Bull Environ Contam Toxicol (2009) 83:497–501
DOI 10.1007/s00128-009-9800-y

Effects of Organic-Rich Sediment and Below-Ground Sulfide Exposure on Submerged Macrophyte, *Hydrilla verticillata*

Juan Wu · Shuiping Cheng · Wei Liang · Zhenbin Wu

Received: 22 December 2008 / Accepted: 15 June 2009 / Published online: 30 June 2009

Abstract The effects of organic-rich sediment and sulfide exposure on *Hydrilla verticillata* were investigated. The organic richness of sediment was simulated by adding sucrose into sediments, and sulfide exposure was conducted by adding sodium sulfide to plant roots. The length, biomass and density of shoot reduced in the sucrose-amended sediments, and the largest reduction occurred in the highest 1.0% addition treatment by 84.2%, 56.7% and 92.4%, respectively. However, the 0.1% addition treatment stimulated the growth of root. The effects of below-ground sulfide exposure on the physiological activities of *H. verticillata* were determined by adding sulfide to the below-ground tissue. Significantly inhibitory effects of sulfide were observed on plant photosynthesis, root carbohydrate and nitrogen synthetic reserves. The net photosynthetic rates, soluble carbohydrate and soluble protein contents in root were reduced by 104%, 71.8% and 49.8%, respectively, in the 0.6 mM sulfide treatment.

Keywords Sediment · Sulfide · *Hydrilla verticillata* · Growth

Widespread declines of *Hydrilla verticillata* have frequently occurred since the late 1990s in China (Yan et al. 1997; Qiu and Wu 1998). Some previous reports on seagrass "die-back" indicate that organic enrichment in sediment and its subsequent effects could be serious stressors on survivals and growths of submerged macrophyte species (Wijcka et al. 1992; Terrados et al. 1999). Organic enrichment of sediment is usually followed by some chemical and biological processes, which consume a large amount of sediment oxygen and result in accumulations of phytotoxins (Wijcka et al. 1992). Submerged macrophytes generally have aerenchyma tissues, which allow oxygen transport to below-ground tissues to support root respiratory processes (Armstrong et al. 1996; Grosse et al. 1996). Oxygen in excess of the respiratory demand of below-ground tissues is released into rhizosphere sediments, thereby oxidizing reduced substances in the rhizosphere, and thus preventing phytotoxic exposure to reduced toxic compounds (Borum et al. 2005). Nevertheless, when the organic enrichments in sediment increase, oxygen released from roots may not be sufficient to oxidize the reduced compounds, then below-ground exposures to phytotoxins, such as sulfide, probably occur. Sulfide is accumulated when sulfate reduction is enhanced by input of labile organic matter in sediment (Thamdrup et al. 1994). This increases the chances of below-ground exposure of seagrasses to sulfide. Some previous studies indicated that sulfide in porewater of marine sediment was the main factor contributing to seagrass declines in eutrophic lakes (Borum et al. 2005). Sulfide has negative effects on seagrass photosynthetic and metabolism activities (Goodman et al. 1995; Holmer et al. 2005). Erskine and Koch's (2000) study showed that *Thalassia testudinum* leaf elongation rates, root carbon reserves and root ATP production reduced significantly under the exposure of sulfide with concentrations of 2.0–10.0 mM. Holmer and Bondgaard (2001) found that survivals of *Zostera marina* were negatively influenced by the presence of sulfide (0.1–1 mM), and photosynthesis and carbohydrate reserves reduced.

J. Wu · S. Cheng · W. Liang · Z. Wu (✉)
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, No.7 Donghu Southern Road, 430072 Wuhan, China
e-mail: wuzb@ihb.ac.cn

J. Wu
Graduate School of the Chinese Academy of Sciences, 10039 Beijing, China

Though increasing organic enrichment in sediment is prevalent in eutrophic lakes in China and freshwater submerged macrophyte "die-back" events are frequently reported (Yan et al. 1997; Qiu and Wu 1998), few studies are conducted on relationship between organic-rich sediment and sulfide exposure and macrophyte declines. To obtain the knowledge of the effects of organic-sediment on *H. verticillata,* Experiment I was conducted. In this experiment, sucrose was used as labile organic carbon source added to sediment to simulate organic-rich sediment. In addition, considering sulfide as a major phytotoxin occurring in organic-rich sediment, the responses of *H. verticillata* photosynthesis, root carbohydrate reserve and nitrogen synthetic to below-ground sulfide exposure were solely determined in Experiment II by adding sulfide to roots with no sediment.

Materials and Methods

H. verticillata shoots and sediment were collected from Yuehu lake (N30°33′, E114°15′), located in Wuhan, China. The sediment was derived from the top surface (<15 cm depth) of lake sediment, which was fertile with organic matter content >5% DW, total nitrogen content 7.23 ± 0.56 mg N g^{-1} DW and total phosphorus content 2.01 ± 0.34 mg P g^{-1} DW). The collected sediment was wet-sieved through a 1 mm sieve to remove plant debris and little rocks for experiment. Plants were uprooted, gently washed free of sediments and transported immediately to the laboratory. Shoots were thoroughly cleaned of epiphytes and debris by lightly scraping and rinsing with distilled water and cut into apices with a length of 10 cm. Some of these apices were selected as material for Experiment I, and others were preincubated in lake water and sediment collected from the same lake site to develop root growth for Experiment II.

At the initiation of the experiment I, the sieved sediment was placed into plastic buckets (35 cm × 30 cm, each with 5 kg sediment), mixed with sucrose to reach the added sucrose percentage of 0 (control), 0.1%, 0.2%, 0.5% and 1%, respectively. The final concentration of organic carbon in the sediment was 0 ± 0.04, 0.92 ± 0.06, 2.10 ± 0.11, 6.37 ± 0.15 and 8.65 ± 0.14 g C kg DW^{-1}, respectively. The buckets were gently filled with filtered lake water from the same sampling site. Each treatment was replicated three times. Fifteen apical shoots of *H. verticillata* prepared above were randomly selected and transplanted in the sediment in buckets. The fresh weight of plants in each bucket was 9.86 ± 0.14 g. These buckets were placed outdoors in a mesocosm experimental base, located in Institution of Hydrobiology, CAS. The incubation condition was natural and temperature ranging between 28 and 37°C.

During the 8-day experiment, sediment redox potential (Eh), pH and sulfide in porewater were measured every 1–2 days. Parameters of pH and Eh were determined (Orion125 A+ pH meter and Triode pH electrode and Eh electrode). Porewater was collected by centrifuging sediment at 6,000 r/min and at 4°C. The supernatant was immediately stabilized with 5% (w/v) $ZnCl_2$ and 10% NaOH to prevent the oxidation of sulfide to sulfate. Sulfide concentrations in porewater were measured using methylene blue photometric method (Cline 1969). At the end of experiment, *H. verticillata* were harvested avoiding mechanical damage, the length and densities of shoot (each branch with a length >5 cm was considered as a shoot) and root from each bucket were determined. Then, the above- and under-ground biomass of the plants were separated and weighted after drying at 110°C for 48 h, respectively.

The effects of below-ground sulfide exposure on *H. verticillata* physiology were assessed by a 10-day experiment (experiment II) with sulfide addition to growing environment of roots. After 15-day incubation for root growth, the apical shoots of *H. verticillata*, described above, were uprooted gently avoiding root damages, washed free of sediment and phytoplankton by distilled water. Two shoots were threaded through two 5 mm-diameter rubber stopper holes and sealed in the stopper holes with sponge and wax around nonphotosynthetic tissue. Shoots in stoppers were placed in a 50-L tank filled with lake water and acclimated for 7 days under 4,000 l× light condition. After this acclimation, stoppers with the two shoots were plugged into cylindrical experimental chambers (height 8 cm × 8 cm diameter; 200 mL), which restricted sulfide exposure to below-ground tissues.

Chambers were randomly assigned to five sulfide treatments (0, 0.1, 0.2, 0.4, 0.6 mM sulfide), each with four replicates. Chambers were filled with deoxygenated lake water and sodium sulfide ($Na_2S{\cdot}9H_2O$), which produced the sulfide treatment levels. Chambers filled with deoxygenated lake water and without $Na_2S{\cdot}9H_2O$, but amended for salinity with NaCl to balance the effects of sodium ion in sulfide treatments, were used as controls. All chambers were placed in an aquarium (80 cm × 30 cm × 50 cm; 120 L), which was filled with lake water. The aquarium was placed indoors under two fluorescent lamps. During the incubation, the deoxygenated lake water amended with sodium sulfide was renewed once two days to keep the sulfide concentration constant. The water temperature and light were monitored throughout the experiment. The mean water temperature was 32 ± 1.6°C, the mean light intensity at surface of plants was 4,135 ± 532 l×.

At the end of experiment II, all plants were harvested. Photosynthesis and respiration rates from plants were determined using a modified light–dark bottle technique (Biebl and McRoy 1971) after the 10-day incubation in the

sulfide treatments. An apical segment of shoots with a weight of 0.2 g from each treatment was cut and placed in a 250-mL productivity bottle with a magnetic stir bar for both photosynthesis and respiration measurements. The bottles (n = 4) were incubated at 4,000 l× light condition for 2 h. Respiration rates were determined from oxygen consumption, and net photosynthesis rates were determined by difference from initial and final oxygen concentrations. All measurements were corrected for the oxygen concentration of blank lake water from the aquarium. Root materials for chemical analysis were separated from whole plants and immediately frozen (−20°C) and analyzed as described below.

The frozen roots were ground to a fine powder with fluid nitrogen. Soluble carbohydrate (SC) and soluble protein were extracted from 0.1 g root powder with 4 mL 80% ethanol at 60°C for 10 min, centrifuged at 10,000*g* for 20 min. The supernatant was used for measurements of SC and soluble protein. SC measurement was conducted using anthrone method according to Yemm and Willis (1954). Soluble protein concentration was measured using Coomassie brilliant blue dye-binding method (Bradford 1976). SC and soluble protein concentrations were calculated on a dry weight basis.

All statistical analyses were conducted using SPSS 13.0. Comparisons among treatments were performed using a one way ANOVA following tests for homogeneity of variances and normality or unimodal distributions. The Pearson product–moment correlation was used for correlation analyses. All significant differences are reported at the $p < 0.05$ level, unless otherwise stated.

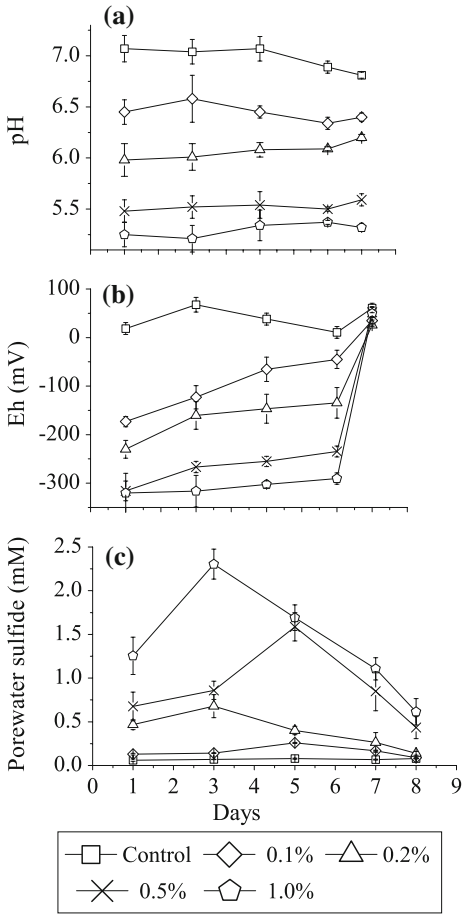

Fig. 1 Time course of pH (**a**), redox potential (**b**) and porewater sulfide (**c**) after sugar addition into the sediments. *Symbols* represent mean (±range/SE, n = 3)

Results and Discussion

In experiment I, pH values significantly decreased with the content of sucrose addition from 6.97 ± 0.12 in the control to 5.32 ± 0.06 in the 1% sucrose treatment (Fig. 1, $p < 0.01$) and correlated linearly with the sucrose addition ($r = -0.984$, $p < 0.01$). The initial Eh showed a negative correlation with sucrose after at the beginning after sucrose addition ($r = -0.616$, $p < 0.01$), but it increased up to similar values (mean 35–60 mV) in all treatments at the 8th day of the incubation. The sulfide concentrations in porewater of all treatments initially increased till 4 or 6 days after sucrose addition, but subsequently declined up to 0.07–0.614 mM at the end of experiment. No significant difference in sulfide was found between 0.1% addition and the control. However, sulfide concentrations were higher in the treatments with sucrose addition >0.1% than that in control ($p < 0.01$), the sulfide concentration in the 1% treatment reached the peak value (mean 2.30 mM) after 4 days of the experiment.

The sucrose addition in this study resulted in significant changes in chemical condition of sediment (reduced pH, Eh and increased porewater sulfide). However, the time course of Eh and porewater sulfide indicated that the anoxic condition simulated by sucrose addition cannot last for a long period, and the fluctuations of sulfide concentration, as a product of anaerobic sulfate reduction, were transient (8 days) and slight (up to 2.3 mM) relative to some in situ marine experiments. The porewater sulfide concentrations in freshwater lake sediments (0.06–2.30 mM, presented in our study) are much lower than in marine sediment. The porewater sulfide concentrations vary extensively with different seasons and sites in marine sediment, and during seagrass mortality, the concentrations could reach 17 mM (Carlson et al. 1994). This discrepancy could be attributed to the sustaining sources of organic matter from plant mortality, which was deficient in the sucrose addition experiment.

The transplants in each treatment were observed alive throughout the experiment, but some shoots from the highest addition treatments showed signs of decay. The

shoot growth of *H. verticillata* reduced significantly by sucrose addition in sediment compared with the control. The length, biomass and density of shoots were reduced significantly by 84.1%, 56.7% and 92.4%, respectively in the highest 1.0% sucrose addition treatment (Fig. 2). However, the below-ground tissue showed some differences in growth from the above-ground tissue. The length, weight and population of roots in the 0.1% sucrose addition treatment were significantly higher than those in the control ($p < 0.01$). Furthermore, the 0.1% sucrose addition treatment had the highest root/shoot ratio due to the relatively well-developed roots.

Net photosynthetic rate of *H. verticillata* in experiment II significantly reduced with below-ground sulfide exposure, it was 104% lower in the highest sulfide treatment than that of control. Respiration rates increased up to 19.8 μmol O_2 h^{-1} g^{-1} in the 0.6 mM sulfide treatment, 3.8 times higher than that of control (Fig. 3). SC and soluble protein contents in root also significantly reduced. SC decreased from 10.9 mg g^{-1} in the control to 3.1 mg g^{-1} under sulfide exposure, it was reduced 71.8%. Soluble protein contents in sulfide treatments were significantly lower than the control, reduced by an average of 49.8%, from 17.55 mg g^{-1} in the control to 5.19 mg g^{-1} in the 0.6 mM sulfide treatment.

H. verticillata showed high susceptibility to organic-rich sediment and sulfide exposure, compared with some marine species. Among marine species, *Thalassia testudinum,* a known seagrass species, also shows high tolerance to sulfide exposures (up to 10 mM) and do not show any mortality signs (Erskine and Koch 2000), but another seagrass species, *Zostera marina*, is susceptible to sulfide exposure, and its photosynthetic activity is reduced when sulfide level is above 0.4 mM (Goodman et al. 1995; Holmer and Bondgaard 2001). Terrados et al. (1999) found that sucrose additions were favorable for increases in shoot density of *Enhalus acoroides* and *Cymodocea rotundata*, but were adverse for growth of *Halodule uninervis*. Comparing with marine species, abilities of *H. Verticillata* species to tolerate sulfide exposure are significantly weaker than that of marine species. This is probably due to their different acclimation to sulfide levels in long terms. Along

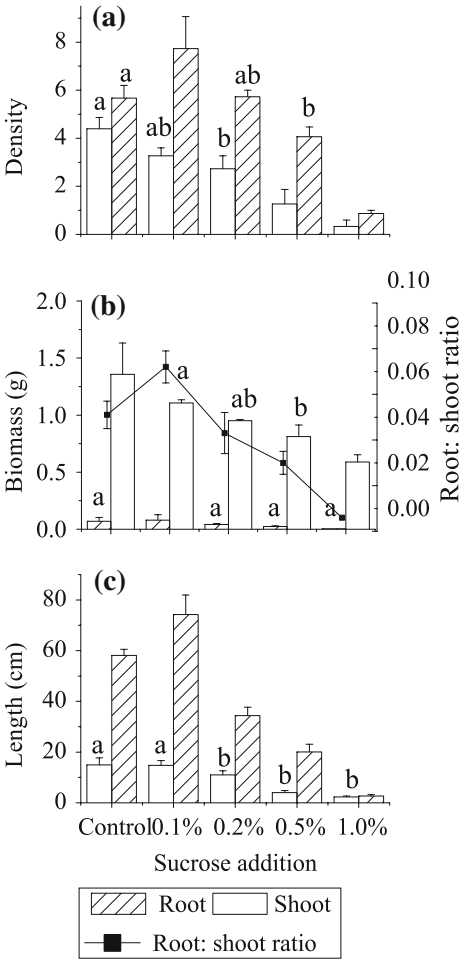

Fig. 2 Length (**a**), biomass (**b**) and density (**c**) of shoots and roots per *Hydrilla verticillata* plant at the end of experiment. The *histogram* represents mean (±SE, n = 3). The *same letter* indicates no significant difference between treatments

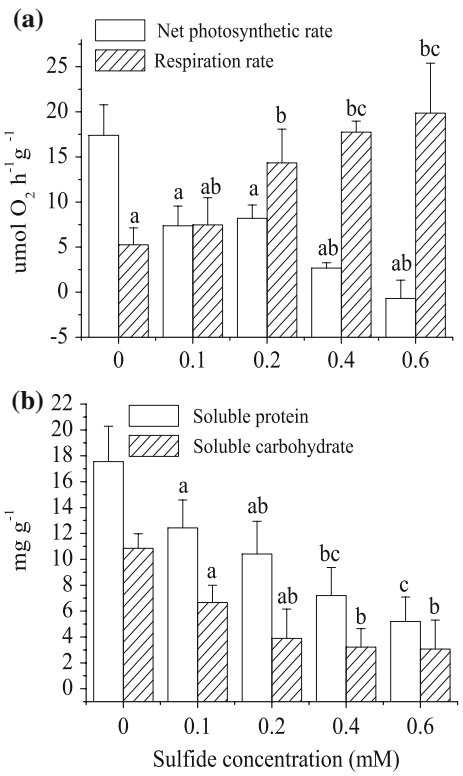

Fig. 3 Effects of sulfide exposure on net photosynthesis and respiration rates (**a**), soluble carbohydrate and protein (**b**) of *Hydrilla verticillata*. The *histogram* represents mean values (±SE, n = 4). The *same letter* indicates no significant difference between treatments

with the environmental acclimatory capacities, the species-specific differences in tolerance to organic-rich sediment and sulfide exposure can, in large part, be attributed to their ability to translocate O_2 (Pedersen et al. 1998) and photosynthetically derived carbohydrates to below-ground tissues (Zimmerman et al. 1995). Nonstructural carbonates are stored in the root-system when the carbon balance is positive at high light intensities and can be used during energy starvation (Burke et al. 1996). In our sulfide exposure experiment, *H. verticillata* showed reductions in SC and soluble protein contents, consistent with the trend of net photosynthesis. In fact, factors, which reduce the photosynthetic capacity of macrophytes, such as low light availability and low temperature, can reduce the supply of oxygen and carbohydrate to roots, then result in reduced tolerance to below-ground sulfide exposure.

The results of the experiments indicate that organic matter addition reduce the pH and Eh values of sediment, increase porewater sulfide concentration, negatively affect the total biomass and ramification of *H. verticillata*. However, some low levels of organic enrichment (e.g. 0.1% sucrose addition) may stimulate the root growth. Porewater sulfide exposure is interpreted as an important factor involving in the declines of *H. verticillata* in organic-rich lakes.

Acknowledgments We would thank Prof. Jianmin Ma, Prof. Yongyuan and Zhang Qiaohong Zhou, for critical review of the manuscript. This research was provided by grants from the Key Project of Knowledge Innovation Program of Chinese Academy of Sciences (KZCX2-YW-426) and Project of National Major Program of Science and Technology (2008ZX07316-004). Comments of anonymous reviewers are greatly appreciated.

References

Armstrong J, Armstrong W, Beckett PM, Halder JE (1996) Pathways of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. ex Steud. Aquat Bot 54:177–197

Biebl R, McRoy CP (1971) Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. Mar Biol 8:48–56

Borum J, Pedersen O, Greve TM (2005) The potential role of plant oxygen and sulfide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*, in Florida Bay. J Ecol 93:148–158

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye-binding. Anal Biochem 72:248–254

Burke MK, Dennison WC, Moore KA (1996) Non-structural carbohydrate reserves of eelgrass *Zostera marina*. Mar Ecol Prog Ser 137:195–201

Carlson PR, Yarbro LA, Barber TR (1994) Relationship of sediment sulfide to mortality of *Thalassia testudinum* in Florida Bay. B Mar Sci 54:733–746

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Erskine JM, Koch MS (2000) Sulfide effects on *Thalassia testudinum* carbon balance and adenylate energy charge. Aquat Bot 67:275–285

Goodman JL, Moore KA, Dennison WC (1995) Photosynthetic responses of eelgrass (*Zostera marina* L.) to light and sediment sulfide in shallow barrier island lagoon. Aquat Bot 50:37–47

Grosse W, Armstrong J, Armstrong W (1996) A history of pressurised gas-flow studies in plants. Aquat Bot 54:87–100

Holmer M, Bondgaard EJ (2001) Photosynthetic and growth responses of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquat Bot 70:29–38

Holmer M, Frederiksen MS, Møllegaard H (2005) Sulfur accumulation in eelgrass (Zostera marina) and effect of sulfur on eelgrass growth. Aquat Bot 81:367–379

Pedersen O, Borum J, Duarte CM (1998) Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. Mar Ecol Prog Ser 169:283–288

Qiu DR, Wu ZB (1998) Ecological studies on aquatic macrophytes in lake Donghu of Wuhan – feasibility for rehabilitation of submerged macrophytes in the lake. Resour Environ in the Yangtze Basin 7:42–47

Terrados J, Duarte CM, Kamp-Nielsen L, Agawin NSR (1999) Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquat Bot 65:175–197

Thamdrup B, Fossing H, Jørgensen BB (1994) Manganese, iron, and sulfur cycling in a coastal marine sediment (Aarhus Bay, Denmark). Geochim Cosmochim Acta 58:5115–5129

Wijcka C, Grootb CJ, Grillas P (1992) The effect of anaerobic sediment on the growth of *Potamogeton pectinatus* L.: the role of organic matter, sulphide and ferrous iron. Aquat Bot 44:31–49

Yan G, Ma JM, Qiu DR, Wu ZB (1997) Succession and species replacement of aquatic plant community in East Lake. Acta Phytoecol Sin 21:319–327

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Zimmerman RC, Kohrs DG, Steller DL, Alberte RS (1995) Carbon partitioning in eelgrass. Plant Physiol 108:1665–1671