ECOPHYSIOLOGICAL STUDIES OF A SUBMERGED MACROPHYTE VALLISNERIA ASIATICA FOR THE ECOLOGICAL RESTORATION OF EUTROPHIC LAKE TAIHU, CHINA

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March, 2015

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By

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CERTIFICATE

The undersigned hereby certify that they have read and recommended to the graduate school of engineering for the acceptance of this thesis entitled, "**Ecophysiological studies of a submerged macrophyte *Vallisneria asiatica* for the ecological restoration of eutrophic Lake Taihu, China**" by **CAIXIA KANG** in partial fulfillment of the requirements for the degree of **Doctor of Engineering**.

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Abstract

Algal blooms in eutrophic water bodies are becoming a worldwide ecological problem. Lake Taihu, the third largest freshwater lake in China, is a well-known case for eutrophication. In view of the serious bad impacts of eutrophication on human beings and the society, the overall objective of this study was to propose ecological restoration strategies to restore or protect submerged macrophytes in eutrophic Lake Taihu, China by analyzing the antioxidant responses of *Vallisneria asiatica* which is one of submerged macrophytes spread over Lake Taihu, to different adverse environmental factors.

Firstly, effects of *V. asiatica* with different biomass levels on the water quality and algal communities were researched. The toxic *Microcystis* spp. declined to $7{,}104 \times 10^5$ cells·30 L^{-1} and $3{,}720 \times 10^5$ cells·30 L^{-1} from the initial value $29{,}217 \times 10^5$ cells·30 L^{-1} when the *V. asiatica* biomass increased to 200 g and 500 g, respectively. These results indicated that *V. asiatica* could control an excess of the main toxic *Microcystis* spp. when the *V. asiatica* biomass was larger than 50 g in the tank with 30 L solution. However, it was found unexpectedly that when there was no *V. asiatica* or just 20 g of *V. asiatica* existed, *Microcystis* spp. totally disappeared or reduced to 60×10^5 cells·30 L^{-1} while *Nitzschia* spp. increased greatly to $2{,}043 \times 10^5$ cells·30 L^{-1} and $2{,}460 \times 10^5$ cells·30 L^{-1}, respectively. It indicated that diatom (mainly *Nitzschia* spp.) can also control the multiplication of *Microcystis* spp. effectively.

Following, effects of the moderate nutrient concentration (NO_3^--N 1.5 mg·L^{-1}; PO_4^{3-}-P 0.1 mg·L^{-1}), excessive NH_4^+-N (3.5 mg·L^{-1}) and PO_4^{3-}-P (0.6 mg·L^{-1}) in the water column on the antioxidant defense system in *V. asiatica* were studied with the 20-day aquarium experiments. The results showed that the moderate concentration of nutrients can promote the metabolism of *V. asiatica* expressed as increased plant chlorophyll a (Chl.a) and protein contents. Either excessive NH_4^+-N or PO_4^{3-}-P could cause the oxidative stress to cells of *V. asiatica*, expressed as decreased contents of plant Chl.a and protein, and the enhancement of catalase (CAT) activities in leaves of *V. asiatica*. In addition, 0.6 mg·L^{-1} of PO_4^{3-}-P caused more oxidative damage to *V. asiatica* than 3.5 mg·L^{-1} NH_4^+-N. The results indicated that the antioxidant defense mechanisms could be activated but still could not prevent the damage of the metabolism system in *V. asiatica* exposed to either excessively high concentrations of NH_4^+-N or PO_4^{3-}-P. Therefore, it is necessary to establish a dual control strategy of nitrogen (N) and phosphorus (P) for the restoration of *V. asiatica* in eutrophic Lake Taihu.

In another 10-day aquarium experiment, this investigation examined the physiological effects of different plant biomass levels and of increasing algal (mainly toxic cyanobacterial) concentrations on *V. asiatica*. Algal stress suppressed the superoxide dismutase (SOD) activity of the plant's leaves and induced the CAT and peroxidase (POD) activities of its roots. The protein content in *V. asiatica* decreased with an increase in algal concentrations, whereas the malonaldehyde (MDA) increased significantly at algal Chl.a concentrations of 222 and 262 $\mu g \cdot L^{-1}$ in water. *V. asiatica* adapted to the stress caused by high algal concentrations by adjusting its antioxidant defense system to remove the excessive reactive oxygen species (ROS) when the algal Chl.a concentration was > 109 $\mu g \cdot L^{-1}$. Additionally, high biomass of *V. asiatica* (2,222 g $FW \cdot m^{-2}$) can inhibit the reproduction of algae more significantly than low biomass (1,111 g $FW \cdot m^{-2}$). High biomass of *V. asiatica* increased the oxidative stress in an individual plant when the initial algal Chl.a concentration in the water reached 222 and 262 $\mu g \cdot L^{-1}$, as expressed by the increased MDA in leaves, compared with low biomass of *V. asiatica*. This provides a basis for controlling algal concentrations and *V. asiatica* biomass for the recovery of *V. asiatica* in eutrophic Lake Taihu.

Then, the physiological responses of *V. asiatica* to three kinds of eutrophic sediment in Lake Taihu were assayed in a 40-day aquarium experiment. The three kinds of the representative sediment were got from East Lake Taihu, Western shore and Meiliang Bay, respectively. The plant Chl.a content stopped increasing from the 10th day and there were no obvious differences in plant Chl.a between the three kinds of sediment. The MDA content in roots had limited changes while the MDA content in leaves of *V. asiatica* growing on the Western shore sediment was significantly higher than that on the other two sediment types from the 20th day. The SOD activity in roots and leaves of *V. asiatica* growing on the Western shore sediment was lower than that on the other two sediment types on the 10th and 40th day, respectively. The CAT activity in roots increased sharply during the early period while the CAT activity in leaves increased with time slightly. The results showed that the *V. asiatica* can adjust itself to the eutrophic sediment in Lake Taihu via antioxidant responses and suffered the oxidative stress.

According to the summary of the growth conditions of *V. asiatica* depending on the results of all the experiments, the Lake Taihu was divided into 4 parts and the corresponding restoration measures of submerged macrophytes were proposed, connecting to the distribution of total nitrogen (TN), total phosphorus (TP) and algal Chl.a, and the contamination degree of sediment in Lake Taihu.

(1) For Wuli Bay, Meiliang Bay, Zhushan Bay and Western shore, after the further managements and physical measures are proceeded to reduce the nutrient loading and algal accumulation, establishing “Eco-engineering dams” was suggested to continuously improve the water quality so that there is a possibility for submerged macrophytes to restore someday.

(2) For Gonghu Bay and Southern shore, some measures are used to reduce the nutrient loadings without dredging and removing algae manually before the restoration of submerged macrophytes.

(3) For Central Lake, the ecological system restores naturally after the ecology of lake shores go back to their normal levels.

(4) For Eastern shore and East Lake Taihu, the harvest of floating-leaved macrophytes during the early growth stage of submerged macrophytes has a positive impact on the submerged macrophytes. Then the management of nutrient sources into the river, removal of sediment, and the improvement of the macrophyte biodiversity to combine the harvest are necessary to prevent or delay lake swamping.

Table of Contents

Abbreviations

ANOVA	Analysis of variance
APX	Ascorbate peroxidase
CAT	Catalase
Chl.a	Chlorophyll a
DO	Dissolved oxygen
DW	Dry weight
Fd_{ox}	Oxidation state of ferredoxin
FW	Fresh weight
Fd_{red}	Redox state of ferredoxin
H_2O_2	Hydrogen peroxide
LEL	Lowest effect level
mC_d	Modified degree of contamination
MCs	Extracellular microcystins
MDA	Malondialdehyde
N	Nitrogen
NBT	Nitro-blue tetrazolium
O_2	Molecular oxygen
$O_2^{\bullet -}$	Superoxide
$OH^{\bullet}$	Hydroxyl radical
OM	Organic matters
P	Phosphorus
POD	Peroxidase
PVI	Plant volume infested
ROS	Reactive oxygen species
SD	Standard deviation
SEL	Severe effect level
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus

CHAPTER 1

INTRODUCTION

CHAPTER

1

Introduction

Abstract

Eutrophication is a worldwide environmental problem, and has drawn much attention from the people all over the world. The water eutrophication is accelerated by human activities involving the development of industry and urbanization, discharge of non-point source pollution, increasing fishery, insufficient wastewater treatment and unsuitable management, and pollutants from inflow-rivers. The overfertilization with nitrogen and phosphorus causes a shift mainly from submerged macrophytes to a dominance of algae, leading to adverse effects on the water quality and the ecosystem function. A series of strategies has been adopted to control the eutrophication and restore the ecosystem, such as the external nutrient loading control, internal source control, manual algal removal, and ecosystem restoration technologies. The restoration of macrophytes has been widely used as one ecological solution to restore the ecosystem of eutrophic water bodies because of their key functions. However, the effects of macrophyte restoration are unstable over the long term and highly case-specific. Thus, it is important to focus on the physiological research on the macrophytes. The eutrophic environments may enhance level of reactive oxygen species (ROS) and cause damage to the macrophytes. Macrophytes possess complex antioxidant defense systems, including superoxide dismutase, catalase, and peroxidase, that scavenge ROS to protect them from oxidative stress. Otherwise excess ROS might result in the disorder of metabolism, including the low photosynthesis efficiency, the decreased protein contents and the increased malonaldehyde contents.

Key words: eutrophication, ecosystem function, control strategy, physiological response, oxidative stress, antioxidant defense

1.1 Lake eutrophication

Eutrophication is described by Harper **(1992)** as “the biological effects of an increase in concentrations of plant nutrients, usually nitrogen (N) and phosphorus (P), on aquatic ecosystems”. Eutrophication is the natural aging process of aquatic ecosystems because of the gradual accumulation of nutrients, which results in an excessive growth of phytoplankton. This undesirable overgrowth of phytoplankton and their subsequent death forms a floating surface bloom and reduces light penetration that restricts the reoxygenation of water **(Beeby, 1995; Rao, 1998)**. The death and decay of phytoplankton produces a foul smell and makes the water more turbid **(Beeby, 1995; Rao, 1998)**.

The eutrophication in freshwater lakes is one of the most severe environmental problems, not only because it would bring significant adverse impacts on ecological functions of lakes, but also because it could influence the ecological services for human society **(Zhao et al., 2005)**, including freshwater supply, fishery, and flood mitigation **(Guo, 2007)**. Thus, the eutrophication in inland lakes has become one of the most widespread environmental and social problems for all countries around the world. For example, lakes such as Victoria in Africa, Okeechobee in the United States, Taihu in China, and the Baltic Sea in Europe were the typical cases **(Duan et al., 2008)**.

China has over 110,000 lakes, which occupy 0.8% of the total area of the country. More than 2,300 of these lakes each cover an area greater than 1 km^2, with a total water storage capacity of 707.7×10^9 m^3 **(Liu and Qiu, 2007)**. About one-third of these lakes are freshwaters, and most lie in the middle and lower reaches of the Chang Jiang River along the eastern coastal area of China **(Qin, 2013)**. Many of the freshwater lakes have become eutrophic with algal blooms **(Qin, 2013)**. In China, the three most eutrophic freshwater lakes are generally referred to as the “Three Lakes”. They are Dianchi Lake in Yunnan province and Taihu Lake and Chaohu Lake in eastern China **(Liu and Qiu, 2007)**. Lake Taihu, the third largest freshwater lake in China, located in the Chang Jiang River delta, one of the more developed areas of eastern China as shown in **Fig. 1.1 (Liu and Qiu, 2007)**. It has an area of 2,338 km^2 and a mean depth of 1.9 m. As shown in **Fig. 1.1**, the administrative regions of the Lake Taihu basin are shared by 14 large cities including Wuxi, Suzhou, and Shanghai **(Liu and Qiu, 2007)**. A total of 35 million people live in the Lake Taihu drainage area and Lake Taihu acts as the main water supply of the surrounding urban and rural areas. The total economic production in this region is one-eighth of that in China **(Pu et al., 1998)**. The eutrophication of Lake Taihu has been greatly concerned because of its significant economic position.

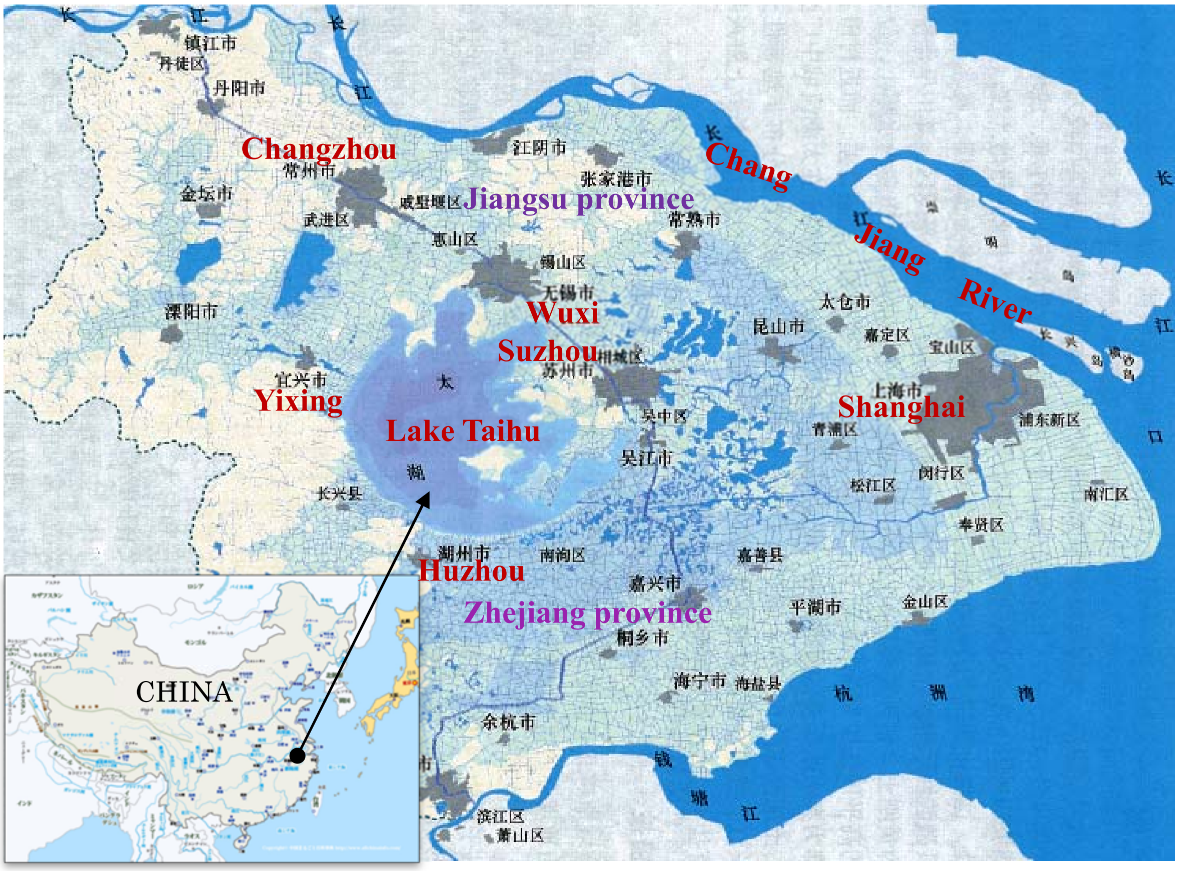


Fig. 1.1 The photo of Lake Taihu.

1.1.1 Causes of eutrophication

In a natural system, nutrients are commonly derived from weathering and leaching from rocks and soils. The eutrophication of lakes is a very slow and natural process and it may take thousands of years for a lake to pass the process naturally **(Liu and Qiu, 2007)**. However, humans strongly influence the aquatic ecosystems and their activities have dramatically accelerated the eutrophication process **(Smith, 2003)**. Nitrogen and phosphorus are the nutrients of most concerning because of their primary role in limiting the growth of algae and aquatic macrophytes in the water bodies **(Novotny and Olem, 1994)**. Excessive input of nutrients, such as nitrogen and phosphorus, greatly accelerates this process with the economic development **(Liu and Qiu, 2007)**. Nutrient sources can be broadly segregated into point sources (such as sewage effluents) and non-point sources (such as the run-off from agriculture land) **(Ongley, 1996)**.

In the past, point source pollution, for example wastewater treatment plant effluents, often formed the major nutrient inputs to water bodies. They are obvious and easily identifiable sources **(Ryding and Rast, 1989)**. Eutrophication control measures are often directly at such effluents. Non-point source pollution occurs when there is no discrete point of discharge and pollution enters the environment by a multitude of pathways **(Whitehead, 2006)**. The main reasons for the eutrophication of Lake Taihu are described in detail as follows.

(1) The increased water use of Lake Taihu: With the development of industry and urbanization in the Lake Taihu drainage area, the water requirements for Lake Taihu have increased, thereby increasing the corresponding wastewater discharge and the amount of pollution entering the Lake Taihu **(Li, 2008)**. For example, water consumption in Wuxi City has increased from just 105 $L \cdot d^{-1}$ in 1980 to 175 $L \cdot d^{-1}$ in 1990, and reached 284 $L \cdot d^{-1}$ in 1999 **(Huang et al., 2008)**. The amounts of annual discharge of industrial and domestic wastewater have a tendency to increase annually. These industries, including textiles, metal smelting, chemical industry, food and tobacco processing, beverage manufacturing, papermaking and machinery production, together used 73% of the total volume of freshwater, and their chemical oxygen demand (COD_{Mn}) discharges accounted for 86% of industrial wastewater for Wuxi City **(Li, 2008)**. The total amount of domestic water use in Wuxi increased from 80 million $kg \cdot d^{-1}$ to 320 million $kg \cdot d^{-1}$ from 1980 to 1999 **(Li, 2008)**.

(2) Changes in land use in the Lake Taihu area: There have been great changes in land use since the policy changes in the 1980s. Large amounts of former agricultural land are used for industry after 1980s **(Huang et al., 2008)**. To compensate for the large loss of cultivated land, the use of chemical fertilizers increased in 1990s resulting in the increasing chemical fertilizer pollution entering into the Lake Taihu with the farmland runoff **(Huang et al., 2008)**.

(3) The developed aquaculture of Lake Taihu: Aquaculture of Lake Taihu is divided into fish pond culture in reclamation areas and the closed net

breeding **(Chen et al., 2008)**. The pond culture fishery existed in the whole reclaimed shoreline areas of the Lake Taihu **(Chen et al., 2008)**. The development of closed net breeding developed rapidly mainly in East Lake Taihu and has had many adverse effects on the aquatic environment, including reduced the purifying ability of the Lake Taihu and contributing to eutrophication **(Li, 2008)**. Such aquaculture requires the input of a large quantity of feed, the amount of which is closed correlated with increases in nitrogen and phosphorus load in the Lake Taihu **(Li, 2008)**.

(4) Insufficient wastewater treatment and unsuitable management in the Lake Taihu area: The handling of industrial effluents still relies mainly on treatments in the factory and the industrial wastewater treatment capacity in the Lake Taihu basin area is insufficient. The urban sewage treatment needs massive investment, and the operating cost is high, so urban sewage treatment has developed slowly in 1990s **(Li, 2008)**. Since 1990, Chinese government has started to close a number of polluting factories and build additional wastewater treatment plants **(Li et al., 2011).** However, the management system fails to manage the municipal sewage effectively and also fails to manage the rivers that bear and receive the sewage discharge **(Li, 2008)**. In fact, only about 40-70% of industrial wastewater in the cities surrounding the Lake Taihu is treated in 1990s **(Liu et al., 2001)**.

(5) Pollutants from inflow-rivers of Lake Taihu: There are about 180 inflow-rivers around Lake Taihu **(Zhang et al., 2008a)**. Input of numerous pollutants (like TN, TP and COD) from rivers is a direct factor in the water quality deterioration of Lake Taihu **(Zhang et al., 2008a)**. A large amount of urban sewage has entered the rivers directly **(Li, 2008).**

1.1.2 Consequences of the lake eutrophication

The eutrophication of shallow lakes is a worldwide ecological problem. The eutrophication of water bodies leads to adverse effects on quality and ecosystem functioning **(Fareed and Abid, 2005)**. The eutrophication process was shown in **Fig. 1.2**. A large amount of nitrogen and phosphorus enters to the lakes through human's activities, which leads to the eutrophication. In the worst case, the eutrophication can result in frequent outbreaks of algal blooms and threaten the reliable supply of drinking water **(Le et al., 2010)**. The overfertilization with nitrogen and phosphorus causes a shift mainly from submerged macrophytes to a dominance of floating algae. This results in anoxic conditions in the water body for the shading effects, and a loss of biodiversity of the lake ecosystem (**Fig. 1.2**). Lake Taihu, being the third largest shallow lake in China, has the typical eutrophication issue of inland water bodies. In 1960s, the water quality of Lake Taihu was good and many kinds of aquatic macrophytes occupied most part of the Lake **(Ye et al., 2007)**. However, the algal blooms have stated to occur since 1970s because of a rapid increase in the development of industry and agriculture **(Ye et al., 2007)**. In the beginning of the 1970s, the algal blooms first appeared in Wuli bay (its site in Lake Taihu was shown in **Fig. 2.1** clearly), and subsequently their scale and frequency constantly increased **(Ye et al., 2007)**. Since 1980s, its water quality has become worse and worse **(Ye, 2007)**. In 1990s, the water blooms took place frequently **(Ye, 2007)**. Recently, the frequency and duration of algal blooms have increased. The algal blooms usually occur in northern bays and extended to the center and south parts of Lake Taihu **(Duan et al., 2009)**. Since 2000, the aquatic macrophyte has declined sharply, and especially submerged macrophyte even disappeared in southwest shores of Lake Taihu **(Cui et al., 2009)**.

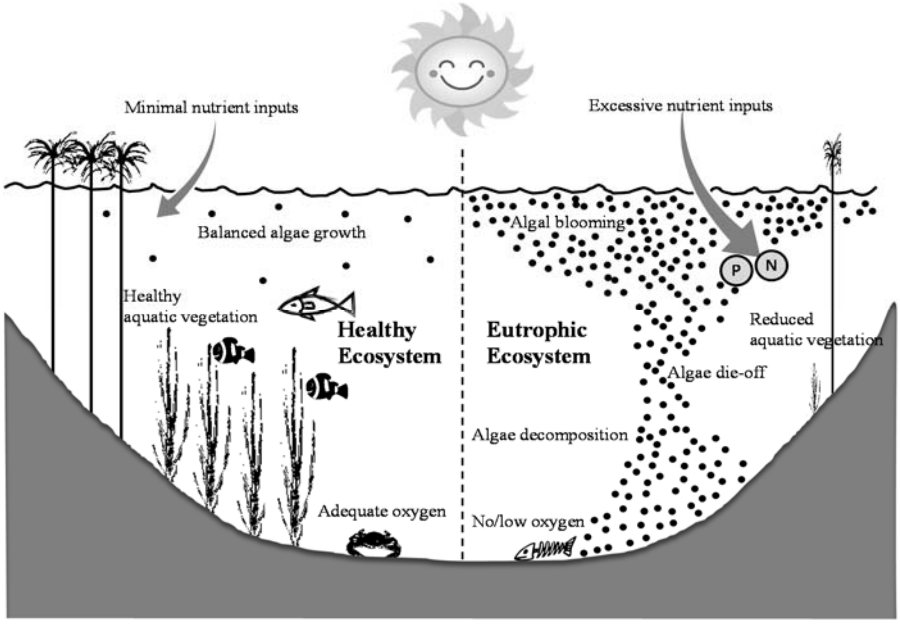


Fig. 1.2 The eutrophication process of lakes.
(http://www.inlandbays.org/about-the-bays/bay-issues/)

1.2 Strategies of controlling eutrophication in lakes

Nutrients such as nitrogen and phosphorus cause algae and other microorganisms to multiply abnormally, which in turn deplete oxygen in water as shown in **Fig. 2.1 (Le et al., 2010)**. This overgrowth of phytoplankton and their subsequent death form a floating surface bloomwhich reduces light penetration that restricts the reoxygenation of water **(Beeby, 1995; Rao, 1998)**, depletes oxygen through the respiration of algae and other organisms, and through the decomposition of the dead algae as shown in **Fig. 2.1 (Zhang et al., 2011b)**. The control of eutrophication and recovery of ecosystem of eutrophic lakes is a complicated and long-term project. In general, restoration of eutrophic lake water should encompass these components: (1) Control of nutrient loads, (2) Manual algal removal and (3) Ecosystem restoration technologies. They all aim at reducing nutrient loads and restoring the lake ecosystem **(Qin et al., 2006; Jobgen et al., 2004)**. Aiming at the problems of Lake Taihu, many efforts concerning the eutrophication control has been conducted as follows.

(1) Control of nutrient loads: As known, the excessive input of nutrients to the water body greatly accelerated its eutrophic process. These nutrients originate chiefly from external sources. For shallow inland lakes, the release of nutrients from sediments is also an important source **(Le et al., 2010)**. The control of nutrient loads is the basic methods for controlling the eutrophication, and mainly includes two parts: the external and internal nutrient source control.

a) External nutrient loading control: There are a great number of industrial enterprises in the Lake Taihu area. The main point pollution sources are industrial and domestic wastewaters **(Liu and Qiu, 2007)**. The point sources are the important external nutrient pollution sources, and are easy and direct to be treated. The government has implemented a series of countermeasures, including the construction of more sewer collection system and wastewater treatment plants, the strengthening of management for industrial drainage, the promotion of clean production in factories, banning the use of phosphorus-containing detergents in some static water areas, and giving full play to environmental standard functions **(Liu and Qiu, 2007)**.

Traditional flooding irrigation and seeping irrigation are the two main sources of non-point source pollution, and are the key reasons for soil erosion and the low utilization rate of fertilizers in China **(Liu and Qiu, 2007)**. The use of pesticides is strictly controlled and water-saving irrigation and ecological agriculture measures are encouraged **(Liu and Qiu, 2007)**. In addition, pollution from livestock and poultry raising should be prevented. Developing an ecological culture by utilizing recycling of culture water and stopping the usage of baits is a good measure for protecting the water **(Zhang et al., 2008a)**.

The pollution from inflow-rivers should be controlled, too. It is a good method to utilize the wet-land in carrying out water purification, basin pollution interception and ensuring regional ecological safety by performing wet-land protection, restoration, and reconstruction because of its sound nutrient removal and ecological protection functions **(Lu et al., 2006a; Lu et al., 2006b)**. The other methods are to construct ecological forests and establish ecological purification projects for inflow-rivers **(Song, 2006)**.

Some other measures are also helpful: improving the efficiency of garbage disposal and making resourceful treatment of garbage; enhancing supervision of waste landfill sites; promoting a better management level of landfill operations; improving seepage control construction of landfill **(Zhang et al., 2008a)**.

(b) *Internal source control*: In some cases, the lake environment cannot be ameliorated effectively in a short period even when the external pollution sources have been controlled efficiently **(Yu et al., 2010)**. It is because that the sediment is the main inner nutrient source and would release nutrients to the overlying water **(Yu et al., 2010)**. The total amount of nitrogen and phosphorus in the sediment of Lake Taihu accounts for 25-35% of the annual total nutrition input **(Liu and Qiu, 2007)**. Several countermeasures are commonly used to control internal source pollution, such as sediment dredging, water flushing, chemical sedimentation, and the aeration technology. Sediment dredging is a key measure in the eutrophication treatment of Lake Taihu **(Qin et al., 2006)**. Aeration is only suitable for small lakes owing to its high running cost. These methods are limited because they just can provide temporary solutions but fail to solve the problems completely **(Le et al., 2010)**.

(2) Manual algal removal: The manual method of removing algae is the simplest and most direct method to remove algae from the lakes and prevent water pollution caused by dead algae. It is necessary to find good technologies to make the dead algae into organic fertilizer, explore the method of power generation from rotted substance, and avoid the secondary pollution **(Zhang et al., 2008a)**.

(3) Ecosystem restoration technologies: There are two alternative stable states in lakes as shown in **Fig. 1.2**. The clear-water state characterizes by the dominance of the submerged macrophytes with low nutrient concentrations, whereas the turbid state dominates by algae and is typical of high concentrations of nutrients **(Scheffer et al., 1993)**. Once the lake existed as the turbid stable state, the turbid lake ecosystem tends to be resistant to recovery after the source control. It is necessary to enforce a shift from the eutrophic status characterized by the high algal biomass and high nutrient concentrations to clear-water states dominated by submerged macrophytes and typical of low nutrient concentrations using quite rigorous methods. The ecological technology is now widely used as a mean of the eutrophication control in shallow lakes because of its low investment **(Liu and Qiu, 2007)**. **Figure 1.2** shows that the key factors controlling which state is present in a water body are nutrients, macrophytes and turbidity **(Scheffer et al., 1993)**. Macrophytes and turbidity oppose each other. The high turbidity prevents the growth of submerged macrophytes by reducing light penetration at high nutrient concentrations, and submerged macrophyes reduces the turbidity by competing with algae for the growth factors including the nutrients, light and dissolved oxygen to inhibit the over reproduction of algae at low nutrient concentrations. Between the two extremes of high and low nutrient concentrations, there exists a region where both stable are possible **(Mclvor, 2004)**. From **Fig. 1.3**, increasing the numbers of algal grazers or increasing

the biomass of submerged macrophytes possibly causes a change from a turbid state to the clear state.

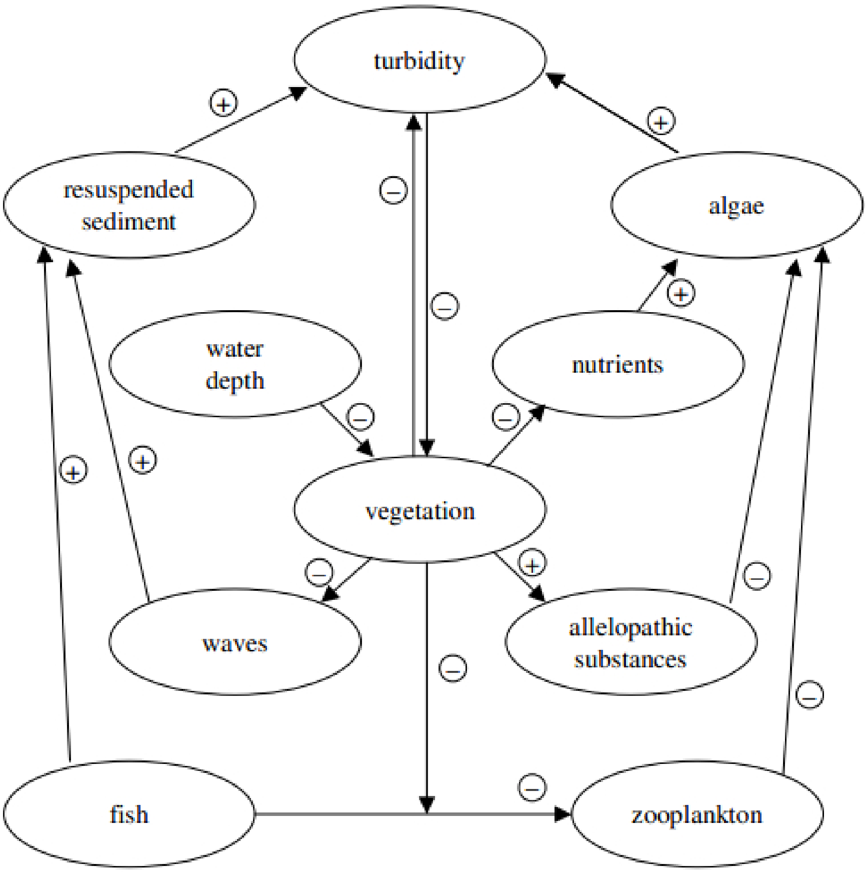


Fig. 1.3 The main feed-back loops believed to be responsible for the existence of alternative stable states in shallow lake ecosystems **(Scheffer et al., 1993)**.

The biomanipulation technology is a method to restore the lake ecosystem through adjusting the food chain of aquatic ecosystem. The term biomanipulation was coined by Hrbáček et al. **(1961)** and has developed through several theories until to today's bottom-up/top-down theory. For example, the reduction in fish populations can reduce the resuspension of sediment caused by benthic-feeding fish, and allow the zooplankton to increase rapidly owing to the reduction of predation by fish. Then the zooplankton feed on the algae to reduce the turbidity **(Perrow et al., 1997)**. The addition of mussels, bivalves or plankton-feeding fish also can reduce the turbidity by feeding directly on phytoplankton **(Ma et al., 2003; Mclvor, 2004; Li et al., 2010)**. All the measures of reducing turbidity can allow the growth of submerged macrophyts to keep the system in the clear state. The importance of submerged macrophyts can be seen from **Fig.1.3**. Thus, the most popular ecosystem recovery technology is the planting of aquatic and/or submerged macrophytes in eutrophic waters.

As primary producers, advanced aquatic macrophyts and algae compete with each other for ecological resources, such as nutrients, light, and living space **(Le et al., 2010)**. Aquatic macrophytes supply food to the first consumers in trophic chains, such as birds, fish and invertebrates **(Lauridsen et al., 1993; Gross et al., 2003)**. Aquatic macrophytes also provide habitats and refuges for periphyton, zooplankton and other vertebrates **(Carpenter and Lodge, 1986; Stansfield et al., 1997; Martín et al., 2005)**. Aquatic macrophytes also can reduce the resuspension of sediment **(Vermaat et al., 2000)**. Aquatic macrophytes play an important role in keeping a good ecological status of aquatic ecosystem because of their key functions **(Bornette and Puijalon, 2011)**. The aquatic macrophytes commonly found in eutrophic water bodies include floating-leaved macrophytes, submerged macrophytes and emergent macrophytes **(Dhote and Dixit, 2009)**. Submerged macrophytes are especially important for the aquatic ecosystem because they can directly absorb the nutrients in the sediment and reduce the resuspension of sediment through roots **(Madsen and Cedergreen, 2002)**. Since the mid-1990s, macrophyte restoration has been widely used as one ecological solution to restore the ecosystem of eutrophic water bodies in China **(Qin, 2013)**.

However, many current results show that the effects of biomanipulation are somewhat unstable over the long term and highly case-specific **(Liu and Qiu, 2007)**. More studies are needed before the results can be used in practical large-scale engineering projects for restoring submerged macrophytes. Thus, the responses of submerged macrophyts to adverse environment of eutrophic lakes should be clear before their restoration.

1.3 Physiological responses of submerged macrophytes to adverse environments

1.3.1 Environmental stress and the production of ROS

The term “plant stress” usually refers to conditions in which plant growth and performance are adversely affected by the environments **(Millar and Whelan, 2000)**. A key sign of such at a molecular level is the increased production of reactive oxygen species (ROS) and the subsequent accumulation of oxidative damage **(Millar and Whelan, 2000)**. As shown in **Fig. 1.4**, in a eutrophic lake, many adverse environments may cause stress to submerged macrophytes, including the high concentrations of nutrients in water, polluted sediment, the high turbidity, large quantity of algae, low dissolved oxygen (DO) concentrations etc.

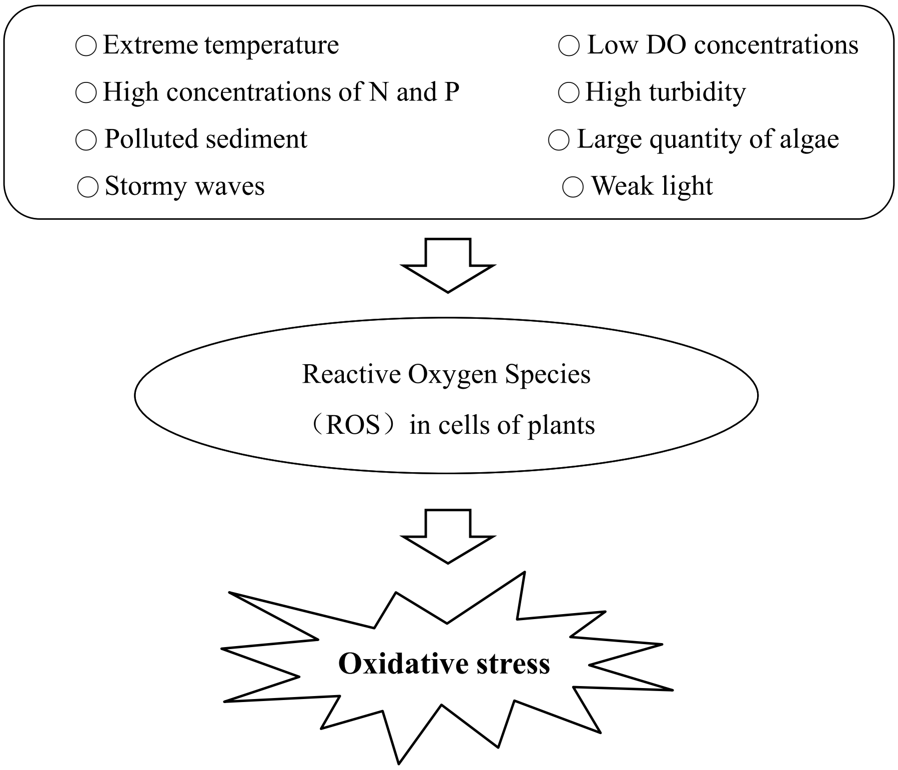


Fig. 1.4 The adverse environments which induce the plant’s ROS in eutrophic lakes.

ROS are chemically reactive molecules containing oxygen. ROS are formed as a natural byproduct of the normal metabolism of oxygen (O_2) and have important roles in cell signaling and homeostasis **(Arora, 2002)**. However, during times of environmental stress, ROS levels can increase dramatically **(Devasagayam, 2004)**. When the level of

ROS exceeds the defense mechanism, the plant is known under the oxidative stress. This may result in harmful effects on the cell of plants.

ROS mainly contain superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$). They are chemically reactive and biologically toxic **(Jackson, 1995)**. The production of ROS is an unavoidable consequence of the operation of the photosynthetic electron transport chain in an oxygen atmosphere **(Arora, 2002)**.

(1) $O_2^{\bullet -}$ is the first reduction product of ground state-oxygen, capable of both oxidation and reduction. The majority of O_2 reduction in vivo is thought to proceed via reduced ferredoxin (Fd_{red}), which reduces molecular oxygen to the $O_2^{\bullet -}$ (Reaction 1.1) **(Arora, 2002)**. The ferredoxin changed from redox state into oxidation state (Fd_{ox}), donating the electron to oxygen.

$$2O_2 + 2Fd_{red} \rightarrow 2O_2^{\cdot -} + 2Fd_{ox} \qquad \text{(Reaction 1.1)}$$

(2) H_2O_2 is not a free radical, but participates as oxidant or reductant in many cellular reactions **(Perl-Tvihai and Perl, 2005)**. Unlike superoxide, H_2O_2 is highly diffusible through membranes and aqueous compartments and it may directly inactivate sensitive enzymes at a low concentration **(Perl-Tvihai and Perl, 2005)**. Much like superoxide, H_2O_2 is rather stable and therefore less toxic than other ROS **(Perl-Tvihai and Perl, 2005)**.

(3) $OH^{\bullet}$ is the most powerful oxidizing species in biological system **(Perl-Tvihai and Perl, 2005)**. It may be generated in all living cells in a reaction catalyzed by the transition metal ions, iron and copper, when superoxide and H_2O_2 are present **(Halliwell and Gutteridge, 1992)**:

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\cdot} + OH^- + Fe^{3+} \qquad \text{(Reaction 1.2)}$$

The ferric ion can be recycled by superoxide, allowing the reaction to continue:

$$O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+} \qquad \text{(Reaction 1.3)}$$

Therefore, this reaction can be summarized as follows which is called Haber-Weiss reaction:

$$H_2O_2 + O_2^{\cdot -} \rightarrow OH^{\cdot} + OH^- + O_2 \qquad \text{(Reaction 1.4)}$$

$OH^{\bullet}$ interacts with all biological molecules and causes subsequent cellular damage such as lipid peroxidation, protein damage, and membrane destruction **(Foyer et al., 1997)**. Because cells have no enzymatic mechanism to eliminate $OH^{\bullet}$, its excess production can eventually lead to cell death **(Pinto et al., 2003)**.

1.3.2 Oxidative damage

The enhanced level of ROS can cause damage to the plants as shown in **Fig. 1.5**. The enhanced level of ROS causes the oxidative damage to biomolecules such as lipid, protein and DNA leading to altered intrinsic membrane properties, ion transport, loss of enzyme activity, protein crosslinking, inhibition of protein synthesis, DNA damage **(Sharma et al., 2012)**. Electron transport chains of chloroplast and mitochondria are main potential sources of ROS in cells so that most of structures of chloroplast and mitochondria are easily disrupted by excessive ROS, ultimately resulting in cell death **(Wang et al., 2008a)**. The final results are that excess ROS might contribute to the disorder of metabolism, including the low photosynthesis efficiency, the decreased protein contents and the increased malonaldehyde (MDA) contents, and thus caused the reduction of plant biomass or even death of the plants **(Wang et al., 2008a)**.

Lipid: Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds when the lipid is under the attack of ROS, resulting in changing and disrupting lipid structure and membrane organization and integrity **(Sharma et al., 2012)**. MDA is one of the final products of lipid peroxidation and is responsible for cell membrane damages **(Halliwell and Gutteridge, 1989)**.

Proteins: The attack of ROS on proteins may cause modification of proteins in a variety of ways, some are direct and others indirect **(Sharma et al., 2012)**. Direct modification involves modulation of a protein's activity, and indirect modification occurs by conjugation with breakdown products of fatty acid peroxidation **(Yamauchi et al., 2008)**. ROS will inhibit some sensitive enzymes, for example, oxidation of iron-sulphur centers by $O_2^{\bullet-}$ is irreversible and leads to enzyme inactivation **(Gardner and Fridovich, 1991)**.

DNA: ROS are the major sources of DNA damage. ROS can cause the oxidative damage to nuclear, mitochondrial, and chloroplastic DNA. The damaged DNA may lead to malfunctions or complete inactivation of the encoded proteins **(Sharma et al., 2012)**. Mitochondrial and chloroplast DNA are more susceptible to the oxidative damage than nuclear DNA due to the lack of protective proteins and histones, and close locations to the ROS producing systems in the former **(Richter, 1992)**.

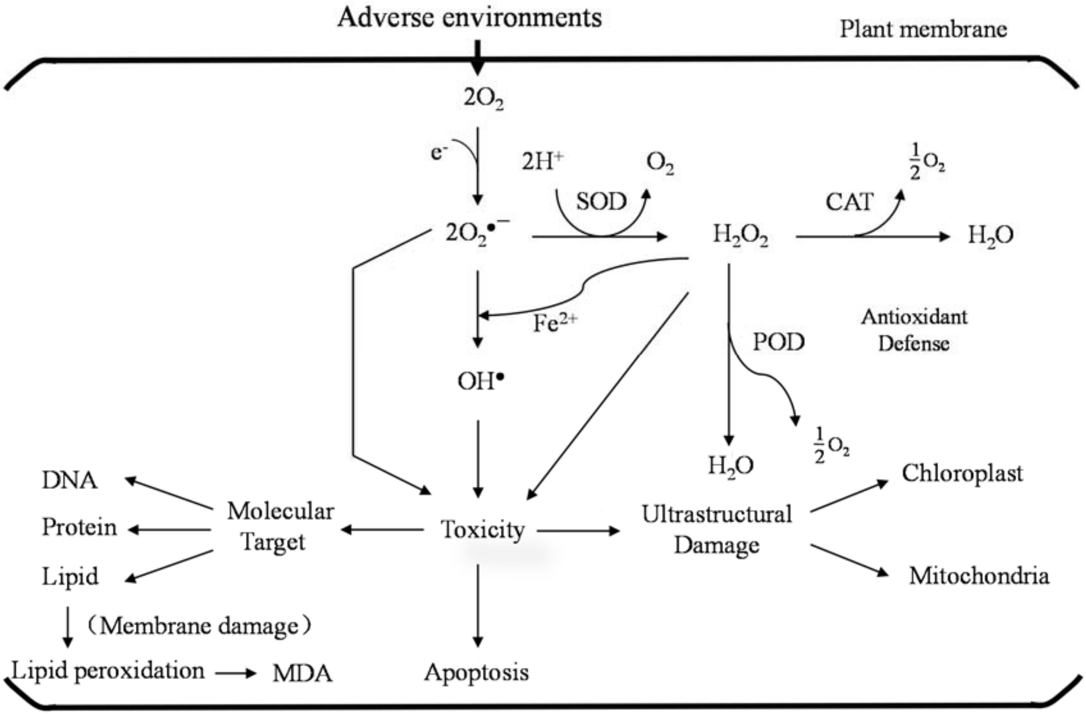


Fig. 1.5 Oxidative stress and antioxidant system in plants **(modified according to Jiang et al., 2011)**.

1.3.3 Defense against oxidative stress

As shown in **Fig. 1.5**, plants possess complex antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), that scavenge ROS to protect them from oxidative stress **(Jiang et al., 2011)**. Under the normal condition, potentially toxic oxygen metabolites are generated at a low level and there is an appropriate balance between production and quenching of ROS **(Noctor et al., 2002)**. This balance may be broken by a number of adverse environments, giving rise to rapid increases in ROS levels, which can induce oxidative damage to lipids, proteins and DNA **(Jiang et al., 2011)**. In order to avoid the oxidative damage, higher plants raise the level of endogenous antioxidant defense **(Sharma et al., 2010)**.

SOD: SOD belongs to the group of metalloenzymes and converts $O_2^{\bullet-}$ to H_2O_2 and oxygen in the following reaction **(Sharma et al., 2012)**:

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2 \qquad \text{(Reaction 1.5)}$$

SOD removes superoxide and hence decreases the risk of hydroxyl radical formation from superoxide via the metal-catalyzed Haber-Weiss reaction (**Reaction 1.4**) **(Arora, 2002)**. SOD is present in most of the subcellular compartments, such as the chloroplast and the mitochondria, which generate ROS **(Sharma et al., 2012)**. Its activity has been reported to respond to various stress conditions, such as the increase to the drought and metal stress **(Sharma and Dubey, 2005; Mishra et al., 2011)**, and the inactivation to the algal blooming stress **(Zhang et al., 2011a)**.

CAT: CAT efficiently scavenges H_2O_2 and does not require a reducing substrate to perform the task **(Sharma et al., 2012)**:

$$2H_2O_2 \xrightarrow{CAT} O_2 + 2H_2O \qquad \text{(Reaction 1.6)}$$

Although plants contain several types of H_2O_2-degrading enzymes, CAT is unique as it does not require a reducing substrate **(Sharma et al., 2012)**. When cells are under the stress and generate H_2O_2 through catabolic processes, H_2O_2 is degraded by CAT in an energy efficient manner **(Mallick and Mohn, 2000)**. The CAT activity increases under various adverse environments, and many studies found that the enhanced CAT indicates ongoing detoxication of H_2O_2 in plants **(Wang et al., 2008a; Jiang et al., 2011)**.

POD: POD is an iron-porphyrin organic catalyst to scavenge H_2O_2 and it catalyzes H_2O_2-dependent oxidation of substrate **(Lu et al., 2008)**. It is widely found in plants and various adverse environments induce the activity of POD **(Wang et al., 2008a; Jiang et al., 2011)**. POD works together in the detoxication of H_2O_2 in plants with CAT, ascorbate peroxidase (APX), etc.

The peroxidatic reaction is the most important of all the reactions catalyzed by POD and its final reaction is shown in reaction 1.7 **(Lamikanra, 2002)**. ROOH can be HOOH or some other organic peroxide, AH_2 is the hydrogen donor in the reduced form, and A is the hydrogen donor in the oxidized form **(Lamikanra, 2002)**.

$\mathrm{ROOH} + \mathrm{AH_2} \rightarrow \mathrm{H_2O} + \mathrm{ROH} + \mathrm{A}$ Reaction (1.7)

Firstly, the essentially involves an oxidative action by way of an initial formation of a complex intermediate with a hydrogen acceptor (reaction 1.8) **(Lamikanra, 2002)**.

$\mathrm{POD} + \mathrm{H_2O} \rightarrow \mathrm{Complex\ I}$ Reaction (1.8)

The transfer of hydrogen from a donor substrate results in a second complex intermediate before the regeneration of the POD enzyme and formation of a reaction product (reaction 1.9, 1.10) **(Lamikanra, 2002)**.

$\mathrm{Complex\ I} + \mathrm{AH_2} \rightarrow \mathrm{Complex\ II} + \mathrm{AH}$ Reaction (1.9)

$\mathrm{Complex\ II} + \mathrm{AH} \rightarrow \mathrm{POD} + \mathrm{A}$ Reaction (1.10)

1.4 Meaning, objective and structure of this Ph.D. thesis

The eutrophication of Lake Taihu has led to ecosystem degradation and water quality deterioration, and has adversely affected the utilization of water resources, socioeconomic developments and human living conditions **(Le et al., 2010)**. Eutrophication has become one of the most important factors in the delay in Chinese economic growth **(Liu and Qiu, 2007)**. It may take a very long time for eutrophic water bodies to return to their original states naturally after the pollution sources are controlled. Thus, since 1990, the Chinese government has undertaken a wide range of activities in an effort to reduce external nutrient loads and improve lake water quality, including closing a number of polluting factories, banning the use of phosphorus-bearing detergents, building additional wastewater treatment plants, and transferring fresh water from the Chang Jiang River (**Fig. 1.1**) to dilute the polluted water in Lake Taihu **(Li et al., 2011)**. However, the lake water quality has not been improved to date **(Li et al., 2011)**.

Ecological restoration techniques are natural ways of establishing a self-regulating ecosystem with low investment **(Liu and Qiu, 2007)**. The most popular ecosystem restoration technology is the planting of aquatic, especially submerged macrophytes in eutrophic lakes because of their effective improving the water quality and reducing algae **(Liu and Qiu, 2007)**. As primary producers, submerged macrophytes can compete for ecological resources with algae, can release chemical substances, so-called allelochemicals, to inhibit algae reproduction, and can absorb nutrients directly helping lakes to transform the former algae dominated ecosystem into a macrophyte-dominated ecosystem as shown in **Fig. 1.2 (Le et al., 2010)**. Different submerged macrophytes require different growing environments and the eutrophic characteristics vary depending on the lake type. Thus, it is necessary to select the optimal plant species and conduct the ecophysiological studies in the ecological restoration of eutrophic Lake Taihu, China.

The introduction of non-native submerged macrophytes which have high tolerance for the eutrophic aquatic environments to Lake Taihu is a kind of methods for improving the aquatic environments. However, the introduction of non-native submerged macrophytes possibly has a high risk of destroying the lake ecosystem by influencing the native biodiversity because of their high invasiveness **(Schultz and Dibble, 2012)**. The restoration of the native submerged macrophytes is the best way to restore the ecosystem for Lake Taihu. *Vallisneria asiatica* is a kind of common submerged macrophyte in Lake Taihu and reducing currently. *Vallisneria* is a genus of submerged plant that can spread by runners. Thus, they multiply readily through the production of daughter plants (**Fig. 1.6**). These daughter plants can be cut away and transplanted once they have established their own roots **(http://en.wikipedia.org/wiki/Vallisneria)**. *Vallisneria* strengthen the absorptive capacity of nutrients through lots of developed root hairs and the adjacent rhizosphere bacteria **(Kurtz et al., 2003)**. Leaves arise in clusters from their roots and have rounded tips and definite raised veins (**Fig. 1.6**). The common *Vallisneria* are tolerant and adaptable, and are not picky about the substrate **(http://en.wikipedia.org/wiki/Vallisneria)**. *Vallisneria asiatica* is widely distributed in some waters in South and East Asia (e.g., India, China and Japan) and likes to grow in mesotrophic-eutrophic lakes **(Riis and Sand-Jensen, 2001)**. Thus, it is probably feasible to restore *V. asiatica* in eutrophic Lake Taihu. Considering its high absorption capacity of contaminants, wide distribution and good resistance for contaminants **(Sun, 1992)**, the submerged macrophyte, *V. asiatica*, was chosen for the dissertation studies. It was of our interest to restore the ecosystem of eutrophic Lake Taihu, China, by restoring the submerged macrophytes owing to their high ability of inhibiting the reproduction of algae and absorbing the nutrients.

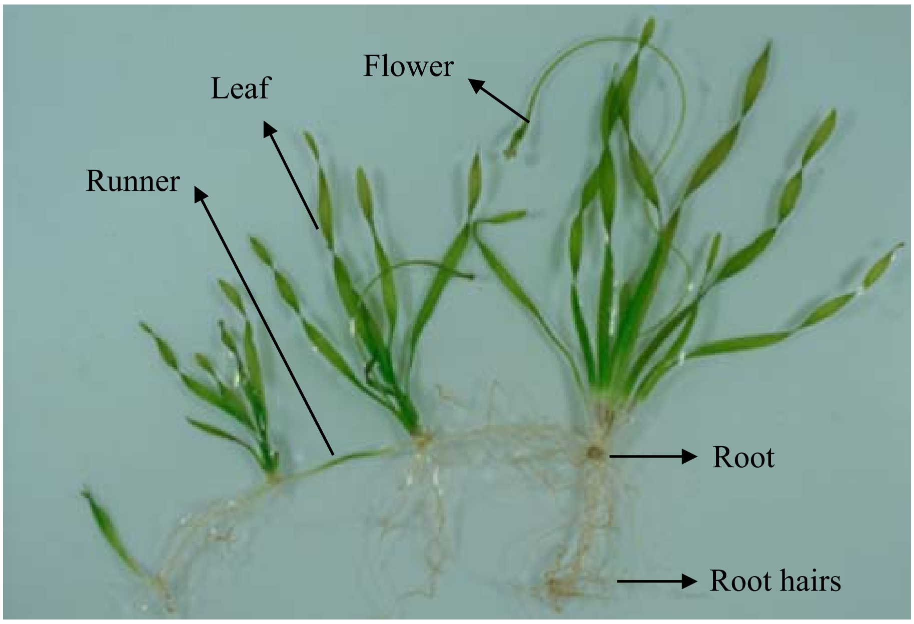


Fig. 1.6 The figure of *Vallisneria asiatica.*
(http://milky.geocities.jp/hamabata1627/nejiremo940913_5030_70.jpg)

The overall objective of this study was to propose ecological restoration strategies and technical measures in eutrophic Lake Taihu, China. The structure of this study is shown in **Fig. 1.7**. The chapter 1 mainly describes the related research background and induces the purpose of this study. The effectiveness of *V. asiatica* in controlling the eutrophication is proved by analyzing the effects of *V. asiatica* on the algal community in chapter 2. Following 3 chapters mainly analyze the physiological responses of *V. asiatica* to different adverse factors (high concentrations of nitrogen and phosphorus, high concentrations of algae and eutrophic sediments) in eutrophic Lake Taihu. Chapter 6 links the aquatic environmental changes in different regions of Lake Taihu to the research results of above 3 chapters, getting the restoration strategies in different regions with different eutrophic degree. Specific objectives addressed in this dissertation include:

- Prove the effectiveness of *V. asiatica* in absorbing the nutrients and controlling the algal blooms.
- Analyze the physiological responses of *V. asiatica* to different concentrations of nitrogen and phosphorus.
- Determine the physiological responses of *V. asiatica* to different concentrations of cyanobacteria and find the optimal biomass of *V. asiatica* for controlling the algal blooms.
- Compare the physiological responses of *V. asiatica* to the sediment from different zones in eutrophic Lake Taihu.
- Analyze the reasons why the submerged macrophytes disappeared in different zones of Lake Taihu and propose the corresponding ecosystem restoration methods by the conservation and restoration of submerged macrophytes.

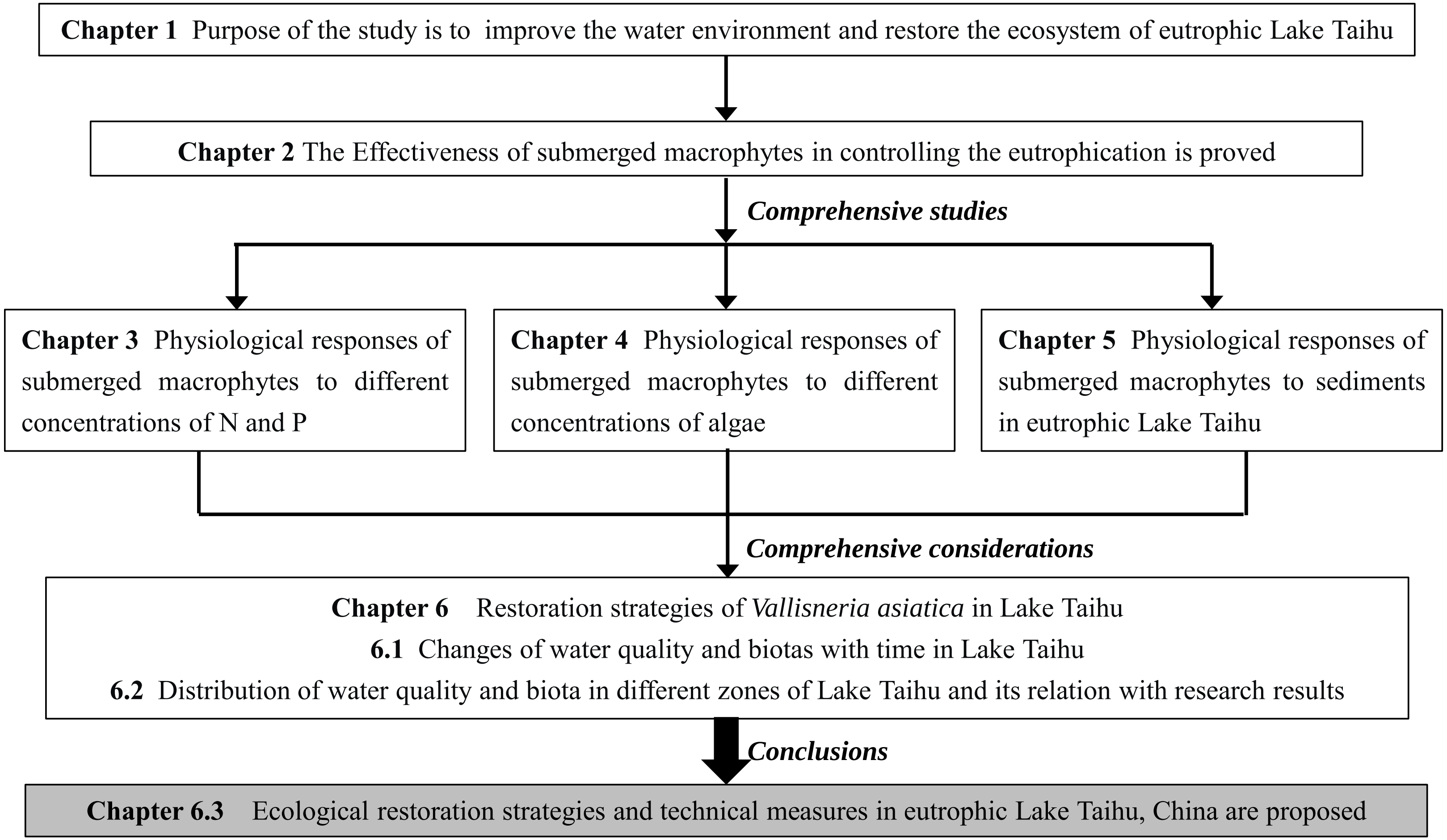


Fig. 1.7 The structure of the Ph.D. thesis.

CHAPTER 2

THE CONTROL OF EUTROPHICATION BY *VALLISNERIA ASIATICA*

CHAPTER

2

The control of eutrophication by *Vallisneria asiatica*

Abstract

To improve the water quality of lakes and control algal blooms, the effects of *Vallisneria asiatica* which is one of submerged macrophytes spread over Lake Taihu, with different weights of biomass on the water quality and algae were researched under the condition that the algal biomass of each tank (contained 30 L solution) was $30{,}926 \times 10^5$ cells. The toxic planktonic *Microcystis* spp. were the dominant species in planktonic cyanobacteria which were the major toxic components of planktonic algae. The results indicated that *V. asiatica* could control an excess of planktonic *Microcystis* spp. when the *V. asiatica* biomass was larger than 50 g in the tank with $30{,}926 \times 10^5$ cells planktonic algae in the laboratory. Planktonic and epiphytic algae responded differently to *V. asiatica*. The presence of submerged macrophyte *V. asiatica* in eutrophic waters has a positive effect on algal compositions. That is, *V. asiatica* could inhibit the growth of planktonic *Microcystis* spp. effectively and was benefited to the planktonic diatom which was the non-toxic algal species on the condition in the laboratory.

Key words: planktonic cyanobacteria, epiphytic diatom, *Vallisneria asiatica*, algal bloom, algal community, *Microcystis* spp.

2.1 Introduction

Recently, along with the economic growth and urbanization, nutrient loadings and eutrophication of Lake Taihu have rapidly accelerated to the point that the algal bloom especially the harmful cyanobacterial (toxic species of algae) bloom is a common feature **(Guo, 2007; Qin et al., 2007a)**. All those bring significant barriers to the growth of aquatic macrophytes and sustainable developments of economies in Lake Taihu.

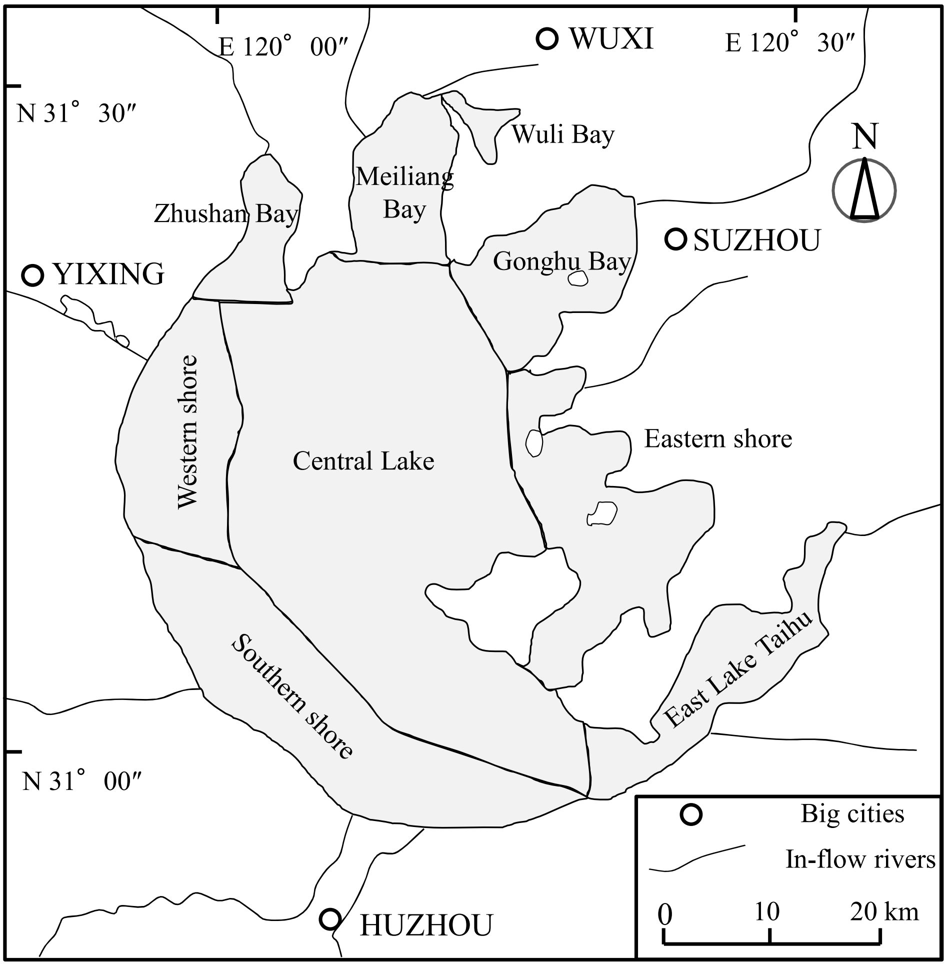


Fig. 2.1 The figure of Lake Taihu.

Correspondingly the area of aquatic macrophytes in Lake Taihu reduced continually, the aquatic macrophyte species decreased constantly even disappeared, and ecological functions degenerated seriously **(He et al., 2008)**. With the structure adjustment of fisheries industry and improvements of crab farming, the community structure of aquatic macrophytes had altered, and dominant species of aquatic plants had also changed greatly **(He et al., 2008)**. Dominant species of aquatic macrophytes included *Hydrilla verticillata*, *Elodea nuttallii*, *Vallisneria asiatica*, *Potamogeton malaianus*, *Trapa maximowiczii* and *Potamogeton crispus* in Lake Taihu **(He et al., 2008; Hao et al., 2010)**. Aquatic macrophytes distributed primarily in East Lake Taihu because of its good water quality, but now are also affected greatly by the water pollution. The East Lake Taihu lies in the southeast of Lake Taihu as shown in **Fig. 2.1**. This bay is 27.5 km long and up to 9.0 km wide, and the water is now less than 1 m deep on average **(Li, 2008)**. East Lake Taihu is an important economy development region because it plays a key role in flood prevention and alleviation, supplies water for Shanghai and the east Zhejiang Province as shown in **Fig. 1.1**, and is one of the most developed areas of aquaculture production in China **(Li, 2008)**.

Aquatic macrophytes can enhance the water clarity by reducing the resuspension of bottom sediment, improve the water quality by absorbing lots of nutrients, and suppress the algal growth by competing for nutrients and releasing allelopathic substances that are toxic to algae **(Takamura et al., 2003)**. Thus, the restoration of aquatic macrophytes has been one of the keys to control the eutrophication of Lake Taihu. Furthermore, algal dynamics should be further researched in relation to aquatic macrophytes because of the importance of controlling algal biomass and species in the water management **(Takamura et al., 2003)**. The submerged macrophyte *V. asiatica* is a kind of common aquatic macrophytes 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 **(Sun, 1992)**. In view of the eutrophication characterized by the algal bloom which is dominated by toxic *Microcystis* spp. in Lake Taihu, effects of *V. asiatica* on the algal biomass (mainly *Microcystis* spp.) and the algal community were researched in this experiment. The experiment also aimed to investigate the competitions among each species of the biotas under different biomass weights of *V. asiatica*. The result will make the improvement to control the algal bloom in Lake Taihu.

2.2 Materials and methods

2.2.1 Materials

The deep-rooted *V. asiatica* was collected from the River Onga in Fukuoka Prefecture, Japan. All plants were thoroughly washed and incubated in dechlorinated tap water for 3 days after the soil and deciduous yellow leaves were removed. Only healthy, evenly sized plants were chosen for the experiment (**Fig. 2.1**). The natural algae were obtained from an algal bloom-infested pond in Fukuoka Prefecture, Japan (**Fig. 2.2**). The pond water in October 2011 contained: 115 $\mu g \cdot L^{-1}$ chlorophyll a, 0.033 $mg \cdot L^{-1}$ NH_4^+-N, 0.058 $mg \cdot L^{-1}$ NO_2^--N, 1.251 $mg \cdot L^{-1}$ NO_3^--N, and 0.094 $mg \cdot L^{-1}$ PO_4^{3-}-P. The toxic cyanobacterial cells (reflected by microcystins) accounted for 99% of the algal cells. Of this percentage, 96% were *Microcystis* spp. The concentration of extracellular microcystins in the pond was 0.35 $\mu g \cdot L^{-1}$.

Fig. 2.1 *Vallisneria asiatica*.

Fig. 2.2 Concentrated algae.

2.2.2 Experimental design

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: 3.0 $mg \cdot L^{-1}$ of NO_3^--N (KNO_3), 0.5 $mg \cdot L^{-1}$ of NH_4^+-N (NH_4Cl), and 0.2 $mg \cdot L^{-1}$ of PO_4^{3-}-P (KH_2PO_4). The concentrated algae with the same volume were added into tanks from No.1 to No.5 expect No.0 and the algal biomass of each tank reached 30,926 × 10^5 cells. All the collected plants were placed into 6 tanks (30 L solution) with 20, 0, 20, 50, 200, 500 ± 5 g respectively from No.0 to No.5. The strengthened glass tanks (L 30 cm × W

30 cm × H 45 cm) 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). The experiment lasted for 15 days. The plants' leaves were chosen and put into sampling bottles with 4% formalin solution for measuring the epiphytic algae.

2.2.3 Measurements

pH, DO, and turbidity were tested by the pH meter (D-52, Horiba, Japan), portable LDO probe and meters (Model HQ10, Hach, USA) and portable turbidimeter (2100P, Hach, USA), respectively. Plant and solution samples which were used for algae analysis were got at the beginning and end of the experiment. NH_4^+-N, NO_3^--N and DTP were determined by salicylate method, chromotropic acid method, and acid persulfate digestion method respectively **(Hach, 2002)**. Algae were identified and counted using a phase contrast microscope (×300 times) **(Nishizawa and Chihara, 1979)**. Leaves of *V. asiatica* were treated by ultrasound for 3 minutes, and then epiphytic algae were identified by the same method as planktonic algae. The extracellular microcystin of the algae in the water body, expressed as the MC concentration in the algal suspensions, was measured by the enzyme-linked immunosorbent assay method by Dr. Sakai at the University of Tokyo.

2.3 Results

2.3.1 pH, DO, and turbidity of solution

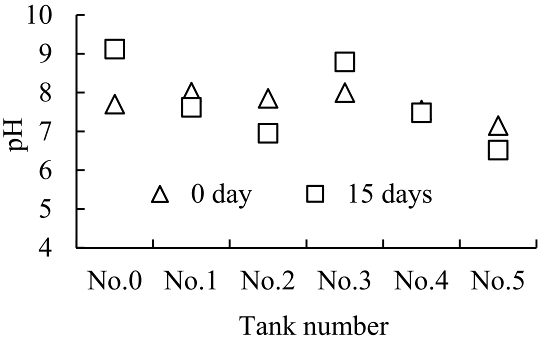


Fig. 2.3 Changes of pH in tanks.

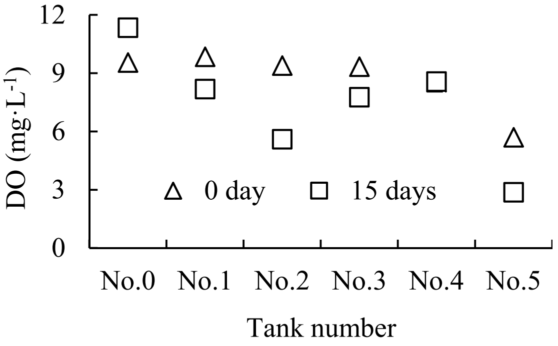


Fig. 2.4 Changes of DO in tanks.

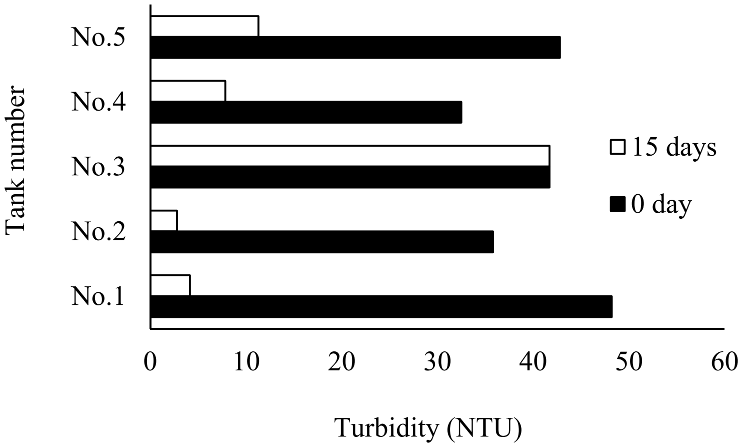
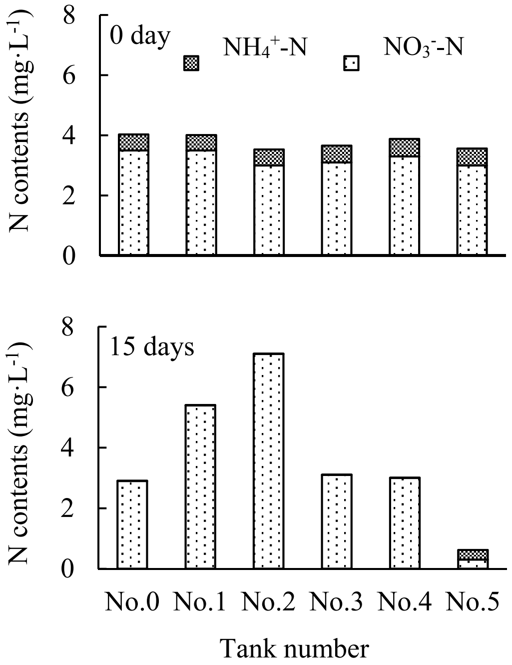


Fig. 2.5 Changes of turbidity in tanks.

In the tanks with algae, the biomass of *V. asiatica* increased from No.1 to No.5 tanks. The No.0 tank without algae contained 20 g *V. asiatica*. pH changed in a range from 6.52 to 9.16 (**Fig. 2.3**). The DO values decreased compared with initial values except No.1. The DO of No.5 was lower than that of other tanks (**Fig. 2.4**). Data shown in **Fig. 2.5** indicated that turbidity decreased except No.3.

2.3.2 N and P concentrations of solution in each tank

The concentrations of NH_4^+-N and NO_3^--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. 2.6**). As shown in **Fig. 2.7**, 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.



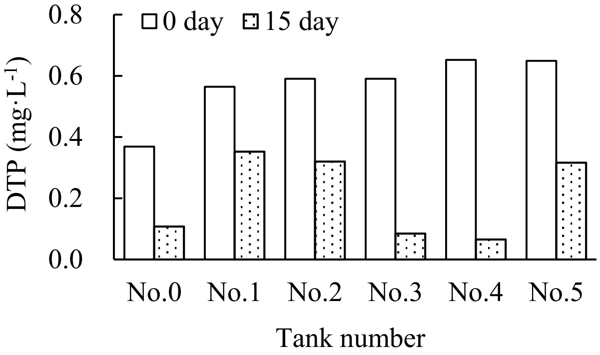


Fig. 2.6 Changes of NH_4^+-N and NO_3^--N.

Fig. 2.7 Changes of DTP.

2.3.3 Analysis results of planktonic algae

At the end of the experiment, the taxa and quantity of planktonic algae in solution of tanks from No.1 to 5 were analyzed comparing with the initial value. The value of shown in **Table 2.1** was the biomass of the planktonic algae in the whole tank (30 L solution). In order to provide more clear changes of each species of planktonic algae, the cell change figures of planktonic *Microcystis* spp., cyanobacteria, diatom and green algae were given from **Fig. 2.8** to **Fig. 2.11**, respectively.

Table 2.1 Species of planktonic algae in each tank.

Tank number			No.1	No.2	No.3	No.4	No.5
Plant biomass (g·30 L^{-1})			0	20	50	200	500
Initial planktonic algal biomass (×10^5cells·30 L^{-1})			30,926	30,926	30,926	30,926	30,926
Algal Taxa		Biomass of each algal taxa (×10^5cells·30 L^{-1})					
		0th day	15th day				
Cyanobacteria	*Chroococcus* spp.	13	-	525	660	-	60
	Microcystis spp.	29,217	-	60	65,620	7,104	3,720
	Phormidium spp.	1,317	-	-	1,200	-	-
	Oscillatria spp.	-	30	12	-	60	9,300
Diatom	*Achnanthes* spp.	-	-	-	-	9	-
	Cocconeis spp.	-	-	360	30	277	105
	Cyclotella spp.	-	-	-	-	-	-
	Cymbella spp.	-	-	-	-	-	-
	Fragilaria spp.	-	-	-	-	-	-
	Gomphonema spp.	1	-	60	15	42	-
	Navicula spp.	-	-	3	-	3	-
	Nitzschia spp.	378	2,460	1,440	60	43	15
	Melosira spp.	-	-	180	-	-	30
	Synedra ulna	-	-	-	-	-	3
Green algae	*Ankistrodesmus falcatus*	-	-	-	12	-	-
	Scenedesmus spp.	-	120	48	192	3	-
	Total cell number	30,926	2,610	2,688	67,789	7,541	13,233

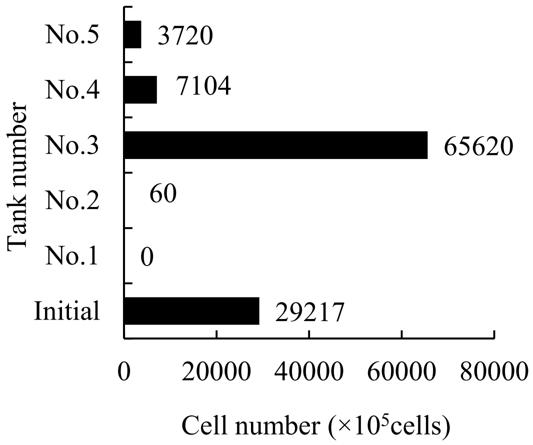


Fig. 2.8 Change of planktonic *Microcystis* spp. cells.

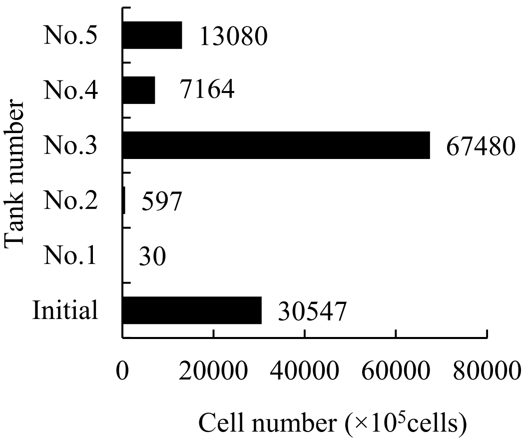


Fig. 2.9 Change of planktonic cyanobacteria cells.

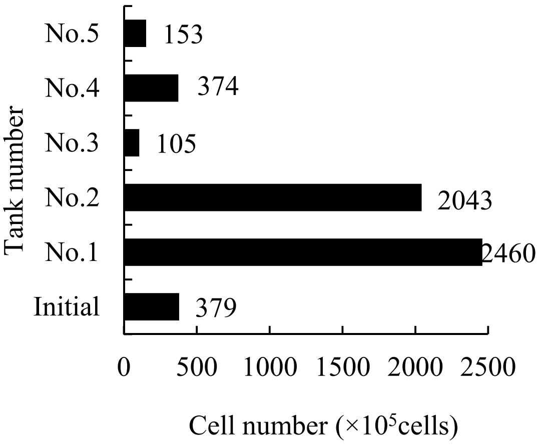


Fig. 2.10 Change of planktonic diatom cells.

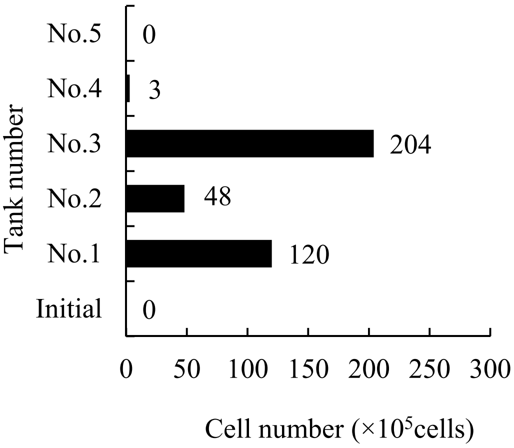


Fig. 2.11 Change of planktonic green algae cells.

At the beginning of the experiment, the planktonic *Microcystis* spp. (99% of cyanobacteria) were the dominant species in the planktonic cyanobacteria (96% of algae) which were the major components of planktonic algae (**Fig. 2.8; Fig. 2.9**). on the 15th day, planktonic *Microcystis* spp. of No.2 decreased sharply, and planktonic *Microcystis* spp. of No.1 even disappeared completely while planktonic diatom (mainly *Nitzschia* spp.) increased (**Fig. 2.8; Fig. 2.10**). The biomass of planktonic green algae in tanks of No.1, 2, and 3 (low biomass of *V. asiatica*) increased also, but the biomass of planktonic green algae was very little and had a small proportion in the whole biomass of planktonic algae (**Fig. 2.11**). The species of planktonic algae became richer after adding *V. asiatica* into solution with algae than before. Especially, the biomass of planktonic *Microcystis* spp. of No.3 tank (50 g of *V. asiatica*) reached a value about 2.2 folds higher than the initial value (**Fig. 2.8**).

Moreover, biomass of planktonic *Microcystis* spp. of No.4 and No.5 (high biomass of *V. asiatica*) decreased with an increase of *V. asiatica* biomass compared with the initial value (**Fig. 2.8**). The biomass of planktonic diatom in tanks No.3, 4, and 5 also decreased with the increasing biomass of *V. asiatica* (**Fig. 2.10**). The biomass of planktonic green algae of No.3 reached the largest value in all tanks. The planktonic green algae of No. 4 and 5 decreased sharply. The whole biomass of planktonic green algae took up very small ratio of planktonic algae (**Fig. 2.11**).

2.3.4 Analysis results of epiphytic algae

At the end of experiment, the taxa and quantity of epiphytic algae attached on leaves of *V. asiatica* in each tank were analyzed. The biomass of epiphytic algae shown in **Table 2.2** was the number of epiphytic algae attached on *V. asiatica* in the whole tank. In order to compare the cell changes of the main epiphytic algal species, the cell number of epiphytic cyanobacteria and the epiphytic diatom in each tank were drawn in **Fig. 2.12** and **2.13**, respectively.

Table 2.2 Species of epiphytic algae on leaves of *V. asiatic* in each tank.

Tank number		No.0	No.2	No.3	No.4	No.5
Plant biomass (g·30 L^{-1})		20	20	50	200	500
Initial planktonic algal biomass (×10^5cells·30 L^{-1})		0	30,926	30,926	30,926	30,926
Algae Taxa		Biomass of each algal taxa (×10^5cells/g)				
Cyanobacteria	*Chroococcus* spp.	243	-	68	-	-
	Microcystis spp.	-	-	-	-	-
	Phormidium spp.	-	-	-	-	-
	Oscillatria spp.	-	-	8	-	-
Diatom	*Achnanthes* spp.	54	-	-	-	-
	Cocconeis spp.	1,935	12	676	4,862	2,094
	Cyclotella spp.	-	-	-	-	-
	Cymbella spp.	59	-	-	-	-
	Fragilaria spp.	49	-	-	-	-
	Gomphonema spp.	65	-	68	90	1,208
	Navicula spp.	92	-	17	-	-
	Nitzschia spp.	43	-	34	-	483
	Melosira spp.	-	-	-	-	-
	Synedra ulna	32	-	-	60	-
	Total cell number	2,572	12	871	5,012	3,785

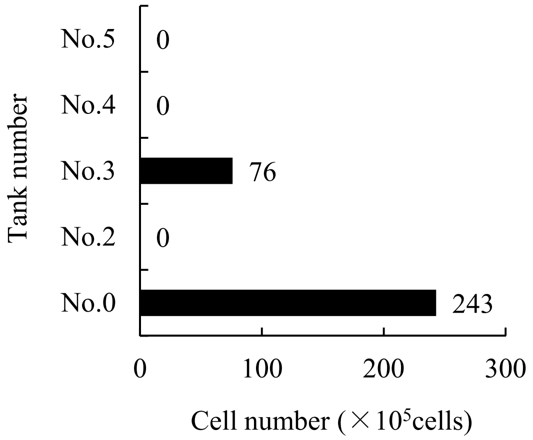


Fig. 2.12 Change of epiphytic cyanobacteria cells.

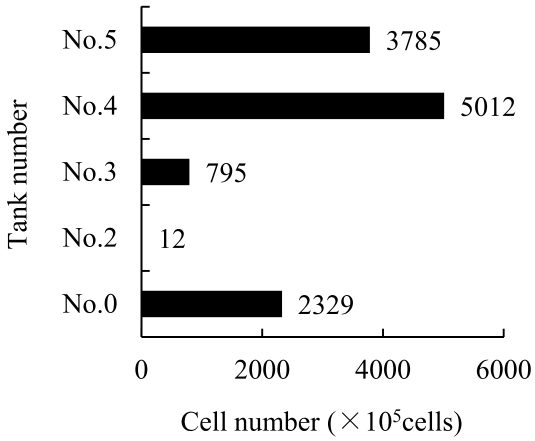


Fig. 2.13 Change of epiphytic diatom cells.

The epiphytic diatom especially *Cocconeis* spp. were the dominant taxon in all epiphytic algae adhered on leaves of *V. asiatica* in each tank during the experimental period. The epiphytic diatom accounted for about 90% in epiphytic algae of No.0 tank.

In comparison with No.0 which was without algae inoculation, there were no epiphytic cyanobacteria attached on the leaves of *V. asiatica* at the end of the experiment except the tank of No.3 with 50 g of *V. asiatica* (**Fig. 2.12**). The epiphytic diatom biomass of No.4 and No.5 tanks (200 g and 500 g of *V. asiatica*) were larger than No.0 tank. The biomass of epiphytic diatom in No.5 tank was about 2 times compared with that of No.0 tank (**Fig. 2.13**). However, the epiphytic diatom biomass of No.2 and No.3 were less than No.0 tank. The biomass of epiphytic diatom in No.2 tank was just 12×10^5 cells (**Fig. 2.13**). The epiphytic *Cocconeis* spp. were also the dominant species in each tank after the experiment compared with the initial result.

2.4 Discussion

2.4.1 Effects of *V. asiatica* and algae on the water quality

The settled planktonic *Microcystis* spp., some of which have died, can release N and P which was the reason for an increase of N and P **(Hanazato, 1989)**. This can explain why the N and P contents of No.2 tank (20 g of *V. asiatica*) 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 20 g (No. 3 to 5 tanks). Furthermore, decayed algae release other nutrients into the water column which can in turn be used again by them since they require not just N and P to grow, but other nutrients as well **(Wang et al., 2010)**. Maybe this is a reason why the concentration of planktonic algal cells in No.3 is high. The turbidity of No.3 was also high. Thus, the high concentration of planktonic algal 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 **(Wang et al., 2010)**. Many researchers studied the release of different elements from decomposing algal debris **(Gobler et al., 1997)** and it is known to be an important internal nutrient source **(Enoksson, 1993)**. For example, Sun et al. in **(Sun et al., 2007)** found that the abundant colloidal, particulate, and dissolved nutrients could be released during the death and decomposition of planktonic cyanobacteria from Lake Taihu. Colloids have been shown to be supplementary sources of nutrients, including N, P, iron (Fe) and other trace elements promoting the growth of algae **(Wang and Guo, 2001)**.

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 *V. asiatica* decayed because of low DO (**Fig. 2.4**) which was caused by the respiration of lots of *V. asiatica* biomass and its shading effects at the end of the experiment. NH_4^+-N and DTP of No.5 were relative high because of the releasing nutrients by rot roots of *V. asiatica*.

2.4.2 Effects of *V. asiatica* biomass on the algal community

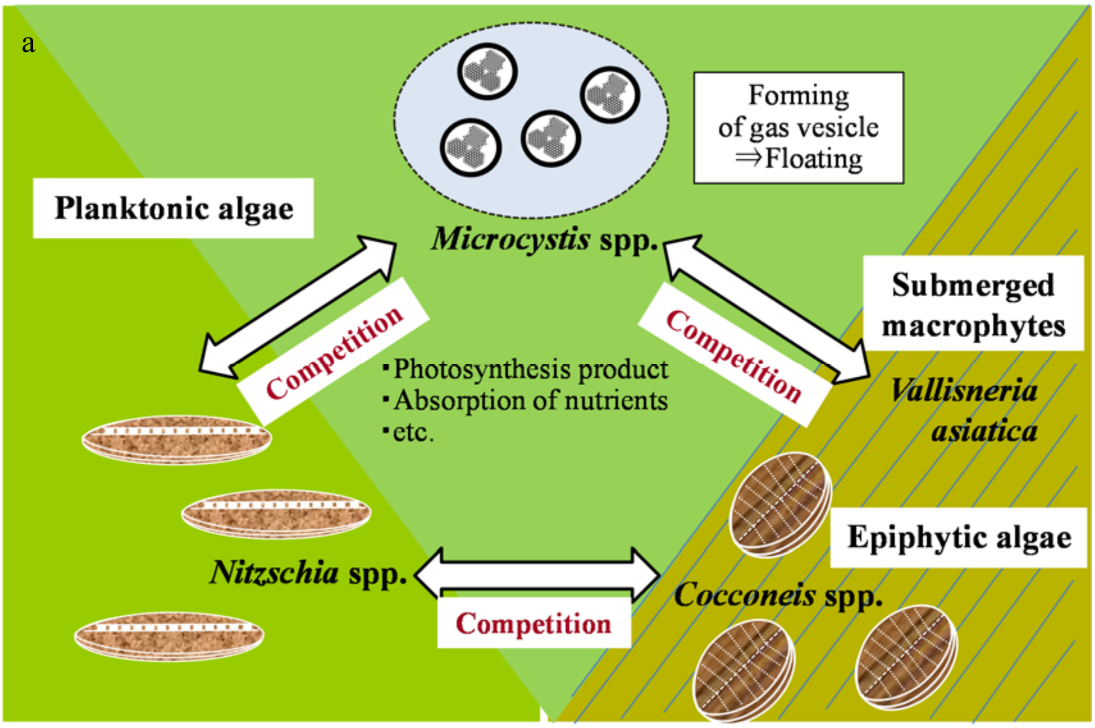


Fig. 2.14a Competitions among planktonic *Microcystis* spp., planktonic *Nitzschia* spp., epiphytic *Cocconeis* spp. and *Vallisneria asiatica* in each tank.

As shown in **Fig. 2.14a**, there are three groups of competitions among the biotas in this experiment. Which competition to be dominant depended on the biomass of *V. asiatica*.

Many studies implied that macrophytes had an inhibitory effect on the growth of algae owing to competition for nutrients, secretion of inhibitory organic compounds and shading **(Wium-Andersen et al., 1982)**. However, the results in this study showed that the planktonic algal biomass increased firstly and then decreased with the increasing of *V. asiatica* biomass. Allelopathy was defined by Molisch **(Molisch, 1937)** 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 **(Saunders, 1980)**. That may be a reason why the small biomass of *V. asiatica* could cause a development of planktonic algal biomass in the No.2 and 3 tanks. When the biomass of *V. asiatica* was larger than 200 g, *V. asiatica* will effectively inhibit planktonic *Microcystis* (**Fig. 2.14b**). Schriver et al. **(1995)** also found that planktonic algal biomass abruptly declined at a threshold level of 15-20% plant volume infested (PVI) for large-scale (100 m^2) enclosures in a shallow eutrophic lake. It seemed that macrophytes have the potential to both stimulate and inhibit the growth of algae in the condition of this study. The final outcome for planktonic algal biomass may depend on both macrophyte species and density. This was consistent with the result that low concentration allelochemicals of aquatic macrophytes promoted the growth of algae and high concentration allelochemicals control the growth of algae **(Xian et al., 2005)**. This would partly explain why the algae biomass of No.3 tank reached maximum value.

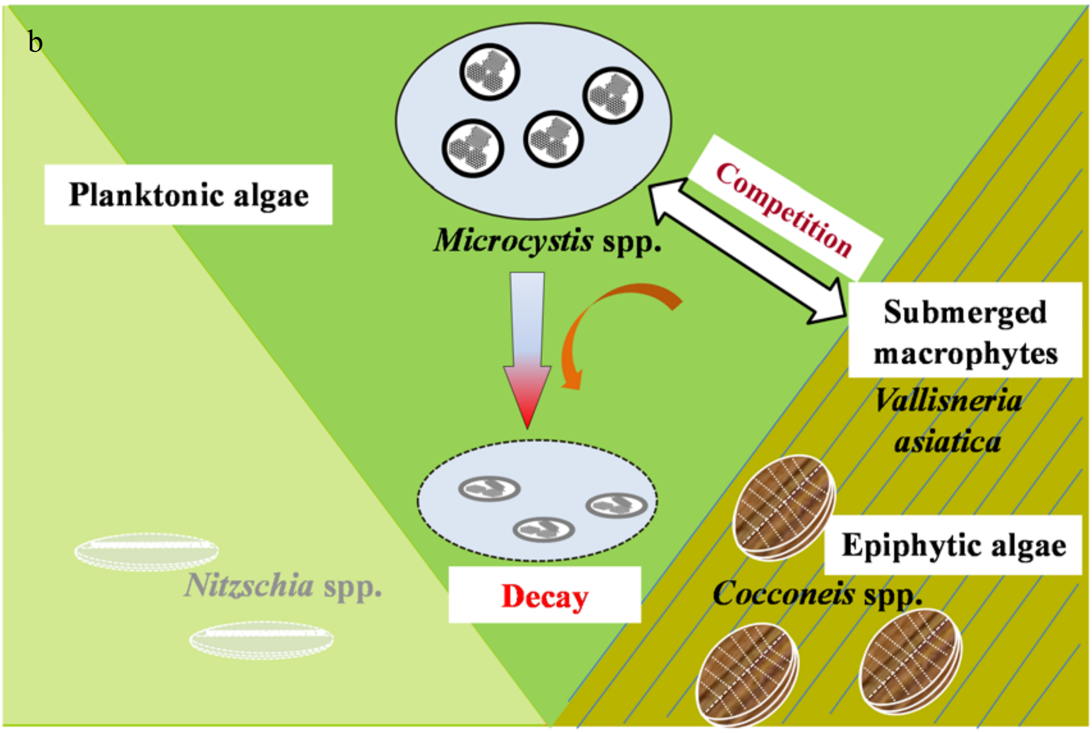


Fig. 2.14b Competitions between planktonic *Microcystis* spp. and *Vallisneria asiatica* in No.3 to No.5 tanks.

In the tanks of No.3, 4, and 5, cyanobacteria were still the dominant taxon of planktonic algae. The buoyant planktonic algal taxa, including harmful cyanobacteria like *Microcystis* spp., tend to be favored during periods with warm weather and weak wind mixing **(Huiaman et al., 2004)**. The biomass of planktonic *Microcystis* spp. declined significantly with the increasing of *V. asiatica* biomass. Planktonic *Nitzschia* spp. declined while epiphytic *Cocconeis* spp. increased obviously with the increasing biomass of *V. asiatica* in tanks of No.3, 4, and 5. The competition between planktonic *Nitzschia* spp. and epiphytic *Cocconeis* spp. possibly was a reason why the large biomass of *V. asiatica* can inhibit the reproduction of planktonic *Microcystis* spp. (**Fig. 2.14c**). Planktonic algae often exhibit differential sensitivity against allelochemicals of macrophytes **(Gross et al., 2003)**. Jasser's research confirmed that the planktonic cyanobacteria are more sensitive to macrophyte-produced allelochemicals than other planktonic algal species **(Jasser, 1995)**.

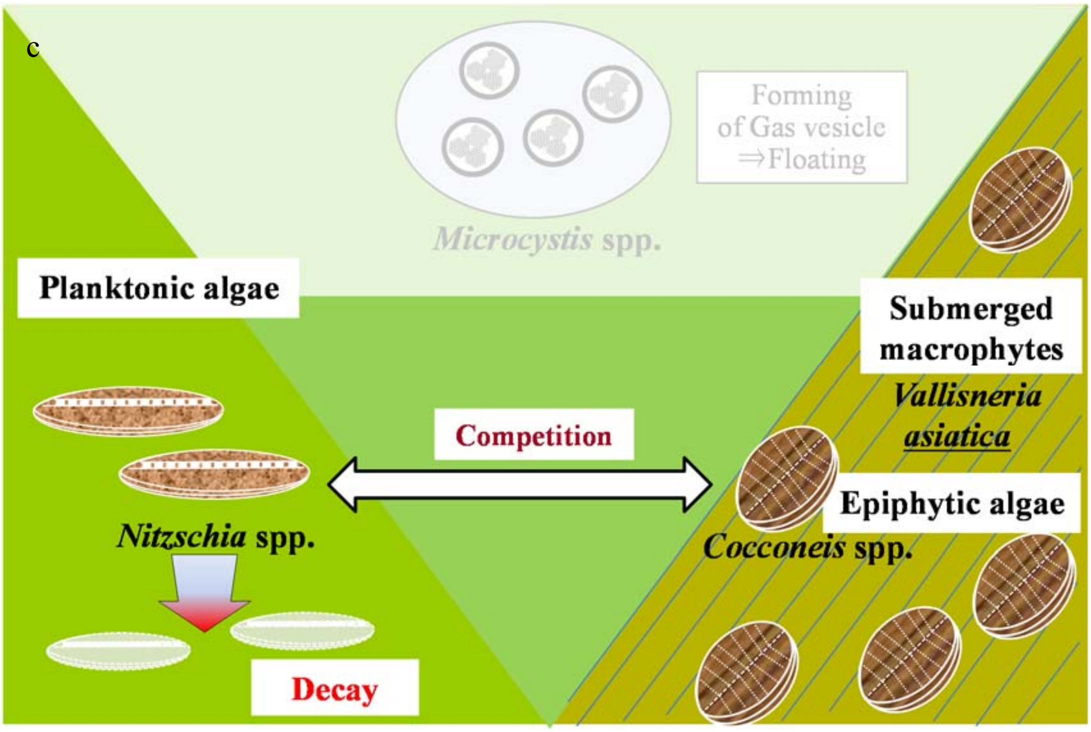


Fig. 2.14c Competitions between planktonic *Nitzschia* spp. and epiphytic *Cocconeis* spp. in No.3 to No.5 tanks.

The epiphytic cyanobacteria were inhibited effectively by *V. asiatica* (**Fig. 2.12**). Although macrophyte *Stratiotes aloides* stimulated or did not affect the growth of epiphytic cyanobacteria **(Mohamed and Shehri, 2010)**, epiphytic *Cocconeis* spp. were the dominant epiphytic algae attached on leaves of *V. asiatica* in our study possibly because the allelopathy differed depending on the species of the macrophytes. 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 **(Hilt, 2006)**, as these substances may provide an advantage for epiphytic species in the competition with planktonic algae 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 planktonic *Nitzschia* spp. and epiphytic *Cocconeis* spp. There was competition between planktonic *Microcystis* spp. and epiphytic *Cocconeis* spp. even planktonic *Nitzschia* spp. were absent **(Sekiya et al., 2010)**. It was hypothesized that after the multiplication of planktonic *Nitzschia* spp. was controlled by the epiphytic *Cocconeis* spp. (**Fig. 2.14c**), *Microcystis* spp. were also controlled by the increasing biomass of *V. asiatica* (**Fig. 2.14b**). This difference in sensitivity of different planktonic algal species to allelochemicals is likely to influence the competitive balance between planktonic cyanobacteria and other algae **(Donk and Bund, 2002)**.

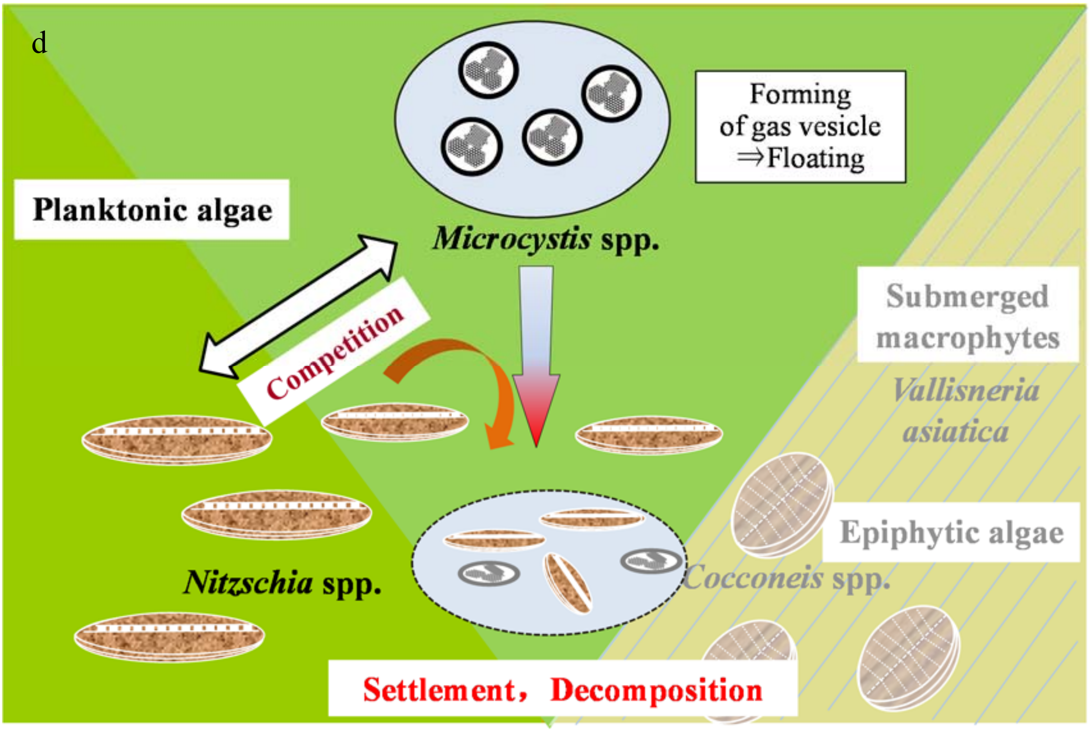


Fig. 2.14d Competitions between planktonic *Microcystis* spp. and planktonic *Nitzschia* spp. in No.1 and No.2 tanks.

It was expected that the planktonic *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 planktonic *Microcystis* spp. of No.1 and No.2 reduced sharply. At the same time, in the tanks of No.1 and 2, lots of planktonic *Nitzschia* spp. appeared while planktonic *Microcystis* spp. declined greatly. It indicated that there was the competition between planktonic *Nitzschia* spp. and planktonic *Microcystis* spp. in the tanks of No.1 and 2 (**Fig. 2.14d**).

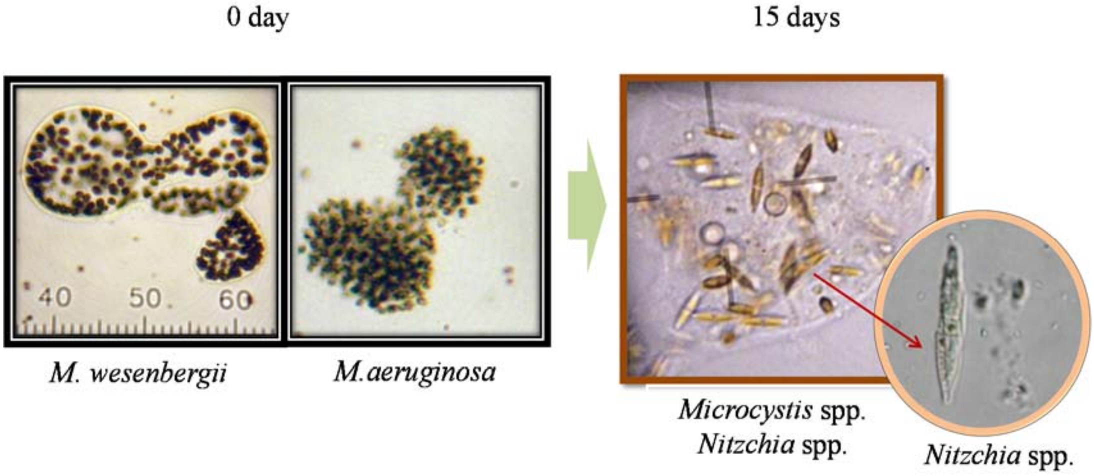


Fig. 2.15 Photos of planktonic *Microcystis* spp. colony's changes during experiments in the tank of No.1 and 2.

It was observed that *Nitzschia* spp. intrude into *Microcystis* spp. colony and multiplied quickly during experimental periods by microscope (**Fig. 2.15**). Maybe the competition of environmental conditions was benefit to planktonic *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 planktonic *Nitzschia* spp. to grow. It has generally been accepted that cyanobacteria have higher temperature optima for growth than other planktonic algal groups. Harmful planktonic cyanobacteria such as *Microcystis* populations have been shown to succeed at, or above 25°C **(Robarts and Zohary, 1987)**. 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 planktonic *Nitzschia* spp. reproduced in the planktonic *Microcystis* spp. colony because of their physical characteristics. The planktonic *Microcystis* spp. cell can adjust its vertical position in the water by buoyancy from the 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 planktonic *Microcystis* spp. cells in the daytime **(Wang et al., 2010)**. Planktonic *Microcystis* spp. rose again because that cell densities decreased due to consuming starch during respiration mainly at night **(Cheng and Qiu, 2006)**. The planktonic *Nitzschia* spp. in planktonic *Microcystis* spp. colony accelerated the rate of decline because of the increasing density of *Microcystis* spp. colony after invaded by *Nitzschia* spp., such as the biomass of *Nitzschia* spp. increased from 378 cells to 2,460 cells in the tank without *V. asiatica*. As a result, lots of planktonic *Microcystis* spp. settled down at the bottom of tanks **(Shapiro, 1973; Watanabe and Harada, 1993)**. In reality, the community structure and dominant taxon of planktonic algae result from mutual competition among various algae **(Zhu et al., 2010b)**. It will be another good method to control planktonic *Microcystis* spp. by changing the environmental conditions of eutrophic water bodies so that the other algal species, such as diatom, replace toxic cyanobacteria as the dominant planktonic algae. The specific factor and mechanism for dominant planktonic diatom were not clear and needed further single-species culture experiment.

In a word, in the case without *V. asiatica*, the dominant planktonic algae altered with the change of environment conditions. After adding *V. asiatica* into the water body, the competition between planktonic algae and epiphytic algae existed also besides the competition between planktonic algae and *V. asiatica*. The relationship among organisms was more complex after introducing *V. asiatica*. *V. asiatica* could inhibit harmful *Microcystis* spp. by the competition and allelopathy when the biomass of macrophyte *V. asiatica* was larger than 50 g in the No.4 and 5 tanks with 30 L solution. Hence it was likely that an introduction of *V. asiatica* in Lake Taihu would be a method to control the algal bloom. However, the biomass of *V. asiatica* should be large enough to control the algal bloom.

2.5 Conclusions

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* spp.

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

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

CHAPTER 3

METABOLIC AND ANTIOXIDANT RESPONSES OF *VALLISNERIA ASIATICA* TO DIFFERENT CONCENTRATIONS OF DISSOLVED INORGANIC NITROGEN AND PHOSPHORUS

CHAPTER

3

Metabolic and antioxidant responses of *Vallisneria asiatica* to different concentrations of dissolved inorganic nitrogen and phosphorus

Abstract

To clarify the reason for the disappearance of submerged macrophytes and supply the necessary theory for the submerged macrophyte restoration in eutrophic Lake Taihu, effects of the moderate nutrients concentration (NO_3^--N 1.50 mg·L^{-1}, PO_4^{3-}-P 0.10 mg·L^{-1}), excessively high NH_4^+-N concentrations (NO_3^--N 1.50 mg·L^{-1}, NH_4^+-N 3.50 mg·L^{-1}) and excessively high PO_4^{3-}-P concentrations (NO_3^--N 1.50 mg·L^{-1}, PO_4^{3-}-P 0.60 mg·L^{-1}) in the water column on the antioxidant defense system in submerged macrophyte, *Vallisneria asiatica*, were studied with the 20-day aquarium experiments. The results showed that the moderate concentration of nutrients can promote the metabolism of *V. asiatica*. Either excessive NH_4^+-N or PO_4^{3-}-P could cause the oxidative stress to cells of *V. asiatica*, expressed as decreased contents of chlorophyll a and protein, and the enhancement of catalase activities in leaves of *V. asiatica*. In addition, 0.6 mg·L^{-1} of PO_4^{3-}-P caused more oxidative damage to *V. asiatica* than 3.5 mg·L^{-1} NH_4^+-N. The results indicated that the antioxidant defense mechanisms could be activated but still could not prevent the damage of the metabolism system in *V. asiatica* exposed to either excessively high concentrations of NH_4^+-N or PO_4^{3-}-P. Therefore, it is necessary to establish a dual control strategy of N and P for the restoration of *V. asiatica* in eutrophic Lake Taihu.

Key words: antioxidant enzyme, metabolism, nutrient, *Vallisneria asiatica*

3.1 Introduction

Plants are continuously producing reactive oxygen species (ROS) during photosynthesis and other metabolic processes. As is often the case, ROS in plant cells are maintained at a low level **(Asada, 1999)**. Environmental stress can induce the production of massive ROS when the changes of environmental factors (such as temperature, light, concentrations of pollutants and nutrients etc.) exceed their required ranges within which plants can grow and breed normally **(Foyer and Noctor, 1999)**. The excessive ROS will prevent the synthesis of chlorophyll a (Chl.a) and protein in plants because of its strong aggressiveness.

To scavenge the excessive ROS, a plant possesses a well-organized antioxidant system to maintain the dynamic equilibrium of ROS **(Pflugmacher et al., 2006)** as shown clearly in **Fig.1.5**. For example, superoxide dismutase (SOD) and catalase (CAT) are important antioxidant enzymes of scavenging ROS in a plant **(Wang et al., 2008a)**. SOD can convert superoxide radical ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) (**Reaction 1.5**), which is then reduced to water and oxygen by CAT as shown by **Reaction 1.6 (Asada, 1999)**. SOD and CAT are generally used as physiological indicators of stress in terrestrial plants and recently are also used in aquatic macrophytes. For example, activities of antioxidant enzymes in *V. asiatica* and *Vallisneria natans* (Lour.) Hara varied under environmental stress, such as the increasing concentration of ammonia and copper ion **(Wang et al., 2008a; Hao et al., 2011)**.

As primary producers, submerged macrophytes are the main natural food of lake fishery and also important regulators of lake ecosystem **(Engel, 1998)**. The water quality of lakes, especial for Lake Taihu in China, has deteriorated rapidly with the development of economy and excess discharge of sewage in recent years, resulting in serious eutrophication problems. Corresponding problems have appeared, as described in chapter 1, the area covered by aquatic macrophyts reduces continually, the macrophyte species decrease constantly even disappear and ecological functions degenerate seriously in Lake Taihu **(He et al., 2008)**. The disappearance of submerged macrophytes in eutrophic lakes is caused by various factors, in which the excessively high concentrations of N and P are the main reason **(Chu et al., 2006)**. It is well known that NO_3^-, NH_4^+ and PO_4^{3-} are three major components of inorganic nutrients in the water of eutrophic lakes. Excessive NO_3^- will be stored in vacuoles so as to be harmless for submerged macrophytes **(Surya et al., 2007)**. Submerged macrophytes are highly sensitive to toxicity of N in form of NH_4^+ at high concentrations. Many researchers hold that high concentrations of NH_4^+ have a direct toxic effect on submerged macrophytes **(Cao et al., 2004; Nimptsch and Pflugmacher, 2007)**. Under the NH_4^+ stress, most plants show slow growth rates and oxidative damage **(Cao et al., 2004; Nimptsch and Pflugmacher, 2007)**. In eutrophic Lake Taihu, the concentration of nutrients is very high in the water column and some restorations of submerged macrophytes are far from success. The NH_4^+-N varied from 3.23 $mg \cdot L^{-1}$ to 3.32 $mg \cdot L^{-1}$ during the period from 2000 to 2002, even reached up to 4.20 $mg \cdot L^{-1}$ in 2003 in Wuli Bay which is shown in the **Fig. 2.1** of Lake Taihu **(Wang et al., 2007)**. The dissolved total phosphorus (DTP) concentration in the surface 20 cm layer water varied between 0.34 $mg \cdot L^{-1}$ and 0.77 $mg \cdot L^{-1}$ in Meiliang Bay of Lake Taihu (also shown in **Fig. 2.1**) during the observed period **(Zhu et al., 2007)**. However, there is little information which illustrates the negative effects of excessively high PO_4^{3-}-P concentrations on the plant. In addition, few comparable studies of excessively high NH_4^+-N and PO_4^{3-}-P concentrations on submerged macrophytes have been conducted. Thus, it will be very important to know the physiological responses of submerged macrophytes to different toxic components of dissolved inorganic nutrients and their tolerance to these nutrients for the restoration of submerged macrophytes in Lake Taihu.

V. asiatica is distributed widely in some waters in South and East Asia (e.g., India, China and Japan) and likes to grow in mesotrophic-eutrophic lakes **(Riis and Sand-Jensen, 2001)**. *V. asiatica* is a kind of common submerged macrophytes in Lake Taihu and reducing currently. Submerged macrophyte can grow well when $6 < pH < 10$; total nitrogen (TN) < 1.24 $mg \cdot L^{-1}$; total phosphorus (TP) < 0.076 $mg \cdot L^{-1}$; the transparency > 0.63 m; 10 °C < temperature < 30°C **(Zhu, 2008; Zhu et al., 2010a)**. Because of its high absorption capacity of contaminants, wide distribution and good resistance for contaminants, it is widely used in the project of vegetation restoration in eutrophic Lake Taihu **(Sun, 1992)**. To understand the mechanisms which lead to the loss of macrophytes in Lake Taihu, effects of the moderate concentration of nutrients (NO_3^--N 1.50 $mg \cdot L^{-1}$, PO_4^{3-}-P 0.10 $mg \cdot L^{-1}$), excessively high NH_4^+-N concentration (NH_4^+-N 3.50 $mg \cdot L^{-1}$) and PO_4^{3-}-P concentrations (PO_4^{3-}-P 0.60 $mg \cdot L^{-1}$) in the water column on the antioxidant defense systems of *V. asiatica* were studied.

3.2 Materials and methods

3.2.1 Materials

The deep-rooted *V. asiatica* was collected in River Onga, Fukuoka, Japan and was pretreated as described in section 2.2.1.

3.2.2 Experimental design

V. asiatica grew well in dechlorinated tap water under the same conditions of this experiment in a preliminary observation for 30 days. Thus, the dechlorinated tap water which contained 0.72 $mg \cdot L^{-1}$ NO_3^--N and 0.02 $mg \cdot L^{-1}$ PO_4^{3-}-P was also used for this experiment. The tap water contained many elements which were necessary for the growth of aquatic plants, such as Na, Fe, Cu, Mn, Zn, Al, B and Cl, depending on the tap water's monitoring data of Fukuoka City Waterworks Bureau. Lake Taihu was in mesotrophic status in 1981 while its trophic status deteriorated severely in the late-1980s **(Zhu, 2008)**. The annual mean value of TN varied between 1.24 $mg \cdot L^{-1}$ and 2.72 $mg \cdot L^{-1}$ while the annual mean TP varied between 0.08 $mg \cdot L^{-1}$ and 1.17 $mg \cdot L^{-1}$ from 1999 to 2006. The NH_4^+-N and TP even reached a peak of 4.20 $mg \cdot L^{-1}$ and 0.77 $mg \cdot L^{-1}$ in 2003 in Wuli Bay and Meiliang Bay (as shown in **Fig. 2.1**), respectively **(Wang et al., 2007; Zhu et al., 2007)**. *V. asiatica* and other submerged macrophytes in Lake Taihu started to decrease since 2000 **(Zhu, 2008)**. Moreover, submerged macrophytes including *V. asiatica* are highly sensitive to the toxicity of N in form of NH_4^+-N at high concentrations which exceed 5 $mg \cdot L^{-1}$ **(Cao et al., 2004; Nimptsch and Pflugmacher, 2007)**. Thus, the N1P1 was set to a moderate concentration of nutrients (NO_3^--N 1.5 $mg \cdot L^{-1}$; PO_4^{3-}-P 0.1 $mg \cdot L^{-1}$) (**Table 3.1**). Then 3.5 $mg \cdot L^{-1}$ NH_4^+-N and 0.5 $mg \cdot L^{-1}$ PO_4^{3-}-P were added into the N1P1 solution respectively, designated as excessively high concentrations of NH_4^+-N (N2P1) and PO_4^{3-}-P (N1P2) (**Table 3.1**). NH_4Cl and KNO_3 were used as the N source, and KH_2PO_4 was used as the P source to adjust the concentrations of N and P in the dechlorinated tap water.

Table 3.1 Concentrations of nutrient at the beginning and end of experiments.

Time	Parameter	Concentrations of nutrient treatments (mg·L^{-1})		
		N1P1	N2P1	N1P2
0th day	NO_3^--N	1.50	1.50	1.50
	NH_4^+-N	—	3.50	—
	PO_4^{3-}-P	0.10	0.10	0.60
20th day	NO_3^--N	1.35	4.34	0.95
	NH_4^+-N	—	0.01	—
	PO_4^{3-}-P	0.06	0.02	0.16

The healthy even-sized plants were divided into 3 groups with 110 ± 5 g fresh weight for each group. Each group was planted in 3 beakers with a volume of 100 mL, which were filled with glass beads as substrates and then put into the strengthened glass tanks. Glass beads were used as a substrate to ensure there were no additional nutrients present that would otherwise occur in a soil substrate. Each tank contained 30 L of treated water solution whose nutrient concentrations were adjusted according to **Table 3.1**. The tanks were placed in a constant temperature room with a temperature of 25°C, illumination intensity of 3,400 lux and photoperiod of 12 h (light):12 h (dark). The experimental device is shown in **Fig. 3.1**. The experiment lasted for 20 days. The plants' leaves were chosen and cut into 2 cm long pieces, and finally transferred into the sampling bottles respectively which were placed in -40°C refrigerator for usage.

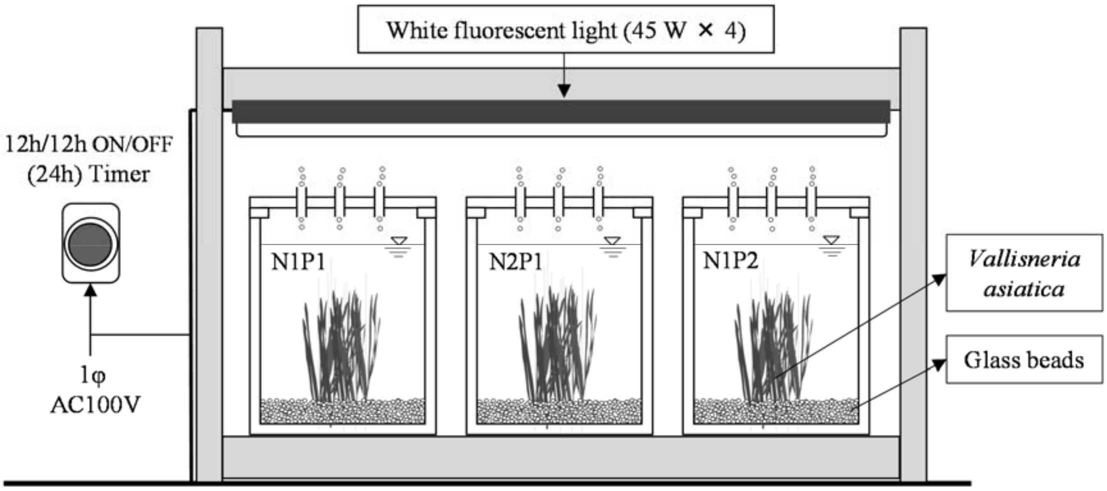


Fig. 3.1 The experimental device.
(Strengthened glass tank: L 30 cm × W 30 cm × H 45 cm)

3.2.3 Measurements

After being filtered through a 0.45μm micropore filter membrane, the concentrations of NO_2^--N, NH_4^+-N, NO_3^--N, and PO_4^{3-}-P in the water were measured by using the Reagent-Free^{TM} Ion Chromatograph System with Eluent Generation ICS-2100 and ICS-1100 for anions and cations, respectively (Dionex, Japan).

Measurements about plant Chl.a, protein, SOD and CAT was referred to Hao et al. **(2011)** and was performed in triplicate. The Chl.a content was measured by the acetone extraction method according to Arnon **(1949)**. A 2 g sample of plant materials was grinded with 10 ml of 90% acetone and then centrifuged at 12,000 × g for 10 min. The absorbance of the supernatant at 645 and 663 nm was measured. The plant Chl.a content was calculated by the formula of Arnon **(1949)**.

2 g of each frozen leaf was ground and homogenized with 8 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 3 mM EDTA and 0.5% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 × g for 10 min at 4°C, and the supernatant was used for measurements of the protein and enzymes. The protein content was measured according to the method of Lowry et al. **(Lowry et al., 1951)**. The 3.6 mL reaction mixture contained 0.6 mL of 20% extractions and 3 mL of mixture reagent which is mixed with 2% Na_2CO_3 in 0.1 M NaOH and 0.5% $CuSO_4$ in 1% sodium tartrate. The reaction mixtures were treated in water baths at 25°C for about 10 minutes before the addition of 50% Folin reagent solution (v/v). 30 min later, the absorbance of the reaction mixtures at 750 nm was measured. The standard curve is obtained by using bovine serum albumin as a standard material. The SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) **(Beauchamp and Fridovich, 1971; Li et al., 2002)**. One unit of the enzyme activity is defined as the amount of enzyme required to generate a 50% inhibition of the rate of NBT reduction measured at 530 nm. The CAT activity was determined by measuring the yield of oxygen in the reaction between extractions and H_2O_2 at the temperature of 24°C and ordinary pressure within 1 min **(Greenfield and Price, 1954)**.

3.3 Results

3.3.1 Changes of pH and nutrient concentrations

There were no obvious differences in pH among these different treatments of nutrients at the beginning of the experiment. The pH value varied between 7.29 and 8.00 at the end of the experiment (**Fig. 3.2**). The total concentrations of N and P declined at the end of the experiment comparing with the initial values (**Table 3.1**). In the treatment of N2P1, the concentration of NH_4^+-N decreased from 3.50 mg·L^{-1} to 0.01 mg·L^{-1} while the concentration of NO_3^--N increased from 1.50 mg·L^{-1} to 4.34 mg·L^{-1}.

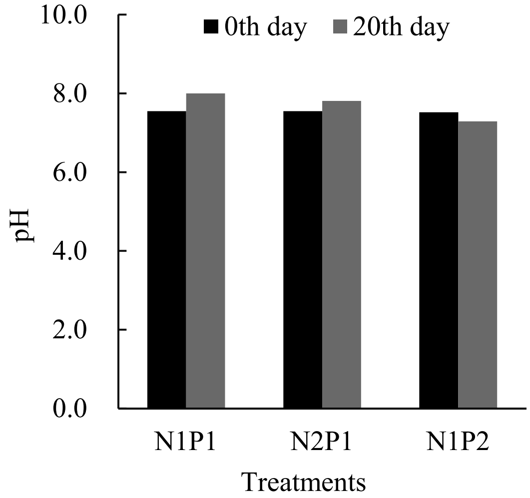


Fig. 3.2 Changes of pH.

3.3.2 Contents of chlorophyll a and protein

The contents of Chl.a increased significantly compared with plants prior to treatments (**Fig. 3.3**). However, the Chl.a content at excessively high concentrations of NH_4^+-N and PO_4^{3-}-P were lower than N1P1 treatment (NO_3^--N 1.50 mg·L^{-1}, PO_4^{3-}-P 0.10 mg·L^{-1}). The Chl.a content at excessively high concentration of PO_4^{3-}-P was lower than NH_4^+-N (**Fig. 3.3**). Treatments with moderate nutrient concentration and excessively high NH_4^+-N concentration increased the content of protein by 14% and 7% respectively, compared with plants prior to treatments (**Fig. 3.4**). However, no obvious increase in protein was found in leaves of plants treated with excessive PO_4^{3-}-P compared with plants before treatments (**Fig. 3.4**).

3.3.3 Activities of SOD and CAT

No notable change in the SOD activity was observed in leaves of plants exposed to treatments of nutrients (**Fig. 3.5**). Exposure of plants to treatments of nutrients caused a significant increase of CAT activity and the CAT activity of plants exposed to the excessively high concentrations of NH_4^+-N and PO_4^{3-}-P was higher than that of plants exposed to the moderate concentration of nutrients (**Fig. 3.6**). The CAT activity in the leaves of plants with the treatment N1P2 was higher than that of N2P1 (**Fig. 3.6**).

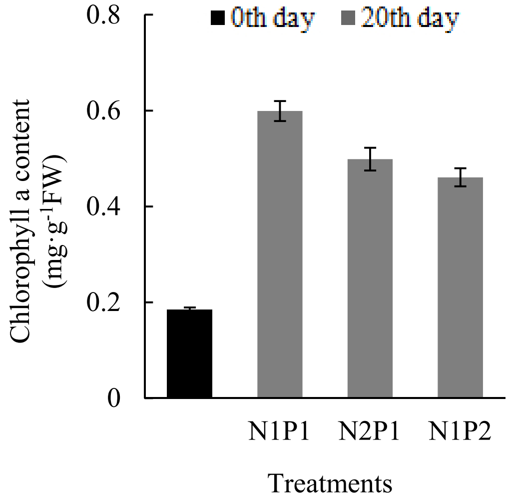


Fig. 3.3 Changes in chlorophyll a contents of *Vallisneria asiatica*.

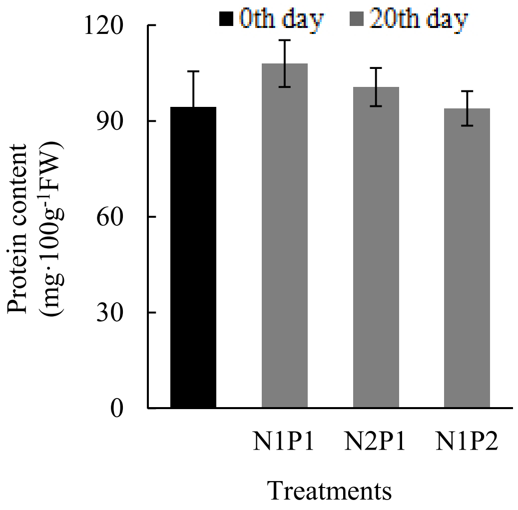


Fig. 3.4 Changes in protein contents of *Vallisneria asiatica*.

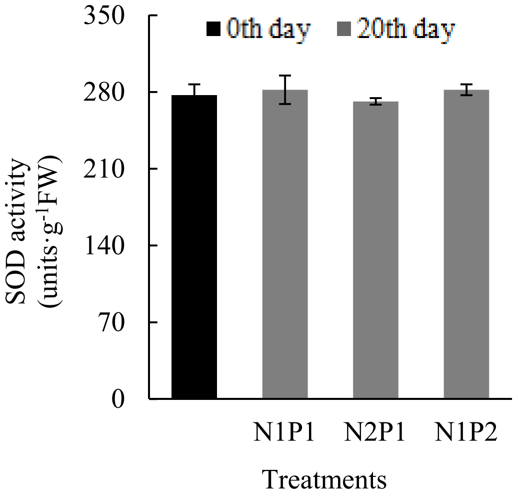


Fig. 3.5 Changes in SOD activities of *Vallisneria asiatica*.

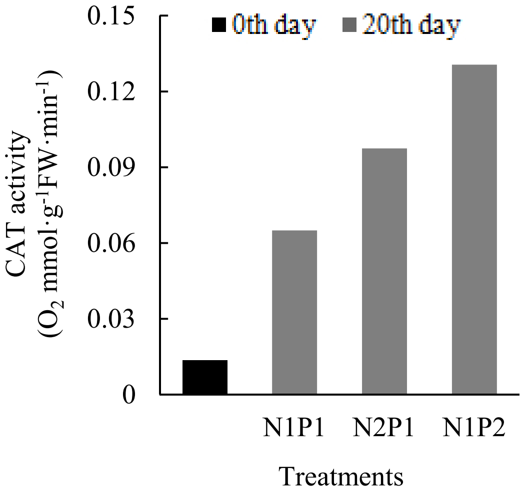


Fig. 3.6 Changes in CAT activities of *Vallisneria asiatica*.

3.4 Discussion

The pH range in which plants can grow is 4 to 12, but the suitable range for their growth is 6 to 10 **(He et al, 2007)**. In this study, the pH varied between 7.52 and 8.00. Thus, the nutrients are considered as main causes of the oxidative stress for *V. asiatica* in this study in the following discussion.

3.4.1 Chl.a and protein responses in *V. asiatica*

Compared with plants prior to treatments, increased contents of Chl.a and protein suggested that the moderate concentration of nutrients (NO_3^--N 1.50 mg·L^{-1}, PO_4^{3-}-P 0.10 mg·L^{-1}) could promote the metabolism of *V. asiatica* (**Fig. 3.3**). However, with 3.5 mg·L^{-1} of NH_4^+-N or 0.6 mg·L^{-1} of PO_4^{3-}-P, the metabolism was influenced a lot expressed as the reduction of Chl.a and protein contents compared with the treatment N1P1. This result indicated that the excessive NH_4^+-N or PO_4^{3-}-P could inhibit the metabolism of *V. asiatica*.

Nitrogen is one of the constituents of plant photosynthetic pigment and also is the important element for the protein. Phosphorus also affects the photosynthesis procedure and an increase of phosphorus supplement will result in significant increase of plant biomass. Chl.a is the major pigment participating in photosynthesis. The content of Chl.a is closely related to the photosynthesis rate of a plant and the photosynthesis rate always increases with the increasing of Chl.a contents in a certain range **(Wang et al., 2008a)**. The significant increased Chl.a content in *V. asiatica* exposed to the moderate concentration of nutrients indicated that the moderate concentration of nutrients promoted the photosynthesis process in *V. asiatica*. However, the photosynthetic system was damaged and photosynthesis efficiency was inhibited in leaves of *V. asiatica* in response to excessively high NH_4^+-N and PO_4^{3-}-P concentrations. It might be because excessively high concentrations of NH_4^+-N and PO_4^{3-}-P have induced the generation of ROS, causing damage to the chloroplast structure or the chlorophyll membrane like *Vallisneria natans* (Lour.) Hara **(Wang et al., 2008a)**. Moreover, exposure of *V. asiatica* to 0.6 mg·L^{-1} PO_4^{3-}-P led to a little lower Chl.a content than 3.5 mg·L^{-1} NH_4^+-N (**Fig. 3.3**). It had been shown that the 0.6 mg·L^{-1} PO_4^{3-}-P caused more photosynthetic damage of *V. asiatica* than 3.5 mg·L^{-1} NH_4^+-N.

In addition, the majority of proteins are various enzymes involved in the metabolism in a plant. Accordingly, the content of protein is an important index which is a reflection of the total metabolism in a plant. Therefore the content of protein can be used for indicating physiological effects of excessively high nutrients concentrations on plants **(Wang et al., 2008a)**. The changes of protein were consistent with the Chl.a contents (**Fig. 3.3, Fig. 3.4**). This also well indicated that the moderate concentration of nutrients benefited the metabolism of *V. asiatica*. However, the metabolism of *V. asiatica* was damaged seriously when the concentration of NH_4^+-N exceeded 5 $mg \cdot L^{-1}$ resulting in the excessive ROS **(Cao et al., 2004)**. This was probably one of the main reasons why *V. asiatica* declined in Lake Taihu. The field investigation in Lake Taihu also showed that the mean values of TN and TP from 1999 to 2001 were 1.71 $mg \cdot L^{-1}$ and 0.08 $mg \cdot L^{-1}$, respectively, and then they increased to 2.34 $mg \cdot L^{-1}$ and 1.17 $mg \cdot L^{-1}$ from 2002 to 2006, respectively **(Zhu, 2008)**. *V. asiatica* and other submerged macrophytes like *Ceratophyllum demersum* L started to decrease since 2000 **(Zhu, 2008)**.

3.4.2 Antioxidant enzyme responses of *V. asiatica*

The increased CAT activity compared with plants before treatments suggested that *V. asiatica* was under the oxidative stress from 3.5 $mg \cdot L^{-1}$ of NH_4^+-N and 0.6 $mg \cdot L^{-1}$ of PO_4^{3-}-P **(Nimptsch and Pflugmacher, 2007)**. The increased CAT activities and invariable SOD activities indicated that *V. asiatica* adapted to the circumstance of excessive nutrient by enhancing the activities of CAT **(Cao et al., 2004)** and CAT was more sensitive than SOD to 3.5 $mg \cdot L^{-1}$ of NH_4^+-N and 0.6 $mg \cdot L^{-1}$ of PO_4^{3-}-P. SOD and CAT, as the important parts of the defense system, can keep the dynamic equilibrium between a production and an elimination of ROS to prevent its harm to submerged macrophytes like *Vallisneria natans* (Lour.) Hara under normal environmental conditions **(Wang et al., 2008a)**. Additionally the superoxide radical which is not transformed by SOD can be transformed to the even more toxic $OH^\bullet$ via the Haber-Weiss reaction (**Reaction 1.4**) owing to the invariable SOD activity **(Yu, 1994)**. In consequence, the toxicity of excessive NH_4^+-N and PO_4^{3-}-P maybe can partly be explained by the oxidative stress response of the excess of $O_2^{\bullet -}$ and H_2O_2 indicated by Cao et al. **(2004)** and additionally by the even more toxic $OH^\bullet$ **(Yu, 1994)**. The activity of CAT in *V. asiatica* exposed to the N1P2 treatment was higher than that of N2P1 treatment (**Fig. 3.6**). It indicated that 0.6 mg·L^{-1} PO_4^{3-}-P would induce more serious oxidative stress to *V. asiatica* than 3.5 mg·L^{-1} NH_4^+-N **(Mishra et al., 2006)**.

Above all, the elevated activity of CAT in *V. asiatica* reflected an increased degree of antioxidant response in plants to resist the oxidative stress of excessively high concentrations of dissolved inorganic N and P. As shown in **Fig. 3.7**, the moderate concentrations of N and P promoted the growth of *V. asiatica* because the increased CAT activities removed the excessive ROS. The excessively high concentrations of N and P inhibited the growth of *V. asiatica* expressed as the declined Chl.a and protein contents because of the excessive ROS (**Fig. 3.7**). Thus, to reduce the oxidative damage of the *V. asiatica*, it is important to control the concentrations of NH_4^+-N and PO_4^{3-}-P in Lake Taihu.

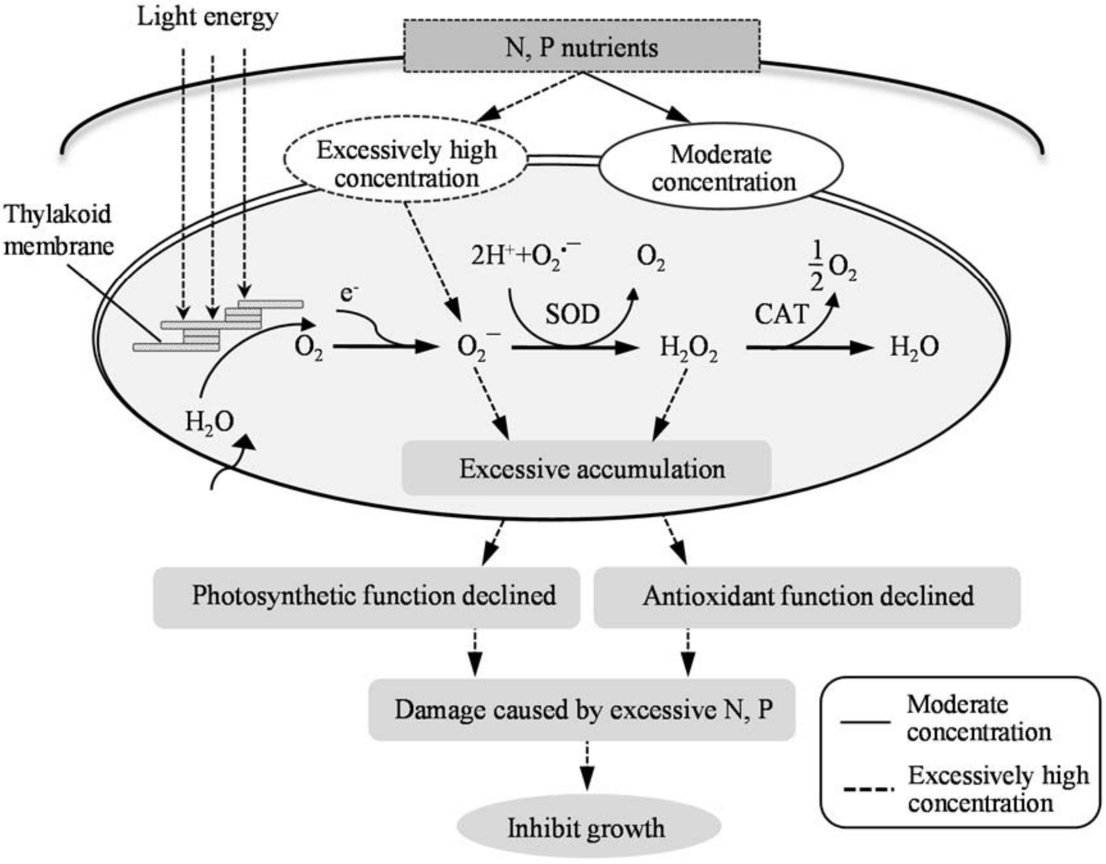


Fig. 3.7 Antioxidant responses of *Vallisneria asiatica* to N, P nutrients.

In addition, in oligotrophic water, submerged macrophytes can obtain nutrients from sediments through roots, whereas filamentous algae like *Cladophora glomerata* and

Spirogyra sp. may be limited by nutrients in water **(Simpson and Eaton, 1986)**. When nutrients in water increased, filamentous algae (mainly *Cladophora glomerata* and *Spirogyra* sp.) have negative effects on the growth of submerged macrophytes by shading and CO_2 limitation **(Simpson and Eaton, 1986)**. There were no filamentous algae in tanks of this study, but special attention should be paid to the growth of filamentous algae when the restoration of *V. asiatica* was in process in eutrophic Lake Taihu.

3.5 Conclusions

To conclude, the antioxidant enzyme CAT in *V. asiatica* was more sensitive to high concentrations of nutrients than SOD. *V. asiatica* could resist oxidative stress from the moderate concentration of nutrients (NO_3^--N 1.50 mg·L^{-1}, PO_4^{3-}-P 0.10 mg·L^{-1}) by activating the antioxidant enzymes in its body. However, either 3.5 mg·L^{-1} of NH_4^+-N or 0.6 mg·L^{-1} of PO_4^{3-}-P were able to trigger oxidative damage of *V. asiatica*, expressing as a decline of Chl.a and protein contents. The results indicated that the antioxidant defense mechanisms were activated but could not prevent the damage of the metabolism system in *V. asiatica* exposed to an excess of NH_4^+-N and PO_4^{3-}-P. These results explained, at least partially, the deterioration reasons of submerged macrophytes like *V. asiatica* in the eutrophic Lake Taihu, China. It also indicated that the restoration of *V. asiatica* needed a nutrient control strategy in eutrophic Lake Taihu.

CHAPTER 4

OXIDATIVE STRESS RESPONSES OF SUBMERGED MACROPHYTE *VALLISNERIA ASIATICA* TO DIFFERENT ALGAL CONCENTRATIONS

CHAPTER

4

Oxidative stress responses of submerged macrophyte *Vallisneria asiatica* to different algal concentrations

Abstract

In a 10-day aquarium experiment, this investigation examined the physiological effects of different plant biomass levels and of increasing natural algal (mainly the toxic cyanobacterial) concentrations on a submerged macrophyte, *Vallisneria asiatica*. Algal stress suppressed the superoxide dismutase activity of the plant's leaves and induced the catalase and peroxidase activities of its roots. The soluble protein content in *V. asiatica* decreased with an increase in natural algal concentrations, whereas the malonaldehyde (MDA) increased significantly at algal Chl.a concentrations of 222 and 262 $\mu g \cdot L^{-1}$ in water. *V. asiatica* adapted to the stress caused through the increment of algal concentrations by adjusting its antioxidant defense system to remove the excessive reactive oxygen species when the algal Chl.a concentration was > 109 $\mu g \cdot L^{-1}$. Additionally, high biomass of *V. asiatica* (2,222 g $FW \cdot m^{-2}$) can inhibit the reproduction of cyanobacteria more significantly than low biomass (1,111 g $FW \cdot m^{-2}$). High biomass of *V. asiatica* increased the oxidative stress in an individual plant when the initial algal Chl.a concentration in the water reached 222 and 262 $\mu g \cdot L^{-1}$, as expressed by the increased MDA in leaves, compared with low biomass of *V. asiatica*. This provides a basis for controlling algal concentrations and *V. asiatica* biomass for the recovery of *V. asiatica* in eutrophic Lake Taihu.

Key words: algal bloom, physiological response, restoration, *Vallisneria asiatica*

4.1 Introduction

Rapid economic growth is usually accompanied by environmental pollution such as the severe eutrophication of water bodies. Algal blooms in eutrophic water bodies are becoming a worldwide ecological problem **(Carmichael, 1992; Codd, 1995)**. Lake Taihu, the third largest freshwater lake in China, is a well-known case of a water body beset by the wide-ranging occurrence of algal blooms. Algal blooms in Lake Taihu have been occurring since the early 1980s and then deteriorated in 2000 onward **(Zhang et al., 2011a)**. The habitable area for aquatic macrophytes in Lake Taihu has been continually reduced, and many macrophyte species have diminished or even disappeared **(He et al., 2008)**.

The disappearance of aquatic macrophytes in eutrophic water bodies, especially submerged macrophytes, is caused by various factors **(Chu et al., 2006; Xie et al., 2005)**. In eutrophic water bodies, cyanobacteria, which are the dominant species in algal blooms, produce different types of toxins which represent the main toxic threat to other organisms **(Carmichael, 1997)**. Algae form floating surface blooms and cause shading effects that inhibit the photosynthesis of submerged macrophytes. They also have allelopathic effects on submerged macrophytes and inhibit their growth and photosynthetic oxygen production **(Pflugmacher, 2002)**. Aside from such effects, algal blooms also degrade the water quality when their period of decay sets in. These harmful effects include the exhaustion of dissolved oxygen and a sharp increase in nutrients, which in turn aggravate the adverse effects of algal blooms on submerged macrophytes **(Buryskova et al., 2006)**.

The reactive oxygen species (ROS) production is a common macrophyte response under both abiotic and biotic stresses **(Miller et al., 2008)**. The mechanism of algal stress on submerged macrophytes can be traced to the formation of ROS during the algal bloom (**Fig. 4.1**). ROS, such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), are formed in many normal cellular reactions in macrophytes **(Bowler et al., 1992)**. However, environmental stress also induces the production of massive ROS or destroys the scavenging system of ROS, resulting in a surplus of ROS when the macrophytes are threatened by environmental adversities **(Foyer and Noctor, 1999)**. To prevent the cellular damage caused by ROS, cells in macrophytes develop a well-organized antioxidant system involving antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). The function of SOD is to convert $O_2^{\bullet-}$ to H_2O_2, which is then reduced to water and oxygen by POD and CAT **(Asada, 1999)**. Thus SOD, CAT, and POD have generally been used as physiological indicators of macrophytes under stress. Malonaldehyde (MDA), the main degradation product of lipid peroxidation, is also commonly used as an indicator of oxidative stress in biological systems. Thus, the increased MDA contents indicated that the cellular membranes in macrophytes were destroyed and the macrophytes have suffered the oxidative stress. Additionally, the majority of soluble proteins are various enzymes involved in macrophyte metabolism. Therefore, the soluble protein content can be used to indicate the physiological effects of adversity affecting macrophytes **(Wang et al., 2008a)**.

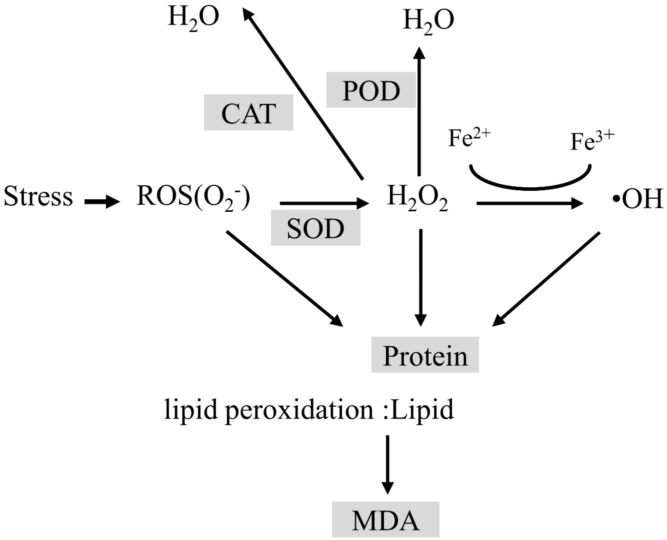


Fig. 4.1 Mechanisms of oxidative cellular damage in *V. asiatica* under the algal stress.

Vallisneria asiatica is a perennial submerged macrophyte that is widely distributed in China **(Wang et al., 2008a)** and some waters in South and East Asia (e.g., India and Japan). This macrophyte has been selected for its high adaptive capability, wide distribution, and easy harvesting in Japan. In Lake Taihu, China, *V. asiatica* is currently diminishing and there are only small amounts of *V. asiatica* in East Lake Taihu (its geographic location can be seen in **Fig. 2.1**). Current research focuses mostly on the abiotic effects of pure pollutants on submerged macrophytes, such as the effect of microcystin-LR (MC-LR) on submerged macrophytes *V. natans* (Lour.) Hara **(Jiang et**

al., 2011) and the effect of copper ion on *V. asiatica* **(Hao et al., 2011)**. Little research studying the damage mechanism of submerged macrophytes caused by biotic factors, such as cyanobacteria or algal blooms, has been conducted. In this study, the natural algae in a pond in Japan and natural healthy *V. asiatica* were used. The aim of this study is to demonstrate the damage mechanism of *V. asiatica* caused by the stress of different algal concentrations that reflected the level of algal blooms. Finally, the experiment also aims to investigate the effects of an increase in *V. asiatica* biomass on the physiology of the individual macrophyte under conditions of different algal Chl.a concentrations in the water. These results will show the occurrence of oxidative stress in *V. asiatica* and assist in the recovery of submerged macrophytes in eutrophic Lake Taihu.

4.2 Materials and methods

4.2.1 Materials

The deep-rooted *V. asiatica* was collected from the River Onga in Fukuoka Prefecture, Japan, and the natural algae were obtained from an algal bloom-infested pond in Fukuoka Prefecture, Japan, which is the same with the text in section 2.2.1.

4.2.2 Experimental design

Table 4.1 The experimental treatments.

Tank number	*V. asiatica* biomass ($g\ FW \cdot m^{-2}$)	Algal Chl.a concentrations ($\mu g \cdot L^{-1}$)
A1	1,111	0
A2	1,111	109
A3	1,111	191
A4	1,111	262
B1	2,222	0
B2	2,222	122
B3	2,222	166
B4	2,222	222

The eight tanks were divided into two groups according to the submerged macrophyte biomass (A: 1,111 $g\ FW \cdot m^{-2}$ and B: 2,222 $g\ FW \cdot m^{-2}$). Approximately 100 ± 5 g fresh weight (FW) of plants with an average of 16 *V. asiatica* was placed in each tank of group A, and 200 ± 5 g FW of plants with an average of 32 *V. asiatica* was placed in each tank of group B. Each group underwent four treatments with different concentrations of algae. The initial concentration of algae in each treatment was expressed as the concentration of algal Chl.a in the solution. The A1 and B1 tanks without algae were used as the controls. The concentrated algae were used to adjust the level of algal concentrations in each treatment according to the algal Chl.a concentrations in Lake Taihu **(Cao et al., 2005; Chen et al., 2003)**. The concentrations of algal Chl.a in group A were 0, 109, 191, and 262 $\mu g \cdot L^{-1}$, designated as A1, A2, A3, and A4, respectively. The concentrations in group B were 0, 122, 166, and 222 $\mu g \cdot L^{-1}$, designated as B1, B2, B3, and B4, respectively (**Table 4.1**). The water from the pond was filtered through a 0.45μm micropore filter membrane to put into tanks A1 and B1. Each treatment of *V. asiatica* was planted in beakers filled with glass beads as substrates, which were then placed in a strengthened glass tank with a volume of 40 L (L 30 cm × W 30 cm × H 45 cm). Each tank, except A1 and B1, was filled with 30 L of pond water. The eight tanks were subjected to a constant temperature of 25°C, an illumination intensity of 3,400 lux, and a photoperiod of 12 h (light):12 h (dark) for 10 days.

4.2.3 Measurements

The concentrations of PO_4^{3-}-P, NO_3^--N, NH_4^+-N, and NO_2^--N in the water were measured with the same method in section 3.2.3. The pH was tested with a pH meter (D-52, Horiba, Japan).

The measurement of algal Chl.a in the water body was conducted following Schalles et al. **(1998)**, with minor modifications. Approximately 50 mL of the water sample was filtered through Whatman GF/C 25 mm filters. Filters for pigment extraction were macerated in 90% acetone and left for at least 5 h but not more than 24 h in the dark at 4°C. Extractions were then cleared by centrifugation and measured spectrometrically.

Additionally, at the end of the experiment, 2 g of leaves and roots were cut from a plant and placed in a sampling bottle. Three plant samples in each tank were chosen randomly for measurements. The measurements of protein, SOD and CAT are the same with the description in section 3.2.3. The MDA content was based on the method of Heath and Packer **(1968)**. Briefly, 2 mL of extraction were mixed with 2 ml of 0.6% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The mixture was heated in boiling water for 20 min, stopped and cooled at once, and centrifuged at 12,000 × g for 10 min. The absorbance (OD) of supernatant was measured by the UV-visible spectrophotometer (UV-1600PC, Shimadzu, Japan) and the MDA content was calculated from the difference in the absorbance at 532 and 600 nm (OD_{532} and OD_{600}) using the equation: $\text{MDA } (\text{nmol}\cdot\text{g}^{-1}\text{FW}) = 8 \times [6.45\,(OD_{532} - OD_{600}) - 0.56\,OD_{450}]/2$. POD activity was determined as oxidation of guaiacol by H_2O_2 with a spectrophotometer **(Kochba et al., 1977)**. The reaction mixture was composed of 0.5 mL enzyme extraction, 0.3 mL of 20 mM guaiacol, 2.7 mL of 50 mM phosphate buffer (pH 7.0) and 0.2 mL of 8 mM H_2O_2. One unit of POD activity was defined as the change of absorbance at 470 nm min^{-1} g^{-1}FW.

4.2.4 Analysis of data

Data were reported as mean ± standard deviation (SD). A statistical analysis was performed by using one-way ANOVA followed by Dunnett's t-test. Significant differences from the plants before treatments are indicated as * ($p < 0.05$).

4.3 Results

4.3.1 Changes in water quality in each tank

The pH and DO were measured under the light condition at the 0th and 10th day. During the experiment, water pH changed from 7.69 to 9.79. The concentration of algal Chl.a in the solution of group A (100 g of *V. asiatica*) increased significantly on the 10th day compared with initial values, while that in group B (200 g of *V. asiatica*) decreased compared with initial values (**Table 4.2**). The dissolved oxygen (DO) concentration was very low when the concentration of algal Chl.a increased to or exceeded the concentrations of A3 and B3 tanks (**Table 4.2**).

Table 4.2 Changes in algal Chl.a and DO concentrations in the solution of each tank.

		A1	A2	A3	A4	B1	B2	B3	B4
Chl.a/μg·L^{-1}	0 d	0	109	191	262	0	122	166	222
	10 d	0	145	230	115	0	76	126	41
DO/mg·L^{-1}	0 d	5.45	10.37	8.88	7.73	4.92	9.64	7.88	6.61
	10 d	9.91	9.80	2.55	3.02	9.16	6.70	4.32	2.49

4.3.2 Physiological changes in leaves of *V. asiatica*

Figure 4.2 shows the SOD, CAT, and POD activities in the leaves of *V. asiatica* in both group A with 100 g of *V. asiatica* and B treatments with 200 g of *V. asiatica*. Compared with the SOD activities of *V. asiatica* prior to treatments, SOD activities in all treatments except A1 and B1 decreased significantly as the concentration of algae increased. No significant promotion of CAT and POD activities was observed in the leaves of *V. asiatica* when placed under stress at high concentrations of natural algae.

Figure 4.3 shows the contents of MDA and protein in the leaves of *V. asiatica* in both group A and B treatments. The MDA content in the leaves of *V. asiatica* was measured and used as a marker for the oxidative stress. Compared with the *V. asiatica* prior to treatments, the MDA content of *V. asiatica* was observed as constant in all tanks except for treatment B4 with 222 μg·L^{-1} of algal Chl.a. The MDA content in the leaves of *V. asiatica* in the treatment B4 increased up to 3.64 μmol·g^{-1} FW from 2.97 μmol·g^{-1}

FW. The soluble protein content in the leaves of *V. asiatica* decreased significantly as algal concentrations increased, except that of treatments A1 and B1 without *V. asiatica*. The soluble protein content in tanks A4 and B4 decreased by 46.5 and 47.2%, respectively, compared with that in the leaves of *V. asiatica* before treatments.

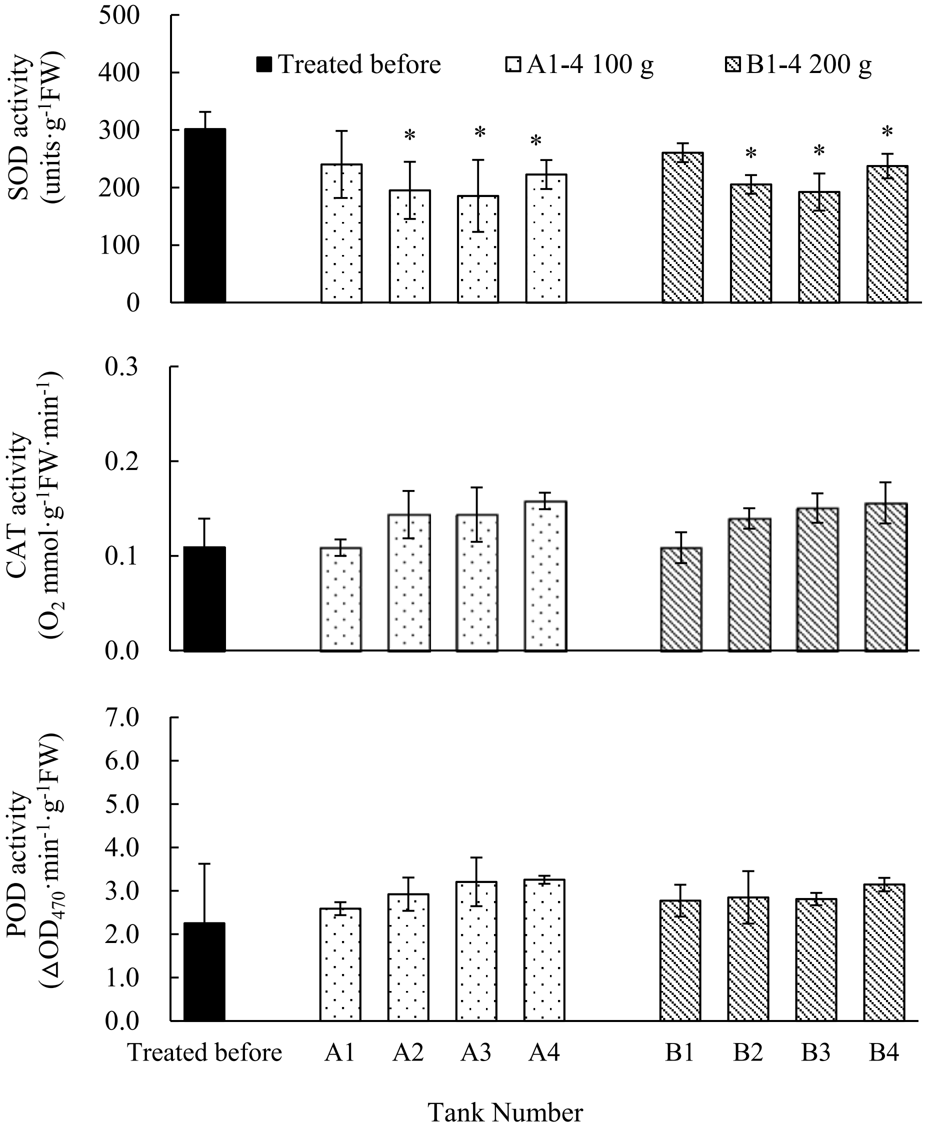


Fig. 4.2 Changes in SOD, CAT, and POD activities in leaves of *V. asiatica* after 10 days of exposure to different algal concentrations. Significant differences from the plants before treatments are indicated as * ($p < 0.05$). (100 g of *V. asiatica* in A1-4; 200 g of *V. asiatica* in B1-4)

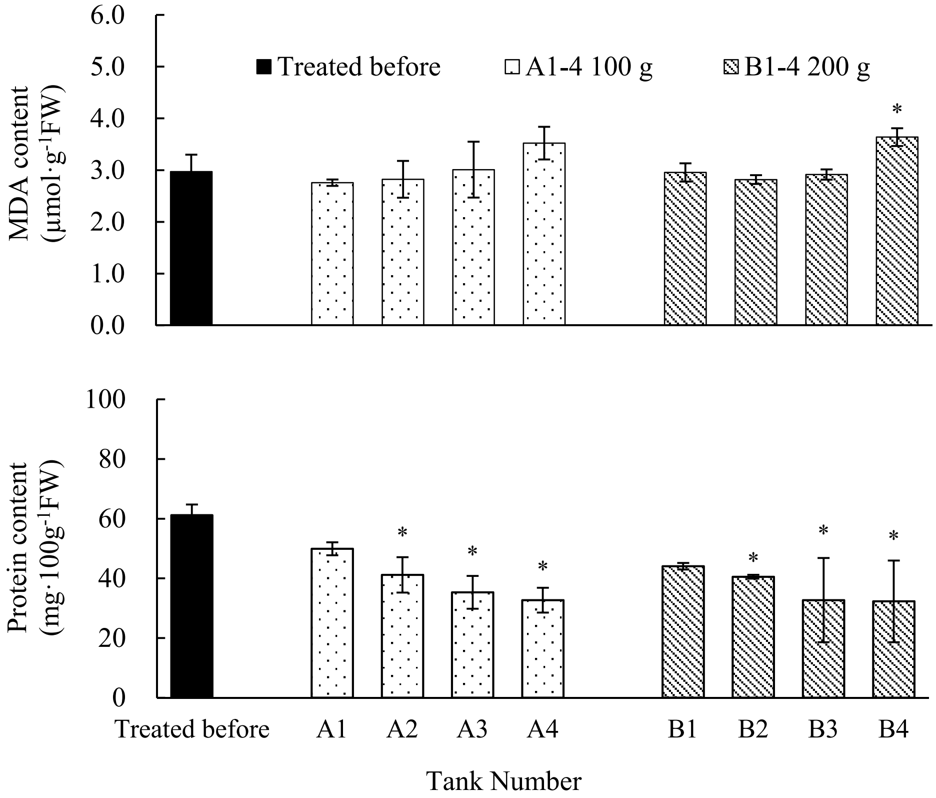


Fig. 4.3 Changes in MDA and soluble protein contents in leaves of *V. asiatica* after 10 days of exposure to different algal concentrations. Significant differences from the plants before treatments are indicated as * ($p < 0.05$). (100 g of *V. asiatica* in A1-4; 200 g of *V. asiatica* in B1-4)

4.3.3 Physiological changes in roots of *V. asiatica*

The changes of SOD, CAT and POD activities in roots of *V. asiatica* for each treatment were shown in **Fig. 4.4**. No significant differences in SOD activities in the roots of *V. asiatica* were found at all treatments of the algal concentration. CAT activities in the roots of *V. asiatica* increased by 100 and 90% for treatments A2 and B2, respectively, after which they decreased with the increment of algal concentrations. Compared with *V. asiatica* before treatments, POD activities for all treatments showed no significant changes except for treatments A4 and B4.

The **Fig. 4.5** showed the changes of MDA and protein contents in roots of *V. asiatica* in the two groups. The soluble protein and MDA content in the roots of *V. asiatica* increased significantly at high concentrations of natural algae in tanks A4 and B4.

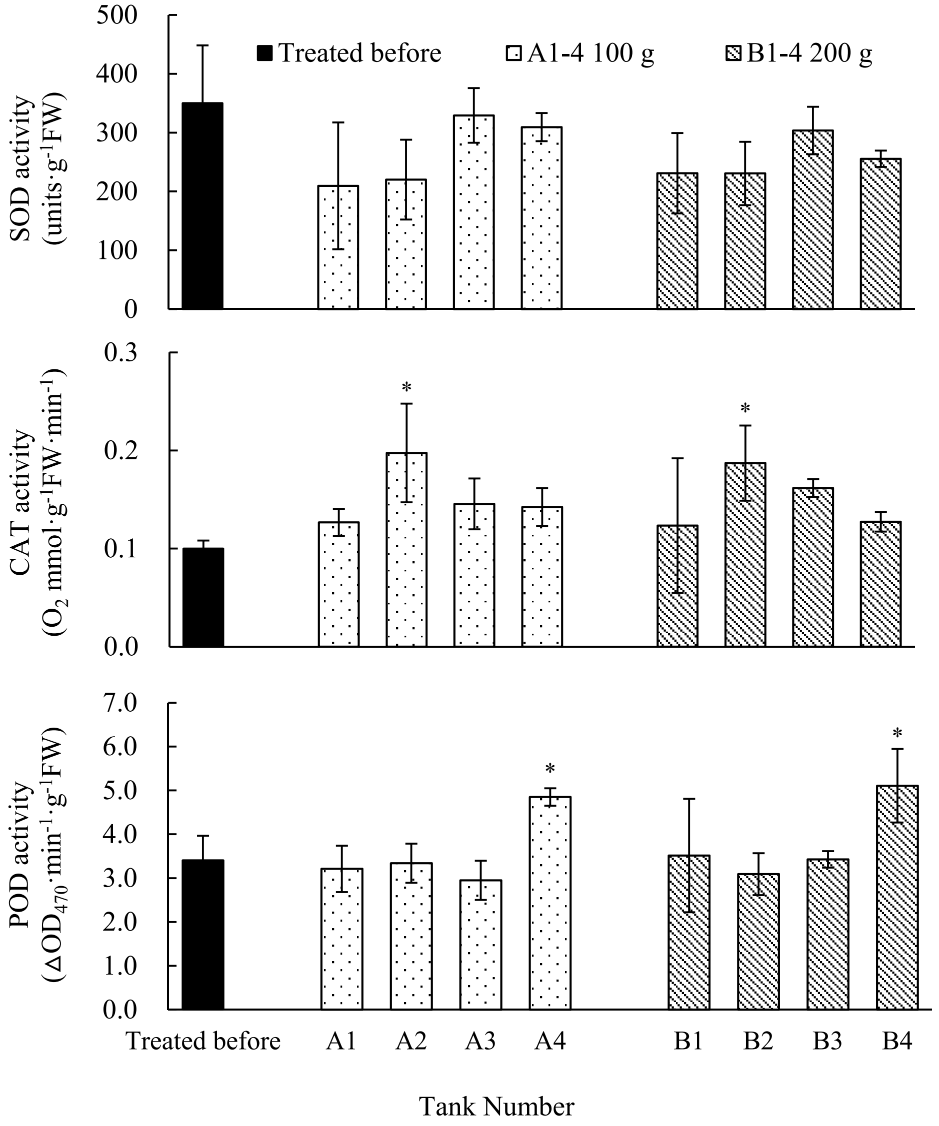


Fig. 4.4 Changes in SOD, CAT, and POD activities in roots of *V. asiatica* after 10 days of exposure to different algal concentrations. Significant differences from the plants before treatments are indicated as * ($p < 0.05$). (100 g of *V. asiatica* in A1-4; 200 g of *V. asiatica* in B1-4)

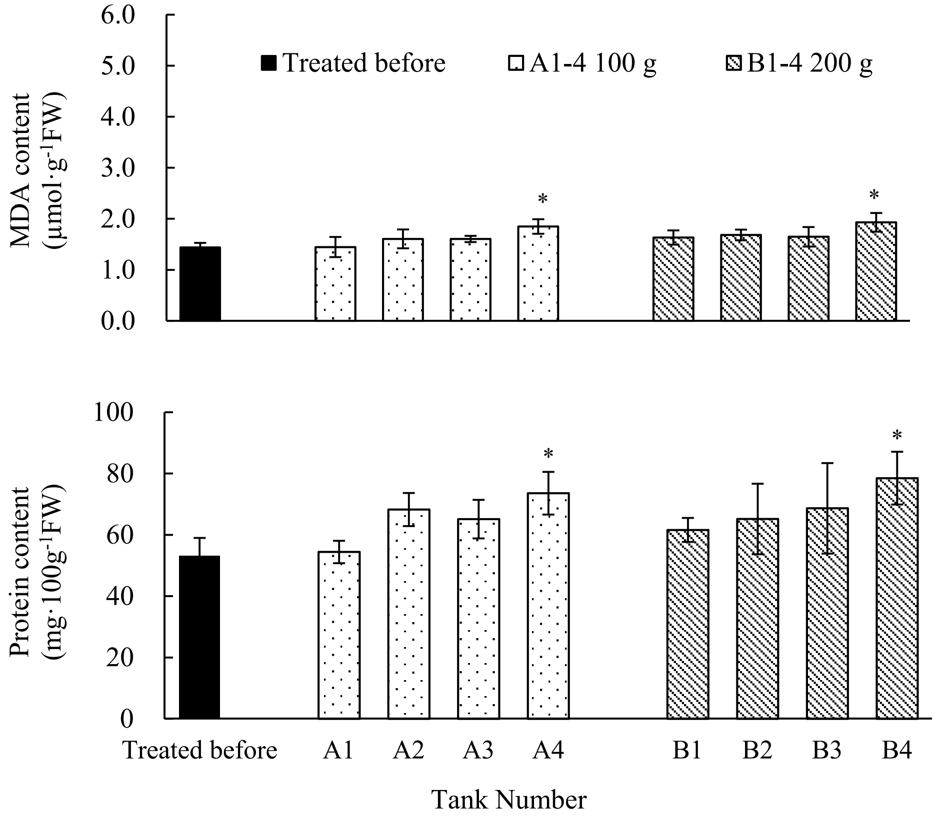


Fig. 4.5 Changes in MDA and soluble protein contents in roots of *V. asiatica* after 10 days of exposure to different algal concentrations. Significant differences from the plants before treatments are indicated as * ($p < 0.05$). (100 g of *V. asiatica* in A1-4; 200 g of *V. asiatica* in B1-4)

4.4 Discussion

Algae always act as a consortium in natural lakes. In this study, natural algae were collected at the same algal bloom-infested pond in chapter 2 and the toxic cyanobacteria were the main species of algae. The natural algae community was used in this study. The physiological responses of *V. asiatica* to the biotic stress caused by algae were researched, which should provide data on useful preconditions for the recovery of *V. asiatica* in eutrophic Lake Taihu. The pH range in which plants can grow is 4-12, but the suitable range for their growth is 6-10 **(He et al., 2007)**. During the experiment, the pH level of the water changed from 7.69 to 9.79 and caused no adverse effects on *V. asiatica*. The high concentration of algae was the main factor that influenced the physiological functions of *V. asiatica*. It is well known that when algal concentrations increase to a certain level, they will affect light conditions, produce toxins, exhaust dissolved oxygen through respiration, and release organic matter, all of which are bad for the growth of aquatic macrophytes **(Li et al., 2009)**. The DO level in Lake Taihu also became much lower than 7.0 mg L^{-1} when the surface algal blooms are dense and begin to decay due to the high summer temperature **(Zhang et al., 2011a)**. Usually, the DO level in clean rivers or lakes is higher than 7.5 mg L^{-1}. DO levels higher than 5.0 mg L^{-1} should be satisfied to ensure the survival of aquatic organisms and the water will stink when the DO level is lower than 2 mg L^{-1}. **Table 4.2** showed that DO levels in the water of A3, A4, B3 and B4 were so low that it is unfavorable for *V. asiatica* to grow normally. For example, Li et al. **(2009)** found that when the absorbance at 665 nm (OD_{665nm} reflected the algal density) of algal blooms is above 0.119, the growth of submerged macrophyte *Ceratophyllum oryzetorum* is influenced, being expressed as a decline in the fresh mass. In this experiment, *V. asiatica* was under the oxidative stress when the algal concentration exceeded 109 $\mu g \cdot L^{-1}$. All adverse influences will increase as algal concentrations rise. The following discussions are concerned with the comprehensive effects of different concentrations of algae on the antioxidant defense systems of *V. asiatica*.

4.4.1 Effects of different algal concentrations on the physiological functions of *V. asiatica*

It is well known that macrophytes respond to abiotic and biotic stress by activating antioxidant mechanisms. The oxidative stress is related to the accumulation of large quantities of ROS under both abiotic and biotic stress **(Peuthert et al., 2008; Miller et al., 2008)**. For submerged macrophytes, survival under stress conditions is possible only if several antioxidant enzymes cooperate and provide a good defense system to maintain the dynamic equilibrium of ROS **(Ge et al., 2012; Pflugmacher, 2004)**.

SOD plays a pivotal role in protecting the plant against oxidative damage and in adjusting the plant's physiological metabolism **(Bowler et al., 1992)**. In this study, compared with *V. asiatica* before treatments, the SOD activity in the leaves of *V. asiatica* was suppressed in all treatments except for A1 and B1. The low SOD activity was also found in the leaves of the submerged macrophytes in Meiliang Bay of Lake Taihu (as shown in **Fig. 2.1**) as a result of very low transparency levels for the algal blooms **(Zhang et al., 2011a)**. It is reported that when superoxide radical $O_2^{\bullet-}$ generation exceeds the elimination ability of SOD, superoxide radical and other oxyradicals like H_2O_2 can inactivate SOD **(Yin et al., 2008)**. Furthermore, the low SOD activity in submerged macrophytes also impairs the scavenging systems of ROS **(Bowler et al., 1992; Alscher et al., 2002; Blokhina et al., 2003)**, causing injury to the plants like submerged macrophytes through the oxidative stress.

CAT and POD are important enzymes of plant defense systems that can convert hydrogen peroxide in plant cells to water and oxygen **(Asada, 1999)**. None of the treatments resulted in observable significant promotion of CAT and POD activities in the leaves of *V. asiatica*. The inactivation of SOD in leaves resulted in the low transformation from superoxide radical into hydrogen peroxide, which in turn failed to activate CAT and POD. The results of the increased activities of CAT and POD in roots also proved that the high concentration of algae causes oxidative stress on *V. asiatica*. The increase in antioxidant enzymes such as CAT and POD in roots can be correlated with the environmental stress caused by the accumulation of ROS **(Pflugmacher, 2004)**. Elevation of antioxidant enzymes indicated the ongoing detoxification process in *V. asiatica*. However, in the roots of *V. asiatica*, the CAT activity first increased and then decreased as algal concentrations rose. It is suggested that once pollution becomes more severe, the activities of antioxidant enzymes in plants will decrease **(Liao et al., 2005)**. The POD activity in roots of treatments A4 and B4 (262μg·L^{-1} and 222 μg·L^{-1} of algal Chl.a) increased significantly, whereas the POD activity of other treatments remained at a constant level compared with *V. asiatica* before treatments. It can be assumed that POD in roots was more tolerant than CAT in roots of *V. asiatica* under the algal exposure.

Several antioxidant enzymes (SOD, CAT, and POD) in plants cooperate to maintain the dynamic equilibrium of ROS under stress conditions as shown in **Fig. 1.5**. However, once the balance of ROS is destroyed under stress, lipid peroxidation occurs under the attack of excessive ROS. Therefore, MDA, the main degradation product of lipid peroxidation, is used to detect the degree of lipid peroxidation and is a useful index to evaluate pollution levels as shown for alfalfa seedlings **(Peutherta and Pflugmacher, 2010)**. MDA also causes considerable damage to cellular membranes and many biological molecules in cells. In this study, the MDA content in leaves and roots of *V. asiatica* increased significantly under the algal stress of treatments A4 and B4. The high MDA contents in leaves could be attributed to the decrease in SOD and insensitive CAT and POD, while the high MDA contents in roots may result from insensitive SOD and inactivation of CAT in roots at high concentrations of algae. The low enzyme activity favored accumulation of superoxide radical and hydrogen peroxide, which could result in lipid peroxidation.

Additionally, much research indicates that excessive ROS contributes to metabolic disorder, including the decreased soluble protein in the leaves of submerged macrophytes like *Potamogeton crispus* L. under environmental stress, such as high NH_4^+ concentrations **(Britto and Kronzucker, 2002; Cao et al., 2004)**. The soluble protein content in the leaves of *V. asiatica* also showed a decreasing trend with an increase in the concentration of algae, partly indicating that the *V. asiatica*'s metabolic capacity was greatly influenced by algal stress compared with *V. asiatica*'s before the treatment. However, the soluble protein content in the roots of *V. asiatica* showed a significant increase when the algal Chl.a concentration was higher than 191 μg·L^{-1}, possibly because the soluble protein transferred from the leaves to the roots is an adaptation to environmental stress **(Bloom et al., 1985)**. The soluble protein content in the roots of *V. asiatica* increased under the algal stress, which is a sign of the positive adaptation of *V. asiatica* to adverse environments. However, this type of adaptation was limited because of the high ratio of leaves to root biomass and the different sensitivities of the soluble protein in leaves and roots of *V. asiatica* to the concentration of Chl.a in water. Thus, a series of negative effects occur in *V. asiatica* under the oxidative stress caused by algae when the algal Chl.a increased up to 109 μg·L^{-1}. These negative effects include the inactivation of SOD in leaves, the increased MDA content in leaves and roots, and the decreased soluble protein content in leaves of *V. asiatica*.

4.4.2 Determination of optimal *V. asiatica* biomass

The physiological indexes of *V. asiatica* in group B1-3 had a similar trend to those of *V. asiatica* in group A1-3 exposed to algae. These results indicated that the increase in *V. asiatica* biomass had no physiological influence on the individual *V. asiatica* when the initial algal Chl.a concentration did not exceed 222 μg·L^{-1} under the conditions of this experiment. Additionally, the stress caused by algae increased with the rise in the initial concentrations of algae in treatments 1-3.

However, when the initial algal Chl.a concentration increased to the values of A4 and B4, the high biomass level of *V. asiatica* (B: 2,222 g FW·m^{-2}) increased the stress in individual *V. asiatica*. The increased stress was expressed as increased MDA in leaves of *V. asiatica* in group B4 compared with A4. It is well known that high densities of both algae and *V. asiatica* will cause a shading effect and exhaustion of dissolved oxygen. The results indicated that high biomass of *V. asiatica* increases these adverse effects after the algae reproduce to a certain quantity under the conditions of this experiment. Most studies also show that a high density of plants inhibits the growth of individual plants because a high density of these plants can influence light reception and photosynthesis like described for *Fucus serratus* (L.) and the land tree **(Creed et al., 1997; Shibata, 2006)**. However, a large biomass of the submerged macrophyte *V. asiatica* was more favorable in controlling the reproduction of algae in this study. The algal Chl.a concentration in water of the tanks with high biomass (2,222 g FW·m^{-2}) of *V. asiatica* declined compared with initial values while the algal Chl.a concentration in tanks with low biomass of *V. asiatica* (1,111 g FW·m^{-2}) increased compared with initial values (**Table 4.2**). Schriver et al. found that algal biomass declines abruptly when the lake plant volume infested (PVI) by the submerged macrophyte population including *Potamotegon pectinatus* L. and *Callitriche hermaphroditica* L. exceeded 15-20% for large-scale (100 m^2) enclosures **(Schriver et al., 1995)**.

Considering the physiological health of individual *V. asiatica* and the controlling effect of algae, under the condition of this experiment, 2,222 g FW·m^{-2} was the optimal biomass to allow the recovery of *V. asiatica*. This occurred when the initial algal Chl.a concentration was ≤191 μg·L^{-1} and a value between 1,111 and 2,222 g FW·m^{-2} was the optimal biomass to allow the recovery of *V. asiatica* when the initial algal Chl.a concentration increased to the levels in A4 and B4. This provides a precondition for controlling algal concentrations and *V. asiatica* biomass for the recovery of *V. asiatica* in eutrophic Lake Taihu. In this real example of the eutrophic Lake Taihu, it has been demonstrated that when working on the submerged macrophyte *V. asiatica* ecological restoration project, appropriate controls of both algae and macrophyte biomass are necessary to ensure healthy growth of the macrophytes.

4.5 Conclusions

In this study, the high concentrations of algae led to oxidative stress in *V. asiatica* when the algal Chl.a concentration was greater than 109 $\mu g \cdot L^{-1}$, shown by changes in the activities of SOD, CAT, POD, the protein content, and the MDA content. The results also indicated that high *V. asiatica* biomass does not increase the oxidative stress in individual *V. asiatica*, but is more helpful in controlling the algal bloom than low biomass when the algal Chl.a concentration did not exceed 191 $\mu g \cdot L^{-1}$. Thus, 2,222 g $FW \cdot m^{-2}$ was the optimal biomass to allow the recovery of *V. asiatica* when the initial algal Chl.a concentration was ≤191 $\mu g \cdot L^{-1}$. Finally, high *V. asiatica* biomass increased the oxidative stress on individual *V. asiatica* when algal Chl.a increased to 222 $\mu g \cdot L^{-1}$, so the optimal biomass to recover *V. asiatica* ranged between 1,111 and 2222 g $FW \cdot m^{-2}$ after algal Chl.a reached 222 $\mu g \cdot L^{-1}$.

CHAPTER 5

ANTIOXIDANT RESPONSES OF *VALLISNERIA ASIATICA* TO THE EUTROPHIC SEDIMENT IN LAKE TAIHU, CHINA

CHAPTER

5

Antioxidant responses of *Vallisneria asiatica* to the eutrophic sediment in Lake Taihu, China

Abstract

Three kinds of the representative sediment were got from East Lake Taihu, Western shore and Meiliang Bay, respectively. The physiological responses of a submerged macrophyte, *Vallisneria asiatica*, to the three kinds of eutrophic sediment in Lake Taihu were assayed in a 40-day aquarium experiment. The plant chlorophyll a (Chl.a) content stopped increasing from the 10th day and there were no obvious differences in Chl.a between the three kinds of sediment. The MDA content in roots had limited changes while the MDA content in leaves of *V. asiatica* growing on the Western shore of Lake Taihu sediment was significantly higher than that on the other two sediment types from the 20th day. The SOD activity in roots and leaves of *V. asiatica* growing on the Western shore of Lake Taihu sediment was lower than that on the other two sediment types on the 10th and 40th day, respectively. The CAT activity in roots increased sharply during the early period while the CAT activity in leaves increased with time slightly. The results showed that the *V. asiatica* can adjust itself to the eutrophic sediment in Lake Taihu via antioxidant responses and suffered the oxidative stress.

Key words: eutrophic sediment, oxidative stress, antioxidant response, *Vallisneria asiatica*

5.1 Introduction

In a healthy lake, submersed macrophytes play an important role in its ecological structure and function **(Engel, 1998)**. Submerged macrophytes can improve the self-purification capacity of a lake ecosystem, such as improve the water quality by inhibiting the growth of algae **(Nakai et al., 2000)**, absorbing excessive nutrients **(Lesage et al., 2007)** and preventing the sediment resuspension **(Madsen et al., 2001)**. It will produce adverse effects on submerged macrophytes when the changes of environmental factors (such as temperature, light, the concentrations of pollutants and nutrients etc.) exceed their required ranges within which the plants can grow and breed normally **(Foyer and Noctor, 1999)**. According to previous studies, the adverse environments like high concentrations of nutrients and heavy metals will cause the visible damage or growth reduction of submerged macrophytes, and it will also induce a series of antioxidant responses in cells because of the excessive reactive oxygen species (ROS) induced by adversities **(Dučić and Polle, 2005; Wang et al., 2009)**. The eutrophic sediment will also induce the production of massive ROS or destroy the scavenging system of ROS, resulting in an accumulation of ROS **(Wang et al., 2009)**. The plants are equipped with a defense system to remove the excessive ROS repairing the damage caused by ROS. Antioxidant enzymes play an important role in this detoxification process. Among the antioxidant enzymes, superoxide dismutase (SOD) acts as the first line of defense against ROS by degrading superoxide to H_2O_2 **(Bowler et al., 1992)** (**Fig. 1.5** and **Reaction 1.5**). Catalase (CAT) can decompose H_2O_2 to water and oxygen **(Pflugmacher, 2004)** (**Fig. 1.5** and **Reaction 1.6**). The content of chlorophyll a (Chl.a) is closely related to the photosynthesis rate of a plant and the photosynthesis rate always increases with the increasing of Chl.a contents in a certain range **(Wang et al., 2008a)**. The lipid peroxidation will occur when the lipid of cell membrane is attacked by ROS because of the deficient antioxidant defense, and as a consequence this damage to cell membranes may lead to a decline in growth rate and biomass **(Kabala et al., 2008)**. Malonaldehyde (MDA), as a secondary end product of lipid peroxidation, is commonly used to detect the degree of lipid peroxidation and oxidative damage in roots and leaves of plants **(Peutherta and Pflugmacher, 2010)**.

The eutrophication of shallow lakes, especial for Lake Taihu in China, has been a worldwide environmental issue in recent years **(Codd, 1995)**. Varieties of aquatic macrophytes have been under decline or even disappear for decades with the eutrophication **(Abe et al., 2006)**. Therefore, to rebuild a healthy lake, the restoration of submerged macrophytes in eutrophic Lake Taihu is very important because of its multiple ecological functions. The decline of aquatic macrophytes is related to the stress of excessive nutrients and pollutants in sediment in eutrophic lakes **(Barrett et al., 1993)**. It is well known that the sediment in eutrophic Lake Taihu contained excessive nutrients (especially N and P) and heavy metals because large amounts of untreated effluents from the industries and agriculture are discharged into Lake Taihu **(Qu et al., 2001)**.

Vallisneria asiatica is distributed widely in some waters in South and East Asia (e.g., India, China and Japan) and likes to grow in mesotrophic-eutrophic lakes **(Riis and Sand-Jensen, 2001)**. Thus, it is probably feasible to restore *V. asiatica* in eutrophic Lake Taihu. At the same time, *V. asiatica* is a kind of common submerged macrophytes in Lake Taihu and reducing currently. The sediment may play a great role in the growth and distribution of submerged macrophytes due to its close relationship with the macrophyte **(Blindow et al., 2002)**. Thus, it is important to investigate responses of *V. asiatica* to the eutrophic sediment of Lake Taihu prior to the restoration or the transplantation of the submerged macrophyte *V. asiatica*. Three kinds of representative sediment were obtained from a macrophyte-dominated bay (East Lake Taihu), and two algae-dominated regions (Western shore of Lake Taihu and Meiliang Bay), respectively. The objective of this study was to compare the antioxidant responses of *V. asiatica* to the sediment from algae-dominated regions and the macrophyte-dominated region. This study also aimed to clear the different physiological responses between leaves and roots of *V. asiatica* to the three kinds of sediment.

5.2 Materials and methods

5.2.1 Materials

The submerged macrophyte, *V. asiatica*, was collected in River Onga, Fukuoka, Japan, and then cultured in dechlorinated tap water for 5 days to remove attachments on plants. Only the plants with healthy growth and even size were chosen for the experiment as in chapter 2-4.

The surface sediment was got from Lake Taihu at the sites shown in **Fig. 5.1**. Site 1 is located in the East Lake Taihu which is the typical region covered by submerged macrophytes, such as *Ceratophyllum demersum* L, *Elodea nuttallii* and *V. asiatica* **(Qu et al., 2001)**. Site 2 is located in the Western shore which is severely polluted and regularly covered by thick algal blooms from late spring into autumn because of the developed agriculture and forestry **(Qu et al., 2001)**. Site 3 is located in the Meiliang Bay which is one of the seriously polluted zones in Lake Taihu **(Qu et al., 2001)**. Years of southeastern winds resulted in serious accumulation of algae at Meiliang Bay.

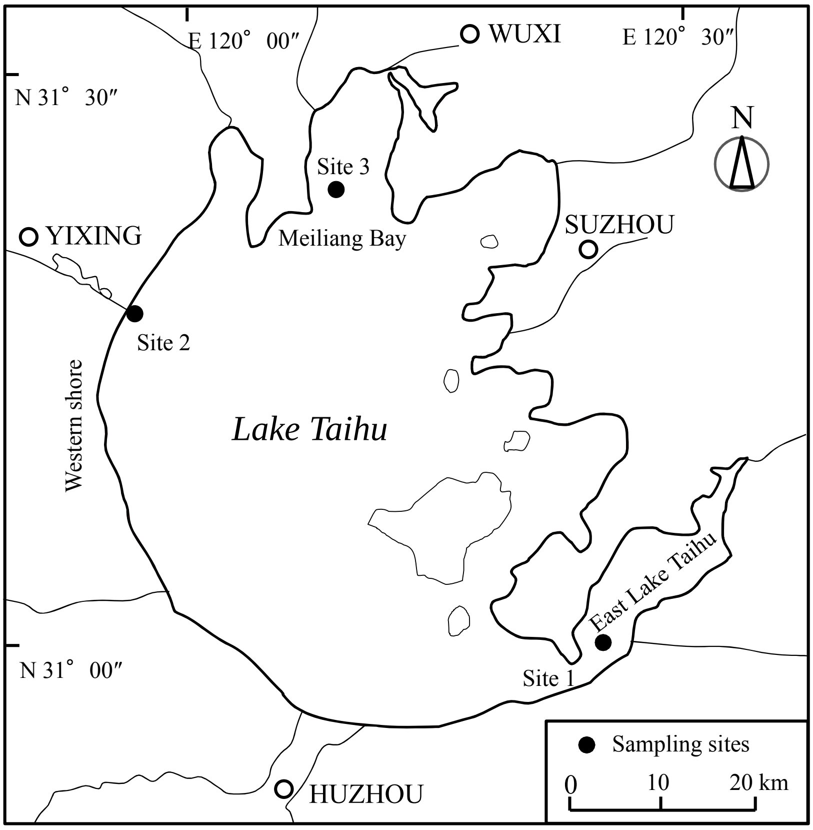


Fig. 5.1 Sampling sites of the sediment in Lake Taihu.

The Sediment Quality Guidelines issued by Water Resources Branch, Ontario Ministry of the Environment established two pollution levels (LEL and SEL) to evaluate the contaminated degree of sediment **(Persaud et al., 1993)**. It is defined that the sediment is considered to be clean to marginal pollution at lowest effect level (LEL) and no adverse effects on the majority of sediment-dwelling (benthic) organisms are expected when the concentrations of the elements were below LEL **(Persaud et al., 1993)**. The sediment is considered to be heavily polluted at severe effect level (SEL) and adverse effects on the majority of sediment-dwelling organisms are expected when the element concentrations exceed SEL **(Persaud et al., 1993)**. The submerged macrophyte, *V. asiatica*, has strong deep roots. Roots of *V. asiatica* were in direct contact with sediment so that it could be influenced by sediment strongly like the majority of sediment-dwelling organisms. Thus, the LEL and SEL were used to evaluate the contaminated degree of sediment in Lake Taihu and its impacts on *V. asiatica*.

Table 5.1 The characteristics of the sediment in Lake Taihu[a].

Indexes	Sampling sites			Sediment quality guidelines	
	East Lake Taihu	Western shore	Meiliang Bay	LEL[c]	SEL[d]
pH	6.53±0.012	6.71±0.015	6.62±0.006		
OM (%)	2.29±0.10	4.40±0.12	1.53±0.23		
TN ($mg\cdot kg^{-1}$)[b]	990±140	3210±180	1830±340	550	4800
TP ($mg\cdot kg^{-1}$)	230±4	390±20	1780±620	600	2000
Cu ($mg\cdot kg^{-1}$)	38.08±7.63	75.00±3.45	34.25±1.24	16	110
Mn ($mg\cdot kg^{-1}$)	1117±118	1165±43	749±83	460	1100
Zn ($mg\cdot kg^{-1}$)	42.06±8.04	217.50±25.82	12.81±2.74	120	820

[a] Cr, Ni, Pb and Cd were not detected in the sediment

[b] The TN of sediment quality guideline is measured by total Kjeldahl nitrogen (TKN)

[c] LEL = Lowest effect level **(Persaud et al., 1993)**

[d] SEL = Severe effect level **(Persaud et al., 1993)**

The basic characteristics of the sediment are summarized in **Table 5.1**. The sediment of East Lake Taihu contained relative low contents of nutrients and heavy metals except Mn. The sediment of Western shore was rich of organic matter (OM), TN, Cu, Mn and

Zn. The sediment of Meiliang Bay contained high contents of nitrogen and TP. The concentrations of the most elements exceeded the LEL. All the concentrations of each element were below the SEL except Mn in the sediment of East Lake Taihu and Western shore.

5.2.2 Experimental design

The three kinds of sediment were sieved (0.2 cm sieve) to remove coarse debris, homogenized, and then placed in beakers (500 mL), respectively. The healthy even-sized plants were divided into 3 groups with 240 ± 5 g (54 *V. asiatica*) fresh weight for each group. Each group of *V. asiatica* was planted in the beakers, which were filled with one kind of sediment as substrates and then put into each strengthened glass tank. Each tank (L 30 cm × W 30 cm × H 45 cm) was filled with 30 L of dechlorinated tap water (containing 0.72 $mg \cdot L^{-1}$ NO_3^--N and 0.02 $mg \cdot L^{-1}$ PO_4^{3-}-P). The three tanks were placed in a constant temperature room with temperature of 25°C, illumination intensity of 3,400 lux and photoperiod of 12h (light):12h (dark). Six *V. asiatica* from each tank was randomly selected for the biochemical analysis during the 0th, 10th, 20th, and 40th day. Three *V. asiatica* was used for the measurement of the Chl.a content and the other three *V. asiatica* was used for analyzing the MDA contents and activities of enzymes.

5.2.3 Measurements

The pH in the sediment was determined by using the portable electrodes with pH meter (D-52, Horiba, Japan), which were pushed into the sediment core. Sediment particles smaller than 100μm were used for all analyses and acid digestion. The proportion of OM in the sediment can be reflected by the loss on ignition (450°C, 3 h) **(Kleeberg et al., 2010)**. To determine the heavy metals and TP, sediment samples were digested by H_2SO_4 and H_2O_2 **(Kleeberg et al., 2010)**. The digestion procedure was modified as follows: heating to 120°C within 1 h, 120°C for 3 h, heating to 300°C within 0.5 h, 300°C for 4 h, cooling to 90°C, and further evaporating to almost dryness (< 90°C), and the residue was redissolved with 25 ml distilled water. Major elements such as P and heavy metals were determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Optima 5300DV, Perkin Elmer, USA). Nitrogen was measured by the total nitrogen analysis **(Hach, 2002)**.

The measurement of Chl.a in *V. asiatica* has been introduced in section 3.2.3. The measurements of MDA, SOD and CAT can be found in section 4.2.3. The results of total plant biomass and root number were determined by 18 plants randomly after harvest at the end of the experiment.

5.2.4 Analysis of data

Data were reported as mean ± standard deviation (n=3). A statistical analysis was performed by using one-way ANOVA followed by the LSD-test, at $p < 0.05$. Different letters indicate significant differences ($p < 0.05$) between three types of sediment by one-way ANOVA test.

5.3 Results

5.3.1 Growth changes of *V. asiatica*

Table 5.2 showed influences of the eutrophic sediment in Lake Taihu on the *V. asiatica* biomass at the end of the experiment. The biomass of *V. asiatica* growing on the three kinds of eutrophic sediment all increased compared with the *V. asiatica* prior to treatments. The total biomass of 18 individuals growing on the East Lake Taihu sediment was higher than that growing on the other two sediment types and the total biomass of *V. asiatica* growing on the Western shore sediment was the lowest one of the three kinds of sediment. The root number of *V. asiatica* growing on the Western shore sediment was obviously fewer than that growing on the East Lake Taihu sediment at the end of the experiment.

Table 5.2 Effects of sediments on the growth of *V. asiatica*. Different letters indicate significant differences ($p < 0.05$) between three types of sediment.

Time	Parameter	East Lake Taihu	Western shore	Meiliang Bay
0th day	Root Number (/plant)	1	1	1
	Weight (g)	80	80	80
40th day	Weight (g)	114	90	102
	Root Number (/plant)	1.83 ± 0.86^{a}	1.33 ± 0.49^{b}	1.67 ± 0.77^{ab}

5.3.2 Physiological changes in roots of *V. asiatica*

As shown in **Fig. 1.5**, plants possess antioxidant enzymes, such as SOD and CAT, scavenging ROS to protect them from oxidative stress. In **Fig. 5.2**, the SOD and CAT activities in roots of *V. asiatica* were shown. SOD in roots showed only limited fluctuation with time. Only on the 10th day, the SOD activity of *V. asiatica* growing on the Western shore sediment was lower than that on the other two sediment types. There was a significant increase in the CAT activity from the 10 day, compared with *V. asiatica* before treatments. There were obvious differences in the CAT activity of *V. asiatica* among the three kinds of sediment on the 10th day while the differences disappeared on the 20th day. On the 40th day, the CAT activity of *V. asiatica* growing on the sediment from Western shore and Meiliang Bay were significantly lower than that on the East Lake Taihu sediment.

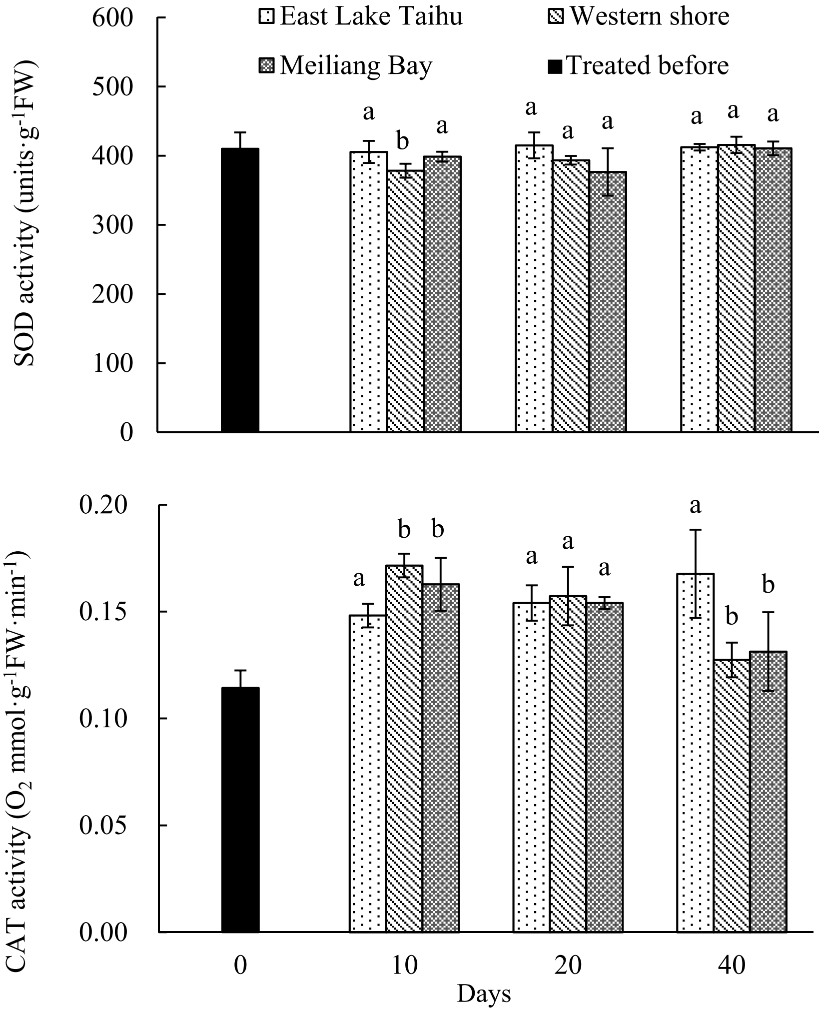


Fig. 5.2 Changes of SOD and CAT activities in roots of *Vallisneria asiatica*. Different letters indicate significant differences ($p < 0.05$) between three types of sediment by one-way ANOVA test.

Once the accumulation of ROS exceeded their scavenging capacity of antioxidant enzymes, the excess ROS might contribute to the disorder of metabolism resulting in the low photosynthesis efficiency, the decreased protein contents and the increased MDA contents. In **Fig. 5.3**, only the limited changes in the MDA content were observed compared with *V. asiatica* prior to treatments except that the MDA content of *V. asiatica* growing on the East Lake Taihu sediment declined on the 40th day compared with *V.*

asiatica before treatments. Moreover, no obvious differences between the three kinds of sediment were noticed at various times.

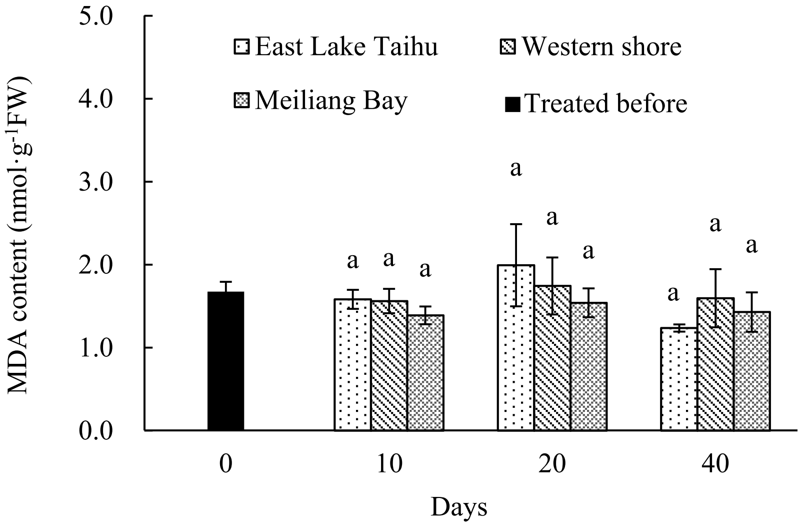


Fig. 5.3 Changes of MDA contents in roots of *Vallisneria asiatica*. Different letters indicate significant differences ($p < 0.05$) between three types of sediment by one-way ANOVA test.

5.3.3 Physiological changes in leaves of *V. asiatica*

The changes of SOD and CAT activities in leaves at different times are shown in **Fig. 5.4**. No significant changes of SOD activities were observed in the leaves of *V. asiatica* except for that growing on the Western shore sediment. The SOD activity of *V. asiatica* growing on the Western shore sediment was significantly lower than that on the other two sediment types on the 40th day. The CAT activity in the leaves of *V. asiatica* for all the three kinds of sediment increased gradually with time. On the 20th day, the CAT activity of *V. asiatica* growing on the Western shore sediment was obviously higher than that on the other two sediment types while it was significantly higher than that on the East Lake Taihu sediment on the 40th day.

Figure 5.5 shows the Chl.a and MDA contents in the leaves of *V. asiatica* growing on the three kinds of sediment at different times. The Chl.a content increased obviously on the 10th day and the increase stopped from the 10th day after the start of the treatment until the end of the experiment. The Chl.a contents between different kinds of sediment were not significantly different during the whole experimental period. There was no obvious variation of MDA content with time except for that on the Western shore sediment. On the 20th and 40th day, the MDA content of *V. asiatica* growing on the Western shore sediment was significantly higher than that on the other two sediment types. Furthermore, for the sediment from Western shore, the MDA content on the 40th day was obviously higher than that on the 20th day.

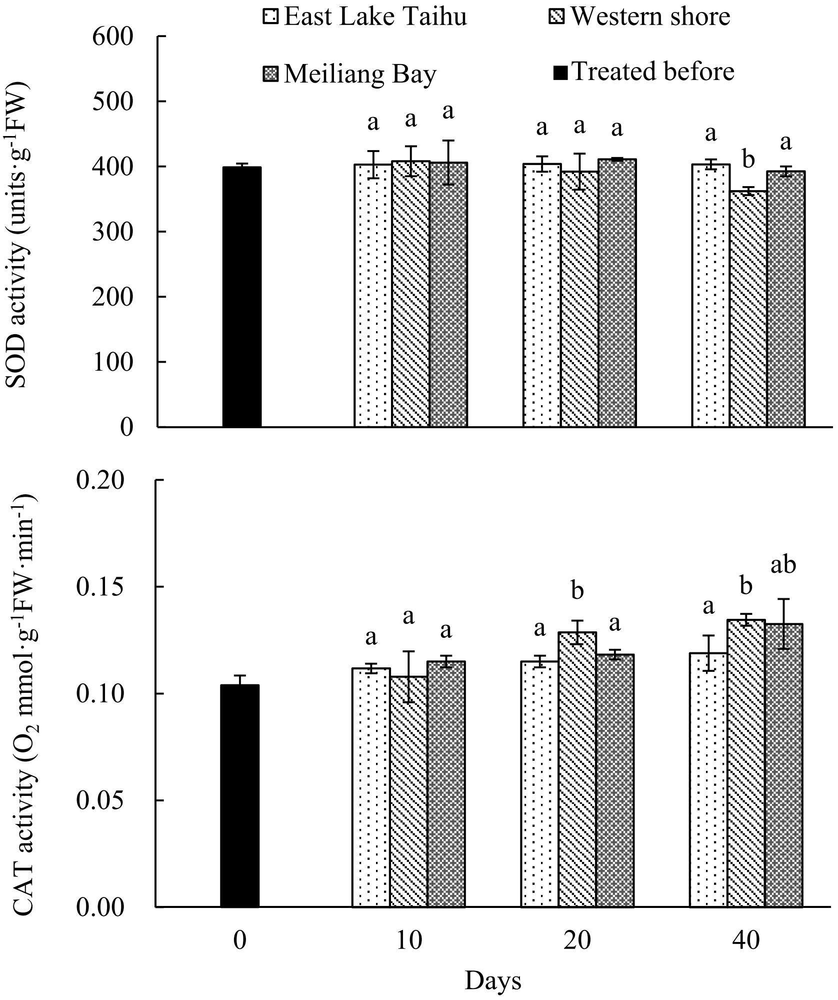


Fig. 5.4 Changes of SOD and CAT activities in leaves of *Vallisneria asiatica.* Different letters indicate significant differences ($p < 0.05$) between three types of sediment by one-way ANOVA test.

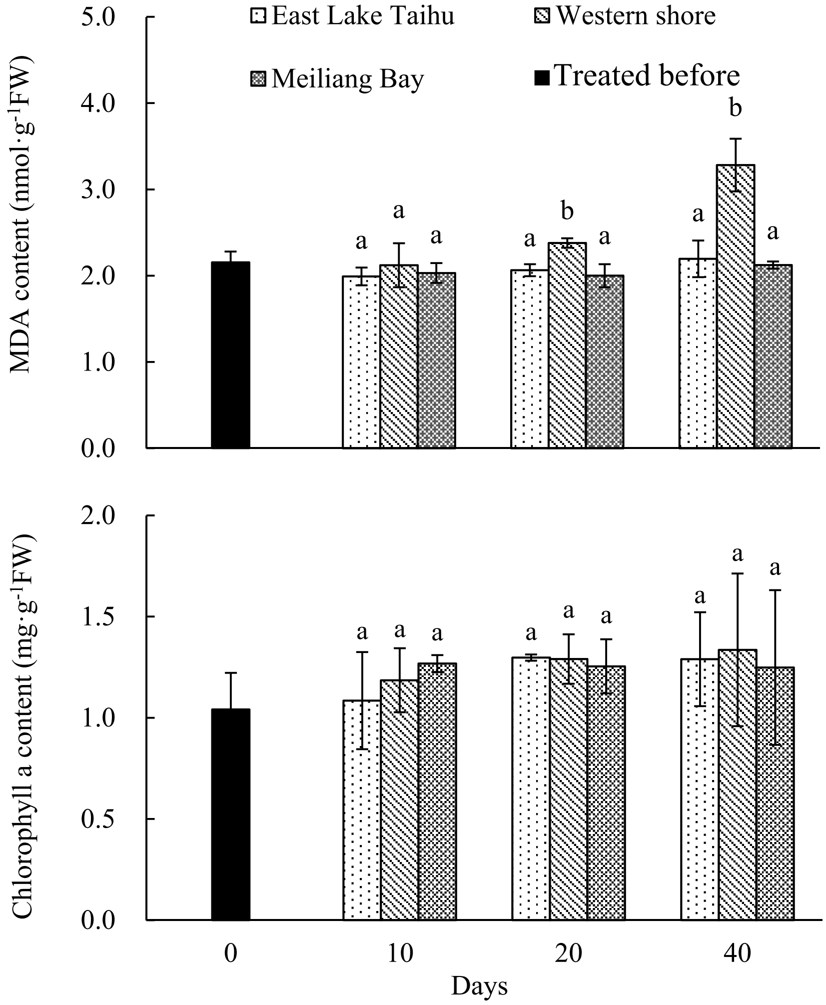


Fig. 5.5 Changes of Chl.a and MDA contents in leaves of *Vallisneria asiatica.* Different letters indicate significant differences ($p < 0.05$) between three types of sediment by one-way ANOVA test.

5.4 Discussion

5.4.1 Effects of sediment on the growth of *V. asiatica*

The high contents of nutrients and heavy metals possibly had some influences to *V. asiatica* because, after all, the contents of some elements in the sediment were much higher than LEL (**Table 5.1**). The influence was expressed as the lower growth rate of *V. asiatica* and reduced root numbers (**Table 5.2**). It is believed that the growth of emergent macrophyte *Canna indica* is affected when the organic matter in the solution with sand as a substrate beyond the plant tolerance limits (TN > 6.4 mg·L^{-1}; TP > 0.48 mg·L^{-1}) **(Zhang et al. 2008b)**. The Western shore sediment contained much higher content of TN and the Meiliang Bay sediment contained much higher contents of TN and TP than East Lake Taihu sediment (**Table 5.1**). This may be one reason why the increased biomass of *V. asiatica* growing on the Western shore sediment and its root number were smaller than that on the East Lake Taihu sediment. Many researchers also have reported that high concentrations of Cu and Zn can inhibit the growth of the floating-leaved plant *Spirodela polyrrhiza* (L.) Schleid and the submerged macrophyte *Hydrilla verticillata* (L.f.) Royle **(Xing et al. 2010; Wang et al. 2009)**. Compared with the East Lake Taihu sediment, the growth of *V. asiatica* growing on Western shore sediment was inhibited because of the high contents of organic matters, nitrogen, Cu and Zn.

5.4.2 Effects of sediment on oxidative stress

The results indicated that the *V. asiatica* was under the oxidative stress from eutrophic sediment in Lake Taihu because of the high contents of nutrients and heavy metals in the sediment. The oxidative stress response in *V. asiatica* to Lake Taihu sediment included the non-increased plant Chl.a content and increased MDA content after treatments for the 10th day. The fertile sediment has typically higher nitrogen and phosphorus contents **(Ye et al., 2009)**. The sediment is considered to be an important nutrient source for the rooted submerged macrophytes **(Ye et al., 2009)**. Compared with the brown clay in other studies, the Lake Taihu sediment was rich in organic matter and nutrients **(Ye et al., 2009; Yu et al., 2010)**. As known, elements like Cu, Mn and Zn are essential nutrients necessary for the normal growth and development of plants **(Dučić and Polle, 2005)**. The sediment in Lake Taihu would play a positive role in forming

Chl.a in the *V. asiatica* in a short time because of their abundant nutrients. However, it is believed that the growth of submerged macrophytes is affected by the sediment when the nutrient in sediment beyond their tolerance limits **(Zhang et al., 2008b)**. Many researchers also have reported that high concentrations of Cu and Zn can inhibit the Chl.a production of aquatic macrophytes **(Xing et al., 2010; Wang et al., 2009)**. The Chl.a content in *V. asiatica* stopped increasing possibly because the contents of some elements in the sediment were much higher than LEL. The Western shore sediment contained much higher content of nitrogen, Cu, Mn and Zn than the other two kinds of sediment. This may be one reason why the MDA content in leaves of *V. asiatica* growing on the Western shore sediment were much higher than that growing on the other two kinds of sediment.

5.4.3 Effects of sediment on antioxidant defense

The eutrophic sediment affects the antioxidant system of *V. asiatica* because of the excessive ROS induced by it. In this study, it is clearly shown that the eutrophic sediment has led to the oxidative stress as well as induced antioxidant defense against it, but the antioxidant response in roots of *V. asiatica* are different from leaves.

During the first period, the roots activated a defense system in cells which can cope with the eutrophic sediment. It is well known that the excessive ROS can induce the antioxidant responses of cells to the oxidative stress, including the enhancement or the suppression of antioxidant enzyme activities **(Jayakumar et al., 2006)**. The result showed that the SOD in roots and leaves was suppressed when *V. asiatica* was exposed to the Western shore sediment for 10 days. It is reported that when the superoxide radical generation exceeds the elimination ability of SOD, the superoxide radical as well as other oxyradicals can inactivate the enzyme SOD **(Yin et al., 2008)**. The induction of antioxidant enzyme CAT in roots and leaves indicated the ongoing detoxification process in *V. asiatica* to resist the oxidative stress from the eutrophic sediment **(Pflugmacher, 2004)**. At later time there were no differences in the SOD activity in roots of *V. asiatica* among the three kinds of sediment in Lake Taihu and the CAT activity remained high. It is obvious that the roots are in direct contact with the eutrophic sediment, and then the elements in sediment are transported to the leaves. Thus, the antioxidant responses of roots to eutrophic sediment are immediately. On the 40th day, the MDA content in roots of *V. asiatica* growing on the East Lake Taihu sediment was obviously lower than that on the other sediment types which may be partly explained by its higher CAT activity compared with the other two sediment types. The low contents of MDA indicated that the membrane structure of root cells was not damaged possibly in part because of the activated antioxidant enzymes. The roots also potentially possess other means of coping with high heavy metal levels, e.g. translocation or complexation **(Dučić and Polle, 2005)**.

In leaves in contrast to roots, the defense system responded to the stress from the eutrophic sediment during the later period. As a consequence, the decreased SOD activity in *V. asiatica* growing on the Western shore sediment was found on the 40th day and the CAT activity increased slightly with time. Furthermore, the low SOD activities will cause injury on *V. asiatica* because of its low ability of scavenging the excessive ROS **(Blokhina et al., 2003)**. Low SOD activities were also found in the leaves of the submerged macrophytes in Meiliang Bay of Lake Taihu **(Zhang et al., 2011a)**. If the cellular antioxidant defense is deficient, the excessive ROS are able to rapidly attack membrane lipids and result in lipid peroxidation **(Peutherta and Pflugmacher, 2010)**. The Western shore sediment caused the sub-cellular damage of membrane structure in *V. asiatica* cells from the 20th day. The negative effects of excessive ROS induced by the eutrophic sediment on leaves of *V. asiatica* mainly include the inactivation of enzyme SOD through reactions with their sulfhydryl or tryptophan residues and the lipid peroxidation through the degradation of polyunsaturated fatty acids in the membrane lipids undergoing the stress **(Bowler et al., 1992)**.

According to the above discussion, *V. asiatica* was under the oxidative stress from the three kinds of eutrophic sediment in Lake Taihu and the sediment in Western shore caused the most serious oxidative damage in *V. asiatica*. The sediment in Meiliang Bay was not as badly as that in Western shore of Lake Taihu was polluted. However, the field investigation in the whole Lake Taihu showed that *V. asiatica* and other submerged macrophytes started to decrease since 2000 with the eutrophication **(Zhu, 2008)**. Only East Lake Taihu is covered by submerged macrophytes, few emergent plants are distributed in Western shore and no aquatic macrophytes are distributed in Meiliang Bay now. The Western shore and Meiliang Bay contain high contents of nutrients in water and lots of algae form a floating surface layer resulting in the low transparency to inhibit the photosynthesis of submerged macrophytes and southeastern winds resulted in serious accumulation of algae at Meiliang Bay **(Zhu, 2008)**. Thus, the eutrophic sediment is just one of these major factors which influence the growth of aquatic macrophytes. The disappearance of submerged macrophytes in eutrophic Lake Taihu is caused by various factors.

5.5 Conclusions

The eutrophic sediment in Lake Taihu caused the oxidative stress and stimulated the antioxidant response in the *V. asiatica*. The growth and antioxidant responses were different depending on the contents of nutrients and heavy metals in sediment. The oxidative damage in *V. asiatica* caused by the Western shore sediment was the most serious of the three kinds of sediment because of the multiple stressors from high contents of nutrients and heavy metals. The East Lake Taihu sediment caused a relative small influence to *V. asiatica*. The results also indicated that the roots of *V. asiatica* responded to the eutrophic sediment immediately because they were in direct contact with sediment. The leaves of *V. asiatica* exhibited their responses to the eutrophic sediment during the later period of the experiment after the transportation of elements in sediment from roots to leaves. The eutrophic sediment was one main reason why the submerged macrophytes disappeared in Lake Taihu according to these results.

CHAPTER 6

ECOLOGICAL RESTORATION STRATEGIES OF LAKE TAIHU, CHINA

CHAPTER

6

Ecological restoration strategies of Lake Taihu, China

Abstract

Lake Taihu, being the third largest shallow lake in China, has the typical eutrophication issue. In 1960s, Lake Taihu was oligotrophic because of the very low N and P concentrations. Then the concentrations of N and P increased sharply with time. The high contents of N and P resulted in increasing coverage of algae and declining coverage of aquatic macrophytes. Only East Lake Taihu is covered by aquatic macrophytes with a large area, and no aquatic macrophytes are distributed in Meiliang Bay now. According to the summary of the growth conditions of *V. asiatica*, the Lake Taihu is divided into 4 parts, connecting to the distribution of TN, TP, Chl.a and sediments in Lake Taihu. (1) For Wuli Bay, Meiliang Bay, Zhushan Bay and Western shore, after the further managements and physical measures are proceeded to reduce the nutrient loading and algal accumulation, "Eco-engineering dams" could be established to continuously improve the water quality. (2) For Gonghu Bay and Southern shore, some measures are used to reduce the nutrient loadings without dredging and removing algae manually before the restoration of submerged macrophytes. (3) For Central Lake, the ecological system restores naturally after the ecology of lake shores go back to their normal levels. (4) For Eastern shore and East Lake Taihu, harvest of floating-leaved macrophytes during the early growth stage of submerged macrophytes has a positive impact on the submerged macrophytes. The management of nutrient sources into the river, removal of silt, and the improvement of the macrophyte biodiversity to combine the harvest are necessary to prevent or delay lake swamping.

Key words: biota, spatial distribution, aquatic environment, ecosystem restoration

6.1 Changes of water quality and biotas in Lake Taihu

In 1960, Lake Taihu was categorized as oligotrophic because the N and P concentrations were very low (TIN 0.05 $mg \cdot L^{-1}$, PO_4^{3-}-P 0.02 mg L^{-1}) **(Qin et al., 2007a)**. The TN and TP increased dramatically until 1980. **Figure 6.1** shows the changes in the annual average concentration of TP and the annual average concentration of TN in Lake Taihu from 1980 to 2008. Between 1980 and 2008, the annual average concentration of TN in Lake Taihu rose approximately four times from 0.65 $mg \cdot L^{-1}$ to 2.42 $mg \cdot L^{-1}$, and the annual average concentration of TP more than doubled from 0.03 $mg \cdot L^{-1}$ to 0.07 $mg \cdot L^{-1}$ **(Qin et al., 2007b)**. Recently, the concentrations of TN and TP have not changed much since 2005 although the Chinese government has undertaken a wide range of activities to improve the water quality of Lake Taihu.

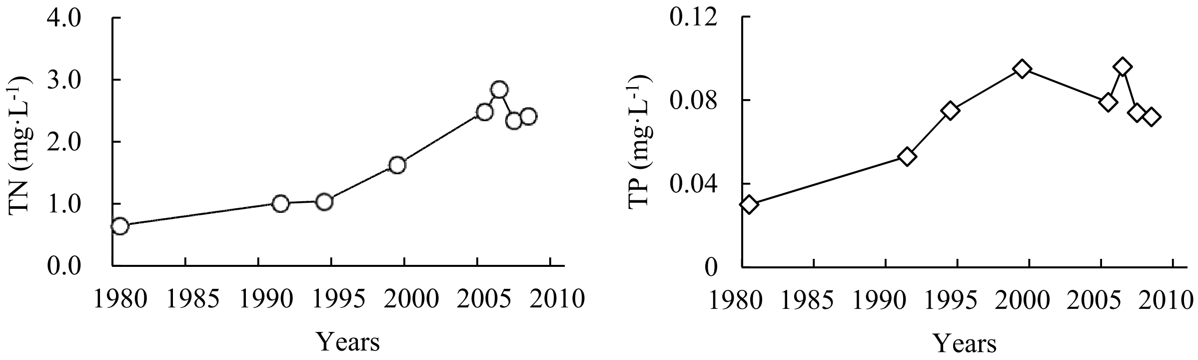


Fig. 6.1 Annual average of TN and TP concentrations.

Figure 6.2 shows the changes of the aquatic macrophytes and algae coverage in Lake Taihu from 1979 to 2006 **(Water resources bureau of Taihu Lake basin, 2008; Qin et al., 2007b)**. From 1993 to 1997, the plant coverage declined sharply from 47% to 26%. The algae coverage continued to increase from 1979 to 1993 and followed a sharp increase from 1993 to 2006. In 1960s, many kinds of aquatic macrophytes occupied most part of the Lake Taihu and no algal bloom occurred **(Ye et al., 2007)**. However, the algal blooms have stated to occur since 1970s because of a rapid increase in the development of industry and agriculture **(Ye et al., 2007)**. Recently, the frequency and duration of algal blooms have increased. The algal blooms usually occur in northern bays and extended to the center and south parts of Lake Taihu **(Duan et al., 2009)**. Since 2000, some species of submerged macrophytes even disappeared in southwest shores of Lake Taihu **(Cui et al., 2009)**.

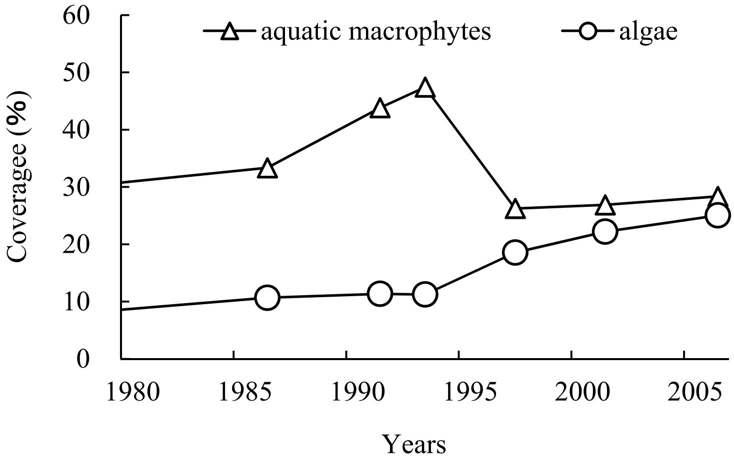


Fig. 6.2. Change of the aquatic macrophytes and algae coverage during different periods in Lake Taihu.

In Lake Taihu, the species of algae is declining and the toxic cyanobacteria were the dominant species **(Water resources bureau of Taihu Lake basin, 2008)**.

Macrophyte species can be classified as emergent, floating-leaved, floating, and submerged. Since 1960, the composition and biomass of the aquatic macrophytes have experienced considerable change **(Chen, 2008)**. From **Fig. 6.2**, it was found that the coverage of aquatic macrophytes increased from 1979 to 1993. However, in the late 1960s and 1970s, human activities caused a decrease in the density of aquatic macrophytes in Lake Taihu, compared with 1960 **(Chen, 2008)**. The increased coverage of aquatic macrophytes possibly mainly attributed to the introduction of the emergent macrophyte *Zizania latifolia* by human **(Chen, 2008)**. Thus, as compared with 1960, the coverage of submerged macrophytes decreased by 49% in 1981, and by 54% in 1996 **(Chen, 2008)**. The rapid development of aquaculture and urbanization in Lake Taihu lead to gradual disappearance of the aquatic macrophytes. In 1960, *Potamogeton maackianus*, *Hydrill verticillata*, and *Vallisneria natans* were the dominant submerged macrophytes **(Chen, 2008)**. Then *P. maackianus* and *Elodea nuttalli* gradually became the dominant species owing to the net-pen enclosures after 1984 **(Chen, 2008)**. Since 2000, submerged macrophytes nearly disappeared, and only a few stands of these submerged species remained in the zone connecting the East Lake Taihu with the open water area of Lake Taihu **(Zhu, 2008; Cui et al., 2009)**.

6.2 Spatial distribution of aquatic environments in Lake Taihu

In 1960, 66 macrophyte species were found in Lake Taihu. These macrophytes are mostly present in East Lake Taihu, and the macrophytes are also abundant in the other littoral zones, such as the Meiliang Bay and Western shore **(Chen, 2008)**. However, during the 1990s, the density of aquatic plants significantly decreased **(Chen, 2008)**. Only East Lake Taihu is covered by aquatic macrophytess, the coverage of aquatic macrophytes on the northern coast of Gonghu Bay, Southern and Eastern shore of Lake Taihu is very low, a few emergent plants are distributed in Western shore and no aquatic macrophytes are distributed in Meiliang Bay now **(Chen, 2008; Water resources bureau of Taihu Lake basin, 2009)**. The disappearance of aquatic macrophytes in Lake Taihu, especially submerged macrophytes, is caused by the decreasing water quality and increasing eutrophication. It is difficult to restore aquatic macrophytes, especially submerged macrophytes, and it would take a long time to restore the original lake ecosystem structure and function. Thus, according to the results in chapter 3, 4 and 5, the effect ranges of environmental factors in *V. asiatica* are summarized in **Table 6.1** to provide useful information for restoring the ecosystem of Lake Taihu. For the sediment of Lake Taihu, *V. asiatica* cannot survive when the mC_d exceeded 3.4 according to the results in chapter 5. This was also proved by the pollution gradations of sediments in **Table 6.2** where the sediment contamination degree has been defined as high when the mC_d is up to 4.

Table 6.1 The summary of the effect ranges of environmental factors in *Vallisneria asiatica* according to above experiment results.

Indexes	Promote growth or slight influence	Influence growth	Cannot grow
TN ($mg \cdot L^{-1}$)	<1.6	1.6~5.0	≥5.0
TP ($mg \cdot L^{-1}$)	<0.1	0.1~0.6	≥0.6
Chl.a ($\mu g \cdot L^{-1}$)	<109	109~220	≥220
Sediments (mC_d)[a]	<1.9	1.9~3.4	≥3.4

[a] mC_d: modified degree of contamination about the sediment **(Abrahim and Parker, 2008)**

$$C_f = \frac{M_x}{M_b} \qquad \text{(Equation 6.1)}$$

C_f: each pollutant of a contamination factor, M_x and M_b: mean concentration of a pollutant in the contaminated sediment and the pre-industrial "baseline" sediment. The M_b values of each element in sediments of Lake Taihu are from Qu et al. **(2001)**.

$$C_d = \sum_{i=1}^{n} C_f^i \qquad \text{(Equation 6.2)}$$

C_d is the sum of the C_f for all the pollutant species in sediment, n = number of analyzed elements.

$$mC_d = \frac{\sum_{i=1}^{i=n} C_f^i}{n} \qquad \text{(Equation 6.3)}$$

The pollution gradations are proposed according to the modified degree of contamination as shown in **Table 6.2**:

Table 6.2 The pollution gradations of sediments **(Abrahim and Parker, 2008)**.

mC_d values	Description of the contamination
$mC_d < 1.5$	Uncontaminated to very low degree of contamination
$1.5 \leq mC_d < 2$	Low degree of contamination
$2 \leq mC_d < 4$	Moderate degree of contamination
$4 \leq mC_d < 8$	High degree of contamination
$8 \leq mC_d < 16$	Very high degree of contamination
$16 \leq mC_d < 32$	Extremely high degree of contamination
$mC_d \geq 32$	Ultra high degree of contamination

According to the spatial concentration of TN, TP and Chl.a from 2000 to 2005 in Lake Taihu, the eutrophic degree from large to small is Wuli Bay, Meiliang Bay and Zhushan Bay, Western shore, Gonghu Bay, Southern shore, Central Lake, Eastern shore, East Lake Taihu **(Li et al., 2011)**. The mC_d of each zone was calculated based on the data of the sediment from 13 sites in Lake Taihu **(Qu et al., 2001)**. Based on the classification of pollution degree in **Table 6.1** and the data of water quality in Lake Taihu **(Li et al., 2011; Wang et al., 2008b)**, the distribution of TN, TP, Chl.a and the contamination degree of sediment is shown in **Fig. 6.3**.

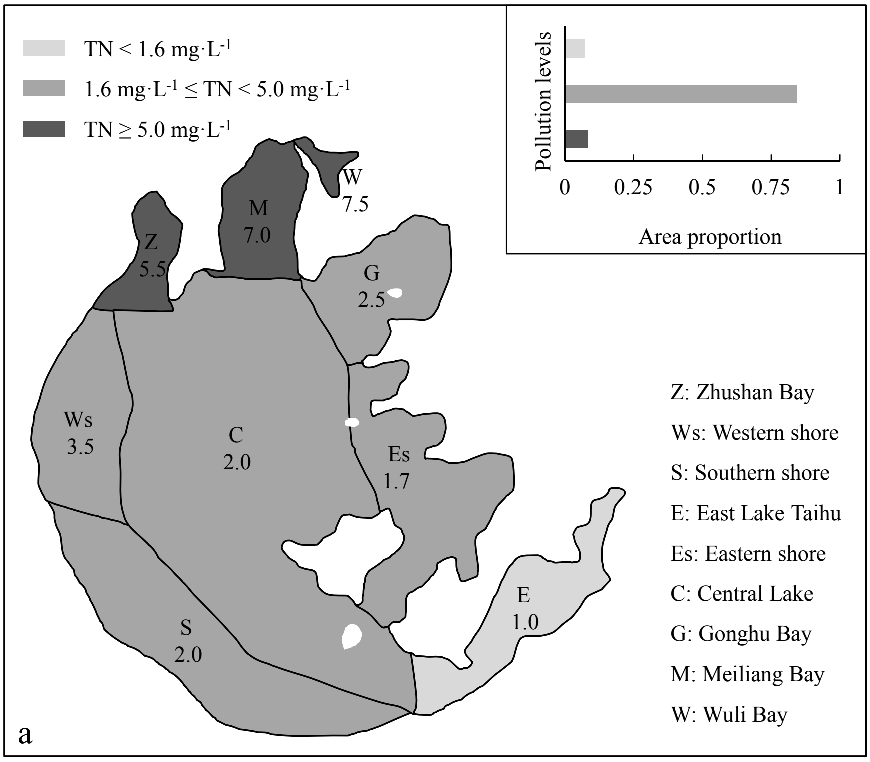


Fig. 6.3a The spatial distribution of TN in Lake Taihu from 2001 to 2012.

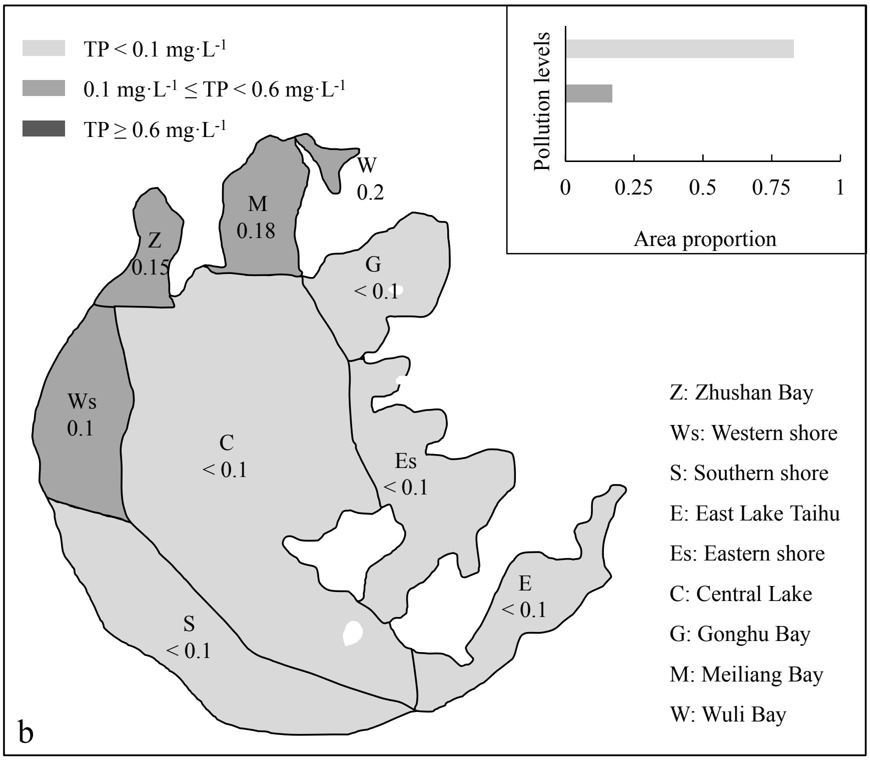


Fig. 6.3b The spatial distribution of TP in Lake Taihu from 2001 to 2012.

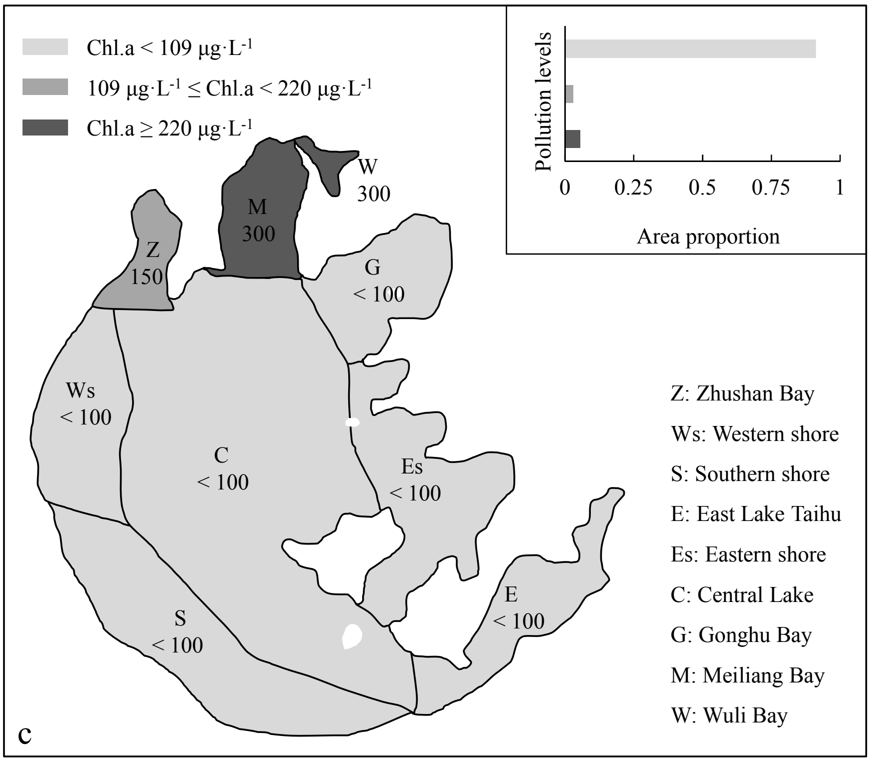


Fig. 6.3c The spatial distribution of Chl.a in Lake Taihu from 2001 to 2012.

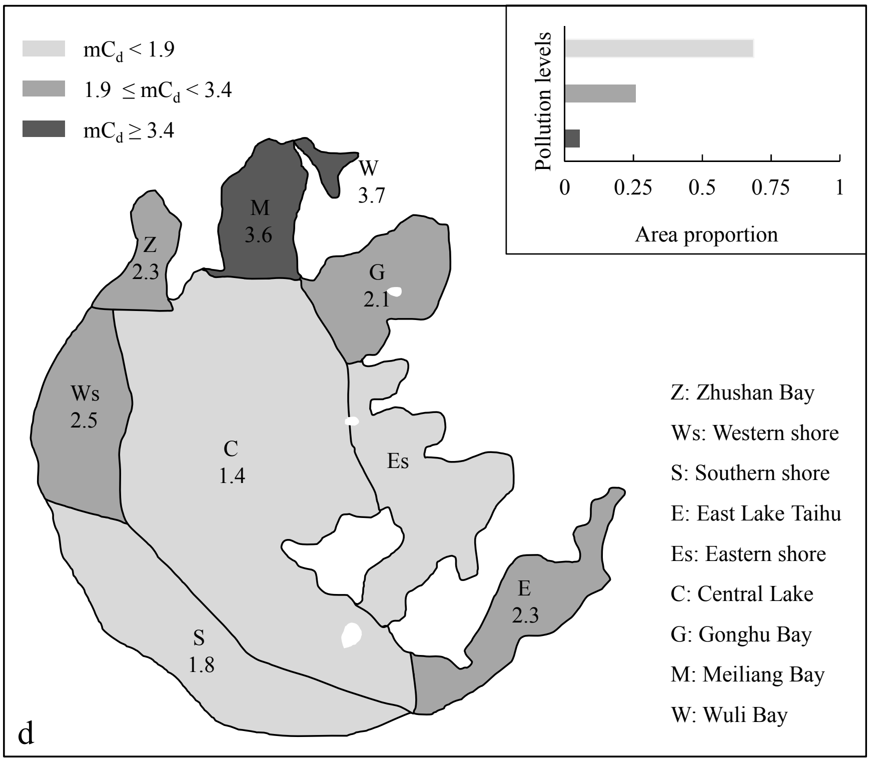


Fig. 6.3d The contamination degree of sediment in different regions of Lake Taihu from 2001 to 2012.

As shown in **Fig. 6.3a-d**, although the comprehensive pollution (including the pollution of the TN, TP, Chl.a and sediments) of Western shore is lighter than Wuli Bay, Meiliang Bay and Zhushan Bay, it was close to them. The Western shore, Wuli Bay, Meiliang Bay and Zhushan Bay were listed as one part to undertake the same measures for the ecological restoration as shown in **Table 6.3**. Connected to our field investigation results **(Hao et al., 2013)** and other researches on Lake Taihu **(Li et al., 2011)**, Gonghu Bay and Southern shore grouped under the same pollution degree, and Eastern shore and East Lake Taihu belonged to one part (**Table 6.3**).

Table 6.3 The summary of the eutrophic degree in each zone of Lake Taihu.

Zones of Lake Taihu	Evaluation indexes				Classified parts of Lake Taihu[b]
	TN ($mg\cdot L^{-1}$)	TP ($mg\cdot L^{-1}$)	Chl.a ($\mu g\cdot L^{-1}$)	Sediment (mC_d)[a]	
Wuli Bay	7.5	0.2	300	3.7	(1)
Meiliang Bay	7	0.18	300	3.6	(1)
Zhushan Bay	5.5	0.15	150	2.3	(1)
Western shore	3.5	0.1	< 100	2.5	(1)
Southern shore	2	< 0.1	< 100	1.8 (< 1.9)	(2)
Gonghu Bay	2.5	< 0.1	< 100	2.1	(2)
Central Lake	2	< 0.1	< 100	1.4 (< 1.9)	(3)
Eastern shore	1.7	< 0.1	< 100	-	(4)
East Lake Taihu	1 (< 1.6)	< 0.1	< 100	2.3	(4)

[a] mC_d: modified degree of contamination about the sediment **(Abrahim and Parker, 2008)**

[b] The Lake Taihu was classified into four parts according to the eutrophic degree from large to small.

6.3 Plans of the ecosystem restoration in Lake Taihu

Based on the summary of the section 6.2, the Lake Taihu was divided into 4 parts. Many field restoration cases in Lake Taihu were proceeded, such as the ecological restoration of Wuli Bay from 1999 to 2010 **(Chen et al., 2013)**, the restoration of submerged macrophytes after reestablishing the floating beds **(Zhang et al., 2010)**, and a biological control project to improve water quality in Lake Taihu by combining physical and biological measures **(Qin, 2013)**. Depending on the summary of the section 1.2 and above field restoration cases in Lake Taihu, ecological restoration methods in each part of Lake Taihu are proposed according to its eutrophic degree, which is shown in **Table 6.4**.

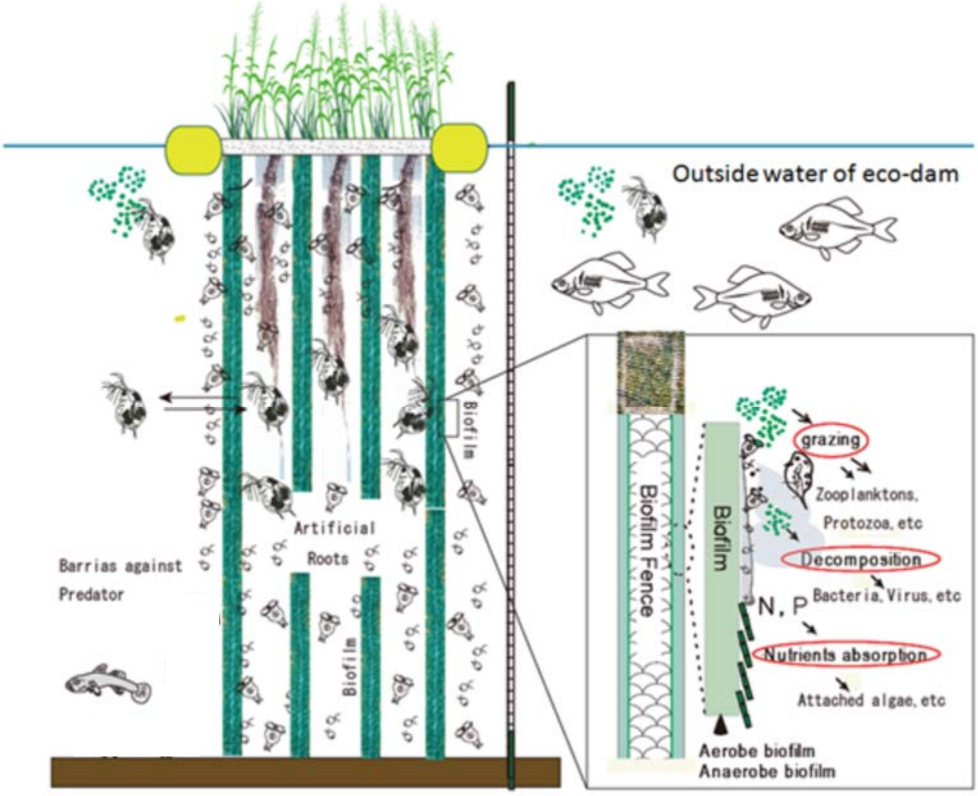


Fig. 6.4 The schematic diagram of "Eco-engineering dams" (cited from **Li et al., 2012**).

The purpose of all the restoration measures is to restore or protect the existing submerged macrophytes, and further to restore the lake ecology. As discussed in section 1.2, the most popular ecosystem recovery technology is the planting of aquatic macrophytes in Lake Taihu. "Eco-engineering dams", a water purification system that can contribute to community stabilization and offers sustained effects on improving the water environment, is proposed by our laboratory as shown by Hao et al. **(2014)**. As drawn in **Fig. 6.4**, "Eco-engineering dams" is a system that improves water environment through recycling contaminants in the water. It works by using the biofilm to build a healthy aquatic ecosystem. The nutrients can be decomposed by microbes attached to the biofilm and absorbed by the plants planting on the surface of the floating bed, and the diversity of communities around biofilm will be further strengthened.

(1) Wuli Bay, Meiliang Bay, Zhushan Bay, Western shore: Most projects of the submerged macrophyte restoration in Lake Taihu has failed because of the serious algal blooms in summer and high concentrations of nutrients **(Zhang et al., 2010; Qin, 2013)**. The results in **Table 6.1** and **Fig. 6.3** indicated that submerged macrophytes cannot grow healthily in these zones. Thus, it is necessary to control the nutrient pollution rather than to restore submerged macrophytes in these serious algal bloom zones including Wuli Bay, Meiliang Bay, Zhushan Bay, and Western shore. After the further managements and physical measures are proceeded to reduce the nutrient loading and algal accumulation, establishing “Eco-engineering dams” is suggested to continuously improve the water quality so that there is a possibility for submerged macrophytes to restore someday.

(2) Gonghu Bay, Southern shore: The restoration of submerged macrophytes is viable at Gonghu Bay and Southern shore of Lake Taihu according to the **Table 6.1** and **Fig. 6.3**. Similarly, some measures are used to reduce the nutrient loadings before the restoration of submerged macrophytes as shown in **Table 6.4**. Unlike the 4 zones with the most serious algal bloom, there is no need to dredge and remove algae manually for the Gonghu Bay and Southern shore. Then, the plan is to restore the submerged macrophytes while the “Eco-engineering dams” are playing a role in reducing the nutrient concentrations in the lake. Firstly, several “Eco-engineering dams” are set up and keep the operation stable. Secondly, in winter and spring (January and February), when the transparency is relatively high, the submerged macrophytes which grow well in winter, such as *Potamogeton crispus* L., *Ceratophyllum demersum*, and *Myriophyllum verticillatum* L., are planted. Thirdly, the other submerged macrophytes, such as *Vallisneria asiatica* and

Hydrilla verticillata, are planted in April to increase species richness and stabilize the plant community. Fourthly, the managements of the restoration project are proceeded to ensure the success.

(3) Eastern shore, East Lake Taihu: The nutrient concentration of Eastern shore and East Lake Taihu are relatively low, especially East Lake Taihu, and the aquatic plants mostly present in East Lake Taihu followed Eastern shore. However, the two zones have been lightly eutrophic, so the measures and managements listed **in Table 6.4** still are needed to reduce the nutrient concentrations. The main task for the two zones is to reduce the nutrient loadings, and further to protect the aquatic macrophytes. As known, East Lake Taihu has been swampy now. The apparent cause of lake swamping is simply overgrowth of the floating-leaved plants, such that they progressively cover the lake surface **(Chen, 2008)**. The actual cause is deposition, causing the lake basin to become shallower, combined with increased nutrient levels **(Chen, 2008)**. Compared to floating-leaved macrophytes, the submerged macrophytes have fewer negative impacts on the function of a lake **(Chen et al., 2009)**. Harvest of floating-leaved macrophytes during the early growth stage of submerged macrophytes has a positive impact on the submerged macrophytes **(Xu et al., 2014)**. Then the management of nutrient sources into the river, removal of silt, and the improvement of the macrophyte biodiversity to combine the harvest are also necessary to prevent or delay lake swamping **(Xu et al., 2014)**.

Table 6.4 Restoration methods for the nine major zones of Lake Taihu.

Zones	Characteristics	Restoration methods
Wuli Bay Meiliang Bay Zhushan Bay Western shore	This area receives a large pollutant discharge. The algae blooms are very serious and submerged macrophytes even almost disappeared.	Pollution control **Restore the submerged macrophytes** Projects for dredging and disposal Manual algal removal Forbid pen-fish
Gonghu Bay Southern shore	Algae blooms have occurred and submerged macrophytes have declined recently	Improve the managements of nutrient sources into the lake **Restore the submerged macrophytes**
Central Lake	Algae blooms have spread to center and south parts of Lake Taihu recently	The ecological system restores naturally after the ecology of lake shores go back to their normal
Eastern shore East Lake Taihu	The sedimentation rate has increased and marshiness has occurred because of the intensive aquaculture	**Submerged macrophyte conservation** Improve managements of nutrient sources into the lake Fish-culture should be limited Suitable harvest of floating-leaf macrophytes is needed for the nutrient export and removal of sediments

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER

7

Conclusions and recommendations

Conclusions

Algal blooms in eutrophic water bodies are becoming a worldwide ecological problem due to its significant bad impacts on ecological functions of lakes and the ecological services. Lake Taihu, the third largest lake in China, is a well-known case for eutrophication. There are so many physical, chemical and biological methods to control the eutrophication and restore the ecosystem of the lake. The most popular ecosystem restoration technology is the planting of aquatic, especially submerged macrophytes in eutrophic lakes because of their effective improving the water quality and reducing algae, and the low cost. The overall objective of this study was to propose ecological restoration strategies to restore or protect submerged macrophytes in eutrophic Lake Taihu by analyzing the antioxidant responses of *V. asiatica* to different adverse factors.

In chapter 2, it was found that the biomass of *Microcystis* spp. reduced with the increase of *V. asiatica* biomass. Therefore, it is possible to control an excess of planktonic *Microcystis* spp. by submerged macrophyte *V. asiatica* in Lake Taihu. At the same time, epiphytic *Cocconeis* spp. appeared while planktonic *Nitzschia* spp. lessened in the tanks with large quantity of *V. asiatica*, which indicated that there was competition between epiphytic *Cocconeis* spp. and planktonic *Nitzschia* spp. It is also a reason why aquatic plants could control the algal bloom. It was found unexpectedly that planktonic *Microcystis* spp. reduced sharply while planktonic *Nitzschia* spp. increased greatly when there was little quantity of *V. asiatica*, which indicated that planktonic diatom (mainly *Nitzschia* spp.) can control the multiplication of algae effectively also.

After the positive impacts of *V. asiatica* on the inhibition of algae were certified, a series of experiments were done to find the growth conditions of *V. asiatica* in chapter 3-5. In chapter 3, it was found the suitable nutrient concentration range (NO_3^--N < 1.5 mg·L^{-1}, PO_4^{3-}-P < 0.1 mg·L^{-1}) for *V. asiatica*. The antioxidant enzyme CAT in *V. asiatica* was more sensitive to high concentrations of nutrients than SOD. *V. asiatica* could resist oxidative stress from the moderate concentration of nutrients (NO_3^--N 1.5 mg·L^{-1}, PO_4^{3-}-P 0.1 mg·L^{-1}) by activating the antioxidant enzymes in its body. However, both excessive N and P (TN > 5 mg·L^{-1}; TP > 0.6 mg·L^{-1}) were able to trigger oxidative damage of *V. asiatica*, expressing as a decline of Chl.a and protein contents. The results indicated that the antioxidant defense mechanisms were activated but could not prevent the damage of the metabolism system in *V. asiatica* exposed to an excess of NH_4^+-N and PO_4^{3-}-P. It also indicated that the restoration of *V. asiatica* needed a nutrient control strategy in eutrophic Lake Taihu.

It was also found that high concentrations of algae led to the oxidative stress in *V. asiatica* when the Chl.a concentration was greater than 109 µg·L^{-1}, shown by changes in the activities of SOD, CAT, POD, protein content, and MDA content. The results also indicated that high *V. asiatica* biomass does not increase the oxidative stress in individual *V. asiatica*, but is more helpful in controlling the algal bloom than low biomass when the algal Chl.a concentration did not exceed 191 µg·L^{-1}. Thus, 2,222 g FW·m^{-2} were the optimal biomass to allow the recovery of *V. asiatica* when the initial algal Chl.a concentration was ≤ 191 µg·L^{-1}. Finally, high *V. asiatica* biomass increased the oxidative stress on individual *V. asiatica* when the algal Chl.a increased to 222 µg·L^{-1}, so the optimal biomass to recover *V. asiatica* ranged between 1111 and 2222 g FW·m^{-2} after the algal Chl.a reached 222 µg·L^{-1}. In this real example of the eutrophic Lake Taihu, it has been demonstrated that when working on *V. asiatica* ecological restoration project, appropriate controls of both algae and macrophyte biomass are necessary to ensure healthy growth of *V. asiatica*.

The eutrophic sediment in Lake Taihu also caused the oxidative stress and stimulated the antioxidant response in the *V. asiatica*. The antioxidant responses were different depending on the contents of nutrients and heavy metals in the sediment. The oxidative damage in *V. asiatica* caused by the Western shore sediment was the most serious of the three kinds of sediment because of the multiple stressors from high contents of nutrients and heavy metals. East Lake Taihu sediment caused a relative small influence to *V. asiatica*. The results also indicated that the roots of *V. asiatica* responded to the eutrophic sediment immediately because they were in direct contract with the sediment. The leaves of *V. asiatica* exhibited their responses to the eutrophic sediment during the later period of the experiment after the transportation of elements in sediment from roots to leaves. The eutrophic sediment was one main reason why the submerged macrophytes disappeared in Lake Taihu according to our results.

According to the summary of the growth conditions of *V. asiatica* in the chapter 3-5, the Lake Taihu is divided into 4 parts, connecting to the distribution of TN, TP, Chl.a and sediment in Lake Taihu.

(1) For Wuli Bay, Meiliang Bay, Zhushan Bay and Western shore, after the further managements and physical measures are proceeded to reduce the nutrient loading and algal accumulation, establishing "Eco-engineering dams" was suggested to continuously improve the water quality so that there is a possibility for submerged macrophytes to restore someday.

(2) For Gonghu Bay and Southern shore, some measures are used to reduce the nutrient loadings without dredging and removing algae manually before the restoration of submerged macrophytes.

(3) For Central Lake, the ecological system restores naturally after the ecology of lake shores go back to their normal levels.

(4) For Eastern shore and East Lake Taihu, harvest of floating-leaved macrophytes during the early growth stage of submerged macrophytes has a positive impact on the submerged macrophytes. Then the management of nutrient sources into the river, removal of sediment, and the improvement of the macrophyte biodiversity to combine the harvest are necessary to prevent or delay lake swamping.

Recommendations and expectations

The suitable growth conditions and different eutrophic factors' effect ranges for *V. asiatica* were summarized in this study. Although only the *V. asiatica* was used in this study. The other submerged macrophytes could also be stored in Lake Taihu. *V. asiatica* is just one species of common submerged macrophytes existed in Lake Taihu. Other species of submerged macrophytes like *Hydrilla verticillata*, *Potamogeton malaianus*, *Myriophyllum spicatum* and *Ceratophyllum demersum* also abundantly existed in East Lake Taihu so that they have the similar growth conditions. These submerged macrophytes also have high adaptive capacity and wide distribution like *V. asiatica*. Thus, the submerged macrophytes *Hydrilla verticillata* and *Potamogeton malaianus* could be planted with *V. asiatica* simultaneously to increase the species richness. In addition, *Hydrilla verticillata*, *Potamogeton malaianus* and *V. asiatica* grow slowly in winter. In winter and early spring, when the transparency is relatively high, the planting of *Myriophyllum spicatum* and *Ceratophyllum demersum* which grow well in winter could stabilize the plant community. Thus, a combination of different species of submerged macrophytes could be conducted for further studies avoiding the disadvantages of seasonal water quality problems.

The restoration technologies of *V. asiatica* for different zones of Lake Taihu were proposed in this study. Lake Taihu is a typical large shallow lake. It has an area of 2,338 km^2, a mean depth of 1.9 m and a maximum depth of 2.6 m. The shallow water depth provides a possibility to restore the submerged macrophytes in Lake Taihu. The differences in depth between shallow and deep lakes resulted in the different lake ecosystems. The corresponding restoration strategies for Lake Taihu could be applied into the other shallow lakes. The other shallow lakes in China, such as Chaohu Lake (depth 1-3 m), also suffered the eutrophication. For Chaohu, even these dominant submerged macrophytes including *Hydrilla verticillata*, *Potamogeton malaianus*, *Myriophyllum spicatum* and *Ceratophyllum demersum* have become scarce. The restoration of submerged macrophytes also is necessary for Chaohu. The summary of the physiological impacts on *V. asiatica* caused by high concentrations of N and P, high algal concentrations and eutrophic sediment could be used as a reference for restoring the submerged macrophytes in Chaohu. Similarly, these results could provide a basis for the restoration of submerged macrophytes in other shallow eutrophic lakes in China even other countries.

In this study, only the effects of high concentrations of N and P, high algal concentrations and eutrophic sediment on *V. asiatica* were done in the laboratory. Other eutrophic factors were considered according to the field investigation before the restoration strategies were proposed in this study. If possible, more extensive studies about the other eutrophic factors are required before the field project. The researches included about the non-algal turbidity, stormy waves, microcystin and the multiple effects of different eutrophic factors.

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