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Antioxidants in Glutathione-Ascorbate Cycle as Early Warning Indicators to Toxicity of Bde-47 in Mangrove Seedlings

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Polybrominated diphenyl ethers (PBDEs) are common brominated flame retardants that have been detected in various environmental matrices and biological samples, and the burdens have been rising rapidly in recent years. PBDEs, the same as other persistent organic pollutants (POPs), are likely to cause the over-production of reactive oxygen species (ROS) leading to the peroxidation, membrane damage and inactivation of enzymes. Higher plants are known to have the ability to to scavenge excess ROS and combat the oxidative stress. The glutathione-ascorbate (GSH-AsA) cycle, involving enzymes such as ascorbate peroxidase (APx), glutathione reductase (GR) and glutathione peroxidase (GPx), is one of the defense systems protecting ROS-mediated oxidative damage in many cellular compartments (Aravind and Prasad, 2005; Caregnato *et al.*, 2008; Anjum *et al.* 2012). The antioxidant parameters in the GSH-AsA cycle were found to be related to the stresses of various toxic metals, metalloids and polycyclic aromatic hydrocarbons (PAHs), but the changes appeared to vary from plant to plant and from stress to stress (Anjum *et al.* 2012). Mangrove plants commonly found in inter-tidal zones along tropical and subtropical coasts provide signficiant ecological services but are subject to various stresses. The GSH-AsA cycle of mangrove plants changed when exposed to environmental stresses producing excessive ROS, such as fluctuating salinity, water logging, heavy metals, PAHs and oil pollution (Song *et al.*, 2012). For instance, the activities of GPx in *Avicennia marina* and *Kandelia candel* were induced by heavy metals, suggesting that GPx could act as an early warning biomarker; however, such stimulation was not found in *Bruguiera gymnorrhiza* (Caregnato *et al.* 2008; Huang *et al.* 2010). How mangrove plants defend themselves against oxidative stresses posed by PBDEs and whether the GSH-AsA cycle-related antioxidants provide a warning to the toxicity of PBDEs in mangrove plants is still poorly understood.

The present study aims to investigate how the GSH-AsA cycle-related antioxidants in the seedlings of *Kandelia obovata* (Ko), a dominant true mangrove plant species in South China, changed under the stress of BDE-47 and identify the early warning biochemical indicator in the cycle to the toxicity of PBDEs. BDE-47 is the main component of a popular commercial product, Penta-BDE, and is one of the most prevalent and toxic PBDE congeners in the environment according to a file from the US Environmental Protection Agency. This congener also has been added to Annex A of the Stockholm Convention, which aims to protect human health and the environment by eliminating toxic POPs. An 8-week hydroponic culture experiment, planted with one-year old seedlings of Ko with five contamination levels of BDE-47, that is, 0, 0.1, 1, 5 and 10 mg/L, was conducted. In the first week, the activities of APx, GR, GPx and the content of dehydroascorbate (DHA), an oxidized form of ascorbate (AsA), in the root and leaf were induced, while the content of AsA was reduced by the two high levels (5 and 10 mg/L). All these enzymes and antioxidants in the seedlings exposed to BDE-47 treatments became the same as the controls in weeks 4 and 8, suggesting a gradual recovery of these antioxidants with time. On the other hand, the content of reduced glutathione (GSH) and the ratio of GSH/GSSG in the root and leaf were enhanced by 5 and 10 mg/L BDE-47 in weeks 1 and 4, while their oxidized glutathione (GSSG) were decreased, but no differences were found in these parameters among all treatments in week 8. This suggests that the BDE-treated mangrove seedling was under an oxidative stress and the plants had to maintain a high content of GSH and a high GSH/GSSG ratio to combat the stress. The gradual return of the antioxidants in the cycle to the same levels as the control in

weeks 4 and 8 implied that the defence mechanism of Ko seedlings might have been broken down after long-term exposure to high levels of BDE-47 contamination.

The growth of mangrove seedlings, in terms of leaf, root and total dry biomass, was suppressed at the two high BDE-47 levels (5 and 10 mg/L) from weeks 4 to 8, implying that plant growth was affected as the GSH-AsA antioxidant defence system of Ko might be inadequate to counter-balance the oxidative stress induced by high levels of BDE-47. Similarly, Caregnato *et al.* (2008) also found that the glutathione antioxidant defence system in *A. marina* was perhaps inadequate to stave off the oxidative stress elicited by zinc at later intervals. It is clear that growth reduction was detected at the end of the 8-week exposure but changes in the enzyme activities and the content of antioxidants in the GSH-AsA cycle, especially those in the root and leaf, were observed almost immediately after exposure, from day 4 onwards. Caregnato *et al.* (2008) also concluded that an accumulated pollutant acted first at the biochemical level and the related responses were later reflected at higher levels of biological organization. Significant positive correlations were found between the antioxidants in week 1 and the final biomass in week 8. The results of stepwise multiple regression further revealed that the most sensitive and important indicators in the GSH-AsA cycle were AsA and GPx. The present study is the first research demonstrating that the GSH-AsA cycle-related antioxidants, particularly AsA content and GPx activity, were sensitive indicators and provided early warning to the toxicity of PBDEs on mangrove plants.

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