Salinity Exacerbates Oil Contamination Effects in Mangroves

Gonasageran Naidoo (✉ naidoogn@ukzn.ac.za)

University of KwaZulu-Natal - Westville Campus <https://orcid.org/0000-0002-0526-2953>

Krishnaveni Naidoo

University of KwaZulu-Natal - Edgewood Campus

Research Article

Keywords: Growth, Ion relations, Photosynthesis, Polycyclic aromatic hydrocarbons (PAHs), Salt secretion, Ultrastructure

Posted Date: February 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-206386/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on July 16th, 2021. See the published version at <https://doi.org/10.1007/s11356-021-15450-9>.

Abstract

The effects of salinity (10 and 50% seawater) and oil in combination on three mangroves, *Avicennia marina*, *Bruguiera gymnorhiza* and *Rhizophora mucronata* were determined. In all species, plant height, number of leaves and CO₂ exchange were generally higher in 10% than in 50% seawater. Salinity and oil decreased plant height, number of leaves, chlorophyll content and CO₂ exchange, with reductions being greater at the higher salinity. In a second experiment, the effects of salinity (0, 10 and 50% seawater) and oil on concentrations of ions, polycyclic aromatic hydrocarbons (PAHs), leaf ultrastructure and salt secretion in *A. marina* were investigated. Salinity and oil in combination increased concentrations of Na⁺ but decreased those of K⁺, Ca²⁺ and Mg²⁺. PAHs damaged cell membranes, disrupted ion concentrations and reduced salt secretion. This study demonstrated that increase in salinity reduces growth of mangroves and that salinity and oiling in combination exacerbate growth reduction. In *A. marina*, oil was absorbed and translocated to the leaves where it disrupted membranes, ion accumulation and salt secretion.

Introduction

Mangroves, which occur in sheltered, intertidal areas of the tropics and subtropics, are economically important as they provide food, timber, and medicine. Mangroves also protect coastlines, attenuate waves, sequester carbon and serve as nursery grounds for fish (Friess 2016; Barbier 2016)). Mangroves occupy low wave energy locations which are usually close to highly populated, industrialized cities. Mangrove habitats are therefore exposed to organic pollutants such as polycyclic aromatic hydrocarbons (Ke et al. 2011; Li et al. 2014). Sources of PAH contamination include oil spills, atmospheric fallout, urban runoff and industrial effluents. Transfer of PAHs from mangroves to other trophic levels, via food chains, causes health problems. PAHs persist in the environment for long periods due to their insolubility in water and low rates of degradation (Lewis et al. 2011).

The uptake and distribution of PAH's and their physiological mechanisms of toxicity in mangroves have been widely investigated (Wang et al. 2014; Veldkornet et al. 2020). Oil coats aerial roots, penetrates sediments and causes oxygen deficiency (Zhang et al. 2007; Cavalcante et al. 2009). Oil has adverse effects on growth as well as ion and water uptake (Ke et al. 2011; Wang et al. 2014). Oiling induces anomalous growth forms (Naidoo et al. 2010), increases mutation (Cavalcante et al. 2009; Veldkornet et al. 2020) and leads to mortality (Ke et al. 2011). Mangrove mortality may lead to their replacement by salt marsh species (Saintilan et al. 2014).

Some mangroves such as *Kandelia* and *Rhizophora* exclude salt by root ultrafiltration, while others such as *Avicennia*, possess glands that excrete salt from their leaves (Ye et al. 2005). Species such as *Bruguiera* and *Rhizophora* accumulate salt in older leaves which are then shed. Salt secreting species like *Avicennia* are more susceptible to oil contamination than non-secreters like *Bruguiera* and *Rhizophora* (Suprayogi and Murray 1999). High salinities decrease growth and productivity of mangroves and increase the activities of antioxidative enzymes (Parida and Jha 2010). The effects of crude oil on mangroves have been investigated in field and laboratory studies (Youssef and Ghanem 2002; Zhang et al. 2007). Few studies, however, have investigated the role of salinity on oil contamination effects (Ke et al. 2011). Oil disrupts cellular membranes

which adversely affect the ionic balance of plants and their ability to tolerate salinity (Gilfillan et al. 1989; Zhang et al. 2007). The volatile fraction of Arabian crude oil was shown to impair salt secretion in *A. marina* (Youssef and Ghanem 2002).

Although the effects of oil on mangroves have been widely investigated, very little information is available on the effects of salinity and oil in combination on growth, ion relations, PAH concentrations, leaf ultrastructure and salt secretion. Of the three species investigated, only *A. marina* possesses salt glands. In this study, the effects of salinity and oil in combination on growth of three disparate mangroves, *Avicennia marina*, *Bruguiera gymnorhiza* and *Rhizophora mucronata* was investigated. In addition, we also determined the effects of salinity and oil in combination on concentrations of ions and PAHs, leaf ultrastructure and salt secretion in *A. marina*.

Materials And Methods

Growth conditions

Propagules of *Avicennia marina* (Forsk.) Vierh, *Bruguiera gymnorhiza* (L.) Lam. and *Rhizophora mucronata* Lam. were collected from the Isipingo Estuary (29°59' S, 30°56' E) Durban, South Africa. Fifty uniform propagules of each species were planted in 24 cm x 21 cm plastic pots. The soil used was a mixture of sand, potting soil and compost (1:2:1 by volume). Pots were watered daily with tap water, once monthly with 10% seawater and maintained in a glasshouse (25°C day and 18°C night) for 12 months. Average maximal photosynthetic photon flux density (PPFD) was 920 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity 70%. Seedlings from this cohort were used in the experiments.

Experiment 1: Effects of salinity and oil on growth of *A. marina*, *B. gymnorhiza* and *R. mucronata*

One-year-old, healthy seedlings of *A. marina* (52 ± 10 cm in height), *B. gymnorhiza* (61 ± 6 cm) and *R. mucronata* (62 ± 7 cm) were selected for the growth experiment. The experimental design was a 3 x 2 x 2 factorial with three species, two salinities (10 and 50% seawater) and two levels of oiling (control and oiled). Pots containing uniform plants were placed in large circular troughs (70 cm diameter x 18 cm height) and subjected to oiling treatments in 10% and 50% seawater (about 3.8 and 19 PSA-practical salinity units respectively) for 12 months. In the oiling treatments, Bunker Fuel Oil 180 (200 ml) was carefully poured onto the soil surface of each pot. Four replicate pots were allocated to each treatment in a completely randomised design, so that there were 16 pots per species. Salinity treatments were introduced gradually at increments of 10% seawater every two days. Pots were irrigated from below and seawater renewed every five days.

Plant height and number of leaves were monitored prior to the commencement of treatments and monthly thereafter. Chlorophyll content was determined at the termination of the experiment with a hand-held chlorophyll absorbance meter (CCM-200, Opti Sciences, Tyngsboro, MA, USA) on the most recently formed mature leaves. Gas exchange measurements were taken with a portable infrared gas analyser (IRGA) (Li-

6400, Li-Cor, Nebraska, USA). Measurements were taken on mature leaves, immediately below the shoot tip (n=4). Average conditions during measurements were as follows: temperature 25°C, incident PPFD 970 $\mu\text{molm}^{-2}\text{s}^{-1}$, CO₂ concentration 400 μL^{-1} and vapour pressure deficit 0.85kPa⁻¹.

Experiment 2: Effects of salinity and oil on salt secretion in *A. marina*

Uniform seedlings of *A. marina*, grown in 0, 10 and 50% seawater for one year, were selected for oiling experiments. The experimental design was a 3x2 factorial with three salinity treatments (0, 10 and 50% seawater) and two oiling levels (control and oiled). Oiling treatments were described previously. Four replicate pots were allocated to each treatment in a completely randomised design, so that there were 24 pots in the experiment. After three weeks of oiling, six mature leaves from each replicate were labelled and washed with distilled water to remove previously secreted salt from the leaf surface. These leaves were labelled and allowed to secrete salt for five days. At the end of five days, secreted salts from each leaf were rinsed for 20 seconds with 20 ml double distilled water and the wash water sealed in air-tight vials (n=4). The rinsed leaves were excised from the plant and fresh mass determined. Leaf areas of the rinsed leaves were determined by photocopying fresh leaves and scanning the images into a computer using image analysis software, SIS Pro Software, version 3.1. The leaves from each replicate were dried to constant mass at 70°C, weighed and milled. Concentrations of Na⁺, K⁺, Ca²⁺ and Mg²⁺ in the leaves and in the secretions were determined by atomic absorption using mid-infrared spectroscopy (Bruker Tensor 27, FTIR Spectrometer with HTS/XT).

Additional one-year-old seedlings of *A. marina*, grown in 10% seawater, were selected to determine whether there were differences in salt secretion between day and night. Pots were placed in troughs and maintained as described previously. Pots were subjected to control and oiling treatments in 10% seawater for 7 days (n=4). In the light experiment, six leaves from each replicate were labelled and rinsed at 06h00 to remove surface salts. The rinsed leaves were allowed to secrete salts during the day. At 17h00, leaves were rinsed to collect secreted salts. In the dark experiment, six leaves from each replicate were rinsed at 17h00 to remove surface salts and labelled. Seedlings were then covered with black plastic bags which excluded light but allowed air movement. Seedlings were then placed in a dark room and allowed to secrete salt. At 06h00 the following morning, leaves were rinsed to collect secreted salts. The rinsed leaves from both light and dark exposure were excised and leaf area and mass determined. Ion concentration in the secretion was determined as described previously.

Transmission electron microscopy (TEM)

After three weeks of oiling treatment, leaf material of *A. marina* (1mm²) was removed from young, fully expanded leaves (n=4) of plants grown in 10% seawater under control and oiling treatments. Leaf segments were fixed under vacuum for 24 h in 2.5% glutaraldehyde (4 °C). Thereafter, the segments were washed three times for 5 min each in phosphate buffer (pH 7.2) and post fixed under darkness in 0.5% osmium tetroxide

for 1 h at room temperature. Leaf segments were then rinsed three times for 5 min each in phosphate buffer (pH 7.2). Subsequently, the segments were dehydrated through acetone solutions (two times for 5 min each in 30%, 50%, 75% and two changes of 10 min each in 100% acetone). Segments were then left to stand for 4 h in equal parts of Spurr (1969) low viscosity resin and 100% acetone. The samples were then embedded in 100% resin for 24 h and placed in a mould with fresh 100% resin. For polymerization, samples were placed in an oven for 8 h at 70 °C. The embedded samples were sectioned transversely (60-100 nm) with a Reichert Ultracut E microtome (Leica, Germany) and collected on grids. Leaf sections were then stained with 2% aqueous uranyl acetate for 10 min and then with Reynolds (1963) lead citrate for 10 min. Grids were placed in a petri dish lined with filter paper containing sodium hydroxide pellets for 5 min. The stained sections were viewed with a Jeol 1010 TEM at 100 kV and photographed with the Megaview 3 system (SIS).

Determination of PAHs

After three weeks of oiling, four leaves of *A. marina* from each replicate were harvested from each treatment. To obtain sufficient material for analyses, the four replicates were pooled in each treatment, freeze-dried and milled for PAH extraction. The milled samples were combined with acetonitrile, buffered with magnesium sulphate, sodium chloride, and citrate salts (pH 5–5.5) and centrifuged. After the addition of formic acid, the resulting extracts were analysed on a HP-5890 gas chromatograph (Hewlett Packard, USA) equipped with an HP-DB 5MS column (60 m×0.25 mm × 0.25µm thick film). The column was coupled to a mass spectrometer (HP 5972) operating in ion monitoring mode (SIM). The carrier gas was high-purity helium (flow rate 1.83 ml min⁻¹). The injector and transfer line temperatures were 290 °C and 300 °C, respectively. The oven temperature was initially set at 60 °C for 1 min, increased to 300 °C at 4 °C/min, and then maintained for 20 min. Before extraction, a known concentration of deuterated PAHs was added to all samples. Quantification was carried out through response factors obtained from PAH standards with deuterated PAHs. At least one deuterated PAH per class of aromaticity was used to determine concentrations of PAHs. Accuracy was monitored by spiking solutions with known amounts of PAHs and determining recovery percentages. Three independent replicates of each sample were prepared and analysed. Sample means and standard errors were calculated after blank subtraction. Ten priority PAH pollutants were determined (USEPA 2008).

Bunker Fuel Oil 180

The characteristics of Bunker Fuel Oil 180 (Light Marine Fuel Oil 180) were described previously (Naidoo and Naidoo 2017). In the glasshouse, the ventilation was elevated to reduce the PAH concentration in the atmosphere due to evaporation from the treatment pots. Preliminary experiments indicated that the oil penetrated the entire soil in the pot within 24 h.

Data analyses

Experiment 1

All data were tested for normality using the Kolmogorov-Smirnov test and then subjected to two-way ANOVA and Tukey's multiple comparisons test ($P \leq 0.05$) using GraphPad Prism, Version 6.05 (GraphPad Software, Inc., USA).

Experiment 2

Data were tested for normality using the Kolmogorov-Smirnov test. Data from ion concentrations in leaves and salt secretion at different salinities were subjected to two-way ANOVA and Tukey's multiple comparisons test ($P \leq 0.05$). PAH analyses in leaves and salt secretion in light and darkness were subjected to unpaired t -tests using MINITAB version 16 (Minitab Statistical Software, MINITAB Inc., USA).

Results

Effects of salinity and oil on growth of *A. marina*, *B. gymnorhiza* and *R. mucronata*

In all three species, plant height, chlorophyll content index (CCI) and CO_2 exchange were generally higher in 10% than in the 50% seawater treatment (Table 1). In *A. marina* there were no differences in the number of leaves or chlorophyll content between the 10% and 50% seawater treatments. In *B. gymnorhiza* and *R. mucronata*, however, the number of leaves was higher in 10% than in 50% seawater. In all three mangroves, CO_2 exchange was higher in 10% and significantly reduced in the 50% seawater treatment. In all three species, salinity and oiling in combination significantly decreased plant height, number of leaves, chlorophyll content and CO_2 exchange, with reductions being greater in 50% than in 10% seawater. Furthermore, in all three mangroves, salinity and oiling in combination caused greater adverse effects than salinity alone.

Table 1

Effects of salinity and oil in combination on plant height, number of leaves, chlorophyll content index and photosynthesis in *A. marina*, *B. gymnorhiza* and *R. mucronata*. One-year-old plants were subjected to salinity and oiling treatments for 12 months. Means $\pm$ standard error are given. Values with different letters are significantly different at $P \leq 0.05$ using two-way ANOVA and Tukey's multiple comparisons test, $n = 4$.

	<i>Avicennia marina</i>				<i>Bruguiera gymnorhiza</i>				<i>Rhizophora mucronata</i>			
	10% seawater		50% seawater		10% seawater		50% seawater		10% seawater		50% seawater	
	C	O	C	O	C	O	C	O	C	O	C	O
Plant height	37 $\pm$ 3.5a	8 $\pm$ 1.2 c	28.3 $\pm$ 3.b	3 $\pm$ 0.1 c	50 $\pm$ 4.5a	21 $\pm$ 1.3b	46 $\pm$ 3.7a	10 $\pm$ 0.5c	25 $\pm$ 3.2a	4 $\pm$ 0.1c	13 $\pm$ 1.1b	3 $\pm$ 0.1 c
No. of leaves	20 $\pm$ 0.6a	10 $\pm$ 0.5b	18 $\pm$ 0.2 a	4 $\pm$ 0.2 c	22 $\pm$ 0.9a	12 $\pm$ 0.4b	16 $\pm$ 0.7b	6 $\pm$ 0.2 c	12 $\pm$ 0.3a	4 $\pm$ 0.1 b	6 $\pm$ 0.1 b	2 $\pm$ 0.1 b
CCI	38 $\pm$ 3.6a	27 $\pm$ 2.6b	36 $\pm$ 2.8 a	16 $\pm$ 1.1c	78 $\pm$ 4.9a	64 $\pm$ 4.6ab	72 $\pm$ 5.6a	42 $\pm$ 2.8b	72 $\pm$ 5.3a	42 $\pm$ 3.2b	64 $\pm$ 6.1a	34 $\pm$ 2.7b
CO ₂ exchange	8.1 $\pm$ 9 a	3.2 $\pm$ 3b	4.3 $\pm$ 0.2b	1.4 $\pm$ 1 c	5.6 $\pm$ 1.1a	2.5 $\pm$ 0.8b	3.1 $\pm$ 0.7b	1.1 $\pm$ 0.3c	4.8 $\pm$ 0.8a	2.4 $\pm$ 0.3b	2.5 $\pm$ 0.6b	1.2 $\pm$ 0.1c

The effects of salinity and oil on salt secretion in *A. marina*

Exposure of seedlings to 0, 10 and 50% seawater for 12 months, followed by three weeks of oiling treatments, caused significant changes in ion concentrations in leaves. In the control treatments, the concentrations of Na⁺, K⁺, Ca²⁺ and Mg²⁺ increased with increase in salinity from 0 to 50% seawater (Fig. 1). Salinity and oiling in combination increased the concentration of Na⁺ in 50% seawater by 41%, while those of K⁺, Ca²⁺ and Mg²⁺ were reduced by 72%, 95% and 96%, respectively, compared to the control. In the salinity treatments alone, Na⁺/K⁺ ratios in leaves were 0.83, 1.36, and 1.91 in the 0%, 10% and 50% seawater, respectively. Salinity and oiling in combination, however, increased Na⁺/K⁺ ratios significantly from 2.3 (0% seawater) to 9.1 (50% seawater).

In the salinity treatments, there were no differences in rates of secretion of Na⁺, K⁺, Ca²⁺, and Mg²⁺ between the 0 and 10% seawater treatments, but further increase in salinity to 50% seawater significantly increased secretion of these ions (Fig. 2). In the 50% seawater treatment, salinity and oiling in combination decreased the rate of secretion of Na⁺, K⁺, Ca²⁺ and Mg²⁺ by 60%, 76%, 68% and 78% respectively, compared to those for salinity alone.

In both light and darkness, rates of secretion of Na^+ , K^+ , Ca^{2+} and Mg^{2+} were higher in the controls and significantly lower by over 50% in the oiled treatments (Table 1). The Na^+/K^+ ratios in the secretion were generally lower in the controls (1.33 to 1.23) and higher (1.5 to 1.75) in the oiled treatments.

TEM indicated that leaves of control plants exhibited typical healthy ultrastructure. In control plants, palisade and spongy mesophyll cells were intact, with a large nucleus, a well-defined nuclear membrane and a distinct nucleolus (Fig. 3). The cytoplasm was appressed against the cell wall and contained numerous intact mitochondria, endoplasmic reticulum and chloroplasts with well-developed grana and intergranal lamellae. Leaves from the oiled treatment exhibited structural aberrations in palisade and spongy mesophyll cells (Fig. 3). Chloroplasts were disorganized and displaced from the plasma membrane, while oil deposits were observed within the cells. Some chloroplasts were dilated, while others showed signs of disintegration. Granal stacks and lamellae were separated and disorganized.

Gas chromatography indicated that the ten main PAHs detected in leaves possessed two to five rings (Table 2). In the leaves, the majority of PAHs were of low molecular weight

(LMW) with 2 to 3 rings. PAHs in leaves included acenaphthene, fluorene and acenaphthylene (two rings), phenanthrene, anthracene, and fluoranthene (three rings), chrysene, benzo[a] anthracene and pyrene (four rings) and benzo[k + b]fluoranthene and benzo[a] pyrene (five rings). The main PAHs in leaves were phenanthrene (30%), chrysene (11%), anthracene (11%) and benzo[k + b]fluoranthene (10%).

Table 2
Concentrations of the ten dominant PAHs ($\mu\text{g/kg}$) in leaves of *A. marina* after three weeks of control and oiling treatments. Means $\pm$ standard error are given. Values with different letters are significantly different at $P \leq 0.05$ using unpaired t-tests, $n=4$, *asterisk indicates undetected in sample.

PAH	# of rings	Control	Oil	%
acenaphthene	2	15 $\pm$ 6a	147 $\pm$ 23b	9
fluorene	2	11 $\pm$ 3a	79 $\pm$ 39b	5
phenanthrene	3	31 $\pm$ 8a	512 $\pm$ 58b	30
anthracene	3	*	191 $\pm$ 24	11
fluoranthene	3	12 $\pm$ 2a	75 $\pm$ 18b	4
pyrene	4	*	118 $\pm$ 19	7
benzo[a]anthracene	4	*	125 $\pm$ 26	7
chrysene	4	*	188 $\pm$ 35	11
benzo[k + b]fluoranthene	5	*	173 $\pm$ 27	10
benzo[a]pyrene	5	*	76 $\pm$ 15	5
Total		69	1684	

Discussion

In *A. marina*, *B. gymnorhiza* and *R. mucronata*, growth was higher in 10% and significantly reduced in 50% seawater, supporting previous studies that optimum salinity for mangroves is below half-strength seawater (Ball 1988). The decline in growth of mangroves with increasing salinity is due to the increased energetic cost of water uptake. Salt tolerance in mangroves is achieved by a variety of mechanisms that include salt exclusion, salt secretion and induction of antioxidative enzymes (Takemura et al. 2000).

In *B. gymnorhiza* and *R. mucronata*, the number of leaves and chlorophyll content were higher in 10% than in 50% seawater probably because these two species accumulate excess salt in older leaves which are then shed to eliminate salt from the plant. In *A. marina*, however, there were no differences in number of leaves or chlorophyll content between the 10% and 50% seawater treatments probably because of more efficient salt

regulation. Several studies have demonstrated that *A. marina* is more salt tolerant than *B. gymnorhiza* and *R. mucronata* (Parida and Jha 2010). The greater tolerance of *A. marina* is attributed to leaf salt secretion and more effective ion exclusion in the roots (Ye et al. 2005). At high salinities, the accumulation of Na^+ and Cl^- reduces cell expansion and growth (Parida and Jha 2010). Salt tolerant plants reduce the amount of salt transported to the leaves by regulating water loss through stomatal control. The decrease in CO_2 exchange in 50% seawater was mediated primarily via stomatal conductance.

Oil contamination of mangrove sediments decreases soil permeability, oxygen concentrations and disrupts ion and water relations (Gilfillan et al. 1989; Suprayogi and Murray 1999). High concentrations of Na^+ in the soil inhibit the activity of oil degrading microorganisms and increase toxicity of PAHs (Lewis et al. 2011). Several studies demonstrated that oil induces the formation of reactive oxygen species which damage membranes of cells and organelles (Zhang et al. 2007; Ye and Tam 2007). Increased oil-induced permeability of membranes increases accumulation of Na^+ and reduces salt tolerance. (Zhang et al. 2007; Ke et al. 2011).

In *A. marina*, salinity and oil in combination caused greater concentrations of Na^+ in leaves, compared to salinity alone, probably due to oil damage to cellular membranes (Gilfillan et al. 1989; Ke et al. 2011). Salinity and oil in combination, however, decreased the concentrations of other ions such as K^+ , Ca^{2+} , Mg^{2+} , probably due to decreased uptake. While Na^+/K^+ ratios were low in the salinity treatments, salinity and oil in combination increased these ratios significantly, resulting in Na^+ toxicity and impaired cell metabolism (Parida and Jha 2010). High Na^+/K^+ ratios in the combined salinity and oiling treatment may be attributed to higher uptake of Na^+ which disrupts absorption of K^+ (Suprayogi and Murray 1999). High Na^+/K^+ ratios have been reported previously for mangroves under salt stress (Waisel et al. 1986). In oil-damaged roots, the concentration of Na^+ increases while that of K^+ decreases, leading to deleterious cellular effects (Gilfillan et al. 1989). High Na^+/K^+ ratios were also reported in sediment oiled *Spartina alterniflora*, a salt secreting grass (Gilfillan et al. 1989). Accumulation of Na^+ and decreased K^+ , Ca^{2+} and Mg^{2+} decrease the quantum yield of PSII and photosynthetic rate (Ball 1988; Naidoo et al. 2010).

Most of the PAHs that are absorbed from the soil are retained within roots (Naidoo and Naidoo 2017). PAHs with high molecular weight ($\text{HMW} > 3$ rings) tend to remain in roots. Those with LMT are transported to the leaves, as these are more water soluble, bioavailable and possess higher vapour pressure than those with HMW (Wang et al. 2014). In our study, phenanthrene which is more water soluble, was dominant in the leaves. In *Kandelia candel*, *A. marina*, and *B. gymnorhiza*, naphthalene and phenanthrene were the most abundant PAHs (Ke et al. 2011; Wang et al. 2014), while in *K. obovata*, *A. corniculatum*, *B. gymnorhiza*, and *A. marina*, naphthalene was dominant (Li et al. 2014). Ultrastructural effects of PAHs in *A. marina* leaves included disorganization of membranes, dilation and distortion of chloroplasts and disintegration of grana and lamellae. Disruption and disorganisation of cellular membranes cause accumulation of Na^+ in leaves (Gilfillan et al. 1989). High Na^+ concentration in leaves leads to decreases in chlorophyll content, energy trapping efficiency, carbon fixation and growth (Parida and Jha 2010; Naidoo et al. 2010).

In *A. marina*, salt glands are important in controlling the ionic composition of leaves, removing about 40% of the salt that enters the shoot (Waisel et al. 1986; Ball 1988). Salt secretion generally increases with increase

in leaf salt content (Waisel et al. 1986). The increase in salt secretion with increase in salinity from 0 to 50% seawater is due to the greater amount of salt entering the leaves (Ye et al. 2005). The multicellular salt glands of *Avicenna* secreted primarily Na⁺ and Cl⁻, and lesser amounts of K⁺, Ca²⁺, Mg²⁺, similar to other studies (Ye et al. 2005). Salinity and oil in combination reduced salt secretion in *A. marina* compared to salinity alone. Oil induced changes in membrane permeability interfered with selective ion absorption leading to accumulation of Na⁺ and lower secretion rates (Youssef and Ghanem 2002; Ke et al. 2011). The dilated, distorted and irregularly shaped chloroplasts in the leaf mesophyll cells were induced by oil. The large spaces observed within grana and chloroplast lamellae suggested breakdown of the photosynthetic machinery and reduced energy transformation. In other plants such as *Arabidopsis thaliana*, oil also caused growth reduction, chlorosis and leaf necrosis (Alkio et al. 2005).

The salt glands of *A. marina*, which comprise of collecting, stalk and secretory cells, contain numerous vacuoles and make them ideal sites for PAH accumulation and thus cytotoxicity (Ball 1988; Ye et al. 2007). In *A. marina*, oil reduced salt secretion by disorganisation of the plasma membranes of the glands (Youssef and Ghanem 2002). Oil probably displaced phytosterols in the plasma membranes of glands leading to disruption of their capacity for salt secretion (Gilfillan et al. 1989; Zhang et al. 2007). Oil accumulation in the vacuoles of salt glands leads to Na⁺ accumulation, dehydration, enzyme denaturation and decreased secretion. In the control treatments, there were no differences in secretion of Na⁺, K⁺, Ca²⁺ and Mg²⁺ between day and night (Table 3). Oiling, however, decreased the secretion of Na⁺, K⁺, Ca²⁺ and Mg²⁺ during the day and night, probably due to oil-damage to the membranes of the salt glands.

Table 3
Salt secretion in leaves of one-year-old *A. marina* seedlings grown in 10% sea water for one year. Seedlings were subjected to 7 days of control and oiling treatments. Secretion was determined in light and darkness. Values are mean ± standard error, n=4. Values with different letters are significantly different at P ≤ 0.05 using unpaired t-tests.

Ion	Light (µmol m ⁻² s ⁻¹)		Dark (µmol m ⁻² s ⁻¹)	
	Control	Oiled	Control	Oiled
Na	0.12±3a	0.07±0.4b	0.16±0.03a	0.06±0.3b
K	0.09±1a	0.04±0.6b	0.13±0.04a	0.04±0.8b
Ca	0.03±0.2a	0.01±0.3b	0.02±0.7a	0.01±0.2b
Mg	0.02±0.3a	0.01±0.2b	0.03±0.4a	0.01±0.2b
Na ⁺ /K ⁺	1.33	1.75	1.23	1.5

Conclusions

This study demonstrated that in *Avicennia marina*, *Bruguiera gymnorhiza* and *Rhizophora mucronata*, the adverse effects of salinity were exacerbated by the presence of oil, resulting in reduced growth in all three species. Salinity and oil in combination decreased plant height, number of leaves, chlorophyll content and CO₂ exchange, with reductions being greater at the higher salinity. In *A. marina*, salinity and oil in combination impaired leaf ultrastructure and the functioning of the salt glands leading to accumulation of Na⁺, decreased K⁺, Ca²⁺ and Mg²⁺ and increased Na⁺/K⁺ ratios. In *A. marina*, oiling reduced secretion of Na⁺, K⁺, Ca²⁺ and Mg²⁺ in light and darkness.

Declarations

- **Ethics approval and consent to participate**

Not applicable

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

"The authors declare that they have no competing interests"

- **Funding**

This research was supported by the National Research Foundation and the University of KwaZulu-Natal (grant number 93560 to G Naidoo).

- **Authors' contributions**

GN and KN contributed equally to the experimental design, data collection, interpretation and discussion of the results and writing of the manuscript.

- **Acknowledgements**

The authors are grateful to A Rajh for technical assistance.

References

Alkio M, Tabuchi, T M, Wang, X, Carmona, CA (2005) Stress responses to polycyclic aromatic hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. *J Exp Bot* 56: 2983-2994

Ball MC (1988) Ecophysiology of mangroves. *Trees* 2: 129-142

Barbier E B (2016) The protective service of mangrove ecosystems: a review of valuation methods. *Mar Pollut Bull* 109: 676–681

Cavalcante R M, Sousa F W, Nascimento R F, Silveira E R, Freire G S S (2009) The impact of urbanization on tropical mangroves (Fortaleza, Brazil): Evidence from PAH distribution in sediments. *J Environ Mgmt* 91: 328-335

Friess DA (2016) Ecosystem services and disservices of mangrove forests: insights from historical colonial observations. *Forests* 7: 183-199

Gilfillan ES, Page DS, Bass AE, Foster JC, Fickett PM, Ellis WG, Rusk S, Brown AC (1989) The use of Na–K ratios in leaf tissues to determine effects of petroleum on salt exclusion in marine halophytes. *Mar Pollut Bull* 20: 272-276

Kang F, Chen D, Gao Y, Zhang Y (2010) Distribution of polycyclic aromatic hydrocarbons in subcellular root tissues of ryegrass (*Lolium multiflorum* Lam.). *BMC Plant Biol* 10: 210-215

Ke L, Zhang C, Wong Y S, Tam N F Y (2011) Dose and accumulative effects of spent lubricating oil on four common mangrove plants in South China. *Ecotoxicol Environ Saf* 7: 55–66

Lewis M, Pryor R, Wilking L (2011) Fate and effects of anthropogenic chemicals in mangrove ecosystems: a review. *Environ Pollut* 180: 345–367

Li F, Zen X, Yang J, Zhou K, Zan Q, Lei A, Tam NFY (2014) Contamination of polycyclic aromatic hydrocarbons (PAHs) in surface sediments and plants of mangrove swamps in Shenzhen, China. *Marine Pollut Bull* 85: 590–596

Naidoo G, Naidoo K (2017) Ultrastructural effects of polycyclic aromatic hydrocarbons in the mangroves *Avicennia marina* and *Rhizophora mucronata*. *Flora* 235: 1-9

Naidoo G, Naidoo Y, Achar, P (2010) Responses of the mangroves *Avicennia marina* and *Bruguiera gymnorhiza* to oil contamination. *Flora* 205: 357-362

Parida AK, Jha B (2010) Salt tolerance mechanisms in mangroves: a review. *Trees* 24: 199-217

Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* 17: 208-212

Saintilan N, Wilson NC, Rogers K, Rajkaran A, Krauss KW (2014) Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global Change Biol* 20: 147-157

Spurr A R (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultra Res* 26: 31-43

Suprayogi B, Murray F (1999) A field experiment of the physical and chemical effects of two oils on mangroves. *Environ Exp Bot* 42: 221-229

Takemura T, Hanagata N, Sugihara K, Baba S, Karube I, Dubinsky Z (2000) Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorhiza*. *Aquat Bot* 68: 15-28

United States Environmental Protection Agency (2008) Polycyclic Aromatic Hydrocarbons (PAHs). Office of Solid Waste, Washington, DC.

Veldkornet D, Rajkaran A, Paul S, Naidoo G (2020) Oil induces chlorophyll deficient propagules in mangroves. *Marine Pollut Bull* 150: 110667

Waisel Y, Eshel A, Agami M (1986) Salt balance of leaves of the mangrove *Avicennia marina*. *Physiol Plant* 67: 67–72

Wang Y, Zhu H, Tam NFY (2014) Effect of a polybrominated diphenyl ether congener (BDE-47) on growth and antioxidative enzymes of two mangrove plant species, *Kandelia obovata* and *Avicennia marina*, in South China. *Marine Pollut Bull* 85: 376-384

Ye Y, Tam NFY (2007) Effects of used lubricating oil on two mangroves *Aegiceras corniculatum* and *Avicennia marina*. *J Environ Sci* 19: 1355-1360

Ye Y, Tam NFY, Lu CY, Wong YS (2005) Effects of salinity on germination, seedling growth and physiology of three salt-secreting mangrove species. *Aquat Bot*: 193-205

Youssef T, Ghanem A (2002) Salt secretion and stomatal behaviour in *Avicennia marina* seedlings fumigated with the volatile fraction of light Arabian crude oil. *Environ Pollut* 116: 215-223

Zhang CG, Leung KK, Wong YS, Tam NFY (2007) Germination, growth and physiological responses of mangrove plant (*Bruguiera gymnorhiza*) to lubricating oil pollution. *Environ Exp Bot* 60: 127-136

Figures

Figure 1

Effects of oil at different salinities (0,10 and 50% seawater) on the concentration of Na⁺ (A), K⁺ (B), Ca²⁺ (C) and Mg²⁺ (D) in *A. marina* after three weeks of oiling treatment, open bars =control, closed bars=oiled. Means ± standard error are given, n = 4. Bars with different letters are significantly different at P ≤ 0.05 using two-way ANOVA and Tukey's multiple comparisons test.

Figure 2

Effects of oil at different salinities (0,10 and 50% seawater) on secretion of Na⁺ (A), K⁺ (B), Ca²⁺ (C) and Mg²⁺ (D) in *A. marina* after three weeks of control and oiling treatments. Other details as for Fig. 1.

Figure 3

TEM of palisade and spongy mesophyll cells in leaf of *A. marina* after three weeks of control and oiling treatments in 10% seawater. (A) Control: note well defined palisade (pc), spongy mesophyll cells (mc), nucleus (arrowhead) and abundance of chloroplasts (arrows). (B) TEM of spongy mesophyll cells (mc) in leaf of *A. marina* from the oiled treatment. Note: displacement of chloroplast from the plasma membrane (arrows), lamellar separation (arrowheads), grana (G) and oil deposits (O).