 to 9 mg O_2/L.

Most aquatic plants and animals require oxygen to survive; fish, for instance, cannot survive for long in water with dissolved oxygen less than 5 mg/L. The low level of dissolved oxygen in water is a sign of contamination and is an important factor in determining water quality, pollution control and treatment process. The DO in a saturated solution varies with the water temperature and elevation.

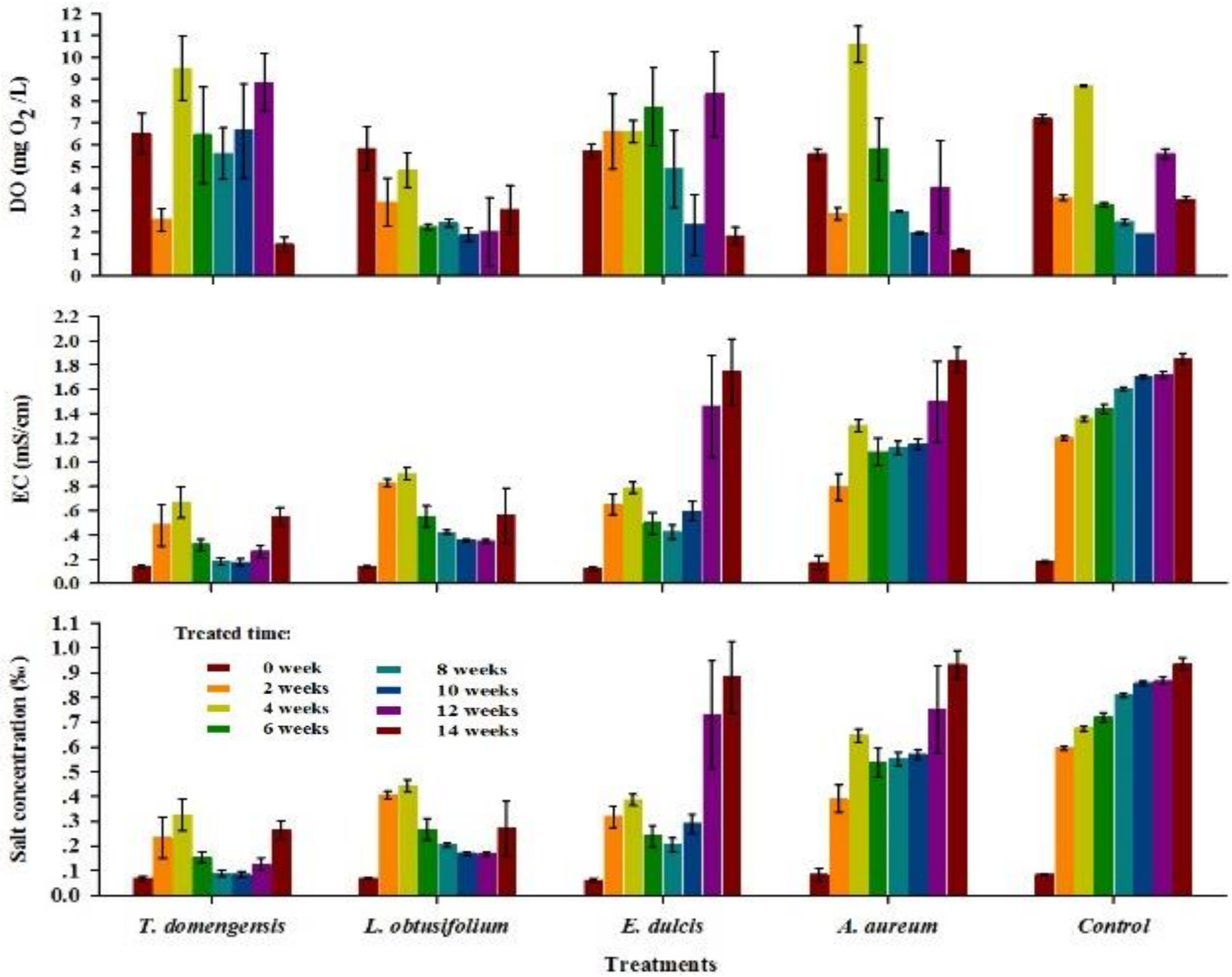

Figure 4 Changes of DO, EC and salt ion contents following the treated time among treatments

Electrical conductivity (EC) shows concentration of ions which exist in water is usually derived from the soluble salt ions such as Na^+, K^+, Cl^-, SO_4^{2-}. A high content of soluble ions would negatively influence and inhibit a growth of aquatic organisms. Salinity in fresh water normally ranges from 0.01 to 0.50‰. The pig wastewater contained a large amount of soluble salt ions with EC of 8.3 mS/cm (Table 2) which corresponded to salinity content of 4.6‰, ten times higher than the normal range of salinity in natural freshwater ecosystems. Due to no vegetation, EC in control treatment accumulatively increased following the treated time resulted in a gradual accumulation of the salt content during the experiment (Figure 4). Species of *T. domengenis, L. obtusifolium*, and *E. dulcis* performed a high absorption of salt ions which resulted in a very low EC and salinity. Salinity of these treatments was always lower than 0.50‰ over 14 weeks of the treated experiment (Figure 4). Exception for *E. dulcis*, since this species grew very quickly and had its shorter growth cycle than those of others, about 10 weeks, when this species died and returned its turnovers into water, and decomposed turnovers quickly (Marois *et al.* 2015, Mitsch *et al.* 2015). This resulted in an increasing of EC and salinity for this treatment in the last stage of the experiment.

Removal Efficacy of BOD_5, COD, N, P, Zn and Cu

As showed in Table 2, the pig wastewater contained a high concentration of organic matters with COD of 31,516 mg O_2/L and biochemical oxygen demand (BOD_5) of 2,687 mg O_2/L. Based on the input load, the retention time and the total volume of each treatment system, initial influent concentrations of COD and BOD_5 in all treatments were 9455.0 and 806.1 mg O_2/L respectively (Table 2). Sixty days after treated, three treatments of *L. obtusifolium, E. dulcis,* and *T. domengensis* removed a large amount of organic matters, and as a result, the COD contents of these treatments reduced to 55.5, 36.0, and 176.0 mg O_2/L respectively (Figure 5). But in 120 days after treated, the COD content of *L. obtusifolium* treatment continued dropping to 46 mg O_2/L, whereas the COD of *E. dulcis* treatment went up to 560 mg O_2/ L, which was explained by due to the 10-month growth cycle of *E. dulcis* species. Similar to effectiveness of COD handling, the treatments of *L. obtusifolium,* and *T. domengensis* showed the best efficacy of BOD_5.

Pig wastewater was polluted with high N and P, where the concentrations of TN and TP are respectively 2207.0 and 1335.0 mg/L, while their influent concentrations at each treatment were 662.0 mg/L (TN) and 400.6 mg/L (TP) (Table 2). Decomposition of organic pollutants by microorganisms associated with treatments of *L. obtusifolium, E. dulcis,* and *T. domengensis* reduced the BOD and COD. This resulted in decomposition and transformation of organic N and P compounds into inorganic compounds for plant uptakes. These three treatments showed the best performance of N and P removal after 60 days treated. The TN contents of *L. obtusifolium, E. dulcis,* and *T. domengensis* were respectively 2.4, 10, and 3.3 mg/L. But after treated in 120 days, TN contents of all treatments increased more than doubled in comparison with those at the treated time of 60 days. Similarly to total N, the content of total P (TP) was also removed significantly after 60 days and increased after 120 days, except for two treatments of *L. obtusifolium* and *T. domengensis* (Figure 5).

Cu and Zn ussualy supplements to food of livestocks, especially in pig foods, but pollution of Cu and Zn in pig wastewater is paid less attention. Concentrations of these two heavy metals in the pig wastewater are so high, approximately 23.4 and 15.4 mg/L for Cu and Zn. Based on input load and retention time on the treatment system, the initial average concentration of Cu and Zn were 4.6 and 7.0 mg/L (Figure 5). Removal capabilities of Cu and Zn are very different among treatments. The treatment of *L. obtusifolium* removed completely Cu and Zn due to plant uptake. As a consequence, concentrations of Cu and Zn in this treatment was nearly equal to zero (non detection) after 60 days treated. In addition to the *L. obtusifolium* treatment, the *A. aureum* also showed a higher efficiency of Cu and Zn removal than others.

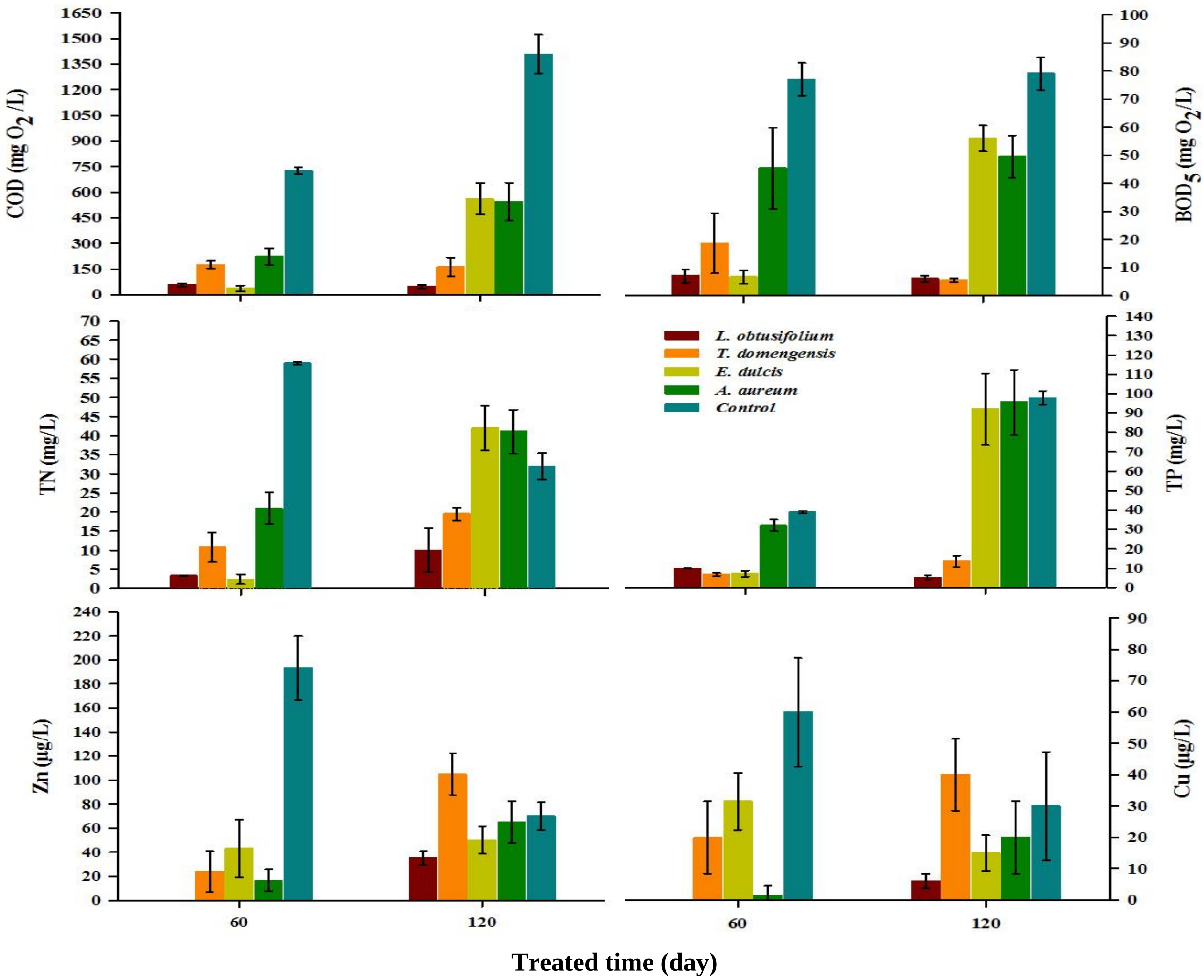

Figure 5 Changes of BOD_5, COD, N, P, Zn and Cu following the treated time among treatments

Accumulation of N, P, Zn, Cu in Soil

According to results of ANOVA analysis shown in Table 3, the initial N and P contents in soil were not different from the treatments ($p = 0.186$ for TN, $p = 0.669$ for TP). Concentration of initial soil TN ranged from 284 to 323 mg N/kg dried soil, whereas the initial TP concentration in soil ranged from 62 to 67 mg P/kg dried soil. Baseline concentrations of TN and TP used for the experiment were very low. However, both N and P concentrations in the soil were increased for all treatments. Due to without vegetation, the control treatment accumulated the lower than those of three other treatments because of its ability of low N transformation and decomposition in water, while the treatment of *L. obtusifolium* had a the highest accumulation of TN and TP contents in soil with 504 mg N/kg and 210 mg P/kg, respectively. This result were corresponded with the lowest TN and TP concentrations of *L. obtusifolium* treatment in water. Initially baseline Cu and Zn concentrations in the soil utilized for the experiment were very low and did not differ among treatments with the Cu concentration under non-detection zero and Zn concentration ranged from 3.1 to 3.9 mg/kg ($p = 0.822$) (Table 3). At the end of the experiment, although accumulation of Cu content in soil increased in all treatments, approximately 6 mg Cu/kg, there was no significant difference ($p = 0.157$). In contrast to Cu accumulation in soil, Zn concentrations were significantly different from the treatments ($p < 0.000$). The treatment of *L. obtusifolium* showed the highest Zn accumulation with 11.4 mg Zn/kg. This is resulted from the high Zn removal efficiency of *L. obtusifolium* treatment in water which assisted by organic matter breakdown associated with microorganisms in this treatment.

Table 3 Accumulation of N, P, Cu and Zn in soil/sediment

Parameter	Treated time (day)	Concentration of treatments (mg/kg dry soil weight) (Mean ± SE)				
		T. domengensis	*E. dulcis*	*A. aureum*	*L. obtusifolium*	Control
TN	0	313 ± 22	301 ± 5	323 ± 3	284 ± 31	304 ± 15
	120	467 ± 21	409 ± 13	386 ± 48	504 ± 9	636 ± 16
TP	0	65 ± 8	63 ± 2	67 ± 1	62 ± 1	63 ± 5
	120	164 ± 8	123 ± 24	140 ± 2	210 ± 19	267 ± 8
Cu	0	nd	nd	nd	nd	nd
	120	6.0 ± 0.5	6.0 ± 1.0	6.2 ± 0.7	6.1 ± 0.8	7.4 ± 0.5
Zn	0	3.8 ± 1.9	3.9 ± 0.9	3.3 ± 0.2	3.1 ± 0.3	3.4 ± 0.5
	120	5.2 ± 2.2	4.1 ± 0.8	4.3 ± 1.0	11.4 ± 2.6	14.6 ± 1.4

nd: none detection

Contents of N, P, Cu and Zn in Plant Biomass

Since the initial N baseline content in foliages and roots are significantly different among treatments (Table 4). The treatment of *L. obtusifolium* contained the highest N concentrations in root (0.97% ± 0.15) and foliage (1.90% ± 0.10). This difference could be explained that because in first month after planting (not input the wastewater to treatments), the *L. obtusifolium* species grew faster than others treatments resulting in higher accumulation of N content in its root and foliage. This corresponded with the lowest initial N content of *L. obtusifolium* treatment in the soil (Table 3). After treated in 120 days, root and foliage N concentrations of all treatments significantly increased in comparison with their initial baseline concentrations (Table 4). The increasing percentage of N absorption rate by vegetation treatments within root and foliage were presented in Figure 6. All treatments showed a high N absorption rate in roots with greater than 50%, but there was no a significant difference of the rate among the treatments. The highest N absorption rate in roots reached 67% for the *A. aureum* treatment while the lowest N absorption rate was 50% for the *L. obtusifolium* treatment. On the other hand, the *L. obtusifolium* treatment showed the highest foliage N absorption with the 28.9%, followed by the treatment of *E. dulcis* (19.6%), from which the rates of two these treatments were significantly different from two other remaining treatments. If combined with the root and foliage absorbed N contents, the treatment of *L. obtusifolium* performed the highest capacity to accumulate nitrogen in its biomass. Generally, under a normal condition, all treatments accumulated lower N in roots than in foliages, but with the addition of N increasing amount from pig wastewater, N tended to be accumulated more in roots than in foliage in order to create a balance of N content in their biomass.

Table 4 Concentrations of N, P, Cu and Zn in plant biomass

Treatment	Bio-mass type	One day before treated				120 days after treated			
		TN (%)	TP (%)	Zn (mg/kg)	Cu (mg/kg)	TN (%)	TP (%)	Zn (mg/kg)	Cu (mg/kg)
T. domengensis	**Root**	0.75 ± 0.05	0.182 ± 0.005	77.1 ± 20.4	19.9 ± 4.7	2.27 ± 0.30	0.907 ± 0.113	154 ± 15.0	19.5 ± 10.5
	Leaf	1.65 ± 0.09	0.240 ± 0.025	nd	nd	1.71 ± 0.02	0.263 ± 0.015	nd	nd
E. dulcis	**Root**	0.55 ± 0.05	0.254 ± 0.048	19.5 ± 5.0	13.7 ± 7.9	1.54 ± 0.24	0.607 ± 0.014	93.5 ± 13.5	26.0 ± 1.0
	Leaf	1.25 ± 0.15	0.314 ± 0.003	nd	nd	1.59 ± 0.41	0.477 ± 0.021	nd	nd
A. aureum	**Root**	0.95 ± 0.05	0.213 ± 0.061	32.1 ± 13.5	26.8 ± 11.4	2.09 ± 0.17	0.689 ± 0.031	45.0 ± 17.0	34.0 ± 16.6
	Leaf	1.85 ± 0.06	0.105 ± 0.005	nd	nd	1.97 ± 0.18	0.272 ± 0.037	nd	nd
L. obtusifolium	**Root**	0.97 ± 0.15	0.329 ± 0.004	73.2 ± 1.2	5.3 ± 0.4	2.00 ± 0.26	1.078 ± 0.017	154 ± 43.8	80.0 ± 6.0
	Leaf	1.90 ± 0.10	0.339 ± 0.094	nd	nd	2.68 ± 0.17	0.486 ± 0.017	nd	nd

nd: none detection

Similar to N status accumulated in biomass, the initial root and foliage P concentrations were very different among treatments. The treatment of *L. obtusifolium* always performed the best ability to uptake P into its biomass, from which the initial P baseline concentration were 0.329% ± 0.004 in root and 0.339% ± 0.094 in foliage and that the P concentration accumulated in its roots and foliage after 120 days were respectively 1.078% ± 0.017 and 0.486% ± 0.017 (Table 4). In contrast to N absorption results, phosphorus mainly accumulated in roots of treatment's plants after the 4-month handling period. If it takes into account of absorbed P rate in biomass, the treatment of *T. domengensis* showed the best performance of P absorption rate in its biomass, about 68% in root and 62% in foliage (Figure 6).

The N/P ratio in plant biomass is an ecological indicator, which is used to assess a level of excess or deficiency of N or P content provided for growth of trees in different ecosystems. N/P ratio of 14-15 reflects a balance between N and P contents in an ecosystem. The N/P ratios in biomass of all treatments in this study were rather low, approximately 3 in root and 8 in foliage for treatments (Figure 6). These results indicated that the pig wastewater was more N polluted than P in a term of N/P ratio for plant uptake.

According to the analyzed results presented in Table 4, both Cu and Zn were only accumulated in roots of treatments. Cu and Zn concentrations in foliage were very low and even under detectable in some treatments. At the same rate of initial Zn and Cu influent loading, two treatments of *T. domengensis* and *L. obtusifolium* accumulated the highest Zn content in their roots, 154.0 ± 15.0 and 154.3 mg/kg ± 43.8 respectively (Table 4), where only the treatment of *L. obtusifolium* showed the highest Cu absorption in its roots with Cu concentration of 80.0 mg/kg ± 6.

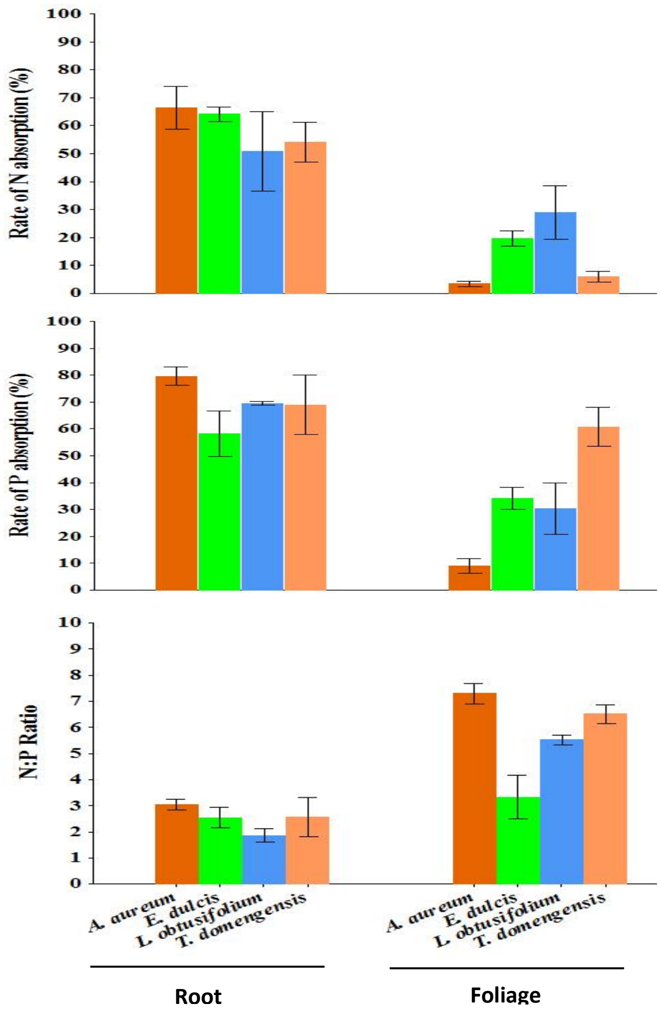

Figure 6 N and P absorption rate and N:P ratio in plant biomass among treatments

CONCLUSION

Three species of *T. domengenis, L. obtusifolium*, and *E. dulcis* performed a high removal efficacy of pollutants accociated with the pig wastewater including salinity, COD, BOD, N, P, Cu and Zn. With its ecological physiology and a fast growing period, the *E. dulcis* species could quickly remove the amounts of BOD and COD. Among these three species, the species of *L. obtusifolium* has most efficiently treated salinity, N, P, and Cu and Zn. The amount of N, P, Cu and Zn tended to be absorped more in foliage than in root. *L. obtusifolium* species was capable of absorbing more Cu and Zn metals than other species. Additionally the treatment of *L. obtusifolium* performed the highest accumulation of N, P and Zn in soils. This could result from the existence of specific microorganisms associated with the root zones of *L. obtusifolium* species having the ability to improve the efficiency of N, P and Zn removal.

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