Original Article

Trace elements in *Halophila ovalis*, *Halimeda macroloba* and their underlying sediment at Lidee island, Thailand♣

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Abstract

Coastal areas are exposed to anthropogenic contamination from industrial activities, sewage, and coastal development. An evaluation of trace metal and heavy metal concentrations in coastal waters can be made from the elemental analysis of seaweed and seagrass samples but limited use has been made of this approach. Here, the seagrass *Halophila ovalis*, the calcified green macroalga *Halimeda macroloba*, and sediment were collected off Lidee island, Satun province, Thailand, and analyzed with X-ray fluorescence (ED-XRF) spectroscopy. Seventeen trace elements were identified: 16 in *H. ovalis*, 14 in *H. macroloba*, and 15 in sediment. Ca was the most abundant element in sediment (33,955 mg/100 g dw), seaweed (28,333 mg/100 g dw) and seagrass (19,569 mg/100 g dw). Mg, Al, P, K, Ti, Mn, Fe, and Zn were all most abundant in *H. ovalis*. Cr and Mn were not detected in *H. macroloba* but were found in *H. ovalis* and sediment, while Zn was only detected in *H. ovalis*. This is the first study of the trace element contents in seaweed, seagrass and sediment conducted in this area. The results confirm the potential of seaweed and seagrass as bioindicators of trace metal contamination and as mineral-rich food sources.

Keywords: Andaman, green seaweed, seagrass, trace elements

1. Introduction

Coastal areas are subjected to anthropogenic pressure from trace element contamination (Chugh *et al.*, 2022; Howarth *et al.*, 2002; Satheeswaran *et al.*, 2019). The sources of contamination are industrial activities, sewage, nutrient runoff from agriculture and aquaculture, and coastal development (Howarth *et al.*, 2002). Various trace elements can accumulate in seawater and soil or sediment until the contamination becomes toxic to marine animals and halophytes such as seagrasses and seaweeds (Jormalainen & Honkanen, 2004). This contamination can then move up the food chain with biomagnification (Bat *et al.*, 2022). The toxicity of trace element contamination in the marine environment is a great concern, but the persistence and ability of these elements to accumulate within living organisms present additional threats. The trace element contamination may affect halophytes by reducing photosynthesis and growth rate, causing chlorosis, inducing phytotoxicity, and increasing mortality (Jothinayagi & Anbazhagan, 2009; Satheeswaran *et al.*, 2019). The significant risks these contaminants pose to ecosystems can have severe consequences (Ali *et al.*, 2022; Bat *et al.*, 2022).

Previous studies reported that trace elements are not only present in the water column and in sediment, but also in seagrass and seaweed (Chakraborty *et al.*, 2014; Chugh *et al.*, 2022). Consequently, seagrasses and seaweeds have been used as bioindicators to evaluate trace metal contamination and pollution (Bonanno *et al.*, 2020; Jeong & Ra, 2022; Nguyen *et al.*, 2017). In Thailand, the seagrass *Halophila ovalis* is abundant in coastal areas. The calcified macroalga *Halimeda*

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macroloba is also abundant along the coast, providing coral engineering, carbon sequestration, and a food source (Mayakun & Prathep, 2019). *H. macroloba* makes a significant contribution to calcium carbonate production (Mayakun & Prathep, 2019). It can uptake dissolved inorganic carbon, converting the uptake into $CaCO_3$ at the rate of 16.6 mg thallus^{-1} day^{-1} (Mayakun, Bunrak, & Kongsaeng, 2014). *H. ovalis*, meanwhile, sequesters carbon at the rate of 0.56 mg ha^{-1} (Stankovic *et al.*, 2017). Like other halophytes, *H. ovalis* and *H. macroloba* are sensitive to changes in environmental conditions, but the responses these species take to some environmental changes might have negative effects on the marine ecosystem and carbon sequestration.

Effects of pollution on the growth rate of *Halophila* and *Halimeda* species have been reported (Malea *et al.*, 2021; Rattanasonboon *et al.*, 2018). Other studies have revealed that heavy metal accumulation reduced the growth rate of seaweeds, preventing their photosynthesis mechanism (Chugh *et al.*, 2022; Zhang *et al.*, 2020). An earlier study of metal accumulation found that heavy metals affected the growth rate of *Gracilaria tenuistipitata* and reduced the uptake of carbon (Collén *et al.*, 2003). Zhang *et al.* (2020) revealed that Cd disturbed the growth rate of *Sargassum fusiforme*. However, little is known about the effects of trace metal concentrations in *Halimeda* species. Rattansasonboom *et al.* (2018) studied heavy metal contents in *H. macroloba* at Tangkhen Bay, Phuket, Thailand, and showed that Pb and Cd were being taken up by the species. However, *Halimeda* species from different locations have not been studied.

This study is the first to investigate trace elements in *H. ovalis, H. macroloba* and sediment from Lidee Island in the Andaman Sea off the coast of Thailand. This investigation of trace element concentrations aims to assess the potential of seaweed and seagrass as rich mineral sources and bioindicators.

2. Materials and Methods

2.1 Study site

The collection site was located in the Andaman Sea at Lidee island (6° 46'58″N, 99° 46'10″ E), Satun province, Thailand (Figure 1A-B). In this area, there are two distinct seasons: a dry season (November to April) and a rainy season (May to October). Lidee island is a pristine location in Mu Koh Phetra National Park, approximately 6 km from the mainland. The island presents diverse habitats including algal and seagrass meadows, mangrove, and sandy beach. The green alga *H. macroloba* Decaisne was the most common species (Figure 1C) at an average density of 76 thalli m^{-2}. Five seagrass species were found: *Cymodocea rotundata* Ascherson & Schweinfurth, *Enhalus acoroides* (L. f.) Royle, *H. ovalis* (R. Brown) J. D. Hooker, *Syringodium isoetifolium* (Ascherson) Dandy, and *Thalassia hemprichii* (Ehrenberg) Ascherson. *H. ovalis* was the dominant seagrass species (Figure 1D) with a coverage of 47.6 ± 29 % (Kongsap *et al.*, 2023).

2.2 Sampling and analytical procedures

Seaweed and seagrass samples were collected within the intertidal zone of Lidee Island. Around 30 shoots of *H. ovalis* and 30 thalli of *H. macroloba* were randomly collected during low tide in October 2023. All seagrass and seaweed samples were kept in cool dark containers, and taken back to the laboratory where they were kept at -20 °C until analysis. Before analysis, all samples were carefully washed with distilled water to remove epiphytes, small particles, sediment, and salt, and dried in an oven at 50 °C until constant weight was reached. The dried samples were ground to a fine powder. Three sediment samples were collected from each vegetative patch. A plastic core 10 cm in diameter and 30 cm long was used to collect the samples at a depth of 10 cm. All sediment samples were dried at 50 °C, sieved through a 250 μm mesh to retain coarse particles and impurities, and then ground to a fine powder.

All halophyte and sediment samples were sent to the Central Analytical Centre, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand for trace element analysis (one-time analysis). Heavy metals were measured using X-ray fluorescence (ED-XRF) spectroscopy to determine percent contents of elements from oxygen through uranium. The detection limit was 0.01% w/w (the Central Analytical Centre, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand). The results are expressed in mg per 100 g dry weight (dw).

3. Results and Discussion

Seventeen trace elements were found: Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Zn, Br, Sr, and I. Sixteen trace elements were found in *H. ovalis*, 14 in *H. macroloba*, and 15 in sediment. Ca was the most abundant element overall. Ca contents were 33,955 mg/100 g dw in sediment, 28,333 mg/100 g dw in *H. macroloba*, and 19,569 mg/100 g dw in *H. ovalis* (Table 1). The highest concentration of Si was found in sediment. Na concentrations ranged from 458 to 1,412 mg/100 g dw, with the lowest concentration in *H. ovalis* and the highest in *H. macroloba*. The highest contents of Mg, Al, P, K, Ti, Mn, Fe, and Zn were detected in *H. ovalis*. Na, K, Mg, P, and Fe, as well as S, are essential trace elements for plant growth and metabolic activities (De Boer, 1981; Therby-Vale 2005). The heavy metals Cr and Mn were not detected in *H. macroloba* but were found in *H. ovalis* and in sediment at concentrations of 9 and 18, and 10.3 and 9.3 mg/100 g dw, respectively. The heavy metal Zn was not detected in *H. macroloba* and sediment. It was found only in *H. ovalis*. Previous studies have suggested that seagrass and seaweed accumulate trace elements at similar rates (Bonanno & Orlando-Bonaca, 2017), but the results have mostly been species-specific and have shown dependence on environmental conditions.

Notably, the results revealed that the heavy metals Cr and Mn were found only in the seagrass and sediment. *Halophila* can take up Cr through the plasma membrane, using the sulfate transporter (Malea *et al.*, 2021) and Cr (III) is less soluble and mostly accumulates in sediment. However, the interaction between Cr and seagrass is unclear but is crucial to the evaluation of the rate of Cr uptake by seagrass, which will enable the use of the plant as a bioindicator of trace element contamination. The different concentrations of Cr and Mn in the seagrass and sediment may be due to factors such as absorption and accumulation rates, uptake rates, the chemical form of chromium taken up, and the movement and cycling of
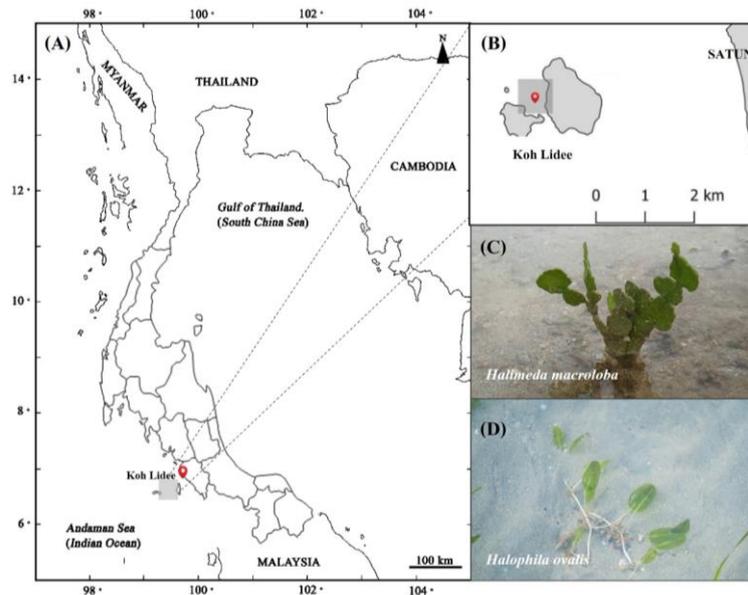
Figure 1. Study site at Lidee island, Mu Koh Phetra National Park, Andaman Sea, Satun province, Thailand (A, B). *Halimeda macroloba* (C) and *Halophila ovalis* (D) are dominant species in this area.

Table 1. Concentrations of elements (mg/100 g dw) in seagrass and seaweed (juvenile and adult) collected from Lidee island, Satun province.

Element	*Halophila ovalis*	*Halimeda macroloba*	Sediment
Na	458	1412	660
Mg	947	287	674
Al	802	74	595
Si	3154	224	4960
P	184	45	23
S	285	450	227
Cl	891	3821	827
K	182	173	138
Ca	19569	28333	33955
Ti	42.6	9	39
Cr	9	ND	10.3
Mn	18	ND	9.3
Fe	9723	46	995
Zn	10	ND	ND
Br	6	85	4
Sr	334	16	553
I	ND	28	ND

ND, not detected

the sediment. Zn was found only in the seagrass. This result implies that the source of Zn in this area is deposition from the atmosphere, which the seagrass takes from the water column and accumulates. Zn can be absorbed and translocated to leaves and stems through passive adsorption in a slow, irreversible uptake (Lyngby *et al.*, 1982).

The results of the present study showed that *H. ovalis* had a greater capacity to accumulate trace elements than *H. macroloba*. This might be because *H. ovalis* has real roots that can take up trace elements from sediment and seawater and bioaccumulate them in leaves, roots and rhizomes. *H. macroloba* has no real roots, so it mainly takes

up trace elements from seawater. Nonetheless, both species can serve as effective bioindicators of trace metal contamination (Jeong & Ra, 2022).

No heavy metals were found in the seaweed from Lidee island. This might be because our study area was within a protected area, and being 6 km from the mainland it is barely impacted by anthropogenic activities. At Tangkhen Bay in Phuket, Rattanasomboon *et al.* (2018) found Pb and Cd in *H. macroloba.* This contamination might have been present because Tangkhen Bay is sheltered and has been exposed to sedimentation, dredging and coastal development (Brown *et al.*, 2019). Moreover, studies conducted at Burung island in Indonesia (Novianty, Herandarudewi, & Suratno, 2017) and Palk Bay in India (Rajaram, Rameshkumar, & Anandkumar, 2020) noted a lot of domestic dumping and sewage, and found traces of Hg, Pb, Cd, Cu and Zn in this species (Table 2).

This study is the first survey of seagrass and seaweed to report heavy metal concentrations determined by ED-XRF spectroscopy. Besides different sources of heavy metal contamination, specific techniques might produce different results. Techniques such as atomic absorption spectrophotometry and inductively coupled plasma mass spectroscopy are commonly used to detect heavy metals, and therefore results obtained using different techniques might provide more definitive results. Nevertheless, our findings indicate that *H. macroloba* and *H. ovalis* could be used to monitor trace metal contamination.

4. Conclusions

A total of 17 trace elements, including Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Zn, Br, Sr, and I, were found in the tropical seagrass *Halophila ovalis*, the calcified green seaweed *H. macroloba*, and their underlying sediment. The heavy metals Cr and Mn were found only in *H. ovalis* and sediment. The seagrass tended to accumulate elements at higher concentrations than the seaweed. Ours is the first report

Table 2.   Reported trace element concentrations (mg/100 g dw) in
            H. macroloba at different locations

Element	Lidee island	Tangkhen bay	Burung island	Palk Bay
	This study	Rattanasomboon *et al.* (2018)	Novianty *et al.* (2017)	Rajaram *et al.* (2020)
Na	1412	71.39	-	-
Mg	287	22.31	-	-
Al	74	-	-	-
Si	224	-	-	-
P	45	24.98	-	-
S	450	-	-	-
Cl	3821	-	-	-
K	173	20.27	-	-
Ca	28333	3923	-	-
Ti	9	-	-	-
Sr	16	-	-	-
I	28	-	-	-
Br	85	-	-	-
Fe	46	-	-	-
Zn	ND	-	-	-
Cr	ND	-	-	-
Mn	ND	-	-	-
Pb	ND	0.028	-	7.36
Cd	ND	0.002	-	0.33
Cu	ND	-	-	7.36
Zn	ND	-	-	8.22
Hg	ND	-	4.22×10^{-3}	-

ND, not detected

of trace metal concentrations in seaweed, seagrass and sediment this area. Based on our results, we concluded that *H. ovalis* and *H. macroloba* have potential as mineral-rich food sources and bioindicators of trace metal contamination. However, the interaction between halophytes and trace elements, and the utility of halophytes as bioindicators require further study.

Acknowledgements

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