# Mangroves- a Potential Phyto-Remediator and Useful Bio-Indicator against Heavy Metal Toxicity

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#### **Abstract**

Mangroves, the tropical and sub-tropical estuarine eco-system is now under severe threat, of which environmental pollution plays an important role in decreasing the species diversity. The present investigation was undertaken to generate information on the effect of some heavy metals (Hg, Pb, and Cd) on two dominant mangroves of sunderban viz., Bruguiera sexangula and Ceriops decandra. In both the mangrove species, roots accumulated more heavy metals than stem and leaf linearly correlated with the reduction in growth parameters and the mode of heavy metal accumulation was in the order of Cd>Pb>Hg. Potentiality of Bruguiera sexangula and Ceriops decandra to grow, establish and thrive within the polluted environment could be effectively used for phyto-remediation, however Bruguiera was found to be more suited. The visible toxicity symptoms i.e. browning of shoot tip in case of Hg, yellowing of leaves for Pb, chlorosis for Cd etc could be efficiently used as bio-marker or bio-monitor or bioindicator in the heavy metal polluted environment. Activity assessment of the most important stress enzyme peroxidase (POD) clearly indicated that a linear relationship between POD activity and leaf tissue metal concentration was found upto concentration 3 (2 times more than the permissible limit) level of all the three heavy metals at 100 DAS for both the mangrove species, but at concentration 4 (4 times more than the permissible limit) a sudden decrease in activity was noticed, which is surprisingly associated with the *visible toxicity* symptoms.

#### 1. Introduction

Mangroves, the vast vegetational swamps are the natural transition between land and sea in tropical and subtropical estuarine ecosystem. They play a vital role in proper maintenance of the coastal ecosystem, e.g. they not only protect the shoreline from natural calamities like hurricanes, tsunami etc. but also are the only escape from environmental pollution of the nearby industrial growth. Mangroves also have significant local economic importance as source of timber, firewood, pulp, honey, tannin, wax and also for natural and manipulated fisheries etc., but this economic and ecological utility of mangrove community is now under severe threat mainly due to demographic pressure, rapid urbanization, industrialization and human interference and random exploitation. While about 500,000 ha of mangrove forest existed in India during the 60's, such area was brought down to 200,000 ha only during the 80's (Blasco, 1977). Similar observation was also reported by Untawale (1986). The causes of such depletion and degeneration are of course numerous, of which environmental pollution including heavy metals [like mercury (Hg), lead (Pb), cadmium (Cd), etc.] play an important role. Moreover, these inorganic contaminants (heavy metals) are getting importance for their non-degradable nature and they often accumulate through tropic level causing a deleterious biological effect. Stiborova et al. (1987) reported shoot and root growth retardation of barley and maize after heavy metal exposure. Similar findings were also observed by Iqbal et al. (1991), Krupa et al. (1993) and Zeid (2001).

In the present experiment it was proposed to generate information on the effect of some identified heavy metals (Hg, Pb and Cd), commonly found in the estuarine eco-system, on two dominant mangroves of Sunderban viz., *Bruguiera sexangula* and *Ceriopas decandra*, regarding their different morphological, physiological and biochemical characters at two growth stages in *ex-situ* condition. The result obtained from this type of study will help us for better understanding of the effect of heavy metals on two dominant mangroves of Sunderban and also help to identify the species which could

be used as *Biomarker* or *Biomonitor* to heavy metal pollution as well as to generate information on phyto-remediation properties of mangroves, if any; thus help to select the potential mangrove species which can grow, establish and thrive within polluted environment.

#### 2. Materials and Methods

Two mangrove species *Bruguiera sexangula* and *Ceriops decandra* were collected from Patharpratima area for the present research work, as they were found to be the most dominant and commonly available species in the Sunderban area. The area of research, i.e. the Patharpratima area of Sunderban, was correctly selected as it was very close to the discharge point of river Hooghly. The concentrations of different heavy metals like Hg, Pb, and Cd in Patharpratima were estimated as 0.573, 40.40 and 25.79  $\mu$ g g<sup>-1</sup> in soil and in water their concentrations were 3.35, 242.50 and 40.50  $\mu$ g l<sup>-1</sup>, respectively. The concentrations of all these heavy metals exceeded permissible limit for crop production (BIS, 1991).

The mature plant propagules of the said mangroves were planted in earthen pots. The sands of the earthen pots were sterilized with 5% Formalin. 30 plants in three replications were planted in the net house of the Department of Seed Sciences and Technology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal. The plants were treated with four different concentrations of three heavy metals viz. Hg, Pb and Cd. Out of four concentrations; one concentration (conc. 2) was prepared as per the permissible limit of heavy metals in drinking water, one concentration was maintained at lower level of the permissible limit (conc. 1) and two other concentrations (conc. 3 & 4) were above that as follows:

| Heavy  | Conc1                 | Conc2                 | Conc3         | Conc4         |
|--------|-----------------------|-----------------------|---------------|---------------|
| metals | (mg l <sup>-1</sup> ) | (mg l <sup>-1</sup> ) | $(mg l^{-1})$ | $(mg l^{-1})$ |
| Hg     | 0.005                 | 0.01                  | 0.02          | 0.04          |
| Pb     | 1.0                   | 2.0                   | 4.0           | 8.0           |
| Cd     | 1.0                   | 2.0                   | 4.0           | 8.0           |

(Source: West Bengal Pollution Control Board websitewww.wbpcb.gov.in)

All the treatments were prepared in distilled water and supplemented with modified Hoagland nutrient solution (Hoagland and Arnon, 1938) at 7 days interval. The control plants were provided with only standard Hoagland nutrient solution. Different plant parts like root, stem and leaves were collected at two different growth stages namely 50 Days after sowing (DAS) and 100 DAS for assessing heavy metal accumulation in each plant part. This was estimated by wet oxidation method with Di-acid mixture without H<sub>2</sub>SO<sub>4</sub> (Jackson, 1973) and the reading was recorded from Varian-AA240 Atomic Absorption Spectrometer. Morphological data and other vis-

ible features were recorded at both the growth stages and the average values from 3 replications. For assessing the activity of stress enzyme peroxidase, leaf samples were collected at both the growth stages and the activity was estimated as per the standard method of Malik and Singh (1980). Statistical analysis was done by augmented two factor factorial ANOVA using Duncan's test (Duncan, 1955).

## 3. Results and Discussion

The results obtained from Atomic Absorption Spectrophotometry indicated that in case of *Ceriops decandra*, root accumulates more (0.83 µg g<sup>-1</sup>) amount of Hg at concentration 4 at 50 DAS than its stem and leaf (Table 1). Similarly in *Bruguiera sexangula*, root accumulated much higher (0.94 µg g<sup>-1</sup>) amount of Hg at concentration 4 after 50 DAS. The trend of more heavy metal accumulation in root area was also noticed at 100 DAS for both the species, which might be due to less mobility of toxic heavy metals within the plant body. Sur et al. (2006) reported such type of less mobility of heavy metals at higher concentrations, in the edible portion of different vegetables. Similar finding was reported earlier by Tam et al. (1997) and Wei et al. (2008). This clearly indicates that roots act as a barrier for heavy metal translocation and protects the sensitive parts of the plant.

The mode of heavy metal accumulation in both Bruguiera and Ceriops was in the order of Cd>Pb>Hg (Table 1). Lin et al. (1997) and Lian et al. (1999) observed similar trend of heavy metal absorption in mangrove species. Hameed et al. (2001) reported that Pb accumulation is much faster and greater than Cu in the leaves of Spinancea oleracea. Ramos and Geraldo (2007) showed that bioaccumulation of heavy metals in three mangrove species like Avicennia schaueriana, Laguncularia recemosa and Rhizophora mangle were in the order of Zn>Pb>Cr>Cu>Cd and concluded that those plants could be efficiently used as biological indicator of heavy metal pollution. It was also observed that there was an increased tendency of hyper-accumulation of heavy metals with increased concentrations and time for both the mangrove species and Bruguiera accumulates more amount of heavy metals than Ceriops; e.g. in Bruguiera, at highest concentration, root accumulated 1.28 μg g<sup>-1</sup> Hg, 11.0 μg g<sup>-1</sup> Pb and 35.12 μg g<sup>-1</sup> Cd after 100 days of growth in pot culture which was much higher than that of Ceriops (Table 1). Similarly, sunflowers and Sesbania drummondii were identified as hyper-accumulator of uranium and lead, respectively, by Dushenkov et al. (1995) and Barlow et al. (2000).

The morphological study when compared between control and treated plants, it was interestingly found that there was a drastic reduction in plant height, fresh and dry weight of shoot and root particularly in *Ceriops decandra* than *Bruguiera sexangula* 

Table 1: Assessment of heavy metal accumulations (µg g<sup>-1</sup>) in different plant parts of two mangrove species at different growth stages

| <u> </u>   |                | Bruguiera sp.     |                |                   |                |                |                | Ceriops sp.    |                |                |                   |                |  |
|------------|----------------|-------------------|----------------|-------------------|----------------|----------------|----------------|----------------|----------------|----------------|-------------------|----------------|--|
| Treatments | 50 DAS         |                   |                |                   | 100 DAS        |                |                | 50 DAS         |                |                | 100 DAS           |                |  |
|            | Root           | Stem              | Leaf           | Root              | Stem           | Leaf           | Root           | Stem           | Leaf           | Root           | Stem              | Leaf           |  |
| Hg 1       | BDLk           | BDLk              | BDLi           | 0.41i             | $0.24^{j}$     | $0.22^{j}$     | BDLk           | BDLj           | BDLk           | BDLl           | BDLl              | BDLl           |  |
| Hg 2       | $0.61^{\rm f}$ | $0.20^{\rm i}$    | $0.16^{\rm g}$ | $0.63^{h}$        | $0.31^{\rm i}$ | $0.28^{i}$     | $0.21^{\rm i}$ | $0.11^{i}$     | $0.08^{j}$     | $0.34^k$       | $0.16^{k}$        | $0.09^k$       |  |
| Hg 3       | $0.81^{e}$     | $0.38^{\rm g}$    | $0.22^{\rm f}$ | $0.91^{\rm g}$    | $0.45^{h}$     | $0.32^{h}$     | $0.64^{g}$     | $0.21^{\rm f}$ | $0.14^{\rm i}$ | $0.81^{j}$     | $0.34^{j}$        | $0.18^{j}$     |  |
| Hg 4       | $0.94^{\rm d}$ | $0.62^{d}$        | $0.34^{e}$     | $1.28^{\rm f}$    | $0.76^{\rm g}$ | $0.53^{\rm g}$ | $0.83^{e}$     | $0.54^{h}$     | $0.28^{\rm g}$ | $1.18^{h}$     | 1.11 <sup>g</sup> | $0.72^{h}$     |  |
| Control    | BDLk           | BDLk              | BDLi           | BDLl              | BDL1           | BDLl           | BDLk           | BDLj           | BDLk           | BDLm           | BDLl              | BDLl           |  |
| Pb 1       | BDLk           | BDLk              | BDLi           | $2.22^{e}$        | $1.76^{e}$     | $1.10^{\rm e}$ | $0.12^{\rm j}$ | BDLj           | BDLk           | $1.91^{\rm f}$ | $1.39^{\rm f}$    | $0.81^{\rm f}$ |  |
| Pb 2       | $1.06^{c}$     | $0.94^{\circ}$    | $0.36^{d}$     | $3.42^d$          | $2.14^{d}$     | $1.26^{d}$     | $0.61^{h}$     | $0.31^{\rm g}$ | $0.22^{h}$     | $2.42^{e}$     | 1.64e             | $0.86^{e}$     |  |
| Pb 3       | $1.26^{b}$     | 1.11 <sup>b</sup> | $0.98^{b}$     | 6.14 <sup>c</sup> | 3.21°          | $2.09^{\circ}$ | $0.76^{\rm f}$ | $0.61^{e}$     | $0.34^{\rm f}$ | $3.44^{d}$     | $1.98^{d}$        | $0.96^{d}$     |  |
| Pb 4       | $3.75^{a}$     | 1.22a             | $1.15^{a}$     | $11.02^{b}$       | $8.75^{b}$     | $6.43^{b}$     | 1.46°          | $0.72^{d}$     | $0.70^{\rm c}$ | $9.94^{b}$     | 2.01°             | $1.26^{\circ}$ |  |
| Control    | BDLk           | BDLk              | BDLi           | BDLl              | BDL1           | BDLl           | BDLk           | BDLj           | BDLk           | BDLm           | BDLl              | BDLl           |  |
| Cd 1       | $0.11^{j}$     | BDLk              | BDLi           | 0.28k             | $0.16^{k}$     | $0.12^k$       | $0.63^{g}$     | $0.60^{\rm e}$ | $0.51^{e}$     | $0.96^{\rm i}$ | $0.79^{i}$        | $0.58^{i}$     |  |
| Cd 2       | $0.15^{\rm i}$ | $0.12^{\rm j}$    | $0.08^{h}$     | $0.32^{\rm j}$    | $0.18^k$       | $0.15^k$       | $1.24^{d}$     | $0.85^{\circ}$ | $0.62^{d}$     | $1.65^{\rm g}$ | $0.96^{h}$        | $0.74^{\rm g}$ |  |
| Cd 3       | $0.34^{h}$     | $0.22^{h}$        | $0.16^{\rm g}$ | $1.28^{\rm f}$    | $1.10^{\rm f}$ | $0.96^{\rm f}$ | $4.38^{b}$     | $2.22^{b}$     | $1.16^{b}$     | $8.74^{c}$     | $2.34^{b}$        | $1.98^{b}$     |  |
| Cd 4       | $0.50^{\rm g}$ | $0.42^{\rm f}$    | $0.40^{\circ}$ | 35.12a            | 31.41a         | 22.63a         | $9.38^{a}$     | $3.42^{a}$     | 2.91a          | $17.88^a$      | $4.03^{a}$        | $3.01^{a}$     |  |
| Control    | $0.001^{k}$    | BDLk              | BDLi           | 0.0021            | BDLl           | BDLl           | BDLk           | BDLj           | BDLk           | 0.001m         | BDLl              | BDLl           |  |

Note: BDL≡ Below Detectable Limit. Similar alphabets denote homogeneous means due to DMRT at 5% level of significance

(Table 2 and 3). These findings of decreased plant height with increased concentrations of heavy metals and time was at par with the findings of Stiborova et al. (1987), Iqbal et al. (1991), Mac Farlane and Burchett (2002), Adhikary et al. (2004), Mahmood et al. (2007) and Rahman et al. (2009) and the reduction in plant height, fresh and dry weight of shoot and root was found to be linearly correlated with increased concentrations of heavy metals and days after plantation. As these growth parameters are the indicator of plant vigour, so it clearly concludes that accumulation of heavy metals in different plant parts of *Ceriops* and *Bruguiera* disrupted plant metabolism.

From the above findings, it could be clearly stated that though retarded plant growth along with decreased fresh and dry weight of root and shoot was observed for heavy metal treatments in both the mangrove species (Table 2 and 3), but they did not interfere with the completion of plant development and survived even under a considerable pollution load. Such observation was supported by earlier findings of Kholodova et al. (2005). This clearly indicates the potentiality of *Bruguiera sexangula* and *Ceriops decandra* in general and the *Bruguiera sexangula* in specific to grow, establish and thrive within the polluted environment. Thus they could be efficiently used as phytoremediator of these heavy metals. The genus *Bruguiera* was identified as the hyper-accumulator of these three heavy metals and is more suited for plantation programme in a polluted area for its sustainable management. Zhou et al. (2011) reported that

mangrove plants can absorb and store non-essential metals in their perennial tissues and thus reduce export of the metals via leaf litter transport and also proposed that the mangroves are excellent candidates for phyto-stabilization of heavy metals in inter-tidal substrates.

Moreover, it was quite interestingly found that some sorts of visible toxicity symptoms were observed at only 100 DAS growth stage in both the mangrove species viz., browning of shoot tip in case of Hg, yellowing of leaves for Pb, chlorosis for Cd etc. (Table 4). But these anomalies were observed only in case of the highest concentrations of each heavy metal treatments. Hence, these two mangrove species could be efficiently used as Bio-marker or Bio-monitor in the heavy metal polluted environment. Rahman et al. (2009) also reported similar kind of toxicity symptoms like chlorosis, necrosis and finally death of the plant due to the effect of high concentration of Cr and explained it as their interference to several metabolic processes. Sharma (2001) reported different pollution indicator plants like Anthoxanthum for Zn, Agrostis for Cu, Festuca for Pb and Impatiens for Cd. A similar finding was also reported by Mac Farlane and Burchett (2001) and Rappe et al. (2011).

The activity measurement of stress enzyme, peroxidase (POD) indicated more value in control plants at 50 DAS than at 100 DAS (Table 5) for both *Bruguiera* and *Ceriops* sp. A linear relationship between peroxidase activity and leaf tissue metal concentration was observed for all the three heavy metals upto

| Treat-  | 50 DAS             |                |                      |                   |                |                      | 100 DAS           |                |                |                |  |
|---------|--------------------|----------------|----------------------|-------------------|----------------|----------------------|-------------------|----------------|----------------|----------------|--|
| ments   | Plant              | Root fresh     | Root dry             | Shoot             | Shoot dry      | Plant                | Root fresh        | Root dry       | Shoot          | Shoot dry      |  |
|         | height             | weight (g)     | weight (g)           | fresh             | weight         | height               | weight (g)        | weight         | fresh          | weight (g)     |  |
|         | (cm)               |                |                      | weight (g)        | (g)            | (cm)                 |                   | (g)            | weight (g)     |                |  |
| Control | 21.65a             | 1.01a          | 0.27 <sup>b</sup>    | 1.65a             | 0.41a          | $33.00^{a}$          | 1.65a             | $0.55^{a}$     | 1.78°          | $0.49^{b}$     |  |
| Hg 1    | $16.75^{d}$        | $0.88^{e}$     | $0.22^{\rm f}$       | $0.98^{\rm g}$    | $0.36^{\circ}$ | 19.52e               | 1.29c             | $0.54^{a}$     | 1.29e          | $0.41^{e}$     |  |
| Hg 2    | 13.21 <sup>g</sup> | $0.93^{\circ}$ | $0.25^{d}$           | 1.39°             | $0.39^{b}$     | $15.83^{\rm f}$      | 1.42 <sup>b</sup> | $0.51^{b}$     | $1.44^{d}$     | $0.44^{d}$     |  |
| Hg 3    | $12.06^{h}$        | $0.97^{\rm b}$ | $0.26^{c}$           | $1.04^{\rm f}$    | $0.22^{g}$     | $14.05^{\mathrm{i}}$ | 1.45 <sup>b</sup> | $0.47^{c}$     | $1.05^{\rm f}$ | $0.26^{i}$     |  |
| Hg 4    | $9.58^{i}$         | $0.78^{\rm f}$ | $0.21^{\rm g}$       | $0.41^{m}$        | $0.11^{j}$     | $10.60^{j}$          | $0.85^{\rm g}$    | $0.3^{\rm f}$  | $0.55^k$       | 0.161          |  |
| Pb 1    | $20.81^{b}$        | $0.90^{d}$     | $0.32^a$             | 1.52 <sup>b</sup> | $0.32^{d}$     | $23.70^{b}$          | $1.17^{d}$        | $0.45^{cd}$    | 2.16a          | 0.51a          |  |
| Pb 2    | 18.50 <sup>c</sup> | $0.77^{\rm f}$ | $0.21^{\rm g}$       | 1.13e             | $0.28^{e}$     | 22.57 <sup>c</sup>   | 1.05e             | $0.44^{d}$     | $1.98^{b}$     | $0.47^{c}$     |  |
| Pb 3    | $16.92^{d}$        | $0.63^{h}$     | $0.17^{h}$           | $0.92^{\rm h}$    | $0.20^{h}$     | $15.23^{gh}$         | $0.83^{\rm gh}$   | $0.33^{\rm f}$ | $0.78^{i}$     | $0.28^{h}$     |  |
| Pb 4    | $14.28^{\rm f}$    | $0.61^{i}$     | $0.17^{\rm h}$       | $0.58^{1}$        | $0.17^{i}$     | $14.97^{\rm h}$      | $0.81^{h}$        | $0.31^{\rm f}$ | $0.63^{j}$     | $0.20^{j}$     |  |
| Cd 1    | 15.70e             | $0.95^{c}$     | $0.25^{d}$           | $1.19^{d}$        | $0.29^{e}$     | $20.14^{d}$          | 1.27°             | $0.45^{cd}$    | 1.28e          | $0.35^{\rm f}$ |  |
| Cd 2    | $12.34^{h}$        | $0.93^{c}$     | $0.23^{e}$           | $0.85^{i}$        | $0.26^{\rm f}$ | 15.33 <sup>g</sup>   | $1.01^{\rm f}$    | $0.37^{e}$     | $0.99^{\rm g}$ | $0.30^{\rm g}$ |  |
| Cd 3    | $9.15^{\rm i}$     | $0.62^{i}$     | $0.21^{\rm g}$       | $0.81^{j}$        | $0.21^{gh}$    | $10.50^{j}$          | $0.66^{j}$        | $0.25^{\rm g}$ | $0.89^{h}$     | $0.25^{i}$     |  |
| Cd 4    | $7.43^{j}$         | $0.69^{g}$     | $0.22^{\mathrm{fg}}$ | $0.65^{k}$        | $0.11^{j}$     | $8.73^k$             | $0.72^{i}$        | $0.27^{\rm g}$ | 0.341          | $0.19^{k}$     |  |

Note: Similar alphabets denote homogeneous means due to DMRT at 5 % level of significance.

| Table 3: Average growth parameters of <i>Ceriops</i> sp. at 50 days and 100 days after sowing (DAS) |                |                |                |                 |                       |                |                |                |                |                      |  |
|---|----------------|----------------|----------------|-----------------|-----------------------|----------------|----------------|----------------|----------------|----------------------|--|
| Treat-  | 50 DAS         |                |                |                 |                       |                | 100 DAS        |                |                |                      |  |
| ments   | Plant          | Root fresh     | Root dry       | Shoot           | Shoot dry             | Plant          | Root fresh     | Root dry       | Shoot          | Shoot dry            |  |
|   | height         | weight (g)     | weight (g)     | fresh           | weight                | height         | weight (g)     | weight         | fresh          | weight (g)           |  |
|   | (cm)           |                |                | weight (g)      | (g)                   | (cm)           |                | (g)            | weight         |                      |  |
|   |                |                |                |                 |                       |                |                |                | (g)            |                      |  |
| Control   | $5.09^{b}$     | $0.25^{a}$     | $0.06^{b}$     | $0.08^{b}$      | $0.025^{a}$           | $7.60^{a}$     | $0.29^{a}$     | $0.10^{a}$     | $0.56^{a}$     | $0.098^a$            |  |
| Hg 1  | $4.76^{d}$     | $0.15^{d}$     | $0.05^{d}$     | $0.07^{\rm cd}$ | $0.019^{c}$           | $6.00^{c}$     | $0.19^{e}$     | $0.05^{\rm e}$ | $0.28^{e}$     | $0.061^h$            |  |
| Hg 2  | $4.23^{\rm f}$ | $0.14^{e}$     | $0.04^{\rm f}$ | $0.05^{\rm f}$  | $0.011^{\rm fg}$      | $5.67^{de}$    | $0.17^{\rm f}$ | $0.04^{\rm g}$ | $0.21^{\rm f}$ | $0.055^{\mathrm{i}}$ |  |
| Hg 3  | $3.90^{h}$     | $0.07^{i}$     | $0.02^{h}$     | $0.04^{\rm h}$  | $0.008^{\rm h}$       | $4.23^{j}$     | $0.10^{j}$     | $0.03^{j}$     | $0.15^{g}$     | $0.031^{k}$          |  |
| Hg 4  | $1.90^{j}$     | $0.06^{j}$     | $0.01^{i}$     | $0.02^{\rm i}$  | $0.006^{i}$           | 2.401          | $0.08^k$       | $0.02^k$       | $0.10^{h}$     | 0.0261               |  |
| Pb 1  | $4.86^{c}$     | $0.18^{c}$     | $0.05^{c}$     | $0.07^{c}$      | $0.023^{b}$           | $5.83^{cd}$    | $0.20^{d}$     | $0.07^{\rm b}$ | $0.39^{b}$     | $0.093^{b}$          |  |
| Pb 2  | $4.28^{ef}$    | $0.14^{\rm f}$ | $0.04^{e}$     | $0.06^{e}$      | $0.013^{e}$           | $5.30^{\rm f}$ | $0.17^{\rm f}$ | $0.06^{c}$     | $0.33^{d}$     | $0.082^{c}$          |  |
| Pb 3  | $4.14^{g}$     | $0.13^{g}$     | $0.04^{\rm f}$ | $0.05^{\rm g}$  | $0.010^{\rm gh}$      | $4.50^{\rm i}$ | $0.15^{h}$     | $0.05^{d}$     | $0.15^{g}$     | $0.038^{j}$          |  |
| Pb 4  | $3.10^{i}$     | $0.03^k$       | $0.01^{j}$     | $0.02^{\rm i}$  | $0.002^{\rm j}$       | $3.77^k$       | $0.10^{j}$     | 0.021          | $0.06^{i}$     | $0.019^{m}$          |  |
| Cd 1  | 5.62a          | $0.22^{b}$     | $0.08^{a}$     | 0.11a           | $0.023^{b}$           | $6.27^{b}$     | $0.27^{b}$     | $0.10^{a}$     | $0.39^{b}$     | $0.080^{d}$          |  |
| Cd 2  | 4.87°          | $0.18^{c}$     | $0.04^{\rm f}$ | $0.07^{c}$      | $0.015^{d}$           | 5.63e          | $0.22^{\circ}$ | $0.04^{\rm f}$ | $0.37^{c}$     | $0.076^{\rm e}$      |  |
| Cd 3  | 4.33e          | $0.13^{g}$     | $0.03^{\rm g}$ | $0.07^{\rm d}$  | $0.012^{ef}$          | $5.10^{g}$     | $0.15^{g}$     | $0.04^{h}$     | $0.36^{c}$     | $0.073^{\rm f}$      |  |
| Cd 4  | $4.14^{g}$     | $0.09^{\rm h}$ | $0.03^{g}$     | $0.04^{\rm h}$  | $0.011^{\mathrm{fg}}$ | $4.77^{h}$     | $0.13^{i}$     | $0.04^{i}$     | $0.20^{\rm f}$ | $0.070^{\rm g}$      |  |

Note: Similar alphabets denote homogeneous means due to DMRT at 5% level of significance.

Concentration 3 (2 times more than the permissible limit) level at 100 DAS for both the species. In *Ceriops*, while the activity was 0.016 unit min<sup>-1</sup> mg<sup>-1</sup> in control at 100 DAS, but it was increased from 0.019 to 0.024 from Hg 1 to Hg 3 and then drastically reduced to 0.013 unit min<sup>-1</sup> mg<sup>-1</sup> in Hg 4 treatment. Moreover, this decrease in peroxidase activity at the Concentration 4 (4 times more than the permissible limit) of all the three heavy metals at 100 DAS was also associated with

visible toxicity symptoms in both the mangrove species. This may be due to the fact that the treatment 4 of all the three heavy metals was with just the double amount of chemicals than in concentration 3. This result corroborates the findings of Mac Farlane and Burchett (2001) and Shevyakova et al. (2003). Zhang et al. (2007) also studied the effect of heavy metal stress on activity of antioxidative enzyme peroxidase in leaves and roots of two mangrove plants and observed that though the

Table 4: List of visible toxicity of mangroves due to heavy metal treatments at 100 DAS

| Treatment | Visible toxicity symptoms |                     |  |  |  |  |
|-----------|---------------------------|---------------------|--|--|--|--|
| Heatiment | Bruguiera sp.             | Ceriops sp.         |  |  |  |  |
|           | Browning of shoot tip     | Stunted grouth      |  |  |  |  |
| Hg 4      | Stunted Growth            | Stunted growth      |  |  |  |  |
|           | Yellowing of leaves       | Yellowing of leaves |  |  |  |  |
| Pb 4      | Broom like shoot tip      | Stunted growth      |  |  |  |  |
|           | Chlorosis                 | Chlorosis           |  |  |  |  |
| Cd 4      | stunted growth            | Bronzing of leaves  |  |  |  |  |

Table 5: Assessment of average peroxidase activity (unit min<sup>-1</sup> mg<sup>-1</sup>) of two mangrove species

| Traatmanta | Brugu           | iera sp.        | Ceriops sp.     |                    |  |  |
|------------|-----------------|-----------------|-----------------|--------------------|--|--|
| Treatments | 50 DAS          | 100 DAS         | 50 DAS          | 100 DAS            |  |  |
| Control    | 0.053k          | 0.011e          | $0.038^{j}$     | 0.016 <sup>h</sup> |  |  |
| Hg 1       | $0.055^{\rm j}$ | $0.018^{b}$     | $0.072^{e}$     | $0.019^{\rm g}$    |  |  |
| Hg 2       | $0.058^{\rm i}$ | $0.019^{b}$     | $0.074^{\rm d}$ | $0.022^{\rm f}$    |  |  |
| Hg 3       | $0.062^{\rm h}$ | $0.021^{a}$     | $0.077^{c}$     | $0.024^{e}$        |  |  |
| Hg 4       | $0.065^{\rm g}$ | $0.008^{\rm f}$ | $0.078^{\rm c}$ | $0.013^{\rm i}$    |  |  |
| Pb 1       | $0.157^{c}$     | $0.012d^{e}$    | $0.074^{\rm d}$ | $0.017^{\rm h}$    |  |  |
| Pb 2       | $0.162^{b}$     | $0.016^{c}$     | $0.077^{c}$     | $0.019^{\rm g}$    |  |  |
| Pb 3       | $0.165^{a}$     | $0.019^{b}$     | $0.081^{b}$     | $0.022^{\rm f}$    |  |  |
| Pb 4       | $0.166^{a}$     | $0.007^{\rm f}$ | $0.083^{a}$     | $0.013^{\rm i}$    |  |  |
| Cd 1       | $0.088^{\rm f}$ | $0.011^{e}$     | $0.052^{\rm i}$ | $0.035^{\circ}$    |  |  |
| Cd 2       | $0.092^{e}$     | $0.013^{d}$     | $0.054^{\rm h}$ | $0.042^{b}$        |  |  |
| Cd 3       | $0.094^{e}$     | $0.016^{c}$     | $0.055^{\rm g}$ | $0.048^{a}$        |  |  |
| Cd 4       | $0.097^{\rm d}$ | $0.007^{\rm f}$ | $0.058^{\rm f}$ | $0.027^{d}$        |  |  |

POD activity was increased first but finally declined and they concluded that POD activity may be served as a biomarker to heavy metal stress.

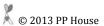
### 4. Conclusion

Peroxidase activity can be efficiently used as a sensitive biochemical indicator of Cd, Pb and Hg stress in *Bruguiera* and *Ceriops species* 

#### 4. References

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