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ARTICLE

Assessing Metal Content in *Halophila stipulacea* Seagrass as an Indicator of Metal Pollution in the Northern Gulf of Aqaba, Red Sea

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Abstract

To assess the utility of the seagrass (*Halophila stipulacea*) for biomonitoring of metal pollution, seagrass samples were collected from four sites along the Jordanian coast of the Gulf of Aqaba between April and July 2017. Concentrations of trace metals (Cr, Mn, Fe, Ni, Zn, and Pb) in leaves, rhizomes, and roots were compared to published data on sediment trace metal and organic carbon content at the same sites to assess the degree of their fidelity in recording local trace metal pollution. The results of this study indicated that the roots of the seagrass *H. stipulacea* accumulated higher metal concentrations of Cd, Pb, Cr, Ni, Zn, Mn, and Fe than rhizomes and leaves. Concentrations in *H. stipulacea* varied significantly between sites for Mn, Fe, Zn, Cd, and Pb but not for Cr and Ni. Higher levels of seagrass trace metals in the Hotels Area and Old Phosphate Port sampling sites compared to other sampled sites are likely related to the sites' proximity to tourist and boating activity and city infrastructure which may contribute to metals accumulating in the tissues of this seagrass. In contrast to other studies, when all the data are considered, no clear trend between sediment metal concentration and seagrass metals is observed, suggesting physiological control on metal uptake by *H. stipulacea* and thus limiting the utility of *H. stipulacea* for biomonitoring of pollution.

Keywords Gulf of Aqaba · *Halophila stipulacea* · Red Sea · Seagrass · Trace metals · Bioconcentration factor · Translocation factor

1 Introduction

Seagrass meadows are among the world's most productive ecosystems and are key constituents in estuaries and the nearshore coastal zones. Seagrass meadows serve as a food source for diverse organisms and provide habitat and nursery areas for many species of invertebrates and vertebrates of commercial and ecological importance (Thayer and Stuart 1974; Thayer et al. 1975; Adams 1976; Brix et al. 1983; Lanyon et al. 1989; Preen 1995; Short and Echeverria 1996; Wahsha et al. 2016). Seagrass ecosystems are found throughout the world's oceans in shallow, nearshore waters where the impact of human activities is most severe, and there is concern over increasing pollution such as concentrations of metals in these systems (Hart 1982; Ward 1989). Seagrasses are known to be good bio-indicators (Lee et al. 2004; Orth et al. 2006) as they are widespread worldwide and sensitive to environmental changes (Walker and McComb 1992;

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Udy and Dennison 1997; Bhattacharya et al. 2003; Ferrat et al. 2003) and are able to integrate ecological conditions and processes over various timescales from weeks to years (Gonzalez-Correa et al. 2005; Madden et al. 2009; Meehan and West 2000).

Seagrasses have been used as bio-indicators in the Mediterranean basin and in Florida, where many complex indices based on seagrass characteristics ranging from the physiological level to the community level have been investigated (Moreno et al. 2001; Romero et al. 2007; Montefalcone 2009; Bennett et al. 2011; Lopez et al. 2011). However, most of the proposed indicator values are specific to areas and species, and cannot therefore, be used at a global scale. The seagrass species *Halophila stipulacea*, native to the Indian Ocean, has spread from the adjacent Red Sea to the Mediterranean through the Suez Canal and has recently migrated to the Caribbean Sea and the South American continent (Sghaier et al. 2011; Willette and Ambrose 2012; Winters et al. 2020). Though invasive, this species may prove useful as a global bio-indicator to monitor pollution in coastal environments due to its broad abundance.

Seagrasses have previously been shown to be indicative of trace metal pollution (Sundelin and Eriksson 2001; Lafabrie et al. 2008), and as this type of pollution is becoming a major threat to coastal ecosystems in rapidly developing countries (Li et al. 2007), there is a strong need for reliable trace metal bio-indicators. Through bioaccumulation, trace metals in seagrasses can also be expected to transfer to higher trophic levels. Their concentrations may, therefore, be used to detect possible threats to ecosystem services such as fisheries. The focus of this study is to assess the utility of *H. stipulacea* for biomonitoring of trace metal pollution. To do so, we measured trace metal concentration in *H. stipulacea* found in the Red Sea along the Jordanian coast and related the metal concentrations in seagrass tissue to metal concentrations in sediment and seawater which, in turn, are related to land use onshore.

Eleven species of seagrasses have been recorded from the Red Sea, including the Gulf of Aqaba and the Gulf of Suez (Jones et al. 1987). *H. stipulacea* is the most common seagrass species in the Jordanian sector of the Gulf of Aqaba and grows at a depth range of 1–45 m with greater abundance below 10 m depth. Previous studies have reported on trace metals in seagrasses in the Gulf of Aqaba (Wahbeh 1983; Wahbeh and Mahasneh 1984) but these studies did not consider multiple sites and land use along the Jordanian coastline.

To evaluate the metal accumulation efficiency in *H. stipulacea*, we used the bio-indices bioaccumulation factor (BCF) and translocation factor (TF). BCF refers to the ability of seagrass to take up and accumulate metals present in the sediment and thus is defined as the proportion of metal concentration in the roots to that in the sediment. TF refers to the ability of seagrass to transfer metals from the roots to other organs of the plant and is defined as the proportion of metal concentration in the shoots (leaves and rhizomes) to the roots (Wahsha et al. 2012). Both BCF and TF must be considered when assessing whether a particular plant is a metal hyper-accumulator, especially if leafy vegetation is a common food source which transfers metals to the trophic web. Plants with both BCF and TF equal to or greater than 1 have been reported to have the potential to use phytoextraction to regulate metal concentrations, and higher values of these indices may suggest a seagrass species is particularly efficient at absorbing metals from the environment and thus may be a useful bio-indicator of metal pollution (Wahsha et al. 2012).

The goal of this study is to assess the utility of *H. stipulacea* in the Gulf of Aqaba as a metal bio-indicator by determining trace metal concentrations in *H. stipulacea* and the relationship between metal concentrations of plant specimens and the surrounding human-impacted coastal environment.

2 Materials and Methods

2.1 Study Area

The Gulf of Aqaba (Fig. 1) is a semi-enclosed body of water in the northern Red Sea and is approximately 180 km long, ranging from 14 to 24 km in width, and reaching a maximum depth of 1815 m (Khalaf et al. 2019). The Gulf exchanges water with the Red Sea via the Strait of Tiran, a narrow, shallow sill of 252 m depth (Al-Najjar et al. 2019). The Jordanian coastline along the Gulf is 27 km long and is characterized by fringing coral reefs found along much of the coastline throughout the Gulf (Sbaihat et al. 2018) as well as human activities, including tourism (hotels, beaches, and boating) and industry (phosphate export and industrial shipping).

2.2 Field Methods and Sample Treatment

Samples of the seagrass *H. stipulacea* and sediment samples were collected from four coastal sites: Hotels Area (HA), Old Phosphate Port (OPP), Public Beach (PB), and Radisson Blue (RB, a resort area) (Fig. 1 and Table 1). Samples were collected by SCUBA diving at a depth range of 15–20 m during the period April–July 2017. None of the seagrass plants collected showed visible signs of toxicity. Seagrasses were washed thoroughly on site with seawater to remove sediments and other debris and transported in plastic jars, on ice, to the laboratory. In the laboratory, seagrass was rinsed again with seawater supplied by a free-flowing seawater pipe and then rinsed with distilled water to remove residual

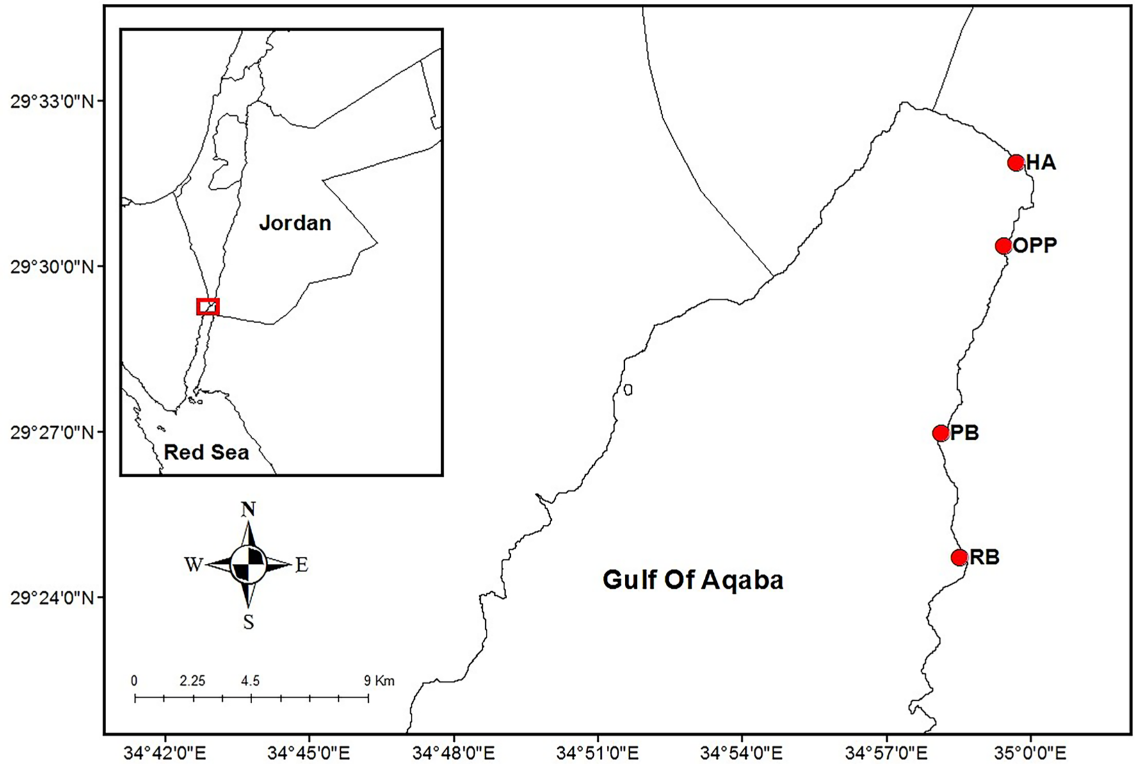

Fig. 1 Sampling site locations from Table 1. From north to south, sites are *HA* Hotels Area, *OPP* Old Phosphate Port, *PB* Public Beach, *RB* Radisson Blue

Table 1 Sample site locations

Site ID	Site	Latitude	Longitude
HA	Hotels Area	29° 53′ 12′′ N	34° 99′ 50′′ E
OPP	Old Phosphate Port	29° 50′ 62′′ N	34° 99′ 07′′ E
PB	Public Beach	29° 44′ 97′′ N	34° 96′ 89′′ E
RB	Radisson Blue	29° 41′ 20′′ N	34° 97′ 53′′ E

sediments and other debris. Seagrass tissues for each sample were then separated into leaves, roots, and rhizomes, and each organ was rinsed with 0.1 N HCl (to remove carbonate grains) and then rinsed three times with Milli-Q (18.2 Megaohm) water. Samples were oven-dried at 50 °C for 24 h to constant weight. After drying, samples were ground into a powder with an agate mortar and pestle and kept in plastic bags for analysis.

2.3 Analytical Methods for Trace Metals

2.3.1 Seagrass

A 0.2 g aliquot of each powdered seagrass sample was added to acid-cleaned ceramic crucibles, covered with aluminum foil, and heated in a muffle furnace at 500 °C for 4 h to burn organic carbon. Upon cooling, samples were dissolved in 3 ml concentrated (16 N) ultra-pure HNO_3 (Optima brand) and left on a shaker for 14–18 h until no solid residue was left. One ml of each solution was filtered through a 0.45 µm glass fiber filter into a 15 ml centrifuge tube (acid-cleaned with 3 N HCl for 24 h) and diluted to 10 ml with 1% ultra-pure HNO_3 containing 100 ppb of rhodium internal standard. Duplicate procedural blanks processed similarly to the samples were used for each batch of digested samples. Total concentrations of trace metals (Cr, Mn, Fe, Ni, Zn Cd, and Pb) in seagrass leaves, rhizomes, and roots were measured by inductively coupled plasma mass spectrometry (ICP-MS Element XR, Thermo Electron Corporation, Franklin, MA, USA) using serial dilution of calibration certified mixed elemental standards with quality assurance based on consistency. The standards were prepared in lab from certified elemental stock solutions from Isotopx Limited (Cheshire, UK). Certified Reference Materials: National Research Council Canada CRMs CASS-5 and SRM 1566a were analyzed at the same time. Comparison of the trace metals concentration measured to those in standard reference materials indicate that the recovery was better than 97% for all metals reported here. No correction was applied to samples as the offset is low. Mean blank was subtracted from each metal, and triplicate analyses of consistency standards were accurate within 6% of known metal values (in ppm) as follows: Cr = 23.9 ± 0.91, Mn = 1,143.58 ± 24.16, Fe = 1,318.14 ± 46.55, Ni = 25.1 ± 1.55, Zn = 235.56 ± 6.75, Cd = 25.32 ± 0.53, and Pb = 23.86 ± 1.57. Seagrass results are reported as μg g^{-1} of metal to seagrass organ dry weight (or ppm).

2.3.2 Sediment

A 0.2 g aliquot of powdered sediment sample was added to 30 ml Teflon beakers and dissolved via sequential treatment with triple-distilled acids (Savillex DST-1000) under class 1000 clean lab conditions at the University of California Santa Cruz. To dissolve the carbonate fraction, 50% (6 N) HCl was continuously added to each of the samples until the reaction ceased. Samples were then dried on a hotplate at 100 °C and the remaining silicate fraction was dissolved by adding 10 ml solution of 50% HNO_3 and 50% HF to each sample, capped, and left on a hotplate at 120 °C for 72 h with regular shaking throughout the day. During heating, concentrated HF was added in ~ 2 ml increments daily until every sample was completely dissolved (~ 6 ml HF addition to each sample). After complete dissolution, the samples were dried at 120 °C and reconstituted in 6 N ultra-pure HNO_3. To analyze, samples were diluted by a 1:100 ratio with 1% ultra-pure HNO_3 containing 100 ppb of rhodium and analyzed by ICP-MS Element XR using lab-prepared calibration and consistency standards from Isotopx stock solutions and certified reference materials (as described above). Duplicate analyses of consistency standards yielded results accurate within 7% with values (in ppm) as follows: Cr = 625.0 ± 42.5, Mn = 625.0 ± 13.2, Fe = 31,250.0 ± 294.3, Ni = 31,250.0 ± 366.1, Zn = 625.0 ± 11.6, Cd = 625.0 ± 40.1, Pb = 625.0 ± 40.8. Sediment results are reported as μg g^{-1} of metal to bulk sediment dry weight (or ppm).

2.4 Bioconcentration Calculations and Statistical Analysis

Bioconcentration factor (BCF) was calculated for plant organs above and below the sediment–water interface, where $BCF_{above} = ([metal]_{leaves} + [metal]_{rhizomes}) / [metal]_{sediment}$ and $BCF_{below} = [metal]_{roots} / [metal]_{sediment}$, respectively. Translocation factor (TF) was calculated as $TF = ([metal]_{leaves} + [metal]_{rhizomes}) / [metal]_{roots}$ to reflect translocation from below to above the sediment–water interface and was also calculated separately for each organ (leaves/roots and rhizomes / roots).

Statistical analyses were performed in R software (v. 3.6.2, R Core Team 2019). For multivariate statistical analysis, trace metal data were converted to ppb (ppm × 1000) and transformed with $\log_{10}$ to reduce non-normal distribution skewness and avoid $\log_{10}$ of values < 1 ppm. The $\log_{10}$-transfored data were analyzed with principal component analysis without scaling to unit variance to preserve the relative magnitude of change in metal concentrations. Log_{10}-transformed data were analyzed with hierarchical agglomerative clustering as both Q-mode (by sample) and R-mode (by metal) using a Euclidian distance matrix and average linkage method (Davis 2002). Cophenetic correlation coefficients were calculated as Pearson's correlation between the distance matrix and dendrogram cophenetic matrix and were used to assess goodness-of-fit between the resulting dendrogram and original multivariate data (higher coefficient means better fit, similar to other correlation metrics). Permutational ANOVA (PERMANOVA, Anderson 2014) was used to assess significant difference among all metals at all sites and among all organs. This method was selected due to the non-normal distribution of metal concentrations but was performed on $\log_{10}$-transformed data to minimize dispersion which can cause a significant result. Kruskal–Wallis One Way Analysis of Variance (ANOVA) was performed on raw metal concentration data to evaluate the statistical significance of variation of metal concentrations among the four sites and among the three seagrass organs. For results significant below $p = 0.05$, a Pairwise Wilcoxon rank sum test with Holm correction was performed to determine differences between pairs of sites. Kendall correlation was used to assess significant correlation of raw metal concentrations between bulk seagrass and sediment and was chosen due to non-normal distribution of data. Lastly, seagrass and sediment data from this study were plotted against sediment and seawater metal concentration data from the literature.

3 Results

3.1 Halophila Stipulacea Metal Concentrations

Roots of the seagrass *H. stipulacea* accumulated higher metal concentrations of Cr, Fe, Ni, Zn, and Pb than rhizomes and leaves (Table 2), but there was no significant difference in metal concentrations among roots, leaves, and rhizomes (Kruskal–Wallis *H* and p, Table 3, Fig. 2a). Among all metals, there was no significant difference among metal concentrations across seagrass organs (PERMANOVA Pseudo *F* and p, Table 3). However, there was a significant difference among all metals in the seagrass *H. stipulacea* collected across the four sampling site locations (PERMANOVA Pseudo *F* and p, Table 3). The concentrations of the metals Mn, Fe, Zn, Cd, and Pb were significantly different among the four sites (Kruskal–Wallis *H* and p, Table 3, Fig. 2b) with Hotels Area (HA) and Old Phosphate Port (OPP) generally different from Public Beach (PB) and Radisson Blue (RB) (Pairwise Wilcoxon p, Table 3). Overall, seagrass

Table 2 Seagrass and sediment trace metal concentrations (μg g^{-1}, or ppm) and summary statistics

[a]Site ID	Sample	Cr	Mn	Fe	Ni	Zn	Cd	Pb
HA	Leaves	0.25 ± 0.06	4.09 ± 0.09	259.63 ± 3.66	0.35 ± 0.12	7.93 ± 3.97	0.05 ± 0.02	1.35 ± 0.08
HA	Roots	0.56 ± 0.10	9.54 ± 0.61	947.92 ± 73.35	0.62 ± 0.18	4.55	0.05 ± 0.04	2.15 ± 0.40
HA	Rhizomes	0.33 ± 0.10	6.18 ± 0.07	431.3 ± 92.97	0.23 ± 0.10	4.31 ± 0.24	0.03 ± 0.01	1.15 ± 0.03
OPP	Leaves	0.47 ± 0.04	7.65 ± 0.30	95.56 ± 16.27	0.31 ± 0.06	3.95 ± 0.10	0.02 ± 0.01	0.11 ± 0.06
OPP	Roots	0.45 ± 0.15	5.86 ± 0.92	214.37 ± 27.60	0.34 ± 0.08	3.56 ± 1.92	0.08 ± 0.06	0.62 ± 0.08
OPP	Rhizomes	0.24 ± 0.01	6.23 ± 0.66	53.6 ± 10.78	0.18 ± 0.01	3.39 ± 0.26	0.03 ± 0.01	0.06 ± 0.01
PB	Leaves	0.29 ± 0.12	2.66 ± 0.01	106.23 ± 62.13	0.24 ± 0.06	1.36 ± 0.22		0.11 ± 0.05
PB	Roots	0.67 ± 0.81	0.84 ± 0.03	139.37 ± 72.74	0.45 ± 0.3	1.19 ± 0.09		0.36 ± 0.20
PB	Rhizomes	0.38 ± 0.20	1.16 ± 0.01	135.1 ± 13.54	0.2 ± 0.13	2.00 ± 0.15		0.17 ± 0.02
RB	Leaves	0.07 ± 0.04	3.75 ± 0.05	153.33 ± 5.64	0.11 ± 0.05	2.45 ± 0.13	0.01 ± 0.01	0.17 ± 0.07
RB	Roots		2.39 ± 0.33	253.12 ± 11.12	0.22 ± 0.19	4.84	0.01 ± 0.01	0.59 ± 0.07
RB	Rhizomes	0.90	15.59 ± 18.17	11.06	1.14	2.06 ± 1.93	0.03	0.16
[b]Grouping								
Leaves		0.25 (0.21)	3.91 (1.52)	149.75 (80.94)	0.26 (0.11)	3.21 (2.16)	0.01 (0.03)	0.15 (0.38)
Roots		0.52 (0.23)	3.92 (5.33)	239.57(225.91)	0.37 (0.27)	3.38 (3.28)	0.02 (0.04)	0.60 (0.44)
Rhizomes		0.26 (0.22)	5.95 (4.00)	125.52 (201.52)	0.18 (0.13)	3.32 (1.66)	0.03 (0.02)	0.16 (0.55)
HA	Seagrass	0.35 (0.20)	6.18 (3.75)	431.30 (508.25)	0.37 (0.21)	4.55 (0.65)	0.03 (0.03)	1.35 (0.54)
OPP	Seagrass	0.39 (0.22)	6.61 (1.30)	95.56 (105.98)	0.27 (0.13)	3.73 (0.69)	0.03 (0.01)	0.11 (0.40)
PB	Seagrass	0.30 (0.27)	1.16 (1.36)	135.10 (51.46)	0.26 (0.08)	1.38 (0.59)		0.17 (0.07)
RB	Seagrass	0.10 (0.43)	3.23 (1.11)	157.32 (95.92)	0.14 (0.27)	2.54 (1.07)	0.01 (0)	0.22 (0.38)
[c]HA	Sediment	0.04 ± 0.01	4.72 ± 0.1		0.07 ± 0.01	0.78 ± 0.01	0.07 ± 0.01	
OPP	Sediment	11.05 ± 0.75	279.9 ± 5.9	13,383.61 ± 126.03	7.23 ± 0.08	21.31 ± 0.39	1.88 ± 0.12	21.11 ± 1.38
PB	Sediment	27.82 ± 1.89	807.69 ± 17.03	24,374.91 ± 229.54	20.1 ± 0.24	98.79 ± 1.83	2.03 ± 0.13	53.74 ± 3.5
RB	Sediment	39.03 ± 2.65	1091.1 ± 23.01	36,040.95 ± 339.4	30.87 ± 0.36	91.37 ± 1.69	3.35 ± 0.21	35.29 ± 2.3

[a]Replicate mean ± standard deviation

[b]Median (interquartile range) of seagrass

[c]Value ± relative standard deviation of lab-prepared standard

metal concentrations were typically highest at the northern HA site and decreased toward the southern RB site, albeit with variability (Table 2).

Difference in seagrass metal concentration among sites, but not organs, is apparent in the principal component analysis (PCA) (Fig. 3). Principal component 1 explains 49% of variance of all seagrass trace metal data, and sites generally cluster together along PCA1. The PCA also indicates metal concentrations generally decrease from north (HA) to south (RB) with no clear trend relating to seagrass organ. Cluster analysis indicates clustering occurs by site with a cophenetic correlation coefficient of 0.81 (Fig. 4a), suggesting a good fit for the observation that RB and PB cluster together and OPP and HA each cluster separately. Across all seagrass metal concentration data, Ni, Cr, and Pb are associated, Zn and Mn are associated, and Cd and Fe have no clear relation to other metal concentrations (Fig. 4b, Table 2).

3.2 Sediment Metal Concentrations

Sediment trace metal concentrations generally increased from north to south with the highest metal concentrations occurring near RB and the lowest values occurring near HA (Table 2). This increasing trend is opposite to the trends seen for seagrass metal concentrations, resulting in negative correlations between seagrass and sediment concentrations of Mn, Ni, Zn, and Cd, though only the Zn correlation was significant (Kendall correlation τ and p, Table 3). Metal concentration trends in sediment from north (HA) to south (RB) are apparent in Fig. 5. There were also no significant correlations between sediment metal concentrations reported in the literature and seagrass sediment concentrations reported here (Kendall correlation τ and p, Table 3). For reference, we have also plotted the sediment weight percent organic carbon and total metal concentration of sediment from Abu-Hilal and Badran (1990) (Fig. 6) to compare to seagrass metal concentrations in Figs. 2b and 3. Sediment Zn, Cd, and Pb exhibit positive relationships to sediment weight percent organic carbon and generally decreased from north (HA)

Table 3 Summary of statistical methods

PERMANOVA		Multivariate (all metals)					
		Degrees of freedom	Sums of squares	Mean squares	Pseudo F	R^2	p
By site ID	Site ID	3	12.3	4.09	7.37	0.58	**0.001**
	Residuals	16	8.87	0.554		0.42	
	Total	19	21.1			1	
By organ	Organ	2	2.07	1.04	0.924	0.098	0.478
	Residuals	17	19.1	1.12		0.902	
	Total	19	21.1			1	

Kruskal–Wallis		Cr	Mn	Fe	Ni	Zn	Cd	Pb
By site ID	H	1.16	14.69	13.14	2.29	14.06	16.30	13.58
	p	0.764	**0.002**	**0.004**	0.515	**0.003**	**0.001**	**0.004**
By organ	H	3.48	0.34	4.18	2.82	0.40	0.21	5.11
	p	0.175	0.846	0.124	0.244	0.818	0.903	0.078

Pairwise Wilcoxon		Cr			Mn			Fe			Ni			Zn			Cd			Pb		
By site ID		HA	OPP	PB	HA	OPP	PB	HA	OPP	PB	HA	OPP	PB	HA	OPP	PB	HA	OPP	PB	HA	OPP	PB
	OPP	1			0.818			**0.013**			1			0.121			1			**0.013**		
	PB	1	1		**0.013**	**0.013**		**0.013**	0.987		1	1		**0.022**	**0.013**		**0.013**	**0.013**		**0.013**	0.987	
	RB	1	1	1	0.195	0.195	0.104	**0.035**	0.987	0.987	1	1	1	0.167	0.329	0.251	0.091	0.091	0.017	**0.017**	0.987	0.987

Kendall		Cr	Mn	Fe	Ni	Zn	Cd	Pb
Literature*	τ	− 0.16	0.302	0.168	0.034	0.268	0.101	0.101
	p	0.579	0.201	0.477	0.887	0.256	0.670	0.670
This study	τ	0.060	− 0.268	0.225	− 0.201	− 0.737	− 0.369	0.096
	p	0.808	0.256	0.437	0.394	**0.002**	0.118	0.739

*Sediment average value derived from Abu-Hilal and Badran (1990), Abu-Hilal (1993), Mourad and El-Azim (2019)

Bold significant p value at 0.05 level

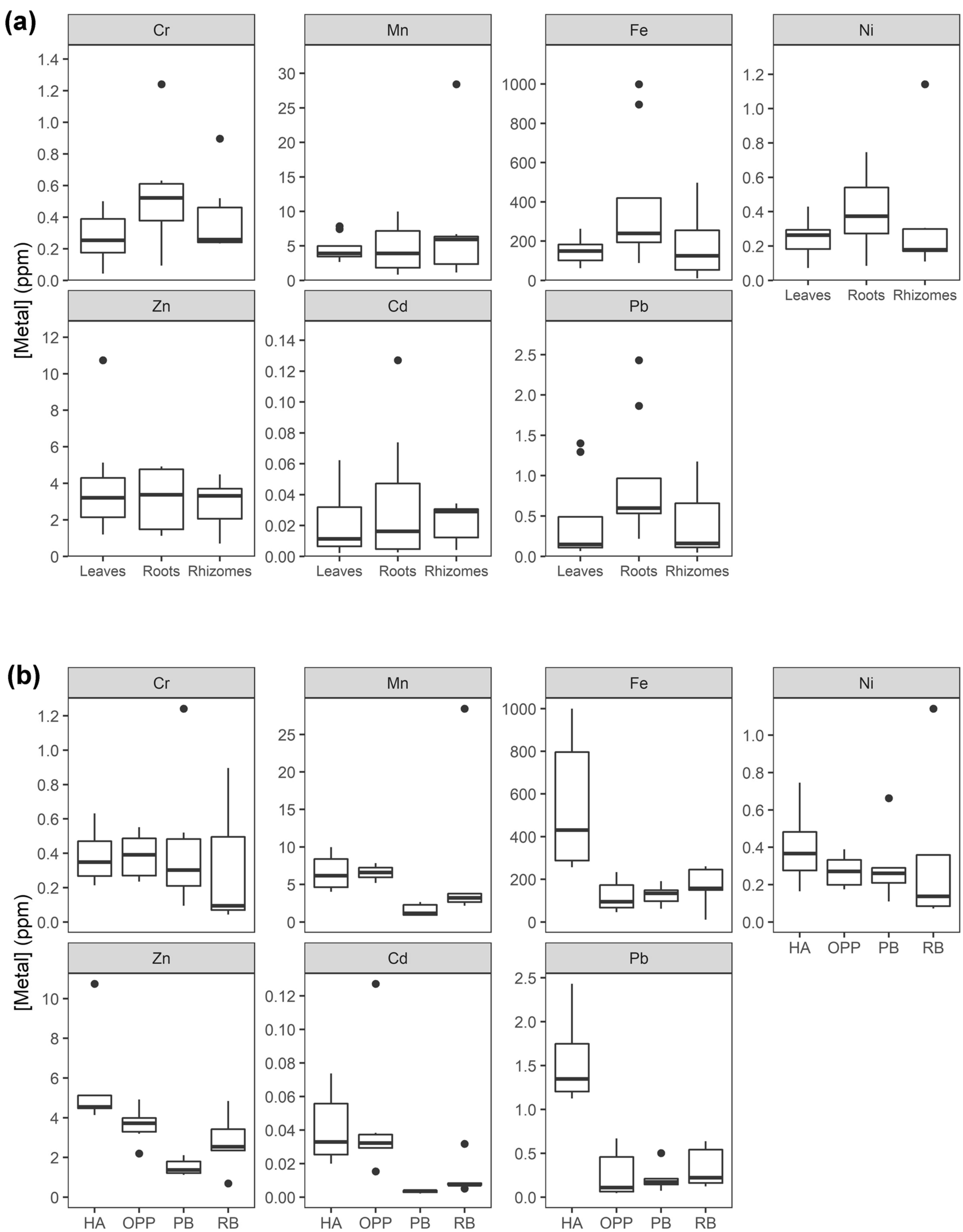

Fig. 2 Boxplots displaying minimum, maximum, median, and interquartile range of *H. stipulacea* data grouped by (**a**) seagrass organ and (**b**) site location. Site location ID is given in Table 1. Values are given in Table 2

to south (RB and IZ, or Industrial Zone 6 km south of RB), similar to trends observed in seagrass metal concentrations across all organs (Fig. 2b).

3.3 Comparison to Seawater

We compared our seagrass data (Table 2) to seawater data reported in Shriadah et al. (2004), Al-Absi et al. (2019), and Mourad and El-Azim (2019). Metal concentrations in

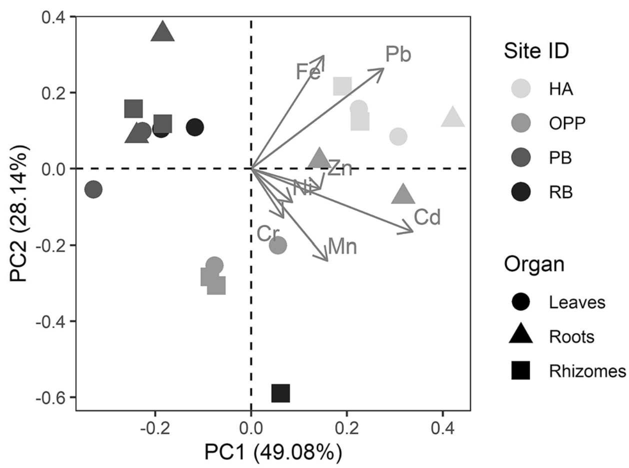

Fig. 3 Principal component analysis of seagrass metals following $\log_{10}$ transformation which preserves relative magnitude of change in concentration (arrow length). Metal concentrations appear to increase from south (RB) to north (HA), and there is no clear relationship between metal and seagrass organ. Statistical relationships represented here are given in Table 3

seagrass exceed seawater concentrations for Mn and Fe at all sites and for Pb at site HA. The other metals (Cr, Ni, Zn, and Cd) are near or below one standard deviation of seawater concentrations reported for this region.

3.4 Metal Accumulation Capability

Bioconcentration factor (BCF) and translocation factor (TF) are given in Table 4. The seagrass *H. stipulacea* accumulated metals both above and below the sediment water interface. BCF below the sediment from this study was ordered from highest to lowest as Zn > Cr > Ni > Pb > Cd > Fe > Mn. BCF above sediment from this study was ordered as Zn > Ni > Cr > Cd > Pb > Mn > Fe. This pattern was true also for sediment metal concentration reported in the literature (Table 4), for all metals except Ni which had higher BCF below compared to above the sediment water interface. Overall, BCF indicates *H. stiupulacea* is most efficient at acquiring Zn from sediments. TF values were typically lowest in the ratio leaves / roots and highest in (leaves + rhizomes) / roots, or organs grouped as above-ground to below-ground (Table 4). TF values for above-ground to below-ground metal concentrations were ordered as Mn > Zn > Ni > Cd > Cr > Pb > Fe. TF values indicate that *H. stipulacea* is most efficient at transporting the metals Mn and Zn from roots to the leaves and rhizomes above the sediment–water interface and least efficient at transporting Pb and Fe (Table 4).

4 Discussion

4.1 Environmental Controls of Trace Metals in H. stipulacea

H. stipulacea from the Hotels Area (HA) and Old Phosphate Port (OPP) have the highest levels for most of the metals with a significant difference for Pb, Ni, Zn, Mn, and Fe from other locations (Fig. 2b). Public Beach and Radisson Blue farther to the south are lower in concentrations of Mn, Ni, Zn, and Cd (Fig. 2b) in seagrass despite higher concentrations of these metals in sediments compared to HA and OPP (Table 2). This contrast suggests bulk sediment is not the important factor that controls metal concentration in *H. stipulacea* and hence this seagrass is not a good

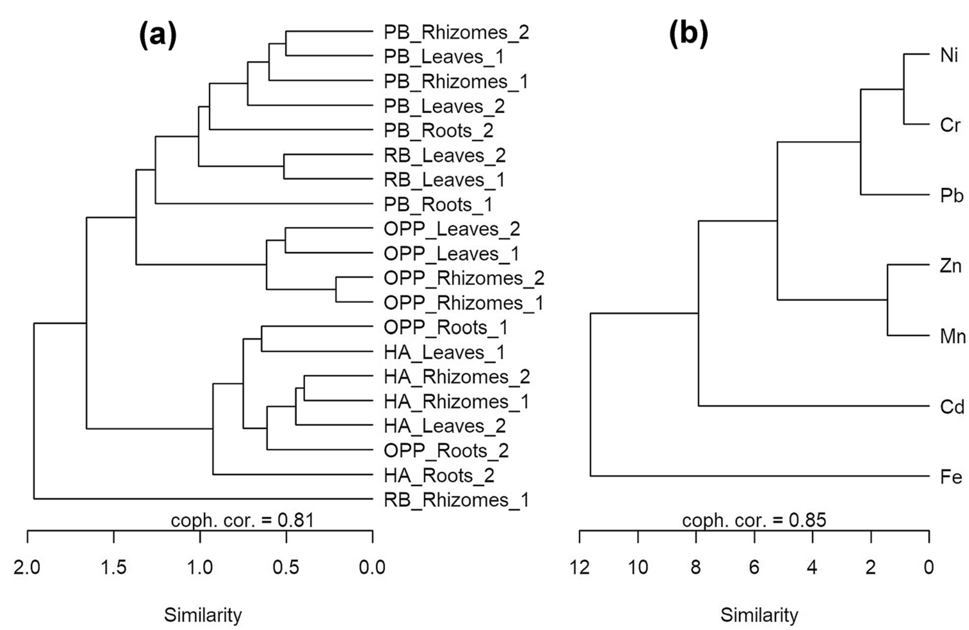

Fig. 4 Agglomerative hierarchical cluster analysis of (**a**) site and organ data across all metals following $\log_{10}$ transformation of metal concentrations. Labels represent site, organ, and replicate, which are included to visualize variability. Data generally clusters by site, grouped by PB and RB, OPP, and HA, which is visually apparent in Fig. 3. RB_rhizomes_1 is an outlier. (**b**) Cluster analysis of metals across all sites and organs using the same $\log_{10}$ transformed data as in (**a**)

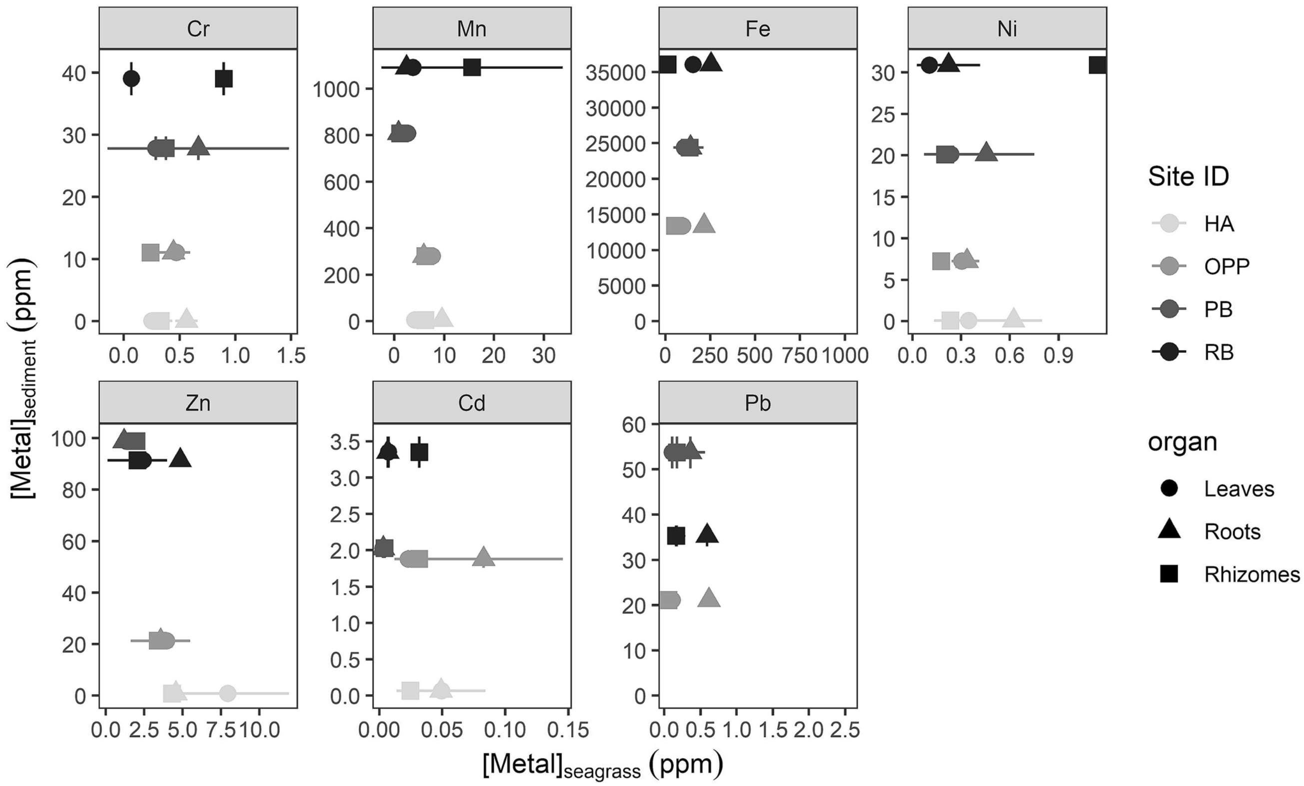

Fig. 5 Metal concentrations in seagrass compared to sediment values derived from this study. Non-parametric Kendall correlations are given in Table 3

biomonitor for sediment pollution, or long-term integrator of pollution. Indeed, bulk sediment geochemistry may not accurately reflect the concentrations of metals that are mobile and available for biological uptake in this region, and specific fractions of sediment such as organic content or fine clay may be more important (Szava-Kovats 2008). Ocean currents change and thus sediment grain size also changes seasonally in the Gulf of Aqaba; however, (Al-Rousan et al. 2016), reported that the range of grain size as follows: HA was 172–154 µm, OPP was 75–92 µm, near PB was 125, and RB was 107–116 µm. None of the reported grain sizes were below the 63 µm threshold defining silts and clays which are thought to be important in metal scavenging (Szava-Kovats 2008). To capture the 63 µm grain size and to understand grain size impact on *H. stipulacea*, a metal accumulation time series study is needed to for the Jordanian coast to better assess the relationship between fine sediments and bioaccumulation of metals in *H. stipulacea.*

Abu-Hilal and Badran (1990) reported the organic carbon fraction present in sediments along the Jordanian coast, and their results reveal strong relationships to metals measured in bulk sediment (Fig. 6). For the metals Zn, Cd, and Pb, bulk sediment metal concentrations were associated with higher weight percent organic carbon values and follow a trend of decreasing metal concentration from north to south, similar to our seagrass results (Fig. 2b). Mn, Fe, and Ni appear to have two distinct distributions, the first characterized by variability in metal concentrations and weight percent organic carbon in the northern Gulf of Aqaba near HA and OPP (Fig. 1), and the second by a positive association between metal concentration and sediment organic carbon in the southern region of the Jordanian coast near RB (Fig. 1). Sediment organic carbon is known to sorb metals from of the water column (Luoma and Davis 1983), thus the organic matter fraction of sediments may have metals more readily bioavailable for accumulation in seagrasses utilizing sediment nutrients than has previously been recognized. We acknowledge that the work of Abu-Hilal and Badran (1990) is 30 years old, and the trends in sediment organic carbon may be different for the modern Jordanian coast, but this underscores the need to potentially pair measurements of sediment organic carbon with seagrass metal data for *H. stipulacea* or other species under consideration as biomonitoring taxa as well as measure water column trace metal data that are of higher spatial resolution and represents differences among sites. With respect to sediments as a source of seagrass metals, future work should consider the organic fraction of sediment and its reactivity alongside the mineralogical composition of sediments to better determine the importance of organic carbon for metal accumulation in *H.*

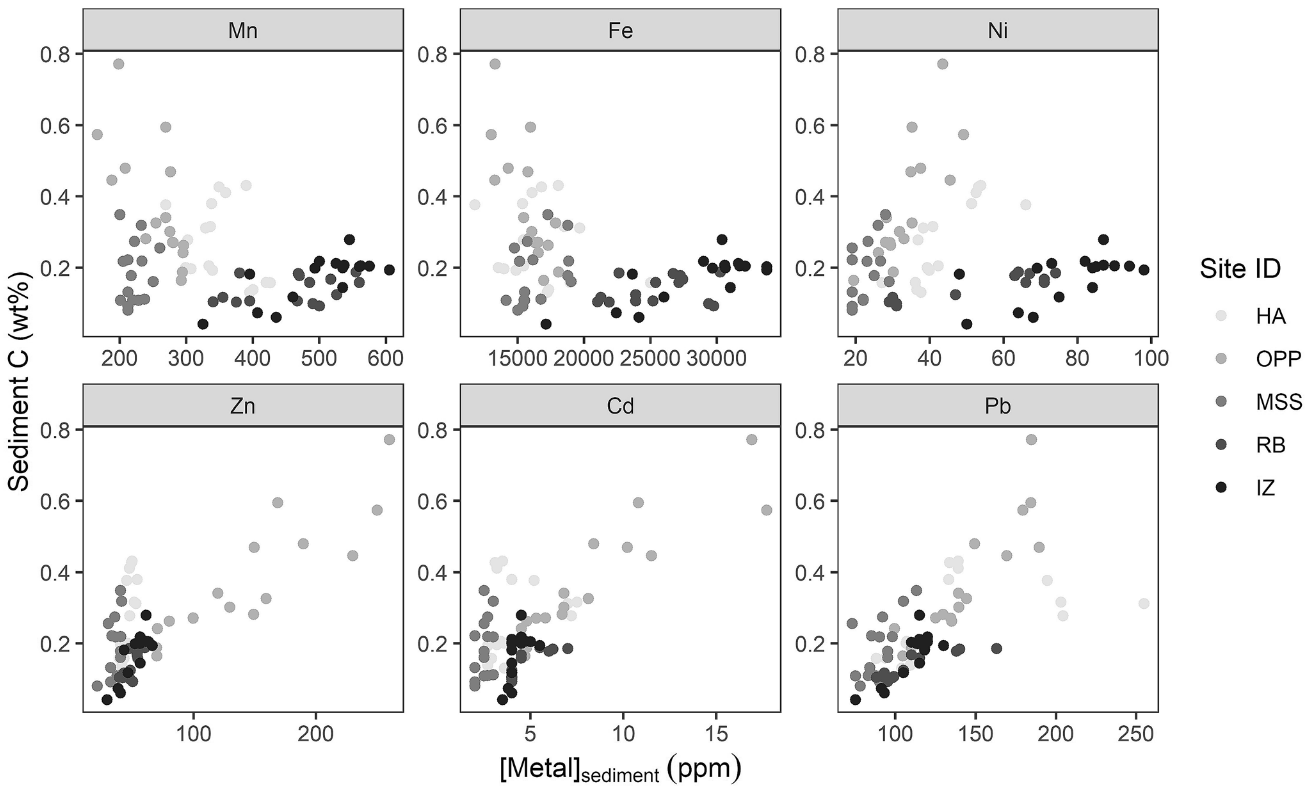

Fig. 6 Sediment total metal concentrations compared to sediment organic carbon weight percent, replotted from Abu-Hilal and Badran (1990) and colored by sample site from north to south (Table 1), where the authors' original "Mamiah" station is replaced with RB here. MSS is Marine Science Station located 2 km north of PB, and IZ is Industrial Zone located 5 km south of RB (see Fig. 1)

Table 4 Bio-indicators calculated from seagrass and sediment

Indicator	Sediment–water interface	Sediment data	Cr	Mn	Fe	Ni	Zn	Cd	Pb
BCF	Above	Literature*	0.0053	0.0213	0.0104	0.0094	0.0514	0.0042	0.0042
	Below	Literature*	0.0081	0.0168	0.0259	0.0112	0.0529	0.0068	0.0096
	Above	This study	0.0188	0.0108	0.0063	0.0236	0.0647	0.0119	0.0112
	Below	This study	0.0287	0.0085	0.0158	0.028	0.0666	0.0193	0.0253
TF	Leaves/Roots		0.484	0.974	0.395	0.609	1.247	0.584	0.468
	Rhiz./roots		0.677	0.622	0.860	0.735	1.334	0.945	1.041
	(leaves + Rhiz.) /roots		1.198	2.538	0.855	1.437	2.181	1.201	0.918

*Sediment average value derived from Abu-Hilal and Badran (1990), Abu-Hilal (1993), Mourad and El-Azim (2019)

stipulacea. Because organic carbon can sorb metals from seawater, temporal, and spatial variability in seawater metal concentrations should also be carefully measured.

Seawater is a possible source of metals in addition to sediments for uptake into *H. stipulacea*, though it is thought to play less of a role in metal accumulation in *H. stipulacea* (Bonanno and Raccuia 2018a). Waters in the Gulf of Aqaba are oligotrophic (Wahbeh 1983) which may result in low biological cycling of metals and thus low levels of metals in leaves and rhizomes. Indeed, concentrations of metals in the seagrass organs are low and are closer to seawater concentrations in the Gulf of Aqaba than to metal concentrations in sediment (Al-Absi et al. 2019), though seawater values presented here represent averages across the northern Gulf of Aqaba rather than at our individual sampling sites (Shriadah et al. 2004; Al-Absi et al. 2019; Mourad and El-Azim 2019). Indeed, work by Bonanno and Raccuia (2018b) indicates that temporal variability in seawater concentrations may influence metal uptake in *H. stipulacea*, and variability was also noted in seagrasses that uptake trace metals from the seawater directly (Malea et al. 2018). The wide range of seawater metal concentrations reported for Cr, Ni, and Zn in the Gulf of Aqaba (Shriadah et al. 2004; Al-Absi et al. 2019; Mourad and El-Azim 2019) compared to the trace metals in *H. stipulacea* indicate seawater variability may be important at our field sites, particularly in relation to the local coastal metal concentrations. Water discharged to these locations from land, or industrial and tourist activity that contribute metals to the coastal environment may be more important sources of metals to *H. stipulacea* than background open ocean seawater levels. Specifically, the Hotels Area (HA) and Old Phosphate Port (OPP) have the highest levels for most of the metals with a significant difference for Pb, Ni, Zn, Mn, and Fe from other locations (Fig. 2b), and these two sites are near the highest concentration of infrastructure in the city of Aqaba, including the hotels and the Yacht club marina. Public Beach and Radisson Blue farther to the south in less urban and developed locations, are lower in concentrations of Mn, Ni, Zn, and Cd (Fig. 2b) in seagrass despite higher concentrations of these metals in sediments compared to HA and OPP (Table 2). To determine if this indeed is the reason for the lack of correlation between the sediment and tissue metal concentration a metal monitoring program in the water column at the site of seagrass collection will be needed. It is possible that *H. stipulacea* could be an efficient biomonitoring agent of seawater metal concentrations and not the long-term accumulation in sediments.

4.2 Biological Mediation of Metal Concentrations

The lack of a clear correlation of trace metals in *H. stipulacea* with environmental concentrations in bulk sediment or seawater may also be a result of strong biological physiological mediation of metal uptake in this species. Our data clearly indicate that metal concentration in sediment is not the only factor responsible for trends in seagrass metal concentrations across sites (Fig. 5).

The overall concentrations of metals and BCF are low for *H. stipulacea* in this study which may indicate either low efficiency of biological uptake of metals (or biological mediation), or low concentrations of the metals in the environment. The latter is not likely given recent data (Al-Taani et al. 2014; Al-Absi et al. 2019), and we do not see a strong correlation between metal concentrations of *H. stipulacea* and sediment, therefore it is worth considering biological controls of metal concentrations in *H. stipulacea*.

The results show that *H. stipulacea* accumulates the trace metals Zn, Ni, and Cr more than Mn and Fe. Fe, Mn, and Zn are important in respiration as activators of enzymes involved in glycolysis and the Krebs cycle (Bidwell 1979; Malik 1980; Wahbeh 1981), but of these, only Zn appears elevated relative to sediments (Table 4) likely due to the high concentrations of Fe and Mn in the sediment (non-limiting). Higher concentrations Zn may also be attributed to higher rates of respiration documented for other species of seagrass in the Gulf of Aqaba by Wahbeh (1983). Conversely, Cd concentrations in *H. stipulacea* were very low (Fig. 2a), and Cd is currently understood to be unessential to plants (Maleci et al. 2014) and possibly excluded by seagrasses. Zn and Cd trends in *H. stipulacea* are in agreement with previous seagrass work (Wahbeh 1981) and thus suggest biological mediation in uptake which adds difficulty in interpreting metal concentrations in specific seagrass organs (e.g., roots or leaves) as bio-indicators of metal pollution of these elements in the environment.

Differences in root morphology among seagrass species may result in differing efficiency in metal bioconcentration and thus variable values of BCF (Table 4). Metal concentrations are lower in *H. stipulacea* compared to three other species of seagrass in the Gulf of Aqaba (Wahbeh 1983; Wahbeh and Mahasneh 1984), and different root morphology was documented to affect differences in the ability of plants to accumulate trace metals (Wahbeh and Mahasneh 1984). *H. stipulacea* has one delicate root compared to the *Halodule universes*, which has 3–4 thin roots at each node (Wahbeh 1983). Larsen and Schierup (1981) suggested that plants with many thin roots accumulate more metals than one with a few thick roots. Plant uptake of metals from sediment may occur either passively with the mass flow of water into the roots, or through active transport from root cells (Wahsha et al. 2012). Lyngby and Brix (1982) suggest that for some metals, the concentrations in the above-ground and below-ground parts of the seagrass give an estimate of bioavailability of these metals in the ambient and interstitial water and sediment, respectively. The transport of certain metals within the plant may, however, play a role in determining whether these relationships exist or not (Brinkhuis et al. 1980). Thus, future research on the mechanism of root uptake and within plant transport and re-allocation of metals in *H. stipulacea* is needed to better determine if this species can be used for biomonitoring across the broad geographic range it inhabits.

Biological control over uptake and transport of metals within *H. stipulacea* may explain the lack of significant difference in metal concentrations across seagrass organs of roots, rhizomes, and leaves (Fig. 2a, Table 3), and it has previously been reported that, while roots can absorb metals from sediment, translocation is low (Drifmeyer et al. 1980; Schierup and Larsen 1981; Lyngby et al. 1982; Wahbeh and Mahasneh 1984). TF values greater than 1 indicate translocation from roots to leaves and rhizomes (Wahsha et al. 2012). Considering leaf/root TF values, only Zn translocated from roots, and considering rhizome TF values, only Zn and Pb translocated from roots (Table 4). Recent work by Malea et al. (2021a, b) suggest that *H. stipulacea* may prove a useful biomonitor of Ni and Cr; however, they note that uptake rates were important in determining overall metal concentration and suggest that *H. stipulacea* has the ability to regulate metal uptake and hence may not be ideal for biomonitoring. Furthermore, physiological kinetics suggest a time delay between metal exposure and uptake. Results from Oscar et al. (2018) further indicate strong physiological controls to regulate salinity, with some evidence of biological exclusion of particular ions again explaining the lack of a clear relation between the metals concentration in *H. stipulacea* and those in the environment (Winters et al. 2020). Moreover, given the strong seasonality at the Gulf of Aqaba, a single grab sample of *H. stipulacea* may be insufficient to track the long-term relation between concentration of metals and environmental parameters.

5 Conclusions

Halophila stipulacea was assessed as a bioaccumulator of the metals Cr, Mn, Fe, Ni, Zn, Cd, and Pb. Roots of this seagrass species were typically elevated in metal concentrations relative to leave and rhizomes, but there was no significant difference in metal concentrations among these organs. Metal concentrations of *H. stipulacea* differed among four sites sampled with generally higher concentrations of metals along the northern coast near the Hotels Area and Old Phosphate Port, located near city infrastructure and tourist and boating activities, and lower values to the south near Public Beach and Radisson Blue resort which are less urbanized. In contrast, sediment metal concentrations increased from north to south, suggesting that bulk sediment is not the major source of metals to this seagrass, but compositional fractions of sediment (e.g., clay or organic content) need more assessment. Zn had the highest bioconcentration factor and translocation factor of the metals studied. This work suggests *H. stipulacea* may be a bioaccumulator of Zn, but we detected no clear relationship between other metals in the sediment and seagrass tissues. Bioaccumulation of select metals and not others have been reported for other seagrasses (Boutahar et al. 2021). Future work should take detailed measurements of sediment organic and inorganic constituents and carefully monitor temporal variability in coastal seawater metal concentrations to assess if variability in metal concentrations of seagrass organs relates to the environment or seagrass physiological control. Overall, this work highlights the need to collect more detailed organic and inorganic geochemistry from sediments in future bioindicator research on *H. stipulacea*.

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