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Effects of used lubricating oil on two mangroves *Aegiceras corniculatum* and *Avicennia marina*

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Abstract

An outdoor experiment was set up to investigate the effects of used lubricating oil (5 L/m^2) on *Aegiceras corniculatum* Blanco. and *Avicennia marina* (Forsk) Vierh., two salt-excreting mangroves. *A. marina* was more sensitive to used lubricating oil than *A. corniculatum* and canopy-oiling resulted in more direct physical damage and stronger lethal effects than base-oiling. When treated with canopy-oiling, half of *A. corniculatum* plants survived for the whole treatment time (90 d); but, for *A. marina*, high mortality (83%) resulted from canopy-oiling within 3 weeks and no plants survived for 80 d. Base-oiling had no lethal effects on *A. corniculatum* plants even at the termination of this experiment, but 83% of *A. marina* plants died 80 d after treatment. Forty days after canopy-oiling, 93% of *A. corniculatum* leaves fell and no live leaves remained on *A. marina* plants. By the end of the experiment, base-oiling treatment resulted in about 45% of *A. corniculatum* leaves falling, while all *A. marina* leaves and buds were burned to die. Lubricating oil resulted in physiological damage to *A. corniculatum* leaves, including decreases in chlorophyll and carotenoid contents, nitrate reductase, peroxidase and superoxide dismutase activities, and increases in malonaldehyde contents. For both species, oil pollution significantly reduced leaf, root, and total biomass, but did not significantly affect stem biomass. Oil pollution resulted in damage to the xylem vessels of fine roots but not to those of mediate roots.

Key words: used lubricating oil; mangrove; *Aegiceras corniculatum*; *Avicennia marina*

Introduction

Oil pollution attracts great scientific attention because of its universality in marine environments and acute or chronic damage to marine life. Mangroves are biological coastlines in tropical and subtropical areas, and vulnerable to oil pollution because: (1) they are usually distributed near oil production sites and sea-borne movement lanes; (2) they are often located in sheltered sites with low wave energy, so that floating oils settle there with the tide; (3) dense vegetation makes it very difficult to clean up oils soaked into sediments or coating trunks and aboveground roots; (4) their unique anaerobic and waterlogged substrates have slow oil degradation rates and oil residues accumulate and persist for many years; (5) their fine grained sediments are easy to absorb oils, and oils can enter deep mud due to frequent burrowing activities of benthic animals; and (6) many mangroves are near cities and towns with heavy traffic and used lubricating oils often enter mangroves from improper disposal and illegal discharge.

The effects of many oil types, such as No.6 fuel (Proffitt and Devlin, 1998); Kuwait crude oil and North West Shelf Condensate (Suprayogi and Murray, 1999); light Arabian crude oil (Youssef and Ghanem, 2002); south Louisiana crude oil (Teas *et al*., 1987; Proffitt and Devlin, 1998); Arabian light crude, Gippsland light crude and Woodside (Duke *et al*., 1998); Bunker C, light Arabian crude and No.2 fuel oil (Getter *et al*., 1985); fresh and aged Bass Strait crude oil and light Arabian oil (Grant *et al*., 1993), or their weathered products, have been tested on mangrove plants. The effects of lubricating oils on mangroves are poorly understood, despite the fact that their discharge accounts for possibly 40% of all oil inputs to harbours and other coastal waterways (Hoffman, 1985; Proffitt *et al*., 1995). Proffitt *et al*. (1995) reported the effects of fresh lubricating oil on seedlings of *Rhizophora mangle* and *Avicennia germinans*. However, lubricating oils in urban runoff will not be the clean, new material originally used, but will be old oils removed from automobiles, and the effects of used lubricating oils on mangroves have not been documented.

We investigated the effects of used lubricating oil on seedlings of *Aegiceras corniculatum* Blanco. and *Avicennia marina* (Forsk) Vierh., two salt-excreting and pioneer mangroves common on South China Sea shores and especially in the Pearl River Estuary. This outdoor experiment

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aimed to (1) compare the tolerance of these two mangrove species to oil pollution, (2) compare the effects of different oiling methods, (3) explore the growth and physiological responses to oil pollution, and (4) investigate the damage to root structure caused by oil pollution.

1 Materials and methods

1.1 Plants and oils

Mature and uniform propagules of two salt-secreting and pioneer mangrove species, *A. corniculatum* and *A. marina*, were collected in September 2000 from Saikeng Mangrove Forest, a typical mangrove habitat in Hong Kong (22°25′ N, 114°16′ E). For each species, four propagules were planted in one plastic pot (18 cm in diameter and 20 cm in height) with 4 kg loamy-sandy soil from the same forest. The established seedlings were cultured in a greenhouse at the City University of Hong Kong for about 1.5 years before experimental treatments began. Each pot was irrigated with 400 ml artificial seawater with a salinity of 15 ng/L (prepared by dissolving a commercial salt purchased from Instant Ocean, Aquarium Systems, Inc., Mentor, Ohio) every 2 d. Each pot had six draining holes (0.6 cm in diameter) at the bottom so that water was able to drain freely by gravitational force. In February 2002, 12 pots with similarly growing seedlings of each species were selected for the oiling experiment, which was conducted outside the greenhouse.

Used lubricating oils for this experiment were obtained from a garage in Hong Kong where abandoned automobile lubricating oils were collected. The oils contained 0.68 g/g of total hydrocarbons, composed of about 75% and 25% aliphatic and aromatic fractions, respectively.

1.2 Experimental design

For each species, a two-factor experiment with four treatments, each with triplicate pots, was set up to examine the growth, physiological and root anatomical responses to oil pollution at different tidally immersed positions. Two positions, i.e. base or canopy of the potted plants immersed, were employed. Four treatment bins had 15 ng/L artificial seawater added to them: in (A) and (B) up to the level of the plant canopy; and, in (C) and (D) only to cover the plant bases. Oil was poured into the oiling bins (Treatments A and C) on the first day of the experiment, with an oiling quantity of 5 L/m^2 of water area in the bin. This oiling dose (5 L/m^2) was a moderate oiling level used in related reports and was based on oil concentrations in mangrove areas following most oil spill accidents (Proffitt *et al*., 1995; Suprayogi and Murray, 1999; Duke *et al*., 2000). During flooding periods, each pot was placed into its corresponding bin for 2 h/d to simulate tidal action. During ebbing periods, each pot was left on a bench to drain. The water level of each bin was maintained frequently with tap water in order to compensate for the amount of water lost by evaporation. The experiment lasted for 90 d.

1.3 Growth analysis

Survival, standing leaf numbers and maximum leaf areas were recorded on day 0, 20, 40 and 90, respectively. Plants with all leaves and buds dead were considered to be dead. At the end of the experiment, all surviving plants were harvested, and biomass partitioning was determined by separating each individual plant into leaf, stem and root portions, which were washed and dried at 65°C, and then the dry weight measured.

1.4 Biochemical assays

Biochemical parameters were assayed on day 20 only on leaves of *A. corniculatum* because most plants of *A. marina* in Treatment A had died before that time.

Pigment contents were determined using the equation given by Lichtenthaler and Wellburn's method (1983). The method used to determine activities of nitrate reductase (NR) was similar to that described by Ross (1974). The extraction and determination for activities of peroxidase (POX) and superoxide dismutase (SOD), and of the malonaldehyde (MDA) contents were done based on the method described by Liu and Zhang (1994).

1.5 Root structure

Anatomical damage resulting from oil pollution of the roots of both species were determined on plants of Treatments C and D on day 90. Typical transections of live mediate and fine roots were observed with an Environmental Scanning Electron Microscope (Philips XL30 Esem-FEG).

1.6 Statistical analysis

Mean and standard deviation values for the growth and biochemical parameters of three replicates were calculated. One-way ANOVA was performed to test significant differences among treatments for each species. Two-way ANOVA was employed to test interactions between oiling and water level. The Student-Newman-Keuls multiple comparison method was used if any significant difference was found among treatments.

2 Results

2.1 Seedling survival

All unoiled plants of *A. corniculatum* and *A. marina* survived the entire experimental period, while oiling treatments, especially canopy-oiling (Treatment A), decreased survival rates. When treated with canopy-oiling, *A. corniculatum* plant deaths were found 40 d after beginning the experiment and only half of the plants survived for the whole treatment time (Table 1). For *A. marina*, high mortality (83%) resulted from canopy-oiling within 3 weeks, and no plants survived for 80 d. Base-oiling had no lethal effects on *A. corniculatum* plants even by the end of the experiment, but 83% of *A. marina* plants died within 80 d of treatment.

Table 1 Effects of used lubricating oil on survival percentages (%) of *Aegiceras corniculatum* and *Avicennia marina* plants

Treatment	*A. corniculatum*					*A. marina*				
	15 d	20 d	40 d	80 d	90 d	15 d	20 d	40 d	80 d	90 d
A	100	100	83	67	50	100	17	17	0	0
B	100	100	100	100	100	100	100	100	100	100
C	100	100	100	100	100	100	100	100	17	17
D	100	100	100	100	100	100	100	100	100	100

A: oiled seawater level with plant canopy; B: unoiled seawater level with plant canopy; C: oiled seawater level with plant base; D: unoiled seawater level with plant base.

2.2 Leaf number and size

Canopy-oiling caused most *A. corniculatum* leaves to fall while still green, while symptoms of burning appeared but the leaves remained on *A. marina* plants. After 40 d treatment, 93% of *A. corniculatum* leaves fell after canopy-oiling (Treatment A), and no live leaves remained on any *A. marina* plants. There were no significant differences in standing leaf number among the other treatments (Fig.1). At the end of the experiment, base-oiling (Treatment C) had caused about 45% of *A. corniculatum* leaves to fall although all plants had live buds, while all of the leaves and buds of *A. marina* were burned and dead.

The size of standing live leaves decreased due to both canopy- and base-oiling treatments for *A. corniculatum* but changed little for *A. marina* (Table 2).

2.3 Biochemical parameters

Biochemical responses of *A. corniculatum* leaves to used lubricating oil were significant (Table 3). Compared to the respective controls, oiling treatments caused de-

Table 2 Effects of used lubricating oil on maximal leaf area (cm^2) of *Aegiceras corniculatum* and *Avicennia marina*

Treatment	*A. corniculatum*			*A. marina*		
	0 d	40 d	90 d	0 d	40 d	90 d
A	15.91±0.24^a	4.83±3.78^d	ND	12.52±0.88^a	ND	ND
B	16.49±1.58^a	26.09±3.44^a	28.25±3.16^a	12.67±1.20^a	13.09±1.66^a	16.07±0.80^a
C	14.73±2.51^a	15.33±1.90^c	12.46±1.86^c	12.56±0.61^a	15.22±0.94^a	ND
D	15.20±1.39^a	18.40±0.60^b	19.82±0.60^b	11.88±0.19^a	13.33±0.95^a	15.53±0.09^a

A, B, C, D are the same as that in Table 1. ND: no data because there were no live leaves. Means and standard deviations are shown, and means in the same column with different superscript letters are significantly different at a level of 0.05.

Table 3 Effects of used lubricating oil on leaf biochemical parameters of *Aegiceras corniculatum*

Parameter	Treatment			
	A	B	C	D
Chl (mg/g dw)	3.292 ± 0.348^d	4.235 ± 0.045^c	4.797 ± 0.270^b	5.430 ± 0.113^a
Chl-*a*/Chl-*b*	8.175 ± 0.218^c	9.480 ± 0.090^b	9.373 ± 0.333^b	10.473 ± 0.523^a
CAR (mg/g dw)	0.560 ± 0.025^a	0.690 ± 0.023^b	0.715 ± 0.045^b	0.785 ± 0.042^a
NR (nmol NO_2^-/((g·h) dw)	278.5 ± 15.5^c	562.5 ± 10.2^a	193.0 ± 44.0^d	401.8 ± 18.5^b
POD (×10^3 U/g dw)	4.742 ± 0.185^d	5.767 ± 0.603^c	7.212 ± 0.365^b	7.895 ± 0.248^a
SOD (U/g dw)	805.0 ± 5.0^b	875.0 ± 1.7^a	873.3 ± 23.3^a	885.0 ± 11.7^a
MDA (μmol/g dw)	235.75 ± 6.82^b	212.50 ± 1.70^c	270.10 ± 10.73^a	232.73 ± 4.83^b

A, B, C, D are the same as that in Table 1. Means and standard deviations are shown, and means in the same row with different superscript letters are significantly different at a level of 0.05.

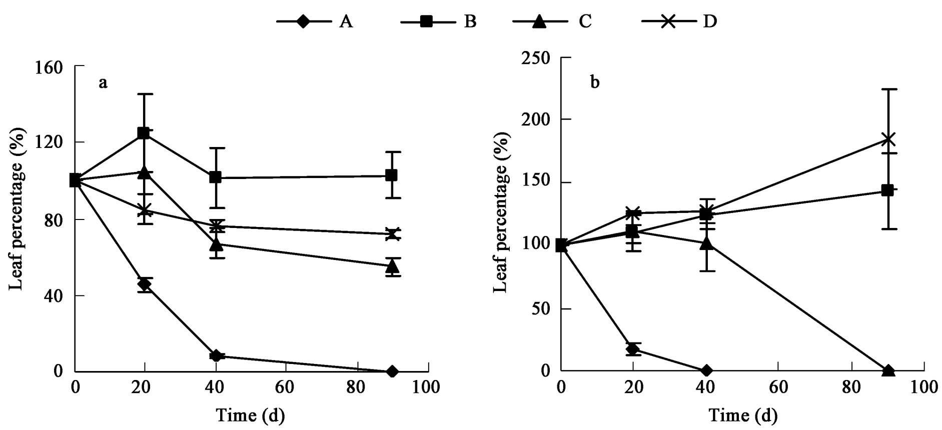

Fig. 1 Effects of used lubricating oil on standing leaf percentages of *Aegiceras corniculatum* (a) and *Avicennia marina* (b). A, B, C, D are the same as that in Table 1.

creases in Chlorophyll content, ratio of Chl-*a* to Chl-*b*, CAR (carotenoid) content, and activities of NR, POD (peroxidase) and SOD, but increases in MDA content. For all of the parameters, no significant interactions between water level and oiling were found (Table 4). Significant differences between canopy and base oiling treatments were found with seawater level but not oiling.

2.4 Biomass

For both species, oil pollution significantly reduced leaf, root, and total biomass significantly, but did not significantly affect stem biomass (Table 5).

2.5 Root structure

Damage by base-oiling was found to the roots of *A. corniculatum* and *A. marina* (Fig.2). The roots of oiled plants were black in colour in contrast to the brownish and whitish root systems in unoiled plants of *A. corniculatum* and *A. marina*, respectively, and a strong oily smell was noted. From scanning electron microscope observation of typical root transections, oil pollution resulted in damage to conducting tissues (xylem vessels) in fine roots but not in mediate roots.

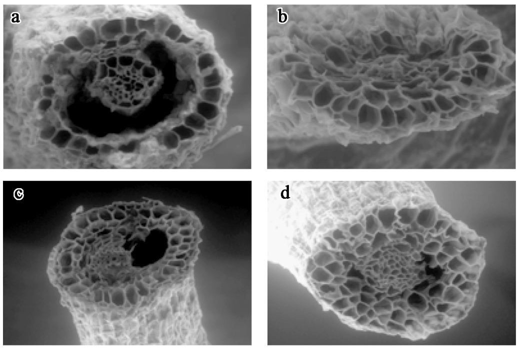

Fig. 2 Effects of used lubricating oil on typical transections of fine roots (Scale: 1:160). (a) *Aegiceras corniculatum* treated with oiled seawater level with plant base; (b) *Aegiceras corniculatum* treated with unoiled seawater level with plant base; (c) *Avicennia marina* treated with oiled seawater level with plant base; (d) *Avicennia marina* treated with unoiled seawater level with plant base.

3 Discussion

Sublethal and lethal effects of used lubricating oil on *A. corniculatum* and *A. marina* were obvious, and were similar to those of other oil types on mangroves (Getter *et al*., 1985; Teas *et al*., 1987; Grant *et al*., 1993; Tam *et al*., 2005). Canopy-oiled plants of *A. corniculatum* and *A. marina* had more rapid mortality than base-oiled ones, indicating that initial and severe toxicity resulted from physical contact of leaves with oils. When oil coats plant leaves, temperature stress may occur, e.g. temperature rises in the leaves because of blocked transpiration pathways (Pezeshki *et al*., 2000), resulting in the burn symptoms to *A. marina* leaves in this study. Another damage of oil fumigation to *A. marina* leaves, reduction of salt secretion, was also reported by Youssef and Ghanem (2002). Oils adhering to leaves due to canopy-oiling did not seem to enter the plants because there were no significant interactions between water level and oiling on biochemical parameters before the leaf defoliation of *A. corniculatum* plants occurred due to oiling. Getter *et al*. (1985) demonstrated that the primary mechanism of toxicity was related to oil entering roots and being drawn up the stems and leaves by transpiration. This may explain the results from our base-oiling treatments whereby biochemical parameters were changed by oiling during the early oiling period (20 d), and much leaf defoliation and root damage were found at the end of this experiment. Destruction of conducting tissues in fine roots will undoubtedly have resulted in the reduction of transpiration, a frequently reported effect of oils on mangroves (Getter *et al*., 1985; Youssef and Ghanem, 2002).

Differences in the effects of oils on mangrove species have been compared mainly between *Rhizophora* and *Avicennia* and the latter was found to be more sensitive to oils than the former. This was attributed to their differences in salt resistance mechanisms (Suprayogi and Murray, 1999). Roots of salt excluding species such as *Rhizophora* can exclude salt and organic compounds including hydrocarbons from oils. On the other hand, roots of salt secreting species such as *Avicennia* can absorb quantities of salt from the sediments and organic matter such as contaminated oils

Table 4 Results of two-way ANOVA for leaf biochemical parameters of *Aegiceras corniculatum*

Variance source	Significance						
	Chlorophyll	Chl-*a*/Chl-*b*	CAR	NR	POD	SOD	MDA
Water level	**	*	*	**	**	*	*
Oiling	*	*	*	**	*	*	*
Water level oiling	NS	NS	NS	NS	NS	NS	NS

* $p < 0.05$, ** $p < 0.01$, NS: not significant.

Table 5 Effects of used lubricating oil on biomass (g/plant) of *Aegiceras corniculatum* and *Avicennia marina*

Species	Treatment	Leaf (g)	Stem (g)	Root (g)	Total (g)
A. corniculatum	C	0.18 ± 0.14^{c}	1.20 ± 0.28^{ab}	1.26 ± 0.23^{b}	2.64 ± 0.63^{c}
	D	1.59 ± 0.36^{a}	1.77 ± 0.38^{a}	2.23 ± 0.61^{a}	5.59 ± 1.34^{a}
A. marina	C	ND	0.92 ± 0.06^{b}	0.65 ± 0.03^{c}	1.57 ± 0.13^{d}
	D	1.08 ± 0.12^{b}	0.97 ± 0.13^{b}	1.74 ± 0.03^{ab}	3.80 ± 0.19^{b}

C, D are the same as that in Table 1. ND: no data because there were no live leaves. Means and standard deviations are shown, and means in the same column with different superscript letters are significantly different at a level of 0.05.

synchronously enter the plants. Both *A. corniculatum* and *A. marina* are salt secreting species, but the ability of *A. marina* to secrete salt is higher than that of *A. corniculatum* (Ye *et al*., 2005). This resulted in the sensitivity of *A. marina* to oils being higher than that of *A. corniculatum* as shown in the present study. Another reason for their difference in sensitivity to oils is that the leaves of these two species have different morphological characteristics. On the dorsal leaf surface of *A. marina* there are dense villi which are prone to oil adherence. Consequently, *A. marina* was considered as the best mangrove species to indicate oil pollution in China's coastal water (Lu and Lin, 1990). This difference can well explain why the canopy-oiled plants of *A. marina* were more sensitive to oils than those of *A. corniculatum*.

In this study, physiologically adverse effects of oils on leaves of *A. corniculatum* were reflected in at least three ways. Firstly, the content of carotenoids (pigments protecting chlorophylls from oxidation) decreased due to oils, resulting in reduction of chlorophyll content. Secondly, the content of MDA significantly increased and the activities of two anti-oxidation enzymes, POX and SOD, significantly decreased due to oils, resulting in lipid peroxidation. Thirdly, activities of NR decreased due to oils, which would result in reduction of N availability. These may be some of the reasons for the growth reduction observed on mangroves treated with oils (Getter *et al*., 1985; Proffit *et al*., 1995; Proffit and Devlin, 1998). Our results showed that these changes in biochemical parameters of *A. corniculatum* were irrespective of oiling position. Youssef and Ghanem (2002) also reported that closed stomata due to fumed oils would prevent these toxic fumes from penetrating the leaves and spreading into the intercellular spaces of the photosynthesizing mesophyll tissue. Like base-oiling, canopy-oiling also resulted in oil contamination in mangrove sediments. Before physical damage due to coating oils were macroscopic, physiological effects occurred, because oils in the sediments entered leaf tissues through transpiration (Getter *et al*., 1985; Proffitt *et al*., 1995), resulting in direct damage due to contact of the organelles with oils, or indirect effects from heavy metals and/or PAHs, usually enriched pollutants in oils, and well known to be harmful to plants (Ke *et al*., 2003; Mishra *et al*., 2006; Rauckyte *et al*., 2006). Furthermore, oiled sediments become poorly oxygenated with strongly reducing conditions (Suprayogi and Murray, 1999), and this will also indirectly affect plant physiology (Ye *et al*., 2003).

4 Conclusions

From this study, we conclude that (1) *A. marina* had a stronger capacity to secrete salts and was thus more sensitive to used lubricating oil than *A. corniculatum*; (2) canopy-oiling resulted in more direct physical damage and had stronger lethal effects on *A. corniculatum* and *A. marina* than base-oiling; (3) used lubricating oil could result in sublethal growth damage including defoliation of *A. corniculatum* leaves, burn symptoms of *A. marina* leaves, and reduction of leaf size of *A. corniculatum*; (4) used lubricating oil could result in physiological damage including decreases in photosynthesis, anti-oxidation and nutrient availability; and (5) used lubricating oil could destroy conducting tissues, especially those in fine roots.

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