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Uptake of polycyclic aromatic hydrocarbons and their cellular effects in the mangrove *Bruguiera gymnorrhiza*

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ABSTRACT

The uptake of polycyclic aromatic hydrocarbons and their cellular effects were investigated in the mangrove *Bruguiera gymnorrhiza*. Seedlings were subjected to sediment oiling for three weeks. In the oiled treatment, the ΣPAHs was higher in roots (99%) than in leaves (1%). In roots, PAHs included phenanthrene (55%), acenaphthene (13%), fluorine (12%) and anthracene (8%). In leaves, PAHs possessed two to three rings and included acenaphthene (35%), naphthalene (33%), fluorine (18%) and phenanthrene (14%). In the roots, oil caused disorganization of cells in the root cap, meristem and conducting tissue. Oil contaminated cells were distorted and possessed large and irregularly shaped vacuoles. Ultrastructural changes included loss of cell contents and fragmentation of the nucleus and mitochondrion. In the leaves, oil caused dilation and distortion of chloroplasts and disintegration of grana and lamellae. Oil targets critical organelles such as nuclei, chloroplasts and mitochondria which are responsible for cell vitality and energy transformation.

1. Introduction

Mangrove ecosystems, which occur in intertidal environments of the tropics and subtropics, have high ecological and conservation value because of their global extent, high productivity and numerous ecosystem services (Li et al., 2014; Naidoo, 2016a). Rapid urbanization and industrialization around mangrove ecosystems make this low-energy environment especially vulnerable to oil contamination, as they receive pollutants from both tidal and freshwater sources. The high productivity, abundant detritus and rich organic carbon make mangroves susceptible to pollutants such as PAHs (Ke et al., 2011; Li et al., 2014). PAHs are ubiquitous soil contaminants that originate from both natural and anthropogenic sources (Tao et al., 2006; Peng et al., 2011). The PAHs that accumulate in mangrove tissues are transferred to other trophic levels via food webs and cause environmental and health problems due to their carcinogenic and mutagenic properties (United States Environmental Protection Agency, 2008; Tao et al., 2006). Mangrove species are differentially tolerant to oil, with toxicity being determined by the type, dosage, and mode of entry into plants (Suprayogi and Murray, 1999). The oil-induced damage is due mainly to the coating of foliage and aerial roots which causes oxygen deprivation and growth irregularities (Zhang et al., 2007; Wang et al., 2014). The light molecular weight (LMW) components of PAHs penetrate plant roots and are transported to shoots, where they cause cellular and metabolic effects such as development of anomalous growth forms (Tam et al., 2005; Naidoo, 2016b), cell membrane damage (Kang et al., 2010), reduced gas exchange (Naidoo et al., 2010) and increased mutation and mortality (Liu et al., 2009).

Oil disrupts cellular lipid membranes in the conducting tissue (Zhang et al., 2007) and damages the xylem vessels of fine roots (Ye and Tam, 2007). The accumulation of PAHs in plant tissues causes different degrees of ultrastructural damage, depending on the chemical structure and concentration of the compounds (Alkio et al., 2005; Liu et al., 2009). Effects of oil on mangroves have been reported under laboratory (Zhang et al., 2007) and field conditions (Sojinu et al., 2010; Wang et al., 2015). Some adverse effects of oil on *Bruguiera gymnorrhiza* are summarized in Table 1. Despite extensive studies on the effects of PAHs on mangroves, information about uptake, distribution, physiology and cellular mechanisms of toxicity effects is lacking (Fismes et al., 2002; Wild et al., 2005; Lu et al., 2005).

The objective of this study was to investigate the uptake and distribution of PAHs, as well as their cellular and ultrastructural effects, in the mangrove, *B. gymnorrhiza* (L.) Lam. In previous studies on mangroves, *B. gymnorrhiza* was reported to be more tolerant to oil than *Kandelia obovata, Aegiceras corniculatum* and *Acanthus ilicifolius* (Zhang et al., 2007; Ke et al., 2011). As far as we are aware, this is the first study on the identity of PAHs in *B. gymnorrhiza* and their cellular and ultrastructural effects.

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Table 1
Adverse effects of oil on *B. gymnorrhiza*.

Oil effect	Reference	Type of oil
Morphology		
Reduction in plant height, leaf number and biomass.	Zang et al. (2007): Naidoo et al. (2010);	Spent lubricating oil Bunker fuel oil 180
Leaf chlorosis, senescence, defoliation, chlorophyll content	Ke et al. (2011); Naidoo et al. (2010); Naidoo (2016b);	Field polluted site Bunker fuel oil 180
Physiology		
Reduction in photosynthesis, chlorophyll fluorescence (ETR, quantum yield, Fv/Fm).	Naidoo et al. (2010)	Bunker fuel oil 180
Increase in free radicals, superoxide dismutase, malondialdehyde.	Zhang et al. (2007): Ke et al. (2011)	Spent lubricating oil Field polluted site

2. Materials and methods

2.1. Growth conditions

Propagules of *B. gymnorrhiza* were collected from the Beachwood Mangroves Nature Reserve (29° 48′ S, 31° 02′ E). Sixty uniform propagules were selected and planted in 24 cm × 21 cm plastic pots containing a mixture of sand, potting soil and compost (1:2:1 volume based). Plants were watered daily with tap water, once monthly with 10% seawater and maintained in a glasshouse for 13 months at 25 °C (day) and 18 °C (night). After one year, 40 uniform seedlings were subjected to control or oiled treatments for three weeks in a completely randomised experiment, replicated 20 times. Plants were selected from this cohort for experiments on chemical staining, PAH determination, and transmission electron microscopy. About 200 mL of Bunker fuel oil 180 were carefully poured onto the soil surface in the oiled treatments. The oil completely covered the surface area of the soil (about 100 cm^{-2}). Preliminary experiments indicated that this volume of oil penetrated the entire soil volume (about 1100 cm^{-3}) after about three days. The characteristics of this oil are indicated in Table 2.

2.2. Fluorescein diacetate (FDA) and propidium iodide (PI) staining

After seven days, four replicates from control and oiled treatments were destructively sampled. Soil was removed carefully from roots by washing pots under running water. Plant roots were harvested, placed between pieces of polystyrene and sectioned (300 μm thickness) with a Vibratome 1000 (Lancer, U.S.A.). Staining was undertaken with the plant cell viability assay kit containing the stains, FDA and PI (Sigma, USA). Stains were thawed in a 30–37 °C water bath and the precipitate formed in the PI solution was dissolved by mixing. Stains (1 μL) were diluted in 99 μL of deionised molecular water (pH 7.4). Sections were stained with PI for 5 min and with FDA for 20 min. Specimens were examined with a Nikon Eclipse 80i, utilizing Nikon filter sets, FITC (fluorescein isothiocyanate) at 510–560 nm and TRITC (tetramethylrhodamine isothiocyanate) at 475–490 nm. Sections stained with FDA were viewed with the FITC band pass filter, while those stained with PI were viewed with the TRITC filter (Truernit and Haseloff, 2008; Lassailly et al., 2010). Photographs were taken with a Nikon sight DS-Fli digital camera.

Table 2
Specifications of Bunker fuel oil 180.

	Unit	Value
Energy content (gross)	kJ kg^{-1}	43,400
Viscosity @ 100 °C	cst	22
Total sulphur as S	mass %	4,0
Pour point	°C	−10
Flash point (PMCC)	°C	70
Water content	mass %	0.30
Density @ 20 °C	kg L^{-1}	0,98
Ash	mass %	0,10

2.3. PAHs

After three weeks of oiling treatment, twelve replicates were selected, and destructively sampled. Plants were washed under running water to remove soil and separated into leaves and roots. Samples were freeze-dried, ground and homogenized. PAHs were extracted from 10 g of leaf and root material. Three replicates were pooled in each case to yield $n = 4$ for PAH determination. The ground samples were combined with acetonitrile, buffered with magnesium sulphate, sodium chloride and citrate salts (pH 5–5.5) and centrifuged. Formic acid was added and the extracts analysed on a HP-5890 gas chromatograph (Hewlett Packard, USA) equipped with an HP-DB 5MS column (60 m × 0.25 mm × 0.25 μm thick film). The column was coupled to a mass spectrometer detector (HP 5972) in the ion monitoring (SIM) mode. The temperature was set at 300 °C and deuterated pyrene and benzo(a)pyrene used as internal standards. Based on the United States Environmental Protection Agency list of priority PAHs (United States Environmental Protection Agency, 2008), fifteen PAHs were identified. Benzo[*k*]fluoranthene and benzo[*b*]fluoranthene were grouped as one compound, benzo[k + b]fluoranthene. Recoveries of PAHs, determined by spiking root and leaf samples, yielded values of 90 ± 4% ($n = 4$).

2.4. Transmission electron microscopy (TEM)

After seven days of treatment, four replicates were destructively sampled. Plants were washed under running water to remove soil. Plants were separated into roots and leaves. Small segments (1 mm^2) of root and leaf material were removed from root tips and the youngest, fully expanded leaves. Root and leaf samples were fixed under vacuum for 24 h in 2.5% glutaraldehyde (4 °C). Samples were washed three times for 5 min each in phosphate buffer (pH 7.2) and post fixed in 0.5% osmium tetroxide for 1 h in the dark at room temperature. Samples were washed three times for 5 min each in phosphate buffer (pH 7.2) and then dehydrated through a graded series of acetone (two times for 5 min each in 30%, 50%, 75% and two changes of 10 min each in 100% acetone). The specimens were kept for 4 h in equal parts of Spurr's (1969) low viscosity resin and 100% acetone and thereafter embedded in 100% resin for 24 h. The specimens were then placed in a mould with fresh 100% resin and allowed to polymerize in an oven for 8 h at 70 °C.

Sectioning was undertaken with a Reichert Ultracut E microtome (Leica, Germany). Sections (60–100 nm) collected on copper grids were stained with 2% aqueous uranyl acetate for 10 min and then with Reynolds' (1963) lead citrate stain for 10 min. All grids were placed in a petri dish lined with filter paper and surrounded by sodium hydroxide pellets for 5 min. Ultra-thin sections were viewed with a Jeol 1010 TEM at 100 kV and images taken with the Megaview 3 system (SIS).

2.5. Data analyses

Data obtained from the PAH analyses were tested for normality using the Kolmogorov–Smirnov test and subjected to analysis of variance and Tukey–Kramer multiple comparisons test ($P \leq 0.05$) using MINITAB version 16 (Minitab Statistical Software, MINITAB Inc. USA). For TEM, the frequencies of occurrence of ultrastructural deformities from several fields of view, and from different replications, were determined and expressed as a percentage relative to the control, to provide quantitative data.

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