International Journal of Bio-resource and Stress Management 2013, 4(4):605-609

Effect of Heavy Metals on Different Anatomical Structures of *Bruguiera sexangula*

Suparna Gupta[1*] and S. K. Chakrabarti[2]

[1]Rice Research Station, Chinsurah, Hooghly, West Bengal (712 102), India
[2]Department of Seed Science and Technology, BCKV, Mohanpur, Nadia, West Bengal (741 252), India

Article History

Manuscript No. c155
Received in 4th December, 2012
Revised in revised form 6th September, 2013
Accepted in final form 24th November, 2013

Correspondence to

E-mail: drsuparnagupta12@gmail.com

Keywords

Mangroves, heavy metal, hyper-accumulation, phyto-remediation, visible toxicity

Abstract

The mangrove ecosystem is claimed to be the most productive ecosystem in the world when gross primary productivity and litter production is considered. In the study, an attempt has been made to generate information on the effect of three identified heavy metals viz., Hg, Pb and Cd, commonly found in the estuarine ecosystem, on *Bruguiera sexangula* by observing some important anatomical characters and heavy metal accumulation potentiality during their growth and development at *ex situ*. There was an increased tendency of hyper-accumulation of heavy metals with increased concentrations and time and the maximum accumulation was observed in the root area followed by stem and leaf. These findings suggest that the root acts as a barrier for metal translocation and protects the sensitive parts of the plants. The mode of heavy metal accumulation was observed to be in the order of Cd>Pb>Hg. From the anatomical study of root and stem, it was observed that the heavy metals at highest concentrations percolated into the hypodermal and stelar region. The stem anatomy of *Bruguiera* at 100 days after sowing (DAS) indicated deformed vascular bundle at treatment 1 and 4 of Hg. Xylem and phloem deformation was also recorded in treatment 1 and 4 of Cd which finally led to visible toxicity. Such detailed study indicated that the mangrove species *Bruguiera* can tolerate and accumulate the aforesaid three heavy metals (Hg, Pb and Cd) even at much higher concentrations. Hence, *Bruguiera* can be designated as hyper-accumulator. It may, therefore, be inferred that it could be effectively used for phyto-remediation purpose.

1. Introduction

Mangroves are the natural transition between land and sea in tropical and subtropical estuarine ecosystem. This vast vegetation plays a vital role in proper maintenance of the coastal ecosystem. They not only protect the shoreline from natural calamities like hurricanes, tsunami etc. but also help to escape from environmental pollution caused by 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 5,00,000 ha of mangrove forest existed in India during the 60's, such area was brought down to 2,00,000 ha only during the 80's (Blasco, 1977). It was estimated that there were 181,399 sq. kilometers of mangrove vegetation which was only about 50% of the past vegetation coverage (Spadling et al., 1997). In 1986, 50% of the mangrove forest area has been depleted and the species diversity was decreased from 58 to 48 (Untawale, 1986). The causes of such depletion and degeneration are of course numerous, of which environmental pollution plays an important role. The coastal zone becomes a dumping area for toxic and hazardous chemicals like halogenated hydrocarbons, oils, different heavy metals like-mercury (Hg), lead (Pb), cadmium (Cd) etc. Among the inorganic contaminants, heavy metals are getting importance for their non-degradable nature and often accumulate through tropic level causing a deleterious biological effect.

In the present programme it was formulated to generate information on accumulation of some identified heavy metals like mercury (Hg), lead (Pb) and cadmium (Cd), commonly found in the estuarine system, in different plant parts of one dominant mangroves of Sunderban i.e. *Bruguiera sexangula* and also the anatomical manifestation of the mangrove species towards those heavy metal pollutants during their (root and stem) growth and development at *ex-situ.*

2. Materials and Methods

The mangrove species *Bruguiera sexangula* was collected from Patharpratima area for the present research programme as this was 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 μg g^{-1}, 40.40 μg g^{-1} and 25.79 μg g^{-1} in soil and in water their concentrations were 3.35 μg l^{-1}, 242.50 μg l^{-1} and 40.50 μg l^{-1} respectively. The concentrations of all these heavy metals exceeded the permissible limit for crop production (BIS, 1991).

The mature plant propagules of the said mangrove were planted in the 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 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. 2) and two other concentrations were above that as follows:

Heavy metals	Conc. 1 (mg l^{-1})	Conc. 2 (mg l^{-1})	Conc. 3 (mg l^{-1})	Conc. 4 (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 website-*www.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_2SO_4 (Jackson, 1973). And the reading was recorded from Varian-AA 240 Atomic Absorption Spectrometer.

For anatomical study, transverse sections (T. S.) of stem and root were taken at both 50 and 100 DAS growth stages by following standard anatomical procedure without using any stain and they were collected from both control and treated plants. But among the treated plant population, only lowest (conc. 1) and highest (conc. 4) concentrations of each heavy metal were considered. The hand sections were prepared with the help of sharp blade and the thinnest section with all desired anatomical characters were selected and placed under the Axioskop 40 (Zeiss) microscope for detailed study and photography. Statistical analysis was done by Augmented two factor factorial ANOVA and Duncan's test results (Duncan, 1955) were included using alphabets where similar alphabets denote homogeneous means.

3. Results and Discussion

The results obtained from Atomic Absorption Spectrophotometry indicated that the amount of Hg accumulated in the root of *Bruguiera sexangula* was much higher (0.94 μg g^{-1}) at concentration 4 at 50 DAS than its stem and leaf (Table 1). The trend of more heavy metal accumulation in root area was also recorded at 100 DAS e.g. at highest concentration, root accumulated 1.28 μg g^{-1} Hg, 11.0 μg g^{-1} Pb and 35.12 μg g^{-1} Cd after 100 days of growth stage which were much higher than their respective stem and leaf accumulation. This 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 also reported by Tam et al. (1997) and Wei et al. (2008). The mode of heavy metal accumulation in *Bruguiera* 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 and Hameed et al. (2001) reported that greater and faster Pb accumulation than Cu in the leaves of *Spinancea oleracea*. It was also observed that there was an increased tendency of hyper-accumulation of heavy metals with increased concentrations and time. Though retarded plant growth was observed for heavy metal treated plants, but this did not interfere with the completion of plant development and they survived even under a considerable pollution load. Hence, this could be stated that these heavy metal treatments did not interfere with the completion of plant development. Such finding was supported by earlier findings of Kholodova et al. (2005).

Similar alphabets denote homogeneous means due to DMRT at 5% level of significance. It clearly indicates the potentiality of *Bruguiera sexangula* to grow, establish and thrive within the polluted environment and thus it could be used efficiently for phytoremediation purpose and as the plant vigour was less affected, so *Bruguiera* is suited for plantation programme in a polluted area for sustainable management of that area. Zhou et al. (2011) also reported that mangrove plants are excellent candidate for phyto-stabilization of heavy metals in intertidal substrate. In the second part of the study i.e. anatomical manifestation of *Bruguiera sexangula* towards the pollutants stated above, following observations were recorded.

3.1. Stem anatomy

The T.S. of stem in control plants of *Bruguiera* at 50 DAS showed single layer epidermis which was thickly cuticularised and the hypodermis with compactly arranged collenchymatous cells, followed by several layers of parenchymatous cells. The last layer of cortex was with compactly arranged barrel shaped cells, known as starch sheath. The stele was composed of well arranged vascular bundles with intervening ray cells (Figure1A). The vascular bundles were collateral and open. Pith was central in position and was made up of parenchymatous cells. The stem T. S. for Cd 1 treatment showed diffusion of that heavy metal upto the pith region. When stem T. S. of Hg 1 and 4 was compared with control, it was observed that the stelar region of Hg 4 treatment was greatly affected. Penetration of Pb in Pb 4 treatment inside the stem starting from epidermis to pith region was noticed (Figure 1B). The stem anatomy of *Bruguiera sexangula* at 100 DAS when taken into account, in the Hg 1 and Hg 4 treated seedlings, deformed vascular structure was noticed (Figure 1C & 1D), which may finally led to visible toxicity like browning of shoot tip in case of Hg, yellowing of leaves for Pb, chlorosis for Cd etc. (Table 2). MacFarlane and Burchett (2001) and Rappe et al. (2011) also reported similar kind of visible toxicity symptoms of plants due to heavy metal pollution stress. Xylem and phloem deformation was also recorded in the treatment 1 and 4 of Cd treated stem sections (Figure 1 E & F).

Table 1: Assessment of heavy metal accumulations (μg g^{-1}) in different plant parts of *Bruguiera sexangula* at different growth stages

Metal Conc.	50 DAS		100 DAS	
	Root	Stem	Root	Stem
Hg 1	BDL^k	BDL^k	0.41^i	0.24^j
Hg2	0.61^f	0.20^i	0.63^h	0.31i
Hg3	0.81^e	0.38^g	0.91^g	0.45^h
Hg4	0.94^d	0.62^d	1.28^f	0.76^g
Control	BDL^k	BDL^k	BDL^l	BDL^l
Pb1	BDL^k	BDL^k	2.22^e	1.76^e
Pb2	1.06^c	0.94^c	3.42^d	2.14^d
Pb3	1.26^b	1.11^b	6.14^c	3.21^c
Pb4	3.75^a	1.22^a	11.02^b	8.75^b
Control	BDL^k	BDL^k	BDL^l	BDL^l
Cd1	0.11^j	BDL^k	0.28^k	0.16^k
Cd2	0.15^i	0.12^j	0.32^j	0.18^k
Cd3	0.34^h	0.22^h	1.28^f	1.10^f
Cd4	0.50^g	0.42^f	35.12^a	31.41^a
Control	0.001^k	BDL^k	0.002^l	BDL^l
SEm±	0.01	0	0.01	0.01
CD (p=0.05)	0.02	0.02	0.03	0.04

BDL: Below Detectable Limit; DAS: Days after sowing

In saltbush (*Artiplex halimus* L.), the cadmium is found to be precipitated in the form of cadmium oxalate crystals in the stems when they were exposed to 0.1 mM Cd in a nutrient solution for 3 week in a fully controlled environment. This may be partly linked to the heavy metal resistance of this species (Lutts et al., 2004).

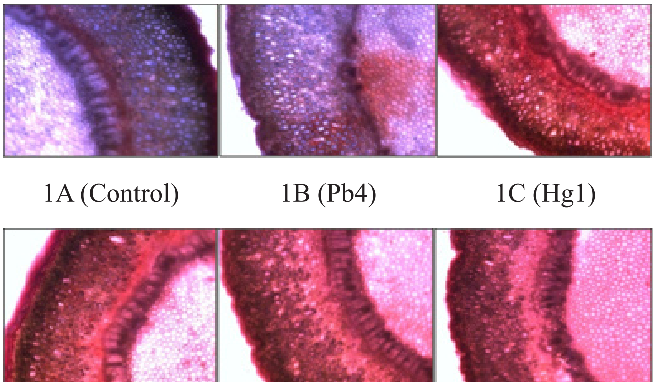
3.2. Root anatomy

The T. S. of the *Bruguiera* root of control plants at 50 DAS indicated uniseriate, thin walled epiblema. The cortex was with homogeneous, oval, thin walled parenchyma. The endodermis was the innermost layer. The stele includes central core of vascular tissues. When the roots of treatment 1 and 4 of Hg were considered, it was seen that the cortex area accumulated Hg and this area was interrupted with cracks (Figure 2A). In Cd treatments an initiation of percolation was observed in Cd 1 (Figure 2B) and finally at treatment 4 the chemical (Cd) reached at the stelar region (Figure 2C) causing great injury to the conduction process. The T. S. of root at 100 DAS of Hg 1 and 4 treatments also showed metal accumulation (Figure 2D). Similar accumulation was also noticed in the treatment 1 and 4 of Pb at 100 DAS (Figure 2E & F).

Similar kind of observation was earlier reported by MacFarlane and Burchett (2000) where the heavy metal accumulation was predominantly found in the root cell walls and the epidermis served as a major barrier to heavy metal transport. It was also observed that the heavy metal movement towards stele was protected by the casparian strip. In Salt cress (*Thellungiella halophila*), a small winter annual crucifer exhibited extra endodermis development in roots during extreme salt stress condition (Inan et al., 2004). Naskar and Palit (2008) indicated the presence of large number of aerenchyma cells in the root zone of *Avicennia* and *Sonneratia*. The root anatomy of the plant *Mesembryanthemum* indicated accumulation of $CdCl_2$

Table 2: Visible toxicity symptoms of *Bruguiera* due to heavy metal treatments at 100 DAS

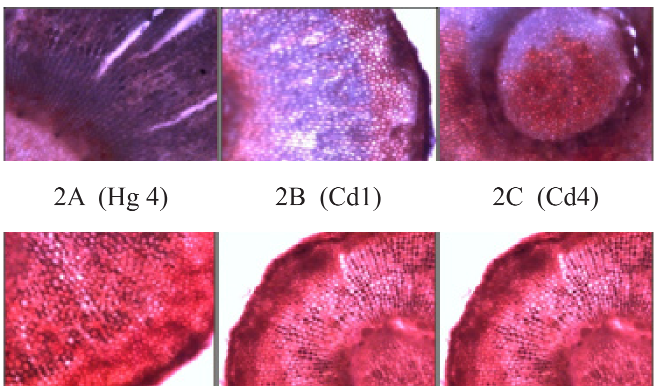
Treatment	Visible Toxicity
Hg 4	Browning of shoot tip Stunted Growth
Pb 4	Yellowing of leaves Broom like shoot tip
Cd 4	Chlorosis stunted growth



1A (Control) 1B (Pb4) 1C (Hg1)

1D (Hg4) 1E (Cd1) 1F (Cd4)

Figure 1: Transverse section of *Bruguiera* stem



2A (Hg 4) 2B (Cd1) 2C (Cd4)

2D (Hg 4) 2E (Pb 1) 2F (Pb 4)

Figure 2: Transverse section of *Bruguiera* root

(cadmium chloride) in the cell walls of endodermis and metaxylem when 0.1 mM of $CdCl_2$ was applied for 48 hrs (Shevyakova et al., 2003). Zhou et al. (2011) reported that mangrove plants can absorb and store non-essential heavy metals in their perennial tissues.

4. Conclusion

Results obtained from this type of study will help us for better understanding of translocation of heavy metals to different plant parts and also anatomical manifestation of this mangrove species towards heavy metal pollutants and help to compute the collected data for assessing the pollution load by both Atomic Absorption Spectrophotometry and anatomical methodology.

5. References

BIS, 1991. Indian standards drinking water specification, Bereau of Indian Standards, Indian Standard 10500.

Blasco, F., 1977. Outlines of Ecology, Botany and Forestry of the Mangals of the Indian Subcontinent, West Coastal Ecosystems. Elsevier Scientific Publishing Company, Oxford, New York, 241-258.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, 11-42.

Hameed, N.Z., Siddiqui, S., Ahmed, S., 2001. Effect of Cu and Pb on germination, accumulation and phenolic contents of *Spinancea oleraecea* and *Lycopersicum esculentum*. Pakistan Journal of Biological Sciences 4, 809-811.

Hoagland, D.R., Arnon, D.I., 1938. The water-culture method for growing plants without soil. California Agricultural Experimentation Station Circular, 347.

Inan, G., Zhang, Q., Li, P., Wang, Z., Cao, Z., Zhang, H., Zhang, C., Quist. T.M., Goodwin, S.M., Zhu, J., Shi, H., Damsz, B., Charbaji, T., Gong, Q., Ma, S., Fredricksen, M., Galbraith, D.W., Jenks, M.A., Rhodes, D., Hasegawa, P.M., Bohnert, H.J., Joly, R.J., Bressan, R.A., Zhu, J.K., 2004. Salt Cress. A halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic analysis of growth and development of extremophiles. Plant Physiology 135(3), 1718-1737.

Jackson, M.L., 1973. Soil chemical analysis. Prentice Hall of India Private Ltd. New Delhi.

Kholodova, V.P., Volkov, K.S., Kuznetsov, V.V., 2005. Adaptation of the common ice plant to high copper and zinc concentrations and their potential using for phytoremediation. Russian Journal of Plant Physiology. 52(6), 748-757.

Lian, Y., Xu, J., Lin, P., Meguro, S., Kawachi, S., 1999. Five heavy metals in propagules of ten mangrove species of China. Journal of Wood Sciences 45(4), 343-347.

Lin, P., Zheng, W., Li, Z.J., 1997. Distribution and accumulation of heavy metals in *Avicennia marina* community in Shenzhen, China. Journal of Environmental Sciences 9(4), 472-479.

Lutts, S., Lefevre, I., Delperee, C., Kivits, S., Dechamps, C., Robledo, A., Correal, E., 2004. Heavy metal accumulation by the halophyte species Mediterranean saltbush. Journal of Environmental Quality 33(4), 1271-1279.

MacFarlane, G.R., Burchett, M.D., 2000. Cellular distribution of copper, lead and Zn in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Aquatic Botany 68(1), 45-59.

MacFarlane, G.R., Burchett, M.D., 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin 42(3), 233-240.

Naskar, S., Palit, P., 2008. Histo-physiology of some salt tolerant plants in Indian Sunderbans. International Symposium to Commemorate the 150th Birth Anniversary of Sir J.C. Bose and Birth centenary of Prof. S.M. Sircar, held on Bose Institute, Kolkata, 24-28th November 2008.

107.

Rappe, R.A., Lajus, D.L., Schreider, M.J., 2011. Heavy metal impact on growth and leaf asymmetry of seagrass, *Halophila ovalis*. Journal of Environmental Chemistry and Ecotoxicology 3(6), 149-159.

Shevyakova, N.I., Netronina, I.A., Arnova, E.E., Kuznetsov, V.V., 2003. Compartmentation of cadmium and iron in *Mesembryanthemum crystallinum* plants during the adaptation to cadmium stress. Russian Journal of Plant Physiology 50(5), 678-685.

Spadling, M., Blasco, F., Field, C., 1997. World mangrove atlas. The International Society for Mangrove Ecosystem, Okinawa, Japan.

Sur, P., Pandit, T.K., Naik, S.K., Mandal, M., Chakrabarty, A., Das, D.K., 2006. Occurrence of some heavy metals in some contaminated soils and crops of West Bengal. Indian Agriculture 50(1-2), 79-84.

Tam, N.F.Y., Wong, Y., Wong, Y.S., 1997. Accumulation and distribution of heavy metals in a stimulated mangrove system treated with sewage. Asia-pacific conference on science and management of coastal environment: proceedings of the international conference held in Hong Kong, 25-28th June 1996. Hydrobologia 352, 67-75.

Untawale, A.G., 1986. Mangrove ecosystem in India. In: Mangroves of Asia and Pacific: status and management. UNESCO, 51-87.

Wei, L., Yan, C.L., Wu, G.R., Guo, X.Y., Ye, B.B., 2008. Variation of delta 13C in *Aegiceras corniculatum* seedling induced by cadmium application. Ecotoxicology 17(6), 480-484.

Zhou, Y.W., Peng, Y.S., Li, X.L., Chen, G.Z., 2011. Accumulation and partitioning of heavy metals in mangrove rhizosphere sediments. Environmental Earth Science 64(3), 799-807.