Accumulation and partitioning of metals and metalloids in the halophytic saltmarsh grass, saltwater couch, *Sporobolus virginicus*

Thi Kim Anh Tran [a,b], Rafiquel Islam [a,c], Diep Le Van [d], Mohammad Mahmudur Rahman [e], Richard Man Kit Yu [a], Geoff R. MacFarlane [a,*]

[a] School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[b] School of Agriculture and Resources, Vinh University, Viet Nam
[c] Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia 7003, Bangladesh
[d] School of Biochemical Technology-Environment, Vinh University, Viet Nam
[e] Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, NSW 2308, Australia

HIGHLIGHTS

- Accumulation & partitioning of Zn, Cu, Pb, Cd & Se was assessed in saltwater couch.
- Essential metals (Zn and Cu) were more mobile in the plant than other metal(loid)s.
- All metal(loid)s were accumulated to tissues with dose (with the exception of Cu).
- High variability in accumulation meant bioindicator utility was limited.

GRAPHICAL ABSTRACT
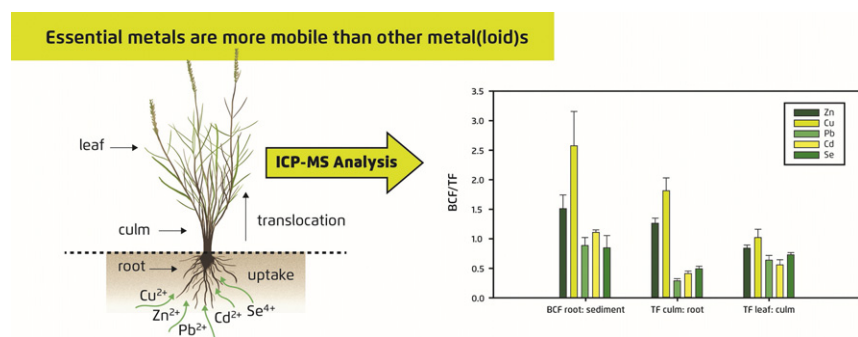
ARTICLE INFO

ABSTRACT

Remnant endangered saltmarsh communities in Australia often occur in urbanised estuaries where industrial processes have contaminated sediments with metal(loid)s. Despite this issue, virtually nothing is known on local plant species exposure to metal contaminants, nor their ability to uptake and translocate metal(loid)s from contaminated estuarine sediment. In the current study, we assessed the accumulation and partitioning of the metal(loid)s Zn, Cu, Pb, Cd and Se in the dominant saltmarsh primary producer, *Sporobolus virginicus*, across three urbanised estuaries in NSW Australia. Lake Macquarie was the most contaminated estuary, while Sydney Olympic Park, Port Jackson exhibited intermediate metal(loid) loadings and Hunter Wetlands exhibited the lowest loadings among estuaries. Essential metals (Zn and Cu) were more mobile, with sediment:root bioconcentration factors (BCFs) greater than unity and translocation among plant organs greater than, or equal to, unity. Other metal(loid)s were less mobile, with BCFs equal to unity and translocation factors among organs much reduced. Despite these barriers to translocation, all metal(loid)s were accumulated to roots with dose, and further accumulative relationships between metal(loid)s in roots and culms, and culms and leaves, were evidenced (with the exception of Cu). Along with sediment metal(loid)s, increases in sediment pH predicted Cu uptake in roots and increases in soil organic matter predicted Se uptake in roots. Although significant positive linear relationships were observed between sediment metal(loid)s and plant organ metal(loid)s(withholding Cu), the variance explained was low to intermediate for most metal(loid)s suggesting employing *S. virginicus* as an accumulative bioindicator would be impractical.

© 2020 Elsevier B.V. All rights reserved.

* Corresponding author at: School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia.
E-mail address: Geoff.MacFarlane@newcastle.edu.au (G.R. MacFarlane).

1. Introduction

Saltmarsh is an ecosystem that occurs in intertidal estuarine environments and provides a number of important ecosystem services. Such services include a floral community that provides habitat for a diverse assemblage of vertebrate and invertebrate taxa; transient habitat and resources for commercially important fish species; vegetation that prevents erosion of soft sediment estuarine shores; significant detrital transport providing resources to adjacent estuarine food webs; and an appreciable carbon sink essential to climate change mitigation (Adam, 2009; Sousa et al., 2010). Despite their inherent ecological importance and value, saltmarsh ecosystems have been subject to significant anthropogenic impacts in terms of reductions in area and distribution (Silliman et al., 2009). As saltmarsh occurs in estuarine settings, often close to coastal urbanisation and industrial activity, saltmarsh has been modified, destroyed or reclaimed through infilling and impediment of tidal flows to create land for agriculture, urban development, waste disposal and industry (Gedan et al., 2008). Further sea-level rise and the landward encroachment of mangroves into areas typically occupied by saltmarsh has resulted in further declines in distribution (Saintilan et al., 2014). Estimates of saltmarsh decline internationally are between 25 and 50% of their historical distribution (Mcowen et al., 2017), while in Australia over 50% of wetlands have been modified or destroyed since European settlement (Saintilan and Williams, 1999). In Australia, acknowledgment of the importance of saltmarsh has resulted in saltmarsh being listed as endangered ecological communities under NSW State (DECC, 2008) and vulnerable under Federal legislation (Department of the Environment and Energy, 2013). Such measures mean that threats and impacts to the vegetation community are managed where possible and that regulation strives to ensure local distributions are maintained. As such, it is necessary to monitor and manage impacting processes. One such potential impacting process is metal contamination of saltmarsh sediments from anthropogenic sources along with resultant impacts to the vegetation community and saltmarsh dependent biota.

Metals enter estuarine saltmarsh sediments from a range sources including effluents from metal smelters and steelworks, overflows from coal-fired power generation ash dams, and industrial, municipal and sewage effluent. More diffuse inputs arise from urban run-off, stormwater, agricultural run-off and boating activities (MacFarlane et al., 2007; Nath et al., 2013, 2014). Resultant metals in estuarine sediments tend to exhibit low mobility and bioavailability due to the binding of metals with negatively charged Si-dominated clay matrices, binding to organic matter and humic substances, exchange with Fe and Mn oxyhydroxides and precipitation of metals as insoluble sulphides attributable to the variably anoxic nature of sediments (Harbison, 1986; Williams et al., 1994). However, periodic oxygenation of sediment from variable tidal regimes, results in decreases in sediment pH, thereby increasing metal bioavailability. Further, increases in sediment salinity increase metal bioavailability due to the exchange of salt-derived cations with metal ions on clay surfaces (Lutts and Lefèvre, 2015). Consequently, resultant bioavailable metals have the potential to accumulate in saltmarsh flora, resulting in sub-lethal effects on the plants themselves and further trophic transfer to saltmarsh dependant biota.

The grasses, in the family Poaceae, are represented by over 7500 species and exhibit a great range in salinity tolerance, from salt-sensitive glycophytes, through to extremely salt-tolerant halophytic species. Salinity tolerance, as assessed by the effective concentration to result in a 50% decline in growth (EC_{50}), ranges from salinities of 150 mM in glycocphytes such as Bouteolua curtipendula, through to >600 mM in halophytes such as the current study species, Sporobolus virginicus (seawater = 550 mM) (Marcum, 2008). The major impediment to growth for grasses in saline sediments is the osmotic inhibition of water uptake. Halophytes adjust osmotically to soil salinity by accumulating Na^+ and 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 Na^+ and Cl^- in vacuoles are balanced by organic solutes in the cytoplasm to maintain osmotic potential (Flowers et al., 2015). Specifically, in a study examining salinity tolerance and ion relations in S. virginicus, plants were exposed to up to 450 mM NaCl and shoot Na^+ and Cl^- uptake was relatively high, accumulating ions to a level required to meet osmotic adjustment (Marcum and Murdoch, 1992).

Despite evidence for accumulation to roots and high translocation to leaf tissue for the monovalent cation Na^+, research on halophytic grasses suggests that they are not hyperaccumulators of divalent metal cations. Most halophytic grasses examined to date, including representatives from the genera Spartina, Triglochin and Phragmites, have been evidenced to accumulate metals to roots at levels approaching unity or greater than surrounding sediment metals concentrations, while barriers exist to acropetal transport, with low metal translocation to the shoot (Otte et al., 1991; Duarte et al., 2010; Almeida et al., 2011; Cambrollé et al., 2011).

Looking more broadly, across all Poaceae, similar patterns are observed, with most species generally not hyperaccumulators of metals, exhibiting the ability to accumulate metals to roots with limited translocation of metals to the aerial portions of the plant (Polechońska and Klink, 2014; Zhang et al., 2014). There are, however, a few grass representatives which deviate from this pattern, and have been found to hyperaccumulate (high shoot metal loading) including Rendlia cupricola and Eragrostis boehmii for Cu, Hordeum spp, Poa pratensis and Triticum aestivum for Hg and Deschampsia cespitosa for Pb, Cd and Zn (Baker and Brooks, 1989; Mahar et al., 2016).

Sporobolus virginicus, (L.) Kunth, commonly known as saltwater couch, is a monocotyledon in the Family, Poaceae. It is a C_4 perennial tussock grass, and is one of the dominant saltmarsh species in the mid-tidal level of South Eastern Australian marshes. It can occur interspersed with other saltmarsh dominants (e.g. Sarcocornia quinqueflora, Suaeda australis, Juncus kraussii and Triglochin striata), or alternatively may occur as a mat-forming monospecific stand. It reproduces asexually/vegetatively through horizontal rhizomes or alternatively sexually through flowering/seed set throughout the year. It occurs in most states of Australia, with populations also present in New Zealand, the Pacific Islands, the Caribbean, Africa, India, China and Indonesia (Adam, 1993). S. virginicus has been found to be the most important saltmarsh species in terms of carbon-based detrital transfer and subsequent fisheries productivity in SE Australian estuaries. Through stable isotope analyses, it was found that S. virginicus had the greatest contribution to consumer diet (25–95%) compared to other saltmarsh species, mangroves and benthic organic matter, for a range of commercial fish, prawn and crab species (Raoult et al., 2018).

Despite its importance as a primary producer, little prior research has examined accumulation of metals in S. virginicus. In the only study published to date, Pulkownik (2000) quantified metals (Cu, Pb, Zn, Fe and Mn) in the shoots of S. virginicus in Australian estuaries and found bioconcentration factors (BCFs: shoot:sediment metal concentrations) generally below unity for all metals examined, suggesting limited acropetal transport of metals. Withholding these preliminary investigations, the relative partitioning of metal(loid)s among plant organs in the species is largely unknown, nor have potential differences in strategy in terms of mobility of essential versus non-essential metal(loid)s been examined in this species. Further, no prior research has focussed on the assessment of patterns of accumulation (increasing tissue concentration with dose) versus regulation (constant concentration in tissue with dose) at key potential cellular and molecular roadblocks in transition zones between various plant organs in the species. Finally, for future biomonitoring initiatives, it would be instructive to ascertain which tissues demonstrate linear accumulated metal(loid) associations with sediment metal(loid) loads, to indicate bioavailability and relative contamination status for future site assessment.

For these various reasons we aim to assess the accumulation and partitioning of metal(loid)s in S. virginicus across an environmental

metal gradient in order to assess metal transport in the plant and relative metal(loid) mobility. Further, we aim to assess the utility of the plant to be employed as an accumulative bioindicator of metal contamination. Accordingly, we pose the following questions in the current study:

1. What is the sediment metal(loid) contamination status of *Sporobolus*-dominated saltmarsh in three urbanised estuaries in New South Wales (NSW), Australia?

2. What is the relative mobility of metals and metalloids (Zn, Cu, Pb Cd and Se) from sediment to root tissue, and further, the relative translocation to culms and leaf tissue in *S. virginicus*?

3. Are metals and metalloids accumulated or regulated at key transition zones between roots, culms and leaves?

4. Do any sediment physiochemical parameters further predict metal (loid) uptake to roots in *S. virginicus*?

5. Do tissue metal(loid) levels correlate with sediment metal(loid) loads and thus can *S. virginicus* be employed as an accumulative bioindicator of metal(loids) in Australian estuaries?

2. Materials and methods

2.1. Study locations

Lake Macquarie (151°35′E, 33°5′S) is a highly urbanised estuarine lake, and further, is the largest saltwater lake in the Southern hemisphere. Lake Macquarie has a long history of industrial development with the most significant impacting processes including a Pb/Zn smelting facility (now decommissioned) and three coal-fired power stations (one now decommissioned at Wangi-Wangi). Towards the Northern end of the lake, was the Pasminco Pb/Zn Smelter, which operated from 1897 to 2003. Consequently, sediments in this end of the lake, especially at Cockle Creek and Five Islands wetland are highly contaminated with trace metals (e.g. Cu, Pb, Zn and Cd) from smelter effluent released during its operation (Batley, 1987; Roach, 2005; Kwon-Rae et al., 2009; MacFarlane et al., 2006; McLean et al., 2009). Towards the Western and Southern reaches of the lake, are two coal-fired power stations, including Eraring (1981-present) and Vales Point power stations (1963-present), respectively. Associated with the power stations are coal-ash dams, laden in metals and metalloids. Historically, the dam at Vales Point would overflow during periods of high dam water levels, but in 1995 operating procedures were updated, with dam water recycled back into the station for cooling (Schneider et al., 2014). Consequently, sediment metals in the West at Dora Creek and to the South in Wyee Bay, although declining with changes in handling procedures, are still elevated predominantly in Se and Cd (Carroll, 1999; Peters et al., 1999; Schneider et al., 2014). Swansea is located at the marine entrance to the lake and is relatively non-contaminated with no direct inputs.

The Hunter Estuary, Hunter Wetlands National Park (Kooragang Island), (151°43′E, 32°51′S) forms part of a wetland mosaic listed as the Hunter Estuary Wetlands Ramsar site. The Kooragang Island wetland is bounded by both urban and industrial developments including the city of Newcastle, a large coal exporting facility, and a decommissioned steelworks with legacy metal and organic contaminants in the sediments of the wetlands (Lottermoser, 1998).

Sydney Olympic Park (SOP) (151°4′E, 33°50′S) is a restored estuarine wetland in Homebush Bay on the Parramatta River, Port Jackson, Sydney. The original wetland complex was reclaimed for industrial development until the restoration of the site for the 2000 Sydney Olympics (Chalmers, 2002). Haslam's flats are a saltmarsh created just prior to the Olympics through elevating an estuarine intertidal creek and remediating with clean topsoil. The Badu saltmarsh, located in Bicentennial Park, was created in the late 1950s, whereby contaminated sediment was dredged from Homebush Bay to create an elevation suitable for saltmarsh colonisation.

A total of four locations were sampled in Lake Macquarie, three locations in Hunter Wetlands National Park, and two locations in Sydney Olympic Park (Fig. 1). Locations were chosen to create a gradient in sediment metal contamination across estuaries to allow assessment of metal accumulation to plant tissues with environmental dose.

2.2. Sampling design and sample preparation

Within each of the 9 locations, plants and sediment were collected at five spatially independent sites, separated by a minimum of 50 m. This was to ensure separate individual plants were sampled and to allow independent assessment of spatial variability in metal load (n = 45 total). At each site, one mature plant (or sample of a plant, as *S. virginicus* spreads vegetatively) was excavated from the sediment using a shovel. Further, a sediment core of 10–15 cm depth (depth *Sporobolus* roots typically descend into the sediment profile) was collected adjacent to the plant using an aluminium trowel. Samples were placed on ice in separate, labelled, plastic zip-lock bags and transported back to the laboratory. Plants were sampled from monospecific stands of *S. virginicus* to avoid sampling roots of other saltmarsh species. All specimens were of adult stature, of healthy appearance with green photosynthetic tissue (i.e. no senescence), growing in full irradiance and had no visible signs of pathogens. To minimise seasonal differences in accumulation with growth, all locations were sampled between the 5th of May and 28th of June 2019. Upon return to the laboratory, random subsamples from each plant of nutritive root tissue, culm (stem) tissue, and photosynthetic leaf tissue were separated, washed in RO water to remove debris and stored at −20 °C until further analysis. Rhizomes were not sampled as the development of this organ varied greatly among locations. Tissue samples were later oven dried at 60 °C and then ground to a fine powder using a barrel mill until the product passed through a 0.18-mm mesh, providing homogenous samples. Sediment cores were stored at −20 °C until further preparation was undertaken.

2.3. Sediment physicochemical analysis

Sediment particle-size distribution was assessed via a wet sieving methodology (Batley and Simpson, 2016), passing approximately 50 g of sediment through two ecologically relevant stainless steel sieves: 2 mm and 63 μm. The fraction >2 mm represented gravel, coarse material, rocks and detritus; the 63 μm – 2 mm fraction captured sand, while <63 μm quantified both silt and clay. Fractions captured in each of these particle size ranges were gravimetrically determined following oven drying at 105 °C and subsequently expressed as percentages of total sediment. Soil organic matter was estimated through determining total organic carbon (TOC) then derivation following the formula, SOM (%) = TOC (%) * 1.72 (assuming that organic matter contains 58% organic carbon). To determine TOC, <2 mm sediment was placed in an aluminium dish and dried at 105 °C before being homogenised with mortar and pestle. A 0.5 g sample of sediment was treated with 1 M HCl dropwise to liberate any inorganic carbon, then redried at 105 °C before undergoing TRUMAC CNS analysis (LECO, USA). A 1:5 ratio of air-dried <2 mm sediment to distilled water was prepared to estimate relative salinity and pH (He et al., 2012). The mixture was then mechanically shaken for 2 h prior to analysis for electrical conductivity (EC) as salinity is composed of predominantly Na with lower contributions of K, Ca and Mg (Hanna edge® EX HI2003) and pH (Hanna edge® pH HI2002). The proportion of clay ranged from 0.5 to 87%, pH 5.6 to 9.1, EC from 0.01 to 2.6 (μS/cm) and SOM 0.5 to 30.5% among locations.

2.4. Metal(loid) analysis

Samples of <2 mm sediment from each entire core were oven dried at 60 °C and then homogenised with mortar and pestle. A 0.5 g dried and homogenised sediment sample was taken and 5 mL of aqua regia (HNO₃/HCL: 1:3, ICP-MS grade, Merck) was added in Teflon digestion
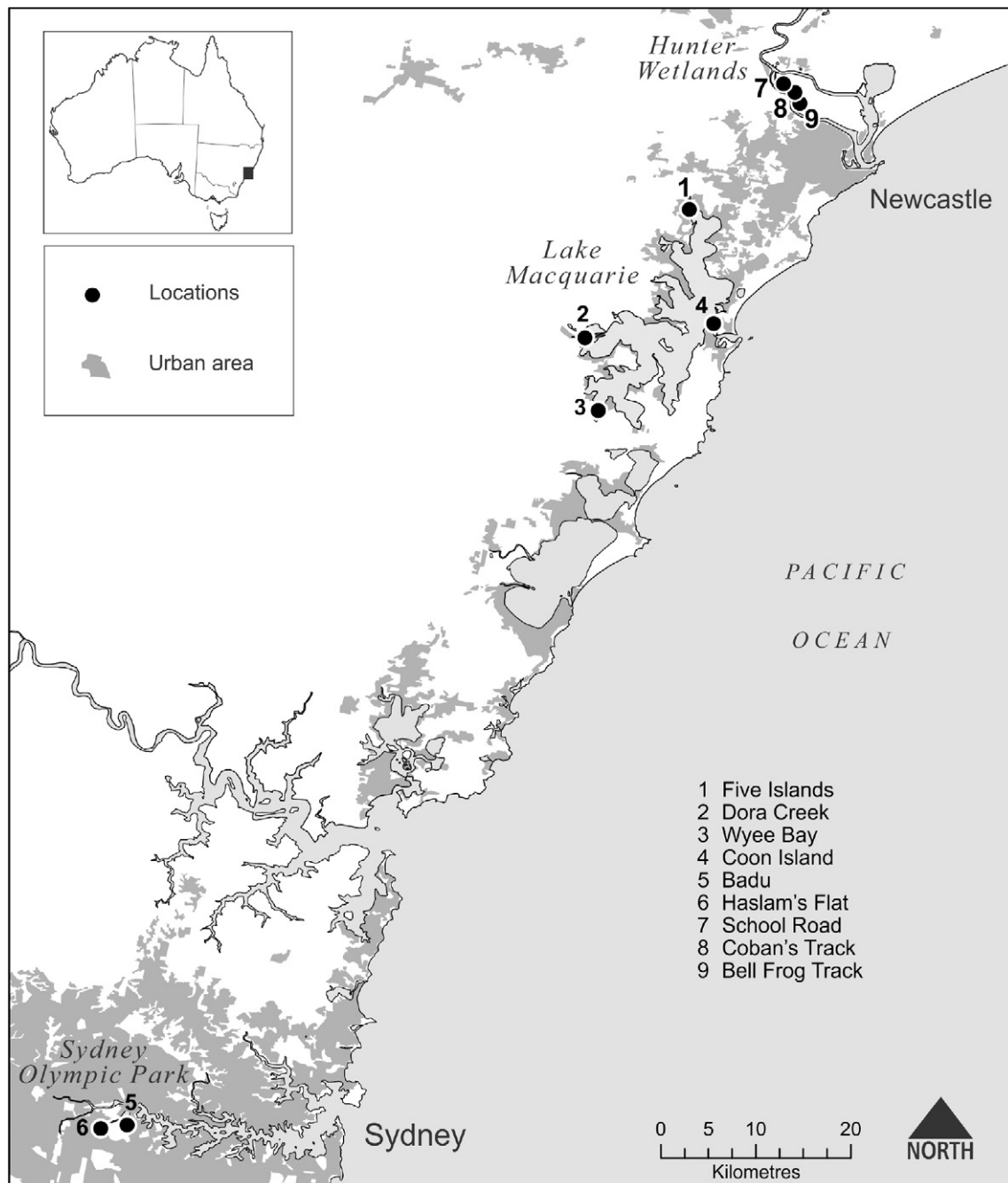
Fig. 1. Sampling locations across three urbanised estuaries, Lake Macquarie, Sydney Olympic Park and Hunter Wetlands National Park (Kooragang Island) in NSW, Australia.

vessels. The resultant mixture was digested using a microwave assisted digestion system (model: MARS 6, CEM, USA) following the US EPA 3051A method (US EPA, 2007), then made to volume (50 mL) and filtered through a 0.45 μm DigiFILTER (SCP Science, 2019). Plant materials were digested as per the procedure of Rahman et al. (2009). A 0.25 g sample of homogenised and dried plant tissue was weighed, to which 5 mL of concentrated HNO_3 (ICP-MS grade, Merck) was added in a 75 mL digestion tube then left in a fumehood overnight. Samples were then digested using the digestion block (Seal Analytical, BD 50), with the following temperature program: 70 °C for 30 min, 90 °C for 30 min, 110 °C for 30 min and 140 °C for 2 h. The digested sample tubes (containing residual liquid ≈ 1 mL) were removed from the digestion block and allowed to cool to room temperature prior to dilution with 0.1% nitric acid (20 mL). The samples were transferred to tubes and filtered through a 0.45 μm DigiFILTER for storage prior to analysis.

Candidate trace metal(loid)s, Cu, Pb, Zn, Cd and Se, were analysed using an Agilent 7900 (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) coupled with an autosampler (SPS 4, Agilent Technologies). While the major element, iron (Fe), was analysed using the dual view (Axial and radial) inductively coupled plasma emission spectrometer (ICP-OES, PerkinElmer Avio 200, USA). Certified reference materials, blanks, duplicates, and continuing calibration verification (CCV) were included in each batch throughout the elemental analysis.

The standard reference materials, marine sediment (PACS−2; N = 6, Canada) and bush branches and leaves (DC 73349, N = 6, NCS Beijing) were used for verifying the recovery of soil and plant digestion methods, respectively (Table 1). Trace metals are usually associated with the fines of sediments, dominated by clay and Fe and Mn oxyhydroxides. Further, the fines can vary greatly among sampling

Table 1
The percentage recovery of trace metal(loid)s based on ICP-MS analysis for certified marine sediment reference material (PACS-2) and bush branches and leaves (NCS DC 73349).

Metal(loid)s	Measured concentration (μg/g)	Certified reference concentration (PACS-2, μg/g dry weight)	Recovery (%)	Measured concentration (μg/g)	Certified reference concentration (NCS DC 73349, μg/g dry weight)	Recovery (%)
Zn	294.85 $\pm$ 2.54	364 $\pm$ 23.00	81.01	43.28 $\pm$ 1.36	55	78.69
Cu	242 $\pm$ 18.60	310 $\pm$ 12.00	78.07	4.67 $\pm$ 0.12	6.6	70.8
Pb	143.5 $\pm$ 5.30	183 $\pm$ 8.00	78.44	40.87 $\pm$ 1.26	47	86.95
Cd	1.89 $\pm$ 0.06	2.11 $\pm$ 0.15	89.94	–	–	–
Se	1.03 $\pm$ 0.06	0.92 $\pm$ 0.22	112.3	0.18 $\pm$ 0.01	0.12	152.03

locations, thus confounding estimates of relative metal loads and assessment of degree of contamination. Therefore, we undertook elemental (Fe) normalisation for total metals in order to further assess location and estuary sediment metal differences (Suh and Birch, 2005). Sediment metal(loid)s were compared to established Australian sediment quality guidelines (ANZECC, 2000; Simpson et al., 2013) to assess whether metal(loid) loads in sediments posed toxic risk to associated biota. Further, concentrations of metals in sediments evidenced to cause toxic effects on terrestrial plants (Kabata-Pendias and Pendias, 1984) were included for comparison (Table 2).

2.5. Statistical analysis

Differences among estuaries and locations in terms of the combined total metal(loid) load were assessed via multivariate analyses. Data were first $\log_{10}$ transformed to generate a nonmetric Multi-Dimensional Scaling (nMDS) ordination employing Euclidian distance to visually assess location and estuary differences in combined metal(loid) load. To further assess whether significant differences in combined total metal(loid) load and Fe-normalised metal(loid)s occurred among estuaries and locations (nested within estuary), a permutational multivariate analysis of variance (PERMANOVA)—a distance-based multivariable routine based on resemblance measures and permutation methods—was performed using $\log_{10}$ transformed data and Euclidian distance (Anderson, 2001 in PRIMER 7 (PRIMER-E, version 7)).

To explore patterns of total metal(loid) accumulation from sediment to roots, patterns of translocation between organs with increasing metal(loid) exposure, and further relationships between sediment and organ metal(loid)s, data were first $\log_{10}$-transformed to achieve normality and homogeneity of variance. Sites were then analysed as independent replicates via linear regression in IBM SPSS Statistic 24. To investigate the influence of physicochemical parameters in predicting metal(loid) accumulation from sediment to roots, multiple regressions were also employed in IBM SPSS Statistic 24. Significance was assessed at the 5% probability level ($p \le 0.05$).

Average bioconcentration factors (BCFs) were calculated as the ratio of root metal(loid) content divided by the sediment metal(loid) content. Similarly, the translocation factors (TFs) were calculated as the ratio of culm metal(loid) content divided by root metal(loid) content (TF culm) and the ratio of leaf metal(loid) content divided by culm metal(loid) content (TF leaves). BCFs and TFs were calculated for each individual plant then averaged across sites (n = 45).

3. Results

3.1. Metal(loid) concentrations in sediments among estuaries and locations

Mean Zn concentrations ($\pm$SE) ranged between 14.2 $\pm$ 1.9 μg/g at Dora Creek, Lake Macquarie, and 90.7 $\pm$ 18.1 μg/g at Five Islands, Lake Macquarie, though no locations exceeded sediment quality guideline values. Similarly, Cu ranged from 2.1 $\pm$ 0.2 μg/g at Coon Island, Lake Macquarie, to 39.3 $\pm$ 1.6 μg/g at Five Island Wetlands, Lake Macquarie, with no exceedances of sediment quality guideline values. Conversely, Pb contamination was evidenced, with values ranging from 8.39 $\pm$ 0.4 μg/g at Coon Island, Lake Macquarie, to 416.5 $\pm$ 34.2 μg/g at Five

Islands, Lake Macquarie, exceeding the sediment quality guideline value (high) at this locale. Further, Pb at Badu Wetlands, Sydney Olympic Park (83.1 $\pm$ 8.1 μg/g), exceeded sediment quality guidelines. Cadmium ranged from 0.18 $\pm$ 0.05 μg/g at Dora Creek, Lake Macquarie, and Bellfrog Track, Hunter Wetlands, to 5.4 $\pm$ 0.5 μg/g at Five Islands, Lake Macquarie, exceeding sediment guideline values for Cd at this location. Selenium concentrations were low and ranged from 0.14 $\pm$ 0.02 μg/g at Coon Island, Lake Macquarie, to 2.5 $\pm$ 0.4 μg/g at Five Island Wetlands. Overall, Five Island wetlands, adjacent to the decommissioned Pb/Zn smelter in Lake Macquarie consistently exhibited the greatest elevations in metal(loid)s in sediment and also exceeded levels of metals in sediment capable of inducing toxic effects on terrestrial plants for Zn and Pb. Gradients established for each metal(loid) across locations included a 6× increase for Zn, 19× for Cu, 52× for Pb, 30× for Cd and 17× for Se, creating appropriate gradients to assess metal accumulation patterns with in *S. virginicus* (albeit Pb was extremely elevated at Five Islands) (Table 2).

When examining total metal(loid) assemblages via nMDS, there was a degree of dissimilarity between Sydney Olympic Park locations and Hunter Wetlands locations. Lake Macquarie locations exhibited the greatest dissimilarity from other estuaries with the largest variance among locations and the most contaminated location, Five Islands, exhibiting the greatest distance from other locations (Fig. 2). Through nested PERMANOVA, there was no significant main effect of estuary (Pseudo F = 0.73, p = 0.62), though significant differences among locations (nested in estuary) were observed for total metal(loid) assemblages (Pseudo F = 46.2, p = 0.001) (Table 3). Turning to Fe-normalised metal(loid)s (which provides a more accurate estimate of anthropogenic source free of effects of granulometry), it was found that estuaries were significantly dissimilar in metal(loid) assemblages (Pseudo F = 20.98, p = 0.007) and further, there were significant location differences within estuaries (Pseudo F = 417.5, p = 0.001) (Table 3). Pairwise post-hoc tests revealed all estuaries were significantly different from each other in terms of Fe-normalised metal(loid) loadings. Lake Macquarie was the most contaminated estuary (and greatest variance in metal(loid) loadings among locations), Sydney Olympic Park exhibited intermediate metal(loid) loadings while Hunter Wetlands consistently exhibited the lowest metal(loid) loadings. Within each estuary, all locations were also significantly dissimilar from one another indicating appropriate location selection for creating a gradient for examining metal(loid) accumulation patterns with dose.

3.2. Metal(loid) uptake and partitioning in S. virginicus

3.2.1. Zinc

Zinc accumulated to root tissue in a linear fashion with increases in sediment Zn loadings (p < 0.0001, R^2 = 0.34) (Fig. 3a). Similarly, Zn in culms (p = 0.002, R^2 = 0.22) and Zn in leaves (p = 0.012, R^2 = 0.14) increased with increases in sediment Zn, though the proportion of explained variance was low (Fig. 3b–c). In terms of accumulation patterns between plant organs, Zn in culms increased as root Zn increased (p < 0.0001, R^2 = 0.52) (Fig. 3d), suggestive of accumulation, and similarly, leaf Zn increased linearly with increases in culm Zn (p < 0.0001, R^2 = 0.46) (Fig. 3e). Roots actively accumulated Zn to 1.52 times greater than sediment loadings, while transport in the plant was relatively

Table 2
Metal(loid) concentrations (μg/g) in sediment and *S. virginicus* roots, culms and leaves across three estuaries in NSW Australia (mean ± SE, N = 5). Sediment metals are compared to Australian sediment quality guideline values (SQGV).

Estuary	Locations		Sediment (μg/g)					Roots (μg/g)					Culm (μg/g)					Leaves (μg/g)				
			Zn	Cu	Pb	Cd	Se	Zn	Cu	Pb	Cd	Se	Zn	Cu	Pb	Cd	Se	Zn	Cu	Pb	Cd	Se
Lake Macquarie	1. Five Islands	Mean	90.67	39.33	416.5	5.44	2.51	75.56	33.79	509.45	6.53	2.81	54.52	16.89	47.58	1.88	0.48	29.94	9.99	26.26	0.34	0.35
		SE	18.07	1.62	34.16	0.47	0.36	8.09	2.96	29.22	0.33	0.10	5.21	3.84	14.11	0.38	0.10	2.75	2.53	14.28	0.08	0.02
	2. Dora Creek	Mean	14.29	6.94	32.64	0.18	1.14	18.76	6.27	10.58	0.22	0.71	21.43	3.56	2.26	0.08	0.19	13.93	6.98	1.03	0.01	0.09
		SE	1.91	0.75	3.04	0.02	0.05	1.62	0.63	2.96	0.03	0.14	4.36	0.21	0.68	0.02	0.04	2.11	1.70	0.25	0.00	0.02
	3. Wyee Bay	Mean	20.48	15.95	14.52	0.27	1.84	13.37	19.71	7.77	0.30	1.67	19.78	18.70	1.49	0.11	0.42	18.63	7.79	0.81	0.03	0.14
		SE	5.74	2.09	1.92	0.07	0.37	2.05	5.88	1.00	0.05	0.37	1.34	3.62	0.26	0.02	0.10	1.98	1.94	0.22	0.00	0.02
	4. Coon Island	Mean	16.61	2.11	8.39	0.42	0.14	31.25	16.40	6.26	0.46	0.30	41.82	86.06	3.02	0.10	0.20	39.86	17.65	0.98	0.05	0.07
		SE	1.95	0.21	0.41	0.13	0.02	9.43	3.79	1.30	0.12	0.17	7.56	25.84	0.37	0.02	0.01	13.16	2.62	0.17	0.04	0.00
	5. Badu	Mean	45.26	9.62	83.09	0.52	0.84	44.17	19.78	21.32	0.54	0.73	33.98	23.79	5.29	0.09	0.20	35.45	13.78	1.84	0.03	0.13
Sydney Olympic Park		SE	3.18	0.61	8.06	0.13	0.04	8.99	4.15	6.07	0.26	0.38	3.64	8.60	1.96	0.03	0.04	1.77	1.53	0.26	0.00	0.02
	6. Haslam's Flats	Mean	55.96	11.34	36.64	0.64	0.90	40.82	24.09	12.75	0.62	0.23	43.85	27.43	1.86	0.38	0.11	30.09	16.45	0.91	0.21	0.08
		SE	4.42	0.34	4.42	0.13	0.03	6.60	6.57	3.70	0.23	0.04	4.01	5.86	0.19	0.20	0.01	2.03	2.14	0.12	0.04	0.01
	7. School Road	Mean	29.12	9.22	32.03	0.22	1.08	39.08	11.77	32.48	0.20	0.23	41.13	19.29	9.18	0.06	0.15	32.66	32.22	4.42	0.02	0.12
Hunter Wetlands		SE	3.29	0.50	1.98	0.06	0.06	4.77	1.62	5.44	0.08	0.02	3.18	2.09	3.54	0.02	0.02	2.34	5.71	0.94	0.00	0.02
	8. Coban's Track	Mean	47.62	12.24	50.68	0.51	1.07	49.71	12.00	27.79	0.57	0.25	59.83	19.87	3.89	0.40	0.16	45.45	14.70	3.48	0.12	0.15
		SE	6.20	0.49	4.30	0.04	0.13	8.48	1.99	6.87	0.05	0.01	10.44	4.69	0.67	0.22	0.02	5.31	1.29	0.85	0.03	0.02
	9. Bellfrog Track	Mean	26.65	5.89	8.91	0.18	0.44	32.31	10.65	9.31	0.21	0.27	36.97	19.83	2.62	0.10	0.15	34.96	16.99	1.21	0.07	0.09
		SE	1.48	0.74	0.98	0.05	0.09	6.08	1.13	1.49	0.05	0.02	4.40	3.22	0.39	0.02	0.02	4.69	4.49	0.36	0.02	0.02
ANZECC (2000)		SQGV	200	65	50	1.5																
Simpson et al. (2013)		SQGC-High	410	270	220	10																
Kabata-Pendias and Pendias (1984)			70–400	60–125	100–400																	

Table 3
Nested PERMANOVA analysis for differences in assemblages of metal(loid)s among estuaries, and locations within estuaries, based on log10(x + 1) transformed data and Euclidian distances for a. total sediment metal(loid)s and b. Fe-normalised metal(loid)s.

Source	df	MS	Pseudo-F	P(perm)	Unique perms
a. total metals					
Estuary	2	12.82	0.732	0.615	678
Location (estuary)	6	17.52	46.182	**0.001**	997
Residuals	36	0.37			
Total	44				
b. Fe-normalised metals					
Estuary	2	262.51	20.984	**0.007**	566
Location (estuary)	6	12.51	417.57	**0.001**	999
Residuals	36	0.03			
Total	44				

P < 0.05 bolded.

3.2.2. Copper

As Cu in sediment increased, Cu accumulated to roots linearly (p = 0.016, R^2 = 0.13), though explained variance was low (Fig. 4a). There was no relationship between Cu in culms and Cu in leaves with sediment Cu (Fig. 4b–c). When examining movement of Cu among plant organs, there was no relationship between Cu in roots and Cu in culms (Fig. 4d), suggesting highly variable uptake across this transition, while Cu in leaves increased as culm Cu load increased (p = 0.03, R^2 = 0.11) (though low R^2) (Fig. 4e). Cu sequestration in roots was much higher than surrounding sediment loads (BCF = 2.58) and unrestricted translocation, equal to unity or greater was observed between plant organs (TF culm:root = 1.82, TF leaf:culm = 1.03) (Fig. 8). Sediment Cu was a strong predictor of Cu in root tissue (p = 0.000), while increases in sediment pH also predicted greater Cu uptake to roots (p = 0.019) (Table 4).

unrestricted with translocation factors around unity between organs (TF culm:root = 1.26, TF leaf:culm = 0.84) (Fig. 8). Zn in sediment was the major predictor of Zn in roots (p = 0.001), with other sediment physicochemical parameters not contributing significantly to explaining variation in root Zn (Table 4).
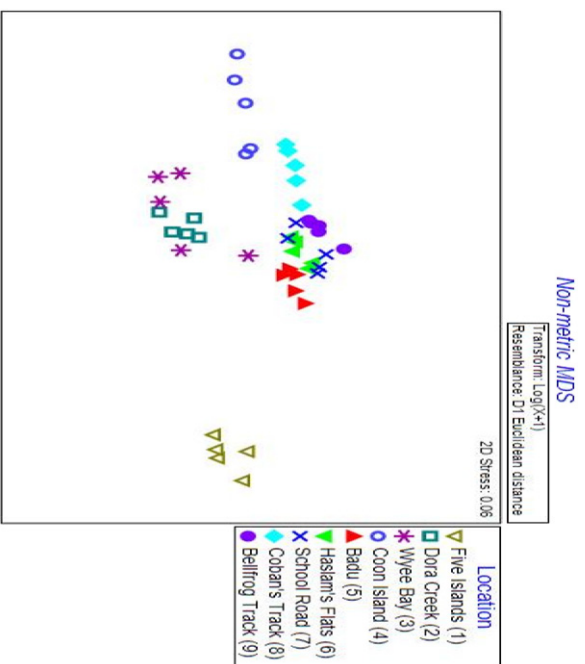
Fig. 2. Non-metric Multi-Dimensional Scaling (nMDS) plots of total sediment metal(loid) assemblages for Zn, Cu, Pb, Cd and Se by location. Log(X + 1) transformed data and Euclidian distance. 2D stress indicates Kruskal's stress, which should be <0.1.
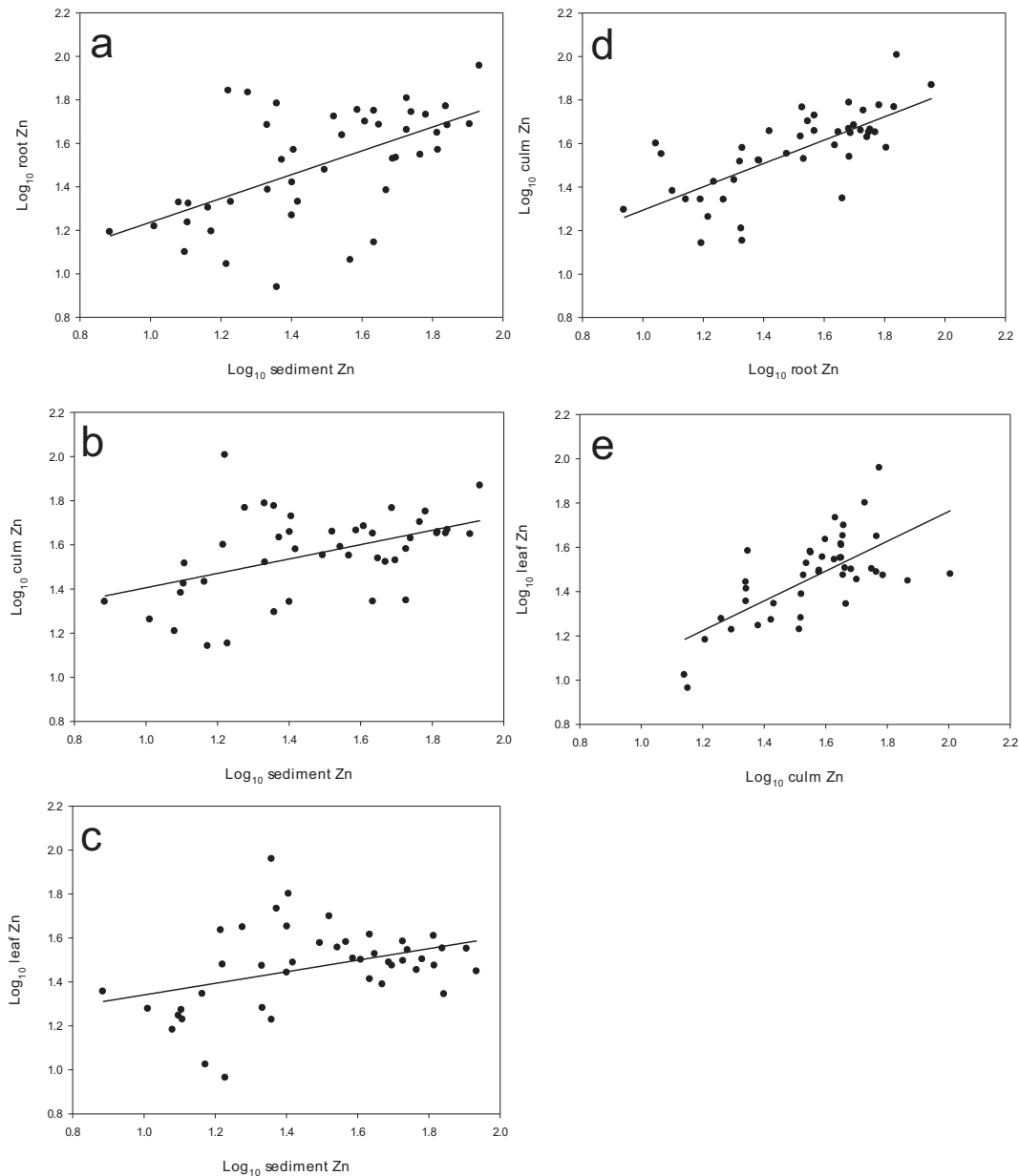
Fig. 3. Bivariate relationships between (a) $\log_{10}$ sediment Zn and $\log_{10}$ root Zn ($\beta = 0.55$, $t = 4.64$, p < 0.0001, $R^2 = 0.34$); (b) $\log_{10}$ sediment Zn and $\log_{10}$ culm Zn ($\beta = 0.33$, $t = 3.40$, p = 0.002, $R^2 = 0.22$); (c) $\log_{10}$ sediment Zn and $\log_{10}$ leaf Zn ($\beta = 0.26$, $t = 2.64$, p = 0.012, $R^2 = 0.14$); (d) $\log_{10}$ root Zn and $\log_{10}$ culm Zn ($\beta = 0.54$, $t = 6.75$, p < 0.0001, $R^2 = 0.52$); (e) $\log_{10}$ culm Zn and $\log_{10}$ leaf Zn ($\beta = 0.67$, $t = 5.94$, p < 0.0001, $R^2 = 0.46$) (N = 44).

3.2.3. Lead

Pb in roots increased with increasing sediment Pb exposure (p < 0.0001, $R^2 = 0.53$) (Fig. 5a). Similarly, Pb in culms (p < 0.0001, $R^2 = 0.39$) and Pb in leaves (p < 0.0001, $R^2 = 0.39$) increased with sediment Pb load (Fig. 5b–c). When examining patterns of accumulation among organs, Pb in culms increased with root Pb (p < 0.0001, $R^2 = 0.72$) and similarly, Pb in leaves increased as culm Pb increased (p < 0.0001, $R^2 = 0.65$) (Fig. 5d–e). Although Pb accumulation patterns with increasing dose were evidenced, there was restriction to translocation among plant compartments. Bioconcentration of Pb to roots from sediment was around unity (TF = 0.89), while strong barriers to translocation were observed with translocation from root to culms (TF = 0.29) and culms to leaves much reduced (TF = 0.64) (Fig. 8). Sediment Pb alone was the best predictor of Pb in root tissue (p = 0.000), with no influence of sediment pH, proportion of clay, soil organic matter or salinity on Pb uptake (Table 4).

3.2.4. Cadmium

The positive relationship between Cd accumulation to roots and Cd load in sediment was an almost prefect linear fit (p < 0.0001, $R^2 = 0.99$) (Fig. 6a). Similarly, Cd in culms (p < 0.0001, $R^2 = 0.68$) and Cd in leaves (p < 0.0001, $R^2 = 0.58$) were strongly linearly related to Cd in sediment (Fig. 6b–c). Patterns of accumulation were maintained across plant tissues, with linear increases in culm Cd with root Cd (p < 0.0001, $R^2 = 0.70$) (Fig. 6d), and increases in leaf Cd with culm Cd (p < 0.0001, $R^2 = 0.47$) (Fig. 6e). Cd accumulated to roots in similar proportions to sediments (BCF = 1.12), while reduced translocation at approximately 50% of the previous compartment was observed between roots and culms (TF = 0.42) and culms and leaves (TF = 0.56) (Fig. 8). As expected, Cd in sediment was the best predictor of Cd accumulated to roots (p = 0.000), with no other soil parameters further explaining variation in Cd accumulation to roots (Table 4).

Table 4
Multiple linear regressions quantifying the influence of $\log_{10}$ sediment metal(loid) concentrations and physicochemical parameters (% clay, pH, soil conductivity (EC, μS/cm), and soil organic matter (SOM%)) on metal(loid) accumulation to roots across sites (N = 44 for Zn, N = 45 for all other metal(loid)s).

	β	t	P	R^2
Log_{10} root Zn				0.657
Log_{10} sediment Zn	0.457	3.760	**0.001**	
clay %	0.002	1.390	0.171	
pH	0.014	0.300	0.765	
EC	−0.080	−0.959	0.344	
SOM %	−0.008	−1.640	0.109	
Log_{10} root Cu				0.632
Log_{10} sediment Cu	0.465	3.957	**0.000**	
clay %	−0.002	−1.039	0.305	
pH	0.111	2.454	**0.019**	
EC	0.062	0.738	0.465	
SOM %	−0.002	−0.332	0.742	
Log_{10} root Pb				0.735
Log_{10} sediment Pb	0.848	6.570	**0.000**	
clay %	−0.001	−0.437	0.665	
pH	−0.073	−0.783	0.439	
EC	−0.106	−0.619	0.539	
SOM %	−0.007	−0.726	0.472	
Log_{10} root Cd				0.993
Log10 sediment Cd	1.068	51.390	**0.000**	
clay %	0.000	0.171	0.865	
pH	0.000	−0.012	0.990	
EC	0.001	0.112	0.911	
SOM %	0.000	0.642	0.525	
Log_{10} root Se				0.775
Log_{10} sediment Se	0.700	3.742	**0.001**	
clay %	−0.001	−0.596	0.555	
pH	0.052	1.883	0.067	
EC	−0.001	−0.016	0.988	
SOM %	0.009	2.451	**0.019**	

P < 0.05 bolded.

3.2.5. Selenium

Se in roots ($p < 0.0001$, $R^2 = 0.52$), culms ($p < 0.0001$, $R^2 = 0.32$) and leaves ($p < 0.0001$, $R^2 = 0.30$) were linearly related to Se in sediments across sites (Fig. 7a–c). Further, accumulative patterns were evidenced across plant organ interfaces with dose, with increases in Se in culms with root Se ($p < 0.0001$, $R^2 = 0.47$), and increasing leaf Se with culm Se ($p < 0.0001$, $R^2 = 0.44$) (Fig. 7d–e). Se was accumulated from sediment to root at concentrations approaching unity (BCF = 0.85), while barriers to translocation were observed at the root to culm interface (TF = 0.50) and culm to leaf transition (TF = 0.73) (Fig. 8). Se in sediment was the best predictor of Se in roots ($p = 0.001$) while increases in soil organic matter further predicted increases in root Se ($p = 0.019$) (Table 4).

4. Discussion

4.1. Metal(loid) contamination status of saltmarsh sediments in three estuaries, NSW, Australia

Through sediment metal(loid) analyses across three urbanised estuaries in NSW, Australia, we observed significant differences in metal(loid) assemblages among estuaries and, further significant differences among locations within estuaries. Lake Macquarie was observed to be the most contaminated estuary, with Five Islands exceeding the sediment quality guideline value (high) for Pb, and the sediment guideline values for Cd, suggesting potential impacts to sediment associated biota at this locale. Lake Macquarie also exhibited the greatest variability in metal(loid) loads among locations. Sydney Olympic Park, exhibited intermediate metal(loid) contamination with Badu Wetlands exceeding the sediment quality guidelines for Pb. Conversely, Hunter Wetlands was found to possess the lowest metal(loid) loadings among estuaries.

Relative metal loading ranks among these three estuaries observed in the current study largely reflect rankings reported previously in the literature. Birch et al. (2015), assessed metal enrichment quotients across 38 estuaries in SE Australia, which represent the normalised surficial concentration of Cu, Pb and Zn divided by their normalised background concentration. Mean enrichment for Cu, Pb and Zn were 6.7, 17.5 and 15.9 respectively for Lake Macquarie, 15.9, 12.3 and 11.7 for Port Jackson (Sydney Olympic Park's estuary) and Hunter River, 1.9. 10.8 and 6.7 respectively, largely reflecting the Lake Macquarie > SOP > Hunter Wetlands trend in the current findings.

Within Lake Macquarie, Five Islands Wetlands, in the north of the lake, was most elevated in metal(loids), largely due to its proximity to the decommissioned Pb/Zn smelter at Cockle Creek. To the south, Dora Creek and Wyee Bay exhibited some elevations in Se likely attributable to coal-ash associated sources from the coal-fired power stations in this vicinity. This largely reflects trends observed in sub-tidal sediments in past studies where the north of the Lake was found to be elevated in Cd, Pb, Hg, Se, Ag and Zn, while the south of Lake was elevated predominantly in Se (Roach, 2005). Despite these trends, when examining past studies which have specifically sampled saltmarsh sediment in the Lake, a trend in declining metals (especially for Pb) has been observed with time since smelter decommissioning. The metal contamination observed in the present study (Cu 2–39 μg/g, Pb 8–417 μg/g, Zn 13–76 μg/g, Cd 0.2–5.4 μg/g) and recent investigations of saltmarsh sediment in the Lake (Cu 25–52 μg/g, Pb 33–311 μg/g, Zn 82–764 μg/g, Cd 0.5–1.9 μg/g) (Vårhammar et al., 2019) are general lower, especially for Pb, when compared to earlier investigations of saltmarsh sediment in Lake Macquarie (Cu 1–81 μg/g, Pb 3–1497 μg/g, Zn 20–563 μg/g, Cd 0.1–7.5 μg/g) (MacFarlane et al., 2006).

At Sydney Olympic Park, Badu Wetlands were elevated predominantly in Pb, while other metals were similar between locations. The Badu saltmarsh was created from contaminated sediment dredged from Homebush Bay in the late 1950s when much industrial and manufacturing activity was present in the estuary (Chalmers, 2002), so Pb elevations here are not surprising. The concentrations of Cu (9–11 μg/g), Pb (36–83 μg/g) and Zn (40–44 μg/g) were generally similar to that found through recent sampling at the same locations i.e. Cu (20–25 μg/g), Pb (27–47 μg/g) and Zn (63–103 μg/g) (Vårhammar et al., 2019) though lower than that found by Suh et al. (2004), which recorded concentrations of 21–138 μg/g for Cu, 44–374 μg/g for Pb and 83–694 μg/g for Zn at around the time of the 2000 Olympic games, again representing a decline in metal load with time. However, Nath et al., 2014, examined metals in mangrove sediments in Homebush Bay more recently and found similar elevations for Cu (10–160 μg/g), Pb (25–535 μg/g) and Zn (90–670 μg/g) to earlier assessments.

Metal(loid) loadings were lowest at Kooragang Island, Hunter Wetlands. Ranges of metals in the current study i.e. Cu (5–12 μg/g), Pb (9–51 μg/g) and Zn (27–48 μg/g) were lower than recent investigations at similar locations on the Island, likely attributable to spatial heterogeneity in sediments across the Island (Cu (27–49 μg/g), Pb (27–54 μg/g) and Zn (85–197 μg/g); Vårhammar et al., 2019). Although these wetlands are in close proximity to a large coal exporting facility, and a decommissioned steelworks, there is attenuated tidal exchange in these wetlands, attributable to historic land reclamation, thus exposure to metals via tidal processes is limited. In more tidally inundated mangrove forests on the Island, Nath et al. (2013) found similar concentrations of metals in sediments between sites affected by acid-sulfate soils (Cu, 18–48 μg/g, Pb 12–46 μg/g and Zn 45–439 μg/g) and control sites (Cu 21–38 μg/g, Pb 12–55 μg/g and Zn 59–272 μg/g) which were significantly higher than upland saltmarsh habitats possibly attributable to increased tidal exchange.

4.2. Metal(loid) accumulation and partitioning patterns in S. virginicus

In S. virginicus, the essential metals (Zn and Cu) were evidenced to be much more mobile than non-essential metals (Pb, Cd) and the
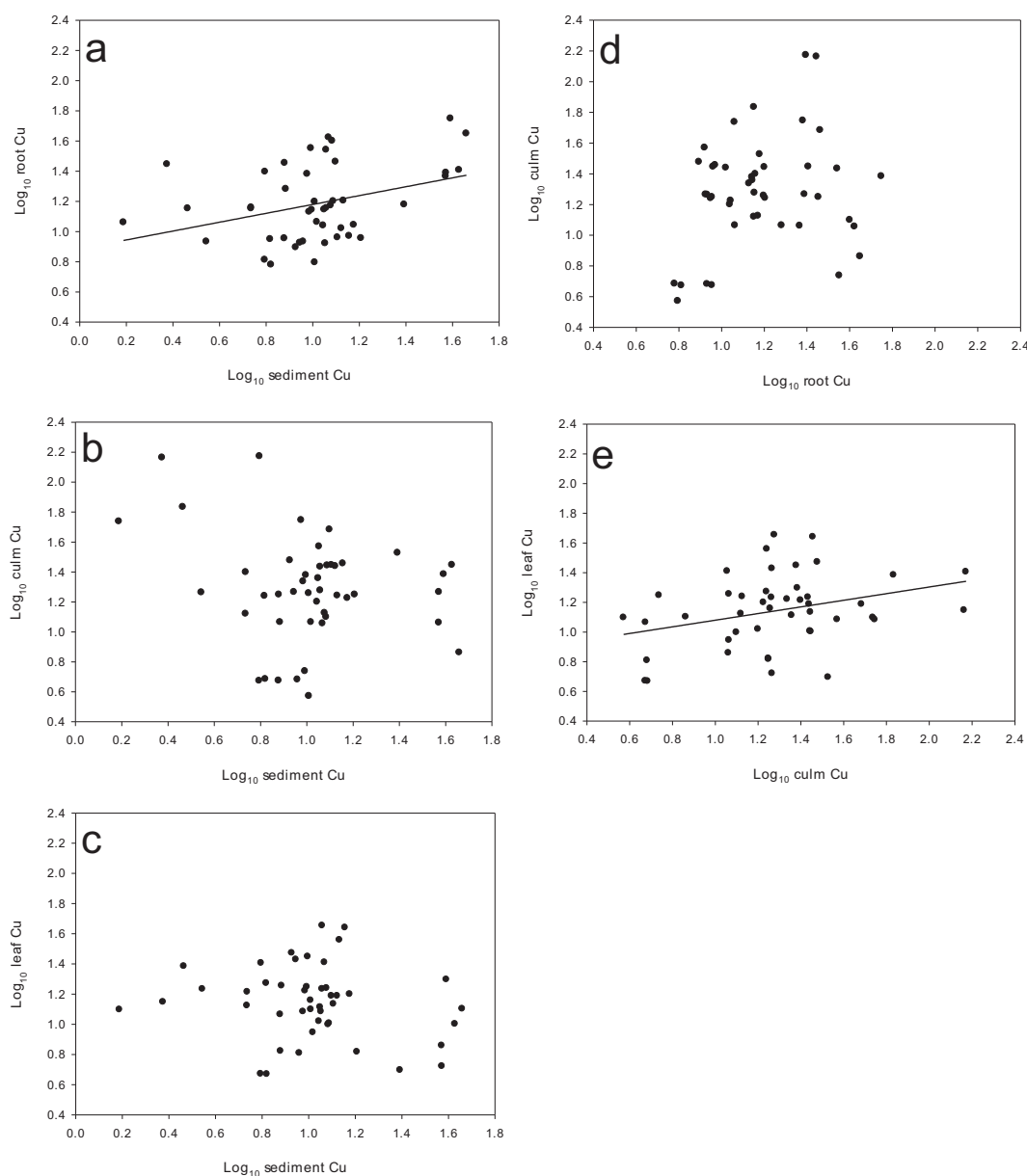
Fig. 4. Bivariate relationships between (a) $\log_{10}$ sediment Cu and $\log_{10}$ root Cu ($\beta = 0.29$, $t = 2.50$, $p = 0.016$, $R^2 = 0.13$); (b) $\log_{10}$ sediment Cu and $\log_{10}$ culm Cu ($\beta = -0.28$, $t = -1.58$, $p = 0.121$, $R^2 = 0.06$); (c) $\log_{10}$ sediment Cu and $\log_{10}$ leaf Cu ($\beta = -0.13$, $t = -1.09$, $p = 0.283$, $R^2 = 0.03$); (d) $\log_{10}$ root Cu and $\log_{10}$ culm Cu ($\beta = 0.33$, $t = 1.59$, $p = 0.120$, $R^2 = 0.06$); (e) $\log_{10}$ culm Cu and $\log_{10}$ leaf Cu ($\beta = 0.22$, $t = 2.26$, $p = 0.03$, $R^2 = 0.11$) (N = 45).

metalloid (Se). Uptake of the essentials, Cu and Zn, to roots was 1.5 to 2.5 times greater than sediment concentrations, with high translocation through subsequent compartments (TFs 0.8–1.82) reflecting metabolic requirements for these metals. Conversely, for the other metal(loid)s, uptake to roots was around unity with sediment loads, and restrictions to translocation were evidenced, especially from root to culm, with culm concentrations approximately 50% of root loads. Transport between the culm and leaf was greater, with TFs between 0.56 and 0.73. We found that the total sediment metal(loid) loads were the most consistent predictors of metal(loid) uptake in roots, and variation in sediment physiochemistry was largely unimportant in further explaining metl(loid) uptake. Exceptions to this general pattern were that increases in sediment pH further predicted Cu uptake in roots and increases in soil organic matter further predicted Se uptake in roots. These patterns are contrary to the established literature, where low pH and high H^+ availability facilitates greater metal bioavailability through H^+ exchanging with metal cations on negatively charged surfaces of clays and hydroxide minerals (Rieuwerts et al., 1998). Similarly,

metal(loid) availability is usually lower at higher SOM content, as different forms of selenium, including selenite (Se^{4+}), elemental selenium (Se^0), selenide (Se^{2-}), selenate (Se^{6+}) and organic Se, such as selenomethionine complex or adsorb to humic and non-humic organic substances (Kabata-Pendias, 2011). It is more probable that these correlations represent type 1 errors, observed by chance, due to the large number of simultaneous comparisons among metal(loid) candidates. Despite evidence of restrictions to translocation within the plant, little evidence of regulation of metal(loid) transport across these transition zones were observed, with most metal(loid)s (with exception of Cu) exhibiting increases in metal(loid) concentration with load from the previous compartment. This strategy contrasts significantly from patterns observed in other dominant Australian saltmarsh halophytes such as the succulent dicotyledon, *Sarcocornia quinqueflora*, in the family Amaranthaceae, where regulation of transport to the stem for Zn, and to the PS stem for Zn and Pb is observed (Vårhammar et al., 2019).

Comparing the patterns evidenced in *S. virginicus* with other halophytic representatives of *Poaceae*, Cambrollé et al. (2011) examined
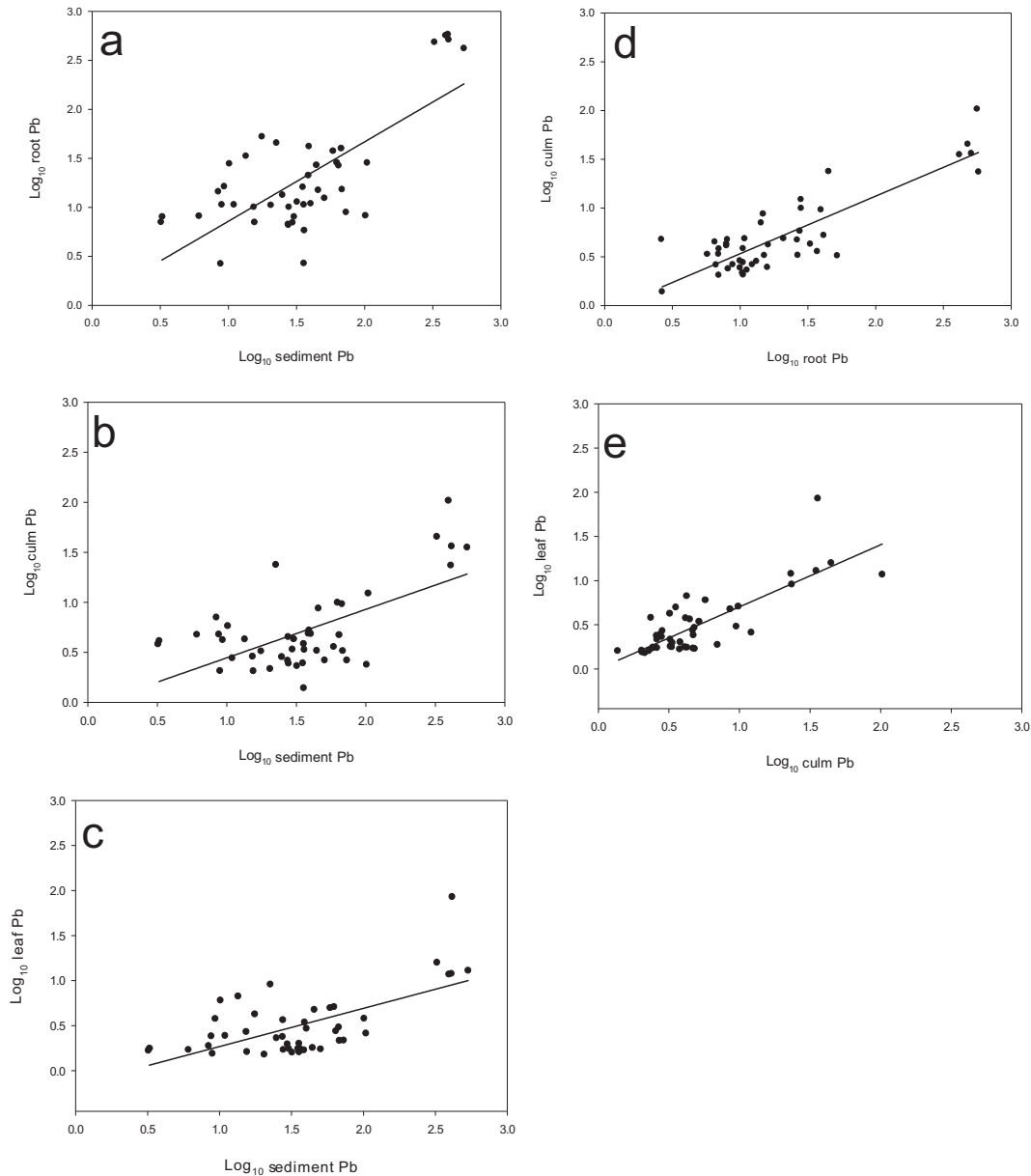
Fig. 5. Bivariate relationships between (a.) $\log_{10}$ sediment Pb and $\log_{10}$ root Pb ($\beta = 0.81$, $t = 6.92$, $p < 0.0001$, $R^2 = 0.53$); (b) $\log_{10}$ sediment Pb and $\log_{10}$ culm Pb ($\beta = 0.48$, $t = 5.26$, $p < 0.0001$, $R^2 = 0.39$); (c) $\log_{10}$ sediment Pb and $\log_{10}$ leaf Pb ($\beta = 0.42$, $t = 5.27$, $p < 0.0001$, $R^2 = 0.39$); (d) $\log_{10}$ root Pb and $\log_{10}$ culm Pb ($\beta = 0.59$, $t = 10.61$, $p < 0.0001$, $R^2 = 0.72$); (e) $\log_{10}$ culm Pb and $\log_{10}$ leaf Pb ($\beta = 0.70$, $t = 8.89$, $p < 0.0001$, $R^2 = 0.65$) (N = 45).

accumulation of Co, Cr and Ni in two *Spartina* species from two contaminated areas in the Odiel and Tinto marshes (SW Spain). Both *Spartina densiflora* and *Spartina maritima* generally exhibited low metal accumulation to roots, with bioconcentration factors from sediment to roots generally below unity (BCF 0.1–0.92). Translocation of these metals to leaf tissue was also low, especially for Co, with translocation factors from root to leaf tissue ranging from 0.002–0.78 (TF). In terms of other metals, Duarte et al. (2010) examined the Cd, Cu, Pb and Zn partitioning and cycling in *S. maritima* in the contaminated Tagus estuary (Portugal). Again, accumulation of metals to roots was low for Cd, Cu and Pb (BCFs 0.16–1.15) and translocation to leaf tissue was also very low, implying barriers to acropetal transport (TF 0.0007–0.006). Zn was much more actively accumulated to roots, with a BCF of 3.9, yet again, mobility within the plant was low (TF 0.004). Turning to other grasses, Otte et al. (1991) examined Cd, Cu and Zn uptake in *Spartina anglica* and *Triglochin maritima*, at 9 locations along the Dutch coast. They found *S. anglica* accumulated greater metals to roots (BCF Cd = 5.4, Cu =

1.6, Zn = 1.3) than *T. maritima* (BCF Cd = 0.9, Cu = 1.2, Zn = 0.6). Again, transport within the plant was limited, with low translocation evidenced for both species (TFs for the three metals ranged from 0.12–0.54). Almeida et al. (2011) also studied metal accumulation and distribution among tissues in a number of representatives from the family *Poaceae* in a moderately contaminated urban estuary in the Lima River (NW Portugal). *Triglochin striata* accumulated significant quantities of metals (e.g. Cd, Cu, Pb and Zn) to root tissue with BCFs ranging between 1.4 and 2.4, but exhibited highly restricted translocation to leaf tissue (TF 0.03–0.07). *Spartina patens* exhibited an intermediate strategy, with high accumulation of these metals to roots (BCFs 0.8–4.0), and some mobility to leaf tissue (TFs 0.23–1.10), while *Phragmites australis*, exhibited greatest mobility among the grasses, with BCFs ranging from 1.2–3.0 and TFs ranging from 0.63–2.08. Generally, uptake and mobility were greater for essential metals than non-essential metals.

In terms of the genus *Sporobolus*, Franco-Hernandez et al. (2010) assessed accumulation of a suite of metals in *Sporobolus indicus*, in
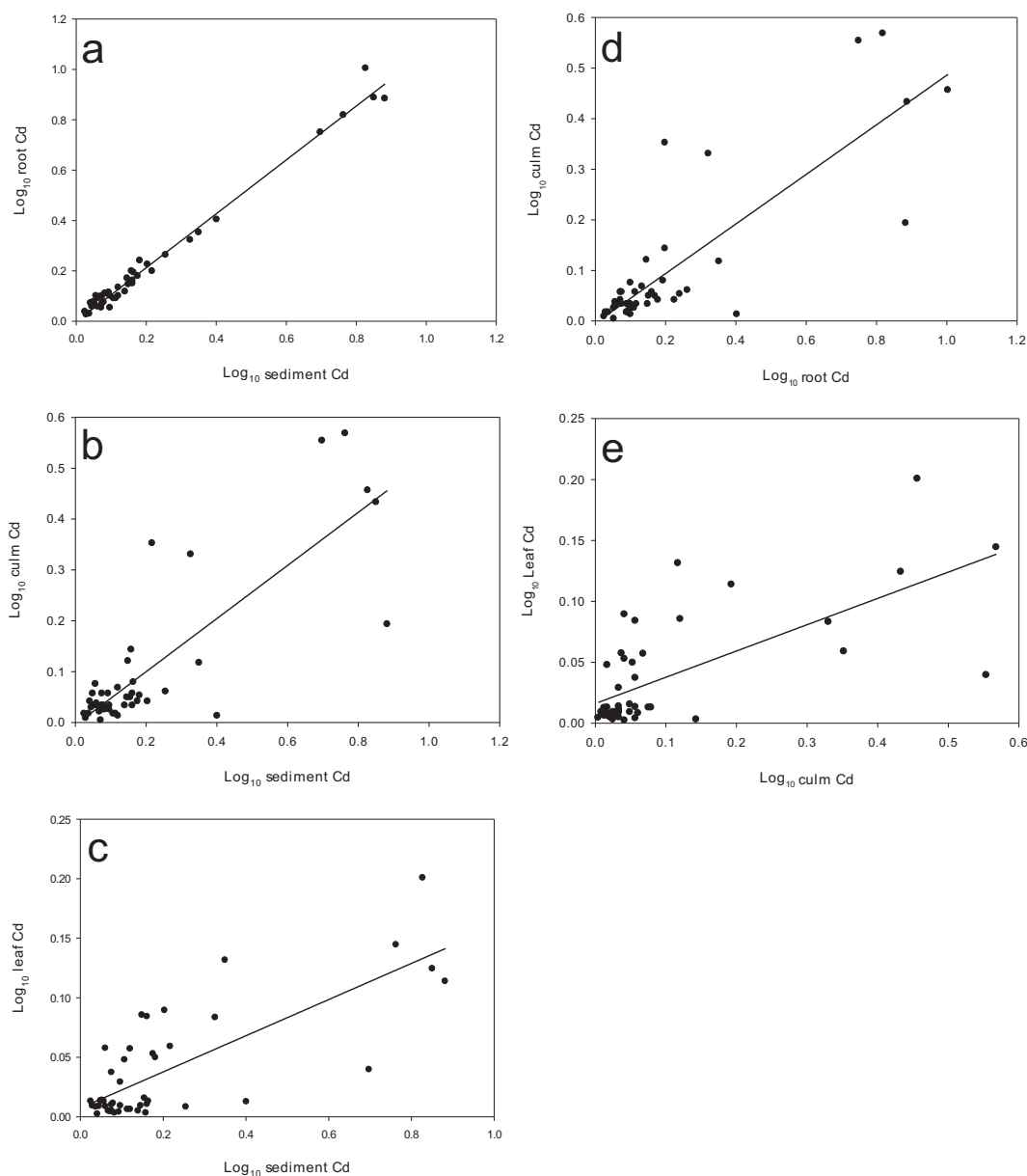
Fig. 6. Bivariate relationships between (a) $\log_{10}$ sediment Cd and $\log_{10}$ root Cd ($\beta = 1.07$, $t = 54.87$, $p < 0.0001$, $R^2 = 0.99$); (b) $\log_{10}$ sediment Cd and $\log_{10}$ culm Cd ($\beta = 0.52$, $t = 9.59$, $p < 0.0001$, $R^2 = 0.68$); (c) $\log_{10}$ sediment Cd and $\log_{10}$ leaf Cd ($\beta = 0.15$, $t = 7.66$, $p < 0.0001$, $R^2 = 0.58$); (d) $\log_{10}$ root Cd and $\log_{10}$ culm Cd ($\beta = 0.49$, $t = 9.96$, $p < 0.0001$, $R^2 = 0.70$); (e) $\log_{10}$ culm Cd and $\log_{10}$ leaf Cd ($\beta = 0.22$, $t = 6.12$, $p < 0.0001$, $R^2 = 0.47$) (N = 45).

heavily contaminated tailings from a mine in San Luis Potosí (Mexico). Cu, Pb and Zn exhibited low accumulation from soil to root tissue (BCF Cu = 0.13, Pb = 0.02, Zn = 0.03). Further, translocation from root to shoot was also low (TF Cu = 0.79, Pb = 0.03, Zn = 0.58). Ogundiran and Osibanjo (2008), examined accumulation of metals in *Sporobolus pyramidalis* at a slag dump site in Lalupon, Ibadan, Nigeria. Accumulation of Pb and Zn from soil to roots tissue was higher for this species, and approached unity (BCF Pb = 0.50–0.93, Zn = 0.23–1.16), while translocation within the plant was generally lower (TF Pb = 0.04–0.58, Zn = 0.20–0.88). Similarly, Mganga (2014) assessed Pb, Zn and Cd accumulation in *S. pyramidalis*, growing around the tailings dam of the North Mara gold mine in Tanzania. Accumulation of Pb and Zn from soil to roots was around unity (BCF 0.9 for both metals), while much higher accumulation of Cd was evidenced (BCF = 4.8). Translocation of Pb and Cd was relatively low (TF Pb = 0.51, Cd = 0.29), while Zn exhibited uninhibited flow from roots to shoots (TF Zn = 1.00). In terms of past research specifically on *S. virginicus*,

Pulkownik (2000) assessed accumulation of metals in this species in SE Australian estuaries, though only measured metals in sediments and shoots. Shoot bioconcentration factors (shoot metals:sediment metals) were generally below unity for Cu (0.14–0.23) Pb(0.00–0.04) and Zn(0.30–0.53), suggesting restrictions to translocation within the plant.

Comparing patterns of metal uptake with other halophytic taxa sampled in NSW estuaries, mangrove trees seem to accumulate higher proportions of metals to roots from sediments than saltmarsh (*Avicennia marina* BCFs Cu = 11.7, Pb = 6.7, Zn = 7.4), yet exhibit similar strong barriers to acropetal translocation, with TFs from roots to leaves very low (TF Cu = 0.06, Pb = 0.003, Zn = 0.04) (Nath et al., 2014).

In *S. virginicus*, metals are most likely taken up in cationic forms, while Se speciation is more complex, with uptake of different forms of Se including selenate, selenite and organic forms of Se such as selenomethionine (Terry et al., 2000; Prasad, 2013). Uptake to roots is predominantly transported via apoplastic pathways with some
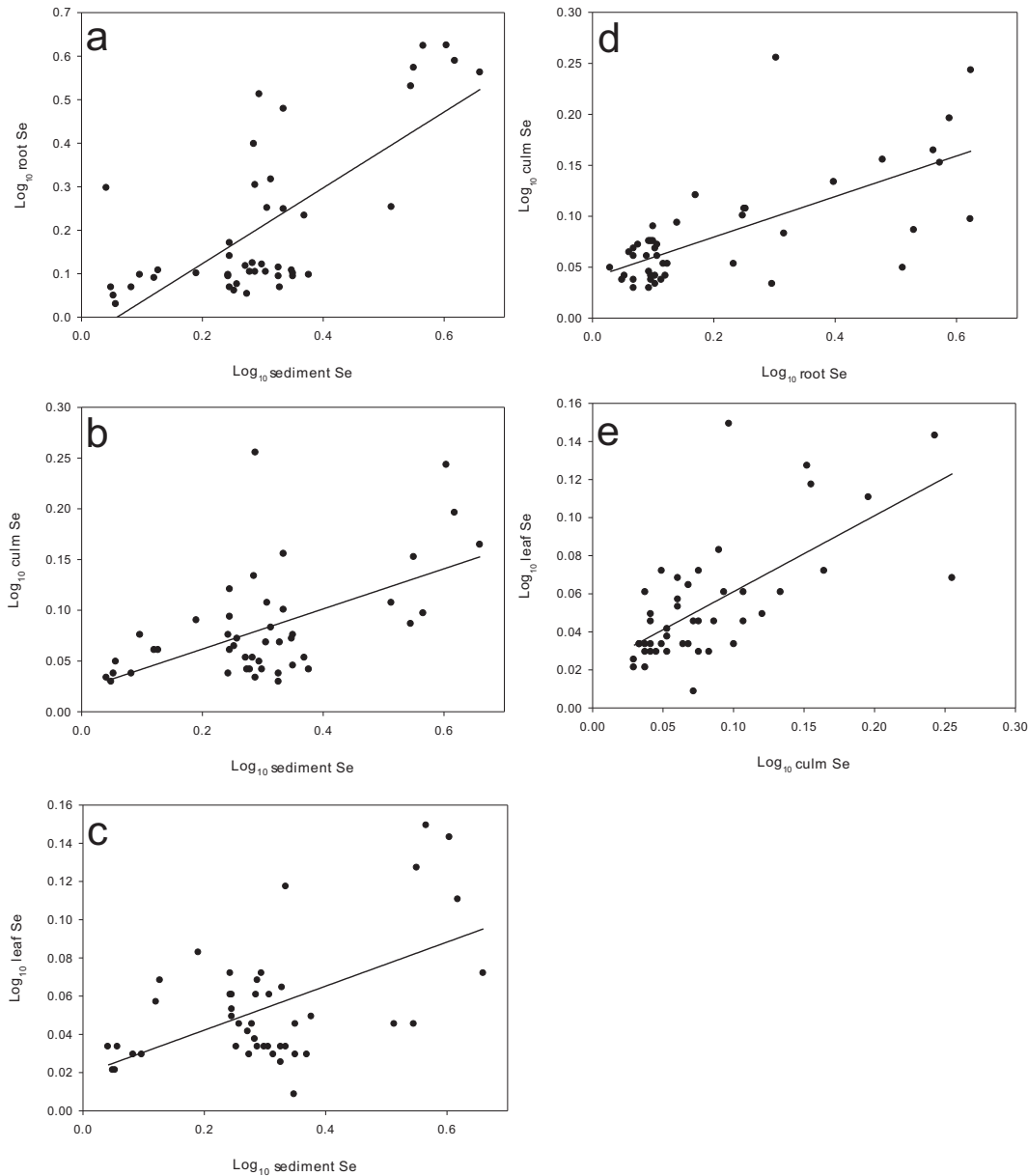
Fig. 7. Bivariate relationships between (a) $\log_{10}$ sediment Se and $\log_{10}$ root Se ($\beta = 0.87$, $t = 6.80$, $p < 0.0001$, $R^2 = 0.52$); (b) $\log_{10}$ sediment Se and $\log_{10}$ culm Se ($\beta = 0.20$, $t = 4.48$, $p < 0.0001$, $R^2 = 0.32$); (c) $\log_{10}$ sediment Se and $\log_{10}$ leaf Se ($\beta = 0.12$, $t = 4.30$, $p < 0.0001$, $R^2 = 0.30$); (d) $\log_{10}$ root Se and $\log_{10}$ culm Se ($\beta = 0.20$, $t = 6.18$, $p < 0.0001$, $R^2 = 0.47$); (e) $\log_{10}$ culm Se and $\log_{10}$ leaf Se ($\beta = 0.40$, $t = 5.86$, $p < 0.0001$, $R^2 = 0.44$) (N = 45).

chelation to cell wall matrices or adsorption to Fe plaques on root surfaces (MacFarlane and Burchett, 2000). However, when accumulation is above unity, compared to surrounding sediment loads, some active uptake across the root plasmalemma is likely. Barriers to transport observed at the root: culm interface is likely due to the endodermis and casparian strip, driving cation transport from predominantly apoplastic to symplastic pathways (Greger, 2004). Movement of metal(loid)s are thus restricted with limited efflux into the stele. Indeed, MacFarlane and Burchett (2000) examined cellular uptake of Cu, Pb and Zn in roots of the halophyte, *A.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, 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. In some halophytic grasses such as *S. virginicus*, excretion of excess ions to maintain osmotic balance is achieved via salt glands on the adaxial and abaxial leaf surfaces (Naidoo and Naidoo, 1998). Such

glands have also been observed to co-excrete metals. In the halophytic dicot, *A. marina*, MacFarlane and Burchett (2000) found leaf washings of both Cu and Zn treated plants contained greater concentrations of these metals than control plants. Further, they found that salt crystals exuded from glandular trichomes were composed of alkaline metals and Zn in Zn-treated plants and Cu in Cu-treated plants. 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 (Kraus et al., 1986; Burke et al., 2000). Such patterns of metal excretion, coupled with barriers to transport at the root:culm interface may explain low metal concentrations evidenced in leaf tissue relative to root load found in the current study species.

4.3. Accumulative indication potential of S. virginicus

The current study also examined whether there were predictive linear relationships between metal(loid)s in sediment and metal(loid)s in plant tissues, with a view to employing *S. virginicus* tissues as surrogate
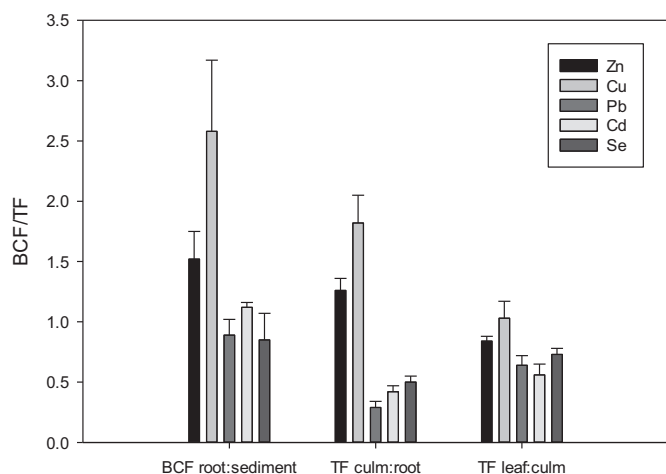
Fig. 8. Average bioconcentration factor (BCF) and translocation factors (TF), examining BCF from sediment to root; TF from root to culm, and TF from culm to leaf for Zn, Cu, Pb, Cd and Se (averaged across all sites, mean ± SE, N = 45).

bioindicators for assessing relative metal(loid) contamination status, and concomitantly bioavailability, in saltmarsh systems. Although all metal(loid)s exhibited significant positive linear relationships between metal(loid)s in sediment and metal(loid)s in roots, the proportion of explained variation was low, making their predictive ability limited, with the exception of Cd in roots ($R^2 = 0.99$). Similarly, although significant linear relationships for culm metal(loid)s and leaf metal(loid)s with sediment metal(loid)s were observed (withholding Cu), there was great variability in the response variable with dose (low R^2). Given these limitations, it would be inaccurate to employ *S. virginicus* tissues as bioindicators to predict sediment metal(loid) loads, with the exception of Cd in root tissue. Such bioindication potential has limited utility, however, as sediment disturbance is required to harvest *S. virginicus* roots and thus has minimal advantages over sampling sediment directly.

5. Conclusions

In summary, metal(loid) accumulation and partitioning in the dominant saltmarsh grass, *S virginicus* was assessed across three contaminated estuaries in NSW Australia. Lake Macquarie was found to be the most contaminated estuary, with Sydney Olympic Park exhibiting intermediate levels of sediment metal(loid) contamination, while Hunter Wetlands possessed the lowest metallic loads. All metal(loids) accumulated to roots in a linear fashion, and further linear accumulative relationships were observed at the major transitions of root:culm and culm:leaf for all metal(loid)s, with the exception of Cu, which was highly variable. Essential metals were more mobile than non-essential metals and metalloids, with essentials (Cu and Zn) accumulating to roots at concentrations greater than unity and further translocation factors at or above unity, while other metal(loids) were accumulated to roots at unity with barriers to translocation from root to culm and culm to leaves. Although linear relationships were observed between sediment metal(loid)s and plant organ metal(loid)s (with the exception of Cu), the low to intermediate explained variance implied that these tissues would be unsuitable for bioindication purposes (withholding Cd in roots). *S. virginicus* thus sequesters the majority of metal(loid) load in the roots, with restricted translocation to shoots, implying minimal transport of metal(loid)s to adjacent estuarine systems through direct detrital transfer or trophic relay, making the species an excellent candidate for phytostabilisation of metal(loid)s in saltmarsh habitats. As such, the species could potentially be deployed through assisted planting in remediation initiatives to stabilise metal(loid)s and prevent metal(loid) transport to adjacent aquatic habitats.

References

Adam, P., 1993. Saltmarsh Ecology. Cambridge University Press https://doi.org/10.1017/CBO9780511565328.

Adam, P., 2009. Australian saltmarshes in global context. In: Saintilan, N. (Ed.), Australian Saltmarsh Ecology. CSIRO, Collingwood VIC, AUSTRALIA, pp. 1–22.

Almeida, C.M.R., Mucha, A.P., Vasconcelos, M.T., 2011. Role of different salt marsh plants on metal retention in an urban estuary (Lima estuary, NW Portugal). Estuar. Coast. Shelf Sci. 91 (2), 243–249. https://doi.org/10.1016/j.ecss.2010.10.037.

Anderson, M., 2001. A new method for non-parametric multivariate analysis of variance. Aust. Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

ANZECC, 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, pp. 1–103.

Baker, A.J.M., Brooks, R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1 (2), 81–126.

Batley, G., 1987. Heavy metal speciation in waters, sediments and biota from Lake Macquarie NSW. Aust. J. Mar. Freshwat. Res. 38, 591–606. https://doi.org/10.1071/MF9870591.

Batley, G., Simpson, S., 2016. Chapter 2. Sediment sampling, sample preparation and general analysis. Sediment Quality Assessment: A Practical Guide, 2nd edition CSIRO Publishing, Clayton South, Vic, p. 2016.

Birch, G.F., Gunns, T.J., Olmos, M., 2015. Sediment-bound metals as indicators of anthropogenic change in estuarine environments. Mar. Pollut. Bull. 101 (1), 243–257. https://doi.org/10.1016/j.marpolbul.2015.09.056.

Burke, D.J., Weis, J.S., 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. https://doi.org/10.1006/ecss.2000.0673.

Cambrollé, J., Mateos-Naranjo, E., Redondo-Gómez, S., Luque, T., Figueroa, M.E., 2011. The role of two Spartina species in phytostabilization and bioaccumulation of Co, Cr, and Ni in the Tinto–Odiel estuary (SW Spain). Hydrobiologia 671 (1), 95.

Carroll, B., 1999. Microbial and Geochemical Aspects of Selenium Cycling in an Estuarine System-Lake Macquarie NSW. PhD Thesis. University of Sydney Australia.

Chalmers, H.O., 2002. Factors Affecting Saltmarsh Regeneration at Sydney Olympic Park. Bachelor (Hons.) thesis, unpublished thesis. University of Technology Sydney.

DECC, 2008. Department of Environment and Climate Change NSW, Best Practice Guidelines for Coastal Saltmarsh. DECC, Sydney.

Department of the Environment and Energy, 2013. Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) (s266B) Conservation Advice for Subtropical and Temperate Coastal Saltmarsh. Department of Environment and Energy, Canberra.

Duarte, B., Caetano, M., Almeida, P.R., Vale, C., Caçador, I., 2010. Accumulation and biological cycling of heavy metal in four salt marsh species, from Tagus estuary (Portugal). Environ. Pollut. 158 (5), 1661–1668. https://doi.org/10.1016/j.envpol.2009.12.004.

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. https://doi.org/10.1093/aob/mcu217.

Franco-Hernández, M.O., Vásquez-Murrieta, M.S., Patiño-Siciliano, A., Dendooven, L., 2010. Heavy metals concentration in plants growing on mine tailings in Central Mexico. Bioresour. Technol. 101 (11), 3864–3869. https://doi.org/10.1016/j.biortech.2010.01.013.

Gedan, K.B., Silliman, B.R., Bertness, M.D., 2008. Centuries of human-driven change in salt marsh ecosystems. Annu. Rev. Mar. Sci. 1 (2008), 117–141. https://doi.org/10.1146/annurev.marine.010908.163930.

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 edition Springer-Verlag Berlin, Heidelberg, Hyderabad, India.

Harbison, P.A.T., 1986. Mangrove muds—a sink and a source for trace metals. Mar. Pollut. Bull. 17 (6), 246–250. https://doi.org/10.1016/0025-326X(86)90057-3.

He, Y., DeSutter, T., Prunty, L., Hopkins, D., Jia, X., Wysocki, D., 2012. Evaluation of 1:5 soil to water extract electrical conductivity methods. Geoderma 185–186, 12–17. https://doi.org/10.1016/j.geoderma.2012.03.022.

Kabata-Pendias, A., 2011. Trace Elements in Soils and Plants. 4th ed. Taylor and Francis Group, Boca Raton https://doi.org/10.1201/b10158 505p.

Kabata-Pendias, A., Pendias, H., 1984. Trace Elements in Soils and Plants. CRC Press, Boca Raton.

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. https://doi.org/10.1016/0141-1136(86)90056-5.

Kwon-Rae, K., Owens, G., Naidu, R., 2009. Heavy metal distribution, bioaccessibility and phytoavailability in long-term contaminated soils from Lake Macquarie, Australia. Australian J. Soil Res. 47 (2), 166–176. https://doi.org/10.1071/SR08054.

Lottermoser, B.G., 1998. Heavy metal pollution of coastal river sediments, north-eastern New South Wales, Australia: lead isotope and chemical evidence. Environ. Geol. 36 (1–2), 118–126. https://doi.org/10.1007/s002540050.

Lutts, S., Lefèvre, I., 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? Ann. Bot. 115 (3), 509–528. https://doi.org/10.1093/aob/mcu264.

MacFarlane, G.R., Burchett, M.D., 2000. Cellular distribution of copper, lead and zinc in the grey mangrove, Avicennia marina (Forsk.) Vierh. Aquat. Bot. 68 (1), 45–59. https://doi.org/10.1016/S0304-3770(00)00105-4.

MacFarlane, G.R., Schreider, M., McLennan, B., 2006. Biomarkers of heavy metal contamination in the red fingered marsh crab, Parasesarma erythodactyla. Arch. Eviron. Con. Tox. 51 (4), 584–593.

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. https://doi.org/10.1016/j.chemosphere.2007.04.059.

Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R., Zhang, Z., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotox. Environ. Saf. 126, 111–121. https://doi.org/10.1016/j.ecoenv.2015.12.023.

Marcum, K.B., 2008. Saline tolerance physiology in grasses. Ecophysiology of High Salinity Tolerant Plants. Springer, Dordrecht, pp. 157–172. https://doi.org/10.1007/1-4020-4018-0_11.

Marcum, K.B., Murdoch, C.L., 1992. Salt tolerance of the coastal salt marsh grass, Sporobolus virginicus (L.) Kunth. New Phytol. 120 (2), 281–288. https://doi.org/10.1111/j.1469-8137.1992.tb05665.x.

McLean, C.M., Koller, C.E., Rodger, J.C., MacFarlane, G.R., 2009. Mammalian hair as an accumulative bioindicator of metal bioavailability in Australian terrestrial environments. Sci. Total Environ. 407 (11), 3588–3596. https://doi.org/10.1016/j.scitotenv.2009.01.038.

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., Fletcher, S., 2017. A global map of saltmarshes. Biodivers. Data. J 5, e11764. https://doi.org/10.3897/BDJ.5.e11764.

Mganga, N.D., 2014. The potential of bioaccumulation and translocation of heavy metals in plant species growing around the tailing dam in Tanzania. Inter. J. Sci. Technol. 3 (10), 690–697.

Naidoo, Y., Naidoo, G., 1998. Sporobolus virginicus leaf salt glands: morphology and ultra-structure. S. Afr. J. Bot. 64 (3), 198–204. https://doi.org/10.1016/S0254-6299(15)30867-X.

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.

Nath, B., Birch, G., Chaudhuri, P., 2014a. Assessment of sediment quality in Avicennia marina-dominated embayments of Sydney Estuary: the potential use of pneumatophores (aerial roots) as a bio-indicator of trace metal contamination. Sci. Total Environ. 472, 1010–1022.

Nath, B., Chaudhuri, P., Birch, G., 2014b. Assessment of biotic response to heavy metal contamination in Avicennia marina mangrove ecosystems in Sydney Estuary, Australia. Ecotoxicol. Environ. Saf. 107, 284–290.

Ogundiran, M.B., Osibanjo, O., 2008. Heavy metal concentrations in soils and accumulation in plants growing in a deserted slag dumpsite in Nigeria. Afr. J. Biotechnol. 7 (17), 3053–3060. https://doi.org/10.5897/AJB08.322.

Otte, M.L., Bestebroer, S.J., Van der Linden, J.M., Rozema, J., Broekman, R.A., 1991. A survey of zinc, copper and cadmium concentrations in salt marsh plants along the Dutch coast. Environ. Pollut. 72 (3), 175–189. https://doi.org/10.1016/0269-7491(91)90098-H.

Peters, G., Maher, W., Krikowa, F., Roach, A., Jeswani, H., Barford, J., Gomes, V., Reible, D., 1999. Selenium in sediments, pore waters and benthic Infauna of Lake Macquarie, New South Wales, Australia. Mar. Environ. Res. 47 (5), 491–508. https://doi.org/10.1016/S0141-1136(99)00027-0.

Polechońska, L., Klink, A., 2014. Trace metal bioindication and phytoremediation potentialities of Phalaris arundinacea L. (reed canary grass). J. Geochem. Explor. 146, 27–33. https://doi.org/10.1016/j.gexplo.2014.07.012.

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

Pulkownik, A., 2000. Ecotoxicology of Metals in Estuarine Plants. PhD thesis. University of Technology Sydney.

Rahman, M.M., Owens, G., Naidu, R., 2009. Arsenic levels in rice grain and assessment of daily dietary intake of arsenic from rice in arsenic-contaminated regions of Bangladesh—implications to groundwater irrigation. Environ. Geochem. Health 31 (1), 179–187.

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. https://doi.org/10.1007/s10750-017-3490-y.

Rieuwerts, J.S., Thornton, I., Farago, M.E., Ashmore, M.R., 1998. Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem. Spec. Bioavailab. 10 (2), 61–75. https://doi.org/10.3184/095422998782775835.

Roach, A.C., 2005. Assessment of metals in sediments from Lake Macquarie, New South Wales, Australia, using normalisation models and sediment quality guidelines. Mar. Environ. Res. 59 (5), 453–472. https://doi.org/10.1016/j.marenvres.2004.07.002.

Saintilan, N., Williams, R.J., 1999. Mangrove transgression into saltmarsh environments in south-east Australia. Glob. Ecol. Biogeogr. 8 (2), 117–124. https://doi.org/10.1046/j.1365-2699.1999.00133.x.

Saintilan, N., Wilson, N.C., Rogers, K., Rajkaran, A., Krauss, K.W., 2014. Mangrove expansion and salt marsh decline at mangrove poleward limits. Glob. Chang. Biol. 20 (1), 147–157. https://doi.org/10.1111/gcb.12341.

Schneider, L., Maher, W., Potts, J., Gruber, B., Batley, G., Taylor, A., Chariton, A., Krikowa, F., Zawadzki, A., Heijnis, H., 2014. Recent history of sediment metal contamination in Lake Macquarie, Australia, and an assessment of ash handling procedure effectiveness in mitigating metal contamination from coal-fired power stations. Sci. Total Environ. 490, 659–670. https://doi.org/10.1016/j.scitotenv.2014.04.055.

SCP Science, 2019. Science Plant Tissue Digestion Procedure Application Note (Canada).

Silliman, B.R., Grosholz, E.D., Bertness, M.D. (Eds.), 2009. Human Impacts on Salt Marshes: A Global Perspective. Univ of California Press.

Simpson, S.L., Batley, G.B., Chariton, A.A., 2013. Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines CSIRO Land and Water Science Report 08/07. CSIRO Land and Water.

Sousa, A.I., Lillebø, A.I., Pardal, M.A., Cacador, I., 2010. The influence of Spartina maritima on carbon retention capacity in salt marshes from warm temperate estuaries. Mar. Pollut. Bull. 61, 215–223. https://doi.org/10.1016/j.marpolbul.2010.02.018.

Suh, J.Y., Birch, G.F., Hughes, K., Matthai, C., 2004. Spatial distribution and source of heavy metals in reclaimed lands of Homebush Bay: the venue of the 2000 Olympic Games, Sydney, New South Wales. Austr. J. Earth. Sci. 51 (1), 53–66. https://doi.org/10.1046/j.1400-0952.2003.01043.x.

Suh, J.Y., Birch, G.F., 2005. Use of grain-size and elemental normalization in the interpretation of trace metal concentrations in soils of the reclaimed area adjoining Port Jackson, Sydney, Australia. Water, air, and soil pollution 160 (1-4), 357–371.

Terry, N., Zayed, A.M., De Souza, M.P., Tarun, A.S., 2000. Selenium in higher plants. Annu. Rev. Plant Biol. 51 (1), 401–432. https://doi.org/10.1146/annurev.arplant.51.1.401.

US EPA, 2007. Method 3051A. Microwave Assisted Acid Digestion of Sediments, Sludge's, Soils and Oils.

Vårhammar, A., McLean, C.M., Yu, R.M.K., MacFarlane, G.R., 2019. Uptake and partitioning of metals in the Australian saltmarsh halophyte, samphire (Sarcocornia quinqueflora). Aquat. Bot. 156, 25–37. https://doi.org/10.1016/j.aquabot.2019.04.001.

Williams, T.P., Bubb, J.M., Lester, J.N., 1994. Metal accumulation within salt marsh environments: a review. Mar. Pollut. Bull. 28 (5), 277–290. https://doi.org/10.1016/0025-326X(94)90152-X.

Zhang, X., Gao, B., Xia, H., 2014. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of bana grass and vetiver grass. Ecotox. Envoron. Saf. 106, 102–108. https://doi.org/10.1016/j.ecoenv.2014.04.025.