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Effect of a polybrominated diphenyl ether congener (BDE-47) on growth and antioxidative enzymes of two mangrove plant species, *Kandelia obovata* and *Avicennia marina*, in South China

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ABSTRACT

The effects of BDE-47 on the growth and antioxidative responses of the seedlings of *Kandelia obovata* (Ko) and *Avicennia marina* (Am) were compared in an 8-week hydroponic culture spiked with different levels of BDE-47, 0.1, 1, 5 and 10 mg l^{-1}. The two highest BDE-47 levels significantly suppressed the growth and increased the activities of three antioxidative enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), of Ko in week 1. However, SOD and POD activities at high levels of BDE-47 became lower than the control in week 8. On the contrary, growth of Am was not affected at all contamination levels, and the activities of three enzymes were enhanced by BDE-47 in weeks 1 and 4, but such stimulatory effect became insignificant in week 8. *Avicennia* was more tolerant to BDE-47 toxicity than *Kandelia*, as its antioxidative enzymes could better counter-balance the oxidative stress caused by BDE-47.

1. Introduction

Mangroves naturally grow along tropical and subtropical coastal regions are subjected to anthropogenic contamination due to their close proximity to urban development and human activities. Mangrove swamps have been used as convenient dumping sites for waste and wastewater (Macfarlane and Burchett, 2001, 2002; Qiu et al., 2010). High concentrations of persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs) and polybrominated diphenyl ethers (PBDEs), have been recorded in mangrove sediments (Tam and Yao, 2002; Bhattacharya et al., 2003; Ke et al., 2005; Binelli et al., 2007; de Souza et al., 2008; Qiu et al., 2010; Zhu et al., 2014). Not only mangrove sediments, Chen et al. (2005) also reported that PBDE concentrations in the sediments of Pearl River Delta, China ranged from 0.13 to 94.72 ng g^{-1} dry weight. The concentrations of PBDEs, as well as the number of PBDE congeners, in sediments showed an increasing trend from the 1970s onwards and peaked in early to middle of the 1990s in Europe and Asia (Choi et al., 2003; Zegers et al., 2003). To our best knowledge, up to 2013, the highest concentration of PBDEs was 16,026 ng g^{-1} dry weight in sediment samples collected from electronic recycling sites in China (Luo et al., 2007). Surprisingly, the latest research on PBDEs in the sediment in urban watershed in Shenzhen, China also showed an extremely high concentration, up to 10^5 ng g^{-1}, and the concentrations exhibited a positive association with the urbanization level of the catchment (Sun et al., 2013). The contamination problem of PBDEs in sediments and their harmful effects on our environment cannot be ignored.

PBDEs pose an oxidative stress to animals through the overproduction of reactive oxygen species (ROS), which causes peroxidation, membrane damage and inactivation of enzymes, leading to poor growth or even death (Fernie et al., 2005; Reistad and Muussen, 2005; Albina et al., 2010; Jin et al., 2010). In plants, POPs other than PBDEs also induce an oxidative stress, and antioxidative enzymes could be stimulated to combat the oxidative stress (Liu et al., 2009; Ke et al., 2011; Song et al., 2011; Ye and Tam, 2007). Liu et al. (2009) reported that the oxidative response to PAH stress in *Arabidopsis thaliana* was rapid and involved many antioxidative enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase. The effects of PBDEs on plants and their antioxidative responses are not clear. The only study by Huang et al. (2013) found that *in vitro* and *in vivo* exposure of ryegrass, pumpkin and maize to PBDEs caused increases in root enzyme activities, such as nitro-reductase and glutathione-transferase. Knowledge on the role of antioxidative enzymes in mangrove plants to remove excess ROS produced by PBDE contamination is scarce.

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Mangrove plants in inter-tidal zones are subject to environmental pressures such as fluctuating oxygen levels, waterlogging, salinities, heavy metals, etc., which could induce the production of excess ROS leading to an oxidative stress. Mangrove plants were found to possess effective antioxidative enzymes, including SOD, POD and CAT, to minimize the harmful effects (Parida et al., 2004; Chen et al., 2005; Jithesh et al., 2006; Ru et al., 2009; Zhang et al., 2007b). *Kandelia candel* (now renamed as *Kandelia obovata*) could tolerate long-term exposure of salt stress by increasing the SOD and POD activities (Ru et al., 2009). Ye et al. (2010) showed that the SOD activities of *K. candel* increased only at 25 parts per thousand (ppt), while POD activities increased with salinity levels ranging from 5 to 25 ppt. *K. candel* was also more tolerant to waterlogging than *Bruguiera gymnorrhiza*, as both SOD and POD in the leaves of *K. candel* increased significantly when the waterlogging period was longer than eight weeks (Ye et al., 2003). However, among the eight true mangrove species in Hong Kong, including *K. obovata*, the highest tolerance to waterlogged sediment was *Avicennia marina* (Pi et al., 2009). In terms of heavy metal stresses, lead stimulated the POD activity in both the roots and leaves of *A. marina*, but the POD activity in *K. obovata* decreased at Day 1 under moderate levels of Pb and Mn, suggesting that antioxidative responses were plant species-specific (Yan et al., 2010; Yan and Tam, 2011). When compare *K. candel* and *B. gymnorrhiza*, the former species had greater stability in the cell membranes of leaves and roots to protect them against the heavy metal stress (Zhang et al., 2007b). These research results revealed that the tolerance of mangrove plants to stresses, as well as the response and effectiveness of the antioxidative system, vary among plant species, the type, duration and intensity of the stress factor. As no research hitherto has focused on the effects of PBDEs on mangrove plants, it is not clear whether mangrove plants could use similar antioxidative enzymes to combat the oxidative stress caused by PBDE contamination and whether this defence system is plant species-specific.

The present study aims to compare the effects of different levels of BDE-47 contamination (0–10 mg l^{-1}) on the growth of the seedlings of *K. obovata* Sheue, Liu & Yong (Ko) and *A. marina* (Forsk.) Vierh. (Am). These two species are typical, true mangrove plant species in Hong Kong and South China, but they are distributed at different tidal positions along the shore. Ko is found at all tidal levels, while Am is a pioneer species colonized in the most foreshore and seaward regions (Tam and Wong, 2000; Pi et al., 2009). The study also investigates the temporal changes of three antioxidative enzymes, SOD, POD and CAT, in the roots and leaves of seedlings exposed to BDE-47 during an eight-week hydroponic culture. Seedlings were used instead of mature plants due to their more sensitive nature; they also potentially respond more rapidly to stresses (Krauss et al., 2008). Roots and leaves are the two plant organs most sensitive to stressors, more so than stems and propagules (Liang et al., 2003; Yan and Tam, 2011). Hydroponics was used instead of a soil culture to examine the intrinsic toxicity of PBDEs to plants because soils are substantial sinks that reduce the bioavailability and toxicity of PBDEs (Mueller et al., 2006). BDE-47 was used as the model PBDE congener as it was one of the most prevalent and toxic PBDE congeners (Li et al., 2009).

2. Materials and methods

2.1. Experiment setup and sampling

Mature propagules of Ko (15–18 cm long), mature seeds of Am (width of 2.2 ± 0.1 cm and length of 2.3 ± 0.1 cm, mean and standard deviation of 54 replicates) and surface sediments (0–5 cm) were collected from Kei Ling Ha Hoi, a typical mangrove swamp (22°25′12″N, 114°16′12″E) in Hong Kong, with little human disturbance and contamination. Propagules and seeds, free of insect damage and fungal infection, were cultivated in experimental pots (each with a dimension of 10 cm in diameter and 14 cm in height) filled with mangrove sediments. The pots were placed in a greenhouse with a photosynthetic active radiation of 800–1400 μmol m^{-2} s^{-1}, temperature of 20–29.5 °C and relative humidity of 60–80%. Plants were irrigated with tap water every day. After about one year, healthy seedlings of Ko (one year old) and Am (one year and three months old) having uniform sizes were harvested from the pots, gently washed under tap water to remove sediment and rinsed in deionised water. The sediment-free seedlings were transferred to 500 ml dark wide-mouth bottles, each containing 250 ml sterilized Hoagland's nutrient solution, prepared by autoclaving at 121 °C and 15 psi for 2 h (Hoagland and Arnon, 1950), with one seedling per bottle.

After a one-month acclimation in nutrient solution, four different contamination levels of BDE-47, purchased from Accustandard (USA), in the presence of 0.3% Tween 20, were added to the nutrient solution. The four levels were low: 0.1, medium: 1.0, high: 5.0 and very high: 10.0 mg l^{-1}. These four levels were chosen to simulate the concentrations in sites with different degrees of contamination, and the highest PBDE contamination in the sediment in urban watershed in Shenzhen, China that was up to 10^5 ng g^{-1} (Sun et al., 2013). The analytical reagent grade Tween 20 obtained from Sigma was added to increase the solubility of PBDEs in nutrient solution, as this is a non-ionic surfactant widely used in the research on insoluble organic compounds (Alcantara et al., 2009). Preliminary experiments showed that 0.3% Tween 20 was sufficient to dissolve 10 mg l^{-1} BDE-47 (up to 89.7% recovery), the highest contamination level in this experiment. Two controls, nutrient solution (positive control) and nutrient solution added with 0.3% Tween 20 (solvent control), were prepared in the same way as the treatment but without BDE-47. Three replicates were established for each treatment and the control. The nutrient solution with different levels of BDE-47 was freshly prepared biweekly to ensure the contamination level of BDE-47 in nutrient solution was maintained at the designed value. At the end of weeks 1, 4 and 8, three replicate bottles from each treatment and the control were retrieved. Seedlings were harvested, washed with tap water, rinsed with deionised water, wiped dry and separated into roots, propagules, stems and leaves. The fresh samples of root and leaf were used for the assays of different antioxidative enzymes (SOD, POD and CAT) and ROS production, that is superoxide radical release (O_2^-) and hydrogen peroxide (H_2O_2).

2.2. Plant growth

The dry biomass (75 °C for 48 h) of each plant organ was measured. The total numbers of the fully expanded leaves in each seedling were counted. Chlorophyll a (Chl a) and chlorophyll b (Chl b) of fresh leaf (0.1 g) were extracted by 10 ml N, N-dimethylformamide (DMF) at 4 °C for one week and the absorbance of the supernatant at 647 and 664 nm, representing Chl a and Chl b, respectively, was determined by a UV–visible spectrophotometer (Schimadzu, model UV-1206) (Inskeep and Bloom, 1985). Chlorophyll a and b contents were calculated by the equations described by Wellburn (1994) in mg g^{-1} fresh weight (FW), and the ratio of Chl a/b was calculated.

$$\text{Chl a} = 12A_{664} - 3.11A_{647}$$

$$\text{Chl b} = 20.78A_{647} - 4.88A_{664}$$

2.3. Assays of antioxidative enzymes in leaf and root

The enzymes were extracted according to Zhang et al. (2007b) with minor modifications. In brief, 0.2 g fresh root or leaf were