INTRODUCTION

Mangroves are intertidal woody communities, common in tropical and subtropical coastal regions. They are one of the most biologically important nature resources on earth, promoting the diversities of terrestrial and aquatic organisms (Lin 1999). Mangrove forests are increasingly impacted by urban and industrial development in the tropical coastal zone, suffering pollution from multiple sources, such as municipal waste, aquaculture, mariculture and shipping as well as onshore industries and urban run-off (e.g. Zheng et al. 1997, Cuong et al. 2005, Vane et al. 2009, Dominguez et al. 2010).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants resulting from incomplete combustion or high-temperature pyrolytic processes, and are thus generated whenever fossil fuels or vegetation are burned. Since these compounds are long lasting, poorly degradable pollutants, they accumulate in soil and sediments, water and the atmosphere (Yang et al. 2000, Zhao et al. 2010), and some of which may exhibit toxic, carcinogenic and mutagenic effects (IARC 2007). Mangrove forests are key ecological habitats that link terrestrial and marine environments. Their unique features, such as high primary productivity, abundant detritus, rich organic matter, and anoxic/reduced conditions, make them a preferential site for uptake and preservation of PAHs from anthropogenic inputs (Bernard et al. 1996, Cavalcante et al. 2010). Elevated concentrations of PAHs have been recorded in mangrove sediments (e.g. Tam et al. 2001, Liu et al. 2005, Medeiros et al. 2005, Ramdine et al. 2012). PAHs are of particular concern because of the persistent and toxic nature of these compounds, leading to adverse effects on mangrove plants and the surrounding ecosystems.

ABSTRACT. – The effect of naphthalene on growth and physiological responses of Kandelia candel (L.) Druce, a dominant species of subtropical mangroves in China, was investigated in sand culture. Root and leaf growth of seedlings were significantly reduced at higher levels of toxicant (1mg/L and above). Stem growth was slightly stimulated at 0.1 and 1 mg/L naphthalene, and was reduced at 10 mg/L toxicant. Chlorophyll content and chlorophyll a/b ratios were increased with increased levels of toxicant. However, the significant declines in net photosynthetic rate were found with increased toxicant exposure. Both transpiration rate and stomatal conductance were stimulated at 0.1 mg/L naphthalene, and were reduced at higher levels of toxicant (1 mg/L and above). It appears that an adverse effect of naphthalene on mangrove K. candel seedlings may be found at the level of 1mg/L and above.

MATERIALS AND METHODS

Experimental set-up: Health and mature propagules of Kandelia candel (L.) Drue, a perforated aluminum pot (14 cm tall and 30cm in diameter) filled with washed river sand. The pot was placed in an aluminum tank containing 1600 mL diluted natural seawater with a salinity of 15 (seawater from the west coast of Xiamen was diluted by tap water). The pots were subjected to four treatments, each in triplicate: control (no naphthalene addition to the culture solution but maintained in every other way the same as the naphthalene treated pots), naphthalene addition to a final concentration of 0.1, 1 and 10 mg/L, respectively. The solid analytical standard naphthalene (Fluka, US) directly added into the culture solution without using any solvent. The culture solution...
was renewed weekly to remain the naphthalene concentration relatively constant during the experimental time. All cultivations were kept in a greenhouse with an air temperature of 25-32 °C for 60 days. None of the PAHs was detected by GC-MS analysis in tap water, seawater and washed river sand used in this experiment.

**Growth analysis:** The stem height of *K. candel* seedlings was measured from the top of the propagule where the stem emerged to the bottom of the most distal opened pairs of leaves. Leaf area was measured by a Portable Area Meter (LI-3000C, LI-COR, USA). After harvested, the number and length of roots emerged from the stem were recorded.

**Photosynthesis and transpiration measurements and contents of pigments in mature leaves:** Photosynthesis and transpiration of mature leaves were measured using a portable photosynthesis system (CIRAS-1, PP system, UK). Ten to fifteen mature leaves for each replicate were randomly selected for the measurement of photosynthetic rate, transpiration rate and stomatal conductance. The measurements were carried out between 9 a.m. and 11 p.m. in the photosynthetically active radiation of 1174 ± 23 µmol m⁻²s⁻¹ and the temperature range of 25-32 °C. Pigment contents were measured following the method of Zhang (1990). About 0.1 g mature leaves was extracted in an ethanol, acetone and H₂O mixture (4.5:4.5:1, v/v/v) in the dark at 4 °C. The absorbance was measured spectrometrically at 645 nm and 663 nm.

**Statistical analyses:** Mean and standard deviation (S.D) values of three replicates were calculated. One-way analysis of variance (ANOVA) was employed to test any difference between naphthalene treatments. If the difference was significant, Tukey multiple comparisons were carried out to determine where the differences were. All statistical analyses were performed using the software, Statistical Package for Social Sciences (SPSS) 13.0 for Window, SPSS Inc., IL, USA.

**RESULTS**

**Growth responses**

Root number, root length, stem height and leaf area of *Kandelia candel* seedlings were measured for evaluating the effect of naphthalene on the plant growth. Root number and root length were not significantly decreased by naphthalene at 0.1 mg/L. A significant decrease by 36.4 % and 54.5 % at 1.0 mg/L and 10 mg/L naphthalene relative to the control seedlings was observed for root number, and by 30.5 % and 86.2% for root length, respectively (Fig. 1). The magnitude of the decrease in root length exceeded that of root number.

Stem height was slightly increased by naphthalene at 0.1 and 1.0 mg/L concentration, but was significantly decreased by 88.5 % at 10 mg/L concentration (Fig. 2).

Leaf area was not significantly affected by naphthalene at 0.1 mg/L concentration, but was significantly decreased by 15.5 % and 83.8 % at 1.0 and 10 mg/L concentration (Fig. 2).

**Chlorophyll content and net photosynthetic rate**

Chlorophyll content and chlorophyll a/b ratios of *K. candel* seedlings were measured (Table I). Chlorophyll a in seedlings at 0.1, 1.0 and 10 mg/L naphthalene was 10.3, 41.4 and 65.5 % significantly higher than that in the control, respectively. Chlorophyll b was not significantly affected by naphthalene at 0.1 and 1.0 mg/L concentration, but was increased by 28.6 % at 10 mg/L. Total chlorophyll was not significantly affected by naphthalene at the lower concentration (0.1 mg/L), but was increased by

Fig. 1. – Effect of different naphthalene concentrations on root number and length of *Kandelia candel* seedlings in sand culture for 60 days.

Fig. 2. – Effect of different naphthalene concentrations on stem height and leaf area of *Kandelia candel* seedlings in sand culture for 60 days.
EFFECT OF NAPHTHALENE ON MANGROVE Kandelia candel

Vie Milieu, 2012, 62 (2)

29.1 % and 53.5 % at 1.0 and 10 mg/L, respectively. It was also found that chlorophyll a/b ratios were significantly increased by 32.5 %, 35.9 % and 27.2 % at 0.1, 1.0 and 10 mg/L naphthalene, respectively.

Net photosynthetic rate decreased gradually as naphthalene concentration increased in sand bed (Fig. 3). Net photosynthetic rate was not significantly decreased by naphthalene at the lower concentration (0.1 mg/L), but was significantly decreased by 34.3 % and 80.8 % at 1.0 mg/L and 10 mg/L naphthalene concentration relative to the control seedlings, respectively.

Transpiration rate and stomatal conductance

Transpiration rate and stomatal conductance of K. candel seedlings followed a similar tendency where they both increased at the lower concentration (0.1 mg/L), and then decreased at the higher naphthalene concentrations (1 and 10 mg/L) (Fig. 4). For example, transpiration rate and stomatal conductance of seedlings increased by 43.6 % and 29.8 % at 0.1 mg/L naphthalene, and then decreased by 34.0 % and 28.6 % at 1 mg/L naphthalene and by 86.2 % and 85.7 % at 10 mg/L naphthalene concentration relative to the control seedlings, respectively.

DISCUSSION

The root and leaf growth of K. candel seedlings were significantly reduced at 1 and 10 mg/L naphthalene (Figs. 1, 2). The stem height was also significantly reduced at 10 mg/L naphthalene. However, naphthalene, at 0.1 and 1 mg/L, was found to stimulate stem growth slightly (Fig. 2), causing an increase in the height of the main stem of mangrove seedlings. This stimulation at low levels of toxicant exposure has been termed as hormesis (Calabrese & Blain 2009). One of the hypotheses that might be applicable to these results is that a plant hormone is aromatic in nature and structurally similar to naphthalene (McCann et al. 2000a). For example, both auxins and gibberellins are known to cause internodal elongation in plants (de Jong et al. 2009) and auxins are known to inhibit root elongation (McCann et al. 2000a).

Chlorophyll content and the chlorophyll a/b ratio are fundamental factors for photosynthetic activity and are often used for detecting and assessing exposure of plants to environmental contaminants (Marwood et al. 2001, Huang et al. 2004). The increase of total chlorophyll content in leaves of K. candel seedlings was induced on exposure to higher levels of toxicant (1 mg/L and above), indicating of a stimulation effect of naphthalene (Table I). The stimulation, coupled with the reduction in growth of root and leaf, indicated that the mangrove seedlings were developing abnormally under stress. Further studies are required to determine the underlying mechanisms for abnormally accumulation of chlorophyll due to the toxicant stress.

A significant increase was also found in chlorophyll a/b ratio over the naphthalene treatment range. It is possibly a potential liability to increase seedling sensitivity to light (Huang et al. 2004). PAHs are known for blocking electron flow from PSII to PSI, resulting in over-saturated PSII reaction centers. This, in turn, induces photochemical oxidation of the light harvesting complexes that bind chlorophyll b (Huang et al. 1997, Marwood et al. 2001). Therefore, an increased chlorophyll a/b ratio can occur when plants are under toxic chemical stress, as was found in the current study.

The increase in chlorophyll content, as a beneficial effect on photosynthesis, did not correspond to greater

Fig. 3. – Effect of different naphthalene concentrations on net photosynthetic rate of Kandelia candel seedlings in sand culture for 60 days.

Fig. 4. – Effect of different naphthalene concentrations on transpiration rate and stomatal conductance of Kandelia candel seedlings in sand culture for 60 days.
photosynthetic rate. The significant declines in net photosynthetic rate were found with increased naphthalene exposure (Fig. 3). The decline in net photosynthetic rate was possibly induced by the damages to the photosynthetic apparatus of mangrove plants, or by the enhancement of respiration under environmental stress. The adverse effect of PAHs on photosynthesis in plants was possibly related to a disruption of the photosynthetic system membranes (Sikkema et al. 1994, McCann et al. 2000b). It also has been reported that PAHs induced oxidative stress, which decreased photosynthesis in plants and evoked increased activities of antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD) (e.g. Liu et al. 2009, Oguntunmisehin et al. 2010, Ahammed et al. 2012).

Both transpiration rate and stomatal conductance in leaves of the mangrove K. candel were reduced at 1 and 10 mg/L naphthalene (Fig. 4). The similar results of mangrove A. marina were also found with exposure to water soluble and volatile fraction of light Arabian crude oil (Youssef & Ghanem 2002). Decreased transpiration rate and increased stomatal resistance could reduce water loss, and concomitantly enhance water holding capacity of the mangrove seedlings. On the other hand, decreased stomatal conductance could also reduce CO₂ input, and subsequently contribute to the declines in net photosynthetic rate. The changes in stomatal behavior were possibly induced by the phytotoxicity of PAHs, or/and served as an adaptive response to fluctuation of leaf water potential (Herppich & von Willert 1995). Further studies are required to determine which mechanisms are mainly responsible for the changes in transpiration rate and stomatal conductance observed.

As shown above, no obvious phytotoxic effect of naphthalene on mangrove K. candel seedlings was observed at level of 0.1 mg/L. When the toxicant level reached at 1mg/L and above, a significant inhibition effect was found on most of the physiological features, such as root and leaf growth, net photosynthetic rate, transpiration rate and stomatal conductance. It appears that a threshold for tolerance of mangrove K. candel seedlings to naphthalene stress may range from 0.1 mg/L to 1 mg/L.

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Table I. – Effect of different naphthalene concentrations on chlorophyll content and chlorophyll a/b ratio of Kandelia candel seedlings in sand culture for 60 days.

<table>
<thead>
<tr>
<th>Naphthalene (mg/L)</th>
<th>Chlorophyll a (mg/g)</th>
<th>Chlorophyll b (mg/g)</th>
<th>Total chlorophyll (mg/g)</th>
<th>Chlorophyll a/b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(CK)</td>
<td>0.58 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.86 ± 0.006</td>
<td>2.06 ± 0.23</td>
</tr>
<tr>
<td>0.1</td>
<td>0.64 ± 0.006*</td>
<td>0.23 ± 0.006</td>
<td>0.87 ± 0.01</td>
<td>2.73 ± 0.06*</td>
</tr>
<tr>
<td>1</td>
<td>0.82 ± 0.01*</td>
<td>0.29 ± 0.006</td>
<td>1.11 ± 0.02*</td>
<td>2.80 ± 0.03*</td>
</tr>
<tr>
<td>10</td>
<td>0.96 ± 0.006*</td>
<td>0.36 ± 0.006*</td>
<td>1.32 ± 0.01*</td>
<td>2.62 ± 0.03*</td>
</tr>
</tbody>
</table>

* Significant difference between control and naphthalene treatments at p < 0.05.

REFERENCES


Vie Milieu, 2012, 62 (2)
EFFECT OF NAPHTHALENE ON MANGROVE KANDELIA CANDEL


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