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Combined effect of water inundation and heavy metals on the photosynthesis and physiology of *Spartina alterniflora*

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ABSTRACT

The frequency and duration of tidal flooding significantly influence the bioavailability of heavy metals (HMs) in sediment and hence exert toxicological effects on coastal wetland plants. In this study, the combined effects of different water inundation times (3, 6, 9, and 12 h) and HMs (Cu, Zn, Pb, and Cr) on the photosynthesis and physiology of *Spartina alterniflora* were investigated under greenhouse conditions. Results showed that *S. alterniflora* was somehow tolerant to the combined HMs treatments, and only the highest level of HM treatment decreased leaf chlorophyll content. Furthermore, the plants did not show any signs of victimization. Different times of water inundation with HMs did not exert any significant effect on the malonaldehyde (MDA) and chlorophyll contents in the leaves of *S. alterniflora* at day 20. Prolonged water inundation time at day 60 significantly reduced leaf chlorophyll content with the decrease in leaf photosynthetic rate, which was accompanied by a significant increase in the intercellular concentration of CO_2. At day 60, abscisic acid dose-dependently increased along the different water inundation times, indicating that this phytohormone is involved in plant responses to flooding stress. Peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX), showed different responses to the combined treatment of water inundation and HMs at different times. At day 20, the long duration of water inundation and HMs treatments (9 h + HMs and/or 12 h + HMs) significantly increased enzyme activity in the leaves compared with the control group (6 h). At day 60, the POD and SOD activities in the leaves of *S. alterniflora* decreased with prolonged water inundation time, and root APX activity significantly decreased compared with the 6 h water inundation treatment.

1. Introduction

With the intensification of industrial and agricultural activities, estuarine wetlands have become seriously polluted by organic and inorganic compounds, including heavy metals (HMs) (Sun et al., 2016). Sediment is a long-term sink for HM cations because its negative redox potential and high amounts of sulfide, iron, and organic matter decrease metal solubility and bioavailability (Harbison, 1986; Smith and Lee, 2015; Calvo-Cubero et al., 2016). In other words, HMs cannot be degraded due to the anoxic nature of estuarine wetland sediment.

Spartina alterniflora L. is a perennial salt marsh plant native to eastern North America and has been introduced to the coastal wetlands of China in the 1990s to stabilize eroding coastal banks. Given its competitive effects on native species, *S. alterniflora* has been considered as an exotic, invasive plant in China (Li et al., 2009a, Smith and Lee, 2015). Nonetheless, *S. alterniflora* has been suggested to play important functions in multiple biogeochemical processes, including the cycling of metal substances in coastal and estuarine wetlands (Otte et al., 1991; Weis and Weis, 2004). Previous studies showed that salt marsh plants, including *Phragmites australis* (Windham et al., 2001; Quan et al., 2007; Weis and Weis, 2004), *S. alterniflora* (Alberts et al., 1990; Windham et al., 2001; Weis and Weis, 2004; Quan et al., 2007; Salla et al., 2011), *Spartina maritima* (Reboreda and Caçador, 2008; Padinha et al., 2000), *Spartina densiflora* (Mateos-Naranjo et al., 2011, 2012), and *Spartina argentinensis* (Redondo-Gómez et al., 2011), can accumulate large quantities of HMs, including Cu, Zn, Pb, Cr, Cd, and As, in the roots while some are transported to the aerial part of the plants. The immobilization of HMs in the rhizosphere of wetland plants is a feasible technical means for the phytoremediation of HM pollution in wetlands. Ideal plants that can be used for phytoremediation must be perennial and can endure barren soil salinity and other adverse environmental conditions (Smith and Lee, 2015).

The distribution and survival of salt marsh plants in coastal wetlands are mainly determined by tidal inundation (Huckle et al., 2000; Winkel et al., 2011; Duarte et al., 2014). Tidal inundation in coastal wetlands varies in frequency and duration with tidal elevation (Huckle

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et al., 2000; Brownstein et al., 2013). Prolonged tidal inundation harms salt marsh plants, including flood-tolerant plants (e.g., *S. alterniflora*) (Naidoo et al., 1992; Smith and Lee, 2015), by reducing oxygen supply. Prolonged flooding could eliminate *S. alterniflora* over several months (Smith and Lee, 2015). The frequency and duration of tidal flooding significantly influence the adsorption and desorption behavior of HMs in sediment by modifying the redox potential, pH, and dissolved organic carbon (DOC), as well as the redox chemistry of Fe, Mn, and S (Zhu et al., 2012; Shaheen et al., 2014; Calvo-Cubero et al., 2016). The extractable HMs in sediment exert major influences on the growth and metal accumulation of plants, and the fractionation of HMs in sediment closely correlates with alterations in the hydrological regimes of sediment (Zhao et al., 2016).

To date, many studies have investigated the tolerances and responses of coastal salt marsh plants to HM stresses (Ye et al., 1998; Mendelssohn et al., 2001; Windham et al., 2001; Redondo-Gómez et al., 2011; Mateos-Naranjo, 2012; Bankaji et al., 2016). Mendelssohn et al. (2001) examined the physiological responses of *S. alterniflora* to increasing levels of Cd. They found that the ratio of aboveground biomass and the regrowth after initial harvest significantly reduce with increasing Cd and that photosynthesis is a sensitive index that responds to Cd stress promptly before visible damages occur. Li et al. (2009b) found that the major antioxidative enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), in *S. alterniflora* are actively increased by low levels of Cd (0.1–2.0 mmol L^{-1}). They also discovered that treatment with a high Cd level of 4 mmol L^{-1} decreases enzyme activity and causes serious and irreversible damages. Redondo-Gómez et al. (2011) found that 1.5 mmol L^{-1} Cr significantly reduces the leaf gas exchange and photosynthetic rate in *S. argentinensis*, and 4 mmol L^{-1} Cr exerts toxic effects on the plant. Chai et al. (2014) investigated the effects of Cu (50–1000 mg kg^{-1}) on the physiology of *S. alterniflora* and found that the species demonstrates relatively high tolerance to Cu stress with unaltered fine root biomass and plant height under the treatment of up to 800 mg kg^{-1} Cu.

Most experimental studies on the toxicological effects of HMs on wetland plants used treatments of constant flooding and sediment HM loading while ignoring the periodic tidal inundation characteristics of coastal wetlands. Periodic tidal inundation determines the physical and chemical properties of sediment, which influences the speciation and bioavailability of HMs with different toxicological effects on plants. In China, the hydrological regime (water inundation or draining) of coastal wetland sediments is considerably influenced by reclamation activities and extreme climate events, which have become frequent due to rapid economic development and climate change (Ma et al., 2014; Duarte et al., 2015). Therefore, the present study investigated the combined effect of tidal factors (e.g., periodic soil inundation) and HMs on the photodsnthetic and antioxidative physiology of the typical coastal wetland plant *S. alterniflora*. The study can provide a theoretical basis for understanding the HM resistance and adaptability of wetland plants under fluctuating tidal conditions.

2. Materials and methods

2.1. Greenhouse water inundation experiments

Seedlings of *S. alterniflora* with comparable size (about 40 cm in height) were collected in Nanhui, Shanghai. Polypropylene (PP) pots with the size of 50 cm (L) × 40 cm (H) × 20 cm (W) were used for the cultivation of the seedlings, and holes with a diameter of 2 cm were punched evenly on the side walls of the pots for convenient water drainage. A total of 30 pots were prepared for the experiment. The collected *S. alterniflora* seedlings were carefully transplanted into the PP pots with the sediments collected from the ambient environment of the seedlings. Each pot had six seedlings and contained about 6 kg of sediments. The pots were placed in greenhouse with a daily temperature of 20–30 °C, a relative humidity of 59–80%, and a light intensity of

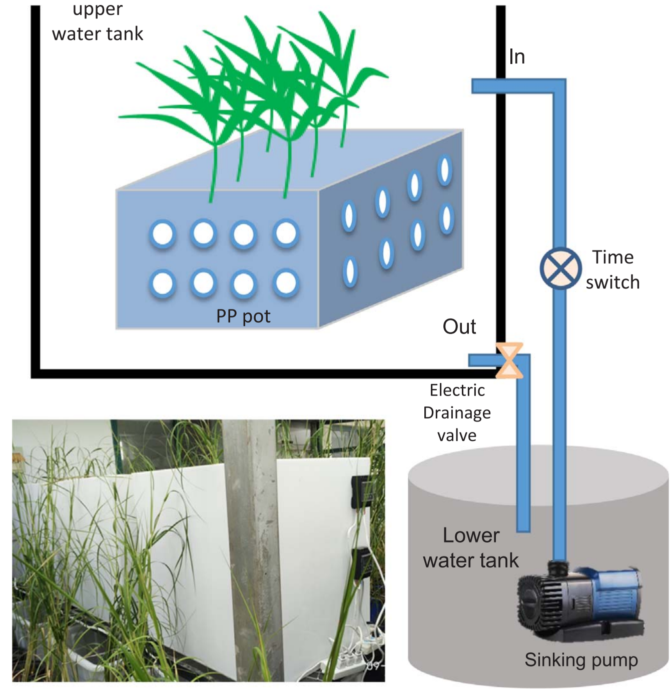

Fig. 1. Diagram of the automatic water inundation system. During the experiment, the treatment solutions in the lower water tank were pumped into the upper water tank automatically controlled by a time switch and kept in the upper water tank for a given period. Then, the solution in the upper tank was drained to the lower water tank through a time-controlled electric valve to complete a water cycle. There were two water cycles every day.

800–1400 μmol photons $m^{-2} s^{-1}$. The seedlings were irrigated with tap water once daily until the start of the treatments.

An automatic water inundation system was designed to mimic the different tidal inundation times in real habitats (Fig. 1). The water inundation system was composed of an upper water tank [75 cm (L) × 60 cm (W) × 75 cm (H)], a PP pot [50 cm (L) × 20 cm (W) × 40 cm (H)], and a lower water tank (with size same as that of the upper water tank). The PP pots sown with the seedlings of *S. alterniflora* were placed in the upper water tank. During the experiment, the treatment solutions in the lower water tank were pumped into the upper water tank automatically controlled by a time switch and kept in the upper water tank for a given period. Then, the solution in the upper tank was drained to the lower water tank through a time-controlled electric valve to complete a water cycle.

After 14 days of rejuvenation, 15 pots of the seedlings with similar height were selected to test the effect of different levels of combined HM stress on the growth and physiology of *S. alterniflora*. The pots were placed into 15 tanks with automatic water inundation control system and randomly divided into five groups, each in triplicate. Four groups were placed into water tanks receiving different levels of combined metal solutions, namely, 1HM, 2HM, 4HM, and 6HM. One group without any metal treatment served as control. For the treatment of 1HM, appropriate amounts of $CuCl_2$, $ZnCl_2$, $PbCl_2$, and $CrCl_3$ were dissolved into salt water (10‰ in salinity) to make the final concentrations of 11.7, 30.4, 9, and 5.6 mg L^{-1} for Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cr^{3+}, respectively. The selection of metals and metal concentrations was based on previous investigation on total bioavailable sediment HMs in Yangtze River estuarine (Chen et al., 2001; Bi et al., 2003; Li et al., 2012). Treatments 2HM, 4HM, and 6HM contained HM concentrations that were 2, 4, and 6 times of 1 HM, respectively. The pots in the water tanks received inundations twice per day by the different levels of HM treatment solution with a total inundation time of 6 h, and the treatment time lasted for 60 days. Another 15 pots of seedlings were also placed into 15 tanks with automatic water inundation control system