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Ultrastructural effects of polycyclic aromatic hydrocarbons in the mangroves *Avicennia marina* and *Rhizophora mucronata*

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ABSTRACT

The effects of polycyclic aromatic hydrocarbons (PAHs) on cell ultrastructure in the mangroves *Avicennia marina* and *Rhizophora mucronata* were investigated. One-year-old seedlings of both species were subjected to sediment oiling with Bunker fuel oil 180. In both species, fresh sections of root tips from the control, stained with fluorescein diacetate, exhibited green fluorescence indicating living cells, while those in the oiled treatment, stained with propidium iodide, exhibited red fluorescence indicating dead cells. In roots of both species, ultrastructural changes induced by oil included disorganization of cells in the root cap, epidermis, meristem and conducting tissue. Oil distorted and disorganised cells as well as their internal structure. Ultrastructural changes included loss of cell contents and fragmentation of organelles such as the nucleus and mitochondria. In the leaves of both species, oil caused dilation and distortion of chloroplasts and disintegration of grana and lamellae. This study demonstrated that in both species, oil damaged membrane integrity and caused disorganisation of 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 areas of the tropics and subtropics, have high ecological and conservation value. These ecosystems are highly productive and provide numerous ecosystem services such as nursing grounds for aquaculture and protection from wave erosion (Lee et al., 2014; Naidoo, 2016a,b). Rapid urbanization and industrialization in close proximity to mangroves make these environments highly vulnerable to oil pollution (Ke et al., 2011; Li et al., 2014). Their intertidal location results in the accumulation of oil pollutants from both tidal and terrestrial sources. Polycyclic aromatic hydrocarbons (PAHs) are nonpolar, hydrophobic contaminants that are of serious environmental concern due to their widespread occurrence and carcinogenic properties (USEPA, 2008). Most PAHs are insoluble in water and degrade very slowly which accounts for their long-term persistence in the environment (Smith et al., 2006).

PAHs originate from either natural seepage or anthropogenic sources such as oil spills from ships or oil wells. Due to their high productivity, mangrove ecosystems contribute abundant detritus, which is rich in organic carbon, to their sediments (Lee et al., 2014). Oil that contaminates mangrove sediments binds strongly to soil organic carbon and is usually transferred to other ecosystems and higher trophic levels via food webs causing environmental and health problems (Wang et al., 2014a,b).

Oil toxicity to mangroves is influenced by the type, dosage, and mode of entry into plants, as well as by the species (Suprayogi and Murray, 1999). The two mangrove species selected in this study exhibit distinct morphological and physiological differences. *Avicennia marina*, a highly salt tolerant pioneer species, possesses salt glands and actively secretes salt. The roots of *Avicennia marina* produce numerous pencil-shaped, negatively geotropic pneumatophores which rise above the soil surface to absorb oxygen for aeration. The second species, *Rhizophora mucronata*, lacks salt glands and its aerial roots arch and suspend the tree over the water, providing extra support and absorbing oxygen. Both species invest considerable resources into root biomass which makes them vulnerable to oil contamination. In most studies on *Avicennia* and *Rhizophora* species, seedlings were exposed to crude oil in the laboratory, greenhouse or *in situ* (Proffitt et al., 1995; Suprayogi and Murray, 1999; Naidoo, 2016a,b).

Several studies that compared oil pollution effects in various species of *Avicennia* and *Rhizophora* are summarised in Table 1. These studies suggested that *Avicennia* seedlings were more susceptible to oil pollution than *Rhizophora*. This was attributed to species differences in root uptake and leaf transpiration (Proffitt et al., 1995) and to differences in root micromorphology (Naidoo, 2016b). In most of these studies, oil reduced growth and biomass accumulation and induced leaf senescence

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Table 1
Adverse effects of different types of oil on *Avicennia* and *Rhizophora* species in previous studies. Reports are in chronological order.

Species	Oil type	Plant parameters measured	Oil tolerance	Reference
R. mucronata, A. officinalis	Diesel, petrol and motor	Stem growth, survival, germination	*A. officinalis* more sensitive	Jagtap and Untawale (1980)
R. mangle A. germinans	Light Arabian crude, No 2 fuel oil, Bunker C, Fresh	Stem and leaf growth, survival	*A. germinans* more sensitive	Getter and Baca (1984)
R. mangle A. germinans	lubricating oil	Stem growth, leaf production, survival	*A. germinans* more sensitive	Proffit et al. (1995)
R. stylosa R. mucronata A. marina	Kuwait crude, North west shelf condensate	Hydrocarbons in leaves, nutrient uptake	*A. marina* most sensitive	Suprayogi and Murray (1999)
A. marina R. mucronata	Bunker fuel oil 180	Stem and leaf growth, chlorophyll content	*A. marina, more sensitive*	Naidoo (2016b)

and abscission. Oil coats aerial roots, damages cellular membranes (Zhang et al., 2007; Wang et al., 2014a,b), reduces photosynthesis (Naidoo et al., 2010), increases mutation, induces anomalous growth forms (Tam et al., 2005; Naidoo, 2016b) and eventually causes mortality (Getter and Ballou, 1985; Teas et al., 1987). None of these studies investigated the cellular and ultrastructural mechanisms of oil toxicity. In this study, we investigated the effects of oil at the ultrastructural level in roots and leaves of the two mangrove species *A. marina* and *R. mucronata.* This was achieved by comparing cell ultrastructure of oil treated seedlings of both species with those of untreated plants. As far as we are aware, this is the first comprehensive study to compare ultrastructural effects of PAHs in these two important and contrasting mangrove species. This study provides novel information on the cellular sites of injury and consequently contributes to a better understanding of the mechanisms of oil toxicity.

2. Materials and methods

2.1. Growth conditions

Propagules of *Avicennia marina* (Forsk.) Vierh. and *Rhizophora mucronata* Lam. were collected from the Isipingo Estuary (29° 59′ S, 30° 56′ E). Twenty five uniform propagules of each species were collected and planted in 24 cm diameter × 21 cm height plastic pots. The soil used was a mixture of sand, potting soil and compost (1:2:1 by volume). Preliminary studies indicated that this soil mixture supported good growth of mangrove seedlings. Plants were grown in a glasshouse at 25 °C (day) and 18 °C (night) for one year. Average maximal photosynthetic photon flux density (PPFD) was 920 μmol m^{-2} s^{-1} while relative humidity was 60% during daylight. All plants were watered regularly with tap water, and once monthly with 10% seawater. Twenty uniform, one-year-old seedlings of *A. marina* (about 60 cm height) and *R. mucronata* (about 43 cm height) were selected for oiling experiments. Pots were arranged in a completely randomised experiment with ten replicates per species in the control and oiling treatments. The sediment was contaminated by carefully pouring 200 mL of Bunker fuel oil 180 onto the soil surface. The oil covered the surface of the soil and completely infiltrated the soil volume (about 5500 cm^3). After seven days of oiling, plants were selected for experiments on chemical staining (n = 5) and electron microscopy (n = 5). The characteristics of this oil have been published previously (Naidoo, 2016b). The glasshouse was adequately ventilated to decrease the PAH concentration in the atmosphere due to evaporation from the treatment pots.

2.2. Fluorescein diacetate and propidium iodide staining

After seven days of oiling, five replicates from control and oiled

Table 2
Adverse effects of Bunker fuel oil 180 on ultrastructural characteristics of roots and leaves of *A. marina* and *R. mucronata*. In roots and leaves of both species, four different fields of view from each of five replicates (n = 5) were randomly selected. In each field of view 20 cells were counted. Means of number of cells with different letters within a row are significantly different at P ≤ 0.05 using 2 × 2 factorial and Tukey's multiple comparisons test, C = control, O = oiled, − = not detected. Results of a multivariate analysis of variance (Manova) are also presented, T = treatment, S = species, T × S = treatment/species interaction, ** = significant at P ≤ 0.01, ns = not significant.

Parameter	A. marina				R. mucronata				Manova		
	Root		Leaf		Root		Leaf				
	C	O	C	O	C	O	C	O	T	S	T × S
Oil present in root cap cells	-a	14b	-a	-a	-a	12b	-a	-a	**	ns	ns
Distorted meristematic cells with oil deposits	-a	10b	-a	-a	-a	6b	-a	-a	**	ns	**
Meristematic cells without cell contents	-a	9b	-a	-a	-a	5b	-a	-a	**	**	**
Fragmented mitochondria and ER in roots	-a	12b	-a	-a	-a	10b	-a	-a	**	ns	ns
Deformed and distorted meristematic cells	-a	13b	-a	-a	0.2a	11b	-a	-a	**	ns	ns
Meristematic cells with oil filled vacuoles	-a	12b	-a	-a	-a	10b	-a	-a	**	ns	ns
Oil deposits close to nucleus in root cells	-a	8b	-a	-a	-a	5b	-a	-a	**	ns	ns
Cells with perforated nuclear membranes	0.2a	6b	-a	-a	0.2a	3a	-a	-a	**	**	**
Invaginated phloem parenchyma cells	0.4a	10b	-a	-a	0.2a	7b	-a	-a	**	**	**
Distorted sieve tube cells	0.2a	7b	0.2a	-a	0.2a	6b	-a	-a	**	ns	ns
Damaged palisade and spongy mesophyll	0.4a	-a	0.4a	9b	0.2a	-a	-a	8b	**	ns	ns
Large and irregular vacuoles in leaf cells	0.2a	-a	0.2a	10b	-a	-a	-a	8b	**	ns	ns
Disorganised and displaced chloroplasts	-a	-a	-a	9b	-a	-a	-a	7b	**	ns	ns
Chloroplasts with oil deposits	-a	-a	-a	9b	-a	-a	-a	8b	**	ns	ns
Dialated and distorted chloroplasts	-a	-a	0.2a	8b	-a	-a	0.2a	6b	**	ns	ns
Few or no starch grains	-a	-a	0.6a	7b	-a	-a	0.4a	5b	**	ns	ns
Spaces between grana and lamellae	-a	-a	0.3a	6b	-a	-a	0.2a	5b	**	ns	ns