Biosciences, Biotechnology Research Asia Vol. **7**(1), 25-32 (2010)

Accumulation and translocation of trace metals in *Halodule uninervis*, in the Kuwait coast

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(Received: March 11, 2010; Accepted: April 16, 2010)

ABSTRACT

Trace metals run-off associated with urban and industrial development showed potential threats to seagrass in the Kuwait coastal ecosystem. Seagrass, *Halodule uninervis* from Doha, Duba'iyah and Nuwaseeb (Sites I-III) representing the power plant, industrial (oil fields) and recreational sites of Kuwait's coastal waters were assessed for trace metals (Copper: Cu, Iron: Fe, Nickel: Ni and Lead: Pb) concentrations respectively. Trace metals concentrations followed an overall trend of: leaves> rhizome in *H uninervis* > sediments and Ni>Cu>Pb>Fe irrespective of the two seasons (summer and winter). Seasonally, trace metals in all the samples showed the sequence of July>Sept.>May> Mar.>Jan.>Nov. Among the three sites, trace metals were high in Site II followed by Sites I and III irrespective of seasons. The overall trace metals bioconcentration factor (BCF) was high in summer than in winter. BCF was higher than the translocation factor (TF) irrespective of the sampled sites and seasons. Among the sampled metals, BCF was high in Cu in both the seasons followed by other metals. However, the overall BCF in the samples was lower than the permissible limits to be defined as hyper-accumulators. Thus, the possibility of trace metals mobilization from contaminated sediment to leaves through rhizomes of *H uninervis*, characterized this species as a bio-indicator to trace metal pollution in the intertidal zone of Kuwaiti waters.

Key words: *Seagrass, metals accumulation, marine pollution*

INTRODUCTION

Trace metals contamination pose deleterious impact on marine life when they are aggravated by their long term persistence in the marine environment. Recent industrialization, discharges from anthropogenic sources and applications involving metals have elevated their levels in the marine environment[1-2]. Seagrass are marine angiosperms that colonize near shore environments[3]. Seagrasses were found to sequester trace metals from the marine environment through both the leaves and root and these concentrations were correlated with the water column and sediments, respectively [4-6]. Earlier investigators[7-8] showed metals sequestered by seagrasses passed from detritus communities to secondary and tertiary consumers and thus lead to the total contamination of the marine ecosystem. Trace metals toxicity in seagrasses were found to be high in low metal tolerant species, sites subjected to domestic or sewage outfalls sites, chemical interaction and ionic speciation from sediment[9-11]. Many plants that accumulated >1000 mg Kg^{-1} of Cu, Ni, Pb or >10000 mg Kg^{-1} of Zn, V, Mn were categorized as metals hyper accumulators[12]. Researchers[2, 13] observed significant trace metals mobilization between rhizome and leaves in seagrasses with translocation factor (TF) >1. Most plants translocated trace metals and nutrients from roots to leaves. Such mobility of metals was found low especially when the pH was high with clay and organic matter[14]. Based on the above findings, we investigated the (a) trace metals concentrations in the leaves, rhizome of *H. uninervis* in relation to its surrounding surface sediment (5cm depth) (b) bioconcentration (BCF) and translocation (TF) factors in *H. uninervis* and (c) interrelationship of BCF and TF in *H. uninervis* to seasonal changes and (d) three different Kuwait sites of pollution importance to characterize *H. uninervis* as potential biological indicators of metal contamination.

MATERIAL AND METHODS

We chose three marine sites namely, (a) Site-I (Doha), a Kuwait Bay site that is significant to industrial and domestic wastewater outfalls from power, desalination and water treatment plants, (b) Site-II (Duba'iyah), the central Kuwait coastal zone with oil wells and oil fields and (c) Site-III (Nuwaseeb), the southern most region of Kuwait observed with seldom industrialization but to certain extent of human recreational activities (Fig.1).

Seagrass, *H. uninervis* was collected during the years 2005-2009 from the three Kuwait marine sites on alternate months and categorized based on Kuwait's seasons (Winter: November, January, March and Summer: May, July September). Sampling within the meadows was restricted to mono-specific areas and homogenous seagrass cover. Patches and disturbed areas were not selected for the study. From each site, a meadow with biomass (15g) was excavated from 2 x 0.25 m^{-2} quadrants following the earlier method[6]. Samples were thoroughly rinsed in seawater to remove all sediment. They were collected in sterile polyethylene labeled bags, frozen on site and transported to the lab and kept frozen at -4°C prior to analysis. The defrosted samples were scrapped with a sterile surgical blade (#11) to remove epiphytes and sections of leaves and root rhizome was rinsed in deionised water. Sub-samples were dried at 50°C to constant weight ground in Agate mortar (Reutch) and sieved in a 0.5mm Nytex mesh to fine homogenous powder.

Sediments were collected by using Van Veen Grab sampler (1000 cm^2) from two quadrants adjoining the meadows samples from the three Kuwait marine sites[5]. Sediments were collected in sterile polyethylene bags and transported to the laboratory. Replicate sediments (20g) from each grab were dried at 50°C in an oven (GallenKamp II) until constant weight. Dried sediments were powdered in the agate mortar, homogenized and sieved in 1.0 mm sieve mesh and stored in sterile vials[4, 6]. Samples (0.2g) were used for trace metal analysis.

H. uninervis leaves, rhizome-root and sediment samples were predigested in aquaregia (Aristar grade HNO_3: HCl-3:1 v/v ratio) in a polystyrene sterile centrifuge tube and left overnight. The sediment samples were treated further in 1% HF for the complete mineralization and digestion. All the samples diluted in de-ionized water (50 ml) and digested in an automatic microwave digester (Spectroprep CEM) was measured in the Analytik Jena, Zeenit-650 to determine the metals concentrations.

Trace metals bio-concentration factor (BCF) in *H. uninervis* was determined by calculating the ratio of metal concentration in the rhizome-roots to that of the sediment as given below:

BCF= Metal concentration in root (μg g^{-1}) / metal concentration in sediment (μg g^{-1}). BCF was categorized further as hyper-accumulators[12] to those samples which accumulated metals above 1000 μg Kg^{-1}. Further, the plant's ability to translocate metals from rhizome to leaves was measured using translocation factor (TF) as given below:

TF= metal concentration in the leaves/ metal concentration in rhizomes. Wherein, TF was categorized as hyper-translocated samples that translocated metals more than 1.

Quality assurance employing replicates, standard trace metals (ICP grade), blanks and standard reference material: Orchard leaves (SRM 1571) for *H. uninervis* and estuarine sediment (SRM 1646A) for sediment samples from National Institute Standard Technology (NIST) assessed the precision of the instrument. Recoveries of samples (90 ±5 %) in agreement with certified values were considered as a part of quality control assessment. Statistically, Pearson's correlation coefficient was used to correlate the significance of trace metals concentrations between the leaves, rhizome and sediment samples.

RESULTS AND DISCUSSION

The mean trace metal concentrations was high in leaves (9.34-68.63 μg $g^{-1}$and 12.32-87.89 μg g^{-1}) followed by rhizome-root (7.35-60.13 μg g^{-1}

Table 1: Trace metal concentrations (ìg/g) in *H. uninervis* parts and surrounding sediment during Winter

Months	Sites	Leaf Tissue				Rhizome tissue				Sediments			
		Cu	Pb	Ni	Fe	Cu	Pb	Ni	Fe	Cu	Pb	Ni	Fe
Nov	Site-I	57.06	16.89	61.91	14.65	39.49	14.20	42.51	7.32	29.51	12.51	38.47	6.80
		±1.97	±1.19	±2.25	±1.02	±2.10	±1.16	±2.09	±0.96	±1.27	±1.18	±2.05	±0.79
	Site-II	74.91	23.34	92.87	15.20	50.21	22.75	91.21	7.97	49.75	20.64	56.32	7.20
		±2.59	±1.24	±4.25	±1.13	±2.13	±1.19	±4.22	±1.02	±1.97	±1.29	±2.15	±0.89
	Site-III	34.61	12.88	34.52	4.48	35.28	11.81	41.14	4.44	14.43	11.08	39.69	4.45
		±1.53	±1.15	±1.63	±0.96	±1.60	±1.14	±1.98	±0.75	±1.02	±1.13	±2.11	±0.67
	Mean	55.53	17.70	63.10	11.44	41.66	16.25	58.29	6.58	31.23	14.74	44.83	6.15
Jan	Site-I	43.46	27.88	76.89	7.75	56.13	31.38	57.18	7.16	26.63	19.62	55.69	6.77
		±2.06	±1.30	±2.92	±1.05	±2.16	±1.56	±2.19	±0.95	±1.28	±1.23	±2.13	±0.78
	Site-II	73.05	29.95	77.10	8.83	76.72	21.22	67.39	8.20	42.32	26.75	66.34	7.97
		±2.45	±1.38	±2.95	±1.09	±2.87	±1.18	±2.26	±1.05	±1.93	±1.32	±2.24	±1.01
	Site-III	39.92	21.12	54.29	5.01	23.03	17.83	55.36	7.13	16.28	12.60	37.54	5.38
		±1.68	±1.21	±2.15	±0.89	±1.20	±1.16	±2.16	±0.87	±1.05	±1.15	±1.76	±0.71
		52.14	26.32	69.43	7.20	51.96	23.48	59.98	7.50	28.41	19.66	53.19	6.71
Mar	Site-I	61.80	28.12	82.54	9.35	49.31	25.29	61.93	7.42	18.10	16.29	50.39	3.42
		±2.20	±1.32	±3.48	±1.12	±2.13	±1.24	±2.21	±0.99	±1.19	±1.21	±2.18	±0.54
	Site-II	79.48	30.16	92.61	11.95	70.99	26.68	92.01	10.01	40.10	25.44	61.85	4.17
		±3.81	±1.41	±4.21	±1.15	±2.28	±1.25	±4.23	±1.10	±1.90	±1.30	±2.21	±0.65
	Site-III	52.10	24.23	44.96	6.88	47.12	19.50	32.41	6.54	17.30	4.96	39.26	3.11
		±2.10	±1.27	±2.09	±0.98	±2.12	±1.17	±1.87	±0.76	±1.15	±0.68	±2.08	±0.46
		64.46	27.50	73.37	9.39	55.81	23.82	62.12	7.99	25.17	15.56	50.50	3.57

Sites I-III: Doha, Duba'iyah, Nuwaseeb, ± values: Standard deviation

Table 2: Trace metal concentrations (µg/g) in *H. uninervis* parts and surrounding sediment during Summer

Months	Sites	Leaf Tissue				Rhizome tissue				Sediments			
		Cu	Pb	Ni	Fe	Cu	Pb	Ni	Fe	Cu	Pb	Ni	Fe
MAY	Site-I	72.76	28.36	97.76	17.89	54.12	16.89	62.65	8.89	26.35	25.78	60.40	6.85
		±2.61	±1.15	±3.86	±0.95	±2.60	±1.12	±2.78	±0.80	±1.40	±1.30	±2.73	±0.65
	Site-II	81.74	30.89	99.95	19.93	71.04	24.32	90.85	8.93	50.92	20.25	75.85	8.51
		±3.10	±1.25	±4.61	±1.04	±2.74	±1.26	±4.20	±0.81	±2.10	±1.50	±2.98	±0.78
	Site-III	60.18	12.87	67.53	6.72	61.35	12.52	57.88	6.69	23.47	11.48	38.20	4.60
		±2.59	±1.12	±2.86	±0.91	±2.60	±1.09	±2.69	±0.68	±1.23	±0.91	±1.61	±0.52
	Mean	71.56	24.04	88.41	14.85	62.17	17.91	70.46	8.14	33.25	19.17	58.15	6.65
JULY	Site-I	74.42	36.42	92.04	10.65	57.07	33.35	76.99	8.77	28.36	19.82	62.97	9.42
		±2.74	±1.58	±4.09	±1.03	±2.46	±1.68	±3.11	±0.79	±1.43	±1.16	±2.86	±0.81
	Site-II	92.37	46.20	105.3	12.58	79.72	38.97	88.79	10.21	43.65	29.06	75.92	8.98
		±4.19	±2.06	±4.76	±1.10	±3.13	±1.72	±3.98	±0.87	±1.98	±1.45	±2.98	±0.79
	Site-III	68.77	35.56	77.70	9.62	59.89	17.85	75.31	7.63	25.56	15.11	41.18	7.55
		±2.78	±1.55	±3.13	±0.87	±2.51	±1.12	±3.08	±0.74	±1.29	±1.10	±1.86	±0.75
	Mean	78.52	39.39	91.68	10.95	65.56	30.06	80.36	8.87	32.52	21.33	60.02	8.65
SEPT	Site-I	74.99	30.52	91.37	11.59	62.31	28.05	83.21	7.30	31.28	17.46	52.83	3.84
		±2.99	±1.32	±3.95	±1.01	±2.68	±1.31	±3.75	±0.72	±1.56	±1.12	±2.76	±0.48
	Site-II	85.37	31.52	93.16	11.98	70.45	28.09	92.43	9.63	41.97	25.51	67.95	4.97
		±3.96	±1.48	±4.10	±1.06	±2.76	±1.35	±4.70	±0.84	±1.95	±1.27	±2.97	±0.69
	Site-III	69.12	25.98	66.24	9.95	59.39	23.41	57.08	7.21	18.51	11.22	41.06	3.82
		±2.85	±1.29	±2.73	±0.98	±2.64	±1.24	±2.50	±0.68	±1.19	±0.97	±1.01	±0.41
	Mean	76.49	29.34	83.59	11.17	64.05	26.52	77.57	8.05	31.59	19.40	53.95	4.14

Sites I-III: Doha, Duba'iyah, Nuwaseeb, ± values: Standard deviation and 8.35-76.13 µg g^{-1}) and sediment (5.47-49.51 and 4.14-53.95 µg g^{-1} µg g^{-1}) during winter and summer respectively (Tables 1-2). Trace metal concentrations in the present study was found higher than the earlier studies in other seagrass [1-3, 10-11], but lower trace metal concentration than the earlier studies [4,6]. Trace metal concentrations in both leaves and rhizome of *H. uninervis* and sediment samples was observed in the sequence of Ni>Cu>Pb>Fe irrespective of the two seasons. The mean bi-monthly analysis showed trace metal concentrations in *H. uninervis* and sediment parts in the sequence of July > September > May > March > January > November. Furthermore, a significant relationship of increasing trace metal concentrations was observed in *H. uninervis* parts (leaves and rhizome) between each bimonthly samples in summer (May-September) and winter (November-March). However, the bimonthly analysis of sediment samples showed varying trace metal concentrations during the two seasons. This attributes to the mobilization of trace metals from sediment to marine flora in the marine environment and supports evidences to the earlier studies [8-9, 12].

Table 3: Pearson's correlation coefficient between trace metals in *H. uninervis* parts and sediment during summer and winter

Samples	L-W	R-W	Sd-W	L-S	R-S
R-W	0.984				
	0.116				
Sd-W	0.980	1.000			
	0.127	0.011			
L-S	0.996	0.963	0.958		
	0.059	0.175	0.186		
R-S	0.963	0.996	0.997	0.934	
	0.174	0.058	0.047	0.233	
Sd-S	0.996	0.995	0.993	0.984	0.982
	0.054	0.062	0.073	0.113	0.120

L: Leaf, R: rhizome, Sd: Sediment, W: winter, S: summer, Cell Contents: Pearson correlation, P-Value in italics

Table 4: Bioconcentration (BCF) and Translocation (TF) factors of trace metals (µg/g) to *H. uninervis* during winter

Months	Sites	Bioconcentration factor (BCF)				BCF	Translocation Factor (TF)				
		Cu	Pb	Ni	Fe	mean	Cu	Pb	Ni	Fe	TF mean
Nov	Site-I	1.33	1.13	1.10	1.07	1.16	1.44	1.18	1.45	2.00	1.52
	Site-II	1.00	1.10	1.61	1.10	1.20	1.49	1.02	1.01	1.90	1.36
	Site-III	2.44	1.06	1.03	0.99	1.44	0.98	1.09	0.83	1.00	1.02
Jan	Site-I	2.10	1.59	1.02	1.05	1.44	0.77	0.88	1.34	1.08	1.14
	Site-II	1.81	0.79	1.01	1.02	1.16	0.95	1.41	1.14	1.07	1.15
	Site-III	1.41	1.41	1.47	1.32	1.40	1.73	1.18	0.98	0.70	1.23
Mar	Site-I	2.72	1.55	1.23	2.16	1.91	1.25	1.11	1.33	1.26	1.12
	Site-II	1.77	1.04	1.48	2.40	1.67	1.11	1.13	1.06	1.19	1.16
	Site-III	2.72	3.93	0.82	2.10	2.39	1.10	1.24	1.38	1.05	1.19

BCF= metals in root/metals in soil, TF= metals in leaves/metals in roots, Sites I-III: Doha, Duba'iyah, Nuwaseeb

Site-wise analysis revealed *H. uninervis* and sediment samples collected from Site-II with high trace metal concentrations followed by Sites-I and III irrespective of the two seasons (Tables 1-2). However, the overall trace metal concentrations were observed high in summer than in winter. Further, Pearson's statistical test revealed significant correlation coefficient between the trace metal concentrations in *H. uninervis* collected during summer and winter (Table 3).

None of the investigated seagrass samples were classified as hyper-accumulators as they showed metal concentrations >1000μg Kg^{-1} in the leaves and supported the observations[10]. However, this species could be labeled for phytoremediation process due to high tolerance limits of this species to trace metal accumulation estimated through BCF and TF. By comparing the BCF and TF we drew conclusions on the ability of the trace metals uptake from soil to rhizome and transfer from rhizome to leaves by *H. uninervis*. A significant relationship existed between BCF and TF. The present study showed an increase in the BCF with decreasing TF and vice versa (Tables 4-5) and supported the earlier findings[2,13]. Analysis showed high TF in Site-I samples during summer (May-September) and winter (November-March) than Sites II and III. Seagrass in Kuwait Bay (Site-I) region showed high trace metals mobilization and bio-concentration when compared to trace metals in seagrass collected from the Kuwait coastal sites. The present study also indicated varying BCF and TF between summer and winter samples thus, indicating that these factors are dependent on seasonal changes in the marine environment. The overall BCF and TF were found high in summer than in winter. Statistical tests by Pearson's correlation coefficient revealed significant BCF and TF between the seasons (summer and winter) respectively (Table 6). The overall TF in the samples were >1, indicating efficient translocation between rhizome and leaves and

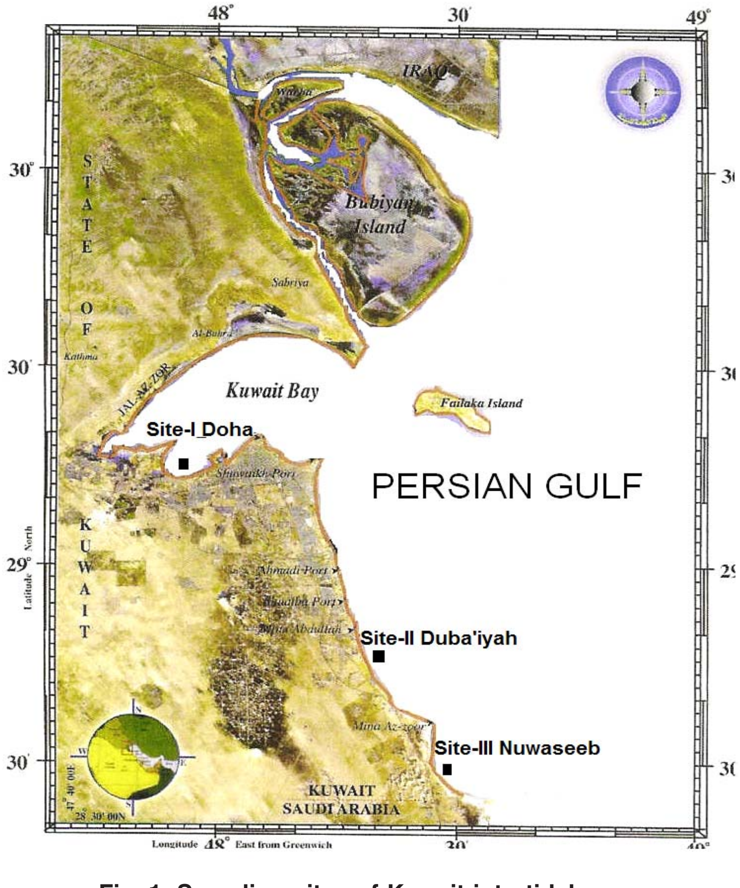

Fig. 1: Sampling sites of Kuwait intertidal zone

Table 5: Bioconcentration (BCF) and Translocation (TF) factors of trace metals (µg/g) to H. uninervis during summer

Months	Sites	Bioconcentration factor (BCF)				BCF	Translocation Factor (TF)				
		Cu	Pb	Ni	Fe	mean	Cu	Pb	Ni	Fe	TF mean
MAY	Site-I	2.13	0.65	1.03	1.28	1.27	1.34	1.67	1.56	2.03	1.65
	Site-II	1.39	1.20	1.19	1.04	1.21	1.15	1.27	1.10	2.23	1.43
	Site-III	2.61	1.09	1.51	1.45	1.66	0.98	1.02	1.16	1.00	1.04
JULY	Site-I	2.01	1.68	1.22	0.93	1.46	1.30	1.09	1.19	1.21	1.20
	Site-II	1.82	1.34	1.16	1.13	1.36	1.15	1.18	1.18	1.23	1.19
	Site-III	2.34	1.18	1.82	1.01	1.59	1.14	1.99	1.03	1.26	1.35
SEPT	Site-I	1.99	1.60	1.57	1.90	1.76	1.20	1.08	1.09	1.58	1.24
	Site-II	1.67	1.10	1.36	1.93	1.51	1.21	1.12	1.00	1.24	1.14
	Site-III	3.20	2.08	1.39	1.99	2.16	1.16	1.10	1.16	1.38	1.20

BCF= metals in root/metals in soil, TF= metals in leaves/metals in roots, Sites I-III: Doha, Duba'iyah, Nuwaseeb

Table 6: Pearson's correlation coefficient between BCF and TF during summer and winter

Samples	BCF-W	TF-W	BCF-S
TF-W	-0.180		
	0.643		
BCF-S	0.922	-0.348	
	0.001	0.359	
TF-S	-0.376	0.915	-0.491
	0.318	0.001	0.180

BCF: bioconcentration factor, TF: translocation factor, W: winter samples, S: summer samples, Cell Contents: Pearson correlation, values in bold: significant correlation, P-Value in italics

supported the earlier studies[2, 13]. The present study showed most samples with BCF and TF >1 to that of the earlier findings[12, 14] indicating the potentiality for phytoextraction of trace metals. Thus, this study revealed that *H. uninervis* can be used as potential tool: (a) to study the pollution levels of intertidal zones, (b) to determine the interrelationship between BCF and TF to seasonal changes and (c) for phytoremediation of contaminated sites in the marine environment.

ACKNOWLEDGMENTS

We express our gratitude to the Research Administration, Kuwait University for the financial support of our project (SC-01/04). We thank the faculty of Science Analytical facility (SAF), Kuwait University, for sample analysis (GS 01/01).

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