Effects of PBDEs on Enzymatic and Non-enzymatic Antioxidants in Seedlings of *Kandelia obovata*

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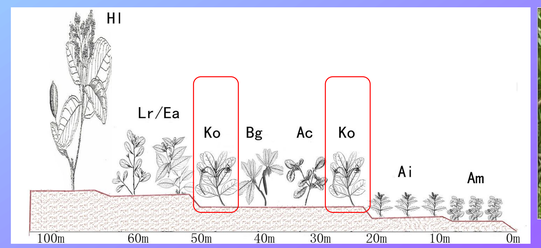
Introduction

- Due to anthropogenic activities, polybrominated diphenyl ethers (PBDEs) are emerging global environmental pollutants. Mangrove ecosystems closed to human settlements are often used as convenient waste/wastewater dumping sites.
- PBDEs are toxic to organisms as they induce oxidative stress through the over-production of reactive oxygen species (ROS). To combat such stress, many organisms develop antioxidative defense mechanisms.
- Mangrove plants are well-known to have high content of tannins and have special adaptations to stressed environment, such as saline and anaerobic conditions. Whether mangrove plants also possess defense systems to minimize the harmful effect of ROS caused by PBDEs have seldom been reported.
- *Kandelia obovata* Sheue, Liu & Yong (Ko) is the most common mangrove plant species in Hong Kong and distributes at all tidal levels (Fig. 1). It is a good model plant to determine the response to PBDEs.



Kandelia obovata

Fig. 1. The location of eight different mangrove species in a mangrove swamp in Kei Ling Ha Hoi, Hong Kong.
(Ac: *Aegiceras corniculatum*, Ai: *Acanthus ilicifolius*, Am: *Avicennia marina*, Bg: *Bruguiera gymnorrhiza*, Ea: *Excoecaria agallocha*, HI: *Heritiera littoralis*, Ko: *Kandelia obovata*, Lr: *Lumnitzera racemosa;* Plant pictures retrieved from Flora of China, 2004)

Objectives

- To investigate the effects of BDE-47, a typical congener of PBDEs, at different contamination levels on the antioxidative defense system of the seedlings of *Kandelia obovata* in hydroponic culture.
- To compare the sensitivity of the antioxidative enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) with the non-enzymatic antioxidants, including total polyphenols (TP), extractable condensed tannins (ECT), protein-bound condensed tannins (PBCT) and fibre-bound condensed tannins (FBCT), in roots and leaves, aiming to identify the potential indicator to PBDE oxidative stress.

Materials & Methods

Kei Ling Ha Hoi (KLHH) mangrove swamp

↓

Collection of seeds/propagules of Ko

↓

Germination in mangrove sediment for three months

↓

Retrieval of 3-month old seedlings from sediment, washing and transferring into hydroponic culture (Fig. 2) spiked with BDE-47 (0.1, 1, 5 and 10 mg l^{-1})

↓ Sampling at weeks 1, 4 and 8

Different plant organs (roots and leaves)

↓ ↓

Antioxidative enzymes (SOD, POD and CAT) | Non-enzymatic antioxidants (TP, ECT, PBCT and FBCT)

Fig. 2. Hydroponic set-up

Results & Discussion

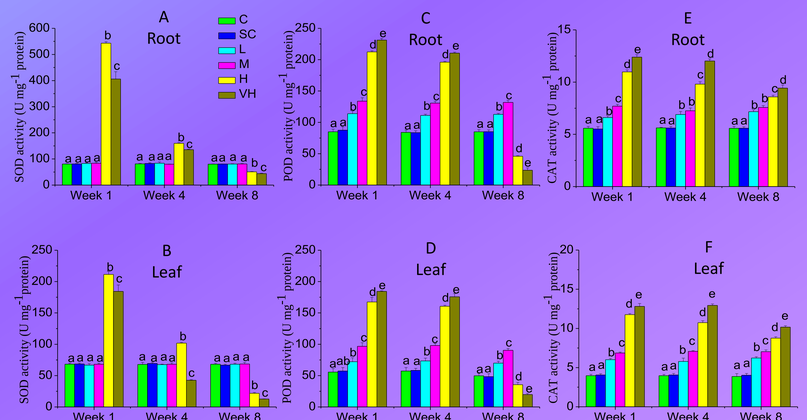


Fig. 3. Effects of BDE-47 on enzymatic antioxidants (SOD, POD, and CAT activities (U mg^{-1} protein)) in roots and leaves of Ko (C: Control, SC: Solvent control, L: 0.1 mg l^{-1} BDE-47, M: 1 mg l^{-1} BDE-47, H: 5 mg l^{-1} BDE-47, VH: 10 mg l^{-1} BDE-47. At each sampling time, bars with different letters are significantly different at $p \le 0.05$ according to one-way ANOVA; mean and standard deviation of three replicates are shown).

Table 1. F-values (degree of freedom and sample size in parenthesis) of two-way ANOVA tests showing the effects of sampling time and concentrations of BDE-47 on SOD, POD and CAT activities in roots and leaves of Ko (*: $p \le 0.05$; **: $p \le 0.01$; ***: $p \le 0.001$).

Parameters \ Sources of variations	Sampling times (2, 54)	Concentration of BDE-47 (5, 54)	Sampling times x Concentration of BDE-47 (10, 54)
SOD in roots	678.16***	130.98***	249.72***
SOD in leaves	1740.15***	279.89***	716.51***
POD in roots	1966.15***	806.24***	794.03***
POD in leaves	819.45***	460.81***	257.38***
CAT in roots	76.86***	1209.13***	47.37***
CAT in leaves	80.08***	1896.62***	39.46***

Antioxidative enzymes

- SOD: The changes of SOD in roots and leaves due to BDE-47 were similar. At high and very high levels of BDE-47, SOD activities were significantly higher than that at medium and low levels which were comparable to the controls in week 1. Such increase was still observed in week 4 but significantly declined in week 8 at high BDE level, while decreases in SOD were found in the very high BDE level from week 4 onwards (Fig. 3A and 3B). These results indicated that the SOD defense system might have been destroyed by long-term exposure to high level of BDE contamination.
- POD: Similar trends as SOD but more sensitive, as significant increases in POD were found in seedlings received even at low level of BDE-47 in week 1. The increase carried on to week 4 and week 8 under low and medium BDE contamination although at a lesser extent, while significant decreases were detected under high and very high contamination levels in week 8 (Fig. 3C and 3D).
- CAT: The changes due to BDE contamination were similar to that of POD, except increases of CAT under different BDE levels lasted till week 8 although the increases became less and less as exposure time (Fig. 3E and 3F).
- The effects of BDE-47 levels and time were significant according to two-way ANOVA, especially POD activity in roots, indicating POD was a more sensitive response than the other two enzymes (Table 1). The interactions of BDE levels and time were also significant, suggesting that the enzymatic responses were both time- and contamination level-specific.

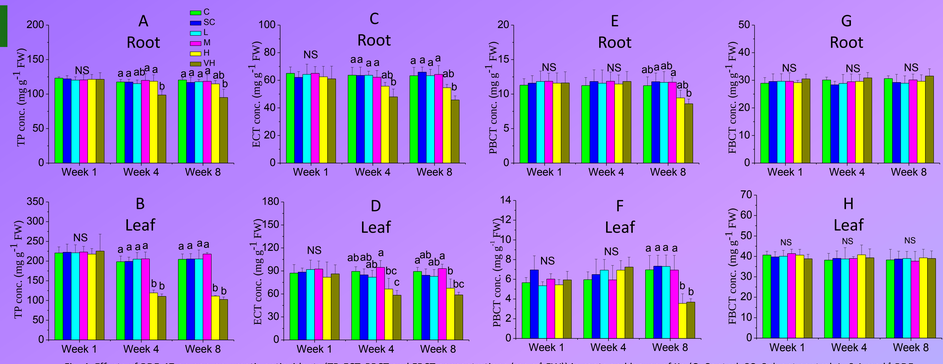


Fig. 4. Effects of BDE-47 on non-enzymatic antioxidants (TP, ECT, PBCT and FBCT concentrations (mg g^{-1} FW)) in roots and leaves of Ko (C: Control, SC: Solvent control, L: 0.1 mg l^{-1} BDE-47, M: 1 mg l^{-1} BDE-47, H: 5 mg l^{-1} BDE-47, VH: 10 mg l^{-1} BDE-47. At each sampling time, bars with different letters are significantly different at $p \le 0.05$ according to one-way ANOVA; NS: not significant; mean and standard deviation of three replicates are shown).

Table 2. F-values (degree of freedom and sample size in parenthesis) of two-way ANOVA tests showing the effects of sampling time and concentration of BDE-47 on TP, ECT, PBCT and FBCT concentration in roots and leaves of Ko (*: $p \le 0.05$; **: $p \le 0.01$; ***: $p \le 0.001$).

Parameters \ Sources of variations	Sampling times (2,54)	Concentration of BDE-47 (5, 54)	Sampling times x Concentration of BDE-47 (10,54)
TP in roots	6.70**	6.36***	1.70
TP in leaves	47.38***	32.79***	8.37***
ECT in roots	3.06*	8.16***	1.32
ECT in leaves	4.62*	9.15***	1.29
PBCT in roots	2.68	1.24	1.09
PBCT in leaves	2.81	2.50*	3.99***
FBCT in roots	0.24	0.82	0.15
FBCT in leaves	0.85	0.14	0.14

Non-enzymatic Antioxidants

- For all types of tannins, the effects of BDE-47, irrespective to the contamination levels, were not significant in both roots and leaves, suggesting that non-enzymatic antioxidants were less sensitive to BDE-47 than antioxidative enzymes.
- TP: TP in roots only showed significant decreases at very high BDE levels in weeks 4 and 8 but decline was found in leaves at both high and very high BDE levels (Fig. 4A and 4B). The decrease in leaves at same BDE level was also more than that in root, indicating that leaf TP was more sensitive than root TP.
- ECT: Same as TP, ECT also decreased at high and very high BDE levels (Fig. 4C and 4D) but the response of root was comparable to that of leaf according to the F values of two-way ANOVA (Table 2).
- PBCT: Not only week 1, there were also no significant changes in PBCT at different BDE levels in week 4 and significant decreases in leaf were observed only in week 8 (Fig. 4E and 4F; Table 2). PBCT was less sensitive to BDE contamination than TP and ECT.
- FBCT: Most insensitive parameter and no significant changes in both roots and leaves at all levels of BDE-47 throughout the experiment (Fig. 4G and 4H; Table 2). It is possible that this bound tannin was more stable than free or extractable tannins, therefore, did not respond to any levels of BDE-47.

Conclusions

- BDE-47, even at a low contamination level (0.1 mg l^{-1}) posed oxidative stress to the seedlings of *Kandelia obovata* in hydroponic culture, as POD and CAT activities in both roots and leaves showed significant increases from week 1 onwards.
- However, at high to very high BDE-47 contamination (5 and 10 mg l^{-1}), SOD and POD activities in week 8 were significantly lower than that at low to medium BDE level which were comparable to the control.
- Ko seedlings also developed non-enzymatic antioxidants under BDE contamination, the concentrations of phenolic compounds, particularly TP and ECT, in high and very high BDE levels were significantly lower than the control.
- Enzymatic responses were more sensitive than the non-enzymatic ones. Among different enzymes, POD appeared to be the most sensitive indicator to BDE oxidative stress.