Marine Pollution Bulletin 133 (2018) 157–172

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Assessment of potentially toxic metal (PTM) pollution in mangrove habitats using biochemical markers: A case study on *Avicennia officinalis* L. in and around Sundarban, India

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ARTICLE INFO

Keywords:
Mangrove
Potentially toxic metal
Biochemical markers
Sundarban
Antioxidative response
Bioaccumulation

ABSTRACT

Spatial distribution of potentially toxic metals (PTMs) and their accumulation in mangrove *Avicennia officinalis* L. were studied along 8 locations in and around Sundarban mangrove wetland, India. Among 8 locations, S3 (Chemaguri) and S5 (Ghushighata) showed higher concentration of PTMs (Cd, Cr, Cu, Ni, Pb, Zn) characterized by higher enrichment factors (3.45–10.03), geo-accumulation indices (0.04–1.22), contamination factors (1.14–3.51) and pollution load indices (1.3–1.45) indicating progressive deterioration of estuarine quality and considerable ecotoxicological risk. Metal concentration in *A. officinalis* leaves showed significant correlation with sediment metals implying elevated level of bioaccumulation. Significant statistical correlation between photosynthetic pigments (Chlorophyll a, Chlorophyll b), antioxidant response (free radical scavenging and reducing ability) and stress enzymatic activity (Peroxidase, Catalase, Super-oxide dismutase) of *A. officinalis* with increasing metal concentration in the contaminated locations reflects active detoxification mechanism of the plant. The study indicates the potentiality of biomonitoring metal pollution using studied biochemical markers in mangrove habitats.

1. Introduction

Over the past two centuries, anthropogenic and industrial activities have led to emissions of toxic metals into the environment at significantly exceeding concentration (Hu et al., 2013; Mireles et al., 2012; Nriagu, 1996; Su, 2014; Vutukuru, 2005; Wei & Yang, 2010; Yaylalı-Abanuz, 2011). Potentially toxic metals (PTMs) including the heavy and trace metals easily get transported to coastal areas, deposited in sediments, become persistent and bioavailable to living organisms due to their bioaccumulation and biomagnification potential (Bryan & Langston, 1992; Zhou et al., 2008). Primary anthropogenic sources of PTM pollution include mining, smelting of metalliferous ores and industrial processing, fossil fuels, automobiles, nuclear fuels, agrochemicals municipal wastes and sewage, fertilizers, domestic sewage etc. (Alloway, 2013; Chary et al., 2008).

Contamination of toxic metals has devastating effects on the ecological balance, density and diversity of biotic communities of the recipient environment, especially in estuarine and marine ecosystem (Farombi et al., 2007; Mountouris et al., 2002; Vosyliene & Jankaite, 2006). They can disrupt metabolic functions and interrupt functioning of vital organs and glands by gradual accumulation. Accumulation of PTMs in plants grown in metal-polluted soil is also very alarming as soils not only provide essential nutrients, but also harbour these toxic elements. Plants have evolved with highly specific mechanisms and biotic responses to take up, translocate and store toxic metals within physiological ranges. Although uptake mechanisms by membrane transporters are generally selective (Lasat, 2002), non-essential elements, like As, Pb or Cd, are also taken up through the same transport systems (Verbruggen et al., 2009). When plants are exposed to elevated metal concentrations, they either undertake exclusion or efflux of metals into the xylem (xylem loading) or intracellular accumulation and sequestration (Montargès-Pelletier et al., 2008). Beside efflux of metal ligands from root into xylem vessels, intracellular detoxification mechanisms potentially leading to plant metal tolerance are linked to metal homeostasis and/or metal-induced oxidative stress (Cuypers et al., 2009; Lasat, 2002; Mishra et al., 2006; Seth et al., 2007). Redox-active metals are able to directly induce Reactive Oxygen Species (ROS) production through Fenton and Haber–Weiss reactions (Halliwell,

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https://doi.org/10.1016/j.marpolbul.2018.05.030
Received 16 August 2017; Received in revised form 9 May 2018; Accepted 16 May 2018
0025-326X/

2006; Schutzendubel & Polle, 2002), whereas non-redox-active metals, such as Cd, Zn and Pb, induce oxidative stress indirectly. However, metal stress also enhances organellar ROS generation by disturbing photosynthesis and photorespiration processes (Heyno et al., 2008; Rodríguez-Serrano et al., 2009). Heavy Metal toxicity in plants grossly interrupt their principal physiological and metabolic processes (Dalcorso et al., 2008; Hossain et al., 2009; Hossain et al., 2010; Rascio & Navari-Izzo, 2011; Villiers et al., 2011). These include inactivation and denaturation of enzymes, conformational modifications and disruption of membrane integrity, inhibition of photosynthesis, respiration, altered activities of several enzymes and disruption in redox homeostasis (Dietz et al., 1999; Schutzendubel & Polle, 2002; Sharma & Dietz, 2009; Sharma & Dubey, 2007). The balance between the reactive oxygen species (ROS) and the quenching activity of antioxidants (AOX) get disturbed in plants exposed to toxic metal stress resulting to redox homeostasis imbalance and oxidative damage, eventually leading to conformational modifications and disruption of membrane integrity, lipid peroxidation, DNA damage or even plant death (Braconi et al., 2011; Hossain et al., 2010; Kandziora-Ciupa et al., 2013; Navari-Izzo et al., 1998; Rascio & Navari-Izzo, 2011; Romero-Puertas et al., 2002; Scandalios, 2005).

Monitoring the PTM induced biotic responses in plants can give an insight to the magnitude of bioavailability of metals in the marine environment and their ecotoxicological relevance (Marchand et al., 2016). Metal bio-monitors in estuarine system need to conform to certain required characteristics, bridging between pollution status of the habitat sediment and consequent response of plant. Mangroves are excellent bio-monitor of metal stress as they have a strong antioxidant activity potential with respect to both enzymatic and non-enzymatic defense systems, which also help them to survive in metal polluted zones (Bandaranayake, 2002; Kathiresan et al., 2013; Ravindran et al., 2012). Their metal specific physiological responses including production of proteins, peroxidase, flavonoids, phenolics and anti-oxidant feedback loop can be exploited as potent biomarkers of pollution as well (Adams, 2002; Apel & Hirt, 2004; Bruns et al., 1997; Butterworth, 1995; Duarte et al., 2013). Mangrove sediments being anoxic, reduced and rich in sulphides and organic matter, enhance the retention of potentially toxic heavy metals transported by the adjacent water system (Bakshi et al., 2017; Lacerda & Abrao, 1984; Tam & Wong, 2000). They also have a large potential to accumulate these pollutants due to their capacity to efficiently trap suspended material from the water column (Furukawa et al., 1997). Metalliferous pollutants are of serious concern in estuarine and coastal region due to their high residence time in sediment which further increases the cumulative ecotoxicological effects followed by penetrating accumulation in living organisms (Banus, 1977; Harbison, 1981; Lacerda & Abrao, 1984; Lacerda et al., 1986). The presence of toxic metals in mangrove dominated estuarine regions has been estimated by several researchers due to their potential ecotoxicological risk (Nath et al., 2013; Nath et al., 2014a; Nath et al., 2014b). Despite of being exposed to metalliferous pollutants largely deposited in the downstream of the estuarine ecosystem, mangroves show high tolerance even at high and multiple metal stresses (Cuong et al., 2005; Macfarlane et al., 2007; Mackey et al., 1992; Ongche, 1999) and can be an indicator of sediment quality. While toxic metals are known to be affecting ROS and antioxidant balance of plants in general, mangroves and their associates are seen to be thriving well in their presence. Hence the effect of elevated metal concentration on antioxidant potential of mangroves and other biochemical parameters deserves to be investigated.

The Sundarban wetland situation at the dynamic estuarine region of West Bengal, India, homes a part of the world's largest mangrove habitat sustained by the vast Hooghly-Matla estuarine system. The extensive industrialization and rapid urbanization have led to severe environmental degradation in this extremely sensitive estuarine system causing from increasing flow of domestic, municipal, industrial effluents containing potentially toxic metals (Antizar-Ladislao et al., 2015; Banerjee et al., 2012a; Banerjee et al., 2012b; Ghosh et al., 2016). Anthropogenic interferences in terms of human habitat, tourism and boating activities, increasing agricultural and aquaculture practices, deforestation and change in land use pattern also resulted unwanted contamination, pollution and disruption in regular ecosystem activities in this region (Antizar-Ladislao et al., 2015). The increasing environmental stress also causes deposition, accumulation and magnification of the toxic metals in the entire ecosystem. Researchers have studied the distribution, bioavailability and contamination status of the toxic pollutants in this dynamic shallow estuarine region (Antizar-Ladislao et al., 2011; Antizar-Ladislao et al., 2015; Ghosh et al., 2016; Sarkar et al., 2012; Watts et al., 2013). Biotic response of the mangroves in and around Sundarban deserves a thorough investigation and can be used as a substantial biomarker to quantify the level of contamination. Toxic response of mangroves exposed to PTMs have been mostly studied in ex-situ system (Cheng et al., 2017; Huang & Wang, 2010; Huang et al., 2010; Yan & Tam, 2011; Zhang et al., 2007), whereas very less reports are found on the natural populations (Caregnato et al., 2008; Harish & Murugan, 2011; Macfarlane & Burchett, 2001). In the present study, PTM contamination and their ecotoxicological risks in 8 locations in this dynamic estuarine region has been assessed along with the consequent biotic response in *Avicennia officinalis* which is a commonly found mangrove species grown throughout this estuarine zone. Though enlisted in the 'least concern' category in the International Union for Conservation of Nature (IUCN) Red Data List, the species is currently facing anthropogenic and climatic pressure and since last 30 years there is a 24% decline in mangrove cover including this species in the range (Duke et al., 2010; Food & Agriculture Organization (FAO), 2007).

2. Materials and methods

2.1. Location of sampling sites

Sundarban wetland located at the Hooghly Matla estuary in the lower stretch of West Bengal consists of continuous and discontinuous mangrove vegetation belonging to the low-lying coastal zone in the northeast of the Bay of Bengal. This highly vulnerable and ecologically sensitive region in this lower deltaic plain of Bengal is typically sustained by a complex system made up of numerous tributaries, distributaries, confluences and tidal creeks. The Hooghly estuary, the first deltaic offshoot of the Ganges is a coastal plain estuary and serves as a lifeline for the people of that region. The Indian Sundarban geographically belongs to the region between the imaginary Dampier and Hodges line in the northwest, Bay of Bengal in the south, in the west river Hooghly and in the east river Icchamati, Kalindi, Raimangal and Harinbari forming the International boundary between India and Bangladesh. Due to vigorous tidal activity in this region, transportation and deposition of the silt in the tidal channels have been gradually transformed into several small islands (Bhattacharya, 1989).

Eight field locations across this estuarine region namely Topoban (S1), Junput (S2), Chemaguri (S3), Kaukhali (S4), Ghushighata (S5), Jharkhali (S6), Bali (S7) and Samshernagar (S8) were chosen based on their contamination category to evaluate the effect of metal contaminant on the biotic response of *A. officinalis* (Fig. 1). The fully matured, healthy leaves were collected from at least 10 different plants and pooled into one sample for each site. Three pooled leaf samples from each site were collected from mature trees of *A. officinalis* with similar height and health condition. Surface sediment samples from rooting zone (sampling depth approximately 10–20 cm) were also collected in triplicate from each of the corresponding sampling locations. Sediment and leaves of the mangrove species were stored in clean plastic zip lock pouch bags, kept in ice box and immediately transferred to the laboratory. The geographical co-ordinates of the sampling locations were marked using a Trimble Juno SD hand-held GPS.