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Metallic mangroves: Sediments and in situ diffusive gradients in thin films (DGTs) reveal *Avicennia marina* (Forssk.) Vierh. lives with high contamination near a lead-zinc smelter in South Australia

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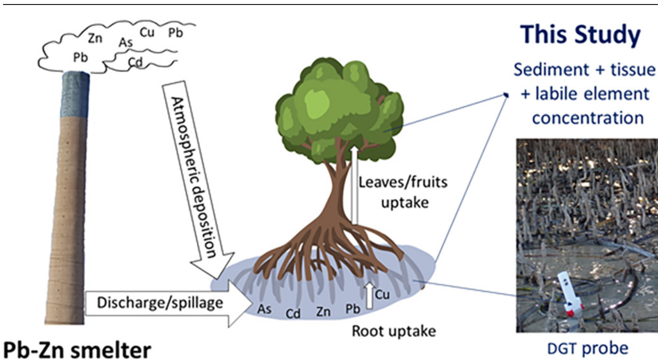
HIGHLIGHTS

- 14,488 mg/kg Pb, 62,097 mg/kg Zn found in mangrove sediment near Port Pirie smelter.
- Highest Pb & Zn values exceeded sediment quality guideline values by 60 & 151-fold.
- *A. marina* leaves closer to smelter contained up to 319 mg/kg Pb; 1033 mg/kg Zn.
- Transfer of non-essential elements (As, Cd, Pb) into fruits were low (≤5.23 mg/kg).
- Labile As, Cd, Cu, Pb, Zn near smelter exceeded 95 % species protection guideline values.

GRAPHICAL ABSTRACT



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ABSTRACT

From 1889, aerial emissions and effluent from a coastal lead-zinc smelter at Port Pirie, South Australia, have led to the accumulation of lead (Pb), zinc (Zn), arsenic (As), cadmium (Cd) and copper (Cu) in the surrounding marine environment. Despite this, extensive stands of grey mangrove (*Avicennia marina*) inhabit coastal areas at Port Pirie, right up to the smelter's boundary. To understand the contamination level the mangroves are living in there, elemental concentrations were measured in mangrove sediments, leaves, pneumatophores and fruits at sites 0.30–43.0 km from the smelter. Plant health was assessed via leaf chlorophyll content at four sites with contrasting contamination, as well as in situ labile elemental concentration using diffusive gradients in thin films (DGT). Sites < 1.7 km of the smelter exceeded Australian and New Zealand Environment and Conservation Council (ANZECC) & Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) (2000) sediment quality guideline values for As (78.3–191 mg/kg), Cd (5.17–151 mg/kg), Cu (80.7–788 mg/kg), Pb (2,544–14,488 mg/kg) and Zn (281–62,097 mg/kg), while sites further away showed less enrichment above background. Similarly, elevated elemental concentrations in leaves and pneumatophores occurred closer to the smelter (up to 319 mg/kg Pb; 1,033 mg/kg Zn), while fruits had little contamination of non-essential elements (≤ 5.23 mg/kg). Relationship between sediment and leaf elemental concentration was isometric for Pb and anisometric for others. Labile As, Cd, Cu, Pb and Zn exceeded the 95% and 80%

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level of species protection in marine water by ANZECC & ARMCANZ (2000) near the smelter, but chlorophyll content did not vary significantly among sites ($p > 0.05$). These results reveal that *A. marina* tolerate high elemental contamination at Port Pirie, contributing to lesser but still high contamination in plants, warranting further investigation into non-lethal impacts on mangroves or additional biota inhabiting this ecosystem.

1. Introduction

Worldwide, mangrove forests occupy large areas of tropical, subtropical and temperate intertidal coastlines, in part, because of their abilities to tolerate extreme variation in salinities, temperatures, inundation, and anoxia (Besley and Birch, 2021; Tonhá et al., 2020). Mangrove forests, tidal marshes and seagrass meadows contribute to blue carbon ecosystem, storing >30,000 Tg C across ~185 million hectares and potentially reducing ~3 % of global carbon emissions (Macreadie et al., 2021). Mangroves are highly productive primary producers, playing a significant role in preventing coastline erosion against natural disasters and serving as nurseries/breeding grounds to many marine species including crustaceans and juvenile fish (Besley and Birch, 2021; Macreadie et al., 2021). Mangroves also offer significant economic benefits directly (approximately $181 billion per year), by providing local communities with fishing and tourism opportunities, medicine, fuel and building materials (Worthington et al., 2020).

Despite providing extensive ecological and economical services, mangroves face considerable threat from a range of stressors including climate change and destruction/modification from coastal developments (Ferreira et al., 2022). Another important and pervasive stressor is pollution via industrial emissions, including effluent, stormwater runoff, agriculture or aquaculture, spillage from shipping, and atmospheric deposition (Besley and Birch, 2021). Unfortunately, due to their extensive and intricate root systems (i.e., wide spreading lateral roots, interwoven with fine nutritive roots and vertical pneumatophores), mangroves act as a natural trap for contaminants in coastal environments. Moreover, the high clay and organic carbon content of the sediments around their roots often create anoxic and sulphidic conditions, which results in mangrove sediments ending up as a sink for many elements (Parida and Kumari, 2021). Potentially toxic element exposure has been shown to cause oxidative/osmotic stress, impaired photosynthesis, reduced biomass/height, inhibit/delay seedling emergence, and may cause mortality in mangroves (Caregnato et al., 2008; MacFarlane and Burchett, 2002).

Avicennia marina (grey mangrove) is the most widely distributed mangrove species worldwide, found in temperate and tropical regions and both in arid and wet environments (Besley and Birch, 2021). This mangrove species is also well-known for its apparent high tolerance to potentially toxic elements (Besley and Birch, 2021; MacFarlane and Burchett, 2002; MacFarlane et al., 2007). At least in part, *A. marina* achieves this tolerance via enhanced iron plaque formation on root surfaces, which acts as a barrier/scavenger of toxic elements and reduces their uptake into the root (Jian et al., 2019; Lin et al., 2018). *A. marina* also selectively excludes non-essential toxic elements (e.g., Pb) at the root epidermis, while restricting movement of others (e.g., Cu, Pb and Zn) using the endodermal casparian strip (MacFarlane and Burchett, 2000). Although *A. marina* may excrete excess nutrients via leaf salt glands (e.g., Zn) as a coping mechanism to high elemental stress (Natarajan et al., 2021), a recent study linked porewater salinity of >100 to a dieback event of *A. marina* in South Australia (~9 ha), indicating physiological tolerance limit of hypersalinity in this species (Dittmann et al., 2022).

A. marina extensively populates Upper Spencer Gulf, South Australia, which also hosts one of the world's largest Pb and Zn smelter at Port Pirie (220 km north of Adelaide, the capital city of South Australia, Fig. 1). Port Pirie has a Mediterranean climate, and the estuary has no riverine input, leading to hypersaline conditions (Taylor et al., 2019; Vandeleur, 2020). An example of the continuous Pb exposure can be cited from the National Pollution Inventory (2018), which reported that approximately 50,000 kg of Pb was released into the atmosphere and 9600 kg into the estuary during 2016–17. Previous studies reported that elevated Arsenic (As), Cd, Cu, Silver (Ag) and Manganese (Mn) are detectable in the marine ecosystem up to 65 km from Port Pirie (Vandeleur, 2020; Ward, 1987; Ward et al., 1984; Ward and Young, 1981; Ward and Young, 1983; Ward and Hutchings, 1996; Ward and Young, 1982). This persistent elevated elemental contamination of Port Pirie's sediments has prompted several investigations into its adverse effect on epibenthic seagrass and benthic infauna (e.g., bivalve molluscs, polychaetes and crustaceans) (Edwards et al., 2001; Ward, 1987; Ward and Young, 1983; Ward and Hutchings, 1996; Ward and Young, 1982). Those studies found that bioaccumulation shows a strong relationship with distance from effluent discharge sites with clear impacts on species richness/diversity (Ward, 1987; Ward and Hutchings, 1996; Ward and Young, 1982). Yet, studies assessing the extent of contamination in the mangrove sediments or its effect on plant health has received limited attention, except for a recent report indicating that mangroves at Port Pirie seemed "healthy and abundant" (Stockbridge, 2017).

The overarching aim of this study was to investigate elemental contamination of mangrove sediments, its bioaccumulation in *A. marina* at Port Pirie, and how these change with distance from the smelter or affect plant health. To achieve this aim, total and labile elemental concentrations in mangrove sediment and plant tissues were assessed, along with differences in leaf chlorophyll content across sites with contrasting contamination. It was hypothesized that total and labile elemental concentration will correlate with distance from smelter and the health of mangroves will be more adversely affected in the vicinity of the smelter.

2. Materials and methods

2.1. Sediment/mangrove tissue collection

Nine sites were sampled around Port Pirie smelter in the upper Spencer Gulf of South Australia according to Fig. 1. Sites were chosen to represent a range of distances (0.34–43 km) from the smelter (−33.169917, 138.011694) and thus were expected to represent a gradient of sediment elemental contamination. Sites 1–5 were 0.3–1.7 km from the smelter (hereafter referred to as 'adjacent to smelter') and expected to contain high contaminant concentrations. Three more locations (sites 7–8) were at a distance between 7.5 and 18.3 km to the north of the smelter (hereafter referred to as 'within influence of smelter') and were expected to have medium to low contaminant concentrations. Lastly, site 6 was 43 km away to the south (Fisherman's Bay), and thus expected to represent 'not influenced' sediment, with background (natural) concentrations of elements. Initial sample collection was conducted in October 2019, with GPS coordinates recorded for future re-sampling. For one tree at each site (~20) leaves and (~10) pneumatophores (aerial roots) from healthy mature plants (>2 m tall) were collected randomly from across the tree, as well as a sample of the surface sediment (0–10 cm depth) directly under the tree. Sediment and plant tissue samples were placed in Ziplock bags and frozen until further processing. Trees from the sites adjacent to the smelter were noted to be of unhealthy appearance, leaves covered with dust (Fig. S1) and without fruiting.

2.2. Sediment processing and analysis

A subsample from each sediment was thawed and particle size analyzed using Mastersizer 2000 (Malvern Panalytical). The remaining material for each sample was oven-dried at 60°C, ground to homogenize and used during subsequent analysis. Total organic matter content (TOC) of the

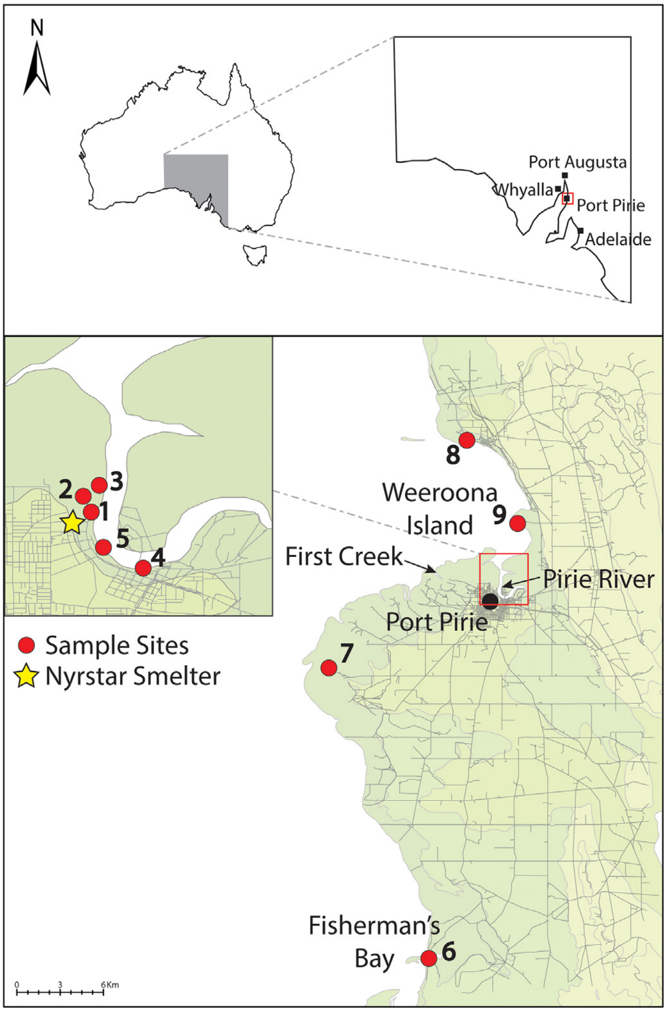

Fig. 1. Mangrove sample site locations in Port Pirie, Upper Spencer Gulf, South Australia. The city of Port Pirie is indicated with the black circle in the bottom panel, while the Pb-Zn smelter is indicated by the yellow star in the insert of the bottom panel.

sediments (0.2 g, n = 3) was analyzed by taking the difference between total carbon and total inorganic carbon after reacting with Sulphurous acid (H_2O_3S) solution (> 6%) in LECO TruMac CNS as detailed in Kastury et al. (2021). Sediments were pre-digested (0.5 g, n = 4) overnight in 5 mL reverse aqua regia [3:1 = 70% Nitric acid (HNO_3): 36% Hydrochloric acid (HCl)], followed by digestion in a Mars6 microwave (CEM) according to USEPA method 3051 (USEPA, 1998). A standard reference material (SRM) 2702 - Inorganics in Marine Sediment, from the National Institute of Standards and Technology (NIST) was used to confirm the accuracy of digestion (n = 5, Recoveries of elements of interest were: As = 71.5%, Cd = 122%, Cu = 76.6%, Fe = 80.0%, Pb = 84.3% and Zn = 78.5%). Following digestion, samples were made up to 50 mL using MQ water (>18 MΩ), syringe filtered (0.45 μm, cellulose acetate) and stored until analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Perkin Elmer Avio 500) according to US EPA method 6010D (USEPA, 2018).

2.3. Mangrove tissue processing and analysis

A. marina leaves and pneumatophores were washed with MQ water to remove particles, oven-dried (60°C) and ground to homogenize. Tissues were pre-digested (0.5 g, n = 4) with 5 mL 70% HNO_3 overnight and digested in Mars6 microwave using method described in Kastury et al. (2021), with post-digestion processing conducted as described above. To assess the accuracy of tissue digestion, NIST SRM 1573a: Tomato leaves was used (n = 4, Recoveries of elements of interest were: As = 106%, Cd = 78.3%, Cu = 84.5%, Fe = 92.6%, and Zn = 91.4%, while certified value for Pb was not available). Elemental concentration analysis was conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent, Triple Quad 8800) using USEPA method 6020A (USEPA, 2007).

2.4. Re-sampling at four sites

Four sites were re-visited in January 2021 to assess mangrove health, contamination in fruits (which were present this time at each site), as well as to assess labile elemental concentrations. Two of these sites were selected from adjacent to smelter: corresponding to site 1 and 3 during 1st sampling. The third site was within influence of smelter (site 8 during 1st sampling), while the last site, Fisherman's Bay (site 6 during 1st sampling), acted as a site not influenced by smelter.

2.4.1. Collection of leaves, fruit and chlorophyll measurement

At each site, three trees (≥2 m tall) were randomly chosen for sampling; shorter trees were excluded to avoid recruits/young trees, as were trees with obvious dieback (dead branches). Up to twenty leaves and fruits were collected from each tree, including all sides of the tree and from branches at the outer, sunnier extremes and inner, shaded areas. Leaves from each tree were put into one bag, mixed, then five leaves from each tree measured for chlorophyll content using SPAD 502 Chlorophyll meter (Spectrum Technologies Inc). Five SPAD measurements were taken from each leaf, starting at the base/to one side and then evenly spaced around the circumference to the base of the leaf on the other side, each measurement set about 7–8 mm in from the edge. Samples were processed and elemental concentration analyzed as Section 2.3.

2.4.2. Labile elemental concentration analysis in the mangrove sediment

Diffusive Gradients in Thin Films (DGT) was deployed (n = 3, ~18–24 h) to measure labile or freely bioavailable elemental concentrations (hereafter referred to as C_{DGT}). Sediment DGT probes (LSPX for metals cationic and oxyanion measurement using chelex and titanium oxide) were purchased from DGT Research (Lancaster, UK). More details regarding DGT characteristics and deployment conditions are given in Supplementary materials. At the end of deployment, DGT gel layers were retrieved from each probe and sliced into fifteen sections (1 cm/section). The 1st and 2nd sections were exposed to the water column and sediment-water interface respectively, and the following thirteen sections exposed to sequentially deeper sediments. Cations from each section was eluted in 1 mL HNO_3 (1 M, baseline, Seastar) for 24 h, and then anions were eluted in 1 mL NaOH (1 M, high purity, Sigma-Aldrich) for another 24 h. The eluents were combined and diluted to a final volume of 5 mL using 2% HNO_3, syringe filtered (0.45 μm, cellulose acetate), and elemental concentration analyzed in ICP-MS (Agilent, Triple Quad 8800) (USEPA method 6020A). During analysis by ICP-MS, at least 7 points were used to construct calibration curve, the lowest point being at least 3× above the calibration blank. Limit of detection for the elements of interest during the analysis using ICP-MS are: As = 1 μg/L, Cd = 0.5 μg/L, Cu = 50 μg/L, Fe = 50 μg/L, Pb = 0.5 μg/L, and Zn = 10 μg/L. All field blank values were below LOD. Based on Fick's first law of diffusion, C_{DGT} was determined according to Eq. (1) (Amato et al., 2014, 2016):

$$C_{DGT} = M\,\Delta g / DtA \tag{1}$$

where M is the accumulated mass of analyte in the binding gel; Δg is the diffusion layer thickness (0.09 cm, including 0.08 cm APA gel layer and 0.01 cm filter membrane); D is the analyte diffusion coefficient in the diffusive layer at the certain temperature; t is the deployment time in seconds; A is the exposed surface area of the DGT device (1.8 cm^2).

2.5. Statistical analysis

All statistical analysis was conducted using SPSS version 25 (IBM) and Graphpad Prism 9.3.0. Normality of elemental concentration across the nine sites was investigated using Shapiro-Wilk normality test ($\alpha = 0.05$). Log_{10} transformation was applied to sediment elemental concentrations for As, Cd, Cu, Fe, Pb and Zn (as the main elements of concern) and distance from the smelter measured on Google maps as the straight-line distance from the site to the center of the Port Pirie smelter. Linear regression was performed between log_{10} distance and log_{10} sediment elemental concentrations to test their relationship.

Regression analysis was also conducted to investigate if sediment elemental concentration is a good predictor of leaf elemental concentration and if the relationship between elemental concentrations in sediment and leaves differ among the elements of interest. Log_{10} transformation was applied to leaf and sediment elemental concentrations for As, Cd, Cu, Fe, Pb and Zn (as the main elements of concern); transformed values passed Shapiro-Wilk Normality test at $\alpha = 0.05$. Analysis of covariance (ANCOVA) was used to test for differences in the slopes of the relationship between leaf and sediment concentrations among the different elements and to test whether the slopes of those relationship were isometric (slope = 1) or anisometric (slopes ≠ 1). Given that there were differences in slopes, the relationship between leaf and sediment elemental concentration was then assessed separately by linear regression to generate confidence intervals for the slope of the relationship for each element. Comparisons of these individual slope confidence intervals suggested that the slope of the relationship in Pb was possibly the only difference among elements and thus the reason for the initial significant interaction (elements x slopes) in the ANCOVA. To test whether this was the only difference among elements, the ANCOVA was re-analyzed excluding Pb, followed by of pairwise comparisons of slopes among elements, using a Dunn Sidak adjustment for multiple comparisons ($\alpha = 0.05$).

To ascertain the impact of elemental bioaccumulation on mangrove health, five chlorophyll measurements from each leaf were averaged and then all the averaged leaf chlorophyll measurements from each tree per site was averaged again, giving an average of chlorophyll measurement for each tree. ANOVA (one factor = site) was used to test for differences in chlorophyll *a* measurement among sites (n = 3 trees per site).

3. Results and discussions

3.1. Elemental concentrations in sediments

Table 1 summarizes distance of each site from the smelter and gives the TOC and concentrations of elements of interest (As, Cd, Cu, Fe, Mn, Pb and Zn) in the mangrove sediments collected during the first sampling period, while Fig. 2 depicts the particle size distribution. The sites adjacent to the smelter (sites 1–5, 0.34–1.72 km), were expected to receive direct smelter stack emissions, evident as dust covered mangrove leaves (Fig. S1), and site 4 received additional effluent discharge into the river. Sites 7–9 (7.46–18.3 km) were not expected to receive direct aerial emissions, but were known to be influenced by sediment contamination in a recent study by Vandeleur (2020). Particularly, site 8 (Weerona Island) was situated near a dredge spoil dump area and may have been influenced via stormwater run-offs. In contrast, site 6 (Fisherman's Bay) was not expected to be influenced by either stack emissions/effluent discharge, or stormwater run-off based on Vandeleur (2020) because of the 43.0 km distance from smelter, and therefore considered to represent the background (natural) elemental concentration in this region. As shown in Table 1, Pb concentrations in sites adjacent to the smelter ranged from 2,544 ± 61.0 mg/kg (site 4) to 14,488 ± 2,728 mg/kg (Site 2). Compared to an assumed background of 9.81 ± 0.29 mg/kg in sediments at site 6 (not influenced by smelter), Pb concentration in sediments at sites 1–5 (near the smelter) were enriched between 128 and 1,477 times. Overall, Fig. S3 showed a statistically significant, negative relationship between distance from the smelter and Pb concentration ($r^2 = 0.95$, $p < 0.0001$), which was also observed for Zn ($r^2 = 0.81$, $p < 0.01$). Zn concentrations near the smelter (sites 1–3) ranged between 3,232 ± 220 mg/kg to 62,097 ± 7,356 mg/kg, while it decreased sharply in sites 4 and 5 to 506 ± 20.1 mg/kg and 281 ± 5.64 mg/kg respectively. Further away, Zn concentrations continued to decline ranging between 39.5 ± 2.22 mg/kg to 67.3 ± 3.78 mg/kg at sites 7–9, down to a background value of 10.7 ± 0.83 mg/kg (site 6). Thus, not surprisingly around a Pb-Zn smelter, there was clear evidence of distance-dependent Pb and Zn contamination in the sediments associated with mangroves at Port Pirie. A similar negative relationship was also observed for As (r^2: 0.77), Cd (r^2: 0.90), Cu (r^2: 0.83); however this relationship was not observed for Fe (r^2: 0.14), suggesting that sediment contamination of As, Cd and Cu may also be associated with the legacy/current smelter activities.

The distribution of particles (Fig. 2) in fine grains [clay (< 2 μm) and silt (> 2 μm and < 63 μm)], medium grains (> 63 μm and < 250 μm) and coarse

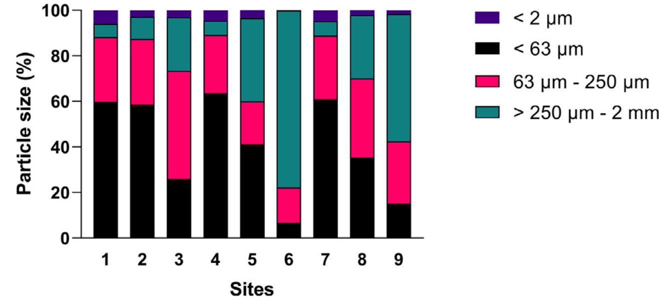

Fig. 2. Particle size distribution of mangrove sediment samples at Port Pirie, Upper Spencer Gulf, South Australia.

Table 1
Mean ± SEM concentrations of As, Cd, Cu, Fe, Mn, Pb and Zn, distance of each site from smelter and total organic carbon (TOC) in mangrove sediment at Port Pirie, Upper Spencer Gulf, South Australia. n = number of replicates; NA = not available.

Site	Expected contamination level	Distance from smelter (km)	n	TOC (%)	As (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
1	Adjacent to	0.71	4	4.28 ± 0.06	191 ± 7.10	39.3 ± 1.94	183 ± 12.8	21,734 ± 1,457	498 ± 38.1	5,205 ± 292	16,495 ± 1,122
2	smelter	0.74	4	1.24 ± 0.04	614 ± 61.6	151 ± 15.9	788 ± 64.5	24,450 ± 3,894	934 ± 109	14,488 ± 2,728	62,097 ± 7,356
3		0.88	4	1.45 ± 0.02	89.4 ± 5.30	9.56 ± 0.61	104 ± 6.71	15,143 ± 1,256	866 ± 60.0	3,367 ± 215	3,232 ± 220
4		1.72	4	2.57 ± 0.07	78.3 ± 1.55	89.3 ± 2.86	80.7 ± 3.33	1,811 ± 77.1	44.4 ± 2.67	2,544 ± 61.0	506 ± 20.1
5		0.34	4	2.51 ± 0.02	132 ± 2.97	5.17 ± 0.13	175 ± 4.04	1,257 ± 24.8	40.3 ± 0.37	5,365 ± 118	281 ± 5.64
6	Not influenced by smelter	43.0	4	6.22 ± 0.13	3.07 ± 0.09	0.06 ± 0.002	1.33 ± 0.07	1,008 ± 33.6	17.8 ± 0.67	9.81 ± 0.29	10.7 ± 0.83
7	Within influence of	18.3	4	6.30 ± 0.03	15.8 ± 0.57	0.26 ± 0.01	7.34 ± 0.25	12,146 ± 822	125 ± 3.95	19.8 ± 0.60	41.8 ± 1.21
8	smelter	17.1	4	5.74 ± 0.09	5.24 ± 0.26	0.41 ± 0.02	2.12 ± 0.10	3,151 ± 145	74.2 ± 3.80	36.0 ± 1.89	39.5 ± 2.22
9		7.46	4	3.37 ± 0.08	2.34 ± 0.11	0.99 ± 0.05	1.41 ± 0.07	777 ± 54.4	43.9 ± 2.12	55.8 ± 3.26	67.3 ± 3.78
ANZECC/ARMCANZ (2000) SQGV low					20	1.5	65	NA		50	200
ANZECC/ARMCANZ (2000) SQGV high					70	10	270			220	410

(> 250 μm – 2 mm) grains were variable among the nine sites. Fig. 2 shows that sites 6 and 9 contained mostly (> 50%) coarse particles, while sites 1, 2, 4 and 7 were dominated by fine particles size fractions (61.5–68.1%). Fine particle size fraction may have a significant role in elemental retention via providing higher surface area for sorption onto clay particles (Parida and Kumari, 2021). However, the high variability of fine particles observed among the 9 sites in this study indicates that fine particle fraction may play a minor role in controlling elemental enrichment in mangrove sediments adjacent to the smelter; presence of phases such as Fe/Mn oxyhydroxides and organic matter possibly exerting a greater effect on elemental retention (Marchand et al., 2016).

For reference, sediment quality guideline values (SQGV) are also given in Table 1 according to Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand (ANZECC/ARMCANZ, 2000). According to the ANZECC/ARMCANZ (2000), elemental concentrations below $SQGV_{low}$ are not expected to cause aquatic toxicity, while concentrations between $SQGV_{low}$ and $SQGV_{high}$ indicate potential toxicity on aquatic life, requiring additional environmental investigations for specific species (Besley and Birch, 2021). However, sediments displaying elemental concentrations above $SQGV_{high}$ suggest possible adverse effects to biological receptors inhabiting this marine environment (Besley and Birch, 2021). This study found that concentrations of both Pb and Zn exceeded the $SQGV_{high}$ limits for sites 1–5 for Pb and sites 1–4 for Zn. Moreover, the greatest concentrations found were up to 60 times the $SQGV_{high}$ for Pb and 151 times the $SQGV_{high}$ for Zn.

Elevated Pb and Zn concentrations at non-mangrove associated sites adjacent to the smelter was reported previously from (non-mangrove associated) muddy sediments around Port Pirie by Vandeleur (2020), Ward and Hutchings (1996), Ward (1987), Ward et al. (1984), Ward and Young (1981), Ward and Young (1983) and Ward and Young (1982). The most recent study among these by Vandeleur (2020) included non-mangrove associated sites adjacent to 1–3 in the present study. Compared to the results by Vandeleur (2020), where maximum of 9,166 mg/kg of Pb and 21,036 mg/kg of Zn was observed, maximum Pb and Zn at mangrove associated sediment in this study were 1.8 and 2.9 times higher (site 2). Higher elemental concentration in this study may be attributed to the higher expected Fe and Mn plaques on the mangrove root surfaces in response to the elevated elemental contamination as a coping mechanism; Fe and Mn plaques providing surface areas for adsorption (Marchand et al., 2016). Accordingly, this study found elevated concentrations of Fe and Mn where Pb and Zn concentrations were the greatest (near the smelter, Table 1). In particular, given background concentration of Fe and Mn (at site 6) of 1,008 ± 33.6 mg/kg and 17.8 ± 0.65 mg/kg respectively, Fe and Mn concentrations were elevated 15–55-fold at sites 1–3 (15,143 ± 1,256 to 24,450 ± 3,894 mg/kg and 498 ± 38.1 to 934 ± 109 mg/kg respectively). Also of note, is a study by Tonhá et al. (2020), who reported a maximum of 20,050 mg/kg of Zn in *Laguncularia racemose* sediments near an electroplating plant in Sepetiba Bay, State of Rio de Janeiro (southeastern Brazil). Among other non-mangrove associated coastal sediment studies reporting highly elevated Pb and Zn concentrations include Boughriet et al. (2007), where maximum concentration of 10,079 mg/kg of Pb and 12,895 mg/kg of Zn was observed in Deûle-canal sediments near Metaleurop smelter (Northern France).

Enrichment of other potentially toxic elements at sites near the smelter included As (89.4 ± 5.30 to 614 ± 61.6 mg/kg), Cd (5.17 ± 0.13 to 151 ± 15.9 mg/kg) and Cu (80.7 ± 3.33 to 788 ± 64.5 mg/kg). Table S1 presents concentrations of other major elements [Aluminium (Al), Calcium (Ca), Magnesium (Mg), Sodium (Na)] and trace elements [Silver (Ag), Cobalt (Co), Chromium (Cr), Molybdenum (Mo), Nickel (Ni), Antimony (Sb), Selenium (Se), Vanadium (V)]. Among these, Ag exceeded the $SQGV_{high}$ of 4 mg/kg at site 1 (9.30 ± 0.54 mg/kg) and site 2 (15.6 ± 0.47 mg/kg), although even the background concentration (1.88 ± 0.17 mg/kg) was higher than $SQGV_{low}$ of 1 mg/kg. Vandeleur (2020) suggested that the most likely source of elevated Ag near the smelter is a historic leak in the silver recovery plant at the smelter which previously allowed Ag to make its way into groundwater and surrounding sediments. Vandeleur (2020) reported that non-mangrove sediments near First creek recorded 0.09–3.11 mg/kg of Ag, while that in the Port Pirie River (directly next to the smelter and the historic leak site) ranged from 0.10 to 41.8 mg/kg. Thus, in contrast to Pb and Zn, there is little evidence that Ag concentrations are higher in mangrove associated sediments than other, adjacent soft sediments.

Although well-known for their high tolerance to inorganic pollutants, the survival of *A. marina* at sites near the Pb-Zn smelter suggests that this species is living in elemental concentrations that far exceed those previous ecotoxicological studies suggested should kill or significantly harm mangroves. In particular, the field results here contrast with the findings of MacFarlane and Burchett (2002), who investigated the effects of Cu, Pb and Zn bioaccumulation and toxicity of *A. marina* propagules in carefully controlled, glasshouse-based studies. Using a combination of single, binary and tertiary mixtures across a range of concentrations of these elements, they found that 800 mg/kg Cu may result in total emergence inhibition and 1,000 mg/kg Zn may lead to 100% mortality in *A. marina*, while these effects were not observed at the highest Pb concentration of 800 mg/kg. These contrasting results may be attributed to the fact that MacFarlane and Burchett (2002) used spiked sediment aged for 2 weeks to assess toxicity in *A. marina* seedlings, whereas this study included field data of mature trees where elements were most likely strongly sorbed to the sediment because of the extended contamination period. Another noteworthy factor is the very high salinity of the waters of the Upper Spencer Gulf, which range between 42 and 47‰ according to Vaz et al. (1990); personal observations by one of the authors (CAS) indicated salinity as great at 54‰ in shallow areas around Port Pirie. High salinity may promote the formation of element-chloride complexations, which are less bioavailable species (MacFarlane et al., 2007). Therefore, Pb/Zn-chloride complexation in the mangrove sediment may have lowered the freely available Pb and Zn concentration for plant uptake in this study.

3.2. Elemental concentration in mangrove tissues and its relationship to sediment

Table 2 presents concentrations of As, Cd, Cu, Fe, Pb and Zn in *A. marina* tissues, including leaves and pneumatophores collected from nine sites during the 1st sampling period, and leaves and fruits collected from four sites during the 2nd sampling period (Tables S2 and S3 details additional detectable elements). Essential elements were higher in sites 6–9 (maximum leaf concentrations of 11.6 ± 0.33 mg/kg of Cu in site 6; 154 ± 5.33 mg/kg of Fe in site 7; and 60.4 ± 2.88 mg/kg of Zn in site 8) compared to the non-essential elements (maximum leaf concentrations of 0.80 ± 0.02 mg/kg of As in site 9, 0.20 ± 0.11 mg/kg of Cd in site 8; and 7.95 ± 0.06 mg/kg of Pb in site 9). A similar trend was observed in Besley and Birch (2021) who reported that essential element uptake into the nutritive roots, leaves and pneumatophores of *A. marina* were higher than non-essential elements across the Sydney estuary site. However, to the best of the authors' knowledge, the maximum Pb and Zn concentrations in the leaf (319 ± 24.7 mg/kg and 1033 ± 78.6 mg/kg respectively) recorded in site 1 in this study has not been previously recorded and reflects the high sediment contamination discussed earlier. High leaf Zn concentration is most likely the result of a synergistic response to elevated Pb and Zn in the sediment, which was first suggested in a six-month laboratory trial using *A. marina* propagules by MacFarlane and Burchett (2002). Notably, translocation of non-essential elements in fruit was lower than both leaves and pneumatophores (maximum concentration of 0.18 ± 0.05 mg/kg As in site 1; 0.05 ± 0.02 mg/kg Cd in site 3; and 5.23 ± 4.12 mg/kg Pb in site 3), showing that *A. marina* exerts an additional level of regulation for the transfer of non-essential element into the fruit.

The overall trend of elemental bioaccumulation in pneumatophores followed the trend of leaf bioaccumulation, however, the magnitude of the former was smaller. This result is in contrast to the finding of Besley and Birch (2021) who reported that elemental bioaccumulation followed the trend of roots > pneumatophores > leaves. However, lower elemental concentrations in pneumatophores compared to leaves can be explained by the high expression of aquaporins in pneumatophores, which allow

Table 2
Mean ± SEM concentrations of As, Cd, Cu, Fe, Pb and Zn in leaves, pneumatophores (aerial roots) and fruits (selected sites) in *A. marina* at Port Pirie, Upper Spencer Gulf, South Australia. n = number of replicates.

Site	Expected level of contamination		n	As (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
1st sampling October 2019									
1	Adjacent to smelter	Leaves	4	11.0 ± 0.81	2.20 ± 0.14	38.8 ± 2.59	548 ± 37.7	319 ± 24.7	1033 ± 78.6
		Pneumatophores	4	1.52 ± 0.07	0.30 ± 0.02	9.43 ± 0.81	205 ± 12.3	94.5 ± 4.78	168 ± 7.85
2		Leaves	4	9.93 ± 0.14	1.76 ± 0.02	27.0 ± 0.10	698 ± 13.0	317 ± 5.04	743 ± 9.79
		Pneumatophores	4	1.52 ± 0.04	0.35 ± 0.01	13.1 ± 0.39	104 ± 0.56	122 ± 1.96	233 ± 3.17
3		Leaves	4	4.80 ± 0.08	1.68 ± 0.01	31.8 ± 0.16	264 ± 2.63	109 ± 1.23	382 ± 6.48
		Pneumatophores	4	0.32 ± 0.04	0.16 ± 0.01	6.23 ± 0.64	46.9 ± 3.82	32.1 ± 2.76	57.1 ± 4.67
4		Leaves	4	1.40 ± 0.01	0.432 ± 0.004	12.8 ± 0.13	138 ± 2.93	32.9 ± 0.24	86.3 ± 0.93
		Pneumatophores	4	0.18 ± 0.02	0.06 ± 0.01	3.76 ± 0.63	18.6 ± 2.38	10.6 ± 1.27	35.8 ± 3.69
5		Leaves	4	4.66 ± 0.06	1.12 ± 0.03	24.4 ± 0.47	231 ± 5.98	108 ± 2.09	244 ± 3.80
		Pneumatophores	4	0.67 ± 0.11	0.30 ± 0.02	7.89 ± 0.65	72.9 ± 6.62	66.8 ± 4.97	86.2 ± 7.19
6	Not influenced by smelter	Leaves	4	0.60 ± 0.01	0.04 ± 0.001	11.6 ± 0.33	64.1 ± 0.88	0.35 ± 0.02	26.0 ± 0.78
		Pneumatophores	4	0.13 ± 0.01	0.04 ± 0.003	8.31 ± 0.31	19.3 ± 0.86	0.217 ± 0	15.9 ± 0.61
7	Within influence of smelter	Leaves	4	0.65 ± 0.02	0.13 ± 0.001	9.54 ± 0.54	154 ± 5.33	0.40 ± 0.01	48.2 ± 0.98
		Pneumatophores	4	0.36 ± 0.04	0.04 ± 0.005	7.49 ± 0.51	114 ± 6.08	0.27 ± 0.02	21.1 ± 1.35
8		Leaves	4	0.75 ± 0.03	0.20 ± 0.11	2.91 ± 0.31	119 ± 5.09	1.30 ± 0.04	60.4 ± 2.88
		Pneumatophores	4	0.09 ± 0.002	0.04 ± 0.003	1.33 ± 0.04	22.4 ± 0.51	0.34 ± 0.02	43.6 ± 1.68
9		Leaves	4	0.80 ± 0.02	0.16 ± 0.003	1.75 ± 0.04	141 ± 1.81	7.95 ± 0.06	56.5 ± 0.88
		Pneumatophores	4	0.32 ± 0.03	0.04 ± 0.006	3.26 ± 0.25	44.3 ± 3.64	3.40 ± 0.29	31.8 ± 3.52
2nd sampling January 2021									
Site 1	Adjacent to smelter	Leaves	3	7.15 ± 1.33	1.17 ± 0.20	14.9 ± 1.34	301 ± 9.41	153 ± 25.8	528 ± 88.6
		Fruits	3	0.18 ± 0.05	0.02 ± 0.001	10.2 ± 0.24	11.8 ± 0.68	0.70 ± 0.18	16.3 ± 0.49
Site 3		Leaves	3	4.80 ± 0.47	1.68 ± 0.37	17.8 ± 1.91	216 ± 12.8	81.2 ± 8.70	187 ± 12.2
		Fruits	3	0.17 ± 0.02	0.05 ± 0.02	17.5 ± 0.03	21.5 ± 2.79	5.23 ± 4.12	1.2 ± 0.38
Site 6	Not influenced by smelter	Leaves	3	0.86 ± 0.02	0.09 ± 0.004	7.24 ± 0.11	142 ± 6.53	1.05 ± 0.03	43.0 ± 7.68
		Fruits	3	0.07 ± 0.001	0.01 ± 0.001	8.52 ± 1.7	12.9 ± 0.89	0.71 ± 0.07	10.7 ± 1.26
Site 8	Within influence of smelter	Leaves	3	0.74 ± 0.05	0.83 ± 0.28	2.58 ± 0.33	153 ± 20.3	7.44 ± 2.05	38.0 ± 1.17
		Fruits	3	0.04 ± 0.002	0.04 ± 0.01	4.79 ± 0.71	12.5 ± 1.56	0.48 ± 0.05	10.8 ± 0.06

them to absorb water, while repelling excess salt (Nelson et al., 2022). It is possible that in addition to salt, aquaporins in pneumatophore also repel excess elements, keeping their overall concentration low in pneumatophores. In contrast, because leaves are adapted in *A. marina* to excrete excess salt via leaf salt glands, excess elements presumably translocate to the leaves in order to be excreted, making their concentration higher in the leaves.

The relationship between leaf and sediment elemental concentrations was further investigated using ANCOVA (α = 0.05) to test for an

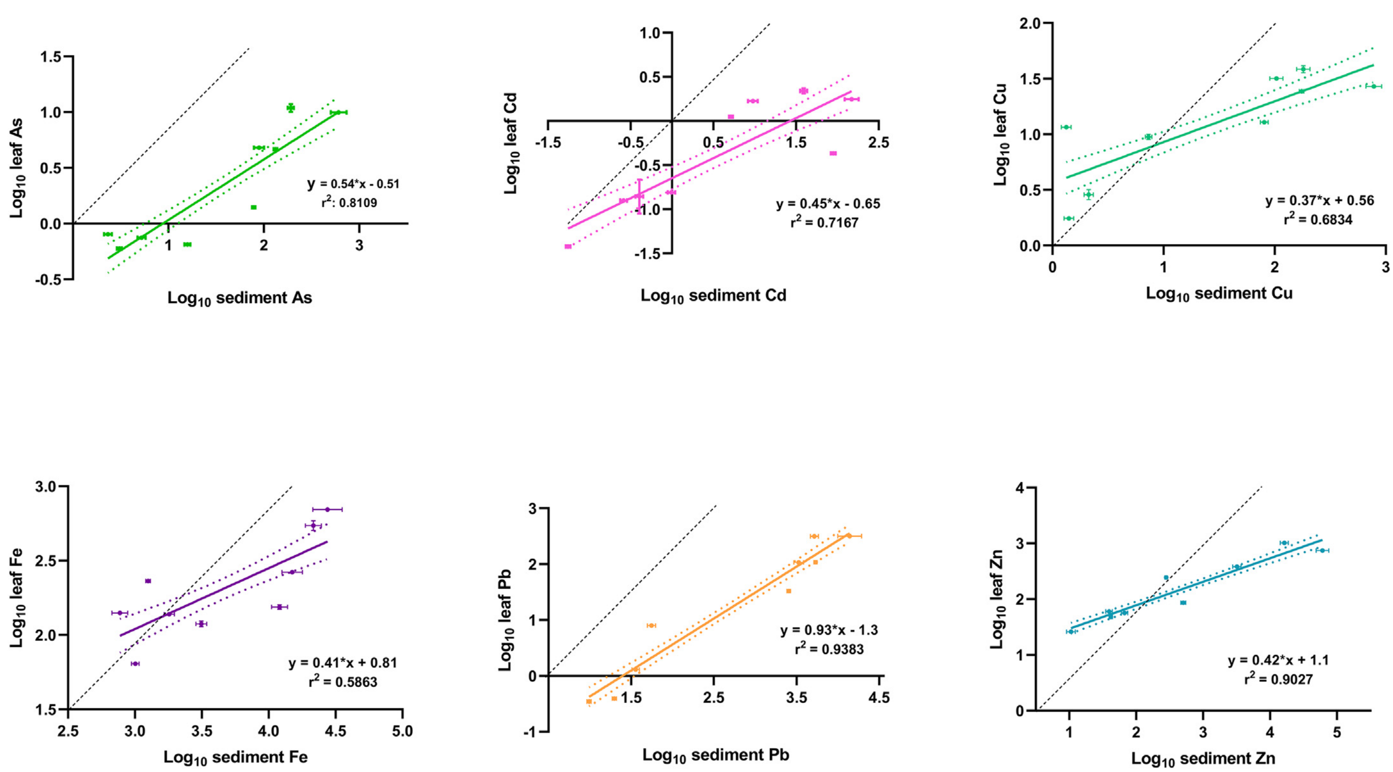

Fig. 3. Linear regression (±95 % confidence interval) of the relationship between $\log_{10}$sediment and $\log_{10}$*A. marina* leaf elemental concentrations at Port Pirie, Upper Spencer Gulf, South Australia using data from 9 sites collected during 1st sampling period. The dashed black line represents a 1:1 relationship for visual comparison.

interaction between the slope of the regression lines for As, Cd, Cu, Fe, Pb and Zn concentrations. A significant interaction ($p < 0.001$) was found (Table S4), indicating that a difference exists among the relationships between leaf and sediment elemental concentrations among these six elements. Therefore, separate regression analysis for each element was performed (Fig. 3). Table S5 summarizes the regression modeling, including slope, y and x intercept and r^2 values. While strong relationships were observed for As (r^2: 0.81), Cd (r^2: 0.72), Cu (r^2: 0.68), Pb (r^2: 0.94) and Zn (r^2: 0.90), the strength of the relationship for Fe may be considered moderate (r^2: 0.59). However, the results highlight that for Pb only, there may be a simple, consistent and isometric relationship between leaf and sediment concentration (slope 0.9296, 95 % confidence interval 0.8465–1.0130). The slope was observed to be within 0.3670–0.5436 for all other elements, indicating that when sediment elemental concentration increases, leaf elemental concentration increases, but the relative amount in leaves decreases as sediment concentration increases (anisometric relationship). To confirm the findings from above, the test for interaction between slope of the relationship between sediment concentration and leaf elemental uptake was re-analyzed without Pb. No significant interaction was found ($p = 0.777$, Table S7), corroborating that only the slope of Pb was significantly different among the six elements tested. This unexpected result suggest that mechanisms involved in regulating Pb uptake may be different to the other elements studied, warranting further investigation.

Post-hoc comparisons among elements (summary in Table S8) showed no detectable difference between As and Cd ($p = 0.22$). Additionally, no difference was observed between Fe and Cu ($p = 0.634$) and between Fe and Zn ($p = 0.226$), although the concentrations of these elements varied widely between sediment and leaf. Notably (excluding Pb), the two non-essential elements (i.e., As and Cd) were present at lower concentrations than the three essential elements at all sites, which also indicates different uptake and regulation mechanisms between essential and non-essential elements. However, pairwise comparison highlights that among the essential elements, there were significant differences (e.g., Cu vs Zn, $p < 0.001$). In addition to very high sediment concentration, elevated Zn in leaves at sites near the smelter may be attributed to the linear trend of Zn leaf bioaccumulation that was observed in the laboratory experiments with *A. marina* propagules in MacFarlane and Burchett (2002). Presence of Pb in sediment was also found to cause a synergistic increase of both Pb and Zn uptake into leaves in MacFarlane and Burchett (2002), which may also be attributed to the higher Zn concentration in the leaves compared to other essential elements such as Cu and Fe.

Overall, these regression relationships show that leaf elemental concentration may be predicted by sediment concentrations, however, the relationship may depend on whether the element is essential or non-essential, or is taken up in a linear fashion or tends to plateau at and above a specific sediment concentration. These results are also important in wider sense in terms of interpreting bioaccumulation factors or ratios, which are routinely reported in many similar studies. Our regressions (and estimates of the slope of the relationship) illustrate that care needs to be taken when interpreting the significance of "bioaccumulation factor/ratio" in other studies as any factor or ratio is likely to be contingent on sediment concentration.

3.3. Assessment of mangrove health

Chlorophyll a fluorescence analysis has been indicated to be a good biomarker for Zn stress, which may lead to inhibition of photosynthesis (MacFarlane, 2003). According to Zhen et al. (2021), leaf chlorophyll content measured by a handheld SPAD 502 m is good indicator of mangrove health and nutritional status and field collected leaf SPAD values yields reliable measurement of relative chlorophyll content. Therefore, a handheld SPAD 502 Chlorophyll meter was utilized in this study (in selected sites) to test whether high elemental load in sediments might affect the health of *A. marina*. No significant difference in leaf chlorophyll content was observed among the four sites during the 2nd sampling period ($p = 0.77$; average SPAD unit of 57.1 at site 6: 57.1, 50.6 at site 8, 51.6 at site 1 and 59.2 at site 3). The absence of any adverse health effect of chlorophyll content at these four sites with contrasting leaf elemental concentration disagrees with the finding of MacFarlane and Burchett (2002) that 100 % mortality can occur at >400 mg/kg Zn in the sediment. The results in this study show instead that mature trees under field conditions may be more tolerant to Zn toxicity than propagules. Shen et al. (2019) found that, Zn and Cu bioaccumulation in *Kandelia obovate* mangrove species may alleviate toxicity of elevated non-essential elements (e.g., Pb). Although similar research has not been conducted in *A. marina,* it is possible that high Zn and Cu bioaccumulation in sites closest to the smelter may play a protective role from the toxicity of non-essential elements in *A. marina* leaves. However, further research using chlorophyll *a*/b ratio and/or antioxidant activity (e.g., reactive oxygen species, peroxidase) may be needed to confirm the extent of potential toxic effects of non-essential elements such as As, Cd and Pb at these sites in the Upper Spencer Gulf (MacFarlane, 2002).

3.4. Labile elemental concentration using DGT

Labile elemental concentration [DGT(C_{DGT})] was measured to elucidate the fraction of freely available elements in the mangrove sediment, which was correlated to toxicity in other biota (e.g., bivalves and amphipods) (Amato et al., 2014, 2016). Vertical profiles of labile As, Cd, Cu, Fe, Pb and Zn C_{DGT} (μg/L) collected at the four sites during the 2nd sampling period are given in Fig. 4 and the corresponding leaf and fruit elemental concentrations are given in Table 2. Toxicant default guidelines values (80% and 95% level of species protection in marine water) for Cd, Cu, Pb and Zn are given as orange and red vertical lines according to ANZECC/ARMCANZ (2000). Because of an absence of a comparable value for As in ANZECC/ARMCANZ (2000), the Canadian Water Quality Guideline for the Protection of Aquatic Life (marine) for As (CCME, 1997) is given by the red vertical line. The results are discussed starting with Fe to provide an overview of redox cycles pertinent to sediment biogeochemistry, and then in the order of exceedance of these limits (i.e., Pb and Zn, As, Cd and Cu).

3.4.1. Fe

Despite having the highest total Fe concentrations in the sediments (Table 1), sites adjacent to smelter resulted in the lowest C_{DGT} values (geomean ± SD factor of 8.14 ± 5.83 μg/L in site 1, 3.52 ± 2.49 μg/L in site 3), compared to the sites further away (91.8 ± 2.08 μg/L in site 8 and 130 ± 2.78 μg/L at site 6). A combination of factors causes daily redox cycles in mangrove sediments, which may be attributed to the variable Fe C_{DGT}. Oxygen (O_2) consumption during microbial organic matter degradation at the sediment surface and low solubility in water during high tide results in anoxic conditions, promoting H_2S formation by sulphate reducing microbes (Araújo et al., 2018). At night, the absence of microbial photosynthetic activity leads to the diffusion of H_2S to the surface, causing reduction of free ions, Fe (and Mn) oxyhydroxides or weakly bound element-organic matter complexes, which precipitate as sulfides (Araújo et al., 2018). However, greater diffusion of O_2 during low tides, microbial/pneumatophore photosynthesis during daytime and bioturbation processes promote oxic conditions of the sediment. Under oxic conditions, a fraction of the sulfides may be re-oxidized by microbial activities, which re-precipitates as Fe (and Mn) oxyhydroxides (Araújo et al., 2018; Machado et al., 2014). Increase in sulfide oxidation rate has been reported in summer (Dang et al., 2015), which coincides with the DGT deployment time for this study. Thus, low labile Fe concentration at the sites adjacent to the smelter may indicate high redox cycling of Fe (i.e., presence of Fe oxyhydroxides during low tide, and FeS in high tide). However, it is noteworthy that despite having lower labile Fe, mangroves leaves during the 2nd sampling period contained marginally higher Fe in sites adjacent to smelter (301 ± 9.41 mg/kg in site 1 and 216 ± 12.8 mg/kg in site 3) when compared to sites further away (153 ± 20.3 mg/kg in site 8 and 142 ± 6.53 mg/kg in site 6), which shows that mangrove plants are highly efficient at taking up this essential element, even at low bioavailable concentrations. Fruit Fe concentrations did not differ according to distance or smelter influence among the 4 sites, ranging from 11.8 ± 0.68 mg/kg (site 1) to 21.5 ± 2.79 mg/kg (site 3).

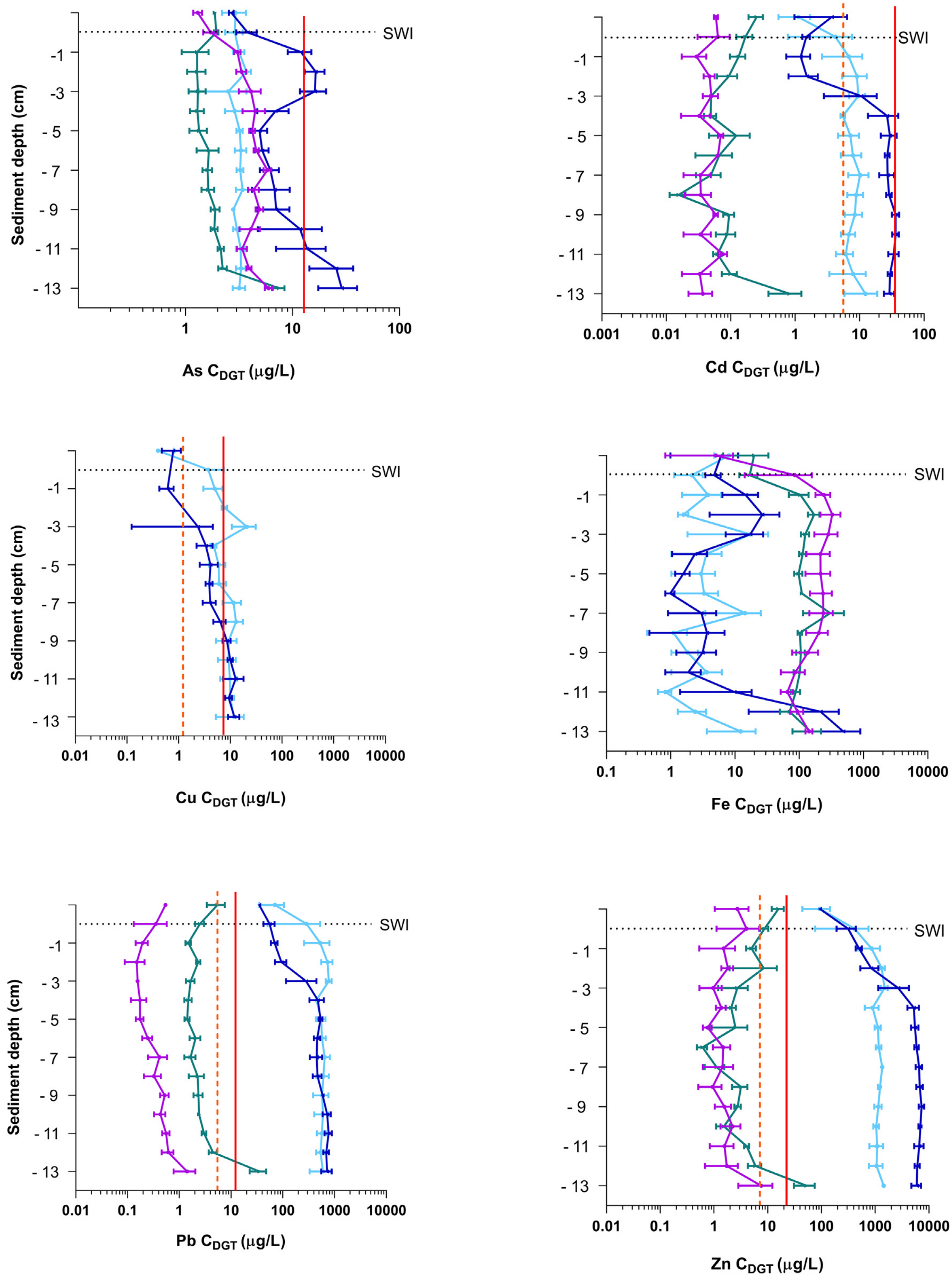

Fig. 4. Vertical profile of elemental concentrations measured using sediment DGT probe (C_{DGT}) in mangrove sediments at four sites at Port Pirie, Upper Spencer Gulf, South Australia. Concentration is expressed as mean ± SEM (μg/L). Sites adjacent to smelter include ■ = site 1, ■ = site 3. Sites within the influence of smelter is represented by ■ = site 8, while site not influenced by smelter is indicated as ■ = site 6. S/W-I refers to sediment-water interface and is given as dashed black line. Toxicant default guidelines values for 95 % and 80 % level of species protection in marine water are given as dashed orange and red vertical lines respectively for Cd, Cu, Pb and Zn according to ANZECC/ARMCANZ (2000). Water quality guidelines for As for marine water is given by the red vertical line according to Canadian Environmental Quality Guidelines (CCME, 1997).

3.4.2. Pb and Zn

Sites adjacent to smelter exceeded the 95% level of species protection of 12 μg/L of Pb and 21 μg/L of Zn according to ANZECC/ARMCANZ (2000) at all depths for sites 1 and 3, while it exceeded this level at a depth of −13 cm only. Geomean ± SD factor for Pb C_{DGT} at sites 1, 3 and 8 (at −13 cm) were 300 ± 2.88 μg/L, 488 ± 1.79 μg/L and 35.3 ± 12.3 μg/L respectively (25–41-fold higher in sites 1 and 3 than the 95% species protection level). The Zn C_{DGT} was 2,710 ± 4.04 μg/L (site 1) and

914 ± 2.01 μg/L (site 3), which are 43–129-fold higher than 95% species protection level, while that was 53.0 ± 21.9 μg/L (site 8 at −13 cm). Higher labile Pb and Zn explains the high bioaccumulation of these elements in *A. marina* leaves at the sites adjacent to smelter (Table 2, 2nd sampling). Leaf Pb concentrations at sites 1 and 3 were 153 ± 25.8 mg/kg and 81.2 ± 8.70 mg/kg respectively, while that of Zn was 528 ± 88.6 mg/kg and 187 ± 12.2 mg/kg respectively. While these values were considerably lower than those observed during the 1st sampling period, high bioaccumulation of Pb and Zn at sites adjacent to smelter shows repeatability across time. Although higher Pb C_{DGT} at site 8 resulted in higher leaf Pb bioaccumulation of 7.44 ± 2.05 mg/kg compared to that of site 6 (1.05 ± 0.03 mg/kg), higher Zn C_{DGT} at site 8 did not cause a difference in leaf Zn bioaccumulation between the two sites, indicating different regulatory mechanisms between essential and non-essential elements which was previously discussed (38.0 ± 1.17 mg/kg and 43.0 ± 7.68 mg/kg respectively). Of particular note, fruit Pb and Zn concentrations all sites were several times lower than that of the corresponding leaf values and did not differ significantly. Fruit Pb concentration ranged from 0.70 ± 0.18 (site 1) to 5.23 ± 4.12 mg/kg (site 3) and fruit Zn concentration ranged from 1.2 ± 0.38 mg/kg (site 2) to 16.3 ± 0.49 mg/kg (site 1). These results indicate that elemental translocation into fruits is regulated in *A. marina*; this mechanism should be further explored in future research.

Using sequential extraction technique, Clark et al. (1998) classified Zn as a Type I element, present in sediments principally as sulfides and complexed to organic matter, indicating low lability. Clark et al. (1998) also classified Pb as a Type II element, predominantly adsorbed onto minerals, clays and organic matter, indicating that they may be weakly bound and show higher lability. In contrast to Clark et al. (1998), results of this study suggests that Zn was present as more labile species in the mangrove sediment than Pb, which was also observed by the rapidly decreasing total Zn concentrations with increasing distance from the smelter (Table 1). It may therefore be possible that Pb is present in the mangrove sediments as mostly sulfide complexes, while Zn may be weakly bound to minerals, clays and organic matter. Nonetheless, both bioaccumulation and labile Pb and Zn concentrations suggest that elemental enrichment at sites adjacent to and within the influence of the smelter may pose considerable risk to the marine species.

3.4.3. As

Higher labile As species was observed at site 1 (geomean ± SD factor = 9.00 ± 1.99 μg/L) compared to the other three sites (site 3: 3.11 ± 1.09 μg/L; site 8: 1.81 ± 1.55 μg/L; site 6: 3.69 ± 1.51 μg/L). Site 1 was also distinct from other sites in terms of displaying a subsurface peak of 16.2 ± 4.43 μg As /L at −3 cm, followed by increase in C_{DGT} with depth to reach 28.8 ± 11.4 μg As/L at −13 cm, which exceeded the Canadian Water Quality Guidelines for the Protection of Aquatic Life (marine) of 12.5 μg/L (CCME, 1997). High labile As species in site 1 may have contributed to its higher leaf bioaccumulation (7.15 ± 1.33 mg/kg). The partitioning of As from solid phase into water may be dependent on As speciation (As^{III} being more mobile than As^{V}), ion displacement, microbial reduction of As^{V} to As^{III}, as well as reductive dissolution of Fe and Mn (oxy)hydroxides (Kumar et al., 2022). Lower than 12.5 μg/L As C_{DGT} values at sites 3, 6 and 8 indicate that As may not be harmful to its aquatic species. In contrast, the mangrove sediment at site 1 may act as a source of As to *A. marina* (and other biota). Similar to Pb and Zn, As concentrations in fruit were similar across the four sites [0.04 ± 0.002 mg/kg (site 6) to 0.18 ± 0.05 mg/kg (site 1)], indicating a regulatory control of this non-essential element during translocation into fruits.

3.4.4. Cd

Similar to Pb and Zn, labile Cd concentration was the highest at sites 1 and 3 (geomean ± SD factor = 13.3 ± 3.68 μg/L and 6.74 ± 1.75 μg/L respectively), exceeding the toxicant default guidelines values for 95% (5.5 μg/L) level of species protection according to ANZECC/ARMCANZ (2000). Lower Cd C_{DGT} was observed for sites further away from the smelter (0.05 ± 1.39 μg/L at site 6 and0.09 ± 2.43 μg/L at site 8). The higher labile C_{DGT} at sites 1 and 3 may be attributed to the higher leaf Cd concentration at these sites (1.17 ± 0.20 and 1.68 ± 0.37 mg/kg respectively), while lowest C_{DGT} at site 6 resulted in very low leaf bioaccumulation of 0.09 ± 0.004 mg/kg. Sediment Cd may be distributed between weakly adsorbed onto clay, organic matter, and mineral phases (e.g., Fe, Al) or strongly bound, non-labile Cd-S complexes (Clark et al., 1998; Gao et al., 2021). Therefore, the elevated Cd C_{DGT} in this study at sites adjacent to smelter may be a combination of oxidation of CdS in oxic conditions or desorption from weakly bound Fe (and Mn/Al) oxyhydroxides. Fruit Cd concentrations were very low across the four sites [0.01 ± 0.001 mg/kg (site 6) to 0.05 ± 0.02 mg/kg (site 3)], indicating restricted translocation mechanisms similar to As and Pb.

3.4.5. Cu

In contrast to all other elements analyzed in this study, Cu C_{DGT} at sites 6 and 8 was below the level of detection of 1 μg/L. However, C_{DGT} values exceeded the 80% and 95% species protection levels of 8 and 1.3 μg/L at lower than −5 cm at site 1 (4.34 ± 2.64 μg/L) and lower than −1 cm at site 3 (6.78 ± 2.46 μg/L). Similarly, low leaf Cu concentrations were observed at sites 6 and 8 (2.58 ± 0.33 mg/kg and 7.24 ± 0.11 mg/kg respectively) compared to that in sites 1 and 3 (14.9 ± 1.34 mg/kg and 17.8 ± 1.91 mg/kg respectively). Fruit Cu concentrations followed the leaf bioaccumulation pattern, indicating that Cu translocation into fruit may not be as tightly regulated as the non-essential As, Cd and Pb. According to Clark et al. (1998), Cu may exhibit a range of speciation in sediments, including Cu-S complexes and sorbed onto clay/TOC/mineral phases. In addition to the potential release of Cu into the water from solid phases during the redox cycling of mineral phases as previously discussed, Cu mobilization near the surface of sediment may also result due to microbial activities (i.e., organic matter oxidation) (Clark et al., 1998).

4. Conclusions

This study assessed total and labile elemental concentrations in mangrove associated sediments at Port Pirie, South Australia, correlating contamination level in *A. marina* with distance from the smelter and translocation into plant tissues. The results showed that the total Pb and Zn concentrations in sediments at sites adjacent to smelter exceed the ANZECC/ARMCANZ (2000) $SQGV_{high}$ values by 60 and 151-fold. Labile concentrations of Pb and Zn were also found to exceed the regulatory guidelines for the 95% protection of marine biota in the mangrove sediments adjacent to the smelter. Although leaf and pneumatophore elemental concentrations correlated with total sedimental concentrations, translocation of elements into fruit differed based on whether the element was essential or not. Despite not finding any adverse effects on mangrove health based on the chlorophyll content of the leaves, further research into the toxicity of these elements (e.g., antioxidant and lipid peroxidation assay) is needed to confirm these results in *A. marina* and other marine species that use this mangrove forest for food and shelter. Additionally, because *A. marina* roots may extend to a depth of 1 m, further research using deeper mangrove sediment layers may be necessary to fully ascertain the implications of elevated labile toxic elements to this ecosystem.

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CRediT authorship contribution statement

Farzana Kastury: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Georgia Cahill:** Investigation, Formal analysis, Visualization.

Ameesha Fernando: Investigation, Formal analysis, Visualization. **Adrienne Brotodewo:** Formal analysis, Visualization. **Jianyin Huang:** Methodology, Supervision, Formal analysis, Writing – review & editing. **Albert L. Juhasz:** Supervision, Writing – review & editing. **Hazel M. Vandeleur:** Conceptualization, Methodology, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Craig Styan:** Investigation, Formal analysis, Visualization, Funding acquisition, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflicting interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159503.

References

Amato, E.D., Simpson, S.L., Jarolimek, C.V., Jolley, D.F., 2014. Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. Environ. Sci. Technol. 48, 4485–4494.

Amato, E.D., Simpson, S.L., Remaili, T.M., Spadaro, D.A., Jarolimek, C.V., Jolley, D.F., 2016. Assessing the effects of bioturbation on metal bioavailability in contaminated sediments by diffusive gradients in thin films (DGT). Environ. Sci. Technol. 50 (6), 3055–3064.

ANZECC/ARMCANZ, 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.

Araújo, D.F., Machado, W., Weiss, D., Mulholland, D.S., Garnier, J., Souto-Oliveira, C.E., et al., 2018. Zinc isotopes as tracers of anthropogenic sources and biogeochemical processes in contaminated mangroves. Appl. Geochem. 95, 25–32.

Besley, C., Birch, G., 2021. Comparison of mangrove (Avicennia marina) metal tissue concentrations to ambient sediment with an extensive range of contaminant levels in a highly-modified estuary (Sydney estuary, Australia). Mar. Pollut. Bull. 171, 112680.

Boughriet, A., Proix, N., Billon, G., Recourt, P., Ouddane, B., 2007. Environmental impacts of heavy metal discharges from a smelter in Deûle-canal sediments (Northern France): concentration levels and chemical fractionation. Water Air Soil Pollut. 180, 83–95.

Caregnato, F.F., Koller, C.E., MacFarlane, G.R., Moreira, J.C., 2008. The glutathione antioxidant system as a biomarker suite for the assessment of heavy metal exposure and effect in the grey mangrove, Avicennia marina (Forsk.) Vierh. Mar. Pollut. Bull. 56, 1119–1127.

CCME, 1997. Water Quality Guidelines for the Protection of Aquatic Life, Marine.

Clark, M.W., McConchie, D., Lewis, D., Saenger, P., 1998. Redox stratification and heavy metal partitioning in Avicennia-dominated mangrove sediments: a geochemical model. Chem. Geol. 149, 147–171.

Dang, D.H., Lenoble, V., Durrieu, G., Omanović, D., Mullot, J.-U., Mounier, S., et al., 2015. Seasonal variations of coastal sedimentary trace metals cycling: insight on the effect of manganese and iron (oxy) hydroxides, sulphide and organic matter. Mar. Pollut. Bull. 92, 113–124.

Dittmann, S., Mosley, L., Stangoulis, J., Nguyen, V.L., Beaumont, K., Dang, T., et al., 2022. Effects of extreme salinity stress on a temperate mangrove ecosystem. Front. For. Glob. Chang. 96.

Edwards, J., Edyvane, K., Boxall, V., Hamann, M., Soole, K., 2001. Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. Mar. Pollut. Bull. 42, 389–396.

Ferreira, A.C., Borges, R., de Lacerda, L.D., 2022. Can sustainable development save mangroves? Sustainability 14, 1263.

Gao, L., Li, R., Liang, Z., Hou, L., Chen, J., 2021. Seasonal variations of cadmium (Cd) speciation and mobility in sediments from the Xizhi River basin, South China, based on passive sampling techniques and a thermodynamic chemical equilibrium model. Water Res. 207, 117751.

Jian, L., Jingchun, L., Chongling, Y., Daolin, D., Haoliang, L., 2019. The alleviation effect of iron on cadmium phytotoxicity in mangrove A. marina. Alleviation effect of iron on cadmium phytotoxicity in mangrove Avicennia marina (Forsk.) Vierh. Chemosphere 226, 413–420.

Kastury, F., Tang, W., Herde, C., Noerpel, M.R., Scheckel, K.G., Juhasz, A.L., 2021. Plumbojarosite formation in contaminated soil to mitigate childhood exposure to lead, arsenic and antimony. J. Hazard. Mater. 418, 126312.

Kumar, N., Noël, V., Besold, J., Planer-Friedrich, B., Boye, K., Fendorf, S., Brown Jr., G.E., 2022. Mechanism of arsenic partitioning during sulfidation of As-sorbed ferrihydrite nanoparticles. ACS Earth Space Chem. 6, 1666–1673.

Lin, Y., Fan, J., Yu, J., Jiang, S., Yan, C., Liu, J., 2018. Root activities and arsenic translocation of Avicennia marina (Forsk.) Vierh seedlings influenced by sulfur and iron amendments. Mar. Pollut. Bull. 135, 1174–1182.

MacFarlane, G., 2002. Leaf biochemical parameters in Avicennia marina (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. Mar. Pollut. Bull. 44, 244–256.

MacFarlane, G., 2003. Chlorophyll a fluorescence as a potential biomarker of zinc stress in the grey mangrove, Avicennia marina (Forsk.) Vierh. Bull. Environ. Contam. Toxicol. 70, 0090–0096.

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

MacFarlane, G., Burchett, M., 2002. Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove Avicennia marina (Forsk.) Vierh. Mar. Environ. Res. 54, 65–84.

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, 1454–1464.

Machado, W., Borrelli, N.L., Ferreira, T., Marques, A., Osterrieth, M., Guizan, C., 2014. Trace metal pyritization variability in response to mangrove soil aerobic and anaerobic oxidation processes. Mar. Pollut. Bull. 79, 365–370.

Macreadie, P.I., Costa, M.D., Atwood, T.B., Friess, D.A., Kelleway, J.J., Kennedy, H., et al., 2021. Blue carbon as a natural climate solution. Nat.Rev.EarthEnviron. 2, 826–839.

Marchand, C., Fernandez, J.-M., Moreton, B., 2016. Trace metal geochemistry in mangrove sediments and their transfer to mangrove plants (New Caledonia). Sci. Total Environ. 562, 216–227.

Natarajan, P., Murugesan, A.K., Govindan, G., Gopalakrishnan, A., Kumar, R., Duraisamy, P., Parani, M., 2021. A reference-grade genome identifies salt-tolerance genes from the salt-secreting mangrove species Avicennia marina. Commun. Biol. 2021 (4), 1–10.

National Pollutant Inventory, 2018. 2016/2017 report for NYRSTAR PORT PIRIE PTY LTD, Nyrstar Port Pirie. Australian Government, Department of the Environment and Energy, Port Pirie, SA. Available at http://www.npi.gov.au/npidata/action/load/individual-facility-detail/criteria/jurisdiction-facility/SA0018/state/SA/year/2017. (Accessed 19 September 2022).

Nelson, D.R., Chaiboonchoe, A., Hazzouri, K.M., Khraiwesh, B., Alzahmi, A., Jaiswal, A., Friis, G., Burt, J.A., Amiri, Khaled, M.A., Salehi-Ashtiani, K., 2022. Tissue-specific transcriptomes outline halophyte adaptive strategies in the gray mangrove (Avicennia marina). Agronomy 12, 2030.

Parida, B.R., Kumari, A., 2021. Mapping and modeling mangrove biophysical and biochemical parameters using Sentinel-2A satellite data in Bhitarkanika National Park, Odisha. Model. Earth Syst. Environ. 7, 2463–2474.

Shen, X., Li, R., Chai, M., Cheng, S., Niu, Z., Qiu, G.Y., 2019. Interactive effects of single, binary and trinary trace metals (lead, zinc and copper) on the physiological responses of kandelia obovata seedlings. Environ. Geochem. Health 41, 135–148.

Stockbridge, J., 2017. Quantifying Mangrove Condition in South Australia, And the Effect on Fish Assemblages. University of Adelaide, South Australia BSc (Honours).

Taylor, M.P., Isley, C.F., Glover, J., 2019. Prevalence of childhood lead poisoning and respiratory disease associated with lead smelter emissions. Environ. Int. 127, 340–352.

Tonhá, M.S., Garnier, J., Araújo, D.F., Cunha, B.C., Machado, W., Dantas, E., et al., 2020. Behavior of metallurgical zinc contamination in coastal environments: a survey of Zn from electroplating wastes and partitioning in sediments. Sci. Total Environ. 743, 140610.

USEPA, 1998. Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils.

USEPA, 2007. Method 6020A (SW-846): Inductively Coupled Plasma-mass Spectrometry, Revision 1.

USEPA, 2018. SW-846 Test Method 6010D: Inductively Coupled Plasma-optical Emission Spectrometry (ICP-OES).

Vandeleur, H., 2020. Port Pirie Phase 1 Project Measuring, modelling and managing legacies of marine pollution as new resources.

Vaz, R.A.N., Lennon, G.W., Bowers, D.G., 1990. Physical behaviour of a large, negative or inverse estuary. Cont. Shelf Res. 10, 277–304.

Ward, T., 1987. Temporal variation of metals in the seagrass Posidonia australis and its potential as a sentinel accumulator near a lead smelter. Mar. Biol. 95, 315–321.

Ward, T.J., Hutchings, P.A., 1996. Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. Mar. Ecol. Prog. Ser. 135, 123–135.

Ward, T., Young, P., 1981. Trace metal contamination of shallow marine sediments near a lead smelter, Spencer Gulf,South Australia. Mar. Freshw. Res. 32, 45–56.

Ward, T.J., Young, P., 1982. Effects of sediment trace metals and particle size on the community structure of epibenthic seagrass fauna near a lead smelter, South Australia. Marine Ecology Progress Series. 9. Oldendorf, pp. 137–146.

Ward, T., Young, P., 1983. The depauperation of epifauna on Pinna bicolor near a lead smelter, Spencer Gulf, South Australia. Environ. Pollut. Ser. A Ecol. Biol. 30, 293–308.

Ward, T., Warren, L., Tiller, K., 1984. Distribution and effects of metals in the marine environment near a lead-zinc smelter, South Australia.

Worthington, T.A., Andradi-Brown, D.A., Bhargava, R., Buelow, C., Bunting, P., Duncan, C., Spalding, M., 2020. Harnessing big data to support the conservation and rehabilitation of mangrove forests globally. OneEarth 2, 429–443.

Zhen, J., Jiang, X., Xu, Y., Miao, J., Zhao, D., Wang, J., Wang, J., Wu, G., 2021. Mapping leaf chlorophyll content of mangrove forests with Sentinel-2 images of four periods. Int. J. Appl. Earth Obs. Geoinf. 102, 102387.