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The growth of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) and water lettuce (*Pistia stratiotes* L.) in domestic wastewater in wastewater treatment plant (WWTP) bojongsong, Bandung, Indonesia

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Abstract

Domestic wastewater containing ammonia and phosphate are the main pollutants of toxin. Its affects the growth of aquatic plants, including of *Eichhornia crassipes* and *Pistia stratiotes*. The plants were placed outlet in anaerob, facultative, and maturation ponds in Wastewater Treatment Plant (WWTP) Bojongsong, Bandung. Each of them consists of 9 individuals, during 14 days, from July to November 2012, with 6 replicates. The results was showed the growth of *E. crassipes* higher than *P. stratiotes*, in anaerobic and facultative ponds. The growth all part are increasing, except the root length. On *E. crassipes*, the range of growth of wet weight of 99.39–99.49 g, stolon length of 13.28–15.97 cm, leaf area of 1.12–8.56 cm² and number of petiole of 17.58–18.84, relative growth rate (RGR) of 0.069–0.072 g/day, and doubling time (DT) of 9.7–10.5 day, and its sequentially on *P. stratiotes* 14.09–25.58, 4.58–6.69 cm, 1.50–2.43 cm², 10.73–18.78, 0.044–0.073 g/day, and 10.0–17.0 day. Most of the highest values its found in the anaerobic pond, that is change of total chlorophyll which increased on *E. crassipes* (96.31 mg/L), but decreased in *P. stratiotes* (-6.03 mg/L).

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Introduction

Domestic wastewater is wastewater mostly from human feces and urine, along with the water used to flush toilets and garbage generated from waste washing, laundry, food preparation and cleaning of kitchen tools (Gray, 2004; Bitton, 2005). Its was increased every year. The composition of domestic wastewater 99.9% water and 0.1% solids consist of 70% organic material composed of 65% protein, 25% carbohydrate and 10% fat; and 30% inorganic material consist of sand or gravel, and metal salts (Tebbutt, 1998). Furthermore, intestinal bacteria was founded, such as *Escherichia coli*, and other organisms, such as Protozoa and Helminthes. Its generally harmful and cause disease in humans (Mara, 2003).

Eichhornia crassipes and *Pistia stratiotes* are floating macrophyte waters. Their lives dependent on environmental conditions. The characteristic of both are most adaptive to the polluted environment and high pollution levels (Haryanti *et al.*, 2004); they are also growth rate, biomass, and high capacity to absorb pollutants and accumulate high nutrients such as nitrate and phosphate (Sood *et al.*, 2012). The ratio of root and stem inversely related to nutrition, especially relating to the availability of nitrogen in water (Coetzee *et al.*, 2009). However, if their growth was not controlled, it can become invasive (Julien *et al.*, 1996).

The growth of *E. crassipes* and *P. stratiotes* have variations during the life in the domestic wastewater. It was influenced by the physical and chemical characteristics of domestic wastewater to the anatomy and physiology (9) (Turner, 1981; Kramer, 1983). Various organic compounds, ions and small molecules dissolved give important effects for plants to survive (Fitter and Hay, 2002). The concentration of chlorophyll in the leaves is usually an indicator of nutrient stress, photosynthetic capacity and stage of development (Curran *et al.*, 1990), such as limited nitrogen (Everitt *et al.*, 1985) can lead the chlorophyll levels to burst and inhibit the growth. Relative

Growth Rate (RGR) and Doubling Time (DT) plants increase along with nitrate and phosphate level in wastewater (Mufarrege *et al.*, 2010).

This research aims to examine the growth of *E. crassipes* and *P. stratiotes* in domestic wastewater. The parameters are including changes the parts of plant, relative growth rate (RGR), doubling time (DT) and leaf chlorophyll content.

Materials and methods

Experimental set up

The weight of *E. crassipes* between 40-70 g with 6 sheet of leaf and *P. stratiotes* 10-25 g with 5 sheet of leaf, obtained from Cibiru, Bandung. Both were acclimatized for 3-5 days in freshwater and stored at floatation device measuring 120 cm x 80 cm, consist of 9 individuals. All material are placed on the outlet an anaerobic, facultative and maturation ponds in Wastewater Treatment Plant (WWTP) Bojongsoang, Bandung, for 14 days.

Observation parameter

The growth was observed including changes in wet weight, root length, stolon length, length width and large leaf, number and length petiole. RGR was used to measure plant growth rate (Taiz and Zeiger, 2010). RGR was calculated using the formula described by Jackson (1980) (15): $RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$, where: w = dry or wet weight (g), t = time of observation (days), 1 = initial (day 0), 2 = end (day 14). DT was the timed dry or wet weight of the organism increased by two times from original weight. DT was determined by using the formula described by (Mitchell (1974) (16): $DT = \ln 2 / RGR$.

Leaf chlorophyll content was calculated by using the method of Arnon (1949) with 96% alcohol solvent and a spectrophotometer (Hasch) at a wavelength of 649 and 665 nm. Chlorophyll content was calculated based on the measurement of absorbance of light on leaf extracts using the equation: Chlorophyll-a = $13,7 \lambda_{665} - 5,76 \lambda_{649}$, Chlorophyll-b = $16 \lambda_{649} - 7,7 \lambda_{665}$, and the Total Chlorophyll = $20,0 \lambda_{649} + 6,1 \lambda_{665}$.

Data analysis

Analysis of data was used analysis of variance (Anova) and then continued by Duncan Mean Range Test (DMRT).

Results

The growth of E. crassipes and P. stratiotes

Base the results of Anova and DMRT, most growth parameters observed were included of wet weight, root length, stolon length, leaf length, leaf width, leaf area, number and length of petiole of *E. crassipes* and *P. stratiotes* significantly. Domestic wastewater ponds shows significance to wet weight, stolon length, leaf area, and length petiole (Table 1).

Changes in wet weight, stolon length, number and length of petiole of *E. crassipes* showed was increased in size, but the root length, length and leaf area was showed a decrease in size. The increasing size of *E. crassipes* was showed a narrow range occurs wet weight of 99.39 to 99.43 g, root length of 0.05 to 1.49

cm, leaf length of 0.07 to 0.68 cm, leaf width of 0.06 to 1.27 cm, number of petiole of 17.58 to 18.15 and petiole length of 2.05 to 3.21 cm, and showed a wide range stolon length of 11.33 to 15.97 cm and leaf area of 1.12 to 8.56 cm². The highest measure changes in an anaerobic pond stolon length of 15.97 cm, leaf width of 1.27 cm, leaf large of 8.56 cm², and petiole length of 3.21 cm; facultative pond wet weight of 99.49 g and number of petiole of 18.84; and maturation pond root length of 0.49 cm and length leaves of 0.68 cm. The growth in wet weight, stolon length, length and large leaf and number of petiole *P. stratiotes* was showed an increase, but the root length and leaves was showed a decrease. The increasing size of *P. stratiotes* was showed a narrow range occurs (-) 5.21 to (-)5.97 cm of root length, 0.44 to 1.15 cm of leaf length, 0.03 to 0.10 cm of leaf width, 1.50 to 1.62 cm² of wide leaves, and occurs in a wide range 14.09 to 25.58 g of wet weight, 5.10 to 6.69 cm of stolon length, number of petiole of 10.73 to 18.78.

Table 1. Changes in the size of the plant parts *E. crassipes* and *P. stratiotes* in domestic wastewater for 14 days.

Domestic wastewater	Wet weighth		Root length		Stolon length		Leaf length		Leaf width		Leaf Area		Number of petiole		Length of petiole
	(g)	(g)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm ²)	(cm ²)	(cm)	(cm)	(cm)	(cm)
	EC	PS	EC	PS	EC	PS	EC	PS	EC	PS	EC	PS	EC	PS	EC
Control	7.73 ^a	3.82 ^a	0.46 ^b	-1.77 ^a	0.67 ^a	3.06 ^a	-1.79 ^a	1.09 ^c	-0.25 ^{bc}	0.42 ^b	-2.32 ^a	6.64 ^b	0.71 ^a	4.73 ^a	2.12 ^a
Anaerobic	99.39 ^c	17.10 ^b	-1.49 ^a	-5.97 ^b	15.97 ^c	4.58 ^b	-0.19 ^a	1.15 ^{ab}	1.27 ^c	0.10 ^a	8.56 ^a	1.62 ^a	18.15 ^b	10.73 ^b	3.21 ^c
Facultative	99.49 ^b	25.58 ^c	-0.05 ^a	-5.78 ^b	13.28 ^b	6.69 ^d	0.07 ^a	0.90 ^{bc}	0.06 ^a	0.18 ^a	1.12 ^a	2.43 ^a	18.84 ^b	18.78 ^c	3.06 ^a
Maturation	93.43 ^{bc}	14.09 ^b	0.49 ^b	-5.21 ^b	11.33 ^b	5.10 ^c	0.68 ^a	0.44 ^a	-0.36 ^{bc}	0.03 ^a	4.80 ^a	1.50 ^a	17.58 ^b	17.57 ^c	2.05 ^{ab}

Notes:

Number followed by the same letter in a column in the table shows no significant using DMRT at the significance level (α) = 0.05 with n = 9. Positive sign (+): change increases; negative sign (-): decreased changes.

EC=*E. crassipes*; PS=*P. stratiotes*

RGR and DT E. crassipes and P. stratiotes

RGR of *E. crassipes* were in a narrow range, the highest one is 0.072 g/days in the anaerobic pond, and the same is 0.069 g/days in facultative and maturation ponds. The highest RGR of *P. stratiotes* is

0.073 g/days in the facultative pond and the lowest one is 0.044 g/days in the anaerobic pond. Physical and chemical characteristics which include turbidity, TSS, ammonia, BOD, NH₃, NO₃ and PO₄ and the temperature and pH of the wastewater do not inhibit

the growth of *E. crassipes* but inhibits the growth of *P. stratiotes*. That Inhibition is in line with the research on *Egeria densa* by Pistori *et al.* (2004).

The DT of *E. crassipes* within a narrow range 9.7 days in an anaerobic, 10.3 days in facultative pond, and 10.5 days in the maturation pond. The DT of *P. stratiotes* in the width range are 17.0 days in an anaerobic pond, 10.0 days in facultative pond and 14.7 days in the maturation pond. The DT of *E. crassipes* is in line with the research of Lindsey and Hirt (1999), which shows that in a period of growth, biomass and RGR of *E. crassipes* can change doubled on day 6 to day 15. But the DT values of *P. stratiotes* in an anaerobic (17.0 days) exceeds the values of research Lindsey and Hirt (1999). DT *E. crassipes* and *P. stratiotes* controls showed a negative value as in fresh water without nitrates and phosphates which are both nutrients.

The Chlorophyll of E. crassipes and P. stratiotes

Changes in leaf chlorophyll content of *E. crassipes* in each pool mostly have decreased on day 14 (Table 2). Increased chlorophyll content of *E. crassipes* occurs in chlorophyll-a 224.19 mg/L and the total chlorophyll is 96.31 mg/L in the anaerobic pond, and chlorophyll-b is 699.89 mg/L in the maturation pond. Similar to *P. stratiotes*, the chlorophyll content has generally decreased, except for chlorophyll-b that is 0.141 mg/L in the facultative pond. The Chlorophyll content of *E. crassipes* and *P. stratiotes* are in line with the color of the leaves in each pool. Leaf color in anaerobic and facultative ponds is green, and yellow it in the maturation pond. The decrease in chlorophyll content of *E. crassipes* shows higher than in *P. stratiotes*. The highest value of *E. crassipes* is in an anaerobic and for *P. stratiotes* is in facultative pond.

Table 2. Change content of chlorophyll of *E. crassipes* and *P. stratiotes* on domestic wastewater during 14 days.

Domestic wastewater	<i>Eichhornia crassipes</i>			<i>Pistia stratiotes</i>		
	Chlo-a (mg/L)	Chlo-b (mg/L)	Chlo-Total (mg/L)	Chlo-a (mg/L)	Chlo-b (mg/L)	Chlo-Total (mg/L)
Control	3392.53 ^b	-1907.86 ^a	1509.43 ^b	-1.382 ^b	-0.313 ^a	-1.705 ^b
Anaerobic	224.19 ^{ab}	-129.51 ^{ab}	96.31 ^{ab}	-2.571 ^b	-1.493 ^a	-4.083 ^{a^b}
Facultative	-0.19 ^{ab}	-0.64 ^{ab}	-0.83 ^{ab}	-1.289 ^b	0.141 ^a	-1.158 ^b
Maturation	-1250.33 ^a	699.89 ^b	-559.57 ^a	-5.169 ^a	-1.102 ^a	-6.309 ^a

Notes:

A number that is followed by the same letter in a column in the table shows no significant use DMRT on $\alpha=0.05$ with $n=9$. Positive (+): changes increased; negative (-): changes decreases.

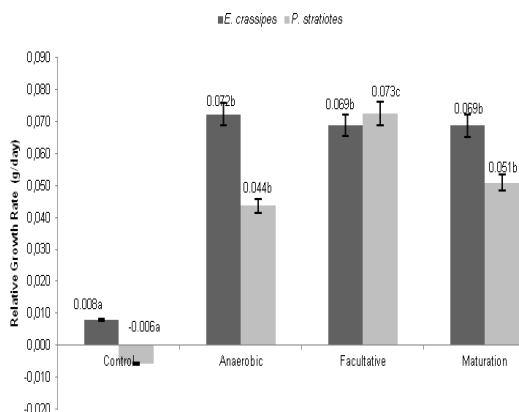


Fig. 1. Relative Growth Rate (RGR) *E. crassipes* and *P. stratiotes* on domestic wastewater during 14 days.

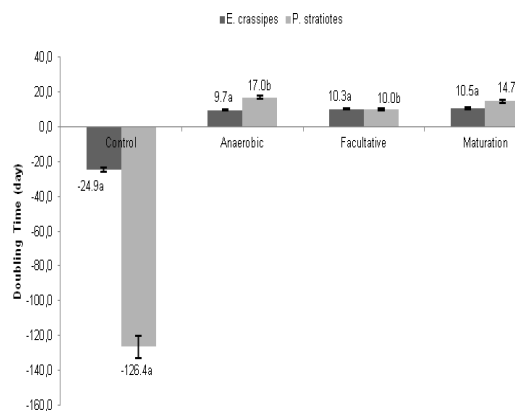


Fig. 2. Doubling Time (DT) *E. crassipes* and *P. stratiotes* on domestic wastewater during 14 days.

Discussion

The growth of E. crassipes and P. stratiotes

The stolon as the vegetative reproduction of *E. crassipes* and *P. stratiotes* more dominant than flowers as the generative reproduction. Ammonia, nitrate and phosphate higher in domestic wastewater caused them stress. These conditions of both survive in two ways, first, stolon elongation in order to reach the sources of nutrients, second, to widen the leaves for photosynthesis boost.

The smallest and the largest changes in the growth of *E. crassipes* and *P. stratiotes* respectively are in the facultative, anaerobic and maturation ponds. This is consistent with the values of turbidity, TSS, BOD, nitrate and phosphate in domestic wastewater from the largest to the smallest, namely (Mangunwardoyo *et al.*, 2013). These five environmental factors trigger the growth of their. Mara (2003) explains that the turbidity in domestic wastewater derived from decomposition of organic particles by bacteria. Nitrate in domestic waste results from the conversion of ammonia to nitrite by *Nitrosomonas*, and nitrite to nitrate by *Nitrobacter*. Phosphate compounds are the result of decomposition of various rocks and human processed products, such as detergents and chemicals that are added to food.

Nitrogen is the key element in domestic wastewater originating from feces and urine, and phosphorus is derived from other ingredients that are in it, including detergents (Mara, 2003). Nitrogen and phosphorus are the macronutrient needs of the growth and development of plants (Sacala *et al.*, 2008). Nitrogen generally affects the production of biomass, growth, enzyme activity and pigment content (Arruda and Azevedo, 2009). Nitrogen absorbed by the root system of *E. crassipes* and *P. stratiotes* occur through domestic wastewater solution. The concentration of $\text{NO}_3\text{-N}$ in the environment affects the level of absorption by roots (Marschner, 1995) and its relationship with enzyme nitrate activity of reduction in roots and stems (Cedergreen and Madsen, 2003). Nitrogen will enter

the cell metabolism of *E. crassipes* and *P. stratiotes* and subsequently is used as a source of energy and protein (Taiz and Zeiger, 2010). Therefore, growth in the domestic wastewater in an anaerobic facultative is higher than in the maturation pond.

The Growth of *E. crassipes* and *P. stratiotes* have improved in all observed parts, except for root length and leaf width. The length of the roots of *E. crassipes* and *P. stratiotes* for 14 days living in domestic wastewater was showed negative changes or reduced. This is due to the nitrogen in the form of ammonia and nitrates in domestic wastewater. Its levels from the smallest value to the largest one of *E. crassipes* is in a sequence in facultative pond, anaerobic and maturation, and while the growth of *P. stratiotes* are in maturation pond, facultative and anaerobic. The greatest depreciation of root length of *E. crassipes* and *P. stratiotes* occurs in anaerobic pond. Nitrogen levels in the pond water causes the water to be acid (the pH less than 6). This value becomes the limiting factor in the growth of the roots of *E. crassipes* and *P. stratiotes*. The Nitrogen content and pH of domestic wastewater in a reduction anaerobic nitrate inhibit the enzyme activity in protein metabolism (27) (Cedergreen and Madsen, 2003). Consequently, there is decreases protein synthesis and the size of cell becomes smaller or the cell division is fail, and although so an increase in wet weight decreased (Qaisar, 2005; Taiz and Zeiger, 2010). The relationship was showed a state of high nutrients in the environment affect to the growth of aquatic plants (Greger *et al.*, 1991).

Reduction of nitrate was affected by the concentration of nitrate in the environment and root absorption rate (Cedergreen and Madsen, 2003). If there is low nitrate concentrations in the environment, nitrate will be reduced in the roots, and if there is high nitrate concentrations in the environment, nitrate will be reduced to the root and the increased activity in the stem (Samuelson *et al.*, 1995). The rate of nitrate uptake is slow, the growth is slow due to lack of

nitrate in roots which is not sufficient to meet the metabolic needs throughout the cell.

If there is rapid absorption of nitrate levels, it was reduced so that the excess in the root (Goujon *et al.*, 1994). In addition, the hormone auxin also affects root growth through metabolism in the cell walls of root cells (Moore, 1989). The concentration of nitrate in facultative and maturation ponds is higher than in the anaerobic pond. The rate of nitrate uptake by *E. crassipes* higher than by *P. stratiotes*, so that the growth of *E. crassipes* is better than of *P. stratiotes* in anaerobic pond.

Parts of *E. crassipes* showed the highest growth changes in an anaerobic including the stolon length and width of leaves, in the facultative pond is wet weight, number and length petiole, and in the maturation pond is root length (lowest decrease) and leaf length. In *P. stratiotes* was showed the highest growth change in an anaerobic including the stolon length, leaf length and width; in the facultative pond is wet weight and number of petiole; and in the maturation pond is root length. These results demonstrate the ability of *E. crassipes* and *P. stratiotes* survive and respond to various of environmental pollutants (Vitória *et al.*, 2010). But both can sometimes growth and thrive in conditions of stress without showing symptoms of poisoning (Pinto *et al.*, 2008). In addition, seasonal differences are influenced by the variability of solar radiation and precipitation, as well as the efficiency of absorption of nutrients which affect the growth of aquatic plants (Redy and Sutton, 1984; Lu *et al.*, 2010), including *E. crassipes* and *P. stratiotes*.

RGR and DT *E. crassipes* and *P. stratiotes*

The RGR and DT of *E. crassipes* and *P. stratiotes* have the relationship with changes in biomass or wet weight. The RGR dan DT of *E. crassipes* is in line to the changes in the wet weight, the highest one is in the facultative pond and the lowest one is in the anaerobic pond. In *P. stratiotes*, the RGR values vary. It is related to different levels of nitrate and

phosphate in domestic wastewater in an anaerobic, facultative and maturation ponds. The RGR and DT was increased with levels of nitrate and phosphate (Mufarrege *et al.*, 2010) in an anaerobic, facultative and maturation ponds. Increased population density of aquatic plants can also be a cause of reduced growth (Henry-Silva *et al.*, 2002). This is related to the opening of space and population as well as parts of the plant to reach the source of nitrate which absorbed (Silvertown and Doust, 1993). *Eichhornia crassipes* and *Pistia stratiotes* in an anaerobic, facultative and maturation, the availability of nitrate is generally evenly distributed in each pool so that RGR and DT are in a narrow range.

The chlorophyll of *E. crassipes* and *P. stratiotes*

Decreased chlorophyll content in the leaves of *E. crassipes* and *P. stratiotes* in facultative and maturation ponds is caused by higher nitrate and phosphate levels than in the anaerobic pond. The High level of nitrate and phosphate can be a limiting factor in the formation of chlorophyll in plant cells. This results shows reduced chlorophyll content compared with normal and chlorate leaves. High nitrate levels causes the environment become acidic and inhibits the activity of the reduction enzyme (NR) and phosphates enzymes which play a role in photosynthesis and protein metabolism (Taiz and Zeiger, 2010).

Nitrogen, particularly from nitrates, is necessary for the growth and survival of plants (Gerendas *et al.*, 1997; Taiz and Zeiger, 2010), including the synthesis of *E. crassipes* and *P. stratiotes* chlorophyll. The different effects occur because of the different effect of levels and forms of nitrogen in an anaerobic, facultative and maturation (Cramer and Lewis, 1993), the level of nitrogen (Evans, 1989) and environmental factors (such as temperature and light intensity), as well as aspects of the sensitivity of plants to nitrate (Errebhi and Wilcox, 1990). Leaf colors in anaerobic and facultative pond green, and yellow in the maturation pond.

Conclusion

Eichhornia crassipes and *Pistia stratiotes* in domestic wastewater have increased in wet weight, stolon length, number and length of petiole, RGR, and DT, as well as the decrease in root length, leaf size and chlorophyll content.

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