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Hydrogen sulfide induced growth, photosynthesis and biochemical responses in three submerged macrophytes

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ABSTRACT

Hydrogen sulfide (H_2S) is a known phytotoxin for submerged macrophytes, which can affect plant species differentially and modify species composition in aquatic environments. To investigate the effects of H_2S on three submerged macrophytes, *Elodea nuttallii*, *Myriophyllum spicatum* and *Potamogeton crispus* were exposed to five treatments containing varying concentrations (0–1.0 mM, depending on species) of sodium hydrosulfide (NaHS) as the H_2S donor. NaHS can produce the desired levels of H_2S for the experiment. All the plants exposed to low concentrations of NaHS exhibited increased plant growth without showing oxidative stress. However, a decrease in growth rate, chlorophyll content, and an increase in hydrogen peroxide (H_2O_2) and malondialdehyde content (MDA) were observed after exposure to high sulfide concentrations, which indicated the presence of increased oxidative stress in the three plant species of interest. For *E. nuttallii* and *M. spicatum*, the activity of guaiacol peroxidase (POD) and ascorbate peroxidase (APX) levels decreased in the presence of 0.5 and 1.0 mM NaHS concentrations, suggesting that the antioxidative enzymes were not able to scavenge the reactive oxygen species responsible for oxidative stress, furthering plant senescence. Compared with *E. nuttallii*, higher antioxidative responses in *M. spicatum* and *P. crispus* exhibited a higher tolerance to NaHS exposure.

1. Introduction

Submerged macrophytes provide many important ecosystem services. They serve aquatic ecosystems by providing food and refuges for fishes, aquatic invertebrates and other aquatic organisms (Costanza et al., 1997; Harborne et al., 2006). They are the source of primary production, and other important services they offer are the control of transport, settling and resuspension of sediment (Madsen et al., 2001; Asaeda et al., 2010). Aquatic macrophytes also play an important role in bio-geochemical cycles and thus purify water in natural water bodies and constructed wetlands (Asaeda and Rashid, 2015). Due to their important services, they have been termed as 'ecosystem engineers' (Asaeda et al., 2010). However, aquatic macrophytes often encounter an array of biotic and abiotic stress factors. Most of these factors, individually or collectively, govern the growth of macrophytes (Ben Rejeb et al., 2014) and determine their distribution (Chambers et al., 2008). Among many other factors, eutrophication and submergence are considered the major drivers for plant growth and species distribution (Zhang et al., 2016).

Hydrogen sulfide (H_2S) is a known phytotoxin in aquatic environments (Lamers et al., 2013). It can be produced either in the course of microbial organic matter decomposition or dissimilatory sulfate reduction in waterlogged soil (Reddy and DeLaune, 2008). In eutrophic waters, it can also be formed by anaerobic decomposition of organic wastes. Sulfides can be trapped in the sediment by precipitation with metal ions. However, some portion of it remains undissociated in the form of H_2S, dissolved in sediment pore water, especially at pH < 7 (Thode-Andersen and Jørgensen, 1989). Despite being present in low concentration, H_2S plays a very important role in the biological, physical and chemical processes in aquatic ecosystems. Among several forms of sulfides, being dissolved in water, it is the only form that can freely penetrate through the cell membrane affecting growth of submerged macrophytes (Koren et al., 2015). In addition to direct interference with plant physiological processes, the production of H_2S can deplete dissolved oxygen (DO) in waters by increasing the sediment oxygen demand rate and thus creates toxicity to aquatic plants (Dunnette et al., 1985). Anoxic

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production of H_2S exerts strong toxicity in submerged plants and reduces growth (DeLaune et al., 1983) by interfering with nutrient uptake (King et al., 1982), photosynthesis and metabolism (Holmer et al., 2005). In addition, high H_2S can be also responsible for the formation of reactive oxygen species (ROS), which can lead to protein degradation and peroxidation of membrane lipids (lipid peroxidation), resulting in production of malondialdehyde (MDA) (Brodersen et al., 2015). However, recent studies reported that H_2S at very low concentrations acts as a gaseous signal molecule and alleviates oxidative damage in plants by antioxidant enzymes (Shi et al., 2015; Li et al., 2016).

Despite the potent toxicity of sulfide, some aquatic macrophytes, such as *Spartina alterniflora* (Lee et al., 1999) and *S. anglica* (Lee, 2003) have adapted to sulfide-rich sediments. Sulfide concentration has been linked to the distribution of emergent macrophytes, *Phragmites australis*, *S. alterniflora* (Chambers et al., 1998) and other species (Ingold and Havill, 1984) in salt marshes. Li et al. (2009) suggested that this kind of macrophyte distribution is due to the differential responses of plants to sediment sulfides. Though we do not have any report that sulfide determines the composition and distribution of submerged macrophytes, its effect on the growth of the same have been studied elsewhere (DeBusk et al., 2015; Pedersen and Kristensen, 2015). Toxicity symptoms appeared in the tropical seagrass *Thalassia testudinum* at 2 mM sulfides (Erskine and Koch, 2000), and 100 μM sulfides in *Potamogeton compressus* (Geurts et al., 2009).

Currently, eutrophication in different water bodies is increasing due to effluent wastewater from factories or agricultural leakage, and submerged macrophyte populations are declining in eutrophic lakes globally (Kemp et al., 1983; Chai et al., 2006). Sulphate enrichment can also lead to eutrophication and cause serious problems in freshwater wetlands (Lamers et al., 1998). On the other hand, anaerobic decomposition in eutrophic waters produces H_2S (Castel et al., 1996). Therefore it is necessary to investigate the effects of dissolved H_2S on the growth and biochemical responses of submerged macrophytes. Though the occurrence of H_2S in aquatic environments is temporary, it can either exert oxidative stress (at higher concentration) by depleting oxygen in the water column (Dunnette et al., 1985) or promote growth of submerged macrophytes at lower concentration (Shi et al., 2015; Li et al., 2016). Although works cited above studied the effects of sulfides in sediment on the growth response of aquatic macrophytes, none of these studies has investigated dissolved H_2S as a stressor for the growth of submerged macrophytes.

Though the tolerance of a plant to abiotic stress factors is a physiological trait, it can also be determined by the morphology of plants (Cedergreen et al., 2004). Likewise, aquatic macrophytes have been reported to adopt phenotypic plasticity to cope with certain abiotic stress (Gratani, 2014). The level of tolerance to sediment sulfide among several species varied greatly as reported by some researchers (Geurts et al., 2009; Wu et al., 2009). Therefore it can be assumed that growth promotion or stress tolerance of aquatic macrophytes to H_2S exposure is species specific, and it depends on the morphology of the plants.

To test this hypothesis, we observed growth rate, chlorophyll content, maximum photochemical efficiency (Fv/Fm), concentrations of indole acetic acid (IAA), contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) as oxidative stress indicators, and activities of antioxidant enzymes, viz. ascorbate peroxidase (APX) and guaiacol peroxidase (POD) of three submerged macrophytes grown under varying concentrations of NaHS (as the H_2S donor). The investigated macrophytes are *Elodea nuttallii* St. John, *Myriophyllum spicatum* L. and *Potamogeton crispus* L. They are very common and abundant species in Japan. These macrophytes have conspicuous differences in the foliar structures (Fig. 1). *M. spicatum* has feather like leaves, composed of more than 10 thin and short (≈1.0 cm) leaflets. Both *P. crispus* and *E. nuttallii* have lamina leaves. However, *P. crispus* leaves are curly and much larger (4–10 cm) than those of *E. nuttallii* (0.5–1.5 cm). *E. nuttallii* and *M. spicatum* leaves are whorled around the stem, whereas *P .crispus* leaves have alternate arrangement.

2. Materials and methods

2.1. Plant materials, growth conditions, and treatment

Plant samples of *E. nuttallii*, *M. spicatum and P. crispus* were collected from Moto-Arakawa River, a tributary of the Arakawa River in southern Saitama, Japan (36° 7′ 30.1″ N, 139° 24′ 20″ E) (Asaeda and Rashid, 2015). Plant materials were cultured in tanks (50 cm × 35 cm × 35 cm) in a growth chamber at a controlled temperature of 23 ± 3 °C and a photoperiod of light: dark of 12 h: 12 h. The photosynthetic photon flux density was maintained at approximately 100–120 μmol photons m^{-2} s^{-1} by using fluorescent lamp tubes. Commercial river sand (90% <1 mm) was used as the substrate, which was purchased from the local market (DIY, DoIt, Japan). Experimental plants were obtained from the culture tanks. After 21 days of acclimation, two apical tips (≈6 cm) were clipped and plugged into silicone sponge clumps and placed in a 500 ml glass beaker. The culture medium was 5% Hoagland nutrient solution (HNS) (Hoagland and Arnon, 1950). The pH of the solution was maintained at 6.0–6.5 using 1 M NaOH or HCl.

A preliminary experiment was set up with different concentrations of NaHS applied to *E. nuttallii*, *M. spicatum* and *P. crispus* and shoot length and vigor (naked eye observation) were observed. It appeared that *E. nuttallii* was weaker in comparison to the other two and could not survive at 1.0 mM NaHS concentration after 15 days. Therefore, on the basis of the observations of this preliminary experiment and previous literature (Geurts et al., 2009; Wu et al., 2009), different sets of treatments were established for *E. nuttallii*, and *M. spicatum* and *P. crispus*. Five treatments were selected for each plant, with three replicates (30 similar sized plants from each species) for each of the following treatments: NaHS concentrations of 0, 0.01, 0.05, 0.1 and 0.5 mM for *E. nuttallii*; and 0, 0.01, 0.1, 0.5 and 1.0 mM for *M. spicatum* and *P. crispus*.

To achieve the desired H_2S concentrations, sodium hydrogen sulfide (NaHS, Sigma) was used as a hydrogen sulfide (H_2S) donor (Ali et al., 2015). Previous studies reported that NaHS can generate the highest amount of H_2S contents compared to other chemicals. Chen et al. (2011) used a series of sulphur- and sodium-containing chemicals, including NaHS, Na_2S, Na_2SO_4, Na_2SO_3, $NaHSO_4$, $NaHSO_3$ and NaAC, and exposing *Spinacea oleracea* to them, and suggested that H_2S rather than the other sulphur-containing compounds or sodium was responsible for the increase in chlorophyll content in *S. oleracea* after NaHS treatment. The targeted H_2S concentrations were achieved by adding 10 mM NaHS via syringe, after deoxygenating the media with inert gas. The beakers were sealed with Parafilm M and disturbed as few as possible to prevent the escape of H_2S gas. The culture medium of each treatment was renewed everyday due to the relatively short half-life of H_2S (Napoli et al., 2006). To maintain the target concentrations of the treatments, a 5 ml portion of the medium from each flask was extracted daily by syringe and H_2S was measured colorimetrically (Cline, 1969).

The experiment was conducted for 7 days, as plants exposed to high H_2S concentrations showed brown discoloration and increased mortality after 7 days. At the end of the experiment, plant samples were collected for physical and biochemical analyses, washed with distilled water, and dried by blotting with laboratory tissue. Final shoot length (length of main stem) was measured with the help of a ruler to calculate the growth rate of plants. The same