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Effects of polycyclic aromatic hydrocarbons exposure on antioxidant system activities and proline content in *Kandelia candel*

Hui Song[1, 2], You-Shao Wang[1, 2,*], Cui-Ci Sun[1, 2], Mei-Lin Wu[1,2], Ya-Lan Peng[1, 2], Chao Deng[1, 2], Qian P. Li[3]

[1]*State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China*
[2]*Marine Biology Research Station at Daya Bay, Chinese Academy of Sciences, Shenzhen 518121, China*
[3]*Scripps Institution of Oceanography, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA*

Key words: Mangrove plant, *Kandelia candel*, PAH stress; antioxidant enzymes, MDA, proline

Abstract

The antioxidant system effects of *Kandelia candel* were investigated under four different levels of PAH stress. The activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), the responses to the change of malondialdehyde (MDA) contents and the accumulation of proline in *K. candel* were determined. Our results suggested that the activities of SOD, CAT, POD increased significantly in leaves and roots of *K. candel* ($p \leq 0.05$) with the increase of the external PAH concentrations, while in stems, the activities of these antioxidant enzymes were all significantly inhibited ($p \leq 0.01$). We also observed an increase of MDA in leaves, stems and roots, and an obvious correlation between MDA content and PAH concentrations in three locations, which showed that the change of MDA content could be used as a biomarker of *K. candel* under PAH stress. The proline content was found remarkably enhanced in leaves, stems and roots. However, a significant inverse correlation was observed between the proline content and SOD ($r=-0.99$, $p \leq 0.01$), POD ($r=-0.95$, $p \leq 0.05$) activities in stems. This study suggested that the antioxidative system of *K. candel* has an obvious organ-dependent feature when exposed to PAH contamination as revealed by discriminant analysis (DA).

INTRODUCTION

Mangrove ecosystems, important components of intertidal estuarine wetlands, are subjected to dual contaminations from both the land and the ocean. Anthropogenic inputs of PAH from oil spills, shipping traffic, urban runoff, wastewater and industrial discharge, as well as atmospheric fallout of vehicle exhaust have led to its significant accumulations in marine environments (Zheng et al. 2002; Zhang et al. 2004; Olsen et al. 2007). The unique features of mangrove, such as high primary productivity, high detritus abundance and organic carbon abundance, make it easier for PAH retention and accumulation. Tam et al. (2001) showed that the concentration of PAH in the mangrove wetland of Hong Kong was 2-10 times higher than in marine bottom sediments. PAHs, which are well known for their persistency, carcinogenesis and mutagenicity, have posed a great threat to both mangrove ecosystems and humans through affecting the nutrient cycles and food webs (Bayen et al. 2005).

Previous studies on PAH contamination in the mangrove systems are generally focused on the sources and types of the pollutants and their spatial distributions (Tam et al. 2001; Zhang et al. 2004; Bayen et al. 2005; Ke et al.2005; Tam 2006). A large number of studies also focused on the potential biodegradation of microbes isolated in the mangrove

* Corresponding author: *yswang@scsio.ac.cn*; Tel/Fax: 8620-89023102(O)

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sediments, and also there is much information about the microbial degradation characteristics of different consortia in relation to different PAHs (Yu et al. 2005a; Yu et al. 2005b; Chen et al.2008; Tian et al. 2008; Zhou et al. 2008; Ke et al. 2009). Responses of mangrove plants to PAH stress in terms of their growth and morphological transmutation have been well addressed (Dutrieux et al. 1990; Klekowski et al. 1994; Duke and Watkinson 2002; Ke et al. 2003a; Ke et al. 2003b), though little is known about their antioxidant system and physiological responses. Toxicity of PAH to plants is directly manifested by their physiological and biochemical behaviors, which can instead serve as a sensitive indicator of PAH contaminant. External environmental stresses could break the balance of plant metabolism and result in the production of reactive oxygen species (ROS) (Ledford and Niyogi 2005). The enhanced production of ROS can pose a threat to cells and therefore ROS content is often used as a symptom of activation of stress-response and defense pathways in plants. Excessive ROS would lead to membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage (Mittler 2002). In order to adapt to the oxidative stress, plants developed defense strategies against oxidative damage by the induction of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) (Mittler 2002; Xin et al. 2009; Liu et al. 2009). The efficient antioxidant defense system provided by proline, betaine, sugars and polyols played an important role in the tolerance of plants to the environmental stress (Hare and Cress 1997; Zhang et al. 2007b). As far as mangrove plants are concerned, a large number of studies have investigated their antioxidant responses to salt stress, waterlogging, oil pollution and heavy metal stress (Liao and Chen 2007; Yong and Tam 2007; Zhang et al. 2007b). However, there are very few studies that addressed the effects of PAH on the changes of enzyme systems and the damage of cell membrane of aquatic macrophytes (McCann et al. 2000; McCann and Solomon 2000; Liu et al. 2002). We still know very little about the oxidative stress of the mangrove plants under PAH stress. It is therefore very necessary to investigate the responses of the antioxidant system of the mangrove plants to PAH treatment, so as to protect and optimize the mangrove ecosystem.

The present study therefore aims at examining the effects of PAH on the antioxidant system of mangrove plants. Pyrene (four-ring) and p-terphenyl (tricyclic) were chosen as the typical PAH according to Tam et al. (2001), which suggested that three and four-ring PAH were dominant in mangrove sediments. With the two types of PAH treatments after two months, we measured the levels of antioxidant enzymes of SOD, CAT, POD and the contents of antioxidants of proline and MDA in a leaf, a stem and a root of mangrove plants of *K. candel* (a dominant mangrove species along the South China coast). The physiology of plants and the multiple factors of their cultivation environment are very dynamic (Landis et al. 1994, Rodríguez-Ortega et al. 2009), so a proper statistical analysis is necessary to explain the complex relationships and extract accurate information from the biological system (Sinha et al. 2009). In this study we applied the analysis of variance (ANOVA), correlation analysis and discriminant analysis to investigate the responses of the antioxidant system of *K. candel* to PAH stress, as to identify the potential biomarkers for PAH contamination.

MATERIALS AND METHODS

PAH treatment and plant growth

The PAH-contamination was artificially prepared according to the modified method of Liu et al. (2007). One control and four levels of PAH-contaminations were defined as follows: free PAH, 1PAH level of the effects range-median of marine water quality standards of pyrene (ERM, 2600ppb dry wt) (Long et al. 1995), 5PAH (13000ppb dry wt), 10 PAH (26000ppb dry wt) and 15PAH (39000ppb dry wt). The mixture of pyrene and p-terphenyl (1:1) dissolved in acetone was first added to 1 kg sand, which was PAH-free and air-dried. The sand was mixed thoroughly and then sat for 5 days to ensure the evaporation of acetone. The PAH-spiked sand was then homogeneously mixed with 8 kg uncontaminated sand to obtain the four different levels of concentration. After 15 days, the sand sample was equally divided into three plastic pots, which were used to plant the seedlings of *K. candel.*

Viviparous seeds of *K. candel* are collected from Dongchong of Daya Bay in Guangdong Province, China. Seeds were planted in free-PAH plastic pots washed with sterilized water. These plants were kept in a greenhouse at the temperature of 25 ±5°C and the light intensity of 480 μmol m^{-2} s^{-1} from natural sunlight. Each spot was irrigated with 0.50 l liquid

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fertilizer (half strength Hoagland's solution with 10‰ NaCl) every 3 days. After two leaves had been developed, the seedlings were divided into five groups, which has been pretreated (three replicates for each group, each replicate has 5 plants). After two months, leaves, stems and roots were harvested for the analysis of the antioxidant parameters.

Enzymes extraction and assays

All biochemical analyses were performed at 4°C, 0.5 g of fresh leaves, stems and roots were extracted in 5 ml of 50 mmol l^{-1} phosphate buffer (pH = 7.8) containing 1.0 mmol l^{-1} EDTA and 4% (w/v) polyvinylpyrrolidone (PVPP) to neutralize the interference effects of phenol in mangrove plants tissues. The homogenates were centrifuged at 3000 g for 15 min., and the supernatant was used for the enzymatic assays. Protein concentrations in the extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

SOD activity was determined by the method of photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971) with minor modifications. The 3 ml reaction mixture contained 50 mmol l^{-1} phosphate buffer (pH 7.8), 0.1 mmol l^{-1} EDTA, 14.5 mmol l^{-1} methionine, 60 μmol l^{-1} riboflavin, 2.25 mmol l^{-1} NBT, and 50 μl supernatant. The reaction was initiated under illumination of 72 μmol m^{-2} s^{-1} for 10 minutes. Non-illuminated/ illuminated reaction mixtures without supernatant served as calibration standards. The photoreduction of NBT was measured at A_{560}. One unit of SOD was calculated as 50% inhibition of the maximum photo-reduction of NBT, and SOD activity was expressed as SOD units per mg of protein.

Catalase (CAT) activity was quantified by measuring the rate of H_2O_2 disappearance at 240 nm (Chen and Wang 2006). The reaction mixture (3 ml) consisted of 1.95 ml 50 mmol l^{-1} phosphate buffer (pH 7.0), 1 ml 0.3% H_2O_2 and 0.05 ml enzyme extract. The reaction was initiated by addition of the extraction and recorded every 30 s for 2 min. One unit activity was defined as an absorbance change of 0.001 unit min^{-1} mg^{-1}.

Peroxidase (POD) was measured spectrophotometrically in a 3 ml mixture containing 1 ml 0.3% H_2O_2, 0.95 ml guaiacol, 1 ml pH 7.0 phosphate buffer, and started with the 0.05 ml enzyme extraction (Chen and Wang 2006). In the presence of H_2O_2, POD catalyzes the transformation of guaiacol to tetraguaiacol (brown product). The POD activity was measured by monitoring the increase in absorbance at 470 nm (the extinction coefficient of 26.6 mM^{-1} cm^{-1}) for 2 min. The unit of POD activity was expressed as POD units per min and mg of protein.

Determination of lipid peroxidation and proline

Malonaldehyde (MDA) was measured by the method of bi-component spectrophotometry to eliminate the interference of the dissolvable salt (D-salt). The absorbance of supernatant was recorded at 532 nm and 450 nm (Zhao et al. 1994). The reaction products of MDA-TBA and D-salt-TBA usually show their absorption peaks at 532 nm and 450 nm. According to Lambert-Beer's law, the extinction coefficient of D-salt-TBA is therefore 85.4 mM^{-1} cm^{-1} at 450 nm and 7.40 mM^{-1} cm^{-1}at 532 nm. The coefficient of MDA-TBA is 0.56 at 450 nm and 15500 mM^{-1} cm^{-1} at 532 nm. The amount of MDA-TBA complex, expressed as μmol g^{-1} fresh weight, could thus be calculated as:

$$C_{MDA} = 6.45 \times OD_{532} - 0.56OD_{450}$$

To determine the amount of free proline, 0.5 g of leaf, stem, root samples from each group were homogenized in 3% (w/v) sulfosalicylic acid; the homogenate was heated for 10 min. at 100°C; the mixture was centrifuged at 4500 g for 10 min. after cooling; the content of free proline in the supernatant was measured using ninhydrin at 520 nm and expressed as μg g^{-1} fresh weight (Zhang et al. 1990).

Statistical analysis

All measurements were carried out in four replicates from two independent experiments (n=8). All data were subjected to one-way analysis of variance (ANOVA), p values ≤0.05 and p values ≤0.01 were considered as significant. In addition, a comparison was made with Pearson's correlation for each antioxidant, enzyme and PAH treatment level. The correlation analysis was tested between various antioxidant enzymes and/or non-enzymatic (MDA and proline content) in three parts of the plant. The changes of various indices in three organs were analyzed with the discriminant analysis (DA). All analyses were carried out with SPSS 10.0.

RESULTS

Superoxide dismutase activity

Except for the treatment 5PAH, our results (Fig. 1) generally show a significant increase in SOD activity with the increased PAH concentrations in leaves and roots ($p \leq 0.01$). However, inhibition of the SOD activity in stems was found under 5PAH and 10PAH treatments. Compared to the control, 50% reduction in the SOD activity was found for these two treatments, but no statistical differences in stems was found for 1PAH and 15PAH treatments.

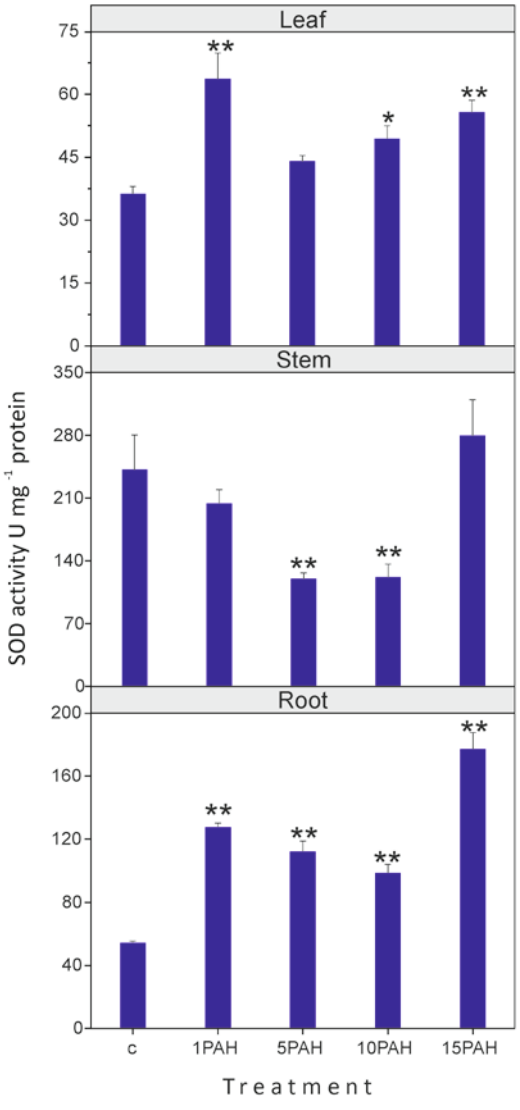


Fig. 1. Changes of SOD activity (U mg^{-1} protein) in leaves, stems and roots of *K. candel*. Results are the means ±S.E., n=8. * and ** indicate that a statistical difference is at $p<0.05$, $p<0.01$ respectively.

Catalase activity

Under four different treatments, the CAT activity in leaves and roots generally increased with PAH concentrations ($p \leq 0.01$), but showed a peak at 10PAH and 15PAH, respectively (Fig. 2). Compared with the control, the CAT activity of stems in 1PAH treatment increased to approximately 234%, no variation of CAT activity was found for stems under the treatments of 5PAH, but that in 10PAH and 15 PAH treatment decreased to 34% and 52% of the control.

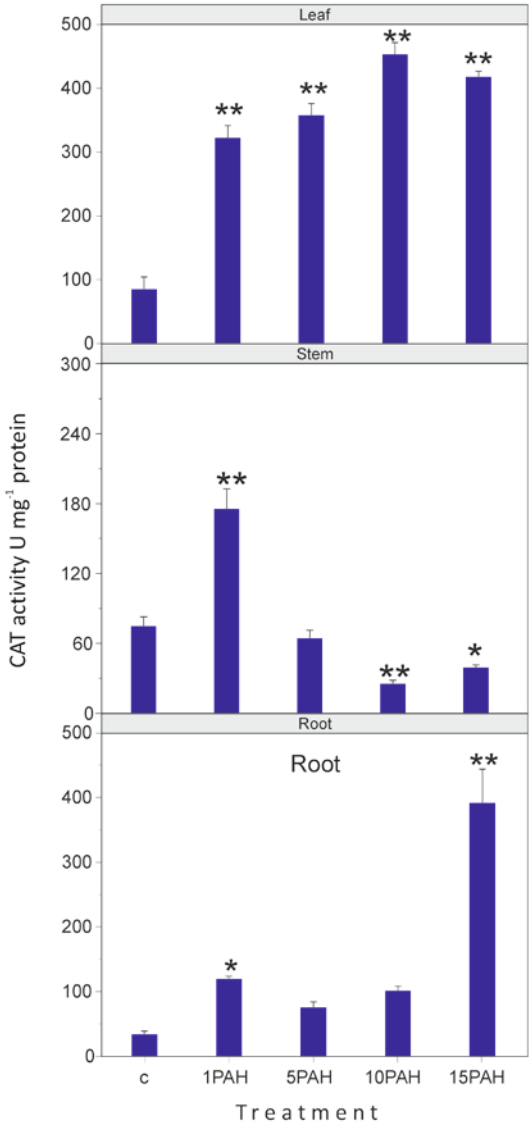


Fig. 2. Changes of CAT activity (U mg^{-1} protein) in leaves, stems and roots of *K. candel*. Results are the means ±S.E., n=8. * and ** indicate that a statistical difference is at $p<0.05$, $p<0.01$ respectively.

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Peroxidase activity

Fig. 3 shows that POD activity in roots of *K. candel* was generally enhanced when exposed to four different PAH levels ($p \leq 0.05$) with the most remarkable increase found from 5PAH to 15PAH ($p \leq 0.01$). However, the changes of POD activity in leaves and stems were not monotonous. POD activity was significantly decreased in stems under 5 PAH and 10PAH treatments ($p \leq 0.01$), but increased under 15PAH stress. In leaves, POD activity showed a decrease at 1PAH and an increase at 10PAH, but no alterations at 5PAH and 15PAH when compared with the control.

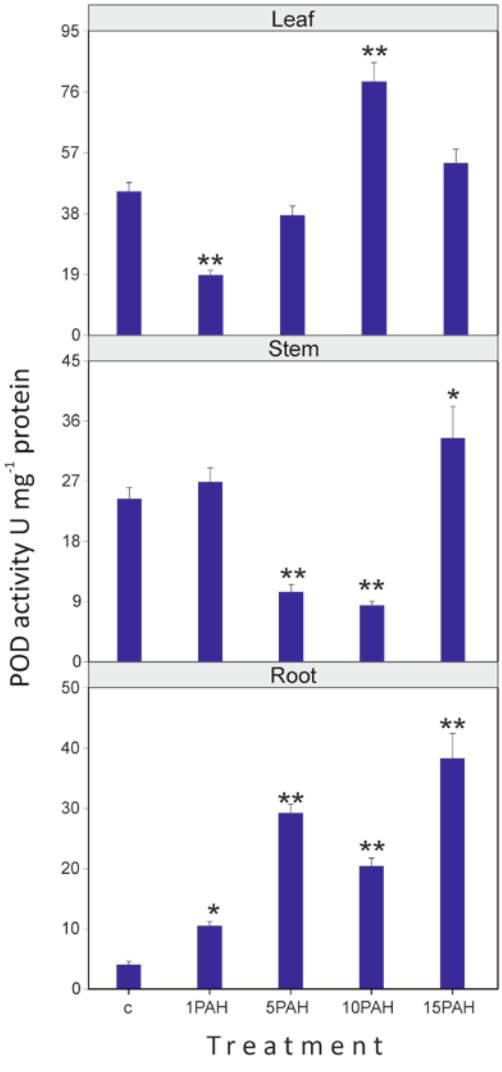


Fig. 3. Changes of POD activity (U mg^{-1} protein) in leaves, stems and roots of *K. candel*. Results are the means ±S.E., n=8. * and ** indicate that a statistical difference is at p<0.05, P<0.01 respectively.

Lipid peroxidation products

Lipid peroxidation in *K. candel* was estimated by determining the concentration of MDA, as presented in Fig. 4. Generally, the content of MDA increased with the amount of PAH. A significant increase of MDA was found from 5PAH in a root, from 10PAH in a stem, but only at 15PAH in a leaf.

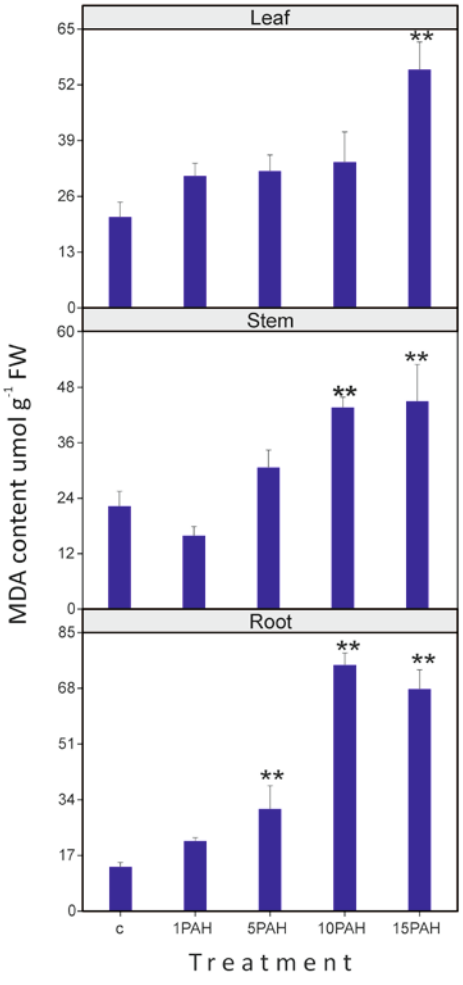


Fig. 4. Changes of MDA content (µmol g^{-1} FW) in leaves, stems and roots of *K. candel*. Results are the means ±S.E., n=8. * and ** indicate that a statistical difference between the control is at p<0.05, p<0.01 respectively.

Proline content

The proline content in leaves and roots increased significantly ($p \leq 0.01$) as presented in Fig. 5. The concentration of proline in stems first increased under 5PAH and 10PAH and then decreased to the control level at 15PAH.

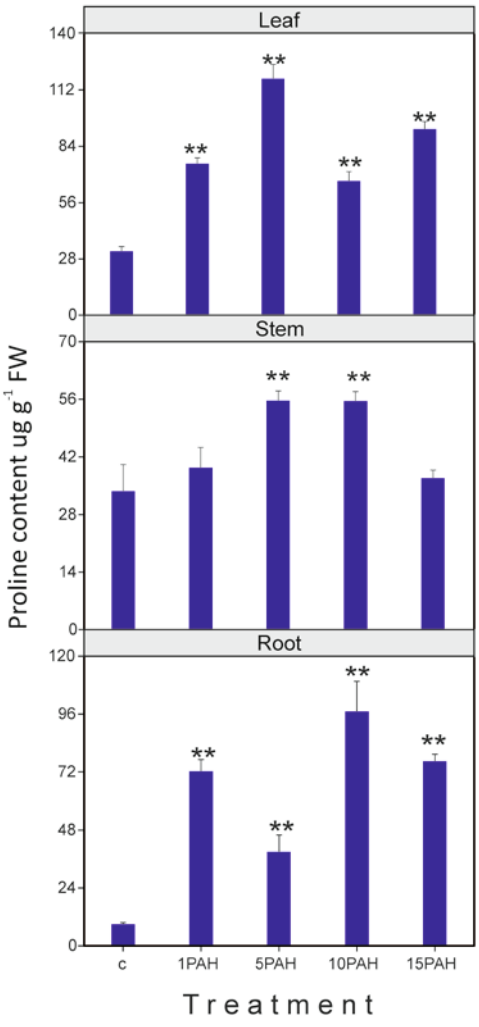


Fig. 5. Changes of the proline content (μg g^{-1} FW) in leaves, stems and roots of *K. candel*.

The relationship between antioxidant enzymes, the content of antioxidants and the PAH treatment levels.

The analysis of Pearson's correlation coefficients indicated that only the content of MDA showed a clear correlation with the PAH concentration (Table 1). With the partial correlation analysis as presented in Table 2, we found high negative correlations between the proline content and the activities of SOD and POD in stems. A high negative correlation was also found between the content of MDA and POD activities in leaves. In order to further study the effect of PAH stress on different organs, DA analysis was performed as presented in Fig. 6, where the data were divided into three parts and assigned 77.5% of cases correctly.

Table 1

Correlation coefficients (r) between the monitoring parameters and PAH treatment levels in leaves, stems and roots.

Index	Leaf		Stem		Root	
	r	p	r	p	r	p
SOD	0.26	0.67	0.12	0.84	0.71	0.18
CAT	0.76	0.14	-0.68	0.21	0.81	0.09
POD	0.63	0.25	0.08	0.89	0.86	0.06
MDA	0.9	0.04	0.94	0.02	0.93	0.03
Pro	0.42	0.48	0.21	0.74	0.64	0.24

Table 2

Partial correlation coefficients between antioxidant and enzymes activity in leaves, stems and roots. The data were presented in the form of r (p).

Index	SOD	CAT	POD	MDA	Proline
Leaf					
SOD	1.00				
CAT	0.60(0.40)	1.00			
POD	-0.62(0.38)	-0.17(0.83)	1.00		
MDA	0.64(0.36)	-0.01(0.99)	-0.95(0.05)	1.00	
Proline	0.26(0.74)	0.59(0.41)	-0.61(0.39)	0.35(0.65)	1.00
Stem					
SOD	1.00				
CAT	0.30(0.70)	1.00			
POD	0.96 (0.04)	-0..55(0.45)	1.00		
MDA	-0.55(0.45)	-0.93(0.07)	-0.76(0.24)	1.00	
Proline	-0.99(0.00)	-0.31(0.69)	-0.95(0.05)	0.53(0.47)	1.00
Root					
SOD	1.00				
CAT	0.77(0.23)	1.00			
POD	0.50(0.49)	0.16(0.84)	1.00		
MDA	-0.52(0.48)	0.72(0.28)	-0.63(0.37)	1.00	
Proline	0.27(0.73)	-0.12(0.88)	-0.32(0.68)	0.68(0.32)	1.00

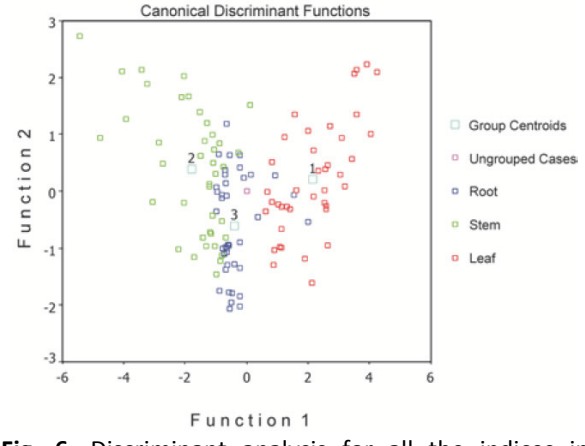


Fig. 6. Discriminant analysis for all the indices in leaves, stems and roots.

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DISCUSSIONS

Previous studies have suggested that PAH were cytotoxic and mutagenic involving in the formation of reactive oxygen species (ROS) in plant tissues (Flowers-Geary et al. 1996). ROS, including superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-) and singlet oxygen (O_2), were responsible for the damage of membrane lipids, proteins and DNA in plants. Their accumulation would result in irreparable metabolic dysfunction, tissues injury and cell death (Imlay and Linn 1988). Higher plants, however, have developed an extensive range of defense systems to counteract the attacks of ROS in cellular compartments including enzymatic and non-enzymatic components (Jaleel et al. 2009). The antioxidant enzymes can directly scavenge ROS, SOD can catalyze O^{2-} into H_2O_2, then CAT and POD catalyze the reduction of H_2O_2. The enhancement of defense systems showed the ability of plants to protect themselves from the damage associated with oxidative stress (Fridovich 1986, Willekens et al. 1997, Lin and Kao 2000). Our results showed also that the antioxidative enzymes of *K. candel* were remarkably enhanced in leaves and roots with the increase of PAH in the sediments. These findings suggested that the oxidative stress in *K. candel* might be caused by PAH, similar to those studies on the response of antioxidant enzymes of mangrove species to salt stress, waterlogging, oil pollution and heavy metal stress (Liao and Chen 2007, Yong and Tam 2007, Zhang et al. 2007a,b).

SOD constitutes the first line of defense against ROS (Alscher et al. 2002). Liu et al. (2009) reported that PAH can cause oxidative stress in *Arabidopsis thaliana* and increase the enzyme activities including SOD, CAT, POD and ascorbate peroxidase (APX). SOD activities in *K. candel* also increased significantly in roots with the increase of PAH ($p \leq 0.01$). Except for the 5PAH treatment, SOD activity in leaves was also significantly enhanced ($p \leq 0.01$, 10PAH is at $p \leq 0.05$), which indicates that *K. candel* has a sensitive protective mechanism to scavenge O^{2-} from cellular oxidative damage in a leaf and a root. While in a stem, the SOD activity was inhibited except for the 15PAH treatment, so the antioxidant enzyme was not involved in a stem, the defense machinery may be different from that of a leaf and a root. This result is in accordance with the theory of Gill and Tuteja (2010) saying that with the varied subcellular location a different defense system was activated to counteract the oxidative stress.

As it is a byproduct of SOD preventing the cellular damage, H_2O_2 can be eliminated by CAT, POD, APX and glutathione peroxidase (GPX) (Mittler 2002). Our results showed that the variation patterns of CAT and POD activities in leaves and roots were consistent with that of SOD, which is generally in agreement with those responses of *Arabidopsis thaliana* under PAH stress (Liu et al. 2009). Therefore, the enhancement of POD and CAT activities in leaves could be a necessary mechanism for plants to scavenge H_2O_2 in peroxisomes and cytosol where H_2O_2 might have diffused from chloroplasts as a result of the enhanced SOD activity (Li et al. 2008). Our study showed a differential response of antioxidative enzymes under PAH stress in roots and leaves. CAT obviously increased in leaves with all PAH treatments, but enhanced only at 5PAH and 15PAH in roots. In contrast, POD showed a general increase in roots, but enhanced only at 10PAH in leaves. These results suggested the coupling of POD and CAT in plants in order to detoxify the stress of H_2O_2 and also suggested that different organs may activate different enzymes. High CAT and POD activities showed at 10PAH and 15PAH in leaves and roots respectively should suggest that the defense system of mangrove was strengthened in response to the enhancement of abiotic stress in order to adapt to the physical environments and to reduce the damage. These data are consistent with researches on behaviors of other plants under oxidative stress (Mittler 2002, Wu et al. 2009).

SOD activity in stems was inhibited under 5PAH and 10PAH treatment ($p \leq 0.01$), which may be due to the accumulation of O^{2-} that suppressed the SOD activities as the defense system in stems didn't have the ability to resist the oxidative stress (Candan and Tarhan 2003). Though no significant inhibition effect for SOD in stems was found at 1PAH and 15PAH, its activity decreased compared to the control. This result implied that PAH stress could interfere with the enzyme systems in stems. Comparing the trends of the responses of SOD, POD and CAT in stems in Fig. 1-3, we can find that all the activities of antioxidant enzymes were inhibited significantly. As indicated by the data in Table 2, there was a strong positive correlation between SOD and POD ($r=0.96$, $p<0.05$) in stems, which suggested that the antioxidant enzymatic system in stems are damaged during PAH treatments. The content of MDA in stems increased significantly due to the inhibition of enzyme protection mechanisms, further suggested

that the defense mechanisms cannot detoxify the damage of ROS and cause membrane peroxidation. This result was in agreement with the reaction of *Sonneratia apetala* with salt stress (Liao and Chen 2007). The content of proline in stems increased with the increased PAH stress and showed negative correlations with the activities of SOD and CAT (see Table 2), which are in agreement with those reported by Singh et al. (2004). The complementary action of enzymatic and non-enzymatic antioxidant systems in stems may support the argument that different plant organs might activate different defensive systems to avoid excessive ROS (Li et al. 2008), and the antioxidant system may have organ-specific feature. This hypothesis was further supported by our results from the discriminant analysis (DA) as shown in Fig. 6. Out results (with the data divided into three parts and 77.5% cases assigned correctly) suggested that all five parameters of *K. candel* under PAH-treatment showed a clear organ-specific feature. This finding is in agreement with the previous study of *Pistia stratiotes* L. under chromium stress (Sinha et al. 2009).

MDA, a decompression product of polyunsaturated fatty acids hydroperoxides, is regularly used as a biomarker for lipid peroxidation (Bailly et al. 1996). Only in leaves at 15PAH treatment, the content of MDA was statistically enhanced, indicating that the antioxidant enzymes cannot detoxify the oxidative stress effectively at higher level PAH stress and thus lead to membrane damage. The content of MDA increased significantly at 5PAH in roots, while at 15PAH in leaves. This result suggested that the defense mechanism of leaves was more effective than that of roots. This may be due to the fact that the root was in the direct position exposed to the PAH stress and it was also the main PAH accumulation organ (Gao and Zhu 2004), so the injury was the most severe. It is similar to changes of biomass in lettuce and radish plants under ANT treatment (Wieczorek J.K. and Wieczorek Z.J. 2007). We also found that a stem was the least sensitive organ in resisting PAH stress, which is consistent with the findings of Li et al. (2008) on the responses of *Dendrobium candidum* to sound wave stress. The correlation analysis in Table 1 indicated that only the content of MDA in leaves, stems and roots showed a generally positive correlation with the increased PAH concentrations ($r=0.90$, $r=0.94$, $r=0.93$, $p\leq0.05$). These results suggested that MDA is a parameter sensitive to the abiotic stress, which may serve as a good indicator of PAH stress. This finding corresponds well with the previous reports of *Bruguiera gymnorrhiza* under heavy metal stress (Zhang et al. 2007).

It is generally believed that proline plays an important role in osmoregulation, protection of enzyme denaturation, regulation of cytosolic acidity (Alia and Matysik 2001). On the other hand, proline was found to reduce the production of the singlet oxygen ($^1O_2$) (Saradhi et al. 1995, Alia et al. 2001). Significant accumulations ($p\leq0.01$) of proline were found both in leaves and roots under all treatments, though it increased only at 5PAH and 10PAH for stems accompanied by the decrease of SOD, POD and CAT activities. The negative correlation between the proline content and the activities of SOD and POD in stems (Table 2) suggested that accumulation of proline may relieve the damage by the stress, and compensate the inhibition of the antioxidant enzymes in stems (Parida et al. 2002, Singh et al. 2004).

In order to further analyze the complex relationship between the antioxidative enzymes and antioxidants, partial correlation analysis was conducted between the antioxidant and/or antioxidative enzymes (Chiang and Lin 2000). As presented in Table 2, the POD activities correlated negatively with the content of MDA in a leaf ($r=-0.9546$, $p=0.05$), which indicated that POD plays an important role in scavenging ROS and thus insufficient enhancement of POD may cause the increase of lipid membrane damage. This finding is consistent with the finding from *Cucumis sativus* L. when exposed to fungicide carbendazim and *Mentha pulegium* under Mg^{2+} deficiency (Candan and Tarhan 2003, Zhang et al. 2007c). POD and SOD activities in stems were positively correlated, but both of them showed negative correlations with the content of proline in stems. In general, we didn't find a significant correlation between the antioxidant enzymes and/or antioxidants in leaves and roots. These results may support the argument that the defensive mechanisms of plants to the abiotic stress might be different in different organs (Li et al. 2008). The covariation of proline with the inhibition effects of the two enzymes in stems may suggest that other defense mechanisms in plants may be involved when exposed to PAH stress, similar to the responses of *Vicia faba* under UV treatment (Shetty et al. 2002, Li et al. 2008). The correlation analysis as shown in Table 1 further supported the thesis that the defense ability in different organs may be different. In the root, the correlation coefficient was much higher than in the leaf and in the stem at a lower

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significance level, though the antioxidant system was enhanced in the root at low PAH treatment. The root was even the most severely damaged point as MDA indicated. As the research reported the root was the organ which is exposed to PAH directly, this may be affected by changes in nutrient transport and the pattern of assimilate distribution within a plant, as induced by stressors (Wieczorek J.K. and Wieczorek Z.J. 2007).

CONCLUSIONS

The present study clearly shows that *K. candel* has a sensitive antioxidant defense mechanism under PAH treatment. Compared to roots and stems, the enzymatic- antioxidant system in leaves is the most effective one, while the system in stems is the least effective one and shows an obvious organ-dependent feature. Our data with the correlation analysis suggested that MDA is the most sensitive index under PAH stress (Table 1) and thus it may be a valuable biomarker of *K. candel* for PAH contamination. Because the mangrove wetland is a complex environment, our preliminary studies focus here just on an ideal unitary PAH-contaminant environment. Further field researches are needed to verify these results and to establish an effective model to identify the PAH contaminant level of the mangrove wetland through the biochemical indices of the mangrove plants.

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