



## Global patterns of accumulation and partitioning of metals in halophytic saltmarsh taxa: A phylogenetic comparative approach

Md Rushna Alam<sup>a,b</sup>, Rafiqueel Islam<sup>a,c</sup>, Thi Kim Anh Tran<sup>a,d</sup>, Diep Le Van<sup>e</sup>,  
 Mohammad Mahmudur Rahman<sup>f</sup>, Andrea S. Griffin<sup>a,g</sup>, Richard Man Kit Yu<sup>a</sup>, Geoff  
 R. MacFarlane<sup>a,\*</sup>

<sup>a</sup> School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia

<sup>b</sup> Department of Aquaculture, Patuakhali Science and Technology University, Dumki, Patuakhali 8602, Bangladesh

<sup>c</sup> Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia 7003, Bangladesh

<sup>d</sup> School of Agriculture and Resources, Vinh University, Viet Nam

<sup>e</sup> School of Biochemical Technology-Environment, Vinh University, Viet Nam

<sup>f</sup> Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, NSW 2308, Australia

<sup>g</sup> School of Psychology, The University of Newcastle, Callaghan, NSW 2308, Australia

### ARTICLE INFO

Editor: Dr. Rinklebe Jörg

#### Keywords:

Bioconcentration factor  
 Halophyte  
 Translocation factor  
 Trace metals  
 Salinity tolerance

### ABSTRACT

The current study represents the first attempt to analyse quantitatively, within a phylogenetic framework, uptake and partitioning patterns of copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in extant saltmarsh taxa globally, and to assess associations of these patterns with various plant traits indicative of their halophytic adaptations. Despite saltmarsh being diverse taxonomically, most saltmarsh taxa accumulate metals to roots at, or above, unity ( $> 1$ ). Further, there is significant translocation from roots to shoot for Cu, Zn and Cd ( $\leq 1$ ), however, Pb is less mobile (TF = 0.65). Patterns of accumulation were similar among families, except greater Cd accumulation to roots in members of Juncaceae. Patterns of uptake to roots and translocation to leaves were broadly similar among plant type, plant form, habitat and photosynthetic mode. Zinc is lower in the leaves of salt-secreting species for some closely related taxa, suggesting some species co-excrete sodium ( $\text{Na}^+$ ) and  $\text{Zn}^{2+}$  through glands in leaf tissue. Salinity tolerance has no relationship to metal uptake and translocation. Translocation of Zn is greater at lower Zn sediment exposures, reflecting its active uptake and essentiality, but such bias does not affect outcomes of analyses when included as a covariate.

### 1. Introduction

Saltmarshes are ecosystems commonly defined as coastal areas, vegetated by a range of herbs, grasses and shrubs adjacent to saline water bodies that receive periodic tidal inundation (Sarika and Zikos, 2020). The vegetation present in saltmarsh is characteristically halophytic (salt-tolerant), with variability in relative salt tolerance among species, and thus clear patterns of zonation in plant taxa throughout the marsh dependent on elevation, the frequency of tidal inundation and consequent salinity (Mcowen et al., 2017).

Saltmarshes provide transitory or permanent habitat for a variety of invertebrate, fish and wading bird taxa (Saintilan, 2009). Saltmarsh plants are primary producers that provide trophic subsidy for dependent commercial and recreational fish and crustacean species (Raoult et al.,

2018). Saltmarsh also acts as a net carbon sink, important in climate change mitigation strategies (Sousa et al., 2010).

Despite their inherent ecological importance and value, saltmarsh has historically been subject to large scale modification and destruction. Saltmarsh occurs in coastal zones, often adjacent to significant urban development and industrial activity. As a consequence, saltmarsh has been modified, reclaimed and destroyed through infilling and impediment to tidal flow to make way for land for urban development, agriculture, waste disposal and industry (Gedan et al., 2009). Estimates of decline internationally are between 25 and 50% of their historic global coverage till this decade, with an approximately 1–2% yearly reduction rate. To place this in context, current area of extant saltmarsh globally is approximately 5 Mha across 43 countries and territories, thus their status is currently perilous (Mcowen et al., 2017).

\* Corresponding author.

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

<https://doi.org/10.1016/j.jhazmat.2021.125515>

Received 1 October 2020; Received in revised form 10 February 2021; Accepted 22 February 2021

Available online 24 February 2021

0304-3894/© 2021 Elsevier B.V. All rights reserved.

Since 1800, the Anthropocene began with the onset of the industrial revolution. Humans and their industrialisation have significantly altered the Earth's biogeochemical processes at an unprecedented pace and scale (Steffen et al., 2007); Within this context, remnant saltmarsh is currently under significant anthropogenically mediated pressures. One particular pressure experienced by remaining marshes in proximity to urban activity is the associated polluting of saltmarsh habitats by contaminants, including metals and metalloids. Metals enter saltmarsh sediments from a variety of sources including effluents from metal smelters and steelworks, overflow from coal-fired power generation ash dams and industrial, municipal and sewage effluents (MacFarlane et al., 2007; Tran et al., 2020). More diffuse non-point source metal contaminant inputs arise from urban and stormwater runoff, agricultural runoff and boating activities (Nath et al., 2013).

Saltmarsh halophytes have evolved over the millennia to adapt to hyperosmotic saline environments and thus typically exhibit the ability to tolerate exposure to high concentrations of toxic ions such as sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) (Flowers et al., 2010). Due to their ability to tolerate high salt concentrations, it stands to reason that this adaptation may also confer a coupled tolerance to other toxic ions, including metal (loid) exposure and/or accumulation (Van Oosten and Maggio, 2015). Metal uptake and translocation in saltmarsh halophytes exhibit high phylogenetic variability, but in general, bioconcentration factors (ratio of metal in root compared to sediment) are at unity or greater. In contrast, translocation factors of transport from root to shoot are generally lower than 1, suggesting substantial barriers to acropetal translocation (Anjum et al., 2014).

Existing qualitative cross-species accounts of metal accumulation in saltmarsh suggest species in the family Amaranthaceae typically exhibit low metal translocation. Grasses in the family Poaceae show great variability in accumulation and translocation patterns among taxa, while rushes in the family Juncaceae tend to exhibit greater metal mobility between roots and shoots (Burke et al., 2000; Weis and Weis, 2004; Anjum et al., 2014). Despite these preliminary trends, no previous study has attempted to quantitatively assess differences in patterns of metal accumulation among families and genera, nor patterns of accumulation with growth form, or the specific niches in which the species reside, which may be expected to influence accumulation strategy.

Saltmarsh halophytes as a group also vary significantly in terms of their uniquely evolved strategies for dealing with the hyperosmotic stress of a saline environment. Halophytes typically adjust osmotically to soil salinity by accumulating  $\text{Na}^+$  and  $\text{Cl}^-$  in roots with high translocation to shoots. Within these tissues, ions undergo intracellular vacuolar compartmentalisation to minimise their interference with cell metabolism. The high concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in vacuoles are balanced by organic solutes in the cytoplasm to maintain osmotic potential (Flowers et al., 2015). As halophytes vary greatly in their relative salinity tolerance, it stands to reason that more tolerant halophytes need to accumulate and translocate greater quantities of  $\text{Na}^+$  to adjust osmotically to higher exposure concentrations to allow sufficient water uptake (Flowers et al., 2015). Little is known about the molecular mechanisms of symplastic metal transport in halophytes, and it is likely that the specific cation transporter assemblages differ for the monovalent cation  $\text{Na}^+$  and divalent metal cations (see Flowers and Colmer, 2008) for implicated  $\text{Na}^+$  transporters and Williams et al. (2000) and Guerinot (2000) for metal-specific transporters). However, there is some evidence that saltmarsh metal accumulation and transport appears to be associated with salt accumulation. Redondo-Gómez et al. (2011), for example, observed that accumulated Zn in the roots and tillers of *Spartina densiflora* increased with increasing NaCl exposure and consequent  $\text{Na}^+$  accumulation. For these various reasons we hypothesise that we may observe elevated patterns of metal uptake and translocation in species with greater salinity tolerance and greater coupled mobility of the monovalent cation  $\text{Na}^+$ .

Finally, some halophytic species manage saline toxicity and ion balance via excreting excess ions through salt secreting organs such as

glands in abaxial and adaxial surfaces of the leaf. As well as the excretion of  $\text{Na}^+$  from glands, these glands have been evidenced to co-excrete metals (Burke et al., 2000; Kraus et al., 1986; MacFarlane and Burchett, 2000). Thus, we predict that lower metal loads may be observed in shoots of salt secreting species compared to non-secreting species due to glandular excretion of metals.

Given these various lines of evidence, we surveyed the published literature, where sediment Cu, Zn, Cd and Pb and subsequent uptake in saltmarsh roots and leaves have been investigated globally, to answer the following questions:

1. What is the pattern of metal uptake and translocation in saltmarsh species across phylogenetically distinct families?
2. Likewise, do saltmarsh taxa show variation in metal uptake and partitioning based on life form, plant type, photosynthetic pathway and habitat?
3. Does accumulation and translocation of metals increase with salinity tolerance?
4. Are relative metal loadings in shoots reduced in species with salt glands and bladders?
5. Does metal uptake and translocation exhibit any pattern across halophyte species with environmental exposure?

As such, the study represents the first assessment of the patterns of metal accumulation in saltmarsh globally and their relationship to plant attributes indicative of salinity tolerance. Although there is data on metal accumulation in saltmarsh and halophytic plants in the literature (e.g. Williams et al., 1994; Weis and Weis, 2004; Anjum et al., 2014), never before has this data been amassed and analysed quantitatively in order to understand general patterns of metal transport behaviour. Further, although there is some evidence that adaptations of these plants to salt tolerance may also confer a coupled tolerance to other hazardous materials such as metals, no prior study has investigated these questions quantitatively. Finally, this is the first study to understand patterns using a sophisticated phylogenetically-based statistical framework, rarely attempted in risk assessment.

## 2. Methods

### 2.1. Data compilation

An extensive survey of the published literature was conducted for saltmarsh species where data on metal accumulation under laboratory and/or field conditions have been investigated. Initially, we accessed the online database eHALOPH (<http://www.sussex.ac.uk/affiliates/halophytes/>) (Santos et al., 2016) to derive a saltmarsh species list on which to base our search. eHALOPH is a database of salt-tolerant plants (halophytes) with records of plant species tolerant of salt concentrations of approximately 80 mM sodium chloride or higher, along with associated data pertinent to plant attributes and salinity tolerance. To generate a list of saltmarsh species for further investigation, a search was conducted using the search term "habitat" to arrive at a subset of halophyte species that occur in coastal saltmarshes, low-mid and upper saltmarshes and inland saltmarshes to arrive at a species list of 138 species on which to base our initial literature search.

The academic search engine Google Scholar was chosen to conduct the literature search. Google Scholar was chosen over other established databases such as Scidirect and Web of Science as Google Scholar records have greater coverage and also include many grey literature references in addition to major journals in the field (Gehanno et al., 2013; Haddaway et al., 2015). So, a survey of the literature was conducted employing Google Scholar to arrive at a total of 28 species (out of 138 species examined) and 41 published studies where the accumulation of the metals Cu, Zn, Cd and Pb have been assessed in saltmarsh taxa under lab and/or field conditions worldwide (Supplementary File S1).

Copper, Zn, Cd and Pb were chosen as representative trace metals

which are essential (Cu and Zn) or non-essential (Cd and Pb) to plant metabolism, as the patterns of accumulation are likely to vary dependant on essentiality (Baker, 1981). Copper, Cd, 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). For each species, a Google Scholar search, sorting by relevance with the search terms “species name” and the keywords “metals, Cu, Pb, Zn and Cd”, was conducted. Each species was also checked for older attribution/classification to ensure all records were located. All the references generated by each search were accessed and examined for relevant data for inclusion.

Only those studies were included where metals were quantified on a dry weight/weight basis (ppm or  $\mu\text{g}\cdot\text{g}^{-1}$ ), or could be calculated from original data. For each study, the average sediment metal, average root metal and average leaf metal in  $\mu\text{g}\cdot\text{g}^{-1}$  were calculated. If studies only report shoot metals (leaves and stems combined) this average value was employed as a surrogate for leaf metals. To obtain the sediment, root and leaf averages, for field-based studies, we averaged various sampling times per location, then averaged among locations for a particular study, which facilitated phylogenetic analysis, as one data point (a species average) is required per species for analysis. For lab-based investigations, averages were obtained across the various exposure treatments for a particular study (excluding controls). Where data were only presented in figures, we took the help of engage digitiser software to estimate concentrations.

As all studies varied in their relative environmental metal contamination levels, we assessed relative uptake ratios and translocation factors in an attempt to normalise data and allow interspecific comparisons among candidate studies. Root bioconcentration factors (BCF: a ratio of root metal to sediment metal concentration) were calculated to assess concentrations in roots relative to environmental loadings. Translocation factors (TF: a ratio of leaf metal to root metal concentration) were calculated to enable assessment of transport of accumulated metal from root to shoot. Root BCFs and TFs of each candidate metal were also represented as averages of original data.

When amassing data from a variety of independent studies, significant problems arise in terms of the validity of the data obtained for comparative purposes. The physicochemical nature of the sediments at the locations sampled can strongly influence uptake of metals, with locales high in sulphur, organic matter, clay and fines exhibiting lower bioavailability than sandier sediments (Harbison, 1986). Thus, the calculated BCFs will be somewhat dependent on sediment physicochemistry of the location under investigation and, in some instances, may be an underestimate of the species ability to accumulate metals to roots. Metal availability may also vary depending on numerous other site-specific or laboratory-specific sediment physicochemical considerations including the cation exchange capacity, pH, redox status, metal speciation and salinity of sediments (Greger, 2004). Indeed, metal bioavailability is likely to be significantly greater under laboratory conditions, where metals are added to sediment with rather short equilibration periods and are usually added to sediments as relatively bioavailable salts. 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. Further, the geographical location where the study was conducted may influence uptake and partitioning, with locations at varying latitudes perhaps exhibiting altered day length, temperature and subsequent plant growth and metabolic differences. Metal uptake may also vary with habitat, with estuarine saltmarsh more frequently inundated than other more inland marshes, with consequences for metal bioavailability and uptake.

In terms of digestion of metals for analysis, different acids and concentrations were employed across candidate studies. Some studies use relatively weak acid digestion protocols, especially for sediment such as

the addition of dilute  $\text{HNO}_3$ . In contrast, other studies apply more complete digestions employing combinations of  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$  and  $\text{HCl}$  at higher molar concentrations. Further, different heating protocols for digestion are used from hotplate digestions to more comprehensive microwave digestion technologies. Although plant tissue is readily digestible and the methodology bears little weight on measured concentrations, the acid digestion methodology will influence the total metal concentration in sediment as some metals are more tightly bound to sediment and require a combination of acids for more complete digestion. 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 higher degree than TFs.

Finally, additional variation among candidate studies exists dependent on the sampling protocols utilised for vegetation sampling. There is great variation among studies in terms of age of leaves sampled, leaf position, age/life stage of plants sampled, position in the marsh, and level of replication. Thus, we acknowledge that numerous biases exist in the final dataset. Nonetheless, for species where metal accumulation has been assessed under lab and/or field conditions, it is important to attempt to ascertain broad accumulation patterns, though the data should be viewed cautiously and these caveats kept in mind.

Along with data generated on metal accumulation patterns for each species, data on plant attributes including habit, morphology, physiology and salinity tolerance were obtained from the eHALOPH database for comparative analyses (Santos et al., 2016). Data selected included current scientific phylogenetic designation of family. Species were categorised into plant type including, hydrohalophytes: comprising of tidal swamp or mangrove and coastal lagoon elements or temperate zone saltmarsh elements; psammophiles: sand-loving plants; xerophytes: drought tolerant/adapted plants; weedy: high degree of adaptability to a range of sites and ability to colonise highly disturbed sites; and xero-halophytes: plants adapted to inland salt and saline habitats. Unknown was assigned when no data from literature exists to classify species in eHALOPH. Data was also included on life form of plants including annuals: plants with an annual life cycle; chamaephytes: small shrubs under 0.5 m height; herbaceous perennial: a herbaceous plant that survives a dormant period but rarely lives longer than a few years; nano-chamaephyte: dwarf shrub under 0.25 m in height; perennial grass: a member of Poaceae that survives periods of dormancy; and shrub: a long-lived woody perennial under 5 m height. The maximum salinity (mM) under which plants of a particular species survive was also included as a relative measure of salinity tolerance. All data were converted to mM to enable interspecies comparisons. The presence or absence of salt glands on adaxial or abaxial surfaces of the leaves and or salt bladders were also recorded as a specific adaptation for dealing with excess salt. The photosynthetic pathway as  $\text{C}_3$ ,  $\text{C}_4$  or unknown was coded for each species. Finally, the habitat was coded for each species including the following habitat designations, coastal salt marshes and saline reed beds; low-mid saltmarshes; mid-upper saltmarshes and saline and brackish reed beds; upper saltmarshes; coastal dunes and sandy shores; permanent inland saline; coastal stable dune grassland (grey dunes); continental inland salt steppes and brackish lakes; and inland saltmarshes.

## 2.2. Statistical analysis

### 2.2.1. Species-level comparisons

We performed all the species level analyses using  $\log_{10}(x)$  data with the help of either statistical software IBM SPSS version 26 or Sigma Plot 13. A series of one-way analyses of variance (ANOVA) were employed for comparing BCFs and TFs for each metal among families, plant types, life forms, salt glands/ bladders presence or absence, photosynthetic pathway and habitat across species followed by the DMRT post hoc test. An independent samples *t*-test was used if the grouping treatments were two. Linear regression was run to assess patterns accumulation to roots and translocation to leaves as a function of maximum salinity tolerance

across species via Sigma Plot 13. A similar statistical approach was employed to explore the relations of BCFs and TFs with sediment metal loading.

As some species actively take up metals (especially essential metals) for nutritional requirements, BCFs and TFs may be elevated at lower sediment exposures, and thus this may confound our analyses depending on exposures where the species BCFs and TFs were observed in candidate studies. Thus to control for this potential confounding variable we conducted multiple regression and analysis of covariance (ANCOVA) where sufficient species were present for analysis. Multiple regression was performed to examine the influence of salinity tolerance on metal uptake and translocation controlling for environmental exposure. Finally, we conducted ANCOVA to assess differences in BCF and TF with photosynthetic pathway (C<sub>3</sub>, C<sub>4</sub> or Unknown) and presence and absence of salt glands/ bladders while controlling for sediment metal exposure. Firstly, we ran an interaction model testing for homogeneity of slopes. If non-significant, we proceeded to the main effects models in order to assess differences in BCF and TF with salt gland presence/absence or photosynthetic pathway (Engqvist, 2005). All other categorical analyses have insufficient species within each category to run reliable ANCOVAs.

### 2.2.2. Phylogenetic comparisons

Most parametric statistical tests assume that each data point is an independent sample. However, closely related species that share a common evolutionary history are not truly independent. When statistical inferences are made about the influence of one trait on another in evolutionary terms, relationships observed among species may not be the outcome of particular trait combinations arising independently in separate lineages during evolutionary history, but may merely represent an artefact of common descent or radiation from a single or few evolutionary events (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). Despite this, comparative analyses of how species' adaptations may influence contaminant uptake in ecotoxicology have rarely, if ever, considered this problem. To our knowledge, this is the one of the first instances of species accumulation patterns being analysed quantitatively within a phylogenetic framework.

We thus conducted a phylogenetically informed analysis to complement species level analyses, employing generalised least-squares regression models (GLS) assuming a Brownian motion model of evolution, where dependence among species is incorporated into the variance-covariance matrix of the residuals during analysis. A hybrid phylogenetic tree (Supplementary File S2) was constructed based on the recent megaphylogenetic topology of vascular plants by Qian and Jin (2016) supplemented with the phylogeny of Schütze et al. (2003) to account for species that were not present in the Qian and Jin (2016) phylogeny. As node heights differed between the topologies, we standardised branch lengths employing 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.

All BCF and TF data were  $\log_{10}(x)$  transformed prior to analysis. For assessment of differences in BCF and TF among categorical variables, we performed ANOVAs in GLS. Post hoc tests were achieved by releveling the intercept of categorical variables in associated categorical regression analyses. Linear and multiple linear least square regressions were run on continuous predictor variables as in species analyses. ANCOVAs were also run following the same procedure as species level analyses. Analyses were performed using R version 2.13.0. with the caper, ape and nlme packages.

## 3. Results

### 3.1. Metal uptake and translocation for saltmarsh as a clade: average BCF and TF across species

Across halophytic saltmarsh, essential metals (Cu and Zn) and the non-essential, Cd, exhibited relatively high uptake from sediment with 2–3 fold higher levels of metals in roots compared with that of sediments, with mean ( $\pm$  se) BCFs of Cu ( $2.34 \pm 0.74$ ), Zn ( $3.08 \pm 0.58$ ) and Cd ( $2.81 \pm 0.96$ ) (Supplementary File S1). In contrast, the non-essential metal Pb was accumulated in root tissue at similar levels to sediments, with a BCF of  $1.41 \pm 0.42$ . In terms of metal mobility and translocation within the plant across species, TFs for Cu, Zn and Cd ( $0.96 \pm 0.49$ ,  $0.75 \pm 0.16$  and  $1.19 \pm 0.51$ , respectively) were at or below unity, while mobility of Pb was lower ( $0.65 \pm 0.14$ ).

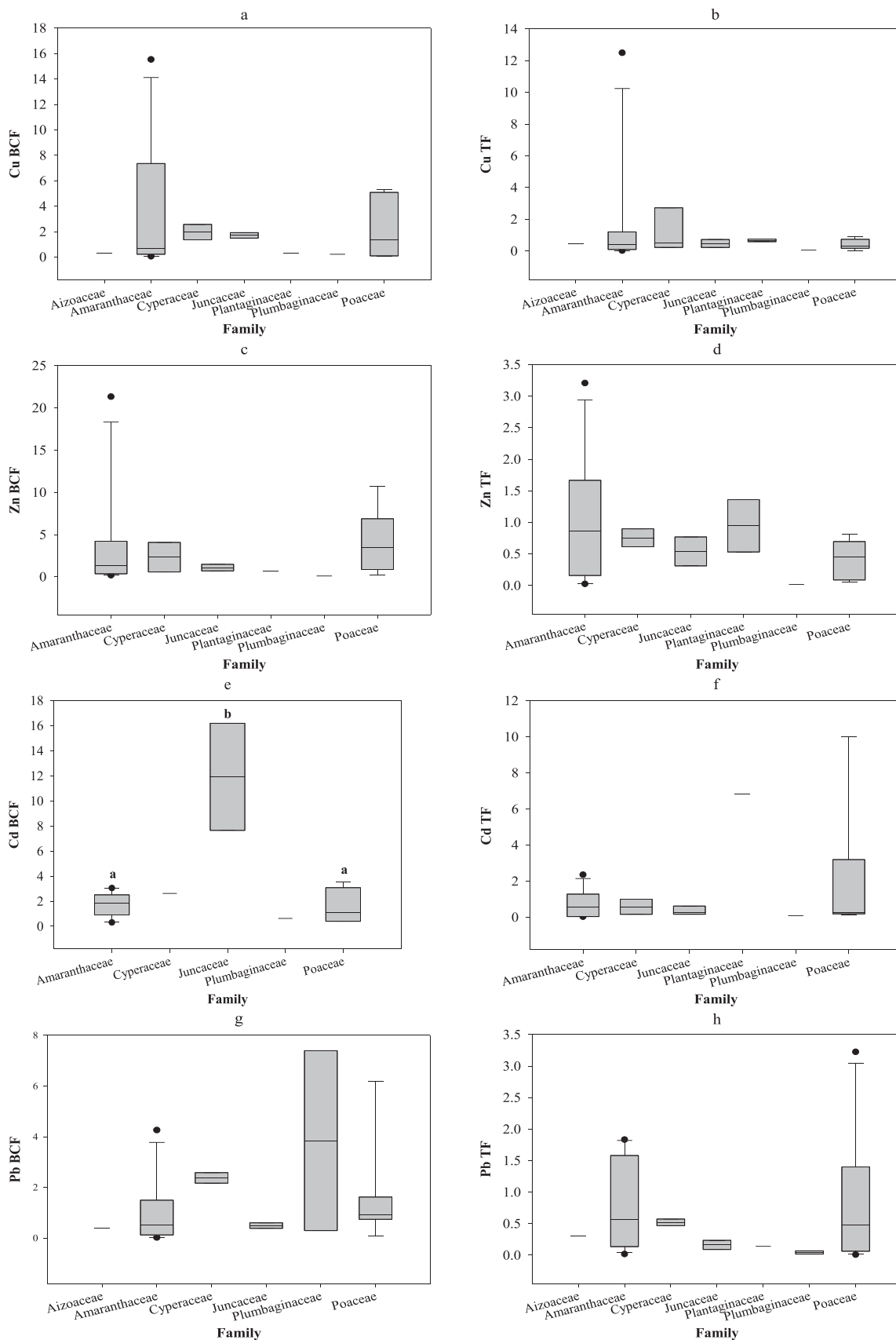
### 3.2. Metal accumulation and partitioning among families

Members of the Amaranthaceae family exhibited a greater BCF of Cu ( $3.27 \pm 1.52$ ) compared with other families (Cyperaceae,  $1.97 \pm 0.35$ ; Juncaceae,  $1.70 \pm 0.21$  and Poaceae,  $2.09 \pm 0.83$ ), however there was no significant difference among families ( $p \geq 0.05$ ) (Fig. 1a). Similarly, BCFs for Zn were not significantly different among families, though it appeared that species of Poaceae and Amaranthaceae showed trends towards greater Zn accumulation in their roots ( $4.10 \pm 1.57$  and  $3.52 \pm 1.87$ , respectively) (Fig. 1c). In contrast, BCFs for Cd exhibited significant variation among families, with Juncaceae having a significantly higher BCF of  $11.92 \pm 4.27$  compared to other families ( $F = 6.399$ ,  $p = 0.012$ ) (Fig. 1e). No significant variation in Pb BCFs among families was observed. Species of Plumbaginaceae showed trends towards greater Pb accumulation compared with other families (BCF of  $3.85 \pm 3.55$ ), followed by Cyperaceae ( $2.37 \pm 0.21$ ) and Poaceae ( $1.66 \pm 0.78$ ) (Fig. 1g).

Translocation factors for Cu, Zn, Cd and Pb did not vary significantly among families (Fig. 1). The highest TF for Cu was observed for Amaranthaceae ( $1.53 \pm 1.10$ ), followed by plants belonging to the Cyperaceae family ( $1.15 \pm 0.79$ ). Copper translocation in the leaves of Juncaceae, Plantaginaceae and Poaceae plants were around 50% of that of roots, with TFs of  $0.47 \pm 0.25$ ,  $0.67 \pm 0.08$  and  $0.42 \pm 0.12$ , respectively. For Zn, Amaranthaceae displayed trends towards greater TFs ( $1.00 \pm 0.30$ ), followed by Plantaginaceae ( $0.95 \pm 0.42$ ). For most families, Cd exhibited limited translocation from root to leaf tissue, while Poaceae revealed a trend to greater translocation ( $1.96 \pm 1.6$ ), followed by Amaranthaceae ( $0.70 \pm 0.21$ ). A similar pattern was observed for Pb, where species of Poaceae family displayed a higher TF ( $0.81 \pm 0.32$ ), followed by Amaranthaceae ( $0.81 \pm 0.22$ ).

### 3.3. Metals accumulation and partitioning among plant types

Copper, Zn and Cd BCFs were not significantly different among plant types in both species level and phylogenetic comparisons (Table 1). Although Pb accumulation in root tissues of different plant types did not vary significantly at the species level, significant variation was observed across plant type, when data were organised phylogenetically. Xerohalophyte species exhibited significantly greater Pb accumulation in root tissues (BCF 7.40) compared to other plant types ( $F = 3.595$ ,  $p = 0.029$ ). However, Pb was accumulated in the roots of Hydrohalophytes ( $1.34 \pm 0.49$ ), Psammophiles (0.93) and Weedy plants ( $0.78 \pm 0.38$ ) at concentrations similar to sedimentary doses. In terms of TFs for Cu, Zn and Pb, no significant variation was observed at both the species and phylogenetic analysis (Table 1). Weedy plants exhibited significantly higher Cd mobility (TF,  $1.43 \pm 0.50$ ) than other plant types ( $F = 3.542$ ,  $p = 0.053$ ) however no difference among plant types was observed after phylogenetic correction.



**Fig. 1.** Box and Whisker plots (median, quartiles and upper and lower limits) for uptake and partitioning of metals among families of saltmarsh halophytes globally, where a. Cu BCF; b. Cu TF; c. Zn BCF; d. Zn TF; e. Cd BCF; f. Cd TF; g. Pb BCF and h. Pb TF (mean  $\pm$  se) and different letters indicate significant differences among families.



**Table 1**

Bioaccumulation factors (BCF) and translocation factors (TF) of metals Cu, Zn, Cd and Pb (mean  $\pm$  se) among halophytic plant types. ANOVAs assessing differences among groups at the species level and after phylogenetic correction. Metals where statistically significant differences among plant types occur are denoted by different letters (*italicised letters for phylogenetic comparisons*).

	Plant type	Plant type						Species-level		Phylogenetic	
		Hydrohalophyte	Psammophile	Weedy	Xerohalophyte	Xerophyte	Unknown	F	P	F	P
BCF	Cu	1.55 $\pm$ 0.45	0.85 $\pm$ 0.52	1.89 $\pm$ 1.09	0.17	0.74 $\pm$ 0.43	5.47 $\pm$ 2.51	0.149	0.961	1.887	0.165
	Zn	2.75 $\pm$ 0.99	0.86 $\pm$ 0.21	6.53 $\pm$ 4.94	–	0.36	2.49 $\pm$ 1.21	0.518	0.675	2.182	0.138
	Cd	3.95 $\pm$ 1.70	0.39	1.78 $\pm$ 0.78	–	–	1.85 $\pm$ 0.39	0.384	0.688	2.003	0.184
	Pb	1.34 $\pm$ 0.49 <sup>a</sup>	0.93 <sup>a</sup>	0.78 $\pm$ 0.38 <sup>a</sup>	7.40 <sup>b</sup>	0.33 $\pm$ 0.15 <sup>a</sup>	1.69 $\pm$ 0.98 <sup>a</sup>	0.744	0.539	3.595	0.029
TF	Cu	0.54 $\pm$ 0.23	0.73 $\pm$ 0.15	0.62 $\pm$ 0.21	1.2	0.44 $\pm$ 0.19	2.27 $\pm$ 2.05	0.362	0.833	1.941	0.151
	Zn	0.51 $\pm$ 0.15	1.09 $\pm$ 0.27	1.27 $\pm$ 0.30	–	0.53 $\pm$ 0.00	0.82 $\pm$ 0.61	1.314	0.301	0.729	0.589
	Cd	1.24 $\pm$ 0.74 <sup>a, b</sup>	0.27	1.43 $\pm$ 0.50 <sup>a</sup>	–	6.85	0.18 $\pm$ 0.06 <sup>b</sup>	3.452	0.053	0.635	0.647
	Pb	0.76 $\pm$ 0.23	0.06	0.95 $\pm$ 0.40	0.07	0.28 $\pm$ 0.10	0.62 $\pm$ 0.33	0.439	0.727	2.512	0.073

### 3.4. Metal accumulation and partitioning among life forms

Levels of Zn, Cd and Pb sequestration from sediment to root tissues and translocation from roots to leaf tissues among life forms were not significantly different at both the species level and after phylogenetic comparison (Table 2). Only Cu BCFs were found to be significantly higher in Chamaephytes (11.08  $\pm$  3.55) compared to other life forms (F = 3.879, p = 0.025) at the species level but this difference was not maintained after phylogenetic evaluation (F = 0.897, p = 0.511). The findings suggest that differences in Cu uptake observed in this group may be attributable to the close kinship of a limited number of halophyte species within this group.

### 3.5. Metal accumulation and partitioning in the presence of salt glands and bladders

All studied metals were found to accumulate to higher concentrations in root tissues of saltmarsh species that do not have salt glands/bladders compared with plant species that possess salt secreting mechanisms (Table 3). The species without salt secretion capacity exhibited metal accumulation to roots at 2–4 times higher than their concentration in sediment. In contrast, species which possess salt secretory mechanisms exhibited a lower uptake from sediments to roots, with approximately a 50% reduction in roots compared to sediments. Despite these trends, only Zn BCFs were significantly higher in non-secreting species compared with secreting species at the species level (F = 3.913, p = 0.037) though this pattern was not maintained after phylogenetic correction (F = 3.422, p = 0.064). Similarly, the findings indicated salt secreting species tend to have lower leaf metal loads compared to non-salt secreting species though again, there was no significant difference observed among groups for Cu, Zn and Pb TFs (Table 3). Conversely, Cd TF indicated greater mobility in salt secreting species than non-salt secreting species, though this trend was also non-significant.

**Table 2**

Bioaccumulation factors (BCF) and translocation factors (TF) of metals Cu, Zn, Cd and Pb (mean  $\pm$  se) among halophytic life forms. ANOVAs assessing differences among groups at the species level and after phylogenetic correction. Metals where statistically significant differences among life forms occur are denoted by different letters.

	Life form	Life form						Species-level		Phylogenetic	
		Annual	Chamaephyte	Herbaceous perennial	Nano-chamaephyte	Perennial grass	Shrub	F	P	F	p
BCF	Cu	0.47 $\pm$ 0.35 <sup>a</sup>	11.98 $\pm$ 3.55 <sup>b</sup>	1.07 $\pm$ 0.24 <sup>a</sup>	0.57	2.09 $\pm$ 0.83 <sup>a</sup>	7.35	3.879	0.025	0.897	0.511
	Zn	0.27 $\pm$ 0.09	3.90 $\pm$ 2.46	2.92 $\pm$ 1.87	1.59	4.1 $\pm$ 1.57	4.21	1.801	0.186	0.331	0.883
	Cd	0.99	2.37	3.80 $\pm$ 1.72	3.05	1.52 $\pm$ 0.74	1.90 $\pm$ 0.26	0.419	0.667	0.456	0.798
	Pb	0.24 $\pm$ 0.16	4.26	1.41 $\pm$ 0.60	1.50	1.66 $\pm$ 0.78	0.26	1.825	0.188	0.359	0.868
TF	Cu	3.64 $\pm$ 2.96	0.06 $\pm$ 0.03	0.67 $\pm$ 0.21	0.34	0.42 $\pm$ 0.12	0.02	2.704	0.071	1.219	0.351
	Zn	1.42 $\pm$ 0.89	0.11 $\pm$ 0.05	0.98 $\pm$ 0.18	0.19	0.42 $\pm$ 0.13	0.02	2.054	0.142	2.348	0.111
	Cd	3.71 $\pm$ 3.14	0.18 $\pm$ 0.15	0.78 $\pm$ 0.21	0.03	1.96 $\pm$ 1.61	0.19 $\pm$ 0.16	1.815	0.170	0.579	0.716
	Pb	0.56 $\pm$ 0.31	0.14	0.57 $\pm$ 0.18	0.02	0.81 $\pm$ 0.32	1.58	0.012	0.988	1.799	0.170

### 3.6. Metal accumulation and partitioning in halophytes having different photosynthesis strategies

We also compared Cu, Zn, Cd and Pb accumulation in roots and partitioning in leaves across the saltmarsh species after grouping them based on their photosynthetic pathway as either C<sub>3</sub>, C<sub>4</sub> or unknown (Table 4). In general, BCF in C<sub>3</sub> species exhibited a trend for greater bioconcentration from sediment to roots than C<sub>4</sub> species, though there were no significant differences for all metals with photosynthetic type at both the species level and after phylogenetic correction. In terms of TFs, there was also no significant difference of metal partitioning in leaves between saltmarsh species having C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways. Non significance was observed at both the species and phylogenetic level.

### 3.7. Patterns of metals accumulation and partitioning in halophytes based on habitat

Metal accumulation to root tissue did not show any significant difference across habitat classes (Table 5). Despite this, some trends were evident. Species residing in mid-upper saltmarshes exhibited very high Cu accumulation to roots (BCF = 7.35) compared to other habitats. There was also a trend for Zn to be taken up by plants in roots at higher levels in species that live in permanent inland saline and brackish lakes (BCF = 7.74  $\pm$  3.01). Though again no significant difference, species dwelling in coastal saltmarshes exhibited a trend of higher Cd accumulation to roots (BCF = 3.70  $\pm$  1.54). There was highest Pb accumulation in roots of plants dwelling in permanent inland saline and brackish lakes (3.47  $\pm$  2.46). Likewise, metal translocation across plants organs were not statistically different among habitat classes (Table 5). Coastal saltmarshes and coastal stable dunes saltmarshes exhibited higher Cu and Zn mobility as 1.44  $\pm$  0.87, 1.36, respectively. Permanent inland saline and brackish lakes species exhibited a trend towards greater Cd translocation (2.67  $\pm$  2.10). Continental inland salt steppes showed the highest Pb mobility (0.83  $\pm$  0.57).

**Table 3**

Bioaccumulation factors (BCF) and translocation factors (TF) of metals Cu, Zn, Cd and Pb (mean  $\pm$  se) among taxa with or without salt-secreting glands or bladders. ANOVAs assessing differences among groups at the species level and after phylogenetic correction. Metals where statistically significant differences among categories occur are denoted by different letters.

		Salt glands and bladders			Species-level		Phylogenetic	
		No	Unknown	Yes	F	P	F	p
BCF	Cu	1.75 $\pm$ 1.15	2.96 $\pm$ 1.02	0.74 $\pm$ 0.31	1.033	0.372	0.138	0.872
	Zn	4.10 $\pm$ 1.89 <sup>a</sup>	3.70 $\pm$ 1.56 <sup>a</sup>	0.44 $\pm$ 0.17 <sup>b</sup>	3.913	0.037	3.422	0.064
	Cd	2.01 $\pm$ 0.43	4.19 $\pm$ 1.66	0.70 $\pm$ 0.15	2.477	0.118	2.400	0.141
	Pb	1.96 $\pm$ 1.43	1.17 $\pm$ 0.30	1.64 $\pm$ 1.16	0.039	0.962	0.056	0.946
TF	Cu	2.04 $\pm$ 1.74	0.64 $\pm$ 0.16	0.35 $\pm$ 0.19	0.495	0.616	0.165	0.849
	Zn	0.91 $\pm$ 0.50	0.68 $\pm$ 0.16	0.76 $\pm$ 0.25	0.147	0.865	1.394	0.280
	Cd	1.49 $\pm$ 1.08	0.59 $\pm$ 0.20	2.09 $\pm$ 1.59	0.363	0.700	0.319	0.732
	Pb	0.79 $\pm$ 0.32	0.54 $\pm$ 0.14	0.77 $\pm$ 0.36	0.114	0.893	0.286	0.754

**Table 4**

Bioaccumulation factors (BCF) and translocation factors (TF) of metals Cu, Zn, Cd and Pb (mean  $\pm$  se) among taxa with differing photosynthetic modes. ANOVAs assessing differences among C<sub>3</sub>, C<sub>4</sub> and unknown at the species level and after phylogenetic correction.

		Photosynthetic pathway			Species-level		Phylogenetic	
		C3	C4	Unknown	F/t	P	F	P
BCF	Cu	2.61 $\pm$ 0.96	1.27 $\pm$ 0.10	1.87 $\pm$ 0.76	0.048	0.954	0.494	0.619
	Zn	3.66 $\pm$ 1.31	0.71 $\pm$ 0.36	1.78 $\pm$ 0.88	0.381	0.688	0.879	0.438
	Cd	3.25 $\pm$ 1.11	0.39	1.57 $\pm$ 0.63	0.693	0.499	2.712	0.115
	Pb	1.64 $\pm$ 0.53	0.51 $\pm$ 0.26	1.17 $\pm$ 0.51	0.475	0.628	0.059	0.942
TF	Cu	1.02 $\pm$ 0.64	0.49 $\pm$ 0.39	0.95 $\pm$ 0.38	0.855	0.438	1.461	0.259
	Zn	0.77 $\pm$ 0.20	0.67 $\pm$ 0.14	0.70 $\pm$ 0.07	0.579	0.569	1.087	0.364
	Cd	0.97 $\pm$ 0.40	5.14 $\pm$ 4.86	0.38 $\pm$ 0.21	1.066	0.362	2.234	0.142
	Pb	0.55 $\pm$ 0.15	0.99 $\pm$ 0.75	0.71 $\pm$ 0.22	0.596	0.558	0.865	0.437

**Table 5**

Metal (Cu, Zn, Cd and Pb (mean  $\pm$  se)) uptake and partitioning (BCF and TF) in plants residing in different habitats (where A2.5 = Coastal saltmarshes and saline reedbeds; A2.52 = Upper saltmarshes; A2.53 = Mid-upper saltmarshes and saline and brackish reed beds; A2.54 = Low-mid saltmarshes; B1 = Coastal dunes and sandy shores; B1.4 = Coastal stable dune grasslands (grey dunes); C1.5 = Permanent inland saline and brackish lakes; D6.1 = Inland saltmarshes; and E6.2 = Continental inland salt steppes). ANOVAs assessing differences among habitats at the species level.

		Habitat								Species-level		
		A2.5	A2.52	A2.53	A2.54	B1	B1.4	C1.5	D6.1	E6.2	F	P
BCF	Cu	2.56 $\pm$ 1.15	0.08	7.35	1.37		0.33	0.93 $\pm$ 0.42	1.94 $\pm$ 1.08	5.32	0.186	0.832
	Zn	2.93 $\pm$ 1.49	0.24	4.21	1.07		0.65	7.74 $\pm$ 3.01	1.30 $\pm$ 0.95	5.57	1.736	0.210
	Cd	3.70 $\pm$ 1.54	0.44	2.16	0.69 $\pm$ 0.30	1.64		2.63 $\pm$ 0.91	2.63		1.012	0.395
	Pb	1.57 $\pm$ 0.59	0.09		0.53 $\pm$ 0.40	0.26		3.74 $\pm$ 2.46	0.88 $\pm$ 0.45	1.21 $\pm$ 0.41		0.708
TF	Cu	1.44 $\pm$ 0.87	0.74	0.02	0.88		0.58	0.30 $\pm$ 0.16	0.46 $\pm$ 0.25	0.58	0.771	0.477
	Zn	0.94 $\pm$ 0.24	0.66	0.02	0.81		1.36	0.28 $\pm$ 0.14	0.58 $\pm$ 0.06	0.1	0.606	0.558
	Cd	1.40 $\pm$ 0.74	0.20	0.03	0.42 $\pm$ 0.15	0.35		2.67 $\pm$ 2.10	0.31 $\pm$ 0.15		0.495	0.690
	Pb	0.69 $\pm$ 0.25	0.20		0.74 $\pm$ 0.68	1.58		0.32 $\pm$ 0.24	0.57 $\pm$ 0.20	0.83 $\pm$ 0.57		0.465

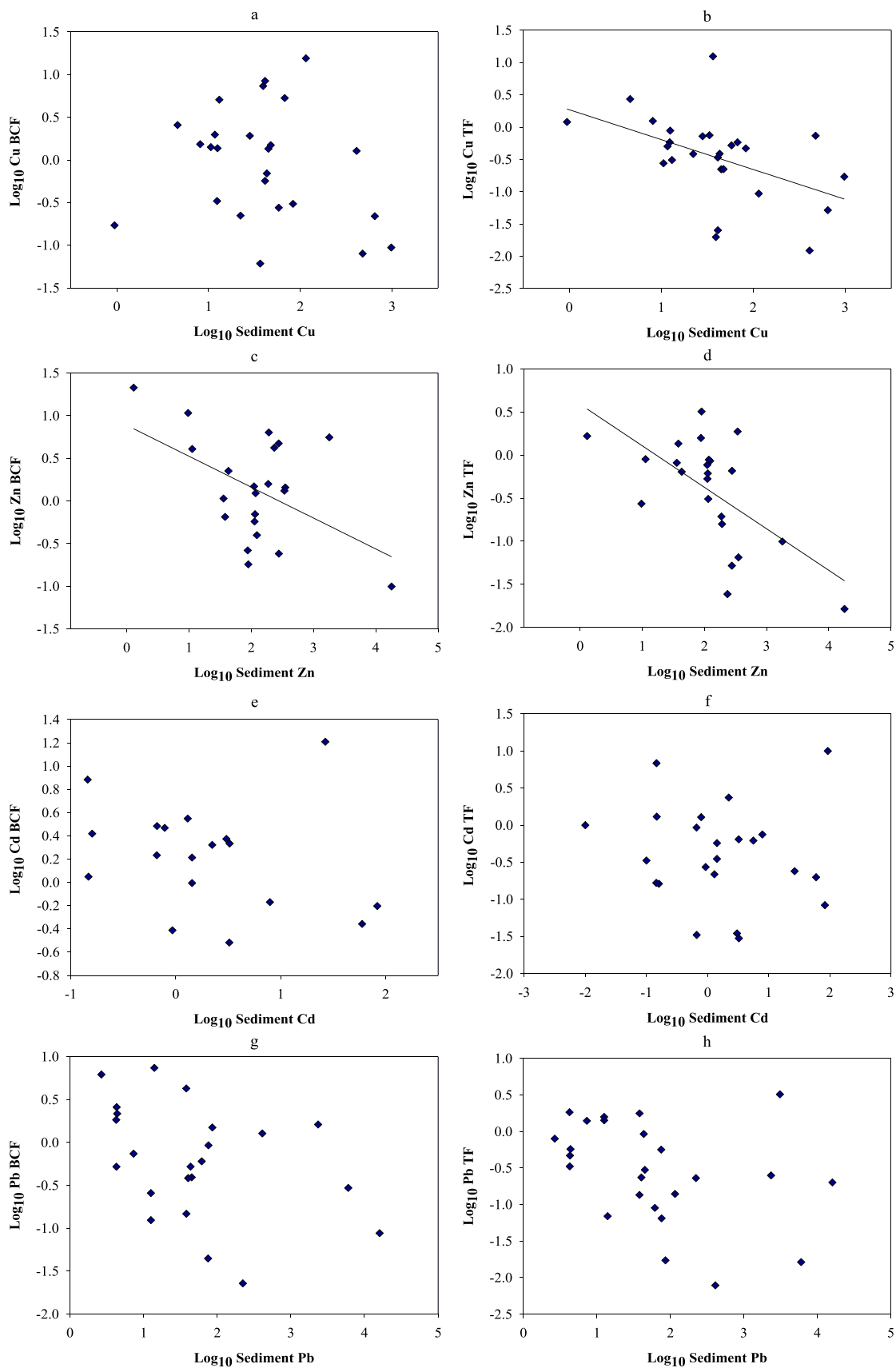
### 3.8. Accumulation and partitioning of metals with environmental dose

Although Cu BCF in halophyte root tissue exhibited no linear relation with environmental Cu exposure at the species level, a negative linear trend of Cu BCF was found with increased sediment Cu after correcting for phylogenetic relatedness with intermediate predictive ability of explaining the dependent variable; ( $\beta = -0.50$ ,  $t = -2.68$ ,  $p = 0.02$ ,  $R^2 = 0.31$ ) (Fig. 2a; Supplementary File S3.1). Cu mobilities across plant organs to leaves were negatively correlated to Cu exposure at the species level with low  $R^2$  ( $\beta = -0.46$ ,  $t = -2.67$ ,  $p = 0.01$ ,  $R^2 = 0.23$ ), but no such relationship was maintained after phylogenetic correction, (Fig. 2b; Supplementary File S3.1). Zn BCFs were higher at lower Zn exposures at the species level, indicating a weak relationship between them ( $\beta = -0.36$ ,  $t = -2.50$ ,  $p = 0.02$ ,  $R^2 = 0.24$ ), but again non-significant after phylogenetic evaluation (Fig. 2c; Supplementary File S3.1). Relatively strong negative relations were observed between Zn TFs and environmental Zn at the species level assessment ( $\beta = -0.8$ ,  $t = -3.59$ ,  $p = 0.00$ ,  $R^2 = 0.38$ ), which was maintained after phylogenetic relatedness was accounted for ( $\beta = -0.56$ ,  $t = -2.90$ ,  $p = 0.01$ ,  $R^2 = 0.38$ ) (Fig. 2d; Supplementary File S3.1). Cd BCFs and Cd TFs

exhibited no relation to environmental Cd exposures (Fig. 2e and f; Supplementary File S3.1). Although no relationship between Pb BCF and TF with sediment Pb was observed at the species level, when we corrected data for phylogenetic kinship, a strong decremental trend for Pb BCF with incremental sediment Pb concentration ( $\beta = -0.34$ ,  $t = -4.31$ ,  $p = 0.00$ ,  $R^2 = 0.54$ ) and Pb TFs with sediment Pb exposure was observed ( $\beta = -0.20$ ,  $t = -2.41$ ,  $p = 0.03$ ,  $R^2 = 0.24$ ) (Fig. 2g and h; Supplementary File S3.1).

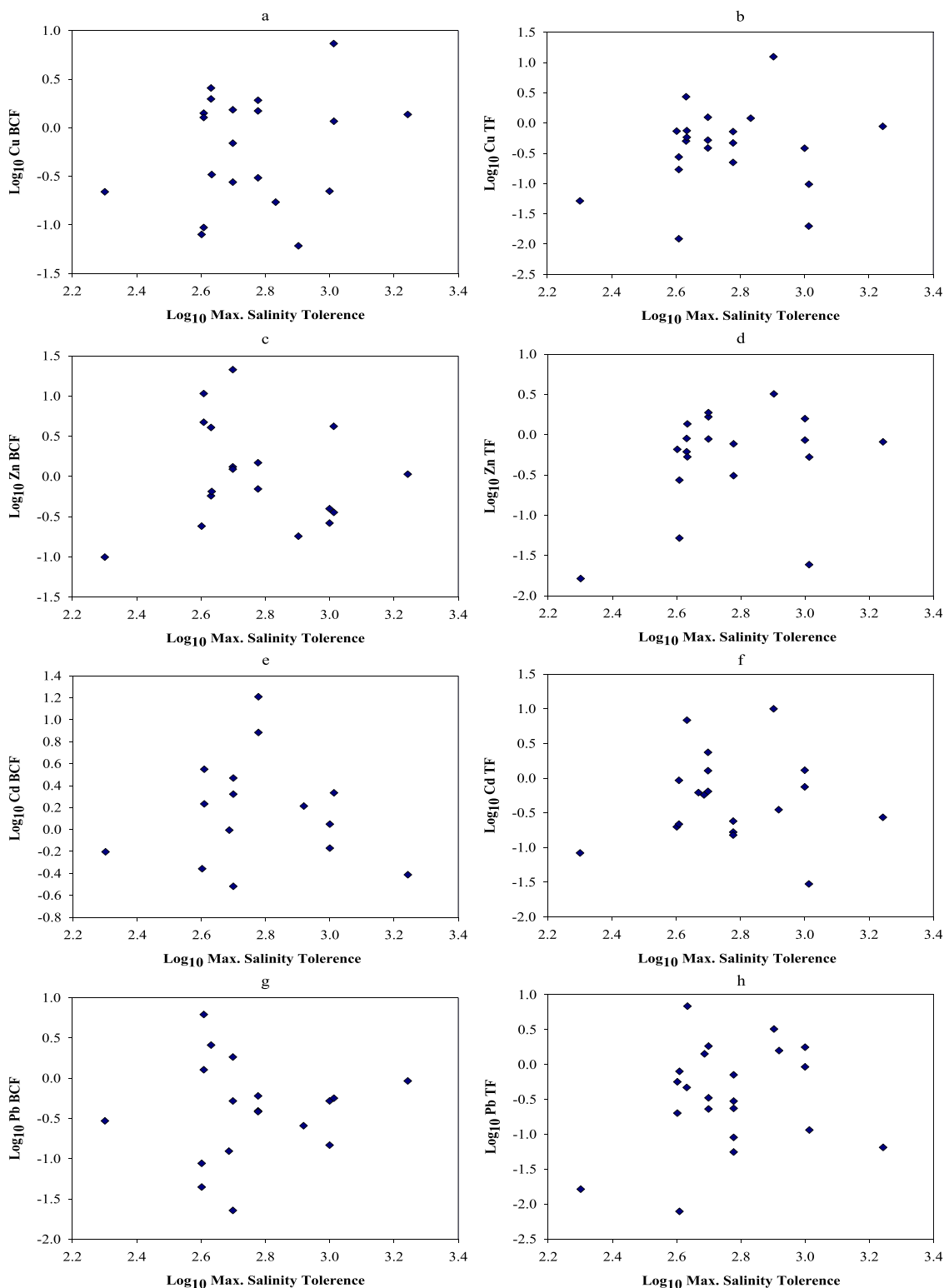
### 3.9. Relationship between metal accumulation and partitioning and species maximum salinity tolerance

Root Cu BCF did not show any linear pattern with incremental species salinity tolerance (Fig. 3a and Supplementary File S3.2). Likewise, no relationship was observed between Cu TF and species salinity tolerance (Fig. 3b and Supplementary File S3.2). Zn BCF exhibited no linear trend with maximum salinity tolerance limit, nor leaf Zn TF (Fig. 3c and d, Supplementary File S3.2). Similarly, Cd accumulation to roots were not linearly linked with salinity tolerance, and no relation between Cd translocation from roots to leaves and salinity limit was observed



**Fig. 2.** Bivariate relationship between (a) log<sub>10</sub> sediment Cu ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Cu BCF; (b) log<sub>10</sub> sediment Cu ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Cu TF; (c) log<sub>10</sub> sediment Zn ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Zn BCF; (d) log<sub>10</sub> sediment Zn ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Zn TF; (e) log<sub>10</sub> sediment Cd ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Cd BCF; (f) log<sub>10</sub> sediment Cd ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Cd TF; (g) log<sub>10</sub> sediment Pb ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Pb BCF; and (h) log<sub>10</sub> sediment Pb ( $\mu\text{g.g}^{-1}$ ) and log<sub>10</sub> Pb TF. Statistically significant regressions have regression line indicated in bold line.





**Fig. 3.** Bivariate relationships between  $\log_{10}$  max. salinity tolerance (mM) and (a)  $\log_{10}$  Cu BCF; (b)  $\log_{10}$  Cu TF; (c)  $\log_{10}$  Zn BCF; (d)  $\log_{10}$  Zn TF; (e)  $\log_{10}$  Cd BCF; (f)  $\log_{10}$  Cd TF; (g)  $\log_{10}$  Pb BCF; and (h)  $\log_{10}$  Pb TF.

(Fig. 3e and f, [Supplementary File S3.2](#)). Further, when we were examining the pattern of Pb uptake to roots and Pb mobility across plant organs with salinity tolerance, no significant relationship was established again (Fig. 3g and h, [Supplementary File S3.2](#)). No significance for all comparisons was observed at both the species level and after phylogenetic correction.

### 3.10. Sediment metal exposure as a covariate in analyses

Given that some BCFs and TFs vary negatively with dose, we included sediment metal as a covariate in analyses where sufficient replicates were possible for regression analyses. In multiple regression, after controlling for metal exposure, no significant relationship was found between BCF or TF with maximum salinity tolerance for all

examined metals, both at the species level and after phylogenetic correction (Supplementary File S3.3).

In the ANCOVA analysis for salt glands, we ran a customised model with an interaction term testing for homogeneity of slopes in terms of relationship between Cu, Zn, Cd and Pb (BCF, TF) and sediment metal loading among categories (presence/absence of salt glands). All were non-significant, except for Cd TF under phylogenetic analysis, thus for this parameter we were not able to move forward to the main effects model. For the main effects model, after controlling for sediment metal exposure, there were no significant differences in BCF and TF for all metals among species with salt gland and bladder presence/absence at both the species level and after phylogenetic correction (Supplementary File S3.4A).

In terms of the photosynthetic pathway analysis, similarly we checked the homogeneity of slopes in an interactive model of Cu, Zn, Cd and Pb accumulation, and translocation with sediment metal exposure between photosynthetic pathway categories. As a significant interaction term was observed for both Cd and Pb TFs in phylogenetic analyses, suggesting heterogeneous slopes, we were only able to test the main effect model for other cases. The main effect model did not exhibit significant difference in metal accumulation (BCF) or translocation (TF) between species having either C<sub>3</sub> or C<sub>4</sub> photosynthetic pathways for all metals examined after correcting for sediment metal exposure in both species level and phylogenetic analyses (Supplementary File S3.4B).

## 4. Discussion

### 4.1. Distribution patterns of metals Cu, Zn, Cd and Pb

Although there was significant variability and heterogeneity among species in terms of uptake and translocation, a number of broad patterns of metal uptake and translocation can be identified for saltmarsh as a clade. Uptake from sediment to root tissue was relatively high for most species, with average BCFs between 2 and 3 for Cu, Zn and Cd. Bioconcentration factors were lower for the non-essential toxic metal Pb, with an average BCF of 1.4. Translocation from root to shoot was at unity or slightly lower for the essential metals Cu and Zn and also the non-essential Cd. Mobility of non-essential Pb was much lower, with concentrations in leaves lower than roots suggesting barriers to translocation from root to shoot.

Patterns of uptake from sediment to root greater than unity, and restricted translocation at unity or lower to leaf tissue is a pattern consistent with most terrestrial vascular plants and suggests few, if any, saltmarsh taxa could be classified as hyperaccumulators (high shoot: root metal ratios) (Salt et al., 1995). Thus, for the majority of saltmarsh taxa, metals are likely accumulated from sediment in their free cationic forms (Prasad, 2013). Uptake of metals in roots is probably transported predominantly via apoplastic pathways with some chelation to cell wall matrices (MacFarlane and Burchett, 2000). However, when accumulation is above unity, which was the case for the majority of metals, some active uptake via transporters across the root plasmalemma is probable. Barriers to transport observed at the root: shoot transition, and consequently lower translocation factors, is likely due to the endodermis and casparian strip, driving cation transport from predominantly apoplastic to symplastic pathways (Greger, 2004). This allows membrane associated transporter specificity and regulation of translocation to the shoot. Movement of metals are thus restricted with limited efflux into the xylem for transport to leaf tissue. Indeed, MacFarlane and Burchett (2000) examined cellular uptake of Cu, Pb and Zn in roots of the halophyte, *Avicennia marina*. Through SEM X-ray microanalysis, it was found that the root endodermis/casparian strip provided barrier and reduced transport of Cu and Zn into vascular tissue. Conversely, MacFarlane and Burchett (2000) found Pb was predominantly accumulated in the epidermal layers of the root with little transport beyond this tissue, most likely being absorbed in outer cell walls of the epidermis and cuticle or being chelated in the rhizosphere. Such differential barriers to metal

uptake may explain lower translocation factors for the non-essential metal Pb compared to other metals examined. More broadly across glycophytes, Cestone et al. (2012) investigated Cu distribution in the roots and leaves of *Brassica carinata* using micro-PIXE. Higher concentrations of Cu were present in the endodermis and inner-cortical cells relative to vascular bundles, suggesting the endodermal layer was an effective barrier to acropetal transport.

Both Cu and Zn, showed higher uptake to roots and translocation to leaf tissue, reflecting their essentiality and the plants' requirement for these metals for active metabolism. Interestingly, Cd also showed high uptake and translocation, mirroring the essentials, despite being a toxic metal with no biological function. It is unlikely specific transporters have evolved to allow regulated traffic of Cd through the plant, so perhaps similar transport mechanisms for this divalent cation are shared with the essentials explaining their similar transportation properties. Clarkson and Lüttge (1989) argue that uptake of Cd appears to be in competition for similar populations of transmembrane carriers as other essential divalent cations (eg. Ca, Mg, Fe and Cu and Zn). Indeed, Bert et al. (2003) observed that both Cd and Zn were co-accumulated in the aerial organs of *Arabidopsis halleri*. These authors suggest that these metals are likely regulated by the same transporters, or if different, are controlled by common regulators. Further, direct evidence exists that Zn-specific transporters, namely the ZNT1 transporter, facilitates not only high affinity Zn transport, but also low affinity Cd transport in the Zn/Cd hyperaccumulator, *Thlaspi caerulescens* (Pence et al., 2000). Thus, it is possible in saltmarsh that transporter assemblages for essential cations also co-transport Cd in certain instances.

Despite saltmarsh being a phylogenetically diverse group, with the saltmarsh element comprising of a number of distinct, distantly related families with largely separate evolutionary histories, the patterns of accumulation and translocation for each metal among families were broadly similar. For all metals, both BCF and TFs were similar among families, suggesting no phylogenetically related differences in uptake strategies. The only exception to this general pattern was that representatives of rushes from the family Juncaceae exhibit significantly greater Cd accumulation to roots than other families. This pattern reflects current qualitative cross species accounts of family differences in metal uptake where *Juncus* is reported to have greater metal accumulation to roots than other saltmarsh taxa (Almeida et al., 2004, 2006; Anjum et al., 2014). Further, no relationships with any plant attributes and accumulation or translocation were observed.

### 4.2. Patterns of accumulation and translocation with environmental dose

For some metals, higher BCFs and TFs were observed at lower environmental exposures. At the species level, Zn BCF and Zn and Cu TF were higher at lower sediment exposures albeit low R<sup>2</sup>. Conversely, after the data was corrected for phylogenetic relatedness, Cu and Pb BCF and Zn and Pb TF were higher at lower sediment metal exposures (R<sup>2</sup> varies from 0.24 to 0.54). Collectively, Zn TF was the only parameter consistently higher at lower Zn sediment exposures in both species and phylogenetic levels of analysis. Thus, it appears for metals such as Zn, that translocation is active to meet metabolic requirements, so at lower exposures the ratio of root to shoot metal is relatively higher. Conversely, a plateauing of accumulation with dose when metabolic demands are met for essential metals is another plausible explanation for the pattern observed. This pattern reflects largely what others have found, with Cu and Zn bioconcentration and transportation in mangroves being higher at lower sediment exposure concentrations (MacFarlane et al., 2007). Mirshekari et al. (2012), also reported greater Zn BCFs at lower exposure concentrations of sediment Zn in glycophytes, *Sorgum* and *Chenopodium* spp., suggesting active uptake for metals with essentiality at lower exposures.

Despite identifying this potential confounding factor as each species environmental exposure varies, when examining patterns of BCF and TF variability with other adaptations and correcting for this bias in both

regressions and ANCOVAs the interpretation of findings was not altered. In other words, no significant differences or relationships between BCF and TF with salt gland and bladder presence/absence, photosynthetic mode or salinity tolerance were observed after including sediment metal as a covariate.

#### 4.3. Patterns of accumulation and translocation with saline adaptations

When examining differences in metal uptake and translocation among species depending on whether species possess adaptations for dealing with excess salt such as salt glands or bladders some patterns were uncovered. It was observed, at the species level, that species which possess salt glands or bladders possess lower leaf proportions of Zn compared to non-secreting species. This may imply that excess Zn is co-excreted with Na in such species, though the patterns were not maintained after phylogenetic correction, suggesting the pattern was characteristic of a few closely related descendent taxa and the pattern is not a relationship that has evolved independently and repeatedly in evolutionary time. In the halophytic dicot, *A. marina*, MacFarlane and Burchett (2000) found leaf washings of Zn treated plants contained greater concentrations of Zn than control plants. They also found that salt crystals exuded from glandular trichomes were composed of alkaline metals and Zn in Zn-treated plants. Further, they found that Zn concentrations in leaf tissue declined from the xylem, through photosynthetic tissue, with a subsequent loading into glandular tissue for excretion. Similarly, the excretion of metals, such as Cu, Hg, Cr, Pb and Zn, from salt glands in the leaves of the monocot salt marsh grass, *Spartina alterniflora* has also been evidenced (Burke et al., 2000; Kraus et al., 1986). It is thus likely that select taxa harness salt secreting tissues to co-excrete metals in excess of nutritional requirements, though it is not a pattern typical of all secreting species.

It was found that species which exhibit higher salinity tolerance, do not necessarily exhibit higher accumulation or translocation patterns for both essential and non-essential metals. So, despite exhibiting greater salinity tolerance, and likely greater accumulation and translocation of the monovalent cation  $\text{Na}^+$  to allow sufficient osmotic potential to allow water uptake in the plant at higher saline exposures, divalent cations do not follow the same accumulation and translocation patterns. Although some past researchers have observed a coupled relationship between saline exposure or  $\text{Na}^+$  accumulation and metal translocation, the patterns are not always consistent. For example, in a lab study, glycophytes, *Elodea canadensis* and *Potamogeton natans* were cultivated in media enriched with Cu, Zn, Cd, and Pb along with incremental salinities of 0.0, 0.5, and 5.0  $\text{gm kg}^{-1}$ . Copper, Zn, Cd bioconcentrations increased with increasing salinity but salinity did not affect Pb accumulation (Fritioff et al., 2005). Further, increasing salinity increased Cd accumulation in the halophyte, *Atriplex halimus L.*, while there were no clear effects of salt on Pb uptake in *Atriplex* tissues (Manousaki and Kalogerakis, 2009). Despite evidence of salinity and metal co-accumulation, others have found no such relationships or the converse. Matijevic et al. (2014), found lower Cu content in the reproductive parts (seeds and pods) of salt treated *Vicia faba* plants (glycophyte). Similarly, NaCl application exhibited no effect on Zn uptake in roots and significantly reduced Zn uptake to the stem of the halophyte *Kosteletzkya virginica* (Han et al., 2012). The discrepancy between Na accumulation patterns and divalent metal accumulation patterns in the current study are likely due to the fact that  $\text{Na}^+$  and metals employ differing and distinct populations of membrane associated transporters to affect regulated translocation.

In terms of monovalent ion transport, several transporters have been found to be implicated in  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  transport and regulation in plants to date (Flowers and Colmer, 2008). For example, cation-chloride co-transporters (CCC) are a family of proteins that couple  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  transport. Further candidates are the cation proton antiporters, including cation/ $\text{H}^+$  exchangers (CHX), which play a role in xylem loading of  $\text{Na}^+$ , as well as the  $\text{Na}^+/\text{H}^+$  exchangers (NHX), which are

localised to the plasma membrane and regulate  $\text{Na}^+$  uptake and transport within the plant. (Munns and Tester, 2008). Little is known mechanistically in terms of how halophytes achieve divalent metal transport regulation, though it seems the transporter populations are different to monovalent membrane associated transport proteins. Several divalent transporters have been implicated in metal transport and regulation in glycophytes, including the heavy metal (CPx-type) ATPases, the natural resistance-associated macrophage protein (Nramp) family, members of the cation diffusion facilitator (CDF) family, and members of the ZIP transporter family (Guerinot, 2000; Williams et al., 2000).

#### 4.4. Limitations of the study

It must be acknowledged that at this point in time the available database of metal accumulation in saltmarsh taxa globally is very limited, with only 20.3% (28 species) of saltmarsh taxa present in the eHALOPH database with available metal accumulation data. This is at the lower limit for reliable phylogenetic analyses. Further, the variability among taxa in BCF and TF for all metals was rather large, resulting in significant heterogeneity of variance and sometimes coupled with low sample sizes among categories may mask any discernible differences. Although attempts were made to account for this heterogeneity, by transforming raw data, resultant data was still heterogeneous, which precludes identifying subtle differences among categories.

In addition, the significant sediment physicochemical differences such as grain size, pH, organic matter and salinity among studies can influence metal bioavailability and thus uptake patterns, contributing to the greater variance seen among taxa. Some other factors, like spatial and temporal differences in metal bioavailability, the dose, i.e., the degree of contamination at the location, and co-existence of multiple metallic contaminants may influence uptake. Further a whole host of location-specific and analytical differences among studies mentioned in the methods may contribute to greater variability among candidate studies, clouding categorical differences.

As the research effort moves forward, we predict that more species will be added to the database with time, which will allow for more robust analyses in the future potentially controlling for these confounding variables. In the meantime, the present analysis provides a valuable assessment of our current state of knowledge in the area and helps identify existing research gaps.

### 5. Conclusions

Saltmarsh taxa as a group tend to accumulate metals to roots at levels above unity (BCF >1), suggesting active accumulation. Translocation from root to shoots is higher for essential metals (Cu and Zn) and the non-essential Cd (TF  $\leq$  1), while translocation for the non-essential metal Pb is much lower (TF = 0.65), exhibiting reduced mobility in the plant. Despite saltmarsh being a heterogenous group and most families being phylogenetically distantly related, patterns of accumulation to root and translocation to leaf tissue are broadly similar among families, with the exception of greater Cd accumulation to roots in members of Juncaceae (BCF = 11.9). Patterns of accumulation and translocation are broadly similar among plant type, plant form, habitat and photosynthetic mode. Zn loads in leaves are lower in salt-secreting species for select taxa, suggesting some species co-excrete  $\text{Na}^+$  and  $\text{Zn}^{2+}$  to maintain cationic balance in leaf tissue. Salinity tolerance and coupled accumulation of  $\text{Na}^+$  bear little relation to metal uptake and translocation, likely due to the fact that  $\text{Na}^+$  and metals are trafficked by different transporter assemblages. Translocation of Zn is greater at lower sediment exposures, reflecting its active uptake and essentiality, but such bias does not affect interpretation of findings for other variables when this is controlled for in analyses.

As more data is added to the international database, variability within taxa will be reduced and finer scale delineation among taxonomic

and plant attribute groups may be availed. Future research should target examining metals in taxa across families at a range of contaminated sites to understand metal accumulation and translocation strategies. Collectively, patterns of high accumulation to roots and limited translocation to leaf tissue means that most saltmarsh taxa are not hyperaccumulators, and as such are not suitable for phytoremediation initiatives of contaminated estuarine locales. Rather most species could be employed as phytostabilisers, sequestering the majority of metal load in roots, preventing transport via detrital export to adjacent estuarine systems.

### CRediT authorship contribution statement

**Md Rushna Alam:** Conceptualization, Methodology, Data curation & Analysis, Writing - original draft preparation and Finalizing the manuscript. **Rafiqueel Islam:** Data curation, Investigation, Writing - review and editing, Finalizing the manuscript. **Thi Kim Anh Tran:** Data curation, Investigation, Writing - review and editing, and Finalizing the manuscript. **Diep Le Van:** Data curation, Writing - review and editing, Investigation. **Mohammad Mahmudur Rahman:** Validation, Writing - review & editing. **Andrea S. Griffin:** Supervision, Validation, Writing - review & editing. **Richard Man Kit Yu:** Supervision, Validation, Writing - review & editing. **Geoff R. MacFarlane:** Conceptualization, Methodology, Data analysis, Validation, Resources, writing - original draft, Writing - review & editing, Supervision and Administration.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

We highly appreciate Mr. Kim Colyvas for his generous assistance in data analysis.

### Authors Declaration

The authors announced that they have no conflict of interest.

### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2021.125515](https://doi.org/10.1016/j.jhazmat.2021.125515).

### References

Almeida, C.M.R., Mucha, A.P., Vasconcelos, M.T.S., 2004. Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environ. Sci. Technol.* 38 (11), 3112–3118.

Almeida, C.M.R., Mucha, A.P., Vasconcelos, M.T.S., 2006. Variability of metal contents in the sea rush *Juncus maritimus* - estuarine sediment system through one year of plant's life. *Mar. Environ. Res.* 61 (4), 424–438.

Anjum, N.A., Ahmad, I., Válega, M., Mohmood, I., Gill, S.S., Tuteja, N., Duarte, A.C., Pereira, E., 2014. Salt marsh halophyte services to metal - metalloid remediation: assessment of the processes and underlying mechanisms. *Crit. Rev. Environ. Sci. Technol.* 44 (18), 2038–2106.

Baker, A.J., 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. *J. Plant Nutr.* 3 (1–4), 643–654.

Bert, V., Meerts, P., Saumitou-Laprade, P., Salis, P., Gruber, W., Verbruggen, N., 2003. Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant Soil* 249 (1), 9–18.

Burke, D.J., Weis, J., Weis, P., 2000. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuar. Coast. Shelf Sci.* 51 (2), 153–159.

Cestone, B., Vogel-Mikuš, K., Quartacci, M.F., Rascio, N., Pongrac, P., Pelicon, P., Vavpetić, P., Grlj, N., Jeromel, L., Kump, P., 2012. Use of micro-PIXE to determine spatial distributions of copper in *Brassica carinata* plants exposed to CuSO<sub>4</sub> or CuEDDS. *Sci. Total Environ.* 427, 339–346.

Clarkson, D.T., Lüttge, U., 1989. Mineral nutrition: divalent cations, transport and compartmentation. *Progress in Botany*. Springer, Berlin, Heidelberg, pp. 93–112.

Engqvist, L., 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim. Behav.* 70 (4), 967–971.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. *New Phytol.* 179, 945–963.

Flowers, T.J., Galal, H.K., Bromham, L., 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. *Funct. Plant Biol.* 37 (7), 604–612.

Flowers, T.J., Munns, R., Colmer, T.D., 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.* 115 (3), 419–431.

Freckleton, R.P., Harvey, P.H., Pagel, M., 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* 160 (6), 712–726.

Fritioff, Å., Kautsky, L., Greger, M., 2005. Influence of temperature and salinity on heavy metal uptake by submersed plants. *Environ. Pollut.* 133 (2), 265–274.

Gedan, K.B., Silliman, B.R., Bertness, M.D., 2009. Centuries of human-driven change in salt marsh ecosystems. *Annu. Rev. Mar. Sci.* 1 (1), 117–141.

Gehanno, J.F., Rollin, L., Darmoni, S., 2013. Is the coverage of google scholar enough to be used alone for systematic reviews. *BMC Med. Inform. Decis. Mak.* 13 (1), 7.

Grafen, A., 1989. The phylogenetic regression. *Philos. Trans. R. Soc. B: Biol. Sci.* 326 (1233), 119–157.

Greger, M., 2004. Metal availability, uptake, transport and accumulation in plants. In: Prasad, M.N.V. (Ed.), *Heavy Metal Stress in Plants from Biomolecules to Ecosystems*, First ed. Springer-Verlag, Heidelberg, Berlin, Hyderabad, India.

Guerinot, M.L., 2000. The ZIP family of metal transporters. *Biochim. Biophys. Acta (BBA)-Biomembr.* 1465 (1–2), 190–198.

Haddaway, N.R., Collins, A.M., Coughlin, D., Kirk, S., 2015. The role of google scholar in evidence reviews and its applicability to grey literature searching. *PLoS One* 10 (9), e0138237.

Han, R.-M., Lefevre, I., Ruan, C.-J., Qin, P., Lutts, S., 2012. NaCl differently interferes with Cd and Zn toxicities in the wetland halophyte species *Kosteletzkya virginica* (L.) Presl. *Plant Growth Regul.* 68 (1), 97–109.

Harbison, P., 1986. Mangrove muds - a sink and a source for trace metals. *Mar. Pollut. Bull.* 17 (6), 246–250.

Harvey, P.H., Pagel, M.D., 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford, UK.

Kraus, M.L., Weis, P., Crow, J.H., 1986. The excretion of heavy metals by the salt marsh cord grass, *Spartina alterniflora*, and *Spartina's* role in mercury cycling. *Mar. Environ. Res.* 20 (4), 307–316.

MacFarlane, G., Burchett, M., 2000. Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquat. Bot.* 68 (1), 45–59.

MacFarlane, G.R., Koller, C.E., Blomberg, S.P., 2007. Accumulation and partitioning of heavy metals in mangroves: a synthesis of field-based studies. *Chemosphere* 69 (9), 1454–1464.

Manousaki, E., Kalogerakis, N., 2009. Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. *Environ. Sci. Pollut. Res.* 16 (7), 844–854.

Matijević, L., Romić, D., Romić, M., 2014. Soil organic matter and salinity affect copper bioavailability in root zone and uptake by *Vicia faba* L. plants. *Environ. Geochem. Health* 36 (5), 883–896.

Mcowen, C.J., Weatherdon, L.V., Van Bochove, J.-W., Sullivan, E., Blyth, S., Zockler, C., Stanwell-Smith, D., Kingston, N., Martin, C.S., Spalding, M., 2017. A global map of saltmarshes. *Biodivers. Data J.* 5, e11764.

Mills, W.B., 1995. *Water Quality Assessment: A Screening Procedure for Toxic and Conventional Pollutants in Surface and Ground Water - Part 1*. US EPA, Georgia.

Mirshakali, H., Hadi, H., Amirnia, R., Khodaverdiloo, H., 2012. Effect of zinc toxicity on plant productivity, chlorophyll and Zn contents of sorghum (*Sorghum bicolor*) and common lambsquarter (*Chenopodium album*). *Int. J. Agric.: Res. Rev.* 2 (3), 247–254.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.

Nath, B., Birch, G., Chaudhuri, P., 2013. Trace metal biogeochemistry in mangrove ecosystems: a comparative assessment of acidified (by acid sulfate soils) and non-acidified sites. *Sci. Total Environ.* 463, 667–674.

Pence, N.S., Larsen, P.B., Ebbs, S.D., Letham, D.L., Lasat, M.M., Garvin, D.F., Eide, D., Kochian, L.V., 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl. Acad. Sci. U.S.A.* 97 (9), 4956–4960.

Prasad, M.N.V., 2013. *Heavy Metal Stress in Plants: From Biomolecules to Ecosystems*. Springer Science & Business Media.

Qian, H., Jin, Y., 2016. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *J. Plant Ecol.* 9 (2), 233–239.

Raoult, V., Gaston, T.F., Taylor, M.D., 2018. Habitat - fishery linkages in two major south-eastern Australian estuaries show that the C4 saltmarsh plant *Sporobolus virginicus* is a significant contributor to fisheries productivity. *Hydrobiologia* 811 (1), 221–238.

Redondo-Gómez, S., Andrades-Moreno, L., Mateos-Naranjo, E., Parra, R., Valera-Burgos, J., Aroca, R., 2011. Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass, *Spartina densiflora*. *J. Exp. Bot.* 62 (15), 5521–5530.

Saintilan, N., 2009. Biogeography of Australian saltmarsh plants. *Austral Ecol.* 34 (8), 929–937.

Salt, D.E., Blaylock, M., Kumar, N.P., Dushenkov, V., Ensley, B.D., Chet, I., Raskin, I., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13 (5), 468–474.

Santos, J., Al-Azzawi, M., Aronson, J., Flowers, T.J., 2016. eHALOPH a database of salt-tolerant plants: helping put halophytes to work. *Plant Cell Physiol.* 57 (1), e10 e10-10.

- Sarika, M., Zikos, A., 2020. Coastal salt marshes: structure and function of plant communities. *Handb. Halophytes: Mol. Ecosyst. Towards Biosaline Agric.* 1–39.
- Schütze, P., Freitag, H., Weising, K., 2003. An integrated molecular and morphological study of the subfamily Suaedoideae Ulbr.(Chenopodiaceae). *Plant Syst. Evol.* 239 (3–4), 257–286.
- Sousa, A.I., Lillebo, A.I., Pardal, M.A., Caçador, I., 2010. The influence of *Spartina maritima* on carbon retention capacity in salt marshes from warm-temperate estuaries. *Mar. Pollut. Bull.* 61 (4–6), 215–223.
- Steffen, W., Crutzen, P.J., McNeill, J.R., 2007. The Anthropocene: are humans now overwhelming the great forces of nature. *AMBIO: J. Hum. Environ.* 36 (8), 614–621.
- Tran, T.K.A., Islam, R., Le Van, D., Rahman, M.M., Yu, R.M.K., MacFarlane, G.R., 2020. Accumulation and partitioning of metals and metalloids in the halophytic saltmarsh grass, saltwater couch, *Sporobolus virginicus*. *Sci. Total Environ.* 713, 136576.
- Van Oosten, M.J., Maggio, A., 2015. Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils. *Environ. Exp. Bot.* 111, 135–146.
- Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environ. Int.* 30 (5), 685–700.
- Williams, L.E., Pittman, J.K., Hall, J., 2000. Emerging mechanisms for heavy metal transport in plants. *Biochim. Biophys. Acta (BBA)-Biomembr.* 1465 (1–2), 104–126.
- Williams, T., Bubb, J., Lester, J., 1994. Metal accumulation within salt marsh environments: a review. *Mar. Pollut. Bull.* 28 (5), 277–290.