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Effect of Phytoremediation of *Eichornia crassipes* (Mart.) Solms and *Marsilea crenata* C. Persl on Reduction of Phosphate Levels in Laundry Waste

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Abstract: Detergent serves as the most widely used cleaner, one of the ingredients for detergent is phosphate. Excessive phosphate in the waters will cause eutrophication and endanger the aquatic biota in it. Therefore, a solution is needed to reduce phosphate in the waters; one such way is by using *Eichornia crassipes* (Mart.) Solms and *Marsilea crenata* C. Presl. The purpose of this study was to determine the effect of plant species and phytoremediation time on phosphate levels in laundry waste. The variables studied were variations in plant species and phytoremediation time. In the plant variety, water hyacinth, water clover, and a combination of the two are used. In the variation of time used 0, 48 and 96 hours. The waste used is waste from a laundry business in Wonokromo, Surabaya. Parameters tested include phosphate, DO, BOD, pH, temperature, and turbidity. The exploration stage is in the form of acclimatisation and range finding tests to determine the concentration of wastewater used in the experimental stage. The results showed that plant species and phytoremediation time affected the decrease in phosphate levels. The correlation between phytoremediation time on parameters of phosphate levels, BOD, and pH will decrease with the duration of phytoremediation, but DO parameters will increase with the duration of phytoremediation.

Key words: Phytoremediation, phosphate, *E. crassipes*, *M. crenata*, laundry waste.

Introduction

Laundry business is a widely accepted form of business, which is developing along with the development of people's lifestyles that are all practical, including in terms of washing clothes. The laundry business is one of the largest producers of waste water (Barambu et al., 2020). Laundry waste water that is disposed of directly into the environment contains detergents that harm the environment (Khery et al., 2022).

One of the ingredients contained in detergents is phosphate. Phosphate functions as a builder, namely to deactivate hardness minerals in water (Zairinayati and Shatriadi, 2019). Excessive nitrogen and phosphate in the aquatic environment cause eutrophication, a condition in which aquatic plants and algae reproduce which causes an imbalance in aquatic ecosystems (Widyarani et al., 2022). Based on PP RI No. 82 of 2001 concerning water quality management and control of water pollution, the quality standard of phosphate in water from class I to class IV is 0.2–5 mg/L.

One method to remove contaminants biologically is by phytoremediation (Naeem et al., 2020). Phytoremediation is a technique that is economically and environmentally beneficial because it utilises plants to retain, absorb or detoxify contaminants from soil or water (Kafle et al., 2022). Aquatic plant species that have the potential for waste management include water

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hyacinth (*Eichhornia crassipes* (Mart.) Solms) and Swater clover (*Marsilea crenata* C. Presl).

Water hyacinth (*Eichhornia crassipes*) is a weed plant that lives in water by floating in deep waters. The surface structure of water hyacinth plants contain carboxyl, hydroxyl, and carbonyl groups, which act as catalysts for the adsorption of water contaminants to vegetable adsorbents (Brown et al., 2020). The composition of the fiber, which is made up of cellulose, in water hyacinth includes a number of hydroxyl groups, these hydroxyl groups are the main determinants of adsorption (Amalina et al., 2022).

Water clover (*Marsilea crenata* C. Presl) has the ability to do phytoremediation. Water clover belongs to the Marsileaceae family. Clover habitats in waters such as swath ponds, swamps, rivers have the potential to act as phytoremediation agents (Musapana et al., 2020). Research by Rulitasari and Rachmadiarti (2021) also stated that *M. crenata* was able to reduce welding detergent levels. As studies by Rachmadiarti and Trimulyono (2019), *M. crenata* plants located in the Benowo wetland, Surabaya is able to survive in a habitat polluted by heavy metal, such as Pb, originating from domestic waste and industrial waste.

The purpose of this study was to determine the effect of phytoremediation with various plant species and the time of phytoremediation to reduce phosphate levels in detergent wastewater.

Materials and Methods

Place and Time of Research

This research was carried out from October to November 2021 at the Green House Biology FMIPA State University of Surabaya. Analysis of water quality parameters was carried out at the Biology Ecology Laboratory of the State University of Surabaya. Analysis of the decrease in phosphate levels was carried out at PT. Envilab Indonesia, Manyar Mas Karimun, Gresik using a UV-Vis spectrophotometer with ascorbic acid refers to SNI 06-6989.31-2005. Plant material *Eichhornia crassipes* (Mart.) Solms and *Marsilea crenata* C. Presl obtained from Sememi Village, Benowo, Surabaya. The sample of detergent waste was obtained from a laundry business in the Jetis Kulon area, Wonokromo, Surabaya.

Research Variable

The independent variables in this study were plant species used as phytoremediation agents and phytoremediation time. The plant species used were water hyacinth, water clover, and a combination of both. While the time used is 48 hours and 96 hours. The response variable in this study was dependent on the level of phosphate remaining in the detergent waste after the treatment process. Supporting parameters were also carried out to strengthen the experimental results, namely DO, BOD, pH, temperature, and turbidity parameters of wastewater.

Research Design

The experiment was carried out with a two-way completely randomised design (CRD), namely the effect of each plant species, such as water hyacinth, clover water, and a combination of both on decreasing phosphate levels and the effect of variations in phytoremediation time of 48 hours and 96 hours in reducing phosphate levels. Each treatment was given control with no treatment and then repeated three times.

Tools and Materials

The materials needed in this research are laundry waste, water hyacinth and water clover plants, 5N H_2SO_4 solution, $K(SbO)C_4H_4O_6.½H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, ascorbic acid solution, $C_6H_8O_{60}$. 1 M, mixed solution, KH_2PO_4. The tools needed in this research are 27 reactors, pH meter (Milwaukee), thermometer, turbidimeter (Hanna Instruments), DO meter (Milwaukee), 36 bright winkler and UV-Vis spectrophotometer (Shimadzu), analytical balance (Denver Instrument), 125 mL Erlenmeyer (Duran), 10 mL measuring pipette (Pyrex), 1000 mL beaker (Duran), and a dropper.

Research Procedure

This research was conducted in two stages, namely the exploration stage and the experimental stage. The exploration stage includes the acclimatisation of water hyacinth and water clover which was carried out for 7 days in a phytoremediation reactor filled with water. Then a range finding test was carried out to determine the concentration of the waste to be used in the experimental stage. Waste concentrations of 0%, 20%, 40%, 60%, and 80% were used in the range finding test. The range finding test was carried out for 4 days. Furthermore, the quality of the wastewater used in the study was tested. At the implementation stage of the experimental stage, a 20% concentration of waste from the RFT test results was used. The research was conducted by making an initial solution with a waste concentration of 20%, then adding 5 liters of wastewater to each reactor. Water hyacinth was chosen as the plant material to be weighed.

Data Analysis

Phosphate reduction data were analysed using two-way ANOVA in the IBM SPSS Statistics 23 program, namely the effect of plant species on the decrease in phosphate levels and the effect of time on the decrease in phosphate levels and their interactions. Then continued Tukey's test with a 0.05 level to find out the highest significant difference. This study was also continued with multiple regression tests to determine the relationship between phytoremediation time and parameters of phosphate levels, DO, BOD, pH, temperature, and turbidity in each plant species. Waste water quality data were analysed descriptively and quantitatively.

Results and Discussion

Exploration Stage

At the exploration stage, the waste characteristics test, plant acclimatisation, and range finding test were done for the further experimental stage. The results of the laundry waste characteristic test are shown in Table 1.

Table 1: Laundry waste characteristics test results

Parameter	*Unit*	*Analysis results*	*Quality standards*
DO	mg/L	5.55	6
BOD	mg/L	1.8	2-12
Turbidity	NTU	52.43	20
Ph	-	9.4	6-9
Temperature	°C	32°C	38°C
Phosphate	mg/L	1.2	5

Table 1 shows that the results of the parameter analysis are not in accordance with the grade III waste water quality standard which refers to the Indonesian Government Regulation no. 82 concerning water quality management and water pollution control. All parameters included in the result of the waste water quality test have not exceeded the quality standard. However, a necessity is deduced to treat wastewater first before being discharged directly into water bodies.

The plant acclimatisation stage was carried out for 7 days. This stage aim that plants are able to adapt to environmental conditions that will be used in the exploration and experiment stages. Plants with good physical condition and not wilting were used in the range finding test and the experimental stage.

Table 2 displays the result of the RFT test, it can be seen that more of the leaf fall occurs in water hyacinth and water clover plants in each concentration.

Table 2: Fallen leaves at each concentration on water hyacinth and water clover

Detergent concentration	*Plant*	*Days to-*			
		1	*2*	*3*	*4*
0%	*E. crassipes*	-	-	-	-
	M. crenata	*	*	**	**
20%	*E. crassipes*	-	*	*	*
	M. crenata	*	**	**	**
40%	*E. crassipes*	-	*	*	*
	M. crenata	*	**	**	***
60%	*E. crassipes*	-	*	**	**
	M. crenata	*	**	***	***
80%	*E. crassipes*	-	*	**	**
	M. crenata	*	***	***	****

Furthermore, the RFT test was carried out with waste concentrations of 0%, 20%, 40%, and 60% in 5 liters of water for 4 days. Figure 1 shows that the results of the RFT test that has been carried out have a good concentration used at the experimental stage, namely a concentration of 20% for both water hyacinth and water clover. At this concentration, the wilting of leaves in plants is only 40%, so it is still possible to do this at the experimental stage.

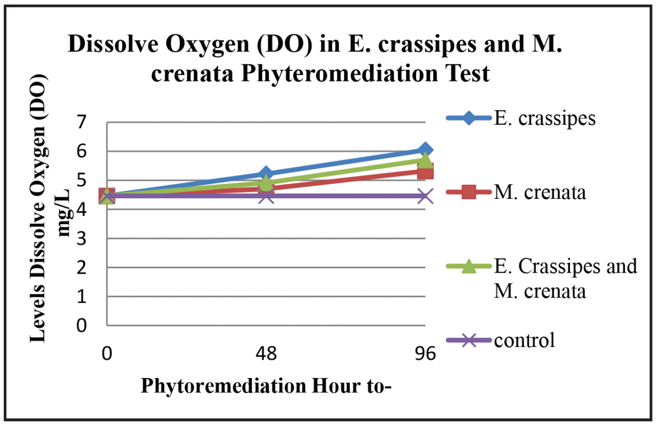

Figure 1: DO in water hyacinth and water clover phytoremediation tests.

Experiment Stage

The percentage decrease in phosphate in phytoremediation of water hyacinth, clover water and a combination of both at 48 hours and 96 hours is presented in Table 3. In Table 3 it can be seen that the highest percentage decrease in phosphate levels occurred in water hyacinth plants at 96 hours phytoremediation time of 78.83% and the lowest decrease occurred in water clover plants at 48 hours phytoremediation time with a decrease of 17%.

Table 3: The percentage decrease in phosphate levels in water hyacinth and water clover plants at 48 hours and 96 hours of phytoremediation

Plant	*Percentage of phosphate reduction (%)*		
	0th hour	*48th hour*	*96th hour*
E. crassipe	0±0	$42.67±5.03^{Ca}$	$78.83±7.50^{Cb}$
M. crenata	0±0	$17±3.26^{Aa}$	$37.67±4.04^{Ab}$
E.crassipe + M.crenata	0±0	$25±4^{Ba}$	$47.33±5.50^{Bb}$
Control	0±0	0±0	0±0

Notes: Numbers followed by different notations indicate different values time for phosphate phytoremediation by plants (a, b) and notation the ability of plants in phosphate phytoremediation (A, B, C), the value of significant differences between each other in the Tukey Test with level 0.05.

The results of the analysis of variance (ANOVA) on the process of decreasing phosphate levels at the experimental stage showed that there was a significant difference caused by the treatment time and plant species. The plant species factor, the time phytoremediation factor and the interaction factor between plant species and time show a sig value of 0.000 <0.05 so it can be seen that the effect of plant species and time phytoremediation decreasing phosphate levels is significantly different, indicating that there is an interaction between plant species and phytoremediation time.

Water hyacinth is a plant that absorbs the most phosphate because of its fibrous root structure, this root arrangement can filter mud or particles dissolved in water (Ahmad, 2008). Water clover roots are rhizome-shaped with adventitious roots at nodes on the underside of the rhizome. The mechanism of plants in absorbing organic and inorganic materials is that when the material enters the cell it will cause the vacuole to swell, then the cytoplasm is pushed to the edge of the cell so that the protoplasm will be close to the cell membrane. Therefore, the exchange or absorption of phosphate between cells and their surroundings is more efficient (Aneta, 2013). Plants show a non-selective absorption system, so both organic and inorganic substances found in water are absorbed directly without being selected.

The combination of water hyacinth and water clover showed a better phytoremediation level than water hyacinth alone. This means that water hyacinth is able to perform phytoremediation optimally even though it is not with other plants. Meanwhile, water clover is more optimal in phytoremediation if it is with other plants. This shows that living things can cooperate with other living things in the environment so as to form a beneficial ecosystem unit.

Figure 1 shows that DO levels from 0 hours to 96 hours continued to increase. The highest increase occurred in water hyacinth plants at 96 hours, namely 6 mg/L and the lowest increase occurred in water clover plants at 48 hours, namely 4.7 mg/L. The data obtained show that the DO value is still in the range that meets PP No. 82 of 2001 concerning water quality treatment and water pollution control. The amount of dissolved oxygen in the water increases with the decrease in phosphate levels in the water so that aquatic organisms can utilise the oxygen in the water. A sufficient amount of oxygen in the waters greatly affects biogeochemical processes and aquatic life in which oxygen is needed by aquatic organisms for respiration (Gadekar et al., 2012).

In Figure 2 it can be seen that the BOD levels decrease as the time increases from 0 hours to 96 hours. The results showed that the level of BOD in the water decreased in line with the decrease in phosphate in the water. Obtaining data shows conformity to the requirements of water quality standards according to Government Regulation No. 82 of 2001 concerning water quality management and water pollution control. The decrease in BOD levels in water is because plants absorb organic substances in the waste, and dissolved oxygen in the water will also increase from the results of plant photosynthesis. The more plants there are, the lower the BOD level, so the water quality will also increase (Fachrurozi et al., 2010).

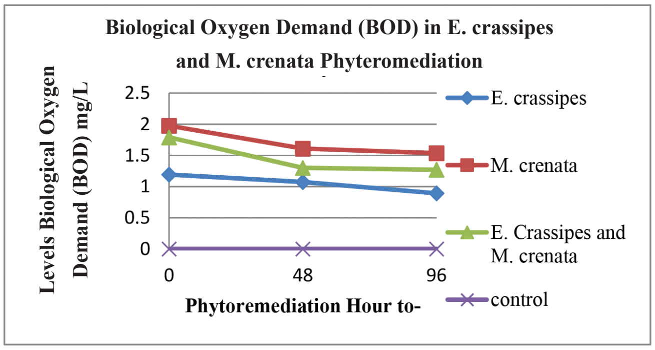

Figure 2: BOD allowance by water hyacinth and water clover plants.

The results of the pH test for each plant species and phytoremediation time are shown in Table 4. It can be seen that the pH from 0 hours to 96 hours decreased to neutral. The lowest and highest pH level occurred in water hyacinth phytoremediation after 96 hours was 7.3 and 7.7, respectively. This indicates that the phytoremediation process of plants can affect the pH value of wastewater to fall closer to the normal direction. Plants have the ability to absorb chemical compounds both organic and inorganic substances through chemical processes, this causes the pH value in the water to be good. This statement is reinforced by Rachmadiarti et al. (2020) who showed that phytoremediation by plants *M. crenata* and *L. adcendens* is able to reduce the pH of the LAS solution.

Table 4: Results of pH test on water hyacinth and water clover phytoremediation

Plant	*(Hours)*		
	0	*48*	*96*
E. crassipes	8.3±0	7.8±0.1	7.3±0.1
M. crenata	8.3±0	8.1±0.08	7.7±0.1
E. crassipes + M. crenata	8.3±0	8.0±0.15	7.5±0.1
Control	8.3±0	8.3±0	8.3±0

Table 5 shows that the temperature measurement results are relatively stable at the initial time the water temperature reaches 32°C but within 48 hours it decreased so that the temperature became 29°C and increased again in 96 hours to 30°C. The temperature in each reactor increased and decreased which was not too significant. This is because each reactor is placed in one location which is influenced by the ambient temperature. The temperature will affect the process of photosynthesis and plant metabolism, the optimum growth temperature for aquatic plants is between 22 and 30°C (Hartanti et al., 2014). The temperature in the reactor is still in the optimum range of phytoremediation so that it does not significantly affect other parameters.

Table 5: The results of temperature measurements of water hyacinth and water clover phytoremediation tests

Plant	*Hours*		
	0	*48*	*96*
E. crassipes	32±0(°C)	29±0(°C)	30±0(°C)
M. crenata	32±0(°C)	29±0(°C)	30±0(°C)
E. crassipes + M.crenata	32±0(°C)	29±0(°C)	30±0(°C)
Control	32±0(°C)	29±0(°C)	30±0(°C)

The results of turbidity measurements in each plant species and phytoremediation time are shown in Table 6. It shows that the turbidity of the water decreased in 48 and 96 hours. There was a drastic decrease in turbidity at 0-48 hours and then an increase in turbidity again at 96 hours. This proves that phytoremediation is able to reduce water turbidity so that it does not harm aquatic biota. The presence of detergent pollutants in the waters will cause plants to experience root loss, the loss of plant roots as a form of plant adaptation in the polluted zone. Rukmi (2013) stated that the effectiveness of water hyacinth absorption was influenced by its own ability, as well as rhizosphere microbes in the roots and supported by high adsorption and root accumulation.

Table 6: Turbidity test results on water hyacinth and clover phytoremediation

Plant	*Hour*		
	0	*48*	*96*
E. crassipes	12.32±0	3.28±0.21	5.53±0.34
M. crenata	12.32±0	5.33±0.08	8.68±0.45
E. crassipes + M. crenata	12.32±0	4.79±0.42	7.67±0.13
Control	12.32±0	12,32±0	12,32±0

The correlation between phytoremediation time on parameters of phosphate levels, DO, BOD, pH, temperature, and turbidity in each plant species were analysed using multiple regression to determine the correlation coefficient and significance of each parameter factor. The results of the regression test on water hyacinth plants can be seen in Table 7.

Table 7: The relationship of phytoremediation time to parameters of phosphate levels, DO, BOD,pH, temperature, and turbidity of water hyacinth, water clover, and a combination both of them

Parameter	*Correlation coefficient*		
	Water hyacinth	*Water Clover*	*Water Hyacinth + Water Clover*
Phosphate Level	-0.943	-0.980	-0.986
DO	0.998	0.965	0.986
BOD	-0.768	-0.909	-0.855
pH	-0.987	-0.949	-0.951
Temperature	-0.665	-0.655	-0.655
Turbidity	-0.720	-0.518	-0.598

Notes: Different values in rows and columns show correlation results based on regression tests with a level of 0.05.

The results of the regression test showed a significance value of <0.05, which means that the phytoremediation time can affect the parameters of phosphate levels, DO, BOD, pH, temperature, and turbidity. Table 7 shows that the correlation is very strong and inversely occurs in the parameters of phosphate levels, BOD, pH, temperature, and turbidity. This means that the longer the phytoremediation time, the lower the parameter value. However, a strong and unidirectional relationship occurs in the DO parameter. This means that the longer the phytoremediation time, the higher the DO level in the water.

Conclusion

The results showed that the type of plant, phytoremediation time, and their interaction affected the decrease in phosphate levels in laundry waste. The most influential plant species was water hyacinth which was able to reduce up to 78%. While the best time to reduce phosphate levels is 96 hrs with a decrease of 78% as well. So that the best interaction between the two in reducing phosphate levels in laundry waste is the water hyacinth plant with a phytoremediation time of 96 hours.

Suggestion

The results of this study can be implemented as a strategy to reduce laundry waste pollution in the home industry scaler or small industry. This research is also used as a reference source in the development of a simple Wastewater Treatment Plant (WWTP) to handle laundry waste before being discharged into the environment by utilising water hyacinth and water clover plants. In addition, this plant can also be cultivated continuously but still must be monitored, so that there is no blooming and disturbing aquatic biota.

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