

Effects of Macrophyte *Vallisneria asiatica* Biomasses on the Algae Community

Caixia Kang, Takahiro Kuba, Aimin Hao, Yasushi Iseri, Chunjie Li, Zhenjia Zhang

Abstract—To improve the water quality of lakes and control algae blooms, the effects of *Vallisneria asiatica* which is one of aquatic plants spread over Lake Taihu, with different biomasses on the water quality and algae communities were researched. The results indicated that *V. asiatica* could control an excess of *Microcystis* spp. when the *V. asiatica* biomass was larger than 50g in the tank with 30L solution in the laboratory. Planktonic and epiphytic algae responded differently to *V. asiatica*. The presence of macrophyte *V. asiatica* in eutrophic waters has a positive effect on algae compositions because of different sensitivities of algae species to allelopathic substances released by macrophyte *V. asiatica*. That is, *V. asiatica* could inhibit the growth of *Microcystis* spp. effectively and was benefited to the diatom on the condition in the laboratory.

Keywords—Algae bloom, algae community, *Microcystis* spp., *Vallisneria asiatica*.

I. INTRODUCTION

RECENTLY, along with the economic growth and urbanization, nutrient loadings and eutrophication of Lake Taihu have rapidly accelerated to the point that harmful cyanobacterial bloom is a common feature [1], [2]. All those bring significant barriers to the growth of aquatic plants and sustainable developments of economies in Lake Taihu.

Correspondingly the area of aquatic plants in Lake Taihu reduced continually, the aquatic plant species decreased constantly even disappeared, and ecological functions degenerated seriously [3]. With the structure adjustment of fisheries industry and improvements of crab farming, the community structure of aquatic plants had altered, and dominant species of aquatic plants had also changed greatly [3]. Dominant species of aquatic plants included *Hydrilla verticillata*, *Elodea nuttallii*, *Vallisneria asiatica*, *Potamogeton malaianus*, *Trapa maximowiczii*, and *Potamogeton crispus* in

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This research was supported by the Research Institute for East Asia Environments, Kyushu University financed by Mitsubishi Corporation in Japan. Thank Mr. Nakata Yoshikaka of Japan Tobacco Inc. for contributing to this research.

Lake Taihu [3], [4]. Aquatic plants distributed primarily in East Lake Taihu because of its good water quality, but now are also affected greatly by the water pollution.

Aquatic plants can enhance the water clarity by reducing the resuspension of bottom sediments, improve the water quality by absorbing lots of nutrients, and suppress the algae growth by competing for nutrients and releasing allelopathic substances that are toxic to algae [5]. Thus, the reparation of aquatic plants has been one of the keys to control the eutrophication of Lake Taihu. Furthermore, phytoplankton dynamics should be further researched in relation to aquatic plants because of the importance of controlling phytoplankton biomass and species in water management [5]. *V. asiatica* is a kind of common aquatic plants in lakes. Because of its high absorption of contaminants, wide distribution, and good resistance for contaminants, it is widely used in the project of vegetation restoration in eutrophic water bodies [6]. In view of the eutrophication characterized by cyanobacterial bloom which is dominated by *Microcystis* spp. in Lake Taihu, effects of *V. asiatica* on the algae biomass (mainly *Microcystis* spp.) and community, which took an engineering view of repairing function of water environments, were researched in this experiment. The result will make the improvement to control the cyanobacterial bloom in Lake Taihu.

II. METHODS

A. Materials and Settings

Tap water exposed to the sun to remove the chlorine-containing disinfectant was treated with an addition of N and P at final concentrations. The N and P elements in solution of tanks were adjusted and reached following concentrations respectively: 3.0mg·L⁻¹ of NO₃⁻-N (KNO₃), 0.5mg·L⁻¹ of NH₄⁺-N (NH₄Cl), and 0.2mg·L⁻¹ of PO₄³⁻-P (KH₂PO₄). The algae were got from a pond where the algae bloom occurred in Fukuoka Prefecture, Japan. The concentrated algae with the same volume were added into tanks from No.1 to No.5 to reach concentrations according to the eutrophication degree in Lake Taihu. The deep-rooted *V. asiatica* were collected in River Onga (in Fukuoka Prefecture, Japan) where the water quality was fine. All the collected plants were thoroughly washed and placed into 6 tanks (30L solution) with 20, 0, 20, 50, 200, 500±5g respectively from No.0 to No.5. The strengthened glass tanks (L 30cm, W 30cm, H 45cm) were placed in a constant temperature room with a temperature of 25°C, illumination intensity of 3,400 lux and photoperiod of 12h (light):12h (dark).

B. Sampling

The experiment lasted for 15 days. pH, DO, and turbidity

were tested by common analytical instruments in the laboratory. Plant and solution samples which were used for algae analysis were got at the beginning and end of the experiment. $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DTP were determined by salicylate method, chromotropic acid method, and acid persulfate digestion method respectively (HACH DR/2400 portable spectrophotometer) for the water quality analysis. Phytoplankton was identified and counted using a phase contrast microscope ($\times 300$ times) [7]. Periphyton was treated by ultrasound for 3 minutes then identified by the same method as phytoplankton.

III. RESULTS

A. pH, DO, and Turbidity of Solution

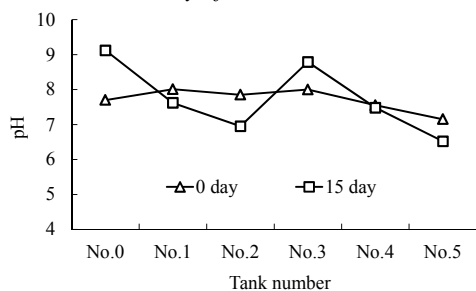


Fig. 1 Changes of pH in tanks

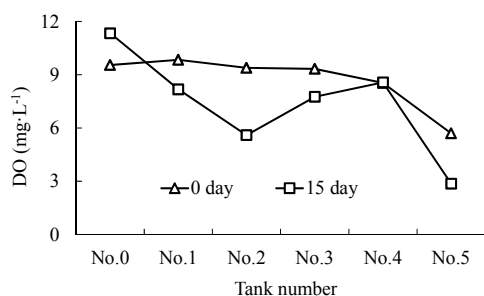


Fig. 2 Changes of DO in tanks

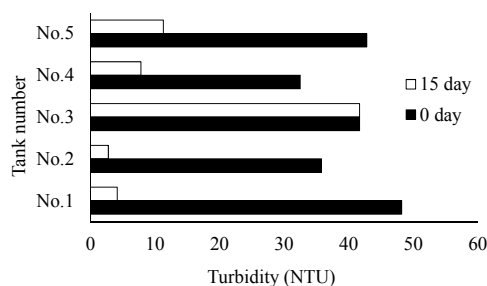


Fig. 3 Changes of turbidity in tanks

pH changed in a range from 6.52 to 9.16. The pH of solution decreased compared with initial values except No.0 and 3 (Fig. 1). The DO values decreased compared with initial values except No.1. The DO of No.5 was lower than that of other tanks (Fig. 2). Data shown in Fig. 3 indicated that turbidity decreased except No.3.

B. N and P Concentrations of Solution in Each Tank

The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in No. 1 and No. 2 were higher than other tanks obviously at the end of the experiment. The N contents of No.0, No.3, No.4, and No.5 decreased sharply after the experiment (Fig. 4). As shown in Fig. 5, DTP of No.0, No.3, and No.4 reduced sharply after the experiment. The DTP contents of No.1, No.2, and No.5 were higher than other tanks.

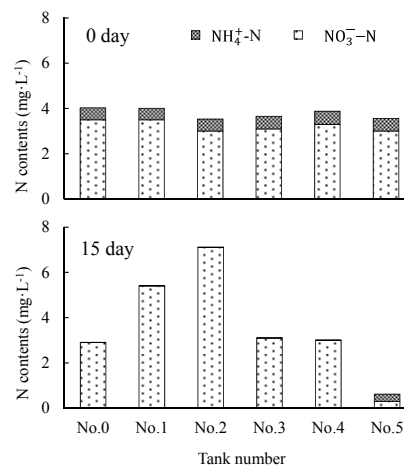


Fig. 4 Concentration changes of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in tanks

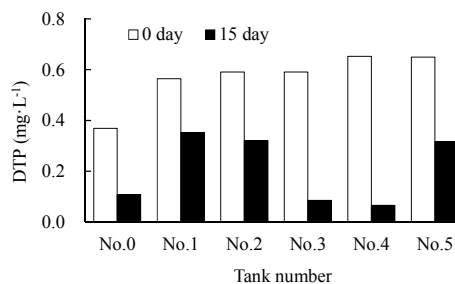


Fig. 5 Concentration changes of DTP in tanks

C. Analysis Results of Phytoplankton

At the end of the experiment, the taxa and quantity of phytoplankton in solution of tanks from No.1 to 5 were analyzed comparing with the initial value. The value of phytoplankton shown in Table I was the biomass in the whole tank (30L solution).

At the beginning of the experiment, *Microcystis* spp. was dominant species in cyanobacteria which were the major components of phytoplankton. After 15 days, *Microcystis* spp. of No.2 decreased sharply, and *Microcystis* spp. of No.1 even disappeared completely while diatom (mainly *Nitzschia* spp.) increased. The biomass of green algae in tanks of No.1, 2, and 3 increased also, but the biomass of green algae was very little and had a small proportion in the whole biomasses of algae. The species of algae became richer after adding *V. asiatica* into solution with algae than before. Especially, the biomass of *Microcystis* spp. of No.3 tank reached a value about 2.2 folds higher than the initial value.

TABLE I
SPECIES OF PHYTOPLANKTON IN EACH TANK

Algae Taxa	Initial	No.0	No.1	No.2	No.3	No.4	No.5	
	Biomass ($\times 10^5$ cells)							
Cyanobacteria	<i>Chroococcus</i> spp.	13	60	-	525	660	-	60
	<i>Microcystis</i> spp.	29,217	42	-	60	65,620	7,104	3,720
	<i>Phormidium</i> spp.	1,317	-	-	-	1,200	-	-
	<i>Oscillatoria</i> spp.	-	-	30	12	-	60	9,300
Diatom	<i>Achnanthes</i> spp.	-	-	-	-	-	9	-
	<i>Cocconeis</i> spp.	-	30	-	360	30	277	105
	<i>Cyclotella</i> spp.	-	210	-	-	-	-	-
	<i>Cymbella</i> spp.	-	-	-	-	-	-	-
	<i>Fragilaria</i> spp.	-	-	-	-	-	-	-
	<i>Gomphonema</i> spp.	1	-	-	60	15	42	-
	<i>Navicula</i> spp.	-	-	-	3	-	3	-
	<i>Nitzschia</i> spp.	378	-	2,460	1,440	60	43	15
	<i>Melosira</i> spp.	-	-	-	180	-	-	30
	<i>Synedra ulna</i>	-	-	-	-	-	-	3
Green algae	<i>Ankistrodesmus falcatus</i>	-	-	-	-	12	-	-
	<i>Scenedesmus</i> spp.	-	-	120	48	192	3	-
Total cell number		30,926	342	2,610	2,688	67,789	7,541	13,233

TABLE II
SPECIES OF PERIPHYTON ON LEAVES OF PLANTS IN EACH TANK

Algae Taxa	No.0	No.2	No.3	No.4	No.5	
	Biomass ($\times 10^5$ cells/g)					
Cyanobacteria	<i>Chroococcus</i> spp.	243	-	68	-	-
	<i>Microcystis</i> spp.	-	-	-	-	-
	<i>Phormidium</i> spp.	-	-	-	-	-
	<i>Oscillatoria</i> spp.	-	-	8	-	-
Diatom	<i>Achnanthes</i> spp.	54	-	-	-	-
	<i>Cocconeis</i> spp.	1,935	12	676	4,862	2,094
	<i>Cyclotella</i> spp.	-	-	-	-	-
	<i>Cymbella</i> spp.	59	-	-	-	-
	<i>Fragilaria</i> spp.	49	-	-	-	-
	<i>Gomphonema</i> spp.	65	-	68	90	1,208
	<i>Navicula</i> spp.	92	-	17	-	-
	<i>Nitzschia</i> spp.	43	-	34	-	483
	<i>Melosira</i> spp.	-	-	-	-	-
	<i>Synedra ulna</i>	32	-	-	60	-
Total cell number		129	1	17	25	8

Moreover, biomasses of *Microcystis* spp. of No.4 and No.5 decreased with an increase of *V. asiatica* biomass compared with the initial value. The biomass of diatom in tanks No.3, 4, and 5 also decreased with the increasing biomass of *V. asiatica*. The biomass of green algae of No.3 reached the largest value in all tanks. The green algae of No.4 and 5 decreased sharply. The whole biomass of green algae took up very small ratio of algae.

D. Analysis Results of Periphyton

At the end of experiment, the taxa and quantity of periphyton attached on leaves of *V. asiatica* in each tank were analyzed. The biomass of algae shown in Table II was the number of periphyton attached on plants in the whole tank.

The diatom especially *Cocconeis* spp. was the dominant

taxon in all periphyton adhered on leaves of *V. asiatica* in each tank during the experimental period. The diatom accounted for about 90% in periphyton of No.0 tank.

In comparison with No.0 which was without algae inoculation, there were no cyanobacteria attached on the leaves of *V. asiatica* at the end of the experiment except the tank of No.3. The diatom biomass of No.4 and No.5 tanks were larger than No.0 tank. The biomass of diatom in No.5 tank was about 2 times compared with that of No.0 tank. However, the diatom biomass of No.2 and No.3 were less than No.0 tank. The biomass of diatom in No.2 tank was just 12×10^5 cells. *Cocconeis* spp. was also the dominant species in each tank after the experiment compared with the initial result.

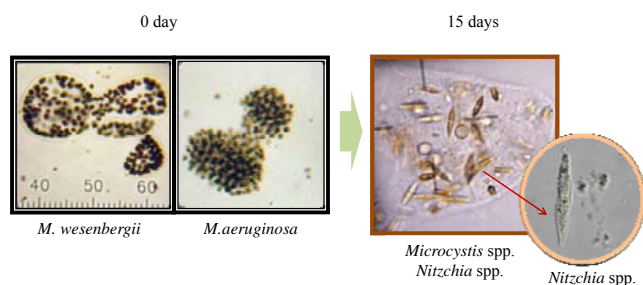


Fig. 6 Photos of *Microcystis* spp. colony's change during experiments in the tank of No.1 and 2

IV. DISCUSSIONS

A. Effects of *V. asiatica* and Algae on the Water Quality

The settled *Microcystis* spp., some of which have died, can release N and P which was the reason for an increase of N and P [8]. This can explain why the N and P contents of No.1 and No.2 in the water column were high after the experiment. The nutrient concentrations at the end of the experiment were lower than initial values owing to the absorption of large amounts of nutrients by the submerged macrophyte when the *V. asiatica* biomass was larger than 20g. Furthermore, decayed phytoplankton releases other nutrients into the water column which can in turn be used again by phytoplankton since phytoplankton requires not just N and P to grow, but other nutrients as well [9]. Maybe this is a reason why the concentration of algae cells in No.3 is high. The turbidity of No.3 was also high. Thus, the high concentration of algae cells was an important reason for a high turbidity in eutrophic lakes. The subsequent release of organic matters and nutrients to the water column from the decomposition of algae leads to a positive feedback cycle providing increasingly more nutrients for the growth of new algae and has become a new type of internal pollutant [9]. Many researchers studied the release of different elements from decomposing phytoplankton debris [10] and it is known to be an important internal nutrient source [11]. For example, Sun et al. in [12] found that the abundant colloidal, particulate, and dissolved nutrients could be released during the death and decomposition of cyanobacteria blooms from Lake Taihu. Colloids have been shown to be supplementary sources of nutrients, including N, P, Fe and other trace elements promoting microalgae growth [13].

In tanks of No.3 and 4, the nutrient concentrations decreased sharply because of the absorption of *V. asiatica* with large biomass. In the tank of No.5, some roots of aquatic plants decayed because of low DO (Fig. 2) which was caused by lots of *V. asiatica* biomass at the end of the experiment. $\text{NH}_4^+\text{-N}$ and DTP of No.5 were relative high because of the releasing nutrients by rot roots of *V. asiatica*.

B. Effects of *V. asiatica* Biomasses on the Algae Community Composition

Many studies implied that macrophytes had an inhibitory effect on the growth of phytoplankton owing to competition for nutrients, secretion of inhibitory organic compounds and shading [14]. However, the results in this study showed that the

algae biomass increased firstly and then decreased with the increasing of *V. asiatica* biomasses. Allelopathy was defined by Molisch [15] to include all biochemical interactions among higher plants and between higher plants and microorganisms, both stimulatory and inhibitory actions. It is possible that the less biomass of macrophyte could secrete organic matters which would quickly decompose to fertilize the water [16]. Therefore, the small biomass of *V. asiatica* could cause a development of phytoplankton biomass in the No.2 and 3 tanks. When the biomass of *V. asiatica* was larger than 200g, *V. asiatica* will effectively inhibit *Microcystis*. Schriver et al. in [17] also found that phytoplankton biomass abruptly declined at a threshold level of 15–20% PVI (plant volume infested) for large-scale (100 m) enclosures in a shallow eutrophic lake. It seemed that macrophytes have the potential to both simulate and inhibit the growth of algae in the condition of this study. The final outcome for algae biomass may depend on both macrophyte species and density. This was consistent with the result that low concentration allelochemicals of aquatic plants promoted the growth of algae and high concentration allelochemicals control the growth of algae [18]. It is not surprising that the algae biomass of No.3 tank reached maximum.

In the tanks of No.3, 4, and 5, cyanobacterial was still the dominant taxon of phytoplankton. The buoyant phytoplankton taxa, including harmful cyanobacteria like *Microcystis* spp., tend to be favored during periods with warm weather and weak wind mixing [19]. The biomass of *Microcystis* spp. declined significantly with the increasing of *V. asiatica* biomasses. *Nitzschia* spp. declined while *Cocconeis* spp. increased obviously with the increasing biomass of *V. asiatica* in tanks of No.3, 4, and 5. The biomass of diatom increased in tanks of No.3, 4, and 5, in general. Phytoplankton species often exhibit differential sensitivity against allelopathic activity of macrophytes [20]. Jasser's research confirmed the macrophyte-produced allelopathic substances mainly restrict the growth of cyanobacteria and phytoplankton cyanobacteria are more sensitive to macrophyte-produced allelopathic substances than other algae species [21].

The epiphytic *Microcystis* spp. was inhibited effectively by *V. asiatica*. Although macrophyte *Stratiotes aloides* stimulated or did not affect the growth of epiphytic cyanobacteria [22], *Cocconeis* spp. was the dominant epiphytic algae attached on leaves of *V. asiatica* in our study maybe because of different the macrophyte. The biomass of epiphytic *Cocconeis* spp. had an increased trend with the increasing of *V. asiatica* biomass in the No.2-5 tanks. Some epiphytic species co-occurring with the plant could even benefit from the production of allelochemicals by the plant [23], as these substances may provide an advantage for epiphytic species in the competition with phytoplankton for nutrients. Consequently, the growth of epiphytic *Cocconeis* spp. increases in the water with macrophyte *V. asiatica* during the study. It was inferred that there was competition between *Nitzschia* spp. and periphytic *Cocconeis* spp. There was competition between *Microcystis* spp. And periphytic *Cocconeis* spp. even *Nitzschia* spp. were absent [24]. We hypothesized that after the multiplication of *Nitzschia* spp. was

controlled by the periphytic *Cocconeis* spp., *Microcystis* spp. were also controlled by the increasing biomass of *V. asiatica*. This difference in sensitivity of different phytoplankton species to allelopathic substances is likely to influence the competitive balance between cyanobacteria and other algae [25].

It was expected that the *Microcystis* spp. of No.1 and No.2 increased during the experimental period because of no or less addition of *V. asiatica* in the tanks. However, the result was in opposition to what is expected. Actually *Microcystis* spp. of No.1 and No.2 reduced sharply. At the same time, in the tanks of No.1 and 2, lots of *Nitzschia* spp. appeared while *Microcystis* spp. declined greatly. It was observed that *Nitzschia* spp. intrude into *Microcystis* spp. colony and multiplied quickly during experimental periods by microscope (Fig. 6). Maybe the competition of environmental conditions was benefit to *Nitzschia* spp. on the condition of our experiment. The temperature of 25°C, the nutrient composition and illumination intensity of 3,400 lux in our laboratory were benefit to *Nitzschia* spp. to grow. It has generally been accepted that cyanobacteria have higher temperature optima for growth than other phytoplankton groups. Harmful cyanobacteria such as *Microcystis* populations have been shown to succeed at, or above 25°C [26]. In addition, the illumination intensity of aquatic environment under full sunlight could reach or even exceeded 90,000 lux which was far higher than that in the laboratory. Consequently, lots of *Nitzschia* spp. reproduced in the *Microcystis* spp. colony because of their physical characteristics. The gas vesicle in *Microcystis* spp. cell can adjust its vertical position in the water by buoyancy form gas vesicle. The buoyancy of gas vesicle cannot offset the increase of cell densities based on a large number of sugars mainly in the form of starch produced during photosynthesis in *Microcystis* spp. cells in the daytime [9]. *Microcystis* spp. rose again because that cell densities decreased because of consuming starch during respiration at night [27]. The *Nitzschia* spp. in *Microcystis* spp. colony accelerated the rate of decline because of the increasing density of *Microcystis* spp. colony after invaded by *Nitzschia*. As a result, lots of *Microcystis* spp. settled down at the bottom of tanks [28], [29]. In reality, the community structure and dominant taxon of phytoplankton result from mutual competition among various algae [30]. It will be another good method to control *Microcystis* spp. by changing the environmental conditions of eutrophic water bodies so that the other species algae, such as diatom, replace toxic cyanobacteria as the dominant algae. The specific factor and mechanism for dominant diatom were not clear and needed further single-species culture experiment.

In a word, in the case without *V. asiatica*, the dominant algae altered with the change of environment conditions. After adding *V. asiatica* into the water body, the competition between phytoplankton and periphyton existed also besides the competition between algae and *V. asiatica*. The relationship among organisms was more complex after introducing *V. asiatica*. *V. asiatica* could inhibit harmful *Microcystis* when the biomass of macrophyte *V. asiatica* was larger than 50g. Hence we inferred that an introduction of *V. asiatica* in Lake Taihu may be a good method to control the cyanobacterial bloom.

However, the biomass of *V. asiatica* should be large enough to control the algae bloom.

V. CONCLUSION

Results from the laboratory experiment in this study implied that the biomass of *Microcystis* spp. reduced with the increase of *V. asiatica* with large biomass. Therefore, it was expected that *V. asiatica* could control an excess of *Microcystis* spp. in Lake Taihu. The biomass control of *V. asiatica* was very important for its inhibitory effect to *Microcystis*.

At the same time, epiphytic *Cocconeis* spp. appeared while *Nitzschia* spp. lessened in the tanks with large quantity of *V. asiatica*, which indicated that there was competition between epiphytic *Cocconeis* spp. and *Nitzschia* spp. It is also a reason why aquatic plants could control the cyanobacterial bloom. Planktonic and epiphytic algae responded differently to *V. asiatica*. The presence of macrophyte *V. asiatica* in eutrophic waters has often a positive effect on algae compositions.

It was found unexpectedly that that *Microcystis* spp. reduced sharply while *Nitzschia* spp. increased greatly when there was little quantity of *V. asiatica*, which indicated that diatom (mainly *Nitzschia* spp.) can control the multiplication of cyanobacteria effectively also by adjusting the environmental condition which was benefit to diatom. Its effectiveness will be further proved in practice.

ACKNOWLEDGMENT

This research was financially supported in part by a grant from Mitsubishi Corporation. Thank Mr. Nakata Yoshikaka of Japan Tobacco Inc. for contributing to this research.

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