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Assessment of Trace Metal Bioaccumulation by *Avicennia marina* (Forsk.) in the Last Remaining Mangrove Stands in Manila Bay, the Philippines

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Abstract Concentrations of lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) were evaluated in the sediments, roots and leaves of a mangrove species (*Avicennia marina*) in Las Piñas—Parañaque Critical Habitat and Ecotourism Area (LPPCHEA), Manila Bay. The concentrations showed a general pattern of Zn > Pb > Cu > Cd in sediments, Cu > Pb > Zn > Cd in roots and Cu > Zn > Pb > Cd in leaves. The trace metal concentrations in both sediments and plant tissues were below contamination threshold levels. Based on computed bioaccumulation indices, *A. marina* could be used for the phytostabilization and phytoextraction of Cu and Cd. The LPPCHEA mangrove ecosystem is an ecologically important ecosystem that will limit the spread of trace metals to the surrounding environment.

Keywords Mangroves · *Avicennia marina* · Pollution · Trace metals · Bioaccumulation · Phytostabilization · Manila Bay

Mangroves have the capacity to contain trace metals by accumulating them in the sediments and plant tissues (MacFarlane et al. 2003). The mangrove genus *Avicennia,* particularly the species *A. marina,* is known to have a higher accumulation capability than other genera (MacFarlane et al. 2007). This is due to its pneumatophores, the upward extensions of an underground root system that obtain oxygen through their lenticels. The pneumatophores provide air to the root zone, oxidizing the rhizospheres. The oxidized rhizospheres stabilize the metals in the sediments and make them available for plant absorption (MacFarlane et al. 2003). The capacity of *A. marina* to accumulate trace metals makes it a potentially valuable species for containing metals through bioaccumulation and possibly for the phytoremediation of metal-contaminated sites (Kularatne 2014).

Bioaccumulation indices can be calculated using the biological concentration factor (BCF), translocation factor (TF), and biological accumulation coefficient (BAC; Pilon-Smits 2005). These values are influenced by the accumulation and distribution of the trace metals between the roots and sediments (referred to as phytostabilization), and between the leaves and sediments (referred to as phytoextraction; Cheraghi et al. 2011). Phytostabilization is a mode of phytoremediation where plants reduce the mobility of the contaminants. Phytoextraction, on the other hand, refers to the ability of the plants to extract contaminants from their tissues (Uppangala, 2010). Despite the long practice of using plants in treating contaminated sediments (see for example Pilon-Smits 2005), such an approach of using *A. marina* has not been thoroughly studied yet, particularly in heavily urbanized and industrialized places like Manila Bay in the Philippines. There is a general perception that highly urbanized and industrialized areas contain high trace metal concentrations (Driscoll et al. 1994). However, mangroves have also been reported to be poor accumulators of trace metals in the sediments (Lacerda 1997) and can tolerate trace metals even at high concentrations (Usman et al. 2013).

The study was conducted in Las Piñas—Parañaque Critical Habitat and Ecotourism Area (LPPCHEA) located east of Manila Bay, the Philippines. It is a protected area from a reclaimed island and is recognized as having the last remaining mangrove forest in southeastern Metro Manila. The four main cities that surround the area have an estimated

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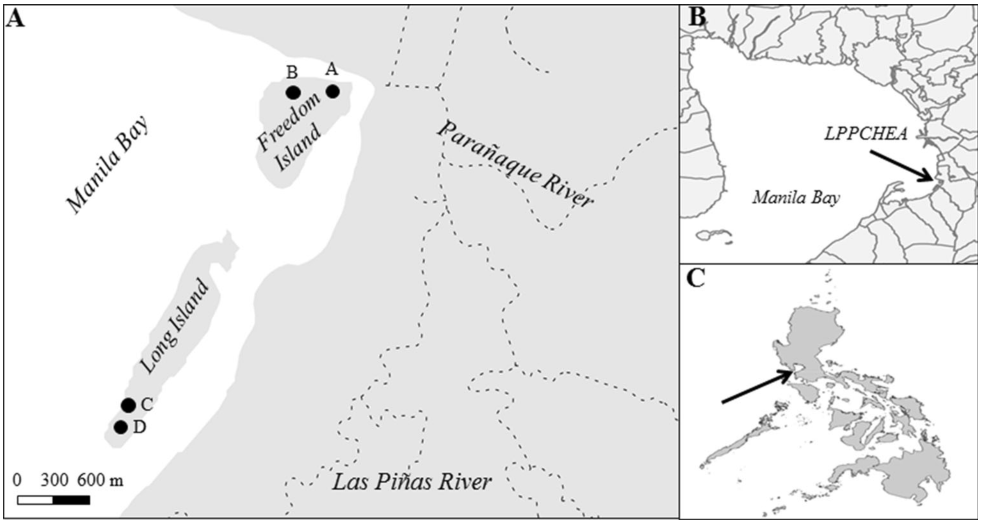

Fig. 1 **a** map of the four sampling sites at Las Piñas—Parañaque Critical Habitat and Ecotourism Area (LPPCHEA) denoting the water bodies (*dashed lines*), **b** map of Manila Bay pointing to LPPCHEA, and **c** map of the Philippines with an *arrow* pointing to the location of Manila Bay

total population of 2,053,784 (Pasay City—392,869; Parañaque City—588,126; Las Piñas City—552,573; and Bacoor City—520,216; Philippine Statistics Authority 2010). The LPPCHEA is adjacent to a highly urbanized and industrialized area that serves as a sink for untreated sewage and effluents (Velasquez et al. 2002). It also serves different activities including fishing, aquaculture, international shipping, and transportation (Prudente et al. 1997).

While Manila Bay is reported and generally perceived to be polluted (see for example Prudente et al. 1994), there are no data that are currently available on trace metal concentrations in the LPPCHEA mangroves. Thus, this study evaluated the concentrations of the trace metals including lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) in the sediments, roots and leaves of *A. marina* in LPPCHEA. From these values, the bioaccumulation and phytoremediation potentials of *A. marina* were also assessed.

Materials and Methods

The study area in LPPCHEA was divided into two parts: Freedom Island (near Parañaque River) and Long Island (near Las Piñas River; Fig. 1). There were four sampling sites that were randomly selected based on the presence and abundance of *A. marina* and the proximity to Parañaque River, which was assumed as the main source of trace metals. Table 1 showed the distance of the sampling sites from Parañaque River and their physico-chemical conditions. The pore water physico-chemical values in all sampling sites were homogenous, except for turbidity (Table 1). These values were obtained through in situ measurements using a Horiba water quality meter (Horiba U-10, Kyoto, Japan) and DO meter (Eutech Instruments, Singapore).

Field sampling was conducted on September 24, 2013 (a wet season in the Philippines). Sediments, roots and leaves of *A. marina* were collected ($n = 3$) at each site. Sediment samples were randomly collected at 15 cm depth using a fabricated polyvinyl chloride (PVC) core sampler (diameter = 5.5 cm). Roots and leaves were collected and composited from five trees for each site (within 5 m radius). Samples were sectioned and separately placed in resealable bags, which were then transported to the laboratory within the same collection date.

In the laboratory, the sediments and plant tissues were oven-dried and homogenized. The homogenized sediments and plant samples were digested following the procedures of Helrich (1990). The resulting digests were then subjected to atomic absorption spectroscopy (AAS; GBC 932, Australia) to determine the Pb, Cu, Zn, and Cd concentrations in the sediments, roots and leaves. The detection ranges are 2.5–20, 1–5, 0.2–1.8 and 0.4–1.5 mg/kg for Pb, Cu, Cd and Zn, respectively. Analytical errors for all trace metals in the standard reference for both sediment and plant tissue samples were below 5 %. Several (i.e. 10 % of the total number of samples) duplicate samples were used to check the precision by analytical splits. All laboratory analyses were conducted in the Department of Chemistry of the Ateneo de Manila University in Quezon City, Philippines.

The bioaccumulation indices were calculated using BCF, TF, and BAC following the methods of Yoon et al. (2006), Cui et al. (2007), and Li et al. (2007) (see equations below).

$$BCF = \frac{concentration\ in\ roots}{concentration\ in\ sediments} \tag{1}$$

$$TF = \frac{concentration\ in\ leaves}{concentration\ in\ roots} \tag{2}$$

Table 1 Physico-chemical characteristics of the pore water and total organic matter (TOM) content in the sediments of each sampling site (mean ± standard deviation)

Site	Distance from Parañaque River (m)	DO (mg/L)	pH	Electrical Conductivity (µS/cm)	Turbidity (NTU)	Temperature (°C)	Salinity (%)	TOM (%)
A	450	3.13	6.74	29.1	15	30	1.81	28.55 ± 11.98
B	751	1.70 ± 0.38	6.42 ± 0.60	35.43 ± 0.96	31.00 ± 5.20	29.93 ± 0.06	2.28 ± 0.01	18.25 ± 3.12
C	2,671	3.92 ± 0.56	6.52 ± 0.22	46.00 ± 0.36	46.33 ± 1.53	31.27 ± 0.06	2.99 ± 0.02	16.42 ± 3.53
D	2,760	4.27 ± 0.53	6.76 ± 0.30	45.67 ± 0.25	90.33 ± 8.39	31.1 ± 4.3e^{-15}	2.97 ± 0.01	30.45 ± 9.19

NTU nephelometric turbidity unit

$$BAC = \frac{concentration\ in\ leaves}{concentation\ in\ sediments} \tag{3}$$

If a plant has a BCF value >1 and TF value <1, it can be used for phytostabilization, whereas if the BAC value is >1, it can be used for phytoextraction (Cheraghi et al. 2011).

Correlation analysis was used to determine if the trace metal concentrations varied with distance from the assumed source of pollutants (Parañaque River). One-way analysis of variance (ANOVA) was used to determine if the trace metal concentrations including the BCF, TF and BAC values varied among sites. A separate one-way ANOVA was done to analyze if the trace metal concentrations varied among sediments, roots, and leaves (per site). Means were compared using the Tukey honest significant difference (HSD) test to evaluate variations in concentrations with compartments and with sites. All statistical tests were implemented using the R statistical software (version 3.0.2; R Core Team 2013).

Results and Discussion

Mean trace metal concentrations in the LPPCHEA were not correlated with distance from the assumed source (Parañaque River). However, the trace metal concentrations varied between the sediments, roots and leaves and with sites (Table 2). There were no significant differences for Cu in the sediments, Zn in the roots and Cd in the leaves across sites ($p > 0.05$). The metal concentrations in the sediments and plant tissues were below contamination thresholds based on standard values prescribed by Kabata-Pandias and Mukherjee (2007). These standards were used for comparison due to the unavailability of soil pollution standards in the Philippines.

Table 2 Concentrations (mg/kg) of Pb, Cu, Zn, and Cd, in the sediments, roots, and leaves per site (mean ± standard deviation)

Trace metal	Site	Sediments	Roots	Leaves
Pb	A	0.65 ± 0.36	nd	0.08 ± 0.10
	B	nd	nd	0.14 ± 0.15
	C	nd	0.04 ± 0.00	0.10 ± 0.07
	D	nd	0.30 ± 0.05	0.06 ± 0.05
	df			3
	MS			0.3 e^{-1}
	P			ns
Cu	A	0.24 ± 0.11	2.23 ± 0.10^{a}	1.19 ± 0.10^{a}
	B	0.10 ± 0.12	1.64 ± 0.18^{ab}	1.63 ± 0.15^{b}
	C	0.56 ± 0.48	1.97 ± 0.15^{ab}	1.02 ± 0.05^{a}
	D	0.33 ± 0.33	1.74 ± 0.07^{b}	2.23 ± 0.09^{ab}
	df	3	3	3
	MS	0.11	0.19	0.88
	P	ns	**	***
Zn	A	2.04 ± 0.75^{a}	0.20 ± 0.06	0.24 ± 0.02^{a}
	B	0.89 ± 0.15^{b}	0.08 ± 0.05	0.20 ± 0.01^{a}
	C	0.82 ± 0.03^{b}	0.14 ± 0.05	0.20 ± 0.01^{a}
	D	0.99 ± 0.15^{b}	0.17 ± 0.04	0.34 ± 0.04^{b}
	df	3	3	3
	MS	0.99	0.01	0.01
	P	*	ns	***
Cd	A	0.01 ± 0.003^{a}	0.05 ± 0.01^{a}	0.10 ± 0.03
	B	0.01 ± 0.004^{a}	0.12 ± 0.02^{b}	0.06 ± 0.04
	C	0.01 ± 0.002^{a}	0.02 ± 0.00^{a}	0.10 ± 0.04
	D	0.004 ± 0.003^{b}	0.02 ± 0.02^{a}	0.05 ± 0.04
	df	3	3	3
	MS	5.89 e^{-5}	0.01	0.02 e^{-1}
	P	*	***	ns

Different letters between and among sites indicate significant difference at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; *ns* not significant; *nd* not detected

The trace metal concentrations (for all metals) ranged from 0.004 to 2.04, 0.02 to 2.23 and 0.05 to 2.23 mg/kg in the sediments, roots and leaves, respectively (Table 2). The Pb concentration in the sediments from Site A was 0.65 mg/kg, while the concentration in plant tissues ranged from 0.04 to 0.30 mg/kg (mean: 0.23 ± 0.16 mg/kg for roots; 0.09 ± 0.09 mg/kg for leaves). No range of concentration could be given for Pb content in the sediments from the other sites because their concentrations were below the limit of detection. The trace metal with the highest concentration in the sediments was Zn (2.04 mg/kg), while Cu was the highest in roots and leaves, containing high concentrations of 2.23 mg/kg in both. The general patterns of accumulation in the sediments were $Zn > Pb > Cu > Cd$. In roots, the order was $Cu > Pb > Zn > Cd$, and in leaves, the order was $Cu > Zn > Pb > Cd$. These patterns were similar to those reported by MacFarlane et al. (2003) in Australia, Parvaresh et al. (2011) in Iran, and Almahasheer et al. (2013) in Eastern Saudi Arabia. Their results also showed that Zn had the highest concentrations in the sediments followed by Pb, and then Cd and Cu. These authors also reported that in their plant samples, Cu had the highest concentrations followed by Zn and Pb. Cadmium had the lowest concentration in all compartments and in all sites.

The pH and organic matter (OM) content in the sediments affects the availability and mobility of trace metals (Matagi et al. 1998; Peng et al. 2009). Since mangrove sediments are generally anoxic and waterlogged, trace metals are precipitated as insoluble sulphides (Lacerda 1997). With high sediment pH and OM content (average across sampling sites; 6.63 ± 0.08 % and 23.42 ± 3.55 %, respectively; Table 1), trace metals are stabilized and undergo complexation with OM resulting in low trace metal bioavailability (MacFarlane et al. 2003). The stabilized sediments limit the absorption of trace metals from the sediments into the plant tissues (Malik et al. 2010). These factors probably explain why the measured trace metal concentrations were surprisingly low (Table 2).

The concentrations of trace metals in the roots and leaves varied with the type of trace metal (Table 2). The concentrations are affected by the mobility and the capacity of the plant to translocate a particular trace metal. Matagi et al. (1998) proposed that the plant tissues in the cell walls absorb trace metals. Hence, mangroves like other wetland habitats can absorb trace metals in plant tissues. Du Laing et al. (2009) proposed that trace metals are accumulated in the roots but have a reduced transfer into the shoots. In this study, Cu had at least four to five times higher concentrations in the roots and leaves than in the sediments (Table 2). The high concentrations of Cu and Zn (classified as essential elements; Kabata-Pandias and Mukherjee 2007) in the plant tissues are affected by the metabolic requirements of the mangrove (Parvaresh et al. 2011). In contrast, Pb and Cd (classified as non-essential elements) are very mobile in the sediments (Kabata-Pandias and Mukherjee 2007). These metals have low translocation in the plant tissues, as these are strongly excluded at the root epidermis of *A. marina* (MacFarlane et al. 2003, 2007). Baker and Walker (1990) proposed that the possible physiological mechanisms that may restrict the uptake and translocation of trace metals in plants include cell wall immobilization, complexation with humic substances, and the presence of barriers at the root endodermis.

Table 3 The biological concentration factor (BCF), translocation factor (TF) and biological accumulation coefficient (BAC) of Pb, Cu, Zn, and Cd in *A. marina* sediments, roots and leaves (mean ± standard deviation)

Metal	Site	BCF	TF	BAC
Pb	A	nd	nd	0.14 ± 0.13
	B	nd	nd	nd
	C	nd	2.97	nd
	D	nd	0.10 ± 0.07	nd
	df			
	MS			
	P			
Cu	A	6.42 ± 2.29	0.89 ± 0.13^{a}	5.67 ± 2.22
	B	36.17 ± 27.95	1.00 ± 0.02^{a}	36.06 ± 27.52
	C	13.98 ± 19.70	0.52 ± 0.04^{b}	7.57 ± 10.88
	D	10.24 ± 8.24	1.29 ± 0.07^{ab}	13.09 ± 10.10
	Mean	16.70 ± 19.27	0.92 ± 0.30	15.60 ± 12.68
	df	3	3	3
	MS	534.10	0.30	587.90
	P	ns	***	ns
Zn	A	0.10 ± 0.03	1.23 ± 0.38	0.13 ± 0.07^{a}
	B	0.09 ± 0.08	3.62 ± 2.13	0.22 ± 0.04^{a}
	C	0.17 ± 0.06	1.60 ± 0.62	0.24 ± 0.01^{a}
	D	0.18 ± 0.05	2.06 ± 0.67	0.35 ± 0.04^{b}
	Mean	0.14 ± 0.06	2.13 ± 1.38	0.24 ± 0.09
	df	3	3	3
	MS	0.01	3.31	0.02
	P	ns	ns	**
Cd	A	2.92 ± 0.94	4.59 ± 2.86	12.71 ± 6.40
	B	8.59 ± 1.85	0.54 ± 0.35	4.31 ± 2.48
	C	1.43 ± 0.50	5.3 ± 0.20	9.19 ± 4.52
	D	5.58 ± 2.16	4.25 ± 3.27	19.33 ± 12.90
	Mean	5.27 ± 3.03	3.34 ± 2.92	11.39 ± 8.67
	df	3	3	3
	MS	21.56	11.53	119.78
	P	*	ns	ns

Different letters between and among sites indicate significant difference at ** $p < 0.01$, *** $p < 0.001$; *ns* not significant

There were no significant differences for the BCF, TF, and BAC means among sites for any of the trace metals except for the TF of Cu ($p < 0.001$) and BAC of Zn ($p < 0.01$; Table 3). The trace metals with BCF values >1 were Cu (mean: 16.70 ± 19.27) and Cd (mean: 5.27 ± 3.03), and the trace metal with a TF value <1 was Cu (mean: 0.92 ± 0.30). This implies that *A. marina* can be used for the phytostabilization of Cu. Consistent with published reports, *A. marina* is known to immobilize Cu from roots to leaves because of its endodermal casparian strip (MacFarlane and Burchett 2000). The trace metals with BAC values >1 were Cu (mean: 15.60 ± 18.41) and Cd (mean: 11.39 ± 8.67) implying that these trace metals can be phytoextracted by *A. marina*. The capacity of *A. marina* to phytoremediate (through phystostabilization and phytoextraction) conforms to several reports (see for example MacFarlane et al. 2003, 2007; Lotfinasabasl and Gunale 2012).

Our computed TF and BAC values are within the range reported for *A. marina,* although the BCF is much higher (see reviews in MacFarlane et al. 2007). The trace metal concentrations in the sediments reported here are extremely low (<2.10 mg/kg). While it may be contested that the bioaccumulation indices may not be applicable because of the low trace metal concentrations in the sediments, we argue however that these indices are intended to assess the actual ratio of trace metal concentrations found between that of the roots and in the sediments, and that of the leaves and in the sediments (Cheraghi et al. 2011). Neglecting these ratios may ignore the important role of mangroves in bioaccumulating metals in the plant tissues.

In summary, this study showed that Pb, Zn, Cu, and Cd were detected in *A. marina* sediments, roots, and leaves. The essential metals, Cu and Zn, had the highest concentrations in the plant tissues. In comparison with trace metal concentrations in the sediments reported near LPPCHEA and around Manila Bay (Prudente et al. 1994; Velasquez et al. 2002), the values reported here were much lower. Hosono et al. (2010) proposed that the increased compliance in environmental regulations after the 1990s may have contributed to the general improvement of the environmental situation, and thus helped to reduce trace metal concentrations in Manila Bay.

We demonstrated that *A. marina* has the potential to contain trace metals (Cu and Cd) through phytostabilization and phytoextraction. Mangroves, just like other wetland habitats, limit the spread and effectively function as long-term sinks of trace metals. This means that if the mangrove trees are removed from LPPCHEA, trace metals may become more readily bioavailable to enter the food chain (Matagi et al. 1998). This is important especially in the study area where coastal residents get most of their food resources from Manila Bay. Thus, aside from providing food and ecotourism benefits, the LPPCHEA is currently performing as an effective phytostabilizer that limits the bioavailability and spread of trace metals in the surrounding environment. These ecological and socioeconomic services may be disturbed under the current threat of a reclamation project (Mayor-Gordove and Aguinaldo 2013).

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