Metals in Tropical Seagrass—Accumulation of Mercury and Lead

Faridahanim Ahmad, Shamila Azman, Mohd Ismid Mohd Said, Lavania-Baloo, Shaikhah Sabri and Salmiati

Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

Abstract: Accumulation of mercury and lead were analysed in seven tropical seagrass species: Enhalus acoroides, Halophila minor, Halophila spinulosa, Halophila ovalis, Thalassia hemprichii, Halodule uninervis and Cymodocea serrulata at Pulai Estuary, Johor Straits, Malaysia. Analyses of all seagrass samples were carried out using Perkin Elmer Atomic Absorption Spectrophotometer Model AAnalyst 400 and Pearson’s correlation coefficients of metal concentrations were determined using SPSS version 16. The concentrations of Hg and Pb in different tissues of the seagrass are in the range of 0.1-69 µg/gDW and 81-249 µg/gDW respectively. There are significant correlations between the accumulation of Pb in roots-rhizomes, rhizomes-leaves and leaves-roots for Enhalus acoroides, Halophila minor, Halophila ovalis, Thalassia hemprichii and Halodule uninervis. Meanwhile for Hg, there are significant correlations among plant parts for Halophila ovalis. Halophila ovalis shows ability in translocation of metals and is capable to absorb metals at high concentration. Therefore Halophila ovalis is selected as the best bioindicator.

Key words: Seagrass • Metal accumulation • Bioindicator • Mercury • Lead

INTRODUCTION

Coastal ecosystem is the interface where land meets ocean and is the most productive and valuable aquatic ecosystem for marine biodiversity and recreation [1-3]. Aside from fisheries, economic growth can be generated from the development of ports and petrochemical resources [4]. Due to increased demands on coastal resources as well as human population growth, coastal ecosystems are more intensely than ever exposed to a wide variety of pollutants such as domestic, industrial and agricultural contaminants, from both atmospheric deposition and point sources [5-7]. Heavy metals can naturally occur in aquatic systems with no ill effect towards aquatic life however industrial development has raised heavy metal concentrations drastically in aquatic ecosystems that is even threatening to human health [8]. Aquatic ecosystems can assimilate heavy metals in response to environmental availability of such metals in seawater and marine sediment [9]. Colonies of seagrasses as a community in aquatic ecosystems can help by reducing metal contamination, act as a buffer zone and provide habitat and food for aquatic animals like fish, dugongs and green turtles [10].

Seagrasses are rooted angiosperm marine plants belonging to the subclass monocotyledoneae. They are widely distributed in large areas known as seagrass beds [11-14]. Seagrass beds play key ecological roles in coastal ecosystems as the most productive plant communities that supply food, nursery and shelter to a wide variety of marine organisms such as sea cucumbers, starfish and seahorses [15-18]. Direct observation of seagrass is becoming a method to determine the overall health of a given aquatic environment [19-21]. The objectives of this study are (1) to determine the accumulations of Hg and Pb in seven tropical seagrass species (2) to determine the correlation analysis for metal content (Hg and Pb) in seagrass tissues and (3) to determine the potential of seagrass to be used as bioindicator.
Step 1: Collection of seagrasses. Sample of seagrasses placed in sealed plastic bags and kept in a portable cooler with crushed ice.

A) Samples will be processed after collection

B) Stored at -20ºC

Seagrass sample thawed at room temperature.

Step 2: Quick rinse the seagrass with tap water.

Step 3: Remove the epiphytes by gently scraping with a razor or with the edge of a glass.

Step 4: Separation of upper and lower tissues: roots, rhizomes and leaves.

Step 5: Dry seagrass samples at room temperature or below 60ºC until a constant weight is achieved.

Step 6: Obtain the dry weight.

Step 7: Grind seagrass samples into homogenous powder using agate mortar.

Step 8: Samples were digested and analyzed using Perkin Elmer Atomic Absorption Spectrophotometer Model AAnalyst 400.

Fig. 1: Location of study area

Fig. 2: Steps taken from collection to analysis of seagrass samples
Table 1: Code names of seagrasses.

<table>
<thead>
<tr>
<th>Code name</th>
<th>Species of seagrass</th>
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<tbody>
<tr>
<td>Ea</td>
<td>Enhalus acoroides</td>
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<tr>
<td>Hm</td>
<td>Halophila minor</td>
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<td>Hs</td>
<td>Halophila spinulosa</td>
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<td>Th</td>
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<tr>
<td>Hu</td>
<td>Halodule uninervis</td>
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<td>Cs</td>
<td>Cymodocea serrulata</td>
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**MATERIALS AND METHODS**

**Sampling Location:** Pulai River Estuary is the waterway that separates Malaysia from Singapore and where a seagrass bed can be found. The seagrass bed in Pulai Estuary is the largest in Malaysia with an approximate area of 3.15 km² and during low tide, the seagrass bed takes the appearances of a football field [22]. Figure 1 illustrates the location of Pulai River, Pulai River Estuary, seagrass bed and development projects around the area.

**Sampling Preparations, Sampling Method and Metals Analyses:** Weather, temperature, wind direction, wind speed, height of wave and monsoon seasons have to be taken into consideration before sampling was carried out. Details were obtained from the official portal of Malaysian Meteorological Department, Ministry of Sciences, Technology and Innovation. The seagrass bed only appears during low tide where the tide level is between 0 - 0.65 m. Seagrass samples were collected from July, 2011 to March, 2012.

Seagrass samples were digested after collection where 0.3g of homogenous seagrass powder were transferred into Pyrex tubes with Teflon closure and added with 5ml HNO₃. It is then kept for 1 hour in a water bath at 100°C, cooled to room temperature, filtered with 0.2μm nylon membrane and diluted in 15 ml deionized water. Metal analyses of all seagrass samples were carried out using Perkin Elmer Atomic Absorption Spectrophotometer Model AAnalyst 400. Figure 2 shows the steps taken from collection of seagrass until it is analyzed. Pearson’s correlation coefficients of metal concentrations were determined using Statistical Package for the Social Science (SPSS version 16).

Seven species of tropical seagrasses were found at the seagrass bed. They were identified as *Enhalus acoroides*, *Halophila minor*, *Halophila spinulosa*, *Halophila ovalis*, *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea serrulata*. The code name of seagrass species is indicated in Table 1 to simplify reference.

**RESULTS AND DISCUSSION**

Figure 3 illustrates the concentration of Hg and Pb in different tissues of the 7 tropical seagrasses. All 7 seagrasses were able to accumulate Hg and Pb metals. The concentrations of Hg and Pb in different seagrass tissues are in the range of 0.1 to 69 µg/gDW and 81 to 249 µg/gDW respectively.

The accumulation of Pb is higher than Hg in tropical seagrass tissues. Pb contamination is usually highest in ecosystems with water sources from industrial areas, pipe production, waste management and landfills [23, 24]. All of these suggest that the high Pb content found in this study may be related to on-going projects in the vicinity. In order to be assimilated in seagrass, Pb is dissolved in seawater [25], then precipitated onto sediment [26-28] and finally assimilates into seagrass tissues. Pb is not essential for plants, in fact, excessive amounts can cause growth inhabitation, increase likelihood of aging, localize to hard tissues as well as reduction in photosynthesis [29]. Moreover, Pb leakage from product use throughout the world has indirectly caused Asia to bear the highest risk of toxicity [30]. Previous studies reported that the concentrations of Pb in seagrass tissues is in the range of 9.7 to 146 µg/L [31, 32].

Fig. 3: Concentrations of Hg and Pb in different seagrass tissues
Table 2: Correlation analysis for Hg and Pb in roots, rhizomes and leaves with r: correlation coefficient; p-value: significance levels at 0.05 (2-tailed). Bold text indicates significant correlation.

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Hg is known to inhabit reproduction of sensitive species of aquatic organisms at seawater concentration of 0.03 to 1.6 µg/L and reduce growth activity of sensitive species at seawater concentration of 0.04 to 1.0 µg/L [29]. Hg is probably present in the study area due to coal-fire plant operations nearby. In incinerators, Hg is volatile and evaporates into the atmosphere via gases, it is then removed from air by wet deposition and later deposited into aquatic environments and undergo methylation [33, 34]. Hg discharged into estuaries can naturally convert into methylmercury compounds [35]. Methymercury is the most hazardous Hg species due to its great stability that allows it to penetrate membranes of living organisms [36]. Sadly, this process can be boosted with the presence of degraded plankton [37], other dissolved metals (Zn, Cd, Pb, As, nutrients, dissolved organic carbon, sulphide) [38], oxidation–reduction conditions of organic matter [39] and organisms of higher trophic levels [40].

Table 2 shows the results of the correlation and significance analysis for metal content between roots, rhizomes and leaves of the seagrass. For Hg, the correlations of roots-rhizomes, rhizomes-leaves and leaves-roots are significant for different seagrass tissues in *Halophila ovalis*. Aside from that, there are significant correlations between the accumulation of Pb for roots-rhizomes, rhizomes-leaves and leaves-roots in *Enhalus acoroides*, *Halophila minor*, *Halophila ovalis*, *Thalassia hemprichii* and *Halodule uninervis*.

Based on the correlation analysis, it can be summarized that there are positive correlations between roots, rhizomes and leaves in *Halophila ovalis*. This indicates that *Halophila ovalis* have active internal tissues to uptake kinetics and to translocate metals to the entire plant system. The correlation between leaves, rhizomes and roots are different because metal accumulation by seagrass is governed by multitude of factors such as the relative metal availability for seagrass uptake, differences of physiology between parts (i.e. uptake kinetics, passive absorption and internal translocation, be it passively or actively, differ by seagrass tissue) [41].

All seagrass species are capable of acting as bioindicators because their tissues are capable of absorbing metal from the ecosystem either from seawater or sediment. Selection of the most efficient seagrass bioindicator, are based on two points: (1) the capabilities of species with the highest amount of metal accumulation and (2) the species with significant correlation in metal translocation within seagrass tissues.

In this study, the highest amount of Hg accumulation was observed in *Enhalus acoroides* rhizomes, followed by *Enhalus acoroides* leaves and *Halophila ovalis* rhizomes. Seagrasses were able to accumulates Pb more than 80 µg/gDW. *Halophila ovalis* shows positive correlation among seagrass parts (leaves, rhizomes and roots) which also translates to its ability in translocation of metals. Therefore *Halophila ovalis* satisfies both desired characteristics and hence is selected as the best bioindicator of the seagrass species found. Seagrass are sensitive to changes in seawater quality and therefore can be valuable indicators of heavy metal and reflect the overall health of an estuarine ecosystem. In future, transplant of the most effective seagrass species can be applied in marine ecosystem to monitor and reduce pollution.
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