Ecotoxicology and Environmental Safety 141 (2017) 290–297

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac)

Shan Jiang[a], Bosen Weng[a,c], Tao Liu[a], Yan Su[a], Jingchun Liu[a], Haoliang Lu[a], Chongling Yan[a,b,*]

[a] Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361005, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China
[c] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

ARTICLE INFO

Keywords:
Polyphenols
Cadmium
Phenanthrene
Translocation
Mangrove plant

ABSTRACT

Polyphenolic compounds are abundant in mangrove plants, playing a pivotal role in the detoxification of pollutants extruded from surrounding environments into plant tissues. The present study aimed to examine the variations of phenolic compounds, namely total polyphenolics, soluble tannins, condensed tannins and lignin, in the mangrove plant *Aegiceras corniculatum* (L.) due to the presence of exogenous cadmium and phenanthrene and to explore the influence of phenolic metabolism on biological translocation of these pollutants from roots to leaves. After a 6-week exposure to cadmium and phenanthrene, significant accumulations of both pollutants were observed. All determined phenolic compounds in both leaves and roots at high dosage levels were enhanced compared to the uncontaminated plant. Elevations of polyphenols in both treatments are possibly a result of stimulation in the activity of phenylalanine ammonia-lyase (PAL) and the enrichment of soluble sugar. Additionally, a significantly positive dosage relationship between polyphenolic metabolism intensity and phenanthrene contamination levels was found, while the trend observed in cadmium treatment was weak since cadmium at high levels inhibited phenolic production. The enrichment of polyphenols led to a decline in the biological translocation of these pollutants from roots to leaves. The immobilization of pollutants in the plant roots is possibly linked to the adsorption potential of polyphenols. These results will improve the understanding of the tolerance of mangrove plants to exogenous pollutants and will guide the selection of plants in phytoremediation because of the variability of polyphenol concentrations among species.

1. Introduction

Mangroves are shrubs or trees with a wide distribution along coasts and estuaries in tropical and sub-tropical regions, covering latitudes of 30°N to 37°S (Feller et al., 2010). Mangrove forests are highly productive; therefore they are one of the most important wetland ecosystems worldwide, providing a wide range of ecological and economic benefits to humans, such as a nursing ground for aquaculture, a source of herbs and protection of banks from erosion (Lee et al., 2014). However, due to intensive human activities in coastal belts, mangroves are subject to a large variety of anthropogenic contaminants, namely trace elements and persistent organic pollutants (Bayen, 2012). Mangrove plants have an extensive root system with a large biomass (Feller et al., 2010). This invasive sediment-root interface may result in a rapid transfer of pollutants from sediment matrix into mangrove tissues, which likely triggers inhibitions of germination, declines of photosynthesis, alterations of mineral nutrition and loss of water balance in plant cells (Kummerová and Kmentová, 2004; Hasan et al., 2009). These biological impacts possibly lead to shrinkage of mangrove coverage and therefore triggering replacement of the dominant species from mangroves to opportunity species, such as cordgrass, in mangrove ecosystems (Yu et al., 2015). Consequently, the biological response of mangrove plants to accumulations of anthropogenic pollutants has received much interest from the scientific community in recent years (Huang and Wang, 2010; Sodré et al., 2013; Zhang et al., 2014b; Jiang et al., 2016).

Plant phenolic compounds are important metabolites with complex structures and large molecular weights, usually ranging from 500 to 3000 Da (Wang et al., 2014), comprising simple phenols, coumarin, lignin, lignan, condensed and hydrolyzable tannins, and flavonoids (Khoddami et al., 2013). These are assumed to actively respond to ecological and physiological stresses, such as pathogen and insect

* Corresponding author at: Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361005, China.
E-mail address: ycl@xmu.edu.cn (C. Yan).

http://dx.doi.org/10.1016/j.ecoenv.2017.03.041
Received 17 October 2016; Received in revised form 22 March 2017; Accepted 24 March 2017
Available online 06 April 2017

attacks, UV radiation and wounding (Khoddami et al., 2013). Phenolic compounds also play an important role in the detoxification of pollutants extruded from surrounding environments into plant tissues. Specifically, studies revealed that the uptake of cadmium and nickel in plant roots can be significantly influenced by phenolic acids (Kováčik et al., 2011). In addition, as reviewed by Kraus et al. (2003), phenols can chelate toxic metal ions with hydroxyl and carboxyl groups and consequently influence the transfer of metals in plant tissues. In terms of their effects on organic pollutants, enzymes, such as lignin peroxidase and manganese peroxidase, involved in the metabolism of phenolic compounds are also assumed to actively participate in the degradation of organic pollutants (Chroma et al., 2002). The adsorption capability of phenols to organic pollutants (e.g., PAHs) via a π–π electron-donor–acceptor interaction may also influence the biological translocation and toxicity of organic contaminants in plants (Zhang et al., 2014a). Polyphenols are also well known for their antioxidant activity, which can be involved in the scavenging of hydrogen peroxides introduced by inorganic/organic pollutants in plant cells (Michalak, 2006; Wang et al., 2014).

In mangrove plants, total phenolic compounds may comprise as much as 5–40% dry weight in leaf and bark tissues (Wei et al., 2010; Wang et al., 2014). The majority of phenols in their tissues is hydrolysable and condensed tannins with a strong antioxidant activity and free radical scavenging potential (Wang et al., 2014), which is possibly linked to the uptake and biological translocation of anthropogenic pollutants in mangroves. Currently, the research literature with regard to systematic and comprehensive studies of the variations of total polyphenolic compounds in mangrove plants due to stresses of metals and organic pollutants are limited. To fill this knowledge gap, in the present study, the role of phenolic metabolism in mangrove plants against inorganic and organic contamination stresses was investigated. *Aegiceras corniculatum* (L.) Blanco (Ac) (hereafter *A. corniculatum*) was selected as the model plant in the current research because of (1) the wide distribution along coastal and estuarine areas in China (Chen et al., 2009), (2) the active production of polyphenols in mangrove tissues (Wang et al., 2014) and (3) a great tolerance to exogenous pollutants, especially metals (Li et al., 2013). Phenanthrene (Phe), a three-ring polycyclic aromatic hydrocarbon (PAH) compound, was applied as the contamination source of the organic pollutants and cadmium (Cd), a typical trace element in mangrove sediments, was used as the source of metals. The selection was based on the known toxic effects and frequency of occurrence in mangrove swamps. The research aimed to determine (1) dosage response of phenolic compounds and metabolisms to both pollutants and potential differences in response; and (2) the relationship between enrichments of phenols and the biological translocation of these pollutants in mangrove plants.

2. Materials and methods

2.1. Exposure experiments

Undamaged propagules of *A. corniculatum* with intact testa were collected from the Caoputou Mangrove Reserve (24°29′N, 117°55′E), Jiulong River Estuary, Fujian Province, China. Afterwards, they were planted in rubber pots. Each pot contained three propagules. The propagules/seedlings were irrigated by Hoagland solution modified for mangrove growth (Xie et al., 2013). The salinity of the culture solution was adjusted by NaCl to 13 PSU (practical salinity units), which is similar with the measurement in the sediment at the sampling site. The solutions were renewed on a weekly basis to prevent nutrient depletion. Sea sands, collected from a sandy beach near the sampling site, were used as the culture medium. The application of sea sands in the culturing can sustain (1) high bioactivity of pollutants since low organic matter reduces the occurrence of adsorption; (2) aerobic environments because high porosity facilitates air exchange between the pore water and the atmosphere. Before planting, the sands were washed with 2% hydrochloric acid solution and rinsed thoroughly with tap water and repeated 6 times in order to reduce Cd and PAHs adsorbed on the particle surface. The culture was carried out in a greenhouse, with an average temperature of 25 °C during the day and 20 °C during the night. The relative humidity ranged from 65% to 85%. After 12 months, plants with similar heights and quantity of leaves were chosen for the following experiments.

The selected plants were randomly divided into two batches. The first batch included five groups and each group contained three pots. The first group was defined as the control, which still received an uncontaminated Hoagland solution. The culture solutions in the remaining groups were spiked by Phe (Sigma–Aldrich Co. Ltd. U.K., purity > 99%) at levels of 0.25, 0.50, 0.75 and 1.0 mg L^{-1}. To improve the dissolution of Phe in the culture solution, methanol was used as a solvent in the preparation of the Phe stock solution. The methanol concentration in the culture solutions was below a level of 0.1% and consequently had limited impact on the growth of mangroves (Zhan et al., 2015). The second batch also contained five treatment groups and three pots were prepared for each treatment. Subsequently, Cd was added as cadmium chloride ($CdCl_2$) at levels of 0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}. These concentrations were selected on the basis of previous experiments conducted by our research group to avoid damaging the mangrove plants. The exposure in the Phe and Cd solutions lasted for approximately 6 weeks. During the exposure, all the plants remained in the sands and the spiked culture solutions were renewed once per week. To prevent any unpredictable effects confounding the results, the plants were rotated randomly in the greenhouse every three days. Moreover, the ventilation in the greenhouse was elevated to the maximum level during the exposure time period in order to decrease Phe concentration in the atmosphere due to evaporation.

2.2. Determination of Phe and Cd

After a 6-week culture, the *A. corniculatum* was carefully removed from the sands and then cleaned with successive tap and Millipore water three times in order to remove loosely adsorbed sandy particles on the root surface. All leaves and roots (containing iron plate on the surface of the roots) were separated from stems and placed on glass Petri dishes. A portion of the samples was freeze dried. The dry samples were then ground using an agate mortar. For the determination of Cd, ground samples were digested using a mixture of HNO_3 and H_2O_2 (2:1, v/v) in a microwave digestion system (MARS 5, CEM™, U.S.). The digestion solution was then diluted with Millipore water and the concentration of Cd was then quantified by inductively coupled plasma–mass spectroscopy (PerkinElmer™, U.K.) on the basis of Nixon and Moyer (1996). A certified reference material prepared from white cabbage (BCR-679, Institute for Reference Materials and Measurements) was used to determine the recoveries of Cd. The average recovery rate was 97.6%. The method detection limit based on replicates of the blank sample was 0.25 μg L^{-1}. The Phe in mangrove tissues was extracted using an accelerated solvent extraction system (ASE-200, DIONEX™, U.S.) with n-hexane/acetone mixture (1:1, v/v). The surrogate Phe-d10 (Sigma–Aldrich, U.K.) was mixed with the samples prior to extraction in order to quantify the systematic recovery rate. Concentrations of the Phe in the extracts were determined by a gas chromatograph-mass spectrometer (HP6890-5975B, Agilent Co., U.S.), equipped with an HP-5MS column, according to Lu et al. (2011). The mean recovery rate of the deuterated surrogate was 94.9% and the detection limit was 2.2 μg L^{-1}.

2.3. Determination of soluble sugar and malondialdehyde (MDA)

Soluble sugar content was analyzed by the method described by Chow and Landhäusser (2004). In brief, 5 mL of 80% ethanol was mixed with 50 mg of ground freeze-dried sample in glass test tubes. The mixture was then incubated in a water bath at 95 °C for 10 min. After