

Article

Contributions of Wetland Plants on Metal Accumulation in Sediment

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Abstract: Wetlands, and especially salt marshes, are well-known sinks of metals, which limit toxic amounts of metals from entering the food chain. This study investigated metal concentrations (Cr, Cu, Fe, Mn, Ni, Pb, Zn) in a highly urbanised estuary, and compared vegetated rhizosediment (*Salicornia tegetaria*, *Spartina maritima*, and *Zostera capensis*) with bare sediment, in a depositional and non-depositional site, in the intertidal zone of the Swartkops Estuary. The samples were collected at two sites along the middle and lower reaches of the estuary and analysed using a Total X-ray Fluorescence (TXRF) spectrometer. It was found that the rhizosediment contained more metals and that metal concentrations in the sediment decreased as follows: *S. tegetaria* > *S. maritima* > *Z. capensis* > bare sediment. Although metal accumulation was similar in bare sediment for the depositional (Site B) and the non-depositional site (Site A), the rhizosediment displayed higher metal accumulation in the depositional site (Site B). However, regardless of site-specific depositional tendencies, rhizosediment displayed higher metal accumulation than bare sediment. These results indicate that vegetated sites and vegetated depositional sites should be the focus of monitoring metals in estuaries around the world.

Keywords: metal pollution; Swartkops Estuary; TXRF; salt marsh; seagrass; wetland plants



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1. Introduction

Estuarine wetlands are primary producers that receive high natural and anthropogenic metal loads, which makes them ideal candidates to monitor ecosystem health in an estuary. Estuaries are often developed and utilised for effluent release of urban and industrial wastewater, tourism, fishing, and bait collecting. The loss of ecosystem health can lead to the loss of ecosystem function, which makes these activities unsafe [1]. Wetlands play an important role in the biogeochemistry of metals in an estuary, by acting as a buffer and sink to metals, they can prevent toxic levels from entering the food chain [2,3]. The human population is disproportionally distributed to favour coastal areas, which further puts pressure on estuarine habitats, through the release of effluent [4]. It was wrongly assumed that the toxic effects of pollutants in effluent would be nullified by the dilution of estuarine and marine water [5]. Instead, the estuarine habitats acted as filters of metal contaminants, capturing metals into their tissues and in the rhizosediment [6]. Metal pollutants are generally accumulated into the sediment close to the source, but the hydrodynamics of the estuarine environment can redistribute the metal-rich particles throughout the estuary [7]. The natural hydrodynamics of an estuary can be changed by man-made structures, creating areas that trap metal-enriched particles flowing downstream.

The extent of metal accumulation in estuarine sediment will depend on the physico-chemical characteristics of the sediment, as well as the biological elements in sediment. Estuarine salt marshes are periodically flooded due to river currents and tidal action. They retain large quantities of suspended particles (natural and anthropogenic of origin), due to the elevated topography and the vegetation structure trapping particles. The salt marshes act as a trap for many contaminants and are important sinks for metal pollutants [2,8]. Once the metals are accumulated and distributed the sediment and the plants of these habitats capture the metals by assimilating them in their biomass [9]. However, salt marsh plants often accumulate metals in their roots, keeping toxic concentrations of metals away from other parts of the ecosystem [6,9,10]. Toxic concentrations of the metals are accumulated away from the sensitive photosynthetic apparatus, allowing the plant to tolerate high nutrient loads, and promoting cycling of metals between the roots and sediment [11]. Strategies for metal tolerance are not uncommon in halophytes, because of the susceptibility of the area to high loads of nutrients [3,12]. Therefore, wetland plants (and habitats) are often used for biomonitoring, to indicate whether an environment has reached toxic levels.

Metal accumulation in sediment is dependent on the chemical and physical attributes of the sediment environment [13]. Vegetation can change the sediment environment in between their roots (i.e., in the rhizosediment) in various ways. Different inundation periods driven by higher surface elevation, coupled with the reduced wave energy from vegetated structures, promotes the deposition of enriched particles during tidal fluctuations [14]. Therefore, vegetation can increase the metal accumulation capacity of sediment by promoting smaller sediment grain sizes and producing and promoting the decomposition of organic matter. Radial Oxygen Loss (ROL) is an adaptation of macrophytes to saturated (waterlogged) conditions and results in the oxygenation of the rhizosphere, which changes the redox environment of the sediment [15–17]. The sediment environment plays a major role in forming either mobile (bioavailable) or immobile metal species [18–20]—grain size, pH, redox organic content, salinity, and Fe and Mn oxides can all influence the metal accumulation capacity of sediment [21]. Metals accumulated into the bottom sediment is in flux with the overlying water column, which indicates that a change in conditions can trigger the dissolution of metals into the water column [22].

The aim of this study was to investigate metal concentrations between dominant salt marsh (*Spartina maritima* and *Salicornia tegetaria*) and seagrass (*Zostera capensis*) rhizosediment, and bare sediment, at the same sites in the Swartkops Estuary. Metal concentrations in rhizosediment and bare sediment within a depositional and non-depositional site, in the middle and lower reaches of the estuary, were compared. The unvegetated (bare) mudflats were near the vegetated areas and would receive similar metal load and have similar hydrodynamic influences. Assessing whether metal concentrations in bare sediment are comparable to concentrations in rhizosediment, assists in promoting vulnerable salt marsh and seagrass habitats for conservation. This study theorises from past research in other estuaries that metal accumulation will be significantly higher in the rhizosediment compared to the bare sediment. The results will remind future researchers to approach metal studies in vegetated areas differently from bare areas.

2. Materials and Methods

2.1. Study Site

Sampling occurred at two different sites in the Swartkops Estuary, a predominantly open estuary located on the warm-temperate south-east coast of South Africa (Figure 1) [23]. The sites were located in the intertidal area of the middle and lower reaches of the estuary. Site A (Markman Canal) was located close to an industrial point source, while Site B (Tippers' Creek) was in a highly urbanised creek (Figure 1). The freshwater inflow into the estuary is restricted by damming and tributaries that reduces freshwater inflow by 5% [24]. It is well mixed and exhibits highly stratified waters only during a high flood event. The salt marshes cover an area of approximately 547.4 ha and are dominated by *S. maritima* in the lower reaches [23]. *Salicornia* sp. are found higher up the tidal gradient of

the saltmarsh, while seagrass beds consisting of *Z. capensis* are found along the subtidal-intertidal fringe [25]. Swartkops Estuary contains some of the largest *Z. capensis* beds [23]. These habitats are essential for nutrient cycling, fish nurseries, and aquatic habitat. The middle and lower reaches of the estuary is surrounded by rural, urban, and industrial zones, which contribute to effluent released into the estuary. The major industrial activities in the vicinity include saltpans, sewage works, sand/clay mining, brickworks, a power station, motor industry, wool industry, tanneries, extraction/beneficiation processes, aquaculture, railway yards, depots, and some agriculture [24,26]. The estuary is therefore subjected to high anthropogenic pressure, with the potential to introduce metal contaminants.

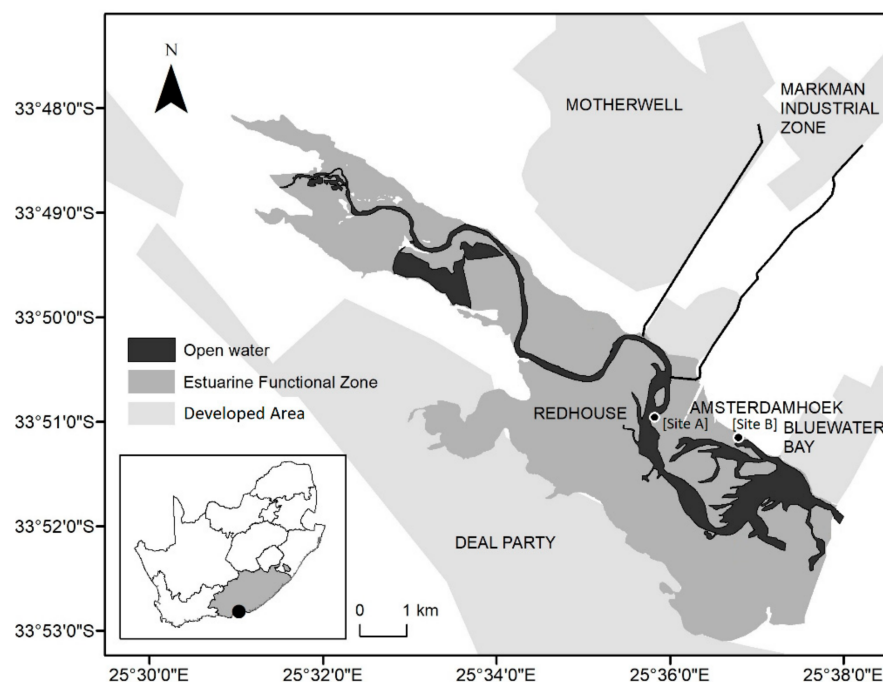


Figure 1. Locations of the sampling sites: (Site A) Markman Canal and (Site B) Tipper's Creek.

2.2. Sampling

Once-off sampling was carried out in winter 2019, during spring low tides. Plants and their accompanying sediment samples were collected at each site in replicates of five. Sediment samples were collected along the shore of the estuary within homogenous patches of *Z. capensis*, *S. maritima*, and *S. tegetaria*, and adjacent unvegetated mudflats. The plants were randomly selected along a vertical line so that the three species were within an intertidal range from each other. Sediment from the surface (0–10 cm) of the rhizosphere was collected and contained within 50 mL plastic jars with a screw cap. A PVC corer (radius = 5.6 cm) was used to remove whole plant biomass from the selected plants, being careful to keep the roots intact (depth = ca. 20 cm). The jars were then contained within sealable ziplock packets. The sediment was transported in a cooled, thermally insulated cooler box and stored at <5 °C until further analysis, according to the specifications in Fourqurean et al. [27]. Once in the laboratory, sediment samples were homogenised. The subsamples were then taken for the analysis of sediment characteristics and for metal extraction. Both subsamples were freeze-dried (Vacutec, V-FD12 Series Freeze Dryer, South Africa) until a constant weight was achieved. All containers and equipment used in handling the samples were acid stripped with 1% HNO₃, rinsed with de-ionised water, and dried in their preparation.

2.3. Analysis of Sediment Characteristics

Grain (particle) size was determined with the freeze-dried sediment samples, according to the Wentworth scale [28,29]. It requires shaking approximately 50 g of dry sample for 5 min, through a graded series of sieves (Clear Edge Test Sieves, SABS ISO 3310 specifications, France) with different mesh sizes, to determine the percentage sand and silt/clay in each sample. Each fraction in a sieve was brushed into the smaller graded sieve to ensure that all smaller particles were correctly placed, and then individually weighed (g).

The Total Organic Matter (TOM) content was determined with the loss-on-ignition method [30,31]. A known mass of approximately 10 g of wet sediment was transferred into porcelain crucibles and dried in an oven at 100 °C for 24 h, or until constant weight (W1). The crucibles were then transferred into an ashing oven for 6 h at 550 °C, or until constant weight (W2). Samples were cooled to room temperature before weighing. The percentage of organic matter content was calculated using the following equation (Equation (1)):

$$\% \text{ TOM} = \frac{[W1 - W2]}{W1} \times 100 \quad (1)$$

Salinity as sediment conductivity was measured using the method outlined in Bernard [32]. Air-dried sediment was saturated with bi-distilled water, shaken overnight, and filtered through a Whatman No.1 filter paper in a Buchner funnel, at a ratio of approximately 50 g:25 mL. The electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) was measured using a YSI, handheld salinity-conductivity-temperature metre (Model 30, USA), after a period of equilibration.

2.4. Analysis of Metal Concentrations through TXRF Spectrometry

The single extraction entailed a conventional overnight wet digestion method [33,34], to extract the total metal concentration. The sediment samples and dissected plant samples were digested for the measurement of Cr, Cu, Fe, Mn, Ni, Pb, and Zn. An aliquot of freeze-dried sediment first was pushed through a 1 mm sieve to remove any unwanted material. The homogenous plant and sediment samples (1.0000 ± 0.001 g) were left in 10 mL HNO_3 (A.R grade, 65%, p.a.) overnight, and then refluxed on a hotplate for ~5 h at 110 °C, using a watch glass, until the production of NO_2 fumes had ceased. After evaporation to near dryness, the sample was diluted with 20 mL of 2% (v/v H_2O) nitric acid and transferred to 50 mL volumetric flasks after filtering (Whatman™ GF/F Filters) to remove particulates and diluted to the mark with ultra-pure water (Milli-Q®, Direct 8 Water Purification System, Germany).

Extractants were analysed for the selected metals using a Total Reflection X-ray Fluorescence Spectrometer (TXRF, Bruker, S4 T-star, Germany). The TXRF is a reliable method of analysis, which is very efficient at using small amounts of samples [35]. The spectrometer is equipped with a molybdenum (Mo) and tungsten-brems (W) X-ray tube. A 10 μL mixture of the following was pipetted in the middle of the circular sample carriers (polished quartz glass): (1) 7000 μL of the digested sample, (2) 70 μL of the 100 ppm internal standard(s) i.e., 1 $\text{mg}\cdot\text{L}^{-1}$, and 70 μL of poly(vinyl) alcohol (for sample homogeneity). Regarding internal standards, Ga and Y were used for plant samples and Se was used for sediment samples. The internal standard method also corrects for variations in sample deposition on the reflective sample carriers [36]. The stocked sample carrier was then transferred to a hotplate and maintained at low heat (ca. 55 °C) until the sample was dry. The TXRF analyses the surface to detect the metal concentrations using X-ray fluorescence. The metals were identified based on their fluorescence $K\alpha$ lines (peaks). The instrument was calibrated according to manufacture indications before each analysis, which entails alignment, and calibration of concentrations using a preset standard. Scan time was defined at 1000 s. Blank runs were performed alongside the samples, which consisted of the HNO_3 acid and the internal standard. The percentage of recovery of metals was calculated by comparing the detected concentrations of certified reference materials (Sigma-Aldrich, Metals in Sewage Sludge, SQC001S-30 g). The detection limits (mg/L) for the selected metals ($\text{Cr} = 0.005$,

Cu = 0.001, Fe = 0.004, Mn = 0.003, Ni = 0.001, Pb = 0.001, Zn = 0.001) were provided by the spectrometer. It is calculated with the area of the target elemental peak and the area of the adjacent (background) peaks (Equation (2)) [37].

$$LLDi = \frac{3 \cdot Ci \cdot \sqrt{N_{BG}}}{N_i} \quad (2)$$

where LLDi = the lowest limit of detection of the element “i” (PPM), Ci = concentration of element “i” in the measured sample (PPM), N_{BG} = background area subjacent to the element “i” K α lines, and N_i = peak area of the element “i” K α lines.

2.5. Statistical Analysis

The analyses of variance (ANOVA, random effects) were used to test for differences in all the data. Tukey’s post hoc test was then used to compare the means where significant differences were found. Differences were significant when the p -value was less than 0.05. The assumption of normality was verified before analysis with the Shapiro–Wilks test, accompanied with the respective histograms, to test for skewness. Variables were log-transformed if the null hypothesis for normality was rejected. Sediment characteristics (i.e., grain size, salinity, TOM) were graphically displayed, and standard error bars were displayed on the graphs. Non-Metric Multidimensional Scaling (NMDS) was performed on the metal concentrations and the environmental factors (i.e., the sediment characteristics) were overlaid as vectors, to show the degree of influence of the environmental characteristics on the metals and the sites. All statistical processing and graphical display were performed in RStudio (3.5.1/4.0.2; RProject) using the packages Vegan, RColorBrewer, Tidyverse, R-commander, mvabund and Geoveg, and Microsoft Excel (365).

3. Results

3.1. Sediment Characteristics

The bare sediment had the most sand and least amount of silt/clay, especially in Site B (Figure 2). The grain size and composition of the different rhizosediment for Site A were very similar, only showing a large difference in composition for *S. tegetaria*, where there was less sand ($p = 0.0016$) and more silt/clay ($p < 0.0001$). The differences between the rhizosediment and bare sediment were more noticeable in Site B (bare = 5.57%, *Z. capensis* = 17.02%, *S. maritima* = 38.32%, *S. tegetaria* = 27.99%), the depositional site (silt/clay; $p < 0.001$, in all cases), than in Site A (bare = 3.80%, *Z. capensis* = 5.66%, *S. maritima* = 6.88%, *S. tegetaria* = 16.02%). Therefore, Site B was generally less sandy than Site A. The bare sediment had similar grain size compositions between the sites (sand; $p = 0.9991$, silt/clay; $p = 0.1449$), but rhizosediments had significant differences (silt/clay; $p < 0.01$, in all cases).

In both sites, TOM increased in the intertidal zone (*Z. capensis* < *S. maritima* < *S. tegetaria*), and bare sediment had the lowest TOM (Figure 3; Site A = 1.46%, Site B = 1.59%). Bare sediment had statistically similar TOM between sites ($p = 0.9926$). Once again, the differences between the rhizosediment and bare sediment were more noticeable in Site B (*Z. capensis* = 5.43%, *S. maritima* = 6.46%, *S. tegetaria* = 12.56%), the depositional site ($p < 0.001$, in all cases). At Site A, however, there was no difference in TOM between *Z. capensis* and the bare sediment in Site A ($p < 0.2173$).

Salinity did not increase with intertidal range, as would be expected, but the highest salinity is instead found for *S. maritima* (Figure 4) though not much statistical difference could be shown between the bare sediment and rhizosediment in Site A ($p > 0.06$, in all cases). Bare sediment, however, showed lower salinity in both sites (Site A = 14.4 μ S, Site B = 14.5), even though bare sediment was collected above the *S. maritima* (highest intertidal) in the case of Site A. At Site B the salinity of the bare sediment was not significantly different to the rhizosediment of *S. tegetaria* ($p = 0.1541$). The bare sediment and the rhizosediment all had statistically similar salinity between the sites (bare sediment; $p = 1.000$ and rhizosediment; $p > 0.5$, respectively).

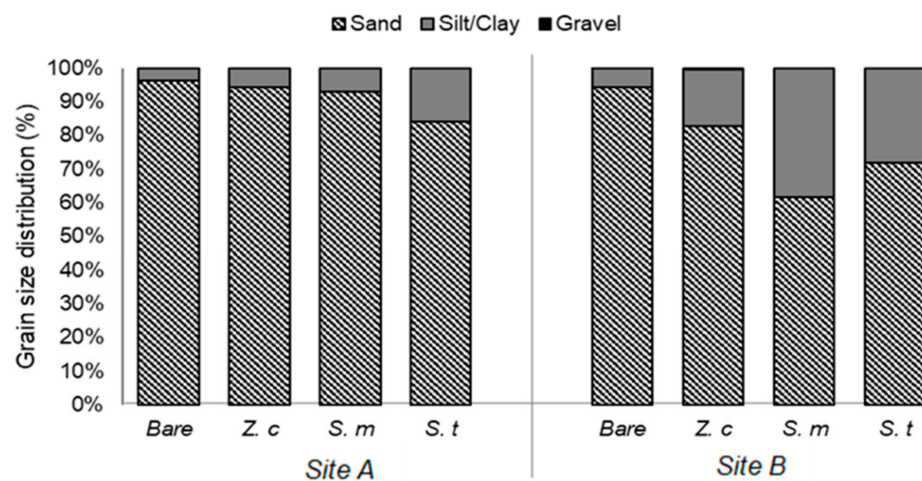


Figure 2. Grain size distribution in rhizosediment (vegetated) and bare sediment (unvegetated) of Site A and Site B; where Z.c = *Z. capensis*, S.m = *S. maritima*, and S.t = *S. tegetaria*.

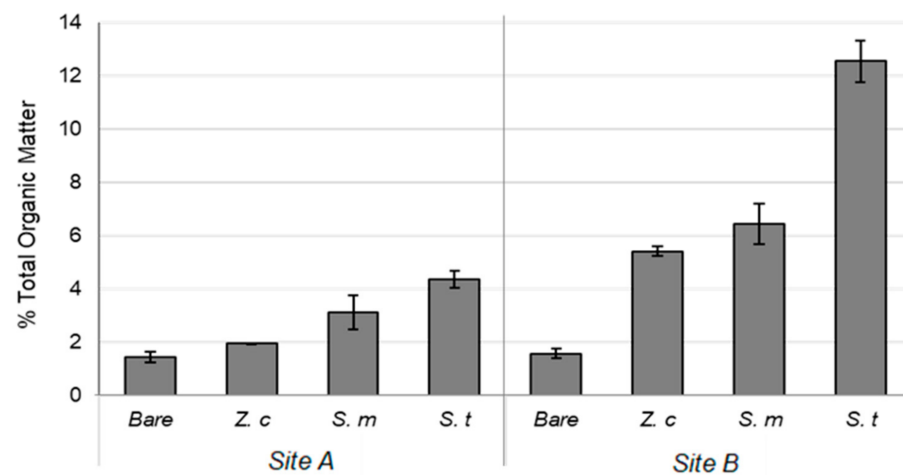


Figure 3. Total Organic Matter (TOM) (%) in the rhizosediment and bare mudflats of Site A and Site B; where Z.c = *Z. capensis*, S.m = *S. maritima*, and S.t = *S. tegetaria* (mean $\pm$ SD, N = 3).

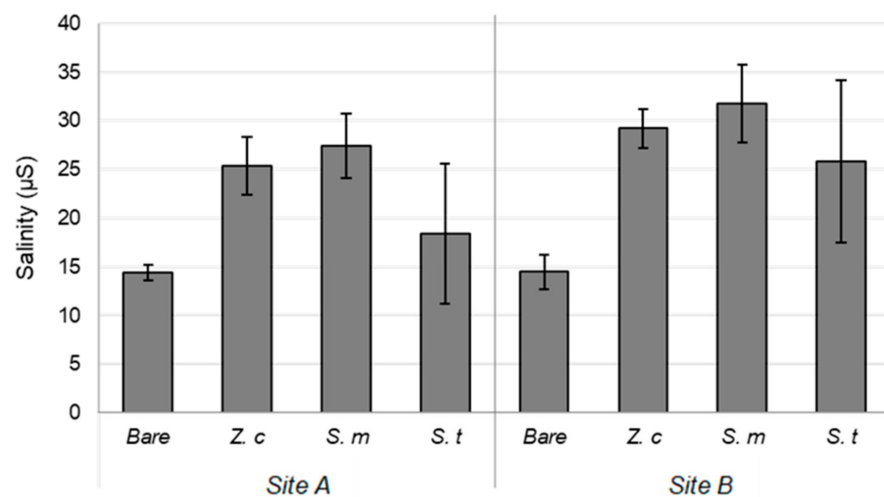


Figure 4. Salinity (μ S) in the rhizosediment and bare mudflats of Site A and Site B; where Z.c = *Z. capensis*, S.m = *S. maritima*, and S.t = *S. tegetaria* (mean $\pm$ SD, N = 3).

3.2. Total Metal Concentrations

Metal concentrations differed between vegetation type, metals, and sites. The different metals had different concentrations (Table 1). Notably, where statistically significant differences occur between concentrations in bare sediment and the other rhizosediment, it was always higher in the rhizosediment than the bare sediment. Differences were more notable in Site B compared to Site A, where only Ni was statistically similar to the bare sediment for *Z. capensis*. However, the margin is very slight as $p = 0.0742$. Regarding Site A, *Z. capensis* rhizosediments was the most similar to the bare sediment, statistically, with the exception of Ni and Cu, which was not the case for Site B ($p = 0.0049$). The notable differences were between Fe and Mn for *S. maritima* and *S. tegetaria*, and Cr for *S. maritima*. Comparably, the metal concentrations in Site A were less than Site B, for the rhizosediment sites. However, there were a couple of exceptions for Ni. *Spartina maritima* also showed statistical similarities for Mn and Zn between the sites, even though the means for Site A was lower than Site B—the similarities could be attributed to an overlap in the range resulting in a high standard deviation ($p = 0.0756$ and $p = 0.1794$, respectively). Metal concentrations between sites for bare sediment were statistically similar, again, with the only exception being Ni ($p = 0.0022$).

Table 1. Average metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight $\pm$ SD) in the surface of vegetated (rhizosediment) and unvegetated (bare) sediment. An asterisk (*) indicates significant differences between the bare mudflat with the different rhizosediments, of the same site and metal ($p < 0.05$; $N = 3$). An arrow indicates if the values are significantly higher ($\uparrow$) or lower ($\downarrow$).

Sites	Metals ($\mu\text{g}\cdot\text{g}^{-1}$)	Unvegetated Bare Mudflat	<i>Z. capensis</i>	Rhizosediment <i>S. maritima</i>	<i>S. tegetaria</i>
Site A	Fe	3468 $\pm$ 385	3152 $\pm$ 171	$\uparrow$ 5407 $\pm$ 985 *	$\uparrow$ 6923 $\pm$ 1222 *
	Mn	31.5 $\pm$ 4.1	30.4 $\pm$ 2.5	$\uparrow$ 63.7 $\pm$ 14.2 *	$\uparrow$ 112.9 $\pm$ 28.6 *
	Zn	48.8 $\pm$ 12.7	38.1 $\pm$ 0.8	57.4 $\pm$ 3.8	53.7 $\pm$ 1.8
	Cr	8.5 $\pm$ 1.1	10.6 $\pm$ 0.5	$\uparrow$ 19.0 $\pm$ 4.0 *	11.6 $\pm$ 1.7
	Pb	3.9 $\pm$ 0.6	2.8 $\pm$ 1.0	5.2 $\pm$ 1.3	7.1 $\pm$ 1.9
	Ni	0.9 $\pm$ 0.5	$\uparrow$ 4.1 $\pm$ 1.7 *	1.2 $\pm$ 0.4	1.2 $\pm$ 0.3
	Cu	2.9 $\pm$ 0.7	$\uparrow$ 1.4 $\pm$ 0.2 *	2.9 $\pm$ 0.7	3.0 $\pm$ 0.4
Site B	Fe	5120 $\pm$ 223	$\uparrow$ 14073 $\pm$ 341 *	$\uparrow$ 19445 $\pm$ 2 850 *	$\uparrow$ 28947 $\pm$ 858 *
	Mn	29.3 $\pm$ 1.8	$\uparrow$ 76.8 $\pm$ 10.4 *	$\uparrow$ 108.6 $\pm$ 21.2 *	$\uparrow$ 367.2 $\pm$ 34.4 *
	Zn	51.2 $\pm$ 2.6	$\uparrow$ 96.7 $\pm$ 16.0 *	$\uparrow$ 81.0 $\pm$ 10.3 *	$\uparrow$ 128.2 $\pm$ 9.0 *
	Cr	10.3 $\pm$ 0.7	$\uparrow$ 36.8 $\pm$ 2.3 *	$\uparrow$ 37.5 $\pm$ 4.1 *	$\uparrow$ 72.1 $\pm$ 1.5 *
	Pb	7.1 $\pm$ 0.9	$\uparrow$ 29.8 $\pm$ 5.5 *	$\uparrow$ 30.3 $\pm$ 5.0 *	$\uparrow$ 59.0 $\pm$ 1.5 *
	Ni	3.5 $\pm$ 0.1	9.6 $\pm$ 0.7	$\uparrow$ 11.2 $\pm$ 1.8 *	$\uparrow$ 21.2 $\pm$ 0.7 *
	Cu	3.5 $\pm$ 0.1	$\uparrow$ 11.6 $\pm$ 0.6 *	$\uparrow$ 8.7 $\pm$ 1.2 *	$\uparrow$ 13.9 $\pm$ 0.8 *

3.3. nMDS Evaluation of Bare versus Vegetated Rhizosediment

The multivariate analysis showed patterns akin to the sediment characteristics, specifically grain size and salinity for the bare sediment, and rhizosediment of *S. maritima* and *Z. capensis*, while TOM was only closely related to *S. tegetaria* in Site B (Figure 5a,b). In Site A it was more difficult to determine, as the sediment characteristics were not as statistically distinct as in Site B. Larger differences between the communities were shown for Site B (Figure 5b) compared to Site A (Figure 5), but each site had well-defined communities. Overlaps occurred for *Z. capensis* and *S. maritima* in Site A and, *S. tegetaria* and *Z. capensis* in Site B.

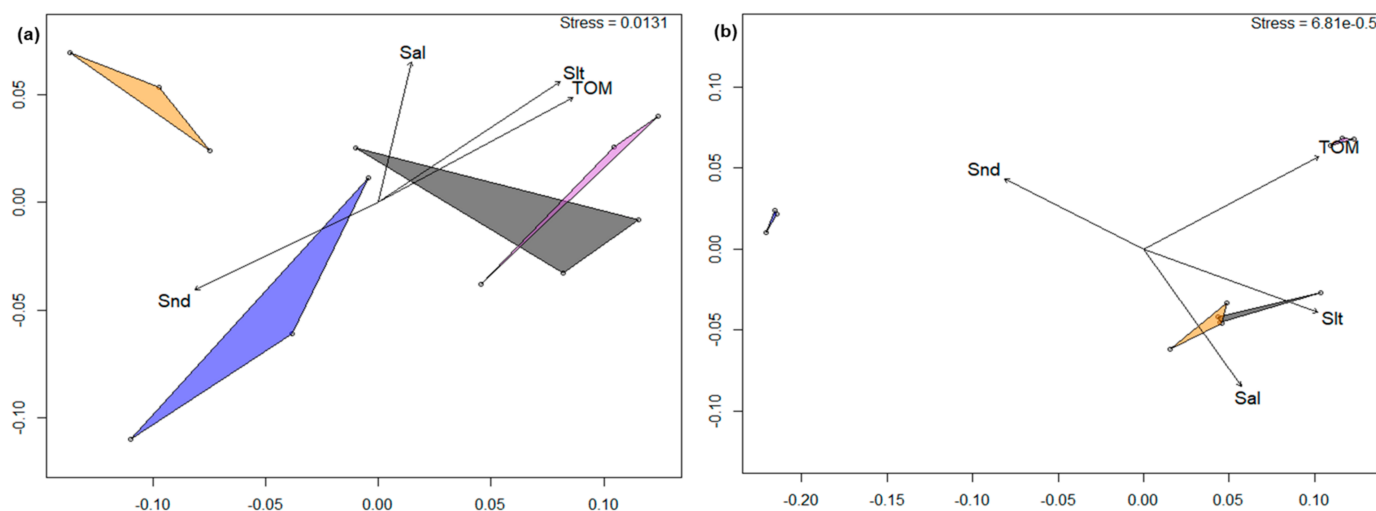


Figure 5. Non-metric multidimensional scaling (nMDS) ordination plots based on Euclidean distances of the vegetation types for bare (■), *Z. capensis* (■), *S. maritima* (■), and *S. tegetaria* (■), at (a) Site A and (b) Site B.

4. Discussion

4.1. Sediment Characteristics

There were distinct differences between the sediment properties of the two sites (Site A and Site B) in the estuary, as well as between the rhizosediment of the vegetation types. Sand was dominant in both sites, but Site A had remarkably more sand than Site B. The finer silt/clay particles were therefore higher in Site B. However, bare sediment showed similar grain size composition between sites. The bare sediment, *Z. capensis* rhizosediment, and *S. maritima* rhizosediment in Site A also showed remarkably similar grain size compositions, while Site B's sediment was more distinct between vegetation types. Nevertheless, bare sediment showed the lowest silt/clay content and the highest sand content in Site B. A large sandbank, most likely succeeding the construction of Wylde Bridge, was largely fluvially driven and deposited by the ebb-directed tidal flow from the sea [38,39]. The natural and man-made geography in this section (around Site B) of the estuary resulted in the slackening of the downstream flow [38], contributing to the build-up of finer-grained sediment in Site B, and a steeper incline between the subtidal and upper intertidal vegetation. The intertidal macrophytes trap more sediment as the flood tide recedes, increasing the presence of fine-grained sediment, particularly for *S. maritima* and *S. tegetaria*, higher up the intertidal zone [40]. The silt/clay fraction in this area is comparatively high, even though there is little input of fine-grained sediment into the estuary [41]. Site B is therefore considered a depositional site, which traps fine particles flowing downstream.

Total Organic Matter (TOM) increased with the intertidal zonation, that is, bare sediment had the lowest content and *S. tegetaria* had the highest content. Similarly to the silt/clay content, TOM was higher in Site B than in Site A, due to the same trapping mechanics of particles at the large sandbank. Site B (Tippers Creek) is a well-known collection site, trapping particles from upstream, and so has been recommended for monitoring [42]. Els reported %TOM for winter, one year prior to this study, and found notably lower TOM for all three of the plant species for the same sites [43]. The exception was *S. tegetaria* in Site B, which instead increased by a factor of approximately 1.5. Watling and Watling and Nel et al. both concluded that Tippers' Creek is incompletely flushed by tidal action, resulting in the build-up of contaminants over time [44,45]. Organic particles deposited by the flood tide may become trapped in the higher intertidal zone colonised by *S. tegetaria*, due to higher surface elevation on the sandbank [46].

Sediment salinity was very similar in the upstream Site A and the downstream Site B. Site B had a larger intertidal range; thus, the vegetation types were further apart. Rhizosediment

ment of *S. tegetaria* in particular had more variability in salinity measurements, which could indicate some extended evaporation periods in some areas in the upper intertidal zone. Higher salinity in the lower intertidal—subtidal zones will be washed more frequently by saline water from the flood tide at lower elevations, while higher intertidal—supratidal species are inundated less frequently and for shorter periods of time [46]. Bornman et al. did not find that sediment salinity was a key driver in their study on sea-level rise and intertidal salt marshes in the Swartkops Estuary since recorded values were very low [40]. However, they expected water column salinity to be lower compared to the natural state, as increasing freshwater input (i.e., stormwater run-off, treated sewerage water) enters into the catchment. This study's values correspond to the range of salinity found in Figure 4, which indicates that salinity is unlikely to be a driver of metal accumulation.

4.2. Metal Concentration

In this study, the vegetated rhizosediment contained more metals than the bare sediment. That is, metal concentrations had the following general trend: bare < *Z. capensis* rhizosediment < *S. maritima* rhizosediment < *S. tegetaria* rhizosediment. This trend has been found in other studies around the world (e.g., Pedro et al. [5]). However, this study also includes a comparison between a depositional and non-depositional site. Studies suggest that wetland plants predominantly accumulate metals within their rhizosphere and that they have an influence on the bioavailability of metals in sediment, so information on metal accumulation in sediment is important to consider in monitoring health in estuaries [2,15,21,47,48].

The metal concentrations were higher in Site B compared to Site A, which was located further upstream. Although Site A is located closer to the point sources, the topography of the two areas is very different. The riverbank in Site A is unmodified followed by Wylde Bridge further away—Site B is located after the bridge where the sandbank splits the channel into a large, but comparatively shallower creek. The jetties, modified embankment, and sandbank located at Site B effectively traps the currently transported particles, slowed down by the bridges. The accretion of sediment in Site B elevates *S. tegetaria* and *S. maritima* on the sandbank and accumulates enriched particles, and with little flushing in the creek, the metals are held in the marsh. Unsurprisingly, various authors have recommended the site for long-term monitoring of metals and other pollutants [42,49]. The results in Table 1 indeed show that the metal load in Site B is much larger than in Site A, particularly on the sandbank where *S. tegetaria* and *S. maritima* was located. The higher metal accumulation in Site B occurred despite the fact that Site A was located close to a point source, and that Site B does not contain a point source.

Our results show significantly fewer metals in bare sediment compared to the rhizosediment. *Salicornia tegetaria* rhizosediments specifically contain 5.6 times more Fe, and 4.8 times more Mn than the bare sediment in Site B (Table 1). Apart from Pb, which had lower concentrations, and Cu with similar concentrations, the Cr, Mn, and Zn concentrations were significantly higher than the concentrations found by Binning and Baird, for the middle and lower reaches of the estuary [26]. Nickel and Cu were in range to the concentrations found in sediments by Nel et al. four to five years prior to this study, in the Swartkops Estuary [44]. However, Fe, Mn, Zn, Cr, and Pb were much higher. Lastly, Phillips et al. found higher concentrations of Pb and Cu and similar Zn concentrations, in rhizosediments, of the Swartkops Estuary [34]. Although older studies indicated an increase in metal concentrations in the Swartkops Estuary [26,45,50], they were not conducted in rhizosediment, but in surface sediment in the channel. Therefore, if a higher concentration is expected in rhizosediment compared to bare or channel sediment (as seen in Table 1), a conclusion on the extent of metal pollution in the estuary cannot be made when compared to the oldest studies, 40 years ago. An enrichment study on the channel sediment is therefore recommended, but our work and that of Phillips et al. indicate that the wetlands in the Swartkops Estuary have been acting as sinks for metal pollution [34].

Bonanno et al. showed that metal translocation and accumulation is often based on the individual species and not their ecological or morphological similarities [51]. Indicating that each plant species should be carefully studied as a potential phytoremediator, biomonitor or metal sink. It is important to consider the metal concentrations in rhizosediment of *S. tegetaria*, *S. maritima*, and *Z. capensis*, due to their dominance in Southern African estuaries, in order to set a baseline of the levels of metals these plants can tolerate. This information is crucial for laboratory-based bioassays, where macrophytes are exposed to different levels of metal concentrations, to determine their tolerance.

Plants can release substances such as root exudates to change the chemical sediment environment and promote metal bioavailability [13]. The uptake and release of exudates highly depend on root structure surface area to volume ratio [52]. Some wetland plants adapt to low-oxygen, waterlogged environments through Radial Oxygen Loss (ROL), by sending oxygen to their rhizosphere [15]. The change from a less reduced environment allows the precipitation of ferric- and Mn-oxide/hydroxides called iron plaque, which often forms a protective layer around the rhizosphere. The oxygenation only extends 0.4–2.5 mm from the rhizosphere and does not affect bulk sediment [53,54]. Iron plaque has been recorded for *S. tegetaria*, *Z. capensis*, and *S. maritima* [15,47,53,55,56], and may contribute to the higher recorded Mn and Fe in the rhizosediments compared to the bare sediment. The precipitation of iron plaque is followed by the co-precipitation of other metals including Zn, Pb, Cu, and Al. This may result in correlations between different metals' concentrations in the sediment. This would account for the considerably higher concentrations of these metals in the rhizosediment compared to the bare sediment. Martins et al. found that the amount of oxygen pumped to the depths can exceed the consumption, and oxygen can spread into the surrounding bulk sediment [56]. The subsequent immobilisation of metals can increase the capacity of the marsh sediment to accumulate more metals, by providing surface area for adsorption [56]. Radical oxygen loss increases the capacity of salt marsh plants as phytostabilisers, the lack of the process in bare sediment (especially in waterlogged areas) will decrease metal content and increase their leaching (and their toxicity). The highly dense root system of *S. maritima* may promote more changes to the metal accumulation capacity of the rhizosediment via root exudates compared to the seagrass, *Z. capensis* and *S. tegetaria*, which has a much less pronounced root system [52].

Another factor contributing to higher metal accumulation in rhizosediment is the total organic matter (TOM). The higher TOM was comparable with higher metal concentration, and lower TOM with the lower metal concentrations (Table 1 and Figure 3). Organic matter can either act as a source or a sink of metals depending on its solubility and the particular metals [57,58]. Williams et al. indicated that high organic matter presents higher metal accumulation in wetlands [8]. In general, the cationic metals in this study should form insoluble complexes with organic matter. Copper and Pb prefer to form bonds with organic matter [11,59,60], and dissolved Fe^{3+} and Cr^{3+} associate well with humic substances. Other metals also adsorb it due to its high specific surface area [61]. High decomposition rates of organic matter can cause the dissolution of Mn and Fe oxides/hydroxides (i.e., dissolve iron plaque), causing rapid anaerobic conditions and higher rates of leaching. Longer inundation times make leaching more prevalent in *Z. capensis* and *S. maritima* habitats in the lower marsh. However, simpler aerenchyma tissue in *Z. capensis* in comparison to more developed aerenchyma tissue in *S. maritima* greatly decreases metal accumulation in *Z. capensis* rhizosediment [19]. Nonetheless, all rhizosediment stored significantly more metals than the bare sediment.

Vegetation greatly reduces the energy of shallow wave-currents, trapping suspended particles in the habitat [14,60]. Suspended particles can contain organic matter and fine-grained sediment. Higher percentages of silt and clays in the sediment increase the metal binding capacity of cationic metals onto the negatively charged surfaces [62]. These bonding sites in clays are created by Al oxides, Mn oxides, and Fe oxides [63,64]. Although higher concentrations of these elements were found in sediments containing high metal concentrations, the results were not as well correlated as the TOM. However, sand being

more prevalent in Site A is probably the reason why metal concentrations are comparatively lower, and why the different rhizosediment had little difference (Figure 5). Bare sediment, *Z. capensis* rhizosediment, and *S. maritima* rhizosediment have similar grain size compositions with >90% sand. However, *S. maritima* has significantly higher Fe and Mn, indicating the possible formation of root plaque. *Spartina spp.* have highly developed aerenchyma tissue, maintaining more effective oxygen demands under waterlogged conditions [15]. While *S. tegetaria* in Site A, the higher clay/silt content played a significant role to sequester higher metal concentrations, in the higher intertidal zone with less frequent anaerobic conditions. The upper intertidal zones (e.g., *S. tegetaria* habitat) are also less frequently inundated and therefore is less flushed compared to the lower intertidal zones (*S. maritima* and *Z. capensis* habitats).

Lastly, sediment salinity did not differ considerably between sites (Figure 4) and vegetation type and was not a driver of metal accumulation, indicated by the direction of the environmental vectors for Site A in Figure 5a. However, higher sediment salinity was associated with *S. tegetaria* rhizosediment in Site B (Figure 5b). Fritioff et al. found that metals become more bioavailable for submersed plants with decreasing salinity and increasing temperature [65]. A decrease in the salinity in the estuary due to increased effluent input may enhance metal toxicity in the future.

5. Conclusions

This study investigated the metal concentrations in the intertidal sediment of the Swartkops Estuary, South Africa. The metal concentrations show a clear pattern of accumulation favouring the rhizosediment over the bare sediment. Metal concentrations were also higher higher up the intertidal range (i.e., in *S. tegetaria*) compared to lower down the intertidal range (i.e., *Z. capensis*). Depositional sites accumulated higher silt/clay content and organic content, but only for the rhizosediment, while bare sediment was similar between depositional and non-depositional sites. Therefore, it can be concluded that the rhizosediment acts as a sink of metals and that the depositional site without point sources captures the metals more efficiently than the upstream non-depositional site, with closer point sources. Our results indicate that it should be a priority to conserve wetland plants that act as metal sinks, considering the high rate of salt marsh loss locally and globally, and their ability to reduce/prevent metal toxicity.

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