Accumulation and partitioning of heavy metals in mangroves: A synthesis of field-based studies

Geoff R. MacFarlane^{a,*}, Claudia E. Koller^a, Simon P. Blomberg^b

^a School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia

^b Centre for Resource and Environmental Studies, Australian National University, ACT, Australia

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Abstract

We report the findings of a comparative analysis examining patterns of accumulation and partitioning of the heavy metals copper (Cu), lead (Pb) and zinc (Zn) in mangroves from available field-based studies to date, employing both species level analyses and a phylogenetic approach. Despite mangroves being a taxonomically diverse group, metal accumulation and partitioning for all metals examined were broadly similar across genera and families. Patterns of metal accumulation were also similar regardless of whether species were classified as salt secreting or non-secreting. Metals were accumulated in roots to concentrations similar to those of adjacent sediments with root bio-concentration factors (BCF; ratio of root metal to sediment metal concentration) of ≤ 1 . Root BCFs were constant across the exposure range for all metals. Metal concentrations in leaves were half that of roots or lower. Essential metals (Cu and Zn; translocation factors (TF; ratio of leaf metal to root metal concentration) of 0.52 and 0.53, and leaf BCFs of 0.47 and 0.51, respectively) showed greater mobility than non-essential metals (Pb; TF of 0.31 and leaf BCF of 0.11). Leaf BCFs for the essential metals Cu and Zn decreased as environmental concentrations increased. The non-essential metal Pb was excluded from leaf tissue regardless of environmental concentrations. Thus mangroves as a group tend to operate as excluder species for non-essential metals and regulators of essential metals. For phytoremediation initiatives, mangrove ecosystems are perhaps best employed as phytostabilisers, potentially aiding in the retention of toxic metals and thereby reducing transport to adjacent estuarine and marine systems.

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1. Introduction

Mangrove ecosystems are diverse intertidal wetlands, commonly situated in tropical, sub-tropical and temperate coastal systems. They are highly productive and play a vital role as a major primary producer within estuarine systems. Mangrove systems serve as habitat and nursery area for many juvenile fish and crustaceans, which have both direct and indirect socio-economic importance. They also provide erosion mitigation and stabilisation for adjacent coastal landforms (Harty, 1997).

Despite their importance, mangrove ecosystems have been exploited and subject to inappropriate management practices in terms of land reclamation and unsustainable forestry, as well as agricultural and aquaculture initiatives (West et al., 1983; Eong, 1995). As a consequence of their close proximity to urban development, they have experienced significant direct contaminant input. Among the major pollutants from anthropogenic inputs are heavy metals (MacFarlane, 2002).

Metallic enrichment of mangrove environments arises from urban and agricultural runoff, industrial effluents, boating and recreational use of waterbodies, chemical spills, sewage treatment plants, leaching from domestic garbage dumps and mining operations (Peters et al., 1997). The metal pollutants most commonly entering estuarine

* Corresponding author. Tel.: +61 24917858; fax +61 249216923.

E-mail address: Geoff.MacFarlane@newcastle.edu.au (G.R. MacFarlane).

systems through industrial sources (based on frequency of detection) are Cu, Pb and Zn (Mills, 1995). These metals consequently occur in high concentrations in many estuaries, with concentrations reaching up to $1000 \mu\text{g Cu g}^{-1}$, $1000 \mu\text{g Pb g}^{-1}$ and $2000 \mu\text{g Zn g}^{-1}$ in contaminated sediments (Irvine and Birch, 1998).

Despite their potential exposure to metal contaminated sediments, mangroves appear to be highly tolerant to heavy metals. Numerous laboratory-based toxicity trials suggest that the concentrations of metals required to impart significant negative effects on mangroves may be several orders of magnitude higher when compared to their aquatic and terrestrial floral counterparts. Thomas and Eong (1984) have found no adverse effects on the growth of seedlings of *Rhizophora mucronata* Lam. and *Avicennia alba* Bl. treated with Zn ($10\text{--}500 \mu\text{g ml}^{-1}$) and Pb ($50\text{--}250 \mu\text{g ml}^{-1}$). In *Kandelia candel* (L.) Druce seedlings, inhibition of leaf and root development was observed only at the highest applied metal concentrations (400 mg kg^{-1} Cu and Zn) (Chiu et al., 1995). Similarly, MacFarlane and Burchett (2002) found Pb ($0\text{--}800 \mu\text{g g}^{-1}$) had little negative effect on *Avicennia marina* (Forsk.) Vierh. seedlings. Copper and Zn showed low relative toxicities in terms of emergence and biomass/growth in *A. marina* with LC_{50} values for emergence $566 \mu\text{g g}^{-1}$ for Cu and $580 \mu\text{g g}^{-1}$ for Zn. The LOEC values for reductions in growth/biomass parameters were $400 \mu\text{g g}^{-1}$ for Cu and $500 \mu\text{g g}^{-1}$ for Zn.

Tolerance has been in part attributed to the ability of mangroves to exclude metals or regulate uptake of metals at the root level and limit translocation to the shoot (MacFarlane and Burchett, 2002). A number of studies have found metals such as Cu, Zn, Pb, Fe, Mn and Cd to be accumulated predominantly in root tissue, rather than in foliage, in numerous mangrove species in the field, such as *Avicennia* spp., *Rhizophora* spp. and *Kandelia* spp. (Peters et al., 1997). Data seem to suggest that for some mangrove taxa at least, concentrations of translocated metals are low, with bio-concentration factors (BCF; ratio of leaf metal to corresponding sediment metal concentration) ranging from <0.01 in *Rhizophora mangle* to 0.06 for other species such as *A. marina* (Lacerda, 1997). Other studies suggest that mangroves may accumulate and translocate some metals with leaf BCFs greater than one, e.g. $1.5\text{--}2.4$ for *A. marina* (Sadiq and Zaidi, 1994), 1.7 for *Aegiceras corniculatum* and 1.2 for *Kandelia candel* (Chen et al., 2003), thereby meeting established criteria for hyperaccumulating species (Baker and Whiting, 2002). To date, little attention has been directed at the systematic examination of cross-species patterns of metal accumulation and translocation in mangroves.

The word 'mangrove' is often employed interchangeably to refer to either the plants (trees/shrubs) which reside in intertidal communities, and/or to the community itself (Tomlinson, 1986). Within the current context, we reserve the use of the term to apply strictly to the constituent plants rather than the community. Mangroves are a heterogeneous group of 80 taxonomically diverse species, in 30 genera and

20 families (Field, 1995). The classification of a plant as a mangrove largely rests on its exclusive distribution within intertidal estuarine environments rather than on any distinct botanical, taxonomic or phylogenetic convention (Tomlinson, 1986). Various taxa have evolved distinct morphological specialisations for dealing with anoxic sediments, periodic inundation and maintaining osmotic balance in (hyper)saline environments. Members of the families Rhizophoraceae possess aerial stilts while members of the families Avicennaceae and Sonneratiaceae possess aerial extensions of the subterranean root system termed 'pneumatophores'. These differing morphologies function to enable gaseous exchange and oxygenation for respiration in an anoxic environment (Hutchings and Saenger, 1987). More landward families inhabiting less anoxic sediments, such as members of Myrsinaceae, possess no aerial roots. The anatomy of the nutritive root varies widely among taxa, even within families (Lawton et al., 1981). Roots of most mangrove species possess two main barriers to the transport of Na^+ and other ions to the vasculature, including layers of the root epidermis and the endodermis. Mechanisms and relative contributions of these tissues to the regulation/exclusion of ion transport vary dependent on whether a species is a salt secretor or a non-secretor (predominantly excluders), with secretors generally showing greater ion mobility and translocation (Kramer and Preston, 1978; Lawton et al., 1981). Heavy metal uptake in mangroves is also regulated at the root endodermis through modifying uptake from predominantly apoplastic to selective symplastic transport. The contribution of each tissue type is dependent on the molecular properties of the plasmalemma (i.e. specific membrane transport proteins) and on the metal in question (MacFarlane and Burchett, 2000; Greger, 2004).

Some genera such as *Aegiceras* and *Avicennia* secrete excessive Na^+ and K^+ through specialised glands or glandular trichomes on abaxial and adaxial leaf surfaces. Such mechanisms are absent in non-secretors, e.g. *Rhizophora* and *Sonneratia* (MacFarlane and Burchett, 1999). Indeed mangroves, and a number of other estuarine halophytes with glandular tissue, have been observed to excrete heavy metals concomitantly with other solutes (Neumann et al., 1995; MacFarlane and Burchett, 2000). It stands to reason that variation in morphology/function of nutritive root tissue and glandular tissue to deal with the challenges of excess cations in saline environments may confer significant implications for metal accumulation, transport, partitioning and excretion among species.

These issues lead us to ask whether consistent patterns of metal accumulation occur across phylogenetically distinct taxa inhabiting contaminated estuarine sediments, or conversely, if mangrove taxa exhibit variation in accumulation strategies. Do species vary in terms of metal accumulation dependent on whether their predominant strategy of dealing with salinity is salt secretion or non-secretion (predominantly exclusion at the root)? Equally, do patterns of metal accumulation vary across taxa with sediment metal loadings?

We chose to investigate these questions using a dataset of published studies from the literature, where metal concentrations have been quantified in mangrove sediments and mangrove root and leaf tissue under field conditions.

2. Methods

2.1. Data collection

A survey of the literature was conducted to arrive at a total of 21 published studies where accumulation of the metals Cu, Pb and Zn have been assessed in mangrove species under field conditions worldwide. Copper, Pb and Zn were chosen as representative trace metals from classes of metals which are essential (Cu and Zn) or non-essential (Pb) to plant metabolism, as the patterns of accumulation are likely to vary dependant on essentiality (Baker, 1981). Copper, Pb and Zn are also metals which occur at high concentrations in many contaminated locales and are considered significant metal contaminants due to high frequency of detection/occurrence (Mills, 1995). Only 'true mangroves' were considered appropriate for inclusion as candidate species. True mangroves exhibit fidelity to the mangal environment, possess morphological adaptations for intertidal environments, display physiological mechanisms for salt exclusion and/or regulation and exhibit taxonomic separation from terrestrial elements. True mangroves may be further delimited into major mangrove elements and minor mangrove elements depending on their ability to form a conspicuous element or monospecific stands within a mangal (Tomlinson, 1986).

Only those studies were included where metals were quantified on a dry weight/weight basis (ppm or $\mu\text{g g}^{-1}$), or could be calculated from original data. Where sampling was conducted at various locations with differing sediment metal levels, ranges were presented. If temporal monitoring of metals in sediment and plant tissue was conducted, values were averaged over the sampling period. Where metals were analysed at varying depths of the sediment profile, an average of horizons was calculated.

Three relative measures of metal uptake were chosen to enable interspecific comparisons of metal uptake/distribution and comparisons among metals. Root BCF (ratio of root metal to sediment metal concentration) and leaf BCF were calculated to assess concentrations in specific tissue types relative to environmental loadings. Translocation factors (TF; ratio of leaf metal to root metal concentration) were calculated to enable assessment of transport of accumulated metal from root to shoot. Root BCFs, leaf BCFs and TFs represent averages of original data.

When amassing data from a variety of unrelated studies, significant problems arise in terms of the validity of the data obtained for comparative purposes. Depending on the location of sampling and the sediment type encountered, uptake of metals may vary significantly as anoxic sediments high in sulphur and organic content may exhibit low metal bioavailability (Harbison, 1986). Thus a specific

BCF will reflect the species ability to accumulate a particular metal in a particular habitat, but this may be an underestimate of the species' ability to accumulate the metal in question under sediment conditions where metals exhibit greater availability. Metal availability may vary depending on numerous other site specific sediment physicochemical considerations including the cation exchange capacity, pH, redox status, metal speciation, nutrient availability and salinity of sediments (Greger, 2004). For many species, data are available across a limited range of sediment contaminant loadings, hence the patterns observed may be skewed and not be representative of a particular species' response in highly contaminated locales. Sample size differs both within and across studies for some species, thus anomalous data may unduly weigh on interpretation.

Analysis methodology for metals varies among candidate studies, in that different acids and concentrations are employed. However, none of sediment metal concentrations came from digestions which included HF, the only method that extracts metals from the silicate matrix. Of the studies that reported results obtained from certified sediment reference materials (SRM), extraction with $\text{H}_2\text{O}_2 + \text{HCl} + \text{HNO}_3 + \text{HClO}_4$ gave results within 10% of the reference material (Silva et al., 1990). Microwave digestion in $\text{HNO}_3 + \text{HCl}$ within 15% of SRM (Marchand et al., 2006) and hotplate digestion in $\text{HNO}_3 + \text{H}_2\text{O}_2$ within 17% of SRM (MacFarlane, 2002; MacFarlane et al., 2003). Other acids used were $\text{HNO}_3 + \text{HClO}_3$ (Peng et al., 1997; Alongi et al., 2003), $\text{HNO}_3 + \text{HSO}_3$ (Ong Che, 1999), and 0.1 N HCl (Chiu and Chou, 1991) or 0.1 M HCl (Lacerda, 1997) to examine the weakly bound fraction. A comparison of digests obtained for four sediment reference materials by aqua regia extraction and $\text{HNO}_3 + \text{H}_2\text{O}_2$ extraction in a microwave and heating block showed consistent recovery of $87 \pm 1.5\%$ for Cu, $78 \pm 0.9\%$ for Zn (except for PACS-1 at $49 \pm 9.2\%$) and lower and more variable recovery for Pb ($62 \pm 4.8\%$). Overall, there was no difference between the three extraction methods tested (Tam and Yao, 1999). Thus estimates of sediment metal loadings among studies may differ somewhat based on the extraction method employed, though this is likely to affect BCFs to a greater degree than TFs, as plant tissues dissolve much more readily than sediments in weak acid digestions.

Finally, significant variation may exist in data depending on the nature of sampling protocols for the collection of vegetation, including age/life stage of individual sampled, leaf age, leaf/branch position, position in the stand and level of replication. Environmental variation among studies in terms of temperature, light and frequency of inundation may also influence metal uptake patterns (Greger, 2004). Thus we acknowledge that numerous biases may exist in the final dataset. Nonetheless, for species where heavy metal accumulation has been assessed under field conditions, it is instructive to attempt to ascertain broad accumulation patterns, though the data should be viewed cautiously.

Table 1

Distribution of the heavy metals: (a) copper, (b) zinc and (c) lead ($\mu\text{g g}^{-1}$) in sediment and in the roots and leaves of 19 mangrove species based on the published literature

Family	Species	Location	Sediment	Roots	Leaves	Root BCF	Leaf BCF	TF
<i>a. Copper (Cu)</i>								
Acanthaceae	<i>Acanthus ilicifolius</i>	Hainan Island, China ⁶ Mai Po, Hong Kong ¹¹	50	41	2.1	0.82	0.07	
Avicenniaceae	<i>Avicennia marina</i>	Saudi coast ¹³ Ting Kok, Hong Kong ² W. Australia ¹ Shenzhen, S. China ²⁰ Shenzhen, China ¹² Karachi, Pakistan ¹⁹ SE Australia ⁸ SE Australia ⁷ N. Australia ¹⁴ Hainan Island, China ⁶	1.8 13 16 36 36 12–56 61 1–102 25–134		4.4 16 7.3 5 5.2 3.2–14 9 3.0–20 9.8–26 6.3		2.42 1.26 0.47 0.14 0.15 0.27 0.15 0.25 0.28 0.46	1.23 0.40 0.36 0.39 0.09
	<i>Avicennia officinalis</i>	E. India ¹⁵ India ¹⁸	6.3 303		2.9 102	0.92	0.58 0.47 0.33 0.40	0.50
Combretaceae	<i>Laguncularia racemosa</i>	Pacific Panama ⁴ SE Brazil ⁹	56 18–80		3.7 7.0–10		0.07 0.25 0.16	
Meliaceae	<i>Xylocarpus granatum</i>	E. India ¹⁵	3.8		2.1		0.57	
Myrsinaceae	<i>Aegiceras corniculatum</i>	Ting Kok, Hong Kong ² N. Australia ¹⁴ Shenzhen, China ¹⁷ Mai Po, Hong Kong ¹¹ Hainan Island, China ⁶	10 34 41 46	16 33	17 9.6 4.1 0.71 7.7	1.62	1.66 0.28 0.10 1.16 0.65	1.03
Rhizophoraceae	<i>Bruguiera cylindrica</i> <i>Bruguiera gymnorrhiza</i> <i>Bruguiera sexangula</i> <i>Ceriops decandra</i> <i>Ceriops tagal</i>	E. India ¹⁵ Hainan Island, China ⁶ Hainan Island, China ⁶ E. India ¹⁵ Hainan Island, China ⁶ N. Australia ¹⁴	2.8 2.8 31–80		1.9 7.8 7.8 1.2 5.4 7.4–17		0.69 0.57 0.56 0.41 0.39 0.28 0.34	
	<i>Kandelia candel</i>	Ting Kok, Hong Kong ² Shenzhen, China ¹⁷ Mai Po, Hong Kong ¹¹ Tamshui estuary, Taiwan ¹³ Hainan Island, China ⁶ Fujian, China ²⁰	8.8 41 42 4.5–55	12 24 35–165	11 4.1 0.58 3.1–10 3.1	1.33 0.58 3.36	1.20 0.10 0.21 0.23 0.07 1.76 1.90 0.04 0.63 0.27 0.47 0.06 0.42 0.18 0.22 1.44 0.49 0.54 0.47	0.90 0.14
	<i>Rhizophora mangle</i> <i>Rhizophora mucronata</i> <i>Rhizophora stylosa</i>	Rio de Janeiro, Brazil ¹⁶ E. India ¹⁵ W. Australia ¹ Yingluo Bay, China ²¹ N. Australia ¹⁴ Hainan Island, China ⁶	2.7 2.8 14 19 39	5.1 1.1	0.1 1.8 3.7 0.6 16 2.4		0.04 0.63 0.27 0.03 0.42 0.18 0.22 1.44 0.49 0.54 0.47	0.02 0.57 0.55
Sonneratiaceae	<i>Sonneratia apetala</i> <i>Sonneratia caseolaris</i> <i>Sonneratia ovata</i>	Guangdong, China ⁵ Hainan Island, China ⁶ Hainan Island, China ⁶			6.8 7.4 Average	1.1	0.47	0.52
<i>b. Lead (Pb)</i>								
Acanthaceae	<i>Acanthus ilicifolius</i>	Hainan Island, China ⁶ Mai Po, Hong Kong ¹¹	220	33	0.03	0.15	0.00	
Avicenniaceae	<i>Avicennia marina</i>	Saudi coast ¹³ N. Australia ¹⁴ Shenzhen, S. China ²⁰ Ting Kok, Hong Kong ² Shenzhen, China ¹⁷ Karachi, Pakistan ¹⁹	12 8.0–27 31 33 34 32–59		6.9 1.1–4.7 1.8 8.0 1.9 11–23		0.58 0.15 0.06 0.24 0.06 0.36	0.53 0.55 0.54

(continued on next page)

Table 1 (continued)

Family	Species	Location	Sediment	Roots	Leaves	Root BCF	Leaf BCF	TF
Combretaceae	<i>Avicennia officialis</i> <i>Laguncularia racemosa</i>	SE Australia ⁸	100	164	5	1.64	0.05	0.03
		SE Australia ⁷	3–414		0.1–2.9		0.04	
		Hainan Island, China ⁶			0.02		0.00	
						0.57	0.17	0.41
		India ¹⁸	1484		168		0.11	
		Pacific Panama ⁴	78		6.1		0.08	
Myrsinaceae	<i>Aegiceras corniculatum</i>	SE Brazil ⁹	20–130		1.0–8.9		0.07	
							0.08	
		N. Australia ¹⁴	12		1.6		0.13	
Rhizophoraceae	<i>Bruguiera gymnorrhiza</i> <i>Bruguiera sexangula</i> <i>Ceriops tagal</i>	Ting Kok, Hong Kong ²	29	27	7.9	0.94	0.28	0.29
		Mai Po, Hong Kong ¹¹	215	30		0.14		
						0.54	0.20	
							0.01	
	<i>Kandelia candel</i>	Hainan Island, China ⁶			0.02		0.01	
		Hainan Island, China ⁶			0.03		0.01	
		Hainan Island, China ⁶			0.03		0.01	
		N. Australia ¹⁴	9.7–20		1.6–6.1		0.27	
							0.14	
		Guangzhou, China ¹⁰	23	67	2.4	2.87	0.10	0.04
		Ting Kok, Hong Kong ²	29	28	11	0.95	0.37	0.39
		Guangzhou, China ¹⁰	32	63	4.8	1.99	0.15	0.08
		Tamshui estuary, Taiwan ¹³	11–36	16–77	1.0–5.0	2.03	0.17	0.11
		Mai Po, Hong Kong ¹¹	162	40		0.25		
		Hainan Island, China ⁶			0.04		0.01	
Fujian, China ²⁰					0.04			
Sonneratiaceae	<i>Sonneratia caseolaris</i> <i>Sonneratia ovata</i>				1.62	0.14	0.15	
		Rio de Janeiro, Brazil ¹⁶	9.9	0.2	0.01	0.02	0.00	0.07
		Yingluo Bay, China ²¹	10	0.9	0.8	0.09	0.08	0.83
		N. Australia ¹⁴	16		2.2		0.14	
		Hainan Island, China ⁶			0.04		0.00	
<i>c. Zinc (Zn)</i> Acanthaceae	<i>Acanthus ilicifolius</i>					0.07		
Avicenniaceae	<i>Avicennia marina</i>	Hainan Island, China ⁶			0.03			
		Hainan Island, China ⁶			0.02			
					Average	0.84	0.11	0.31
Combretaceae	<i>Laguncularia racemosa</i>	Hainan Island, China ⁶			60		0.57	
		Mai Po, Hong Kong ¹¹	321	207		0.64		
		Saudi coast ¹³	7.3		11		1.56	
Meliaceae	<i>Xylocarpus granatum</i> <i>Aegiceras corniculatum</i>	W. Australia ¹	34	16	14	0.48	0.42	0.88
		Ting Kok, Hong Kong ²	55	16	15	0.29	0.28	0.96
		Shenzhen, S. China ²⁰	99	70	23	0.71	0.23	0.33
		Shenzhen, China ¹²	106	79	23	0.75	0.22	0.29
		N. Australia ¹⁴	46–210		16–52		0.31	
		SE Australia ⁸	243	295	25	1.21	0.10	0.08
		SE Australia ⁷	10–289		12–46		0.35	
		Karachi, Pakistan ¹⁹	35–67		10–19		0.29	
		Hainan Island, China ⁶			20		0.70	
						0.69	0.45	0.51
Combretaceae	<i>Laguncularia racemosa</i>	E. India ¹⁵	1.5		1.2		0.8	
		India ¹⁸	764		120		0.16	
							0.48	
Meliaceae	<i>Xylocarpus granatum</i> <i>Aegiceras corniculatum</i>	Pacific Panama ⁴	105		36		0.34	
		SE Brazil ⁹	27–610		14–28		0.31	
							0.33	
Rhizophoraceae	<i>Bruguiera cylindrica</i> <i>Bruguiera gymnorrhiza</i> <i>Bruguiera sexangula</i>	E. India ¹⁵	0.9		0.47		0.55	
		Ting Kok, Hong Kong ²	53	33	17	0.63	0.31	0.50
		N. Australia ¹⁴	97		30		0.31	
		Shenzhen, China ¹⁷	146		85		0.58	
		Mai Po, Hong Kong ¹¹	281	83		0.29		
Rhizophoraceae	<i>Bruguiera cylindrica</i> <i>Bruguiera gymnorrhiza</i> <i>Bruguiera sexangula</i>	Hainan Island, China ⁶			27		0.95	
						0.46	0.54	
							1.23	
Rhizophoraceae	<i>Bruguiera cylindrica</i> <i>Bruguiera gymnorrhiza</i> <i>Bruguiera sexangula</i>	E. India ¹⁵	0.9		1.2		1.23	
		Hainan Island, China ⁶			5.7		0.20	
		Hainan Island, China ⁶			30		1.10	

Table 1 (continued)

Family	Species	Location	Sediment	Roots	Leaves	Root BCF	Leaf BCF	TF
	<i>Cerriops decandra</i>	E. India ¹⁵	0.9		0.7		0.75	
	<i>Cerriops tagal</i>	Hainan Island, China ⁶			6.8		0.24	
		N. Australia ¹⁴	85–118		13–29		0.21	
							0.23	
	<i>Kandelia candel</i>	Ting Kok, Hong Kong ²	47	29	23	0.61	0.49	0.80
		Shenzhen, China ¹	146		70		0.48	
		Tamshui Estuary, Taiwan ³	40–164	49–340	15–20	1.90	0.77	0.27
		Mai Po, Hong Kong ¹¹	277	123		0.44		
		Hainan Island, China ⁶			9.8		0.34	
		Fujian, China ²⁰					0.19	
						0.99	0.45	0.53
	<i>Rhizophora mangle</i>	Rio de Janeiro, Brazil ¹⁶	19	20	7.2	1.04	0.38	0.36
	<i>Rhizophora mucronata</i>	E. India ¹⁵	0.9		0.9		1.05	
	<i>Rhizophora stylosa</i>	W. Australia ¹	29	15	6.6	0.53	0.23	0.43
		Yingluo Bay, China ²¹	47	6.2	5.9	0.13	0.13	0.95
		N. Australia ¹⁴	53		26		0.48	
		Hainan Island, China ⁶			5.7		0.20	
						0.33	0.26	0.69
							0.65	
	<i>Sonneratia apetala</i>	Guangdong, China ⁵					1.30	
	<i>Sonneratia caseolaris</i>	Hainan Island, China ⁶			36		0.68	
	<i>Sonneratia ovata</i>	Hainan Island, China ⁶			19			
					Average	0.69	0.51	0.53

¹Alongi et al. (2003); ²Chen et al. (2003); ³Chiu and Chou (1991); ⁴Defew et al. (2005); ⁵Han et al. (2004); ⁶Lian et al. (1999); ⁷MacFarlane (2002); ⁸MacFarlane et al. (2003); ⁹Machado et al. (2002); ¹⁰Miao and Chen (1998); ¹¹Ong Che (1999); ¹²Peng et al. (1997); ¹³Sadiq and Zaidi (1994); ¹⁴Saenger et al. (1990); ¹⁵Sarangi et al. (2002); ¹⁶Silva et al. (1990); ¹⁷Tam et al. (1995); ¹⁸Thomas and Fernandez (1997); ¹⁹Zahir et al. (2004); ²⁰Zheng and Lin (1996); ²¹Zheng et al. (1997).

Values in bold represent averages for each species.

With these limitations in mind, and on the basis of the aforementioned criteria, a final dataset of 19 species from seven families across a range of geographical locations and heavy metal contaminant profiles were examined (Table 1a–c).

2.2. Statistical analyses

2.2.1. Species level analyses

Relationships between sediment metal loadings and BCFs across species were assessed via the Spearman's correlation coefficient. Species were grouped in terms of their dominant adaptation for dealing with Na⁺ and other ions in order to maintain osmotic balance within saline environments. Representatives from the families Acanthaceae, Avicenniaceae and Myrsinaceae were grouped as salt secretors (presence of salt glands), while families without salt glands were grouped as non-secretors (absence of salt glands in leaves is often associated with exclusion, i.e. barriers to salt accumulation at the root level) (Tomlinson, 1986). Differences between secretors and non-secretors in terms of root BCFs, leaf BCFs and TFs for each metal separately were assessed with the two sample unbalanced *t*-test (Zar, 1999).

Where sufficient data were available (i.e. for leaf BCFs) differences for each metal were compared among family and genera employing a one way unbalanced ANOVA (Zar, 1999). These analyses were performed in Statistica for Windows (StatSoft, 1995). Data for all species were

compared among the three metals for root BCFs, leaf BCFs and TFs to assess if patterns varied among metals employing mixed model ANOVAs with metal as a fixed effect and species as a random effect (Pinheiro and Bates, 2000). In this context, a mixed model ANOVA is conceptually similar to a paired *t*-test, except that there are three groups (metals), instead of the two in a paired *t*-test. Comparisons among metal BCFs and TFs were made within species, thus making corrections for phylogenetic effects unnecessary. Mixed models were fitted using the nlme package for R (Pinheiro et al., 2006).

2.2.2. Analyses within a phylogenetic context

Usual statistical methods assume that each data point is an independent sample. However, closely related species may not be statistically independent as they share a common evolutionary history thus biasing any cross-species analysis of broad scale patterns (Harvey and Pagel, 1991). Recent studies suggest that this lack of independence is an important property of virtually all cross-species datasets and their analysis (Freckleton et al., 2002; Blomberg et al., 2003). Despite this, analyses of species responses to contaminants in ecotoxicology have rarely, if ever, considered this problem. To our knowledge, this is the first instance of species accumulation patterns being analysed quantitatively within a phylogenetic framework. Approaches to the analysis of cross-species datasets have mainly employed phylogenetically independent contrasts (Felsenstein, 1985) or equivalently, generalised least squares (GLS) linear modelling

methods (Martins and Hansen, 1997; Garland and Ives, 2000; Rohlf, 2001). Where sufficient data were available to allow analysis within a phylogenetic context, we employed phylogenetic GLS methods to examine differences between secretors and non-secretors in terms of leaf BCFs for each metal separately.

A matrix of fixed correlations specifying dependence among species observations was derived from shared branch lengths (fixed branch lengths = 1) based on the phylogenetic topology of Parani et al. (2002) above the family level. Relationships among genera and among species within genera were based on Parani et al. (2002) where possible, supplemented with a composite of several phylogenies (i.e. Rhizophoraceae (Lakshmi et al., 2002; Shi et al., 2002), *Rhizophora* spp. (Duke, 2002; Duke et al., 2002) and *Sonneratia* spp. (Shi et al., 2000)). Significant differences among parameter estimates for each category of secreting or non-secreting species were assessed via the t statistic. Analyses were performed using R 2.3.1 (R Development Core Team, 2006) with the Analyses of Phylogenetics and Evolution package (ape 1.8-4 (Paradis et al., 2004)) and the nlme package (nlme 3.1-75 (Pinheiro et al., 2006)).

Assigning branch lengths equal to 1 assumes that all speciation events are present in the phylogeny, and that traits change only at speciation events. In order to explore the effect of different branch length assumptions on our analysis, we reanalysed our data using Grafen's branch lengths ($\rho = 1$) (Grafen, 1989), which sets the node height proportional to the number of taxa above it in the phylogenetic tree. There was a high concordance in the results obtained between the two techniques. We thus chose to report the results for unit branch lengths, as the differences between the two approaches did not alter the interpretation of results.

3. Results

Copper was accumulated in root tissue to levels similar to environmental concentrations, with an average root BCF of 1.1 ± 0.2 (SE). Translocation of Cu was limited, with concentrations of accumulated Cu 50% lower in leaves than roots. Species exhibited an average Cu leaf BCF of 0.47 ± 0.07 , and translocation factor of 0.52 ± 0.09 (Table 1a). Copper root BCFs remained constant across sediment loadings (sediment vs. root BCF, Spearman $r = -0.33$, n.s., $p > 0.05$); leaf BCFs and TFs tended to be lower at higher sediment Cu concentrations suggesting regulation of translocation (sediment vs. leaf BCF, $r = -0.54$, $p < 0.05$). No significant differences were observed between salt secreting and non-secreting species in terms of Cu root BCF ($t_{12} = -0.32$, $p > 0.05$), leaf BCF ($t_{38} = 0.73$, $p > 0.05$) or TF ($t_9 = 0.58$, $p > 0.05$). Copper leaf BCFs were similar among families and genera (leaf BCF, 0.34–0.58; family $F_{5,33} = 1.12$, $p > 0.05$; genera $F_{8,30} = 0.89$, $p > 0.05$) (Table 1a).

Lead was accumulated in root tissue to levels similar to environmental concentrations with an average root BCF of 0.84 ± 0.21 . Translocation of non-essential Pb was minimal and the lowest of all metals with concentrations of accumulated Pb reaching leaf tissue only 30% that of roots. Species exhibited an average Pb leaf BCF of 0.11 ± 0.02 , and an average TF of 0.31 ± 0.07 (Table 1b). Root BCFs were similar across sediment loadings (sediment vs. root BCF, $r = 0.09$, n.s., $p > 0.05$). Lead leaf BCFs and TFs were consistently low, suggesting exclusion of Pb from acropetal transport (sediment vs. leaf BCF, $r = -0.11$, n.s., $p > 0.05$). No significant differences were observed between salt secreting and non-secreting species for Pb root BCF ($t_{12} = -1.21$, $p > 0.05$), leaf BCF ($t_{30} = 1.34$, $p > 0.05$) or TF ($t_9 = 0.92$, $p > 0.05$). Lead leaf BCFs were similar among families and genera (leaf BCF, family $F_{5,26} = 0.80$, $p > 0.05$; genera $F_{8,23} = 0.76$, $p > 0.05$) (Table 1b).

Zinc was accumulated in root tissue of mangroves to concentrations around 70% of environmental concentrations with an average root BCF of 0.69 ± 0.1 . Translocation of Zn was similar to the essential metal Cu with concentrations of accumulated Zn in leaf tissue 50% that of roots. Species exhibited leaf Zn BCFs of 0.51 ± 0.05 , and Zn TFs of 0.53 ± 0.07 (Table 1c). Root BCFs were similar across sediment loadings (sediment vs. root BCF $r = 0.05$, n.s., $p > 0.05$). Zn leaf BCFs were lower at higher Zn sediment/root concentrations, indicative of regulation to acropetal Zn transport (sediment vs. leaf BCF $r = -0.63$, $p < 0.05$). No significant differences in Zn leaf BCFs were observed among family or genera (leaf BCF, 0.34–0.58, family $F_{5,33} = 0.89$, $p > 0.05$; genera $F_{8,30} = 0.94$, $p < 0.05$). Similarly, no significant differences were observed between salt secreting and non-secreting species for Zn root BCF ($t_{12} = -0.42$, $p > 0.05$), leaf BCF ($t_{37} = -0.50$, $p > 0.05$) or TF ($t_9 = -0.34$, $p > 0.05$) (Table 1c).

When the raw (species level) data were corrected for phylogenetic relatedness, similar results were observed suggesting patterns in data were not an artefact of correlated data due to the close relationships of some species. Leaf BCFs for Cu, Pb and Zn were similar regardless of whether species were classified as salt secreting or non-secreting species (Cu, $t_{16} = -0.99$, $p > 0.05$; Pb, $t_{12} = -0.67$, $p > 0.05$; Zn, $t_{16} = -0.85$, $p > 0.05$).

When comparing patterns among metals, significantly lower Pb was accumulated in roots than Cu ($t_{10} = -2.92$, $df = 10$, $p < 0.05$). There were no differences between Zn and Cu BCFs ($t_{10} = -2.04$, $p > 0.05$) nor any difference between Zn and Pb BCFs across mangrove species ($t_{10} = 0.88$, $p > 0.05$). Mangroves accumulated significantly lower levels of Pb in leaves compared with both Cu ($t_{22} = 3.69$, $p < 0.01$) and Zn ($t_{22} = 5.39$, $p < 0.01$). There was no significant difference in leaf BCFs between Cu and Zn ($t_{22} = 1.70$, $p = 0.10$). Translocation factors among species were broadly similar for all metals (Cu vs. Pb, $t_8 = -1.25$, $p > 0.05$; Cu vs. Zn, $t_8 = -0.06$, $p > 0.05$; Pb vs. Zn, $t_8 = 1.19$, $p > 0.05$).

4. Discussion

Despite the true mangroves being a taxonomically diverse group, metal accumulation and partitioning patterns for all metals examined were broadly similar across genera and families. Metal accumulation patterns were also similar regardless of whether species were secretors or non-secretors.

In conventional statistical methods, data are assumed to be sampled randomly and independently. When comparative data such as accumulation factors are sampled across a phylogeny from what is available in the literature, and differences tested among taxa groups, values may not be independent due to closely related species with a common ancestry sharing similar traits (i.e. ability to accumulate a particular metal or salt secretion), thereby biasing analyses and interpretation (Harvey and Pagel, 1991). Similar metal accumulation strategies among secretors or non-secretors were maintained after phylogenetic correction of data, suggesting the lack of differences observed among groups from species level analyses were not unduly influenced by relatedness among species.

Copper, Pb and Zn were accumulated in root tissue to concentrations equal to or less than adjacent sediment concentrations (root BCFs 0.69–1.1). Little translocation was observed, with metal concentrations up to an order of magnitude lower in leaf tissue. Essential metals (Cu and Zn; TFs of 0.52 and 0.53, and leaf BCFs of 0.47 and 0.51, respectively) showed greater mobility than non-essential metals (Pb; TF of 0.31 and leaf BCF of 0.11). Levels of Cu, Pb and Zn in tissues largely reflect trace metal concentrations found in most terrestrial plant species $\text{Cu} = 10 \mu\text{g g}^{-1}$; $\text{Pb} = 1 \mu\text{g g}^{-1}$; $\text{Zn} = 50 \mu\text{g g}^{-1}$ (Markert, 1993). Thus mangroves as a group tend to operate as excluder species for non-essential metals, i.e. Pb, and regulators of essential metals, i.e. Cu and Zn (Baker, 1981).

In terms of patterns in metal accumulation with variations in sediment metal loading, relative proportions of metal accumulated from sediment to roots were similar regardless of environmental concentration for all metals examined. Leaf BCFs for the essential metals Cu and Zn decreased as environmental concentrations increased. These patterns perhaps suggest that at low metal concentrations plants translocate from root to shoot to meet metabolic requirements, while at higher concentrations metal translocation is restricted to prevent toxicity (Baker, 1981). Non-essential metals such as Pb are excluded from leaf tissue regardless of environmental concentrations.

A number of possible mechanisms may account for the low observed metal accumulation in mangroves. The initial bioavailability of sediment metals in estuarine situations is often low. Due to sediments generally being anoxic, waterlogged and of low pH, metals are often precipitated as insoluble sulphides (Lacerda, 1997). Metals may be adsorbed on ion exchange sites of fine silts/clays, incorporated into the lattice structure of clay, or adsorbed within iron and manganese colloidal oxide compounds (Harbison,

1986). The high organic content of detritus rich sediments facilitates complexation with refractory organics, and as such is a major contributor to low metal availability (MacFarlane et al., 2003). Additionally, high salinity facilitates the formation of metal-chloride complexes which are less bioavailable for uptake than free metals (Greger, 2004).

Possible physiological mechanisms responsible for variation in uptake and translocation at the root level include processes at the root's rhizosphere. Small-scale variation in uptake among families/genera may be attributed to the adaptation of aerial root structures such as pneumatophores with lenticels for gaseous exchange in some species and the absence of these adaptations in others. An oxidised rhizosphere within the anoxic soil environment may confer a reduction in complexing sulphides, a lowered stability of iron plaques, resulting in higher concentrations in the exchangeable form for some trace metals (Lacerda and Abreu, 1984; Lacerda, 1993). Indeed, trends towards higher root BCFs seem to be characteristic of species with pneumatophores (i.e. *Avicennia* spp.) and species that inhabit less frequently inundated habitats where sediments are less anoxic (i.e. *Kandelia* and *Aegiceras* spp.).

The accumulation of Cu, Pb and Zn by roots (root BCFs ~ 1) and restricted translocation of Cu, Zn and especially Pb across species found in the present survey, reflect patterns of accumulation and distribution from laboratory studies with *A. marina*. MacFarlane and Burchett (2000, 2002) found Cu, Pb and Zn in *A. marina* roots at concentrations similar to sediment loadings. Through SEM X-ray microanalysis, it was found that metals were mainly localised in cell walls (i.e. apoplastic transport implicated through cationic exchange with cell wall associated carboxylic groups). The epidermal layers operated as a barrier reducing the transport of Pb while the endodermis restricted movement of Cu, Pb and Zn into the stele (i.e. Casparian strip mediated delimitation of apoplastic transport).

Non-secreting species (e.g. *Sonneratia*, *Bruguiera* and *Rhizophora* spp.) typically possess low concentrations of salt (i.e. NaCl) in the xylem with Na^+ concentrations around one hundredth that of sea water and uptake being regulated predominantly at the root endodermis (Tomlinson, 1986). Salt secreting species (e.g. *Avicennia* spp.) exhibit a greater mobility of Na^+ ions with concentrations approximately one tenth that of seawater in xylem sap with salt glands reducing overall Na concentrations in leaves (Lawton et al., 1981; Tomlinson, 1986). Despite these physiological differences in Na uptake among species, patterns of metallic cation (Cu^{2+} , Pb^{2+} and Zn^{2+}) uptake by roots and translocation to the shoot were broadly similar for both salt secreting and non-secreting species. It is probable that metal influx and regulation of transport in mangroves are likely to be achieved through a variety of pathways in addition to those responsible for sodium influx/transport.

MacFarlane and Burchett (2000, 2002) found that Pb translocation to leaves in *A. marina* was low. Accumulated Pb was largely immobile, cell wall bound and/or

sequestered predominantly in the epidermal layers of the root. Zinc (presumably chelated with organic acids) and Cu (primarily amino acid associated) were translocated to leaf tissue, though concentrations were an order of magnitude lower than in roots ($TF < 0.1$). Indeed, these patterns of translocation broadly reflect cross-species trends despite differences in salt regulation strategies. Although mangroves have been shown to excrete metals (Cu and Zn, *A. marina*; MacFarlane and Burchett, 2002) from salt glands concomitantly with Na, secretion does not significantly alter overall distribution patterns of metals in leaf tissue among families, or when comparing secretors to non-secretors.

For phytoremediation initiatives, phytoextraction of contaminants from the sediment requires translocation from root to shoot to enable harvest and removal of contaminants (Kumar et al., 1995). For phytoextraction to be viable, 'hyperaccumulation' of contaminants is required by species tolerant to high concentrations of bioavailable metal, i.e. leaf BCFs and TFs greater than one (Ma et al., 2001b; Baker and Whiting, 2002). When comparing leaf BCFs in the current survey to established hyperaccumulators such as the fern *Pteris vittata* (Ma et al., 2001a) with As BCFs greater than 100, the BCF values in mangroves are relatively low. However, leaf BCFs for Zn in other hyperaccumulating terrestrial plant species are lower than for As, i.e. up to 40 in *Thlaspi caerulescens* (Zhao et al., 2003). Generally, results are presented in absolute values rather than as BCF or TF. Plants with $>500 \mu\text{g g}^{-1}$ Cu or $10000 \mu\text{g g}^{-1}$ Zn in their tissue have been classified as hyperaccumulators (Brooks, 1998), whereas the Pb hyperaccumulator, *Brassica juncea*, has been reported as accumulating $18812 \mu\text{g g}^{-1}$ Pb in shoots (McGrath, 1998). Mangroves exhibit uptake and translocation of metals well below levels found in established hyperaccumulators. Thus mangroves in remediation initiatives are perhaps best employed as phytostabilisers (Salt et al., 1995), functioning as long-term sinks for metallic contaminants. Not only do mangrove sediments reduce many metals in anoxic sediments, thereby limiting bioavailability and mobility, the presence of mangrove trees stabilises sediments physically, thereby reducing sediment (and metal) export to surrounding waters. Furthermore, levels of metals exported from mangroves through leaf litter decomposition are estimated to be very low i.e. 0.01, 0.02 and $0.005 \text{ mol ha}^{-1} \text{ year}^{-1}$ for Cu, Zn and Pb, respectively, for *R. mangle* (Silva et al., 2006), suggesting much of the metal budget is retained within mangrove systems affording protection for adjacent estuarine/marine systems.

5. Conclusions

Mangroves are a phylogenetically heterogeneous group with differing adaptive strategies for dealing with the challenge of highly anoxic and saline environments. Despite these differences, metal accumulation and partitioning for Cu, Pb and Zn were found to be similar across genera and families in the current study. Equally, patterns of metal

accumulation were also similar regardless of whether taxa were classified as salt secreting or non-secreting species. Metals tended to be accumulated in roots to concentrations similar to those of adjacent sediments while metal concentrations in leaves were half that of roots or lower. Essential metals were more mobile than non-essential metals suggesting mangroves tend to operate as excluder species for non-essential metals and regulators of essential metals. In a phytoremediative context, mangrove ecosystems are more appropriate candidates for phytostabilisation, retaining metallic inputs and thereby reducing transport to adjacent systems.

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