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EXPERIMENTAL AND FIELD STUDY ON ACCUMULATION OF HEAVY METALS IN SEAGRASSES (*Halodule pinifolia and Halophila minor*) IN SETIU WETLAND, TERENGGANU

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Abstract: The aims of this study were: (i) to determine the content of copper and cadmium into seagrass *Halodule pinifolia and Halophila minor* from the Setiu Wetland and Setiu River estuary, Terengganu and (ii) to study the bioaccumulation response of the seagrass towards the metals. The contents of the two metals in leaves and root-rhizomes of the seagrasses collected from the field study samples were determined. In laboratory experiments, the two seagrass were exposed to sediments spiked with four different concentrations of copper (56.44 µg/g, 112.87 µg/g, 225.74 µg/g and 451.49 µg/g) or cadmium (36.00 µg/g, 72.00 µg/g, 144.00 µg/g and 288.00 µg/g). The exposure period was for 8 weeks during which plants were sampled weekly and the metal concentrations in leaves and root-rhizomes determined.

The content of heavy metals was measured by AAS. Pearson Correlation were used to determine the relationship between the accumulation of heavy metals in root-rhizomes and leaves. In the exposure experiments, Cu and Cd were bioaccumulated, with tissue concentration generally increased with duration of exposure and increase in sediment metal concentrations. Metal concentrations in root-rhizomes were higher than in leaves for both species. In *Halodule pinifolia*, the mean copper in root-rhizomes and leaves were 120.86 ± 6.79 µg/g and 96.99 ± 8.58 µg/g respectively while the mean cadmium concentration in root-rhizomes was 38.40 ± 4.71 µg/g and in leaves was 36.08 ± 3.21 µg/g. In *Halophila minor,* the mean copper concentration in root-rhizomes and leaves were 49.53 ± 7.67 µg/g and 35.40 ± 6.52 µg/g respectively. The mean cadmium concentration in root-rhizomes was 30.34 ± 3.15 µg/g and in leaves was 29.71 ± 2.64 µg/g. Generally, the metals in root-rhizomes showed strong correlation with the metals in leaves. *Halodule pinifolia* and *Halophila minor* exposed to high concentrations of copper were dead in week 4 and 5 respectively, while exposure to high concentrations of cadmium resulted in death of plants in week 4. Oxygen produced by both seagrass species also began showed negative values in week 4. For the field study, the highest concentrations of Cd and Cu in *Halodule pinifolia* and *Halophila minor* were found for the month of October 2004 and month of November respectively.

KEYWORDS: *Halodule pinifolia; Halophila minor;* Heavy metals; Root-rhizomes; Leaves

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Introduction

Heavy metal are natural constituents in nature, usually occurring in low concentration under normal condition. Pollution of coastal environment by trace and heavy metal contamination may occur via input from point sources (industrial and urban), and diffuse sources (natural run-off and atmosphere deposition) (El-Hasan *et al.*, 2006). Trace and heavy metals contamination of aquatic ecosystem has increased worldwide (Ruangsomboon and Wongrat, 2006). These metal elements deposited in coastal systems can become incorporated into the environment and may influence chemical and biological processes in the water column, sediments and biota (Hart, 1982).

The metals can be classified into two categories i.e. intermediate transition and metalloid. Intermediate transition metals are essential to organism and show their toxicity at high concentration only. For example copper, iron and manganese are essential for plant growth, while metalloid metals are not needed in metabolism and have demonstrated their toxicity at low concentrations of cadmium and lead (Kennish, 1996). These metal are released into environment from a wide range of sources and tend to accumulate in benthic sediment. Sediments are considered as a sink for metal contaminant (Burton *et. al,* 2006)

Toxicity of these metals is stimulated by several factors such as temperature, oxygen, pH and carbon dioxide (Helawell, 1989; Borgman, 1983). Toxicity will increase if environmental temperature increases (Newmann and Jagoe, 1996). In marine sediments, Acid Volatile Sulphide (AVS) has been investigated for some years in relation to biogeochemical cycling (Morse *et al,* 1987) and in relation to metal bioavailability and toxicity. Such studies show that these are seasonal and spatial variation of AVS in both marine and freshwater systems. This variability has been recognized as a problem in applying AVS normalization to metal bioavailability in lake and inter-tidal seasonal where seasonal changes and sediment spatial heterogeneity are also commonplace (Leonard *et al*, 1993).

If littoral sediments became a permanent sink for particle-bound metals, then particle trapping by macrophytes would be an effective way to remove metals from aquatic systems. Subsurface peaks in metal concentrations typically occur in the top 10 cm corresponding to a large portion of the rooting zone of many rooted aquatic macrophytes. Littoral sediments represent a buried reserve of elements that may be exported from the sediments or recycled by rooted aquatic macrophytes. Root uptake of elements with subsequent translocation to above-ground tissues has been shown for a variety of elements, including metals (Jackson *et al.*, 1994), and may be an important vector out of the sediments for elements that would otherwise remain buried. The importance of macrophytes in element cycling depends upon the fate of elements contained within plant tissues. Potential fates include: 1 return to sediments bound to shoot fragments (Carpenter,1983); 2 transfer to attached epiphytes (Wetzel and Manny, 1972; Wium-Andersen and Wium-Anderson, 1972b; Carignan and Kalff, 1982; Jackson, 1992) ; 3 transfer to herbivores by direct grazing (Carpenter, 1980; Lodge, 1991) ; and 4 release to the surrounding water during senescence (Hough and Wetzel, 1975; Carpenter, 1980,1983; Moore *et al.*, 1984). The first fate is an internal loop within the littoral zone. The second and third fates also occur within the littoral zone, but may extend to the pelagic zone if grazers and herbivores migrate between the littoral and pelagic zones. The fourth fate is a source of internal loading to the pelagic zone. Cycling within the littoral zone is likely to be of minimal importance to whole lake metal dynamics, but could be biologically important to invertebrates and grazers within the littoral zone if the metals involved are required trace elements e.g. Fe, Cu or toxic e.g. Pb, Cd. Invertebrates also provide a route of entry to littoral food webs for sediment bound metals that are retrieved from the sediment profile by macrophytes (Jackson, 1998).

Seagrasses are marine angiosperms that colonize near-shore environments that covering about 0.1-0.2 of the global ocean floor (Duarte 2002) and concern has arisen over increasing concentration of metals in the system. Seagrasses perform several important ecological and physical function (Short *et al.*, 2001). Seagrasses most often grow in highly reduced sediments with high concentrations of potentially toxic compounds (Terados *et al.*, 1999; Hemmingga and Durte, 2000). Seagrasses sequester trace metals from the marine environment via both the leaf and root-rhizomes and these concentrations can be correlated with the water column and sediments, respectively (Pulich, 1980; Lyngby and Brix, 1984, Neinhuis, 1986; Ward, 1989). These bindings highlight the potential of these plants as biological indicators of metal contamination. Besides, seagrass, being primary producers, may be utilized as the first level indicator to monitoring trace metal levels in the coastal environments whereby, seagrass ecosystems support important grazing and detrital food webs (Short and Wyllie-Echeverria, 1996). Metals sequestered by seagrasses may be passed through trophic links to higher level consumers, including, dugongs (Dugong dugon) and green turtles (Chelonia mydas) which are the dominant consumers in tropical ecosystems (Lanyon et al, 1989; Denton *et al.*, 1980). Seagrasses also have particular promise in the detection of specific factors that may influence both short-and long-term changes in the near shore aquatic ecosystems. There are fourteen major species of seagrasses recorded in Malaysia. However the most dominant species is *Halodule pinifolia* (Japar *et al.*, 2006)

Heavy metal accumulations within seagrass leaf tissue appear to be influenced more by the levels of biologically available metals within the surrounding water column, rather than by the sediment load, where the heavy metals are generally complexed or precipitated (Batley, 1987; Bond *et al.*, 1988; Ward, 1989). The biological availability of heavy metals is dependent upon a wide diversity of environmental conditions, including the sediment cation exchange capacity, water and sediment pH, redox potential, phosphorus level, water temperature, salinity, organic content, irradiance and the concentration of other heavy metals, especially Fe (e.g. Brinkhuis *et al.*,1980; Wahbeh, 1984; Ward, 1989).

In the photosynthesis process of seagrass, photosystem II photochemical efficiency was used to assess the physiological changes within the plant whereby, free amino acid content and PS II photochemical efficiency may provide early indications of sub lethal toxicity of metals (Prange and Dennison, 2000).

An issue to be considered is the ability to accumulate different specific metals shown by each biological species. Nicolaidou and Nott (1998) showed that *P. caerulea* was the strongest accumulator for Cd, *P. pavonica* for Cr and Pb, *M. turbinata*, *P. oceanica* and *P. pavonica* for Cu, and *P. oceanica* for Zn. *P. oceanica* is located at the base of the food web in the Mediterranean and is thus probably the main source of metals for many animals grazing on its leaves, while *P. caerulea* is a commonly consumed seafood in many Mediterranean countries. Thus, the investigation of trace metal concentrations in the tissues of these species may provide useful information on the transfer of potentially toxic elements from abiotic compartments (water, sediments) to higher consumers, including man (Nicolaidou and Nott, 1998).

In Malaysia, the research on the accumulation of heavy metals in seagrass species and their roles in the biogeochemical cycling of metals in the marine ecosystem is extremely limited and to date no data has been published. Most previous studies on seagrass more to distribution of seagrass.

Material and Methods

Preparation of samples

Clean sediments were collected from the Setiu lagoon at Gong Batu, Terengganu. The sediments were first sieved and left to settle in tanks. Organic carbon, silt and clay contents were analyzed to characterize the sediment. The sediments were then spiked with Cd and Cu solutions separately, prepared from their salts and left for 2 weeks (with periodic stirring) for the metals to bind to the sediment. The spiked sediment were then used as ‘stock” for the preparation of the experimental exposure concentrations of contaminated sediments. Seagrasses were also collected from the Setiu lagoon randomly and left to acclimate to laboratory conditions at UMT in large glass tanks. Ambient light (20, 000 luxs) was provided.

Exposure Experiments

A 5 cm thickness of sediment (control clean sediment and four series of concentrations of spiked sediment) was placed in each aquaria. The series of concentration of sediment which spiked with copper was 56.44 µg/g, 112.87 µg/g, 225.74 µg/g and 451.49 µg/g. The series of concentration of sediment spiked with cadmium was 36.00 µg/g, 72.00 µg/g, 144.00 µg/g and 244.00 µg/g. Two replicates were provided for each metal. The seagrasses were then planted into the sediment in each test aquarium so as to fill up most of the sediment area for their growth. Seawater (35 – 39 ppt and 7.19 – 8.97 pH) are provided by marine hatchery had filled in each aquaria with 28 cm depth from bottom of aquaria.

At 1 week intervals, for 8 weeks, 6 plants with their leaves and root system, were taken from the sediment, rinsed with clean seawater and stored in a freezer until required for analysis.

Morphological Measurement

Morphological characteristics were recorded such as length and colour of leaves. Oxygen produced by seagrasses were measured using BOD meter. Where 6 plants were put in BOD bottle contained seawater for 3 hours. Light also provided. The data were recorded before and after 3 hours.

Monthly field analyses of metals in seagrass

At monthly intervals for 12 months seagrasses were sampled for metal analyses. The purpose of this is to investigate natural variations of metals in the plants and their ambient environment and to relate this to normal physiological development of the plants.

Digestion of seagrass for metal analysis

The samples (0.2 g) were cut and divided into leaves and rhizomes – root and then put in petri dishes and dried in an oven at 95 – 105 ºC overnight. The samples were then kept in a desiccator. Samples of the leaves and roots in beakers or boiling tubes were then digested in nitric and sulphuric acids in a ratio of 0.5:1 w/w basis. For the digestion procedure, the Canadian DOE method for trace metal was followed. The mixture was heated at 80 ºC for 4h in an aluminum – heating block. The digest was then filtered through 0.45 µm Millipore filters to separate particles and diluted to 50 ml with deionised water.

Heavy metal analysis

The heavy metal concentrations in the digests were analyzed using flame AAS. Low concentrations of cadmium was analyzed by graphic furnace AAS. Cadmium did not analyzed using flame AAS because the flame cannot detect the low concentration of metal accurately.

Metal concentration formula:

$$\text{Metal Concentration} = \frac{\text{Dilution Volume (V) (ml) X AAS Reading } (\mu g/ml)}{\text{Sample Dry Weight (g)}}$$

$$= \mu g \text{ of heavy metal / g of dry weight}$$

Preparation of standard reference material (Tomato Leaves 1573a)

As a reference, standard reference material was analyzed in order to check the methodology and to calibrate the measurements. 0.2g tomato Leaves (1573a) from the National Institute of Standard and Technology was prepared in a similar manner to the actual samples and metal concentrations analyzed. The results were used for calculated percentage of recovery by the formula below:

$$\text{Percentage of recovery} = \frac{\text{Reading from AAS x 100}}{\text{Reference reading}}$$

$$= > 80\% \text{ (the measurement can be accepted)}$$

Statistical analysis

Data was analyzed using SPSS (Pearson Correlation design).

Results

Recovery Analysis

Table 1 showed the readings of standard reference material. Percentage of recovery was 91.13%. Hence, all the heavy metal measurement in this study can be accepted

Table 1 : Percentage of Recovery

Heavy metals	**Reference value (µg/g)**	**Reading (µg/g)**	**Percentage (%)**
Tomato Leaves (1573a)			
Cu	71.60+1.6	65.25	91.13
Cd	2.48+0.08	2.26	91.13

Exposure Experiment

Heavy Metals in Seagrass

Concentrations of Cu and Cd in seagrass samples were measured in control before commencing exposure to sediment spiked with for 8 weeks (Table 2). The table showed Cu concentration was higher than Cd concentration in *Halodule pinifolia* and *Halophila minor*.

Table 2 : Metal concentrations in control seagrasses

Heavy metals	**Part of Seagrass**	**Metal Concentration in Seagrass Species (µg/g)**	
		Halodule pinifiolia	***Halophila minor***
Copper (Cu)	Root-rhizomes	8.32	15.12
	Leaves	7.21	12.21
Cadmium (Cd)	Root-rhizomes	0.21	0.15
	Leaves	0.09	0.07

Copper in Halodule pinifolia

The concentration of Cu in *Halodule pinifolia* generally increased with duration of exposure (Table 3, Figure 1). Cu concentrations were generally higher in root-rhizomes than in leaves. Overall, the mean copper concentration in root-rhizomes and leaves of *Halodule pinifolia* were 120.86 ± 6.79 µg/g and 96.99 ± 8.58 µg/g respectively.

Some weekly sample concentration in leaves showed that Cu concentration were higher in leaves than in root rhizomes. In week 1, 4, 5, 6, 7 and 8, the higher concentration in leaves showed in samples which planted in 56.44 µg/g sediment Cu concentration. In week 2 and 3, higher mean Cu concentrations in leaves than in root-rhizomes were found in samples planted in 56.44 µg/g and 225.74 µg/g ; control and 56.44 µg/g sediment Cu concentration respectively. Cu concentration in root-rhizomes and in leaves generally increased with increase in sediment Cu concentration except in week 1 and 2. Whereby, a decrease in Cu accumulated in week 1 and 2 was observed at 56.44 µg/g sediment Cu concentration for leaves and a decrease in Cu accumulated in week 2 was observed with 112.87 µg/g sediment Cu concentration for root-rhizomes.

Table 3 : Copper concentration in *Halodule pinifolia* in week 1 to 8

Week	Part	Sediment Cu Concentration (µg/g)				
		Control	**56.44**	**112.87**	**225.74**	**451.49**
1	Root-rhizomes	7.05± 2.97	20.91± 14.35	46.95± 7.05	97.84± 2.03	266.68± 15.31
	Leaves	5.21± 0.16	31.63± 2.21	25.89± 11.35	31.40± 32.92	153.79± 62.95
2	Root-rhizomes	13.24± 0.12	32.08± 4.38	66.53± 13.97	37.69± 5.53	245.39± 12.73
	Leaves	8.29± 0.04	42.08± 4.91	34.20± 0.87	74.30± 4.24	166.55± 14.32
3	Root-rhizomes	14.26± 1.15	32.40± 9.26	79.00± 3.22	88.40± 12.92	271.13± 14.27
	Leaves	14.36± 0.48	44.58± 5.13	60.13± 4.08	77.85± 6.26	221.48± 9.44
4	Root-rhizomes	14.91± 0.57	33.61± 1.91	93.98± 14.05	97.04± 17.01	312.23± 3.68
	Leaves	14.53± 0.44	46.86± 16.95	69.35± 11.21	84.95± 21.25	221.76± 20.70
5	Root-rhizomes	18.95± 0.02	37.06± 0.03	97.20± 0.00	152.95± 0.03	337.60± 0.00
	Leaves	16.54± 0.07	52.76± 0.01	73.16± 0.02	130.65± 0.02	228.80± 0.00
6	Root-rhizomes	20.36± 4.15	40.90± 20.33	112.31± 2.62	158.63± 35.99	345.34± 0.16
	Leaves	19.53± 1.87	53.18± 0.11	76.06± 8.34	134.60± 11.63	307.39± 26.00
7	Root-rhizomes	22.45± 0.30	47.50± 5.09	114.84± 3.89	180.40± 2.23	424.30 0.87
	Leaves	19.73± 0.35	56.13± 7.92	78.69± 7.62	141.85± 17.75	353.28± 19.87
8	Root-rhizomes	23.66± 0.60	51.25± 9.88	119.25± 7.32	185.60± 2.40	472.53± 19.02
	Leaves	20.59± 1.75	74.59± 1.70	90.00± 1.27	162.94± 1.10	359.89± 6.10

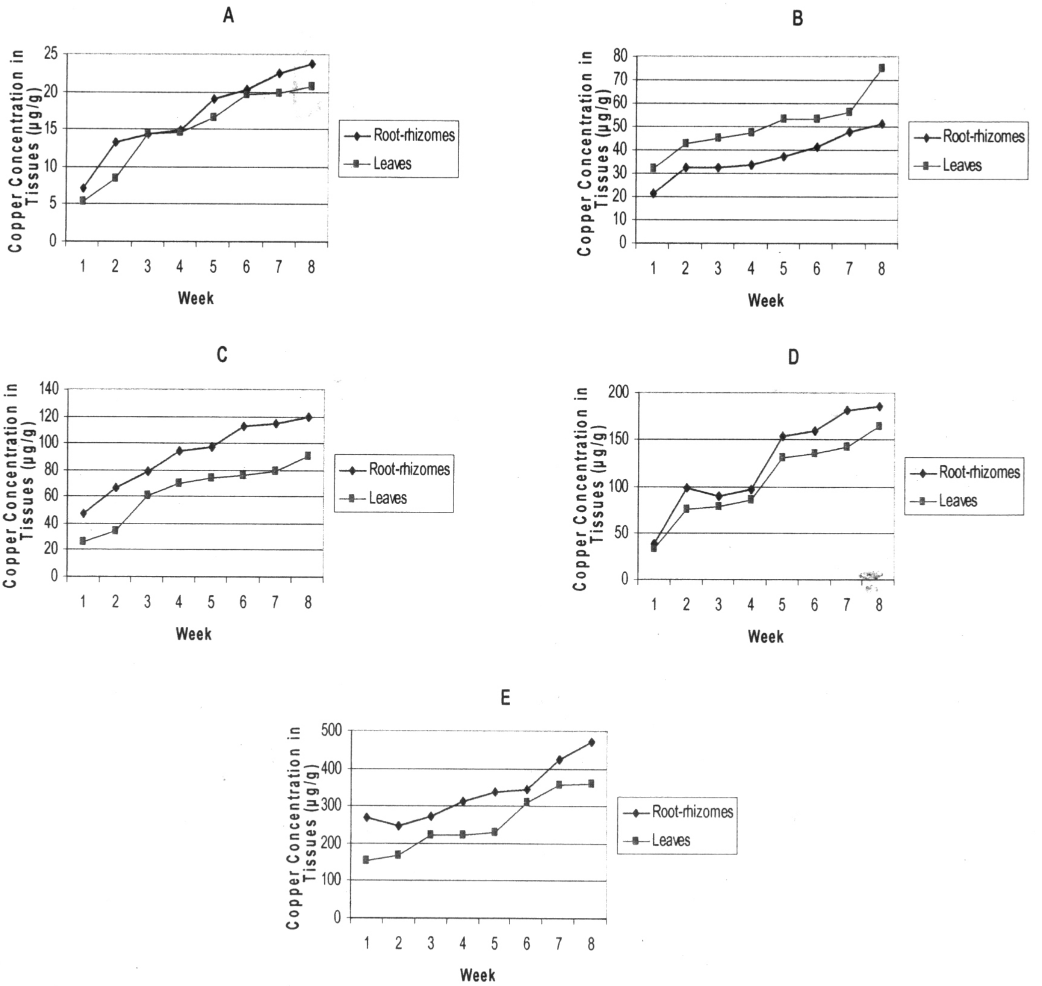


Figure 1: Copper concentration in root-rhizomes and leaves in *Halodule pinifolia* from A (control), B (56.44 µg/g), C (112.87 µg/g), D (225.74 µg/g) and E (451.49) sediment Cu concentration in week 1 until week 8.

Copper in Halophila minor

Copper bioaccumulation in *Halophila minor* varied with duration of exposure (Table 4, Figure 2). Cu concentration were higher in root-rhizomes compared to leaves for *Halophila minor* from week 1 till week 8. Overall, the mean of copper in root-rhizomes and leaves of *Halophila minor* were 49.53 ± 7.67 µg/g and 35.40 ± 6.52 µg/g respectively.

Cu concentration in root-rhizomes and leaves from week 1 till 8 generally did not consistently increased with increase in sediment Cu concentration. A decrease was observed from week 1 till 7 in 56.44 µg/g sediment Cu concentration for both plant part. A decrease also was observed in week 8 in 225.74 µg/g and 56.44 µg/g sediment Cu concentration for root-rhizomes and leaves respectively.

Table 4: Copper concentration in *Halophila minor* in week 1 to 8

Week	Part	Sediment Cu Concentration (µg/g)				
		Control	**56.44**	**112.87**	**225.74**	**451.49**
1	Root-rhizomes	3.58± 3.61	15.63± 5.46	10.18± 4.99	12.03± 3.16	34.70± 4.56
	Leaves	3.45± 3.98	6.01± 8.86	3.96± 5.37	9.70± 7.80	9.55± 8.66
2	Root-rhizomes	5.05± 0.28	31.00± 0.32	15.59± 0.88	21.94± 0.46	35.81± 0.65
	Leaves	4.83± 0.09	24.31± 0.16	10.40± 0.02	12.66± 1.63	30.54± 0.42
3	Root-rhizomes	6.50± 0.19	38.94± 2.16	22.05± 1.75	32.11± 0.37	111.35± 2.05
	Leaves	5.21± 0.18	33.01± 1.11	20.46± 0.81	20.78± 1.11	44.89± 0.65
4	Root-rhizomes	8.44± 0.32	43.04± 11.14	23.54± 1.87	36.64± 5.20	150.36± 14.73
	Leaves	5.73± 0.32	34.21± 13.81	21.21± 5.32	23.79± 4.45	59.06± 13.65
5	Root-rhizomes	13.91± 0.02	50.49± 0.01	24.18± 0.01	39.91± 0.00	158.91± 0.01
	Leaves	6.00± 0.05	38.88± 0.01	23.35± 0.00	31.21± 0.02	110.68± 0.01
6	Root-rhizomes	20.73± 2.14	50.54± 2.17	30.53± 0.88	40.14± 0.21	162.40± 7.83
	Leaves	16.28± 2.63	43.06± 0.02	26.40± 2.58	33.41± 1.96	123.30± 4.53
7	Root-rhizomes	20.74± 0.11	53.33± 12.00	54.55± 0.83	42.83± 1.36	166.68± 1.04
	Leaves	20.16± 0.18	43.51± 0.05	30.55± 0.80	39.96± 7.97	128.00± 24.64
8	Root-rhizomes	24.33± 3.58	58.93± 60.49	75.19± 75.19	63.08± 39.91	171.24± 34.70
	Leaves	21.71± 3.45	52.66± 38.88	52.49± 30.55	55.90± 33.41	134.78± 30.54

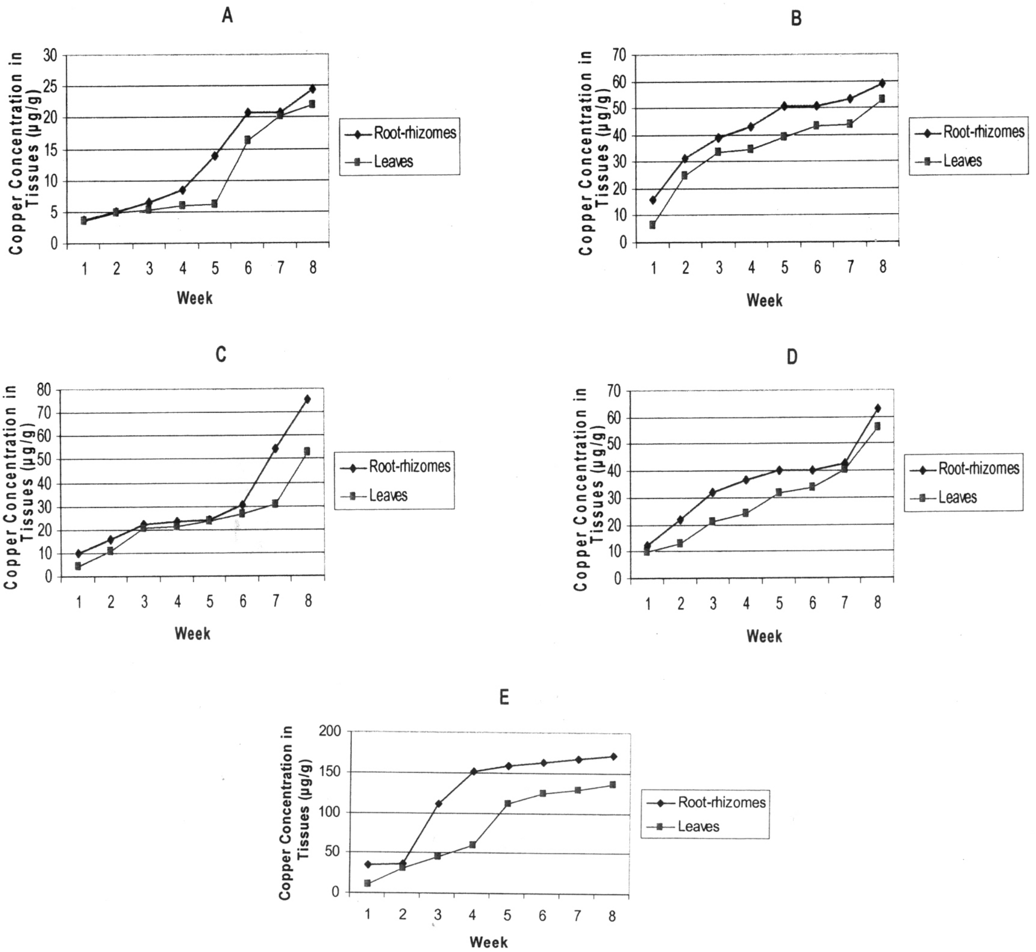


Figure 2: Copper concentration in root-rhizomes and leaves in *Halophila minor* from A (control), B (56.44 µg/g), C (112.87 µg/g), D (225.74 µg/g) and E (451.49) sediment Cu concentration in week 1 until week 8.

Cadmium in Halodule pinifolia

The concentration of Cd in *Halodule pinifolia* generally increased with duration of exposure (Table 5, Figure 3). Cd in *Halodule pinifolia* was higher in root-rhizomes than in leaves with mean value 38.40 ± 4.71 µg/g and 36.08 ± 3.21 µg/g respectively.

Some weekly samples had shown higher concentration of Cd in leaves than in root-rhizomes. In week 1, *Halodule pinifolia* only samples in 36.00 µg/g and 288.00 µg/g sediment Cd concentration showed that Cd concentration in leaves higher than in root-rhizomes. In week 2, concentration of Cd higher in leaves than in root-rhizomes in control , 36.00 µg/g and 72.00 µg/g sediment Cd concentration. In week 3, most samples showed higher concentration in leaves than in root-rhizomes except samples which planted in 144.00 µg/g sediment Cd concentration. In week 4 showed the higher concentration of Cd in leaves than in root-rhizomes were observed in control, 36.00 µg/g and 72.00 µg/g sediment Cd concentration. While in week 5, the higher concentration of Cd in leaves than in root-rhizomes were observed in 36.00 µg/g, 72.00 µg/g and 288.00 µg/g sediment Cd concentration. In week 6, only samples in 72.00 µg/g and 288.00 µg/g sediment Cd concentration showed that Cd concentration in leaves higher than in root-rhizomes. In week 7, only samples in control and 72.00 µg/g sediment Cd concentration showed that Cd concentration in leaves higher than in root-rhizomes. By contrast, in week 8 most samples showed higher concentration in root-rhizomes than in leaves except in 288.00 µg/g sediment Cd concentration.

Cd concentration in root-rhizomes and leaves generally increased with increase in sediment Cd concentration except in week 1, 6 and 7 where the decreased of Cd accumulated by root-rhizomes was observed in 36.00 µg/g sediment Cd concentration.

Table 5: Cadmium concentration in *Halodule pinifolia* in week 1 to 8

Week	Part	Sediment Cd Concentration (µg/g)				
		Control	**36.00**	**72.00**	**144.00**	**288.00**
1	Root-rhizomes	3.63± 1.24	3.63± 6.72	13.25± 6.89	19.00± 21.74	44.63± 19.95
	Leaves	2.75± 0.18	4.00± 0.35	5.63± 7.42	11.13± 0.88	46.38± 1.41
2	Root-rhizomes	3.63± 1.59	7.88± 4.07	17.25± 12.73	33.25± 3.89	44.88± 24.22
	Leaves	6.38± 1.94	8.25± 1.41	8.63± 5.83	12.00± 3.89	51.75± 13.44
3	Root-rhizomes	4.88± 0.53	15.25± 0.53	18.25± 2.47	37.13± 4.60	50.75± 8.49
	Leaves	6.38± 1.41	16.63± 0.35	23.38± 0.18	35.13± 0.18	56.75± 7.87
4	Root-rhizomes	6.13± 1.06	19.13± 0.35	20.25± 2.12	48.88± 1.77	64.13± 6.19
	Leaves	6.75± 0.00	22.75± 1.59	30.00± 0.35	38.13± 6.19	61.00± 1.41
5	Root-rhizomes	8.88± 3.54	26.50± 1.94	30.13± 2.83	69.13± 2.83	67.25± 6.36
	Leaves	7.00± 0.18	27.88± 0.53	30.25± 6.19	41.75± 8.66	97.75± 0.53
6	Root-rhizomes	9.25± 1.94	31.50± 0.53	30.13± 4.77	74.00± 3.18	83.25± 0.71
	Leaves	7.13± 1.06	29.63± 5.83	33.00± 3.89	46.50± 5.48	110.25± 2.12
7	Root-rhizomes	10.00± 2.30	35.38± 3.18	35.13± 0.88	76.00± 6.54	119.50± 7.07
	Leaves	10.13± 0.18	31.13± 3.01	36.50± 6.01	67.13± 3.18	110.63± 7.78
8	Root-rhizomes	11.13± 0.11	37.13± 1.38	71.13± 3.19	113.25± 3.18	121.75± 0.65
	Leaves	10.88± 1.87	33.25± 3.69	68.25± 7.21	76.38± 3.93	114.00± 0.87

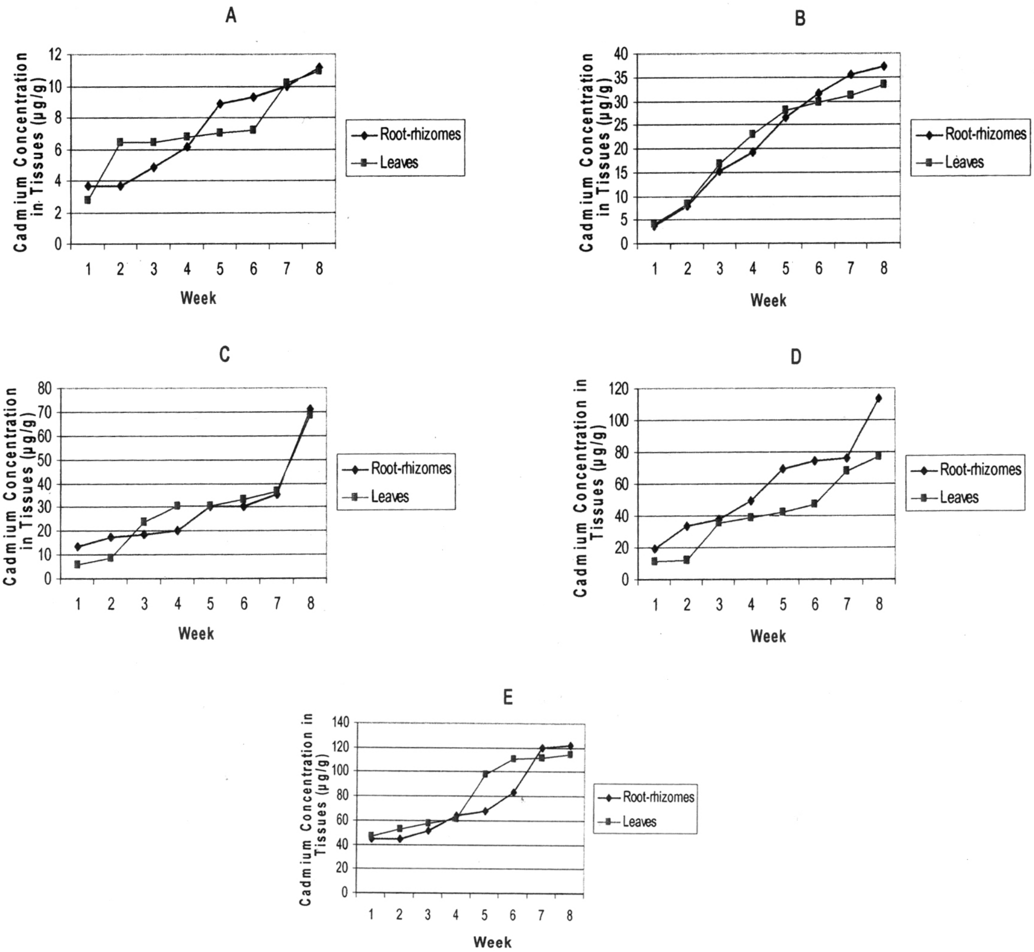


Figure 3: Cadmium concentration in root-rhizomes and leaves in *Halodule pinifolia* from A (control), B (36.00 µg/g), C (72.00 µg/g), D (144.00 µg/g) and E (288.00 µg/g) sediment Cu concentration in week 1 until week 8.

Cadmium in Halophila minor

The concentration of Cd in *Halodule pinifolia* generally increased with duration of exposure (Table 6, Figure 4). Cd in Halophila minor were generally higher in root-rhizomes than leaves with mean value 30.34 ± 3.15 µg/g and 29.71 ± 2.64 µg/g respectively.

In week 1, most samples showed higher concentration in root-rhizomes than in leaves except samples which planted in 72.00 µg/g sediment Cd concentration. In week 2 and 3, Cd concentration was higher in root-rhizomes than in leaves showed in control and 72.00 µg/g sediment Cd concentration. In week 4, the higher concentration in leaves were observed in control, 36.00 µg/g and 144.00 µg/g sediment Cd concentration. In week 5, most samples showed higher concentration in leaves than in root-rhizomes except in 288.00 µg/g sediment Cd concentration. In week 7 and 8 most samples showed higher concentration in leaves than in root-rhizomes except samples which planted in control and 144.00 µg/g sediment Cd concentration; control and 36.00 µg/g sediment Cd concentration respectively.

Cd concentration in root-rhizomes and leaves generally increased with increase in sediment Cd concentration except in week 1 and 2. In week 1, only Cd concentration in root-rhizomes decreased at 36.00 µg/g and 144.00 µg/g sediment Cd concentration. In week 2, Cd concentration in root-rhizomes decreased at 36.00 µg/g sediment Cd concentration.

Table 6: Cadmium concentration in *Halophila minor* in week 1 to 8

Week	**Part**	**Sediment Cd Concentration (µg/g)**				
		Control	**36.00**	**72.00**	**144.00**	**288.00**
1	Root-rhizomes	2.75± 0.53	13.38± 6.72	6.13± 1.59	14.63± 1.41	14.13± 2.83
	Leaves	1.25± 0.71	4.63± 1.77	12.88± 3.89	13.13± 4.24	13.88± 5.66
2	Root-rhizomes	4.63± 0.00	13.63± 0.53	11.38± 0.53	21.25± 2.65	22.75± 2.83
	Leaves	5.25± 0.18	11.25± 0.00	16.00± 1.41	20.63± 1.77	21.88± 1.06
3	Root-rhizomes	5.00± 0.18	13.63± 0.18	14.75± 0.88	21.63± 3.71	24.63± 8.84
	Leaves	5.38± 0.71	12.75± 0.18	18.25± 0.35	21.25± 0.18	23.50± 5.83
4	Root-rhizomes	5.75± 3.18	14.13± 6.19	21.13± 5.30	21.88± 3.54	43.88± 4.77
	Leaves	6.38± 4.42	16.25± 5.48	19.38± 3.89	23.50± 0.18	24.88± 2.12
5	Root-rhizomes	6.13± 1.94	19.75± 0.18	22.50± 1.41	23.38± 5.13	54.13± 4.42
	Leaves	6.63± 0.18	20.25± 1.41	22.75± 1.24	26.00± 2.47	34.75± 7.07
6	Root-rhizomes	7.25± 1.24	22.38± 1.24	22.50± 5.13	34.75± 13.08	56.00± 3.89
	Leaves	7.00± 0.53	26.38± 1.94	28.00± 5.66	30.88± 5.48	36.75± 0.71
7	Root-rhizomes	9.88± 1.41	32.00± 2.83	70.75± 2.12	86.63± 4.77	98.00± 3.54
	Leaves	7.88± 1.41	33.38± 3.01	72.00± 4.95	84.63± 4.24	101.25± 8.13
8	Root-rhizomes	13.63± 0.64	39.63± 4.49	72.88± 1.08	104.50± 7.88	106.00± 3.32
	Leaves	9.00± 0.10	37.38± 0.49	74.50± 7.84	105.00± 4.35	131.75± 0.32

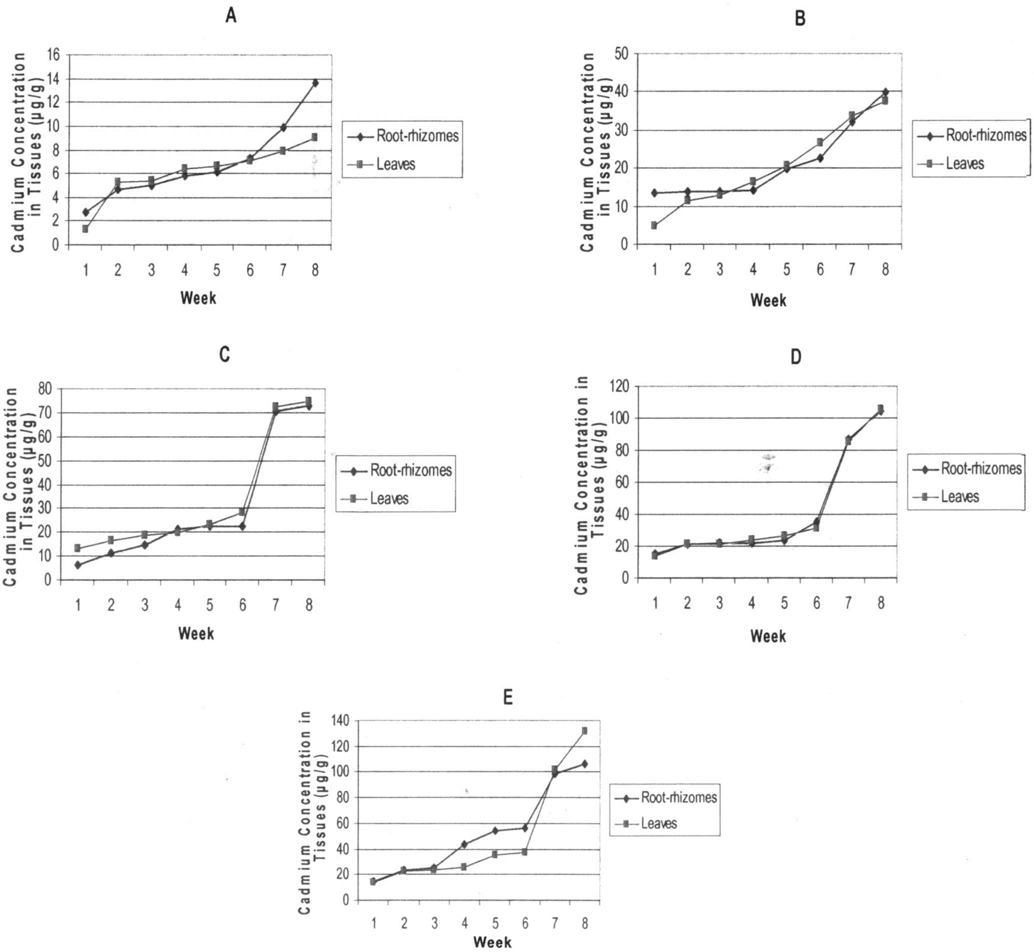


Figure 4: Cadmium concentration in root-rhizomes and leaves in *Halophila minor* from A (control), B (36.00 µg/g), C (72.00 µg/g), D (144.00 µg/g) and E (288.00 µg/g) sediment Cu concentration in week 1 until week 8.

Correlation between Metal Concentration in Root-rhizomes and Leaves

Root-rhizomes Cu concentrations of *Halodule pinifolia* were strongly correlated with leaves Cu concentrations from week 1 untill week 8 (Table 7). While, most Root-rhizomes Cu concentrations of *Halophila minor* were strongly correlated with leaves Cu concentrations except in week 1. Mostly, root-rhizomes Cd concentrations of *Halodule pinifolia* were strongly correlated with leaves Cd concentrations except in week 2, 5 and 6. While, root-rhizomes Cd concentrations of *Halophila minor* were strongly correlated with leaves Cd concentrations except in week 1, 4 and 6.

Table 7: Correlation between copper and cadmium concentrations in root-rhizomes and leaves in week 1 untll week 8

Week	Copper		Cadmium	
	Halodule pinifolia	***Halophila minor***	***Halodule pinifolia***	***Halophila minor***
1	0.991**	0.696	0.970**	0.521
2	0.983**	0.969**	0.819	0.934*
3	0.995**	0.905*	0.987**	0.970**
4	0.994**	0.950*	0.955*	0.841
5	0.989**	0.995**	0.800	0.919*
6	0.992**	0.999**	0/870	0.877
7	0.996**	0.987**	0.998**	0.999**
8	0.994**	0.993**	0.956*	0.983**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Morphological Characteristics and Photosynthesis

The morphological characteristics (color and size) and photosynthesis (oxygen produced) of seagrass species exposed with Cu and Cd for 8 weeks are presented in Table 8. The amount of oxygen produced from *Halodule pinifolia* and *Halophila minor* were decreased with increase of sediment metal concentration. Generally, the amount of oxygen produced showed a decreasing pattern with increase in exposure time.

In the Cu exposure study, *Halodule pinifolia* showed the negative value of oxygen produced on week 4 till 8. At this time, the color of leaves become yellow-green and the size of leaves decreased with exposure time. For *Halophila minor*, the negative value of oxygen produced were recorded on week 5 till 8. At this time, the color of leaves become transparent and the size of leaves decreased by exposure time and increasing of sediment metal concentration.

In the Cd exposure study, the negative value of oxygen produced were recorded in week 4 till 8 for both species. At this time, the leaves color of *Halodule pinifolia* had changed from green to dark-brown and leaves color of *Halophila minor* had changed from brown to transparent. Besides, the size of leaves for both species decreased with exposure time and increasing of sediment metal concentration.

Table 8 : Morphological characteristics (color and size of leaves) and photosynthesis (oxygen produced) of *Halodule pinifolia* and *Halophila minor* which exposed to heavy metals (Cu, Zn, Cd, Pb) from week 1 till week 8

Week	Species	Tanks*	Copper			Cadmium		
			Color of Leaves	**Size of Leaves (cm)**	**O_2 mg/L**	**Color of Leaves**	**Size of Leaves (cm)**	**O_2 mg/L**
1	*Halodule pinifolia*	5.00	green	15.43	0.74	green	15.10	0.48
		4.00	green	14.77	1.18	green	15.55	0.93
		3.00	green	15.63	1.56	green	14.31	2.18
		2.00	green	15.30	1.76	green	15.67	0.38
		1.00	green	14.97	1.97	green	15.90	1.29
	Halophila minor	5.00	brown	0.57	1.03	brown	0.52	0.30
		4.00	brown	0.67	0.91	brown	0.55	0.50
		3.00	brown	0.65	1.24	brown	0.49	0.57
		2.00	brown	0.75	0.87	brown	0.64	0.59
		1.00	brown	0.72	1.24	brown	0.66	0.92
2	*Halodule pinifolia*	5.00	green	15.28	0.55	green	14.32	0.80
		4.00	green	14.92	0.68	green	15.11	0.93
		3.00	green	15.28	0.80	green	15.62	0.02
		2.00	green	15.13	0.87	green	15.58	0.60
		1.00	green	14.97	1.27	green	16.20	1.88
	Halophila minor	5.00	brown	0.63	0.15	brown	0.55	0.35
		4.00	brown	0.68	0.18	brown	0.54	0.57
		3.00	brown	0.67	0.51	brown	0.61	0.45
		2.00	brown	0.72	0.88	brown	0.60	0.66
		1.00	brown	0.70	1.05	brown	0.67	0.94
3	*Halodule pinifolia*	5.00	green	15.27	0.83	green	14.00	1.15
		4.00	green	14.85	1.09	green	14.21	1.65
		3.00	green	15.65	1.36	green	14.53	1.70
		2.00	green	15.90	1.90	green	15.66	0.85
		1.00	green	14.92	2.76	green	15.70	2.80
	Halophila minor	5.00	brown	0.63	0.12	brown	0.51	0.20
		4.00	brown	0.62	0.30	brown	0.56	0.70
		3.00	brown	0.55	0.37	brown	0.63	1.00
		2.00	brown	0.68	0.46	brown	0.66	0.80
		1.00	brown	0.75	1.35	brown	0.70	1.35
4	*Halodule pinifolia*	5.00	yellow-green	13.22	-3.11	dark brown	11.00	-0.56
		4.00	yellow-green	11.85	-3.11	dark brown	11.36	-0.95
		3.00	yellow-green	15.75	-3.04	dark brown	13.21	-0.50
		2.00	yellow-green	15.78	-3.79	dark brown	14.20	-0.22
		1.00	yellow-green	16.42	-0.76	dark brown	15.90	-1.49
	Halophila minor	5.00	transparent	0.62	-1.50	transparent	0.50	-0.13
		4.00	brown	0.57	1.19	transparent	0.55	0.49
		3.00	brown	0.68	1.91	transparent	0.60	-0.17
		2.00	brown	0.70	4.25	transparent	0.63	-0.89
		1.00	brown	0.70	5.00	transparent	0.63	0.06

Week	Species	Tanks*	Copper			Cadmium		
			Color of Leaves	Size of Leaves (cm)	O^2 mg/L	Color of Leaves	Size of Leaves (cm)	O^2 mg/L
5	*Halodule pinifolia*	5.00	yellow-green	14.33	-1.76	dark brown	12.29	-2.05
		4.00	yellow-green	14.97	-1.69	dark brown	13.32	1.10
		3.00	yellow-green	16.30	-1.26	dark brown	13.69	-0.50
		2.00	yellow-green	16.68	-1.22	dark brown	14.72	-0.15
		1.00	green	16.78	1.49	dark brown	15.07	1.30
	Halophila minor	5.00	transparent	0.47	-1.75	transparent	0.37	-4.35
		4.00	transparent	0.52	-1.01	brown	0.32	5.35
		3.00	transparent	0.58	-0.68	brown	0.39	5.60
		2.00	brown	0.55	0.20	transparent	0.42	0.00
		1.00	brown	0.63	0.38	brown	0.51	0.85
6	*Halodule pinifolia*	5.00	yellow-green	14.38	-2.22	dark brown	12.32	-2.55
		4.00	yellow-green	13.63	-1.54	dark brown	12.72	-0.10
		3.00	yellow-green	15.58	-1.41	dark brown	13.48	-2.05
		2.00	yellow-green	15.78	-1.31	dark brown	14.04	-0.95
		1.00	yellow-green	15.72	-1.04	dark brown	15.01	-1.25
	Halophila minor	5.00	transparent	0.45	-0.77	transparent	0.31	-8.05
		4.00	transparent	0.52	-0.73	transparent	0.33	-5.05
		3.00	transparent	0.58	-0.47	brown	0.43	-2.85
		2.00	transparent	0.60	-0.42	transparent	0.51	-6.35
		1.00	transparent	0.62	-0.37	brown	0.61	-0.05
7	*Halodule pinifolia*	5.00	yellow-green	13.92	-1.91	dark brown	11.42	-1.25
		4.00	yellow-green	12.68	-1.86	dark brown	11.21	-0.15
		3.00	yellow-green	14.63	-1.70	dark brown	12.37	-0.45
		2.00	yellow-green	15.38	-1.54	dark brown	13.26	-1.20
		1.00	yellow-green	13.92	-1.18	dark brown	14.99	0.60
	Halophila minor	5.00	transparent	0.48	-3.49	transparent	0.37	-6.80
		4.00	brown	0.50	-1.42	transparent	0.32	-3.35
		3.00	transparent	0.50	-1.00	transparent	0.42	0.10
		2.00	transparent	0.60	-0.31	transparent	0.50	-4.70
		1.00	brown	0.58	1.27	transparent	0.57	3.45
8	*Halodule pinifolia*	5.00	yellow-green	13.27	-2.32	dark brown	11.45	-0.40
		4.00	yellow-green	13.15	-2.58	dark brown	11.24	-0.10
		3.00	yellow-green	15.10	-4.54	dark brown	12.21	-2.00
		2.00	yellow-green	15.07	-3.11	dark brown	13.33	-1.20
		1.00	yellow-green	14.77	-4.85	green	13.35	1.40
	Halophila minor	5.00	transparent	0.40	-2.78	transparent	0.37	-5.70
		4.00	transparent	0.45	-2.13	transparent	0.33	-1.80
		3.00	transparent	0.48	-0.68	transparent	0.41	-7.90
		2.00	transparent	0.55	-0.47	transparent	0.43	-4.60
		1.00	transparent	0.57	-0.64	brown	0.51	8.00

* Tank 1, 2, 3, 4 and 5 refer to difference concentration of sediment Cu concentration. The value are different between selected metals.

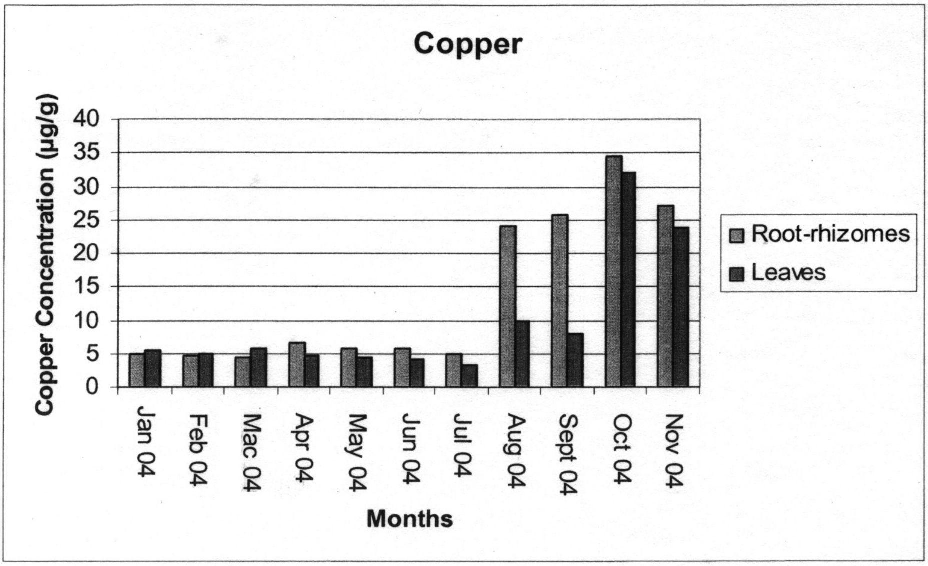
Cu – 1 (control), 2 (56.44 µg/g), 3 (112.87 µg/g), 4 (225.74 µg/g), 5 (451.49 µg/g)

Cd – 1 (control), 2 (36.00 µg/g), 3 (72.00 µg/g), 4 (144.00 µg/g), 5 (288.00 µg/g)

Field Study

Copper and Cadmium in Halodule pinifolia

Based on our result on Figure 5, the descending rank of selected metal was Cu > Cd. In root-rhizomes, the mean of Cu was 13.51 µg/g and Cd was 0.13 µg/g. While in leaves, the mean of Cu was 9.65 µg/g and Cd was 0.08 µg/g. The metals showed higher concentration in root-rhizomes than leaves.



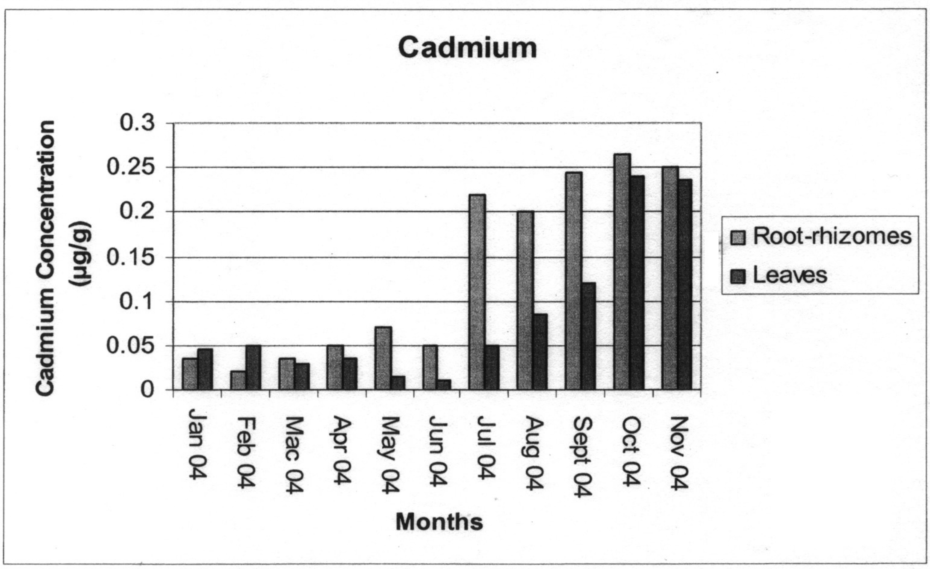


Figure 5: Metal concentration in *Halodule pinifolia* from January 2004 until November 2004

Copper and Cadmium in Halophila minor

Based on Figure 6, Heavy metals in Halophila minor also showed the same ranking with *Halodule pinifolia* which was Cu > Cd. In root-rhizomes, the mean of Cu was 0.75 µg/g and Cd was 0.11 µg/g. While in leaves, the mean of Cu was 0.65 µg/g and Cd was 0.11 µg/g. The metals showed higher concentration in root-rhizomes than leaves.

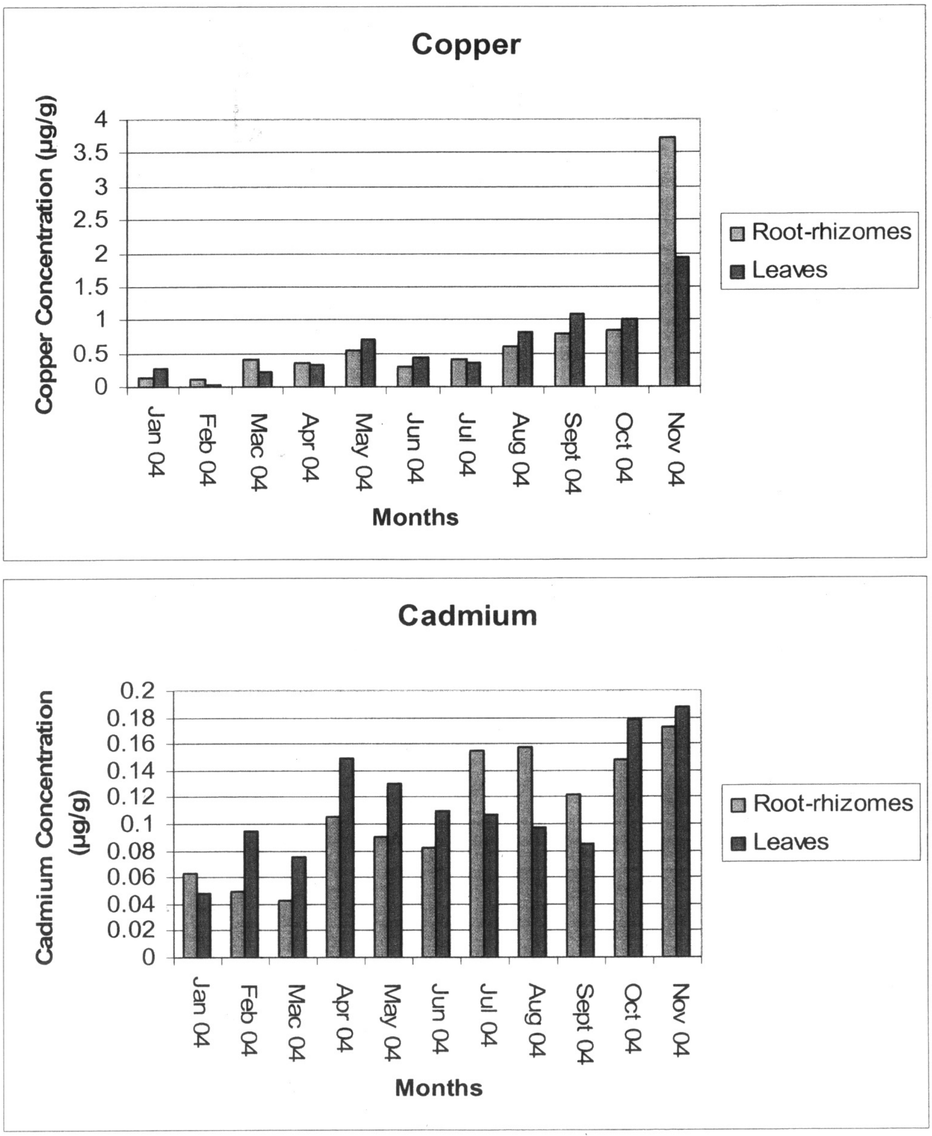


Figure 6. Metal concentration in *Halophila minor* from January 2004 until November 2004

DISCUSSION

General Discussion

Metal concentrations in seagrasses are influenced by several factors. According to Ledent *et. al.* (1995) metal concentration shows a significant decrease with age, where metal concentration tended to be higher in young leaves than in adult leaves. This is because, in adult leaves, part of the mineral content has been re-translocated or leached. Besides, mature leaves require few additional nutrient for growth, however and therefore they mainly serve as conduits for nutrient supply to young, growing leaves (Stapel *et al.*, 1997). Manzenera *et al.* (1995) and Mills and Fonseca (2003) reported significant mortality of shoots of the seagrass in response to experimental over sedimentation even at moderate burial.

Metals are actually not accumulated at levels much above those required for normal cell functioning but that there generally is a fairly close coupling between internal metal pools and leaves growth (Hemminga and Duarte, 1999). According to the Free Ion Activity Model (FIAM) proposed by Morel (1983), the interaction between heavy metals and aquatic organisms should be explained through chemical reaction of free and weakly complexed ionic species with geological membranes (Morel, 1983). Beside that, the concentration patterns in marine organisms are often varying specifically as can be predicted by toxicokinetic modeling (Luoma and Rainbow, 2005). As a result, different species of seagrass has greatly varying metal concentrations in the same body of ocean water (Kahle and Zauke, 2003). Besides, according to Anderson and Morel (1978) in their study on phytoplankton, heavy metals are commonly inhibitors of phytoplankton growth.

Seagrass are able to absorb nutrients and heavy metal both from water and sediment and to translocate some of these elements among the different parts of the plant (William, 1984; Lynby and Brix, 1984). Nevertheless, detailed studies on the nutrient uptake are lacking but as in other plants active process is involved in plant transportation (Hemminga and Duarte, 1999) or lack of metal absorption to fixed or soluble chelators in the root or perhaps due to exchange with the Ca, Mn and Zn moving through the roots (John, 1976). The chelators in nutrient solutions can help in metal uptake (Francis and Rush, 1974). The active process, requires the involvement of cellular metabolism for the provision of chemical energy. Besides, conventional wisdom holds the accumulation of heavy metals in marine and estuarine operates via passive and active process including types of ion-exchange through binding to surface polysaccharides (Veroy *et al.*, 1980). The degree of upward translocation is dependent on the species of plant, the particular metal and a number of environment conditions (Judith and Peddrick, 2004). In study on heavy metals by phytoplankton, normally the uptake of heavy metals occurs in two stages which are short term uptake which involves physical absorption on cell surface and long term uptake which involves intracellular accumulations (Ruangsomboon and Wongrat, 2006). Metal concentrations often higher in sediment. so, it would be seem to make it obvious that the root system plays the dominant role in metal uptake (Hemminga and Duarte, 1999). According to Jalali and Khanlari, (2006), in their research on terrestrial plant, high loadings of metals in soil may cause increase in plant uptake of the metals. At the same time, at high concentrations, it is phytotoxic and may reduce the productivity of the land (Jalali and Khanlari, 2006). In seagrass the metal absorption not only occur in root-rhizomes but also in leaves. This is one of the advantage of seagrass compared to terrestrial plants. These both parts of seagrass have special anatomy to allow heavy metals or nutrient absorption. Leaves of seagrass have greatly in thickness and anatomy, from bearing only 2 cell layers in thin (91µm) (Enriquez *et al.*, 1992). This are of great functional significance for they influence the mechanical properties of the leaves (Cf-Niklas, 1992, 1994). Leaves cuticle also is porous or perforated. So that, metals can be transferred (Kuo and Mc-Comb, 1989). Leaves readily take up metals when bathed in incubation media containing these compounds, whereas the root-rhizomes complex has been shown to take up metals. This double capacity for metal is a general feature and occurs in subtropical and tropical species (Hemminga and Duarte, 1999). Besides, the metal uptake by the leaves is concentrations dependent and the flux of metals from the water column to the vegetation due to leaves uptake is thus most probably less important in metal poor water than in water with higher metal loads (Hemminga and Duarte, 1999). According to our result shows that both metal content was higher in root-rhizomes than in leaves. This is because, the seagrass more exactly reflect the bioavailable metal level in the sediment (Sanchiz *et al.*, 1999) and the roots have been considered as the main organ for nutrient/ metals uptake (Hartin and Thorne Miller, 1981; Wear *et al.*, 1999). The same result also showed in study toward *Zostera marina*, whereby, the study has proved that root play a large role in metals uptake (Pederson and Borum, 1992). Otherwise, the full range between the extremes of metal uptake dominated either by roots or by leaves can probably be found in two other studies. First, study on *Phyllospodix torrey* and *Amphibolis antarctica*, whereas the species can grow on rocky substrates without or little sediment around. The result showed that metal uptake rates in these plants are take placed by the leaves (Pedersen *et al.*, 1997; Terrados and William, 1997). Second, study on *Zostera marina* whereas, these plants developed from seeds in early spring and may had a relatively high leaves to roots biomass ratio (Hemminga and Duarte, 1999). While, according to Stapel *et al.* (1996) in their study on *Thallassia hemprichi*, if no mass flow of pore water occurred in the sediment and the supply of the metals thus would be dependent on the diffusion from the pore water to the root, its mean that the metal uptake would primarily be determined by the diffusion limitation and not by the uptake capacity of the roots (Hemminga and Duarte, 1999). Recently, evidence has been found that in the seagrass rooting zone, the opposite process may also occur whereby, the root system release the metals (Jensen *et al.*, 1998). That's may caused the higher concentrations in leaves than root-rhizomes as certain results that we found in this study. Low nutrient availability also one of the factor, the higher concentrations higher in root-rhizomes than leaves (Short *et al.*, 1985) as in terrestrial plants. It is likely that the plants thereby increase their capacity to acquire metals from the sediment pool (Hemminga and Duarte, 1999).

In terrestrial plants, can establish absorption from soil to root, leaves or fruits (Wasserman, 1989). On the other hand direct leaf absorption from atmospheric spray has been observed. Although metal absorption in marine phanerogams can occur through leaves and through root, the rate vary with the consider metal (Wasserman1 and Masserman2, 2002). However, the type of metal and its chemical form are important in determining the part way it will be enter in plant (Ernst, 1987), after being absorbed, the phanerogams are able to transport the metals through tissues, metabolically placing it where the plant mostly need it (Schroeder and Thorhaug, 1980). In seagrass plants, the lose metals/ nutrients at high rates is implied as short leaves longevity. Thus, have rely more strongly on uptake of metals from environment than many terrestrial angiosperm do (Hemminga and Duarte, 1999). As long as connections are intact, the metals are transported from one point to another in seagrass. Finally, the nutrient uptake capacity of both leaves and root-rhizomes is advantageous for the acquisition of nutrient, but the physiological efficiency of nutrient used by seagrass compares unfavorably with that of many terrestrial angiosperms (Hemminga and Duarte, 1999). In this study, we found that some metal concentrations in seagrass increased by exposure time in spite of during senescence. This is because of metals resorption efficiency of plant leaves. Whereby, the resorption in defined as the amount of metal resorbed during senescence and is expressed as the percentage reduction of metals between mature green and senescent leaves (Aerts, 1996). These metals from senescing tissues resorpted by young growing tissues (Hemminga and Duarte, 1999). Even so, the resorption efficiencies of seagrasses are very low compared with those of perennial terrestrial plants. However, metals resorption from senescing leaves apparently is not a pronounced conservation strategy in seagrass (Hemminga and Duarte, 1999). According to Peterson

(1971), some marine organisms accumulate nonessential metals in their tissues to high concentrations or essential metals at concentrations much higher than required. However, in most cases the reason for the accumulation are not known (Neff, 2002).

Metals introduced in soluble or colloidal forms into estuarine and coastal marine water tend to either precipitate or absorb to suspended particulate matter and colloidal or dissolved organic matter (Salomons and Fostner, 1984). These precipitate, absorbed or complexed metals may be deposited in more or less labile, bioavailable form in surficial sediments. Based on this fact, it proved that most metal concentrations from our finding were higher in surficial (upper layer) than other layer of sediment due to upper layer of sediment tested rich with suspended particle and organic matter. The organic matter come from dead root-rhizomes and leaves. This fact is supported by Hemminga and Duarte (1999). These results were similar to result observed by Jalali and Khanlari (2006). Hinz and Selim (1994) discussed the processes of heavy metal interaction with soils including adsorption on clay and organic matter with very high-binding energies. The increasing solubility of the metals with dissolved organic matter and colloids that expected from solubility product of sulfides (Lyons and Fitzgerard, 1983) whereas, metal speciation in sediment layers controlled by sulfide that produced by microbial oxidation of organic matter to form metal sulfides. The metal sulfides may be mobile and bioavailable in the sediment. The mobility of metal sulfides depends not only on the total concentration in soil / sediment but also soil / sediment properties, metal properties and environmental factors (He *et al.*, 2004). Beside, the diagenic reactions in sediment layers may result of the deposited metals back into the overlaying water column or into sediment pore water (Neff, 2002). According to Tsai *et al.* (2002), the concentrations of heavy metals in pore water tended to fall as depth increased. The distribution of heavy metals in sediment is reportedly influenced by the force of adsorption onto and desorption from sediment-associating matter.

From our finding, seagrasses which exposed with heavy metals mostly lost their pigment in week 4. Oxygen produced values also showed negative values whereby, the seagrass lost their capability in photosynthesis as a result of pigment disappearance. Thus, roots have not received enough oxygen supply cause of the damage to root system. This damage fact had been supported by Hemminga and Duarte (1999) whereby according to him, oxygen important to support root respiration. He also suggesting that heavy metals may lead to a decrease photosynthesis performance.

Copper

Various nonpolar or cationic forms of metals including essential metals such as Cu, penetrate cell membranes more easily than anionic, polar forms (Sunda, 1994). Because of this, the most bioavailable forms of Cu are the inorganic hydroxide complexes ($CuOH^+$ and $Cu(OH)_2$, $Cu(OH)_3$ and $Cu_2(OH_2)$) (Zamuda and Sunda, 1982). Besides, the free ion (Cu^{2+}) also is bioaccumulated rapidly in marine phytoplankton, apparently by an active transport or facilitated diffusion mechanisms (Phinney and Bruland, 1994). Dissolved reactive Cu is toxic to marine plants and animals. Free ionic Cu at concentration as low as 0.3 µg/L decreases production on several species of oceanic phytoplankton (Brand *et al.*, 1986).

Cu as an essential micronutrient for all plants or animals is actively bioaccumulated from the ambient medium. However, tissue concentrations are tightly regulated until concentrations in ambient medium get very high Cu in food is likely to pose a hazard to mobile marine animals in the vicinity to offshore produced water discharges. Because Cu is an essential micronutrient, most marine organisms have evolved mechanisms to control concentration of the free ion in tissues in the presence of variable concentrations in the ambient water, sediment and food. Besides, concentrations in apparently healthy marine organisms from clean environment vary widely, it is difficult to identify a Cu concentrations in soft tissues of marine organisms that is associated with adverse biological effects. Cu regulation may break down when ambient concentrations rise to high level (Neff, 2002)

Concentrations of Cu in various tissues of marine organisms vary seasonally, probably due to seasonal changes in the requirement for the essential macronutrient in different tissues (Neff, 2002)

The concentrations of dissolved Cu found in laboratory test to cause lethal and sublethal effects in marine organisms are in the range of Cu concentrations frequently encountered in ocean waters and particularly in estuary near center of human population (Neff, 2002) there was more than 90% of the total dissolved Cu in natural marine waters is complexed to dissolved organic ligands and is not readily bioavailable and is not vary toxic to marine organisms. Most of the unbound Cu in solution is complexed with various inorganic ligands than vary in their bioavailability and toxicity to marine organisms (Neff, 2002). However, the laboratory tests do indicated that it may be possible to encounter dissolved Cu concentrations in coastal and estuarine waters that are high enough to be toxic to some sensitive marine organisms (Neff, 2002).

From our laboratory study, we found that Cu concentrations in *Halodule pinifolia* were 129.86 ± 6.79 µg/g (root-rhizomes) and 96.99 ± 8.58 µg/g (leaves). Cu concentrations in *Halophila minor* were 49.53 ± 7.67 µg/g (root-rhizomes) and 35.40 ± 6.52 µg/g (leaves). While from our field study, we found that Cu concentrations in *Halodule pinifolia* were 13.57 µg/g (root-rhizomes) and 9.65 µg/g (leaves). Cu concentrations in *Halophila minor* were 0.75 µg/g (root-rhizomes) and 0.65 µg/g (leaves). Concentrations of Cu in tissues of marine organisms from throughout the world showed Green Algae (*Caulerpa toxifolia*) from French, Mediterranean contain 2.0 – 5.6 µg/g (Gnassia Barelle *et al.*, 1995). Red Algae from Greece contain 2.2 – 407 µg/g of Cu (Malae *et al.*, 1994). Brown Algae (*Fucus vesiculosus*) from Greenland contain 1.3 – 3.3 µg/g of Cu (Riget *et al.*, 1995). While Cu concentrations in seagrass (*Posidonia oceanica*) from NW, Mediterranean were 7.9 – 22.0 µg/g (Warnau *et al.*, 1995b). If compared with our results, Cu concentrations from ours for both species from laboratory study showed the concentrations were higher than three species above except Red Algae. In field study, Cu concentration in *Halodule pinifolia* was higher than Cu concentration of *Caulerpa toxifolia* and *Posidonia oceanica* only. While Cu concentration in *Halophila minor* was lower than Cu concentration of three species above. In other study recoded that marine plants from food web of the Caleasieu estuary, Louisiana and the Texas outer continental shelf contain 2.8 – 500 µg/g of Cu. Cu concentrations are lower in phytoplankton and microalgae than in periphyton and seagrass. This is because of phytoplankton are less sensitive than other oceanic species toward Cu. In research by (Neff, 2002) on Posidonia oceanica from the Northwestern, Mediterranean contain up to 22 µg/g of Cu. This finding was quite same with our results.

In determination of short - term Cu toxicity in microalgal by Yu *et al.* (2005), they found that with longer exposure periods (8, 24h), esterase activity decreased as Cu concentration increased. At 24h Cu exposure, this inhibition was substantial. Besides, Cu began to inhibit pigment concentration when the esterase activity of M. aeruginosa disappeared (after 24h exposure; >21 µg/L). from our finding, the green pigment of *Halodule pinifolia* had changed in week 4 exposure. While the brown pigment of Halophila minor had changed in week 5 exposure.

In week 2, 5 and 7 oxygen produced showed negative relationship with copper uptake by root-rhizomes and leaves of *Halodule pinifolia* (Table 9). In week 3, oxygen produced also showed negative relationship with copper uptake by root-rhizomes of Halophila minor. Thus, the increase of copper uptake by root-rhizomes or leaves or both these parts resulted in decreased of oxygen released during photosynthesis.

In week 2, size of leaves showed negative relationship with copper uptake by root-rhizomes and leaves *of Halodule pinifolia*. Thus, the increase in copper uptake by root-rhizomes or leaves resulted in the decrease of seagrass growth especially leaf size.

Nevertheless, Health Standards for Cu concentration in fishery product consumed by men vary from one country to another and range from 50 – 500 µg/g dry wt (Melzian, 1990).

Table 9: Correlation between copper uptake by seagrasses with morphological characteristic (size) and photosynthesis (oxygen produced)

Week	Parameter	*Halodule pinifolia*		*Halophila minor*	
		Root-rhizomes	Leaves	Root-rhizomes	Leaves
1	Oxygen Produced (mg/L)	-0.702	-0.711	-0.702	-0.711
	Size (cm)	0.349	0.467	0.349	0.467
2	Oxygen Produced (mg/L)	-0.991**	-0.975**	-0.991**	-0.975**
	Size (cm)	-0.000**	-0.983**	-0.000**	-0.983**
3	Oxygen Produced (mg/L)	-0.960**	-0.962**	-0.960**	-0.962**
	Size (cm)	0.248	0.303	0.248	0.303
4	Oxygen Produced (mg/L)	-0.877	-0.856	-0.877	-0.856
	Size (cm)	-0.724	-0.691	-0.724	-0.691
5	Oxygen Produced (mg/L)	-0.967**	-0.931*	-0.967**	-0.931*
	Size (cm)	-0.795	-0.828	-0.795	-0.828
6	Oxygen Produced (mg/L)	-0.812	-0.798	-0.812	-0.798
	Size (cm)	-0.712	-0.626	-0.712	-0.626
7	Oxygen Produced (mg/L)	-0.992**	-0.981**	-0.992**	-0.981**
	Size (cm)	-0.216	-0.137	-0.216	-0.137
8	Oxygen Produced (mg/L)	0.762	0.719	0.762	0.719
	Size (cm)	-0.568	-0.540	-0.568	-0.540

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Cadmium

The concentrations of Cd in the whole tissues of marine plants generally fall in the range of 0.001 – 277 µg/g dry wt. This concentrations increased with increasing trophic level (Neff, 2002). From another laboratory studies, suggest that ambient concentrations of dissolved Cd in seawater may be high enough in some contaminated marine environments to cause harm to marine organisms (Neff, 2002). Whereby, short exposure to high concentration of dissolved Cd in the laboratory may lead to accumulation of high concentrations of labile Cd in tissues, whereas chronic exposure to low concentrations of Cd in water and food in the natural environment may lead to high tissues residues of Cd, most of which is tightly bound in a nontoxic form to tissue macromolecules (metallothionein) or granules (Neff, 2002). Therefore, it is possible that benthic communities in some areas with Cd contaminated sediments are adversely affected by the Cd in the sediments. So, Cd in sediment near platform is unlikely to harm local bottom living marine communities (Neff, 2002).

Dillon and Gibson (1985) reviewed the published scientific literature on relationships between toxic responses (mostly sub lethal effects on reproduction) and tissues residues of metals and organic contaminants in fresh water and marine organisms. Tissues residues in whole fish and invertebrates ranging from 3.5 – 33, 077 µg/g of Cd are associated with adverse effects on reproduction (Neff, 2002).

Based on recent publication about concentrations of Cd in the whole tissues of marine plant from throughout the world which summarized by Neff (2002), Cd concentrations in phytoplankton in the range of 0.04 – 4.6 µg/g dry wt, Cd concentration s in macroalgae was 0.1 – 29.8 µg/g dry wt and Cd concentration in seagrasses was 1.0 – 4.9 µg/g dry wt. Compared to concentration of Cd in our laboratory study, concentrations of Cd above were lower than ours. While Cd concentration in our field samples were lower than those species. Whereby Cd concentration in Halodule pinifolia from our laboratory study and field study were 38.40 ± 4.71 µg/g (root-rhizomes) ; 36.08 ± 3.21 µg/g (leaves) and 0.13 µg/g (root-rhizomes); 0.08 µg/g (leaves) respectively. While, concentrations of Cd in Halophila minor in both studies were 30.34 ± 3.15 µg/g (root-rhizomes); 29.71 ± 2.64 µg/g (leaves) and 0.11 µg/g (root-rhizomes); 0.11 µg/g (leaves) respectively.

From our finding above, Cd concentrations in root-rhizomes for laboratory study was higher than leaves. In this study, Cd was accumulated in the Halodule pinifolia to significant level mainly from the sediment having concentration of Cd higher than 72 µg/g. Experiments with *Halophila minor* showed that Cd was more readily accumulated showing an increase with exposure time.

Bioconcentration of Cd by marine plant is dependent as the concentrations of dissolved Cd and the ration of Cd to phosphorus in the ambient medium (Kudo *et al.*, 1996). Besides, the accumulation is more rapid in sunlight than in the dark, suggesting that Cd bioacumulation is at least partly energy dependent (Neff, 2002).

In week 2, oxygen produced showed negative relationship with cadmium uptake by root-rhizomes of *Halophila minor* (Table 10). Thus, the increase in cadmium uptake by root-rhizomes or leaves or both these parts resulted in the decrease of oxygen released.

In week 2 and 5, size of leaves showed negative relationship with cadmium uptake by root-rhizomes of *Halodule pinifolia*. While in week 4 and 6, size of leaves showed negative relationship with cadmium uptake by root-rhizomes of *Halodule pinifolia*. In week 7, size of leaves showed negative relationship with cadmium uptake by root-rhizomes and leaves of Halodule pinifolia and root-rhizomes of *Halophila minor*. In week 3, size of leaves showed negative relationship in zinc uptake by root-rhizomes and leaves of *Halodule pinifolia* and *Halophila minor*. In week 8, size of leaves showed negative relationship with cadmium uptake by both plant parts of *Halodule pinifolia* and Cd uptake by leaves of *Halophila minor*. Thus, the increase in cadmium uptake by root-rhizomes or leaves or both part of seagrass resulted in the decrease of seagrass growth especially leaf size.

Table 10: Correlation between cadmium uptake by seagrasses with morphological characteristic (size) and photosynthesis (oxygen produced)

Week	Parameter	*Halodule pinifolia*		*Halophila minor*	
		Root-rhizomes	Leaves	Root-rhizomes	Leaves
1	Oxygen Produced (mg/L)	-0.209	-0.146	-0.671	-0.804
	Size (cm)	-0.480	-0.571	-0.803	-0.514
2	Oxygen Produced (mg/L)	-0.522	-0.841	-0.918*	-0.815
	Size (cm)	-0.885*	-0.723	-0.707	-0.838
3	Oxygen Produced (mg/L)	-0.542	-0.659	-0.878	-0.917*
	Size (cm)	-0.963**	-0.917*	-0.960**	-0.988*
4	Oxygen Produced (mg/L)	-0.427	-0.550	-0.021	-0.272
	Size (cm)	-0.947*	-0.964*	-0.819	-0.980*
5	Oxygen Produced (mg/L)	-0.589	-0.725	-0.218	-0.425
	Size (cm)	-0.975**	-0.863	-0.842	-0.720
6	Oxygen Produced (mg/L)	-0.430	-0.280	-0.793	-0.781
	Size (cm)	-0.937*	-0.938*	-0.945	-0.812*
7	Oxygen Produced (mg/L)	-0.618	-0.641	-0.719	-0.754
	Size (cm)	-0.953*	-0.965**	-0.882*	-0.875
8	Oxygen Produced (mg/L)	-0.194	-0.309	-0.447	-0.586
	Size (cm)	-0.951**	-0.893*	-0.817	-0.887*

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Field Study

Halodule pinifolia : Cd concentration in root-rhizomes seems like increased with month. However Cd concentration in leaves showed a decrease in April and increase on Jun. A big differences between Cd concentration in root-rhizomes and in leaves apparently observed from May to September. The increasing of Cu concentration did not show apparently in January to July. However a big differences of Cu concentration in root-rhizomes and leaves observed from August to September.

Halophila minor : Only Cu concentration seems like increased with monthly sampling. Mostly, concentration in root-rhizomes higher than leaves. However concentration of Cd, Cu showed in root-rhizomes were very high than concentration in leaves (big differences). For Cd, most monthly samples showed a big differences between Cd concentration in root-rhizomes and leaves. For Cu, samples from November showed a big differences between Cu concentration in root-rhizomes and Cu concentration in leaves. However the highest peak of Cd and Cu uptake by seagrass achieved on November.

December was a senescence period for seagrass. So, no samples to study. The senescence caused by unsuitable conditions (monsoon period). However, the seagrasses began to grow on January. Thus, the metal also began absorpted by seagrasses and accumulated in the tissues until November.

Measurements of metals in the seagrasses collected from the lagoon throughout the year showed that most of metal showed higher concentration in root-rhizomes than leaves. In other studies aquatic plants has been shown to uptake metals via its leaves as suggested by Drifmeyer *et al.* (1980) for copper in *Z. marina*. According to Neff (2002), marine plants absorb 2.8 to nearly 500 µg/g Cu.

The metal concentrations in root-rhizomes and leaves of *Halodule pinifolia* and *Halophila minor* decrease in order : Cu > Cd. In C. nodosa (Nicolaidou and Nott, 1998) and *Zostera marina* (Brix and Lyngby, 1983) the metal concentration decrease in the same order. While Tsai *et al.* (2002) result towards four selected metals, where the metals decrease in order: Cu (16.1 µg/g) > Cd (0.095 µg/g). Concentrations of metals in various tissues of seagrasses vary seasonally due to seasonal changes in the requirement for this essential micronutrient in different tissues (Neff, 2002). According to Erftemeijer and Lewis III (2006), seagrass response to such increases may depend on typical local conditions and vary between seasons. Otherwise, uptake in the field, is not only dependent on the ambient nutrient concentration, but also on flow rates, affecting the thickness of the diffusion boundry layer and the nutrient supply rate. This may affect uptake by both the leaves and the roots (Hemminga and Duarte, 1999).

Non essential metal may be accumulated to high concentrations in the tissues of organisms from natural sources in the environment (Simkiss and Taylor, 1989). The continuous accumulation of metals from apparently natural concentration in the environment may result in a positive correlation between metal concentration in the tissues and the size (mass) of the organisms (Hemminga and Duarte, 1999). While, several of the essential trace nutrient metals are considered important environment contaminants because of their toxicity and potential to be mobilized by man activities (Iyengar, 1991).

In the field, all the metals with potential of becoming pollutants are present at trace or ultratrace concentrations in seawater and all are present at high concentration in uncontaminated marine sediment and the tissues of marine organisms (Neff, 2002).

This study shows that concentrations in *H. pinifolia* and *H. minor* show major concentration changes in the field and that they readily bioaccumulate these metals in sediment exposure experiments. Presumably these two seagrasses play a role in the biogeochemical cycling of metals in the Setiu lagoon and it would be of benefit to carry out further studies to elucidate the function of aquatic plants in tropical lagoons with respect to the metals.

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