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Nutrient bioextraction and microalgae growth inhibition using submerged macrophyte *Myriophyllum spicatum* in a low salinity area of East China Sea

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ABSTRACT

Myriophyllum spicatum was cultivated in a low salinity area of Hangzhou Bay (salinity 5.8–6.5), from August to October in 2016, to evaluate the abilities of its nutrient bioextraction and microalgae growth inhibition. During the 72-day cultivation period, *M. spicatum* had a specific growth rate (*SGR*) of 6.23% day^{-1} and increased 20-fold in biomass (wet weight). Tissue C, N and P assimilation quantities of *M. spicatum* were found to be 3279.39 kg, 360.61 kg and 26.97 kg, respectively. The concentration of NH_4-N, NO_3-N, NO_2-N and PO_4-P after *M. spicatum* cultivation was decreased by 47.92%, 58.28%, 36.40% and 55.57%, respectively. The phytoplankton density was decreased from 1064.60 × 10^4 cells L^{-1} to 12.85 × 10^4 cells L^{-1}. These results indicated that cultivation of *M. spicatum* can help in nutrient bioextraction and microalgae growth inhibition in low salinity marine water bodies.

1. Introduction

Nutrient addition in estuaries and coastal water bodies is a natural process that can be attributed to geological weathering and ocean upwelling (Bricker et al., 2009). However, in the past few decades, the rapid increase in human population and developmental activities has exponentially multiplied the amount of nutrient input in these water bodies. The abrupt increase of nutrient levels further increases the risk of eutrophication, which is one of the greatest threats to the ecological balance and biodiversity of coastal ecosystems (Victor et al., 2002). Eutrophication has been linked to a variety of environmental problems, including low dissolved oxygen, deterioration of water quality and biodiversity of coastal rivers and bays, loss of critical habitats and growing prevalence of toxic blooms of algae (Boyer and Howarth, 2008; Rose et al., 2015). It is also considered as a major factor that can impose negative influence on commercial fishing zones around the world, such as the Baltic, Kattegat, Black Sea, Gulf of Mexico and East China Sea (Fei, 2004; Diaz and Rosenberg, 2008; Strokal et al., 2014; Andersen et al., 2015). Considering all these factors, it is evident that eutrophication is a critical factor of disrupting sustainable economic development of coastal regions.

In an effort to ameliorate the symptoms of eutrophication and improve water quality in such coastal areas, point and nonpoint source pollution reduction management measures are being tested (Tedesco et al., 2014). However, it has been realized that the current high nutrient load status of coastal water bodies cannot be resolved immediately by mere source reduction. This is due to the fact that other forms of nutrient input, such as submarine groundwater discharge and benthic nutrient recycling will continue to impose their effects even without source overloading (Grenzll et al., 2000; Moore, 2010). The use of seaweed aquaculture as an in-situ remediation methodology to minimize the impact of nutrient addition in offshore and coastal waters has received increasing attention in the recent past (Xiao et al., 2017). Wu et al. (2015) reported that large-scale cultivation of red seaweed *Porphyra yezoensi* could be used to alleviate eutrophication and control harmful algal blooms in open sea. Kim et al. (2014) indicated that *Gracilaria* aquaculture has also been proposed as a useful technique for nutrient bioextraction in urbanized coastal waters. In addition, a large number of macroalgal species are being proposed to be used as biofilters to reduce aquaculture derived inorganic nutrients (Klinger and Naylor, 2012; Handå et al., 2013). This strategy is believed to be highly beneficial, especially in purification of water bodies for sustainable development of integrated multi-trophic aquaculture (IMTA) systems (Kim et al., 2014). Although seaweed aquaculture technologies have

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developed dramatically, not all coastal areas are suitable for marine macroalgae cultivation. Low salinity (< 10) estuarine areas, which usually have higher nutrient loads, are one such type that is unfit for macroalgae cultivation. Therefore, it is essential to develop new technologies for nutrient bioextraction in low salinity sea areas.

Myriophyllum spicatum is a fresh water submerged macrophyte and plays an important role in biogeochemical cycles of lake and river ecosystem by absorbing nutrients from both sediment and water (Planas, 1981; Angelstein et al., 2009). This species can tolerate salinity values of 10.0 to 16.6 under controlled indoor conditions (Haller et al., 1974). Hempel et al. (2008) reported that *M. spicatum* may influence epiphytic bacterial community by release polyphenols in wild brackish water of Schaproder Bodden. But to our knowledge, the research on nutrient bioextraction in low salinity sea areas using *M. spicatum* has not yet been reported.

The purpose of this study was to cultivate *M. spicatum* in a low salinity sea area of East China Sea and evaluate its performance of nutrient bioextraction and microalgae growth inhibition. Results of this study may help in devising newer and better methods to alleviate eutrophication and control harmful algae blooms in low salinity coastal areas.

2. Materials and methods

2.1. Study area

This study was conducted in an enclosure of Jinshan sea area (121°34′73.57″E, 30°70′86″N), which located at the coastal region of northern Hangzhou Bay and is adjacent to the Yangtze River in the East China Sea (Fig. 1). The total area of the enclosure is around 138.0 ha and it is separated into two parts (east and west) by a 4.05-km long dam. The west part of the enclosure, which area is around 19.4 ha with the depth from 1.5 to 2.5 m, was selected for *M. spicatum* cultivation, while the east part of the enclosure, which area is around 118.6 ha with the depth from 4.0 to 6.0 m, was used as the control. Nine monitoring sites (1–9) were established in the cultivation area and the control area (Fig. 2). The experimental period of present study was from August to October in 2016, because the coastal region is in the period of high occurrence frequency of eutrophication and harmful algae blooms.

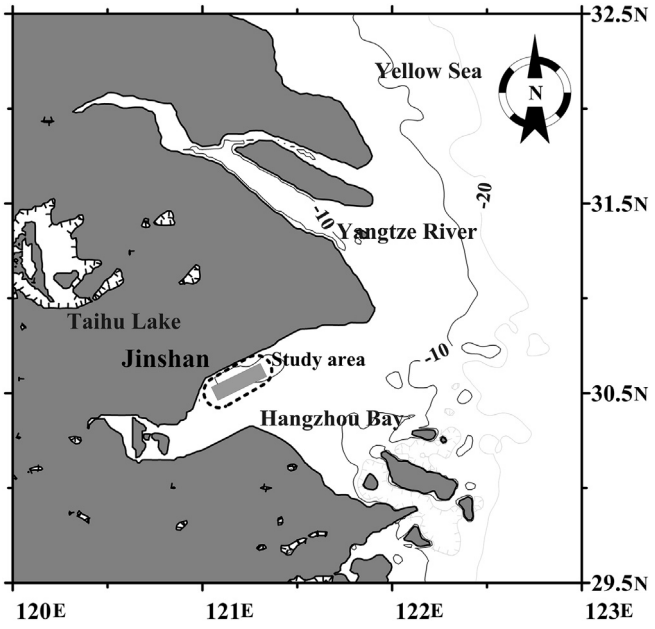

Fig. 1. Map showing the study area in the Hangzhou Bay.

2.2. M. spicatum cultivation

M. spicatum was transferred from some adjacent rivers. A total of 5.0 tonnes (fresh weight) of *M. spicatum* were cultivated in this study. 4.5 tonnes were cultivated through the suspension-cultivation method. This was achieved with 2 × 3 m nets with a mesh size of 10 cm. *M. spicatum* were tied to the nets. The nets were tied to two ropes and kept 0.2–0.3 m below the surface of water, so as to avoid any damage that may be induced due to exposure to strong light intensities. The ropes were fixed on the surface of the water with the help of floaters and anchors. It was noted that the distance between the ropes was maintained at 2 m (Fig. 2). The other 0.5 tonnes *M. spicatum* were tied to bricks and sunk, so that they could be cultivated on the bottom of the water body (Fig. 2). The total areas of suspension- and bottom-cultivation were 6000 m^2 and 4000 m^2, with the density of 0.750 kg m^{-2} and 0.125 kg m^{-2}, respectively.

2.3. Growth rate and tissue nutrient content of M. spicatum

M. spicatum cultivation was began on August 21, and the biomass of *M. spicatum* was measured on October 30. 12 Sample stands were selected for the collection of *M. spicatum* samples by following the quadrat sampling method (Johnson and Newman, 2011). The *M. spicatum* samples were weighed after removing the superficial water to yield wet weight, after which the final biomass of *M. spicatum* was calculated. Specific growth rate (*SGR*) of *M. spicatum* was estimated according to the following equation:

$$SGR\ (\%\ d^{-1}) = \frac{\ln W_e - \ln W_b}{T} \times 100$$

Where *SGR* represents specific growth rate (% day^{-1}), W_e and W_b represent the biomass of *M. spicatum* at the end and beginning of cultivation, respectively, and *T* represents elapsed time.

M. spicatum were dried in an oven at 55 °C to reach a constant weight and were later ground with the help of a grinder (Gaoxin, China) to obtain a uniform size of powder. The contents of nitrogen (N) and carbon (C) in the tissue were determined using a Vario MAX CN elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Tissue phosphorus (P) content was analyzed using the ammonium molybdate colorimetric technique (Allen et al., 1989).

2.4. Water sample collection and measurement of nutrient concentrations

During the cultivation period, water samples were collected twice a month. On each sampling site, water samples were collected in triplicates using Niskin bottles, preferably from 10:00 a.m. to 12:00 a.m. It was also noted that the water samples from each sampling site were collected 0.5–1.0 m below the sea surface. Temperature and salinity were measured simultaneously at 1.0 m depth using an YSI multiparameter water quality meter (YSI, Yellow Springs, OH, USA). Water clarity was measured using a Secchi disk. Dissolved inorganic nutrient measurements were done after the water samples were filtered through GF/F glass-fiber filters (0.45 μm). Ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and inorganic phosphate (PO_4-P) concentrations were determined using a segmented flow analyzer (SKALAR, Breda, Netherlands).

2.5. Collection and determination of phytoplankton

Phytoplankton composition was determined using the seawater samples (1000 mL) that were collected in Niskin bottles from each sampling site (15–20 cm below the water surface). The samples were immediately fixed with formaldehyde solution at a final concentration of 1%. The phytoplankton genera were determined and counted after a settling period of around 24 h. The dominance index (*Y*), diversity index (*H′*) and evenness index (*J*) of the phytoplankton were calculated