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Oxidative stress indices in natural populations of *Avicennia alba* Blume. as biomarker of environmental pollution

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ABSTRACT

Effects of multiple pollutants including heavy metal on oxidative stress indices were measured in leaves and roots of *Avicennia alba* Blume. collected from three coastal locations in Kerala—Cochin, Kollam and Chetua. We observed significant activities in lipid peroxidation, root oxidizability, electrolyte leakage and antioxidant enzymes. Conversely, ascorbate and reduced glutathione showed low levels suggesting that these may be serving as a biomarker of heavy metals for monitoring pollution.

1. Introduction

Mangrove ecosystem possessing great ecological and commercial value, are diverse communities in inter-tidal zones of tropical and subtropical regions and have been widely used as sites, where effluents are discharged and solid wastes are dumped and has a large capacity in retaining heavy metals. Only a few studies are available on their physiological and biochemical mechanisms under multiple heavy metal stress (Defew et al., 2005; Tam and Wong, 2000). In fact, mangrove plants are growing in a complicated environment including multiple heavy metals. Hence, it is necessary to study the correlation between mangroves and heavy metals at different sites for the purpose of improving mangrove ecosystem. In the present study, we describe changes in oxidative stress responses in *Avicennia alba* collected at sites with varying pollution status. Our results will provide useful parameters for biomonitoring studies.

2. Materials and methods

Kalamukke in Cochin (9°97′N–76°23′E), Oachira in Kollam (8°53′–9°52′N) and non-polluted munamakalle in Chetua (76°31′–76°41′E), India were the sites selected for the study. Water and plant samples were analyzed for copper, iron, zinc, lead, manganese and cadmium by acid digestion and quantified using an atomic absorption spectrophotometer (Perkin Elmer 2280 atomic absorption spectrophotometer at slit 0.7).

The quantification limits (pg/g) for copper, iron, zinc and manganese were 0.01, for lead it was 0.02, whereas for cadmium and mercury it was 0.008 (Spevackova et al., 1997). The average recovery for cadmium, zinc and mercury were 86%, for copper, iron, magnesium and manganese it was 90% and for lead it was $>83\%$. Accepted recoveries ranged from 85% to 105%, and batches with recoveries less than 85% were rerun. Precision and accuracy of analysis were also ensured through repeated analysis of samples against National Institute of Standards and Technology standard reference material (SRM 1570) for all the heavy metals. The results were found to be within $\pm 2\%$ of the certified value. Standards used were matrix matched and international tissue/sediment standards (standard reference materials; NBS 1646/estuarine sediment, NBS 1572/citrus leaves) were used to check percentage recovery of metals. Sample digestion for the determination of mercury was done by cold atomic spectrophotometer in a mercury analyzer.

Oxidative stress was evaluated in terms of root oxidizability, membrane integrity for roots, lipid peroxidation and hydrogen peroxide accumulation in leaves and roots of *A. alba*. Root's oxidizing ability was determined as the amount of red triphenyl formazan and the absorbance was read at 485 nm (Singh et al., 2007). The effect of heavy metal on root membrane integrity was studied in terms of ion leakage from roots and was calculated as: $EL=(E_1/E_2)\times 100$, where EL is the ion leakage, E_1 and E_2 are the initial and final conductivities, respectively (Sharma et al., 2009).

Lipid peroxidation was measured in terms of malondialdehyde content (Heath and Packer, 1968). The malondialdehyde content was calculated using extinction coefficient (ε) of 155 $mM^{-1}\ cm^{-1}$. Hydrogen peroxide content was determined as per the methodology of Velikova et al. (2000). The amount of hydrogen peroxide was determined using $\varepsilon=0.28\ \mu M^{-1}\ cm^{-1}$.

Fresh leaf and root samples of *A. alba* collected from different localities were used for enzyme analysis. All assays were done at 4 °C. Total soluble protein contents of the enzyme extracts were determined according to Bradford (1976).

Super-oxide dismutase activity assay was based on the method of Beauchamp and Fridovich (1971), which measures the inhibition in the photochemical

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reduction of nitroblue tetrazolium at 560 nm. Catalase activity was determined as the rate of disappearance of hydrogen peroxide at 240 nm using $\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$ (Cakmak and Marschner, 1992). Ascorbate peroxidase was assayed as the decrease in absorbance at 290 nm due to oxidation of ascorbic acid using $\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$ (Nakano and Asada, 1981). Guaiacol peroxidase activity was determined in terms of increase in absorbance at 470 nm due to oxidation of guaiacol (Egley et al., 1983). Glutathione reductase activity was measured in terms of oxidation of nicotinamide adenine dinucleotide phosphate at 340 nm using $\varepsilon = 6.224\ mM^{-1}\ cm^{-1}$ (Foyer and Halliwell, 1976).

Glutathione S-transferase activity was measured using chloro-dinitro benzene as a substrate, according to the methodology of Habig et al. (1974).

Tissue homogenate was prepared with 5% (w/v) trichloro acetic acid and centrifuged at 1000 g for 30 min. The deproteinised supernatant was used in the assay of reduced glutathione (Ellman, 1959), and ascorbic acid, following the stoichiometric reduction of phosphomolybdate by ascorbic acid (Mitusi and Ohata, 1961).

2.1. Statistical analysis

Results are expressed as mean ± standard deviation. Differences were considered statistically significant when $P < 0.05$.

3. Results

Heavy metal concentration in the water bodies from three localities selected for this study clearly showed that almost all the metals tested remained as the major pollutants (Table 1). Levels of the metal concentration in *A. alba* from polluted and non-polluted sites are listed in Table 1. Leaves accumulate more metal ions compared to roots. Bioaccumulation of minerals in the tissues from polluted sites was in par with concentration of metals in water. Thus, *A. alba* from polluted sites had an average of almost 3–4 times more metal ions than the control sites.

Significant levels of malondialdehyde were seen in the leaves of *A. alba* from Kalamukke in Cochin than Oachira (Kollam) and Munamakalle (Chetua) study sites (Table 2). But malondialdehyde level in roots was higher in *A. alba* from Cochin, followed by Kollam and Chetua (Table 2).

Parallel to malondialdehyde content, root oxidizability also increased significantly ($P < 0.05$) in *A. alba* roots in response to heavy metals (Table 2). It was increased from 0.9 to 2.9 $g^{-1}\ h^{-1}$. The observed increase in root oxidizability further suggests the oxidative stress induced in roots. *A. alba* growing at different sites of heavy metal contaminations caused excessive ion leakage from roots i.e., 52–204% compared to control (Table 2). The maximum electrolyte leakage was noticed at Cochin (Table 2). The increased electrical conductivity of the bathing medium containing roots strongly suggests the disruption of membrane integrity by heavy metals.

Super-oxide dismutase activity in leaves and roots showed a little difference between different locations and was lowest at Chetua (Table 2). The mean super-oxide dismutase value at Kollam was significantly higher than Chetua (Table 2). In case of roots, super-oxide dismutase activity increased significantly at Cochin and Kollam than at Chetua. The same trend was noticed for ascorbate peroxidase activity also. It was 2.6- and 3-fold higher in leaves and roots in *A. alba* collected from Cochin compared to Chetua (Table 2).

Catalase in leaves, as well as in roots showed much higher activity at Cochin and Kollam than at Chetua (Figs. 1 and 2).

Significant variation in guaiacol peroxidase activity was seen in leaves at the different locations, with the highest value at Cochin, followed by Kollam and Chetua. Guaiacol peroxidase activity in roots was also significantly higher at Cochin than at Chetua.

Glutathione S-transferase activity in leaves remained low at Chetua compared to Kollam and Cochin (Fig. 2). In roots, enzyme activity significantly increased at Cochin than at Chetua. Significantly higher glutathione reductase activity was observed in leaves and roots in *A. alba* from Cochin and Kollam compared to those from Chetua (Fig. 2). Similarly, reduced glutathione content was low in leaf and root tissues from Cochin and Kollam, compared to those from Chetua (Table 2). Significantly reduced ascorbic acid values were seen in leaves from Cochin and Kollam,

Table 1
Heavy metals in water sample as well as leaf and root tissues of *A. alba* from the study sites. Each value in mg/kg fresh weight of dissolved metals represents the concentration measured in a composite sample resulting from combining three samples collected from different areas apart. Data are expressed as mean ± SD. Significant at $P < 0.05$.

	Surface water			Leaves			Roots		
	Cochin	Kollam	Chetua	Cochin	Kollam	Chetua	Cochin	Kollam	Chetua
Copper	92.5 ± 10	73.4 ± 2	35.6 ± 1	61.6 ± 4	49.5 ± 5	14.1 ± 3	53.6 ± 7	43.5 ± 2.2	12 ± 0.4
Cadmium	22.2 ± 5.8	18.4 ± 1	11 ± 0.7	6.2 ± 0.6	4.2 ± 0.6	1.4 ± 0.1	4 ± 0.4	2.9 ± 0.03	1 ± 0.5
Lead	69.6 ± 9.2	60.3 ± 1	37 ± 5	4.6 ± 0.2	3.9 ± 0.8	1.7 ± 0.03	3.3 ± 0.09	4 ± 0.8	0.09 ± 0.02
Mercury	0.25 ± 0.5	0.11 ± 0.3	0.03 ± 0.1	0.09 ± 0.01	0.05 ± 0.01	0.004 ± 0.01	0.04 ± 0.04	0.02 ± 0.06	0.001 ± 0.03
Zinc	369.7 ± 10	302.2 ± 9	242 ± 9.4	2 ± 0.2	1.3 ± 0.03	0.89 ± 0.02	1.2 ± 0.01	1 ± 0.09	0.5 ± 0.01
Iron	53.4 ± 4.2	41 ± 0.8	17.3 ± 4.6	42.2 ± 6.2	31.4 ± 1.9	12.3 ± 6	33.4 ± 0.9	27 ± 4.6	8.5 ± 0.05
Manganese	421 ± 11	435.7 ± 8	215 ± 12	5.9 ± 0.01	4 ± 0.8	0.45 ± 0.4	3.4 ± 0.02	3.1 ± 0.02	0.22 ± 0.04

Table 2
Roots oxidizability, Lipid peroxidation, electrolyte leakage, antioxidant compounds and activities of scavenging enzymes in *A. alba* leaves and roots from the different study sites. NA indicates not applicable. Experimentals are done in triplicate. Data are expressed as mean ± SD. Significant at $P < 0.05$.

	Leaves			Roots		
	Cochin	Kollam	Chetua	Cochin	Kollam	Chetua
Root oxidizability ($g^{-1}\ h^{-1}$)	NA	NA	NA	2.9 ± 0.6	1.9 ± 0.7	0.9 ± 0.9
Malondialdehyde (nmol g^{-1} FW)	6.3 ± 2.3	5.2 ± 1.2	3.1 ± 0.7	5.8 ± 2.4	3.4 ± 1.3	2.1 ± 0.97
Electrolyte leakage (%)	NA	NA	NA	204.9 ± 8.6	149.2 ± 4	52.8 ± 11.2
Reduced glutathione (μmol/g wt. tissues)	0.9 ± 0.45	1.1 ± 0.5	1.4 ± 0.2	1.6 ± 0.9	1.2 ± 0.98	2.5 ± 2.1
Ascorbic acid (μg/g wt. tissues)	14 ± 4.6	16.3 ± 8.7	28.5 ± 10.3	29 ± 6.7	22 ± 3.3	30.4 ± 10.5
Super-oxide dismutase (unit/mg protein)	24.2 ± 10.3	17.4 ± 9.5	13.5 ± 5.2	14.6 ± 7.8	12.1 ± 9.2	7.4 ± 3.1
Ascorbate peroxidase (unit/mg protein)	33.3 ± 9.7	27.3 ± 10.2	10.2 ± 4.3	23 ± 10.6	20.2 ± 8.8	9.7 ± 4.2