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Table: 1, Figs : 3

Combined allelopathic potential of aquatic plants species to control Algae

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ABSTRACT

In field investigations, we discovered that in areas of high diversity of aquatic plants species, there was low density of harmful phytoplankton (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*). The β diversity and evenness of aquatic plants communities in all sites reduced the phytoplankton population(*Microcystis aeruginosa* and *Chlorella pyrenoidosa*]. Major reason for the algal inhibition, was combined effects of allelopathic potential from all macrophytes. In greenhouse tests, the combinations of 2 or >2 plant species [*Alternanthera philoxeroides*, *Ranunculus sceleratus*, and *Trapa incise*], showed >50% suppressive capacity of two representative phytoplankton (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*). The synergistic effects in the combined plants allelopathy depended on kinds of plants species, their ratios, test period and presumed allelochemicals, etc. The aquatic plant species diversity based on comprehensive allelopathy would play an important role in blocking harmful algae bloom. So it was feasible for macrophytes biodiversity to eliminate the cyanobacteria in some shallow lakes.

Key words: Plant diversity, algicial effect, joint allelopathy, phytoplankton, ecological sustainability.

INTRODUCTION

In recent years, nutrients pollution (nitrogen and phosphorus) of natural shallow surface water bodies (lake, river, pond, brook, reservoir) has led to severe eutrophication and cyanobacteria outbreak in these water bodies. Hence the control of water bloom algae is necessary. Now chemical, physical and biological methods are available for their control (8-10). Recently many studies investigated the algicidal effects of aquatic macrophytes such as emergent, floating, submerged plants. The allelopathic inhibition of macrophytes on algae, the novel and environment-friendly method to control algal blooms has drawn much attention. The aquatic extract of *Arundo donax* L. (giant reed) inhibits the growth of *Microcystis aeruginosa* and reduces its cell size with increase in extract concentration (6). The allelopathic effects of extract of *A. dona* on algae were strain-specific (6). Wang *et al.* (2012) cultured three submerged macrophytes *Lindernia rotundifolia*, *Hygrophila stricta* and *Cryptocoryne crispatula* in raw water of Guishui Lake. They found that these species inhibits the cyanobacterial growth (13) and simultaneously

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improves the water quality in the microcosms than control. It resulted from typical allelochemicals besides phenolic compounds involved in algal inhibition and water purification (1). These findings suggested that aquatic macrophytes offered the potential of low-effort and sustainable management for the freshwater ecosystem to reduce excessive algal growth.

The individual aquatic macrophyte synthesized and released less allelochemicals, their allelopathy showed specific selectivity in algal control and persisted for short period to kill the harmful phytoplankton. These shortcomings have limited the wide application of aquatic macrophytes. There was little information on algicidal effects of plants diversity or their combinations. Up to now, only a report from Chinese scientists designed four aquatic plant communities and concluded that the culturing water of four aquatic plants communities had the strong inhibiting effect on *M. aeruginosa* growth (12). Accordingly in present study, some indexes of aquatic plants diversity were surveyed in the field, the allelopathic effects of various combinations of three typical macrophytes were studied on harmful phytoplankton growth under laboratory conditions. Based on field investigation and laboratory tests, allelopathic effects of aquatic plants diversity were studied on the representative phytoplankton.

MATERIALS AND METHODS

Ⅰ. Plot design and plant diversity survey in the field

In July and August, 2010 (the best period for aquatic plants growth), some growth parameters of herbal plants species in 10 tributaries of Chaohu Lake were investigated, due to low density of shrub and woody plants (Fig.1). In each tributry, based on disturbance style and environmental rank, 15 plots on the upper, middle, down parts of the branch were screened randomly, whose community was characterized by its dominant species. In a plot, 10 quadrats along the three sampling lines, paralleling with the river bank, were designed and separated in certain distance. So there were 150 sampling locations in a tributary. In every location, the investigation covered many parameters such as kind, density, coverage rate, abundance rate and frequency of aquatic plants and phytoplankton density.

Ⅱ. Algal inhibition of test plants

The tested axenic phytoplankton strains of *M. aeruginosa* and *Chlorella pyrenoidosa* were obtained from the Freshwater Algae Culture of Hydrobiology Collection (FACHB) China. Three typical aquatic macrophytes (*Alternanthera philoxeroides*, *Ranunculus sceleratus* and *Trapa incise*) were collected from the watershed of Chaohu Lake. Algal culture conditions and algicidal bioassay of single macrophyte have already been reported (15).

Similarly, all combinations of three macrophytes based on biomass ratio were discussed and analyzed for their algicidal effects. Each of three combinations consisted of 5- relative ratios: (i) *A. philoxeroides* + *R. sceleratus* (9:1, 7:3, 5:5, 3:7 and 1:9), (ii) *A. philoxeroides* + *T. incise* (9:1, 7:3, 5:5, 3:7 and 1:9), (iii) *T. incise* + *R. sceleratus* (9:1, 7:3, 5:5, 3:7 and 1:9). The combinations of *A. philoxeroides* + *R. sceleratus* + *Trapa incise* were studied in 5-ratio series (1:1:1, 1:2:1, 1:2:2, 1:2:3 and 1:2:4). Regardless of ratio, the total concentration of combined herbal extracts was kept at 10 mg·ml^{-1}. The culture

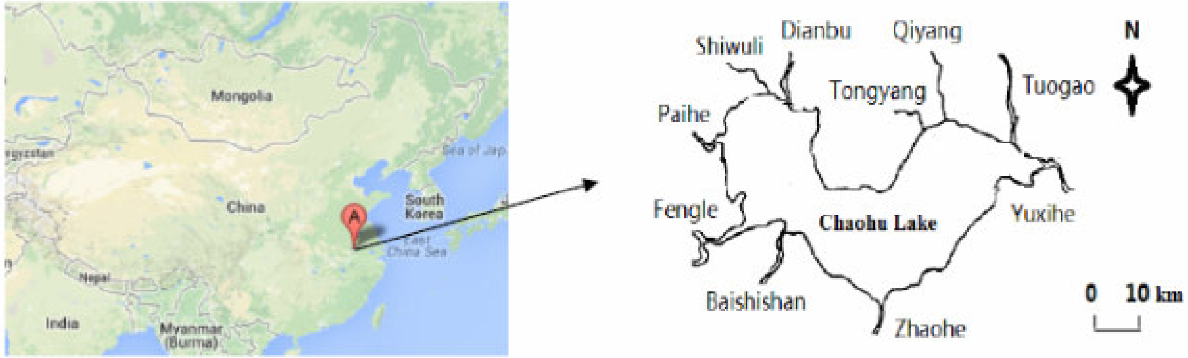

Figure 1. Watershed of Chaohu Lake, Southeastern Anhui Province, China (117°16´54˝–117°51´46˝E and 31°25´28˝–31°43´28˝N). It showed severe eutrophication. Ten main/branch rivers bring water to this lake (A: Chaohu lake location from Google Maps).

Table 1. Plant diversity and algal growth status in investigated areas

Locations (river)	Species abundance	Diversity		Ecological dominance	Community Evenness	Phytoplankton density ($*10^5$/L)	Blue algae Density ($*10^5$/L)	Green algae Density ($*10^5$/L)	Dominant aquatic species
		α	β						
Zhaohe	20b	3.16c	0.09d	0.07a	0.95a	88.13c	75.28c	9.34de	*Phragmites australis*
Qiyang	17c	3.69a	-0.07d	0.07a	0.43f	196.51b	79.12c	78.23a	*R. sceleratus*
Fengle	16cd	3.01d	-0.13c	0.06b	0.89b	212.54b	189.15b	20.75b	*A. philoxeroides*
Paihe	17c	2.58e	-0.07d	0.06b	0.70d	76.18d	29.34e	20.85b	*A. philoxeroides*
Yuxihe	19bc	3.01d	0.04e	0.06b	0.81c	50.23e	31.56e	15.16c	*A. philoxeroides*
Baishishan	21ab	3.34b	0.15c	0.06b	0.56e	23.26	8.96	7.68e	*Potamogeton recurvatus*
Tongyang	15cd	3.71a	-0.18b	0.06b	0.89b	76.48d	65.46d	10.12d	*Typha angustifolia*
Shiwuli	23a	3.76a	0.26a	0.05c	0.85bc	15.29g	5.72g	3.24g	*Nymphoides peltatum*
Tuogao	13d	3.45ab	-0.29a	0.05c	0.68d	325.68a	298.34a	21.65b	*Sagittaria trifolia*
Dianbu	22a	3.52b	0.20b	0.05c	0.93a	26.19f	18.16f	5.14f	*T. incise*

Note: Small letters indicate significant difference at $P < 0.05$ between ten sites (In same column).

condition and algal density determination was same to the treatment of the individual aquatic macrophyte.

Ⅲ. Statistical analyses

As per Zuo *et al.* (16), the diversity of aquatic plants and algal resistance was expressed by the index such as alpha diversity (α), beta diversity (β), between-habitat diversity as Whittaker index (βW), ecological dominance (C), community Evenness (JP) and inhibitory rate (IR)(16).

Analysis of variance (ANOVA) was done based on the Least Significant Difference (LSD), or a t-test. A multiple comparisons analysis was done using SPSS 15.0. Significant differences ($P<0.05$) of pair-wise comparisons between both treatments (represented by small letters in the figures), were detected using the Student's t-test.

RESULTS

I. Field studies

In 10- locations, plant species abundance ranged within 13 – 23, with a mean of 19. The most and least plant species appeared in Shiwuli and Tuogao tributories respectively. In most surveyed areas, their dominant species were floating aquatic plants (*Nymphoides peltatum*, *A. philoxeroides*, *Sagittaria trifolia* and *T. incise*). The mean value of α-diversity among 10-locations was 3.32 and the index of nine habitats showed a value over 3.0.

Based on β-diversity, these 10-sites can be divided into two categories: (i). High heterogeneity in Shiwuli, Baoshishan, Zhaohe, Yuxihe, and Dianbu locations and (ii). Low heterogeneity, or a high homogeneity in the rest. By relationship analyses, there was significant positive correlation between the species abundance, α-diversity and community evenness. But a markedly negative relationship existed between the species abundance and β-diversity.

In all investigated regions, the phytoplankton density varied considerably. The density in Tuogao branch was over 20-folds more than Shiwuli. Among all phytoplankton, blue algae covered 37.4 - 91.6% and green algae covered 6.6 - 39.8%. Meanwhile, *M. aeruginosa* and *C.pyrenoidosa* were the predominant species in phytoplankton assemblage. Under field conditions, there was negative relationship between the aquatic plants diversity and phytoplankton amount. Similarly, it was deduced that β-diversity and community evenness would significantly decreased the phytoplankton density (Table 1) .

II. Laboratory studies

The inhibition rates of phytoplankton on *M. aeruginosa* from three species viz.; *A.philoxeroides*, *R.sceleratus*, and *T.incise* varied were : 38 - 80%, 52 - 80%, and 25 - 49%, respectively [Fig.2 (1), Fig.2 (3), Fig.2 (5)].. In tested periods, the longer the test period, the stronger was the algicidal effect. Three typical macrophytes showed decreasing order of algicidal effects *R.sceleratus* > *A.philoxeroides* > *T.incise*. Similarly, these 3-species showed less suppression effect (< 50% inhibition) of *C. pyrenoidosa*. Tthe inhibitory effects of these species on *C. pyrenoidosa* did not increase significantly with the tested period [Fig.2 (2), Fig.2 (4), Fig.2 (6)].

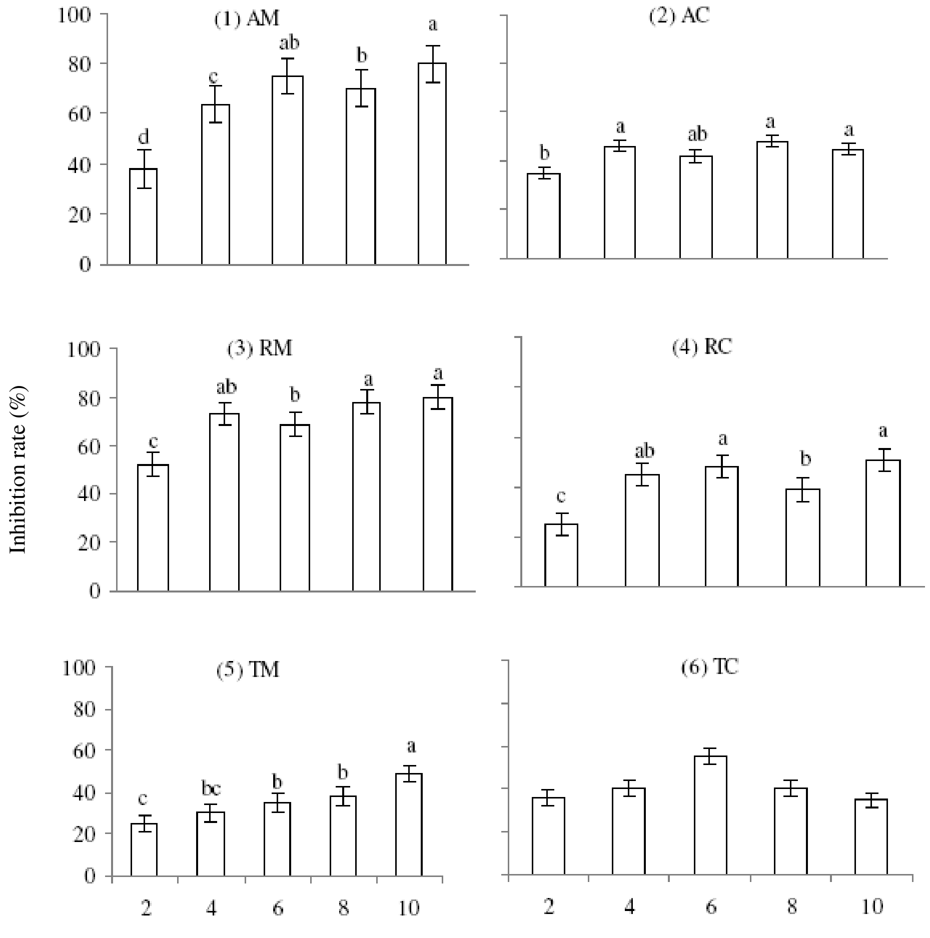

Figure 2. The algicidal effects of individual plant species on 3-typical macrophytes (*Alternanthera philoxeroides*, *Ranunculus sceleratus* and *Trapa incise*) at various time periods (2d,4d,6d,8d,10d). 3-aquatic macrophytes (*A. philoxeroides*, *R. sceleratus* and *T. incise*). were assessed for algicidal effects based on two water-bloom phytoplankton receptors (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*) at vatious days after Treatment. A: *Alternanthera philoxeroides*, C: *Chlorella pyrenoidosa,* M: *Microcystis aeruginosa*, R: *Ranunculus sceleratus*, T: *Trapa incise.* Small letters indicate significant difference at P <0.05 between the five treatments at the different test periods.

(ii). Combined inhibitory allelopathic effects of 3-aquatic macrophytes on algal growth

In all ratios, the algicidal effects of combination *A. philoxeroides* + *R. sceleratus* on *M. aeruginosa* was stronger than individual species and was closely correlated with the test time. In 1:9 ratio, the algicidal capacity of combination was strongest [Fig. 3 (1)].

Similarly, the algicidal effect of combination *A. philoxeroides* + *R. sceleratus* on *C. pyrenoidosa* was stronger than either of both species. The 9:1 ratio of *A. philoxeroides* and *R. sceleratus* had maximum algicidal effect [Fig. 3 (2)]. The combination of *A. philoxeroides* and *R. sceleratus* was more inhibitory to the *M. aeruginosa* than to *C. pyrenoidosa*

The inhibition potential of combination *A. philoxeroides* + *T. incise* on *M. aeruginosa* was stronger than the individual macrophytes. Only in 1:9 ratio, the algicidal capacity of the combination increased significantly with the tested time. While in 9:1 ratio, the algicidal potential of combination was strongest [Fig. 3 (3)]. Similarly, the inhibition rates of combination *A. philoxeroides* + *T. incise* on *C. pyrenoidosa* were > 50%. The 9:1 ratio of *A. philoxeroides* and *T. incisa* displayed strong inhibition [Fig. 3 (4)]. The combination of *A. philoxeroides* + *T. incisa* inhibited the *C. pyrenoidosa* more than *M. aeruginosa*.

The combination *T. incise* + *R. sceleratus* caused greater inhibition of *M. aeruginosa* than single macrophyte. Irrespective of the ratio, the test time positively affected the algicidal effects. The algicidal capacity was strongest in 1:9 ratio. After 4 days, the inhibition rate was < 50% [Fig. 3 (5)]. Similarly, the combination *T. incise* + *R. sceleratus* showed increased algicidal effects on *C. pyrenoidosa* compared with individual. In 5:5 ratio, the combination exhibited the strongest inhibition [Fig. 3 (6)]. The inhibitory potential of combination *T. incise* + *R. sceleratus* on *C. pyrenoidosa* were weaker than on *M. aeruginosa*.

The combination *A. philoxeroides* + *R. sceleratus* + *T. incise* were most inhibitory to *M. aeruginosa* than either individual species or the combinations of both species. Among all treatments, the algicidal effect was positively related to the test time. In 1:2:4 ratio, the algicidal potential was strongest. After the mid-term of test period, the inhibition rate significantly surpassed 50% [Fig. 3 (7)]. Similarly in 1:1:1 ratio, the combination of *A. philoxeroides* + *R. sceleratus* + *T. incise* on *C. pyrenoidosa* displayed strongest inhibition. The inhibition rates of combination of three aquatic macrophytes on *C. pyrenoidosa* were stronger than *M. aeruginosa*. [Fig. 3 (8)].

DISCUSSIONS

Biodiversity is a major determinant of ecosystem function. Species-rich communities often use resources more efficiently, thereby improving the performance of community. The increasing vegetation biodiversity in the agroecosystems reduces the impact of pests and diseases (11). Wortman *et al.* (14) concluded that the mixture of diverse cover crops provided more weed control. In our field investigation, macrophytes abundance would reduce the density of phytoplankton assemblage. Meanwhile, a high β-diversity and community evenness would significantly reduce the outbreak of harmful phytoplankton. So the aquatic macrophytes diversity would significantly suppress the water-bloom algae in field or laboratory. Similarly in a microcosm, the functional combinations of aquatic macrophytes regulates the water quality parameters like water temperature, pH, and etc, and affect the phytoplankton quantity present in the surface water (2). Therefore, the plants diversity shapes the impact of local biotic and abiotic processes.

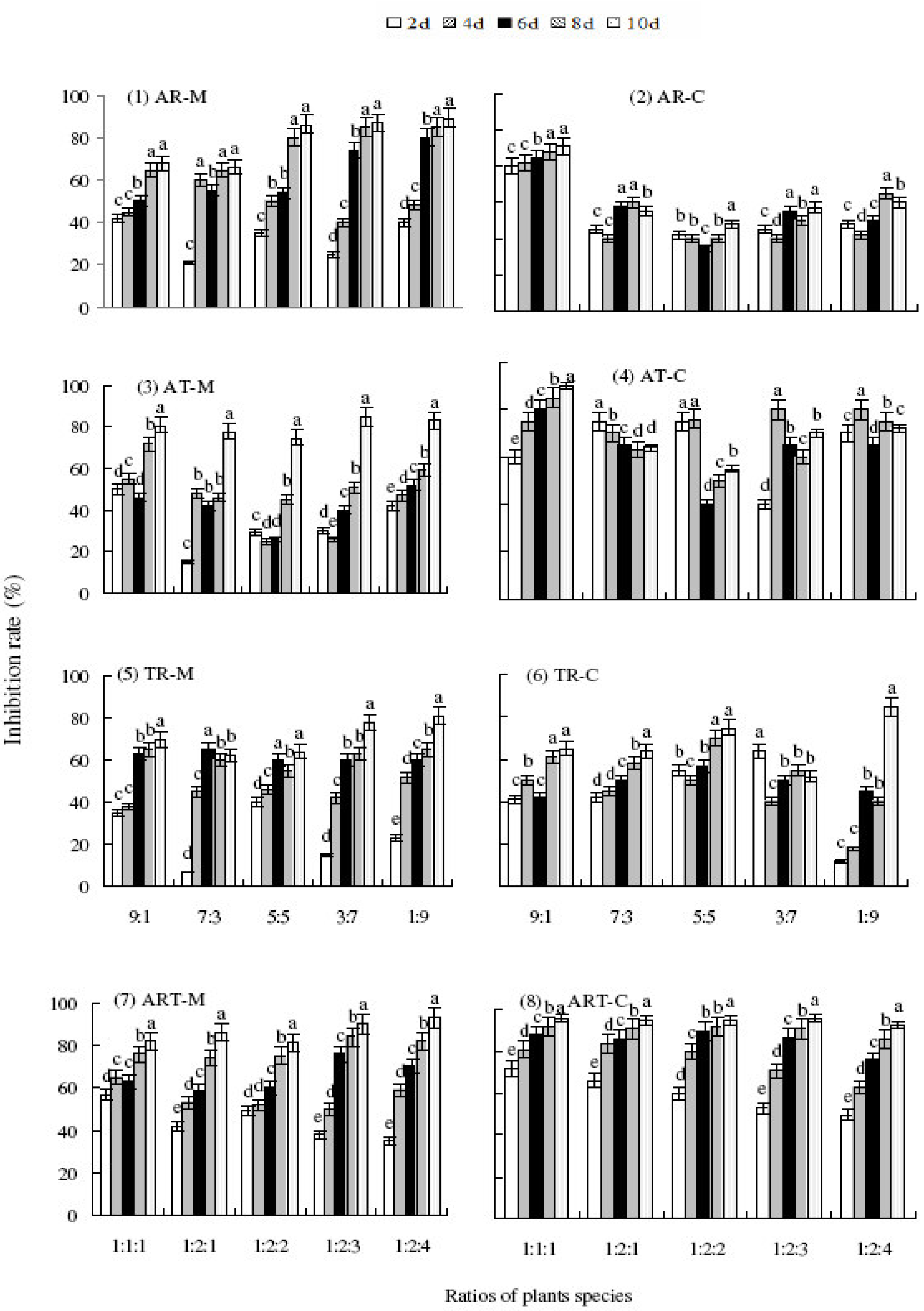

Figure 3. Combined algicidal effects of three representative aquatic plants. The sub-figures (1) – (6) showed the algical effect of three combinations of two species from three typical macrophytes in same ratio. Sub-figures (7)-(8) showed algal inhibition of one combination from these three plants. A: *Alternanthera philoxeroides*, C: *Chlorella pyrenoidosa,* M: *Microcystis aeruginosa*, R: *Ranunculus sceleratus*, T: *Trapa incise.* Small letters indicate significant difference at $P < 0.05$ between five treatments at various test periods and under certain ratio.

The aquatic macrophytes diversity helps to combat the harmful algae. Perhaps, there were all kinds of mechanisms such as competition, shade effects and the inhibitory effects. In addition, allelochemicals produced by aquatic macrophytes decreases the harmful algae bloom (5). In this study either *A. philoxeroides* or *R. sceleratus* or *T. incise* would reduce > 50% quantity of *M. aeruginosa* than control. So it was possible that allelopathically active submerged macrophytes can stabilise the clear-water states in shallow lakes (4). If these species were combined in certain ratios, a synergetic effect in allelopathic potential of diverse aquatic macrophytes would occur. In this study, we concluded that the inhibition potential of phytoplankton was related to number of macrophyte species with algicidal effects. In individual plant extracts, *C. pyrenoidosa* was less sensitive. *C pyrenoidosa* became more sensitive to combination of spp. The algal inhibition of combinations differed and relied on the ratios and the species of aquatic macrophytes involved. So it can be hypothesized that all allelopathic compounds released by these combinations would have synergetic effects on the harmful phytoplankton. The joint action of phenolic acid mixtures stimulates the allelopathic potential of donors on receptors (7). These compounds may be involved in the environmental complex of managed or natural ecosystems and their sustainability. However, these combinations showed a limited use in harmful phytoplankton control, because of the antagonistic effects between allelochemicals. He and Zhang (2012) used the mathematical models to discuss the joint algal growth inhibition of two selected allelochemcials pyrogallic and linolenic acids (3). Therefore, it was feasible for plant species diversity to control harmful algae bloom based on the avoidance of the antagonism among the presumed allelochemicals.

In this study, plants combination provided strong allelopathic inhibition of harmful phytoplankton. So beside space and light competition, nutrients removal, de-nitrification, the additional allelopathic potential would lead to a comprehensive effect in ecological restoration of disturbed aquatic ecosystem (17). The artificial wetland and ecological floating island by utilizing the principle were typical good cases. Although plant biodiversity displayed an unique property in suppressing the algal bloom, it was very crucial to choose the optimum combination of emergent, floating and submerge plants, to design the size and optimal structure of the community, and to erect the ecological process and the stable flowing of biomass and energy in the future.

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REFERENCES

1. Dziga, D., Suda, M., Bialczyk, J., Czaja-Prokop, U. and Lechowski, Z. (2007). The alteration of *Microcystis aeruginosa* biomass and dissolved microcystin-Lr concentration following exposure to plant-producing phenols. *Environmental Toxicology* **22**: 341-346.
2. Gardner, L.M., Bouchard, V. and Frey, S. (2004). Effects of macrophyte functional group and functional group diversity on surface water parameters. *Ohio Journal of Science* **104** : 37.
3. He, Z.X. and Zhang, T.T. (2012). Model development and optimization on the combined algal growth inhibition effects of two allelochemicals. *Acta Scientiae Circumstantiae* **32**:1894-1901. (Chinese).
4. Hilt, S. and Gross, E.M. (2008). Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology* **9**: 422-432.
5. Hong, Y., Hu, H.Y., Xie, X. and Li, F.M. (2008). Responses of enzymatic antioxidants and non-enzymatic antioxidants in the cyanobacterium *Microcystis aeruginosa* to the allelochemical ethyl 2-methyl acetoacetate (EMA) isolated from reed (*Phragmites communis*). *Journal of Plant Physiology* **165**:1264-1273.
6. Hong, Y. and Hu, H.Y. (2007). Effects of the aquatic extracts of *Arundo donax* L. on the growth of freshwater algae. *Allelopathy Journal* **20**: 315-326.
7. Inderjit, J., Streibig, C. and Olofsdotter, M. (2002). Joint action of phenolic acid mixtures and its significance in allelopathy research. *Physiologia Plantarum* **114**: 422-428.
8. Klemencic, A.K. (2010). The efficiency of ultrasound on algal control in a closed loop water treatment system for cyprinid fish farms. *Fresenius Environmental Bulletin* **19**: 919-931.
9. Murray, D. (2010). The impact of barley straw conditioning on the inhibition of Scenedesmus using chemostats. *Water Research* **44** : 1373-1380.
10. Murray Gulde, C.L., Heatley, J.E., Schwartzman, A.L. and Rodgers, J.H. (2002). Algicidal effectiveness of clearigate, cutrine-plus, and copper sulfate and margins of safety associated with their use. *Archives of Environmental Contamination and Toxicology* **43** :19-27.
11. Ratnadass, A., Fernandes, P., Avelino, J. and Habib, R. (2012). Plant species diversity for sustainable management of pests and diseases in agro-ecosystems: a review. *Agronomy for Sustainable Development* **32**: 273-303.
12. Tian, R., Sun, X. and Wei, Y. (2010). Allelopathic effects of four aquatic plants communities on *Microcystis aeruginosa*. *Ecology and Environmental Sciences* **19** : 2149-2154. (Chinese).
13. Wang, H., Zhong, G., Yan, H., Liu, H., Wang, Y. and Zhang, C. (2012). Growth control of cyanobacteria by three submerged macrophytes. *Environmental Engineering Science* **29**: 420-425.
14. Wortman, S.E., Francis, C.A., Bernards, M.A., Blankenship, E.E. and Lindquist, J.L. (2013). Mechanical termination of diverse cover crop mixtures for improved weed suppression in organic cropping systems. *Weed Science* **61**: 162-170.
15. Zuo, S.P,, Mei, H., Ye, L., Wang, J. and Ma, S. (2012). Effects of water quality characteristics on the algicidal property of *Alternanthera philoxeroides* (Mart.) Griseb. in an aquatic ecosystem. *Biochemical Systematics and Ecology* **43**: 93-100.
16. Zuo, S.P., Ma, Y.Q. and Shinobu, I. (2008). Ecological adaptation of weed biodiversity to the allelopathic rank of the stubble of different wheat genotypes in a maize field. *Weed Biology and Management* **8**: 161-171.
17. Zuo, S.P., Wan, K. and Ma, S.M. (2014). Environmental restoration effects of *Ranunculus sceleratus* L. in a eutrophic sewage system. *Biochemical Systematics and Ecology* **55**: 34-40.