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# The Effectiveness of *Scirpus grossus* and *Limnocharis flava* as Fitoremediation Agents of Nitrate-Phosphate to Prevent *Microcystis* Blooming in Fresh Water Ecosystem

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## ABSTRACT

The aim of this study was to reduce the concentration of dissolved nitrate-phosphate, because it can prevent the occurrence and inhibit the growth of *Microcystis* bloom waters. The study was conducted experimentally in the laboratory. The *Microcystis* isolation was carried out in Sutami Reservoir. Then, remediation treatment with hydromacrophyte (*Scirpus grossus*, *Limnocharis flava* and combination of both hydromicrophyte) were done during the 15 day incubation period. Abiotic factors were measured on day 0, 6, 12 and 15, but the abundance of *Microcystis* cells was counted daily. The productivity of hydromacrophyte was measured at the end of the research. The research results showed that hydromacrophytes were effective to reduce nitrate and phosphate concentrations. The combination of *S. grossus* and *Microcystis* reduced nitrate concentration up to 99.89 %, while the highest reduction of dissolved phosphate (98.22 %) was resulted from the combination of *L. flava* and *Microcystis*. The combination treatment of *L. flava* and *S. grossus* was capable to prevent *Microcystis* growth rate and produced the carying capacity of 65 cells.L-1.day-1 and 6.93 x $10^4$ cells/mL.

**Keywords:** *hydromacrophyte, Microcystis, nitrate- phosphate reduction*

## INTRODUCTION

Aquatic ecosystem is an organism environment that is easy to be contamined. The decline of water quality is caused by pollutants from human activities such as residential trash, sedimentation, fertilizers and pesticides. The pollutans can cause euthropication which can be a triger of alga blooming. The alga blooming phenomenan is caused by the changes of concentration and imbalance nutrients ratio [1]. The differences of nitrat ratio and phospat can cause the growth of several types of algae, such as *Microcystis*, which tends to dominate. The statement is supported by research of Retnaningdyah et al [2] which shows that the groth of *Microcystis* is influenced by the level of nitrate in waters.

*Microcystis* is a kind of blue-green algae (Cyanobacteria) that generally grows on the surface of the water. At normal conditions, *Microcystis* is not harmful to other organism or humans. In certain conditions, such as high temperature of air with high nutrients (especially nitrate), *Microcystis* is able to grow rapidly that is commonly called as "algae blooms". The blooming conditions can cause *Microcystis* to produce toxins, called microcystin [3][4]. Microcystin has a high level of toxicity to both plants and animals, and can cause death [5].

Varoius efforts can be done to prevent the blooming *Microcystis*, either biologically, chemically, or mechanically. However, chemical and mechanical methods have a negative impact to the aquatic ecosystems; thus, it is necessary to develop biological methods to resolve the issue. One of them is to reduce the levels of nitrate through denitrification of waters emlpyoying the macrophyte, or is known as phytoremediation system. Phytoremediation is an attempt to use of plants and their parts to solve waste and other environmental pollution problems. Therefore the aim of the study was to solve the *Microcystis* blooming phenomenon by reducing the nitrate

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and phosphat level through bioremediation suppressing the growth and preventing the occurance of *Microcystis* blooming.

## MATERIALS AND METHODS

The research was conducted from December 2011 to April 2012. The sample of *Microcystis* was taken from Sutami Reservoir, Malang, East Java. Hyromacrophyte and acquired land was taken from the wetland area around Malang, and the experiments were conducted in the Glasshouse Laboratory of Ecology and Animal Diversity and Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Sciences, Brawijaya University, Malang.

### *Isolation of Microcystis cell*

*Microcystis* samples were taken horizontally and parallel on the water surface of Sutami Reservoir, Malang, East Java, Indonesia using a one-litre-capacity water sampler. The water was then filtered using aplankton net to get *Microcystis*. Sample was then counted to obtain $5x10^8$ cells.mL$^{-1}$.

### *Determination of Potential Hydromacrophyte*

This study was an experiment study conducted using a complete randomized block design. The first factor was the type hydromacrophyte (*S. grossus*; *L. flava*; combination of *S. grossus* and *L. flava*, and without the addition hydromacrophyte). The second factor was with and without the addition of *Microcystis*. The system used for the experiments employed bacth culture in a tub and aquarium, with the addition of Sutami Reservoir water as much as 15 L for the tub and 5 L for the aquarium. The amount of soil added was as much as 20 kg / tub and 6 kg / aquarium. Water was enriched by phosphate ($K_2HPO_4$) 0.4 ppm.L-1 and nitrate ($NaNO_3$) 16 ppm.L-1. The hydromacrophyte acclimatization was done in two weeks. After the acclimatization, the hydromacrohyte was added to the composition that had been modified according to the treatment (the addition of nitrate, phosphate, and *Microcystis*). The amount of each plant used was based on extensive research of plant closures by 25 %. Each treatment culture was then incubated in a glasshouse for 15 days.

### *Measurement of Nitrate and Phosphate*

Abiotic factors observed in this study included the concentration of dissolved nitrate-phosphate which was measured in every five days. Nitrate concentrations were measured using brusin-colorimetric method. Dissolved phosphate concentrations were measured using stannous chloride-colorimetric method [6].

### *Calculation of Abundance Microcystis*

The number of *Microcystis* cells was counted every day for 15 days to get the abundance sample of *Microcystis*. The sample was taken by filtering 100 mL of the treatment water using a plankton net having 406 pores per inch. Samples taken were boiled for 6 minutes, cooled and then counted for the number of cells using a 1x10-4 cm3 haemocytometer and binocular microscope at 400x magnification, and the density of *Microcystis* (cells/mL) was calculated using Formula 1 [7].

$$\frac{\text{Cells number counted x volume concentration (ml)}}{1x10^{-4}\ \text{cm}^3 \text{ x volume filtered (100 ml)}} \quad (1)$$

### *Data analysis*

Nitrate and phosphate concentrations on the treatment media were measured using ANOVA followed by a T-test on SPSS 16.00 for Windows. The data of Mycrocystis were used for calculating the rate of the individual growth. The calculation of the growth rate was done using Formula 2 [8].

$$g = (\ln N_t - \ln N_0)/t \quad (2)$$

Note:
g : Value growth rate
Nt : The highest *Microcystis* population after incubation
N0 : The initial number of *Microcystis* population
t : The time required to achieve the highest *Microcystis* population

To find the difference of the growth rate (g) and the maximum abundance of *Microcystis* (K) between treatments, ANOVA was conducted followed by Tuckey HSD test at the significance level of 5% with SPSS 16.00 for Windows.

## RESULTS AND DISCUSSION

### *Interaction between Microcystis, Scirpus grossus, and Limnocharis flava to reduce the concentration of Nitrate-Phosphate in the Media*

The concentrations of nitrate and orthophosphate on all types of treatment media

of hydromacrophyte decreased on the value of over 95 %. The result of concentration measurements of nitrate and ortophospate above 95 % was shown on day 12th and 15th (Figure 1 and 2). Based on those pictures, it can be concluded that the decrease of the nitrate concentration and ortophosphate occured on treatment media with or without *Microcystis* addition.

Monoculture (*S. grossus* and *L. flava*) and polyculture treatment (combination of *S. grossus* and *L. flava*) showed significant results (p> 0.05) in decreasing the concentration of nitrate and orthophosphate in the media, this was indicated by the different notations (Figure 1 and 2). Decrease in nitrate concentration after fifteen-day incubation showed a decrease in nitrate maximum concentration value from 0.04 to 0.09 ppm.L$^{-1}$. The decline in orthophosphate after fifteen-day incubation showed a maximum concentration of 0.01 ppm.L-1. Thus, it was clear that virtually all of the plants were able to reduce nitrate and orthophosphate levels in aquatic media during the incubation period.

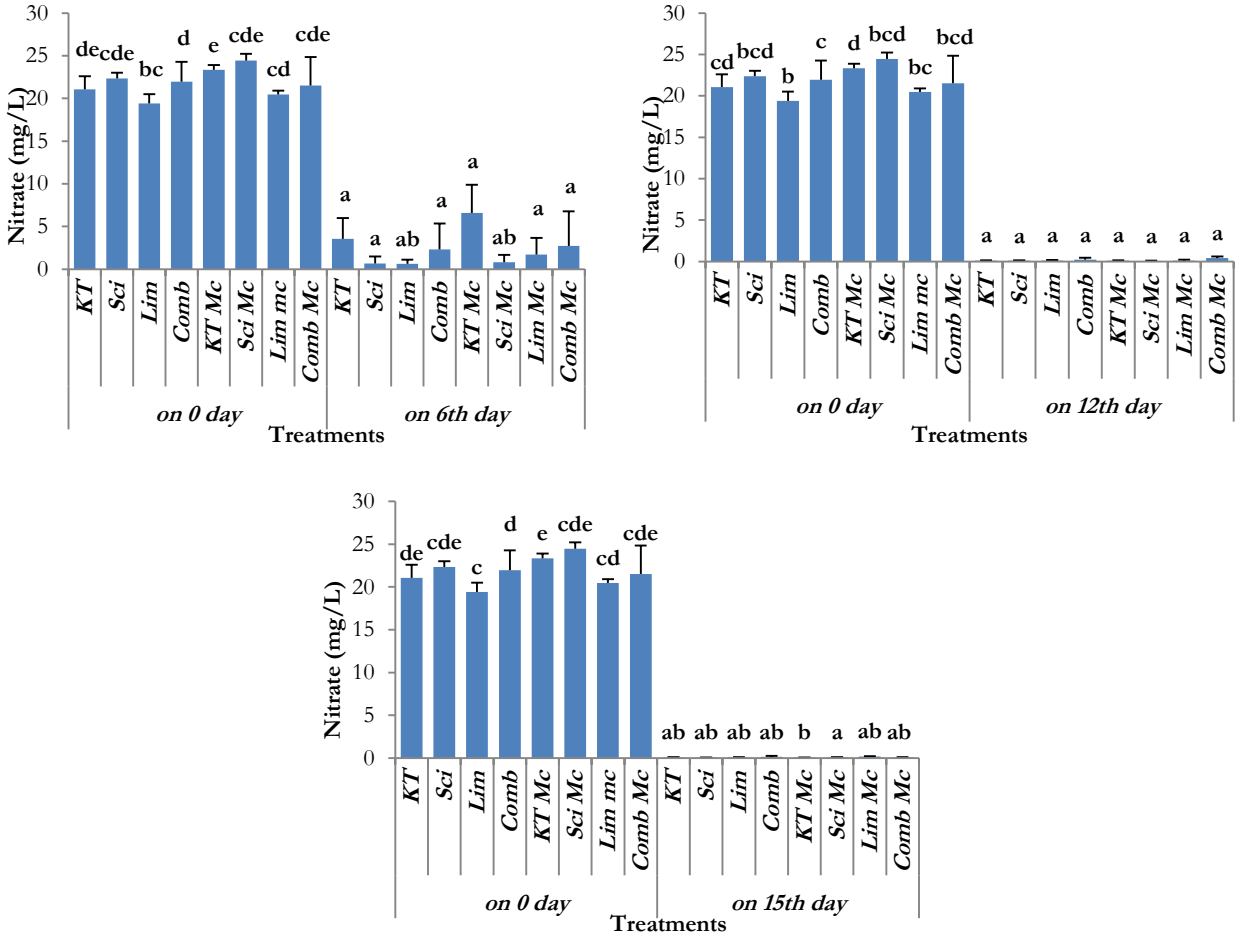


Figure 1. Comparison of nitrate concentration between day 0 and 6 (a), 0 and 12 (b), and 0 and 15 (c) after incubation. KT: control; Sci: S.grossus; Lim: L.flava; Comb: *S. grossus-L. flava*; KT Mc: control *Microcystis*; Sci Mc: S.grossus *Microcystis*; Lim Mc: *L.flava Microcystis*; Comb Mc: *S. grossus-L. Flava Microcystis*. The same notation show non significant difference based on ANOVA and T test

The decrease in concentration of nitrate and orthophosphate was used by hydromacrophyte as both a nutritional source of growth and a way of reducing bacteria interaction with organic compounds in the media. The bacteria couls be naturally derived from the soil and the water reservoir used as media. It is known that the ratio of the absorption of nutrients by the organisms between the nitrogen and phosphor is equal to 16:1, therefor when the phosphor content is high but is not supported by high levels of dissolved nitrogen, the phosphor can't be utilized for growing hydromacrophyte on media treatment [3].

Decrease in the concentration of nitrate and orthophosphate could also occur in the control group. The levels of nitrate and orthophosphate in the controls declined more than 90% on day

12th. This was caused by the fact that the control media were also covered in algae of that genus (*Cladophora* and *Phitophora*). Both types of algae are parts of Cladophoraceae Family. Nitrate and orthophosphate can be utilized by hydromacrophyte and algae as a source of nutrients for growth. In general, algae can grow by utilizing the organic material in the media; and if there is no competition for nutrients with other plants of this type, then the algae can thrive.

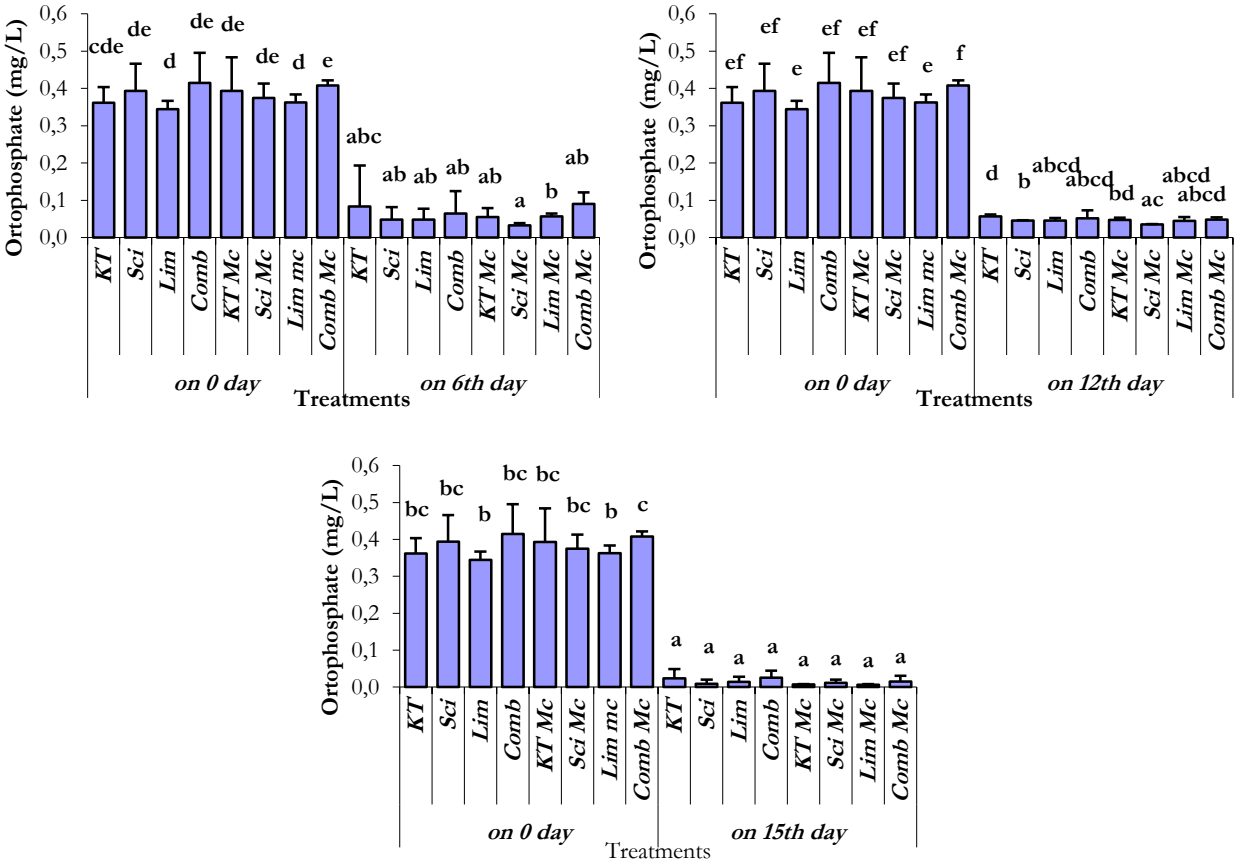


Figure 2. Comparison of Orthophosphate concentration between day 0 and 6 (a), 0 and 12 (b), and 0 and 15 (c) after incubation. KT: control; Sci: S.grossus; Lim: L.flava; Comb: *S. grossus-L. flava*; KT Mc: control *Microcystis*; Sci Mc: S.grossus *Microcystis*; Lim Mc: *L.flava Microcystis*; Comb Mc: *S. grossus-L. Flava Microcystis*. The same notation show non significant difference based on ANOVA and T test

Nitrogen is an essential element required for the synthesis of protein by animals and plants. Plants can't utilize nitrogen directly. With the help of microorganisms, nitrogen can be converted to other compounds such as ammonium, nitrate or other organic compounds. One of the function of nitrate utilization is as an agent for protein synthesis. The high concentration of nitrate in aquatic environment then can be utilized as a source of nutrients to produce new cells and colonies [9].

Phosphorus is a limiting factor for nutrients needed for the growth of hydromacrophyte and *Microcystis*. Media treatments added with soil can be used directly by hydromacrophyte mixed with nitrate and orthophosphate, so that their concentration could decrease every day. The phosporus element is an essential nutrient for the growth of organisms. In cell activity, elemental phosphor is needed to produce energy, making up nucleic acids and phospholipids of cell membranes [4].

### *Growth response of Microcystis in the process of Phytoremediation*

The results of daily calculation of the number of *Microcystis* cells in the treatment and control media is shown in Figure 3. Based on the figure, it can be said that the growth of *Microcystis* was not trough a lag phase or an adaptation phase. *Microcystis* growth entered the exponential phase immediately, and was followed by the death phase. This happened on day 4 until day 5 of the observation when the maximum amount of

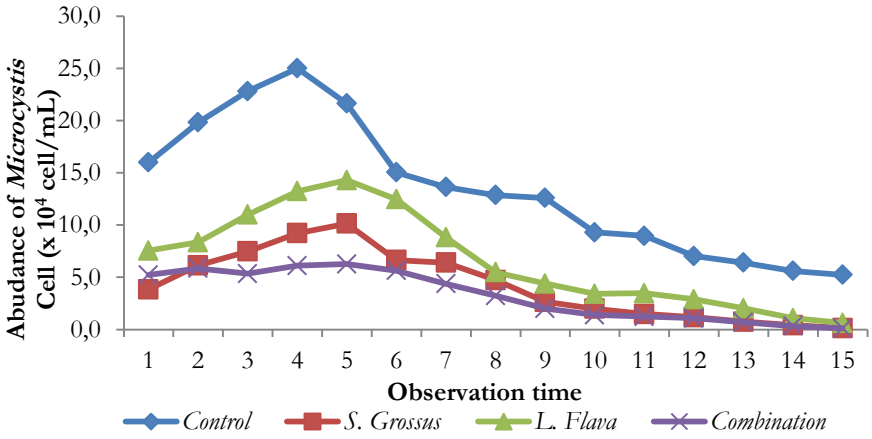


Figure 3. Growth pattern of *Microcystis* during observation.

growth of *Microcystis* could be supported by environmental resources.

Death phase is characterized by the declining number of *Microcystis* cells because nutrients started to run out. This was found during day 5 and 6 of the observation. The decrease in the nutrient content and in the capacity of available space could also affect the growth of *Microcystis*. The growth of *Microcystis* required high light intensity and nutrients. The death of Microcyst was caused by the lack of nutrients in the media and accumulation of toxic, which caused the media to be not conducive for growth. Furthermore, the numbers of dead Microcysti cells could be influenced by nutrient conditions, environment condition, as well as types of microorganisms.

All hydromacrophyte had same potential in reducing the abundance of *Microcystis*, but the ability of each plant to grow was different in lower abundance. The results showed that a combination treatment of *S. grossus* and *L. flava*significantly inhibited the growth of *Microcystis* until 72 % after six days of incubation. The other treatments had lower effect than the combination treatment.

### *Growth rate and maximum abundance of Microcystis cells*

The growth rate and carrying capacity of *Microcystis* in the treatment and control media could be determined by the value of cell density. Based on statistical analysis, it was known that K and g value were significantly different (p> 0.05) between control media and K and g value of the hydromacrophyte treatment (Figure 4a). The highest growth rate of *Microcystis* was for the treatment media of *S. grossus* and the lowest value of *Microcystis* growth rate was found in the combination treatment of *S. grossus* and *L. flava*.

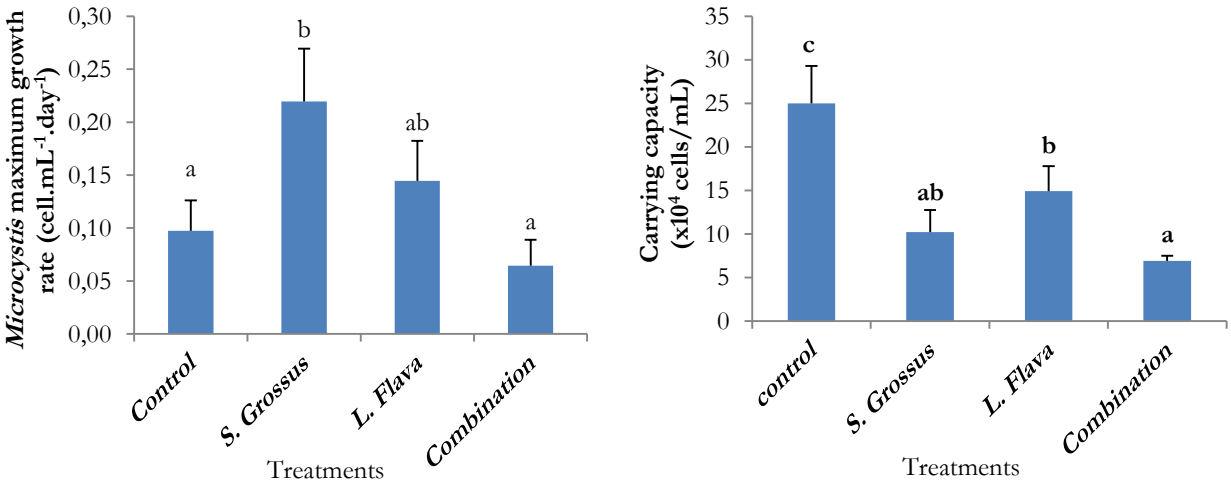


Figure 4. Maximum growth rate (a) and carrying capacity of *Microcystis* during observation (b). Notes: The same notation show non significant difference based on ANOVA and T test

The result of the carrying capacity in each treatment which showed that the highest abundance of *Microcystis* was related to the environmental conditions presented in the control treatment, and was equal to 25 x $10^4$ cells/mL, while the lowest abundance of *Microcystis* presented in the combination treatment of 6.93 x 104 cells/mL. The control treatment showed a high carrying capacity because of the absence of competition to obtain nutrients. Directly, *Microcystis* contained in the media resource control could utilize N and P, although it was noted that the media controls were also covered by other types of algae which was *Cladophora* and *Phitophora*.

Overall, the combination treatment could inhibit the growth of *Microcystis* due to the competition for nutrients. The lowest value growth rate and the lowest carrying capacity of *Microcystis* was found in treatment combination of *S. grossus* and *L. flava*. This could be due to competition in the acquisition of nutrients by hydromacrophyte. In addition, the canopy structure of *S. grossus* and *L. flava* also caused *Microcystis* to unable to grow because the light was blocked by the canopy of the plants.

## CONCLUSION

The results showed that all hydromacrophyte (*S. grossus* and *L. flava*) had similar potential to reduce the nitrate and orthophosphate level until 90 % after six-day incubation. Hydromicrophyte was also able to prevent *Microcystis* blooming in the fresh water ecosystem. The carrying capacity of *Microcystis* 25 x $10^4$ decreased up to 6.93 x 104 cells/mL after six-day incubation or it can be said that the carrying capacity of *Microcystis* decreased up to 72 % after six-day of incubation.

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