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Research Article

Metallothionein (MT) Expression and SEM-EDX Mapping on *Cymodocea serrulata* Seagrass

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Abstract

Lead is a very dangerous heavy metal for organisms because it is carcinogenic, can cause mutations, takes a long time to decompose and has unchanging toxicity. This study on the seagrass *Cymodocea serrulata* aimed to determine the metallothionein expression quantitatively using the Elisa (Enzyme-Linked Immunosorbent Assay) method and describe the surface structure of *C. serrulata* roots, rhizomes, and leaves that were exposed to lead using SEM and EDX Mapping methods. The results showed that metallothionein was abundant in *C. serrulata* tissues (leaves>roots>rhizomes) in both South Bangka and Ketawai Island. SEM showed changes in the morphology and cell size of *C. serrulata* exposed to lead. EDX and Mapping showed the substance elements found in seagrass tissue. Pb was detected in C. *serrulata* roots (0.22%) and leaves (0.6%) in South Bangka. This indicates that the seagrass plants are able to absorb heavy metals into their body tissues.

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1. Introduction

Metallotionein (MT) is a protein (polypeptide) with small molecular mass (4-8 kDa), and its main properties contain 26-33% amino acid cysteine (Cys) and it does not have aromatic amino acids or histidine (Frankenne *et al*., 1980; Engel and Brouwer, 1984; Bayne *et al*., 1985; Rand and Petrocelli, 1985; Binz and Kägi, 1999).

The MT classification by Cobbet and Goldsbrough (2002) divided MT into four types based on the Cys amino acid sequence composition. MT type 1 is composed of six Cys-X-Cys motifs (-X- is amino acids other than cysteine) which are equally distributed in two terminals. MT type 2 has two N-terminal and C-terminal ends that are rich in Cys, with the composition of the Cys-Cys motif on the third and fourth amino acids of the amino acid sequence. In addition, MT type 2 has the Cys-Gly-Gly-Cys motif at the N-terminal end and the Cys-X-Cys motif at the C-terminal end. MT type 3 consists of only four amino acids of Cys at the N-terminal end.

The first three Cys form the Cys-Gly-Asn-Cys-Asp-Cys motif. While the fourth Cys forms its own motive, namely Gln-Cys-X-Lys-Lys-Gly. MT type 4 has three regions, each of which has 5 to 6 Cys which usually form the Cys-X-Cys motif so that the MT type 4 is different from MT in other MT plants. MT plants has a longer separation between N-terminals and C-terminals, which are around 20-40 amino acids, in addition to Cys (Yu *et al*., 2000), compared to MT mammals that has a high similarity to MT plants.

MT in plants was first identified in 1987, as EcMT (*Early cysteine* MT) in wheat embryos (*Wheat*) (Lane *et al*., 1987). Currently, more than 140 MT sequences have been recorded from a variety of plan species (Guo *et al*., 2003; Zhou *et al*., 2005), includin some plants such as soybeans (Kawashima *et al*., 1991), beans (Evans *et al*., 1990), corn, *Arabidopsis* (Zhou an Goldsbrough, 1995), rice (Zhou *et al*., 2005), tobacco and alfalfa (Robinson *et al*., 1992). Gen MT has been isolated from many plant species and is expressed in various tissues and organs such as the roots, stems, leaves, flowers, fruits, and seeds of various different plant species (Liu *et al*., 2002).

MT is also used as an indicator of pollution because of its sensitivity and accuracy. This is based on a natural phenomenon where metals can be locked in an organism body tissue, this is possible due to the presence of MT. Metallothionein is a metal-binding protein that functions and plays a role in the binding or confining of metals in tissues of every living organism (Noël-Lambot *et al*., 1978; Langston and Zhou, 1986; Bebianno *et al*., 1993).

SEM (Scanning Electron Microscopy) is a powerful technique that can be used to determine metal binding. SEM allows us to evaluate morphological changes on the surface such as changes in the composition of cell wall after metal binding. Moreover, when it is combined with EDX techniques, it can provide valuable information about the content of elements found on the surface of the sample (Baruah *et al*., 2012). Data obtained from SEM-EDX methods can be analyzed both qualitatively and quantitatively because, if the types or elements of minerals contained in one sample can be identified, then the quantity or proportion of each type of mineral or element obtained can be analyzed and acquired.

Previous research using SEM has been performed on *Hypericum perforatum* L (Ghelich and Zarinkamar, 2013), *Eichhornia crassipes* (Mart.) (Malar *et al*., 2014), *Eichhornia crassipes* (Baruah *et al*., 2012), *Salvinia natans* (Lima *et al*., 2014), *Calotropis Gigantea Linn* (Ramamurthy and Kannan, 2009), *Avicennia marina* (forsk.) Vierh (Arisandy *et al*., 2012), *Ruditapes philippinarum* (Inoue *et al*., 2012), *Zostera marina L.* (Sugiura *et al*., 2009). However, there is still limited study on metallothionien as metal binding protein in seagrass plant body tissue. This study on the seagrass *Cymodocea serrulata* aimed to determine the metallothionein expression quantitatively using the Elisa (Enzyme-Linked Immunosorbent Assay) method and describe the surface structure of *C. serrulata* roots, rhizomes and leaves exposed to lead using SEM and EDX Mapping methods.

2. Materials and Methods

2.1 Sample Collection Site, Time and Methods

The research was carried out in October 2017. The retrieval of *Cymodocea serrulata* seagrass was carried out in Bangka Belitung Province. Determination of seagrass sampling based on survey methods. Seagrass samples were collected from two sites, namely Ketawai Island in Central Bangka Regency and the coastal waters of South Bangka Regency. Seagrass samples were taken from the highest and lowest Pb concentrations based on data from previous studies (Bidayani *et al*., 2017; Rosalina *et al*., 2018a, 2018b; Rosalina *et al*., 2019a, 2019b). *Cymodocea serrulata* samples were brought to be tested for seagrass histology tissue on seagrass body parts (roots, rhizomes, and leaves) and cell characteristics due to Pb exposure in the field. The samples needed for histological observation using SEM EDX were six samples and cell characteristics using

an electron microscope as many as six samples from two research locations, each research location took one type of seagrass with there body tissues (roots, rhizomes and leaves). Secondary data were obtained from scientific literature.

2.2 The Procedure for Observing Metallothionien in Seagrass Cymodocea serrulata against Heavy Metal Lead (Pb) Quantitatively

Observation of metallothionien in *Cymodocea serrulata* seagrass was quantitatively carried out using the ELISA (*Enzyme-Linked Immunosorbent Assay*) method conducted in the Laboratory of Physiology, Faculty of Medicine, University of Brawijaya, Malang. According to Linde and Garcia-Vazquez (2006), the stages taken to determine the level of metallothionein quantitatively were the sampling stage, which were: taking 0.5 grams of root organ, rhizome and seagrass leaves of *Cymodocea serrulata*; washing them with PBS for three times; inserting the sample into plastic bag with ice cubes (maximum 4 hours for homogenization process). If the sample would be homogenized more than 4 hours, then the sample must be frozen immediately at -20°C. Next are the homogenization stage, extraction stage, metallothionein purification and quantification stage, and finally, the estimation stage with the ELISA method, namely the ELISA plate plan and the coating buffer.

The plan was based on the sample code. Coating buffer was made freshly. The antigen coating with the antigen content used was (1:40) diluted with a buffer coating and incubated at 4ºC overnight. The next day, the plate was washed using PBS Tween of 0.2% as much as 100 µl and repeated as many as six times. Then, 100 µl of anti-MTT primary antibody (1:400) was added in the buffer assay. Incubating the ELISA plate was done at room temperature for two hours while dispersing with a shaker of ELISA plate. Then, it was washed with PBS Tween of 0.2% as much as 200 µl and repeated as many as six times. Added it with 100 µl of biotin IgG secondary antibody (1:800) in the buffer assay then incubated at room temperature for one hour while dispersing. Then, it was washed with PBS Tween of 0.2% and repeated as many as six times. Then, added with 100 µl of SAHRP solution (1:800) in the buffer assay and incubated at room temperature for one hour while dishing out. Next, it was washed with PBS Tween of 0.2% as much as 200 µl and repeated as many as six times. Then, added with 100 µl of each hole sure blue of TMB microwell substrate and incubated it for 20-30 minutes in a dark room. If there was a reaction between antigen and antibody, it would turn into blue. Added it with 100 µl of HCl 1 N as a stop reaction. At this stage, the blue solution turned yellow. Read with an ELISA reader with a wavelength of 450 nm. The absorbance results were converted by a standard curve and the metallothionein value was gained.

2.3 Procedure for Observing the Histology of Cymodocea serrulata Seagrass Tissue using SEM-EDX Mapping

Histology in seagrass *Cymodocea serrulata* using SEM-EDX Mapping was carried out at the Central Laboratory of the Faculty of Mathematics and Science, State University of Malang. Coating Samples for SEM observations, namely samples that have been placed on the cover glass then adjusted to the SEM holder; then the holder provides a carbon tip to attach the cover glass to the holder. After that, it is coated with AUPD (palladium gold) to make it conductive; then, after the sample prep is finished, it is inserted into the sputtering coating tool. Leave it for ± 60 minutes to vacuum, after that the new sample is coated for ± 3 minutes until the sample is purple in color.

Tissue samples of approximately 5 mm in length were taken from the roots, rhizomes, and leaves of each *C. serrulata* sample. Sridhar *et al*. (2005) stated that preparation technique for SEM-EDX consisted of several steps, namely: fixing the tissue sample using 1-3% glutaraldehyde buffer with a pH of 7.2-7.4, then washing and posting fixation in 1-2% osmium tetroxide buffer with a pH of 7.2-7.4. Next is washing using distilled water Then dehydrating (10 minutes for each specimen) in 30%-50%-70%-90%-95%-100%-100%-100% acetone solutions (specimens should not be contaminated with air). This was followed by washing 8-10 times with liquid CO_2. Critical point drying was then conducted slowly at 73.8 bar and 31°C (here the largest phase directly changed to gas phase to avoid the surface tension of the specimen), until the CO_2 disappeared. The specimen was then fixed in the specimen holder using colloidal silver solution and placed on the sputter coating machine so that the cathode ray splashed gold. The specimen was then placed on the SEM (JEOL-JSM-6390 LV model with an attached dispersive X-ray energy unit) with an acceleration voltage of 20 kV.

Analysis using SEM-EDX mapping in seagrass root samples on Ketawai Island was carried out to evaluate the surface morphology of seagrass roots. In order to properly observe seagrass root morphology, the analysis was carried out at 1500x and 2000x magnification, 100x and 1000x magnification for rhizomes, and 1000x and 1500x magnification for seagrass leaves.

3. Results and Discussion

3.1 Metallothionein (MT) Expression in Cymodocea serrulata Seagrass Quantitatively

Metallothionein (MT) levels in *Cymodocea serrulata* seagrass in South Bangka and Ketawai Islands, Bangka Belitung Island Province were obtained from measurements using the ELISA (*Enzyme-Linked Immunosorbent Assay*) method. Metallothionein levels in South Bangka and in Ketawai Island were found to be highest in the leaves, followed by the roots and lowest in the rhizomes (Table 1).

Metallothionein levels in seagrass *Cymodocea serrulata* in South Bangka and Ketawai Island, Bangka Belitung Islands Province were obtained from measurements using the ELISA (Enzyme-Linked Immunosorbent Assay) method. The content of MT in South Bangka in leaves is 0.5778 ng/ml, roots is 0.3071 ng/ml, and rhizomes is 0.2114 ng/ml, and Ketawai Island in leaves is 0.4785 ng/ml, roots is 0.3528 ng/ml, and rhizome 0.0964 ng/ml. This shows that leaves have a higher value than roots and rhizomes. Because the greater the metallic lead (Pb) that is absorbed by the body parts of the seagrass will also increase the metallotionein in the seagrass. In accordance with the opinion of Rosalina *et al.* (2019b) and Lafabrie *et al.* (2008) which said the higher the concentration of heavy metals, the higher the seagrass will accumulate metals in its body tissues so that it will increase the metallothionien content in seagrass.

Metallothionien acts as metal binding protein and can reduce heavy metals on plants (Rumahlatu *et al.*, 2012). MT protein is known to have two main functions, namely detoxification of heavy metals and free radical scavengers. Metallothionien is most useful for various stress responses to heavy metal detoxification, due to its role in metal homeostasis and binding of a number of heavy metal ions (Leszczyszyn *et al.*, 2013).

Metallothionein contain large amounts of Cys residues needed to detoxify heavy metals by binding to cations from transition metals (Kägi, 1991). It is supported by Murphy *et al.* (1997) and Lee *et al.* (2004) that metallothionein have functions such as homeostasis, detoxification of heavy metals and antioxidant defense of cells. This indicates that MT as a protein is involved in heavy metal metabolism which is important in carrying out cell functions of an organism. Therefore, MT not only binds to metal in a cell, but also restores the functional ability of proteins that are inactivated by cadmium metal. MT is considered to have an important role in the development of tolerance to heavy metal toxicity. Tolerance has been shown to cadmium-induced lethality (Probst *et al.*, 1977; Baer and Benson, 1987). MT is responsible for the binding of heavy metals Pb, Cd, and Hg (Hertika *et al.*, 2018).

Metallothionein is most useful for various stress responses to heavy metal detoxification, due to its role in metal homeostasis and binding of a number of heavy metal ions (Leszczyszyn *et al.*, 2013), into metal-binding cysteine-rich storage proteins (Miranda *et al.*, 1990). Under normal environmental conditions MT plays a role in the storage and mobilization of essential metals (Hall, 2002). Metallothionein (MT) is a protein induced by metal exposure and has been widely used a biomarker for metal contamination. The physiological response of seagrass to heavy metal Pb has not been studied much. Anatomically, plants respond to heavy metals by modifying their tissue histology (Gomes *et al.*, 2011). The expression of plant MT genes is regulate by various factors, including different stresses such a injury (Choi *et al.*, 1996), pathogen infection (Choi *et al.*, 1996; Butt *et al.*, 1998), symbiotic interactions (Laplaz *et al.*, 2002; Hall, 2002), leaf senescence (Bhalerao *e al.*,2003), and heavy metals (Usha *et al.*, 2007; Huang and Wang, 2009; 2010). This suggests that MT may be expressed in response to general stresses as mentioned by Cobbett and Goldsbrough (2002).

Table 1. Metallothionein (MT) levels in *Cymodocea serrulata* seagrass

Number	Code		Metallothionein concentration in seagrass		
			Roots	Rhizomes	Leaves
1		St1	0.2571	0.2114	0.5778
2	BS	St2	0.3071	0.1742	0.4278
3		St3	0.1149	0.1292	0.1171
4		St1	0.2278	0.0964	0.4785
5	PK	St2	0.2185	0.0528	0.3356
6		St3	0.3528	0.0471	0.1392

Note: SB = South Bangka, KI = Ketawai Island; St = Station

3.2 Histology of Cymodocea serrulata Seagrass from Two Different Locations: Tissue Analysis (Roots, Rhizomes, and Leaves) using SEM-EDX Mapping

3.2.1 Cymodocea serrulate roots

Analysis using SEM in seagrass root samples on Ketawai Island was carried out to evaluate the surface morphology of seagrass roots. So that the seagrass root morphology could be seen clearly, the analysis was carried out at 1500x and 2000x magnification. There are differences in both exodermis and endodermis within seagrass roots from Ketawai Island and South Bangka (Figures 1 and Figure 2). SEM found that the structure of exoderm and endodermis of the roots in South Bangka, is still clearer than exodermis and endodermis in Ketawai Island. This is most likely due to higher heavy metal content in the roots of the seagrass from Ketawai Island than in those from South Bangka, causing changes in the outer surface structure of the seagrass roots. A similar result was reported from the transverse analysis of *E. crassipes* roots by Baruah *et al*. (2012) who found that Pb accumulated in a higher proportion on the root surface (epidermis) and decreased in concentration towards the center.

3.2.2 Cymodocea serrulata seagrass rhizomes

SEM analysis of seagrass rhizome samples from Ketawai Island and South Bangka was conducted to evaluate the surface morphology of seagrass rhizomes through 100x and 1000x magnification. The SEM-EDX results clearly show differences in both exodermis and endodermis between seagrass rhizomes from Ketawai Island and those from South Bangka (Figures 3 and Figure 4). SEM found that the structure of epidermis and endodermis of the rhizome in South Bangka is still clearer than the exodermis and the endodermis in Ketawai Island. This is thought to be because the heavy metal content was higher in the seagrass rhizomes from Ketawai Island than in those from South Bangka, causing changes in the outer surface structure of the rhizomes.

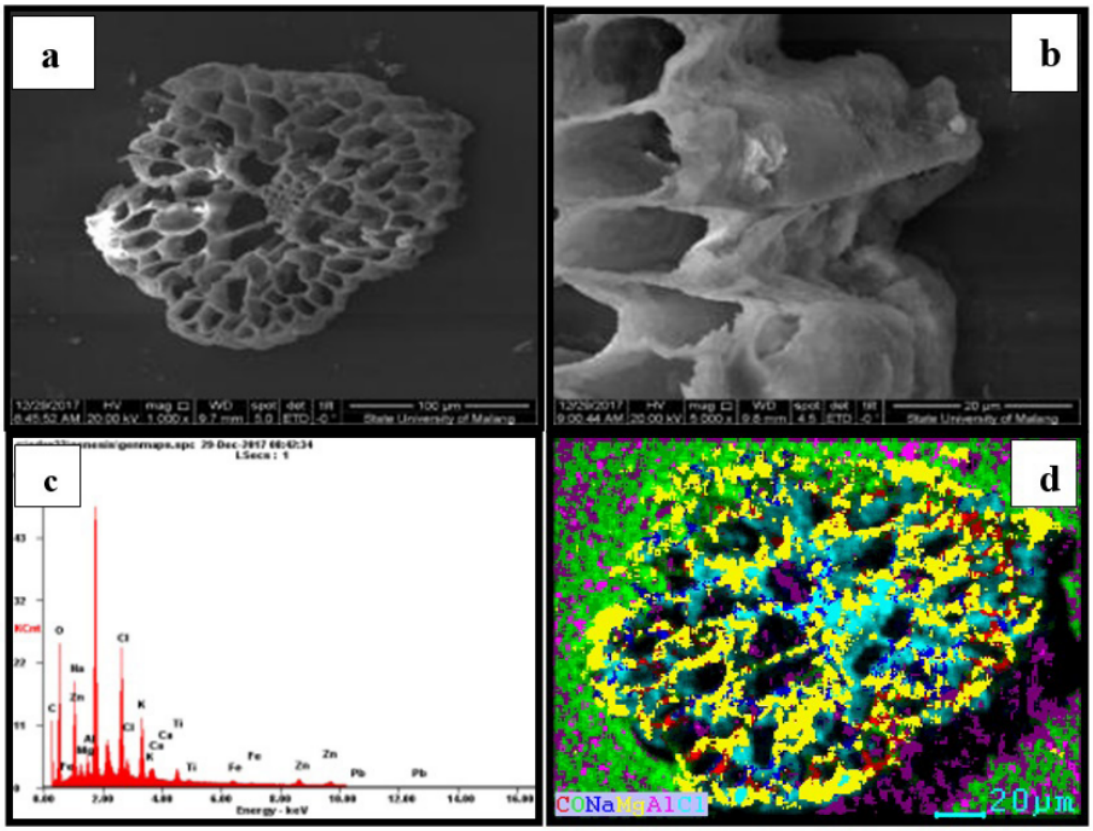

Figure 1. SEM-EDX mapping of Ketawai Island *Cymodocea serrulata* roots: root cross-section (a); root epidermis (b); SEM-EDX graph (c); and root mapping (d)

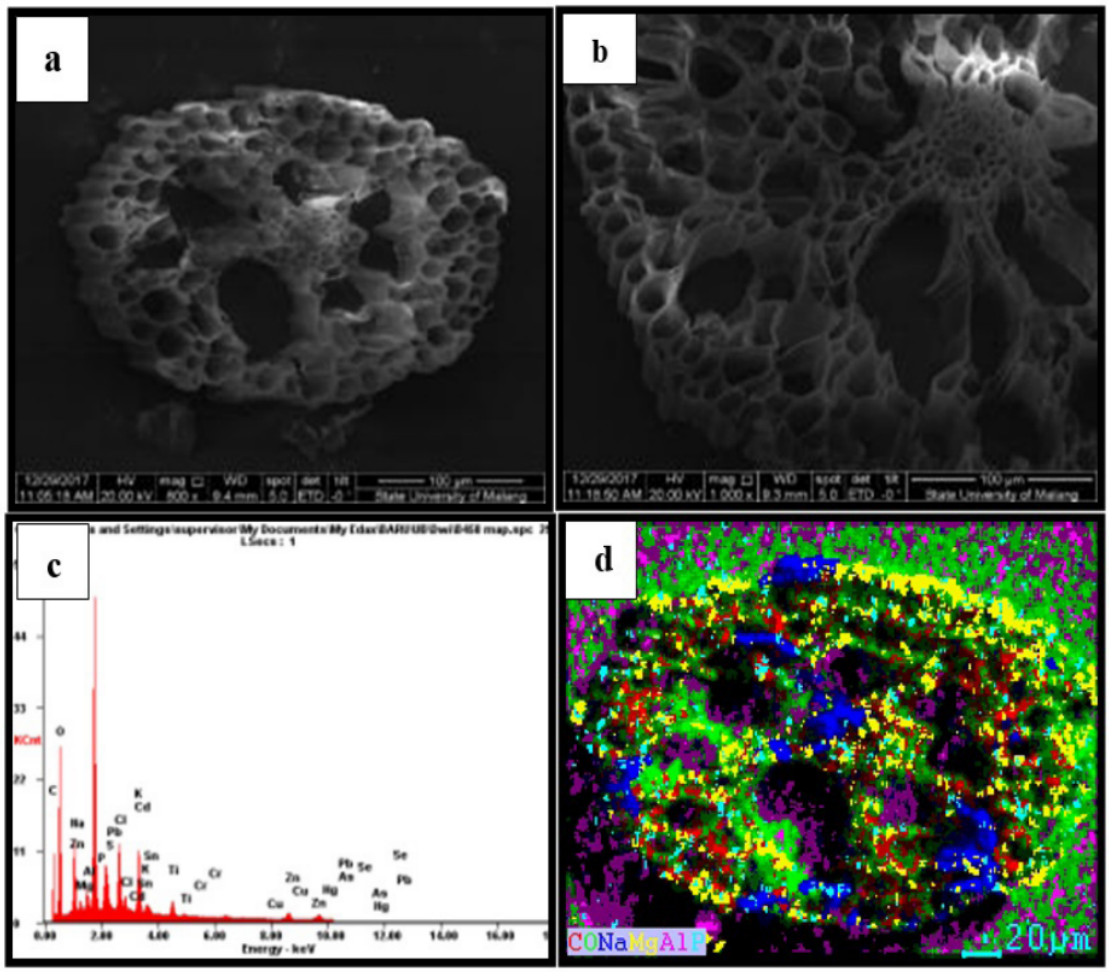

Figure 2. SEM-EDX mapping of South Bangka *Cymodocea serrulata* roots: root cross-section (a); root epidermis (b); SEM-EDX graph (c); and root mapping (d)

3.2.3 Cymodocea serrulata seagrass leaves

Analysis using SEM in seagrass leaf samples in Ketawai Island was carried out to evaluate the surface morphology of seagrass leaves so that the seagrass leaf morphology could be seen clearly; the analysis was carried out with 1000x and 1500x magnification. There are differences in both the epidermis and cuticle in the seagrass leaves on Ketawai Island and South Bangka (Figures 5 and Figure 6). The SEM showed that the epidermis and cuticle leaves in South Bangka to be of clearer structure compared to the damaged exodermis and endodermis on Ketawai Island. This is because the result of the heavy metal content in the seagrass leaves on Ketawai Island is greater than South Bangka, causing changes in the outer surface structure of the seagrass leaves. Plants undergo rapid morphological, anatomical, and metabolic changes in facing metal stress. The visible symptom of metal toxicity in plants is the expression of metal-induced changes at the structural and ultrastructural levels. These changes at the cellular, tissue and organ levels, in turn, are the result of the direct interaction of toxic metals with the structural components of these tissues (Singh and Sinha, 2004). Leaves are more sensitive but more flexible to environmental stresses (Shi and Cai, 2009).

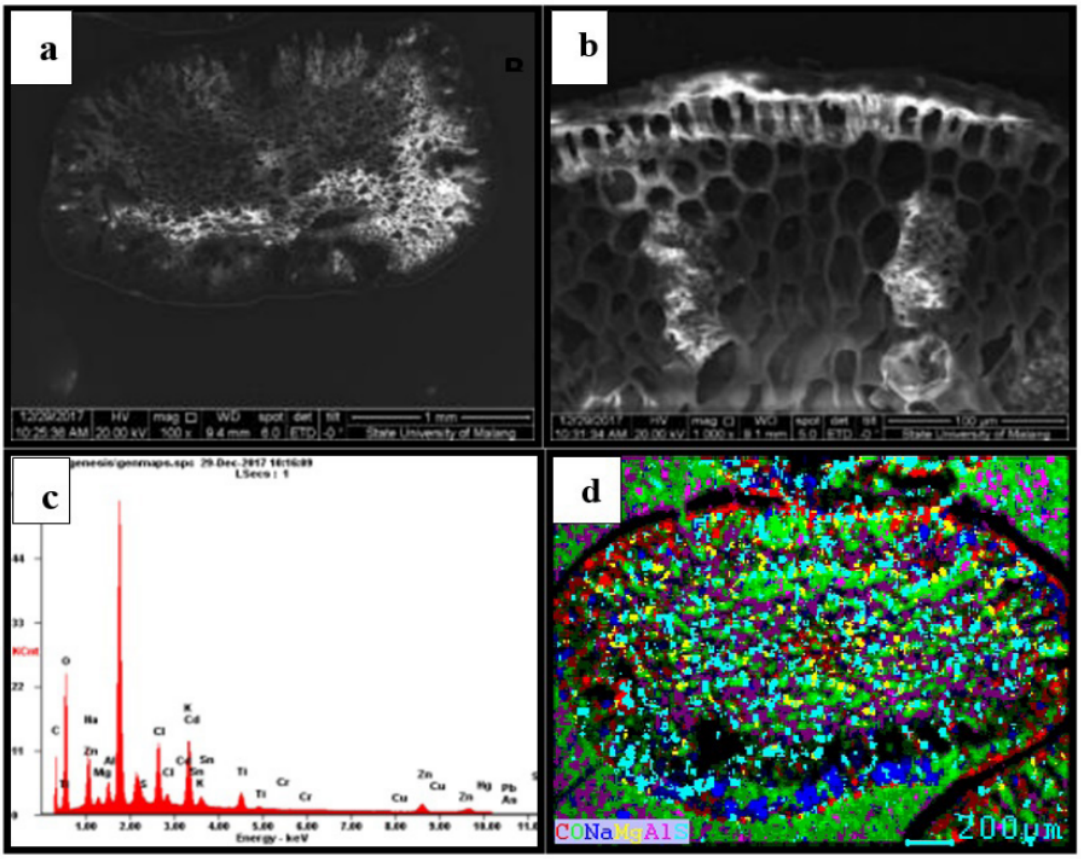

Figure 3. SEM shows the transversal part of the seagrass rhizoma *Cymodocea serrulata* overall rhizome section on Ketawai Island (a), seagrass epidermis rhizoma (b); graph of SEM-EDX (c) and mapping (d) on seagrass roots of *Cymodocea serrulata* on Ketawai Island.

3.3 Quantitative analysis of Metallothionein (MT) Expression in Cymodocea serrulata Seagrass

Metallothionein acts as a metal binding protein and could reduce toxic effects on plants (Rumahlatu *et al*., 2012). Carpenè *et al.* (2007); Pearce *et al.* (2000) stated that MT protein was known to have two main functions, namely heavy metal detoxification and free radical scavenger. This indicated that MT as a protein was involved in the metabolism of heavy metals which was important in carrying out cell functions of an organism. Therefore, MT was not only binding the amount of metal in a cell, but also returning the ability of the protein to function which was inactive due to cadmium metal. MT was considered to have an important role in developing tolerance to heavy metal toxicity. Tolerance had been shown against cadmium-induced (Probst *et al*., 1977; Baer and Benson, 1987). MT was responsible for the absorption of heavy metals Pb, Cd and Hg (Hertika *et al*., 2018).

MT gene expression in plants is regulated by a variety of factors, including various stresses such as openness (Choi *et al*., 1996), pathogenic infections (Choi *et al*., 1996; Butt *et al*., 1998), symbiotic interactions (Laplaze *et al*., 2002), leaf senescence (Bhalerao *et al*., 2003), and heavy metals (Usha *et al*., 2007; Huang and Wang 2009; Huang and Wang, 2010). This indicates that MT could be expressed as a general stress response as mentioned by Cobbett and Goldsbrough (2002). It is suspected that MT strength functioned as an antioxidant and plays an important role in plasma membrane repair (Hall, 2002), in addition of being responsive to toxic metal stresses.

3.4. Histology of Cymodocea serrulata Seagrass from Two Different Locations: Tissue Analysis (Roots, Rhizomes, and Leaves) using SEM-EDX Mapping

3.4.1 Cymodocea serrulata seagrass roots

Analysis results with 1500x magnification (Figure 2a) showed that seagrass roots formed cavities (not homogeneous). To clarify the SEM analysis on roots, 2000x magnification (Figure 2b) was able to show two important types of information, namely particle shape and size in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of seagrass root surface and to identify the elements found on the surface of the root sample. The results of EDX analysis obtained the most elements, namely C, O, Na, Mg, Al, and Cl, with the relative mass percentage of O element of 33.10%, C element of 25.03%, Na element of 10.01%, Cl element of 09.77%, Al element of 02.60%, and Mg element of 02.27% (Figure 2c and Figure 2d).

Analysis results with 1000x magnification indicated that seagrass roots formed cavities (not homogeneous) (Figure 3a and Figure 3b). It showed two important informations, namely particle shape and size that were in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of the seagrass root surface and to identify the elements found on the surface of the root sample. The results of EDX analysis obtained the most elements, namely C, O Na, Mg, Al, and P, with the relative mass percentage of O element of 32.29%, C element of 30.43%, Na element of 10.47%, Mg element of 1.85%, Al element of 1.18%, and P element of 0.61% (Figure 3c and Figure 3d). And the root samples in South Bangka had Pb element of 0.22%. According to Baruah *et al*. (2012), a transverse analysis of the roots of *E. crassipes* showed that Pb was accumulated in a higher proportion on the root surface (epidermis) and decreased in concentration towards the center.

3.4.2 Cymodocea serrulata seagrass rhizomes

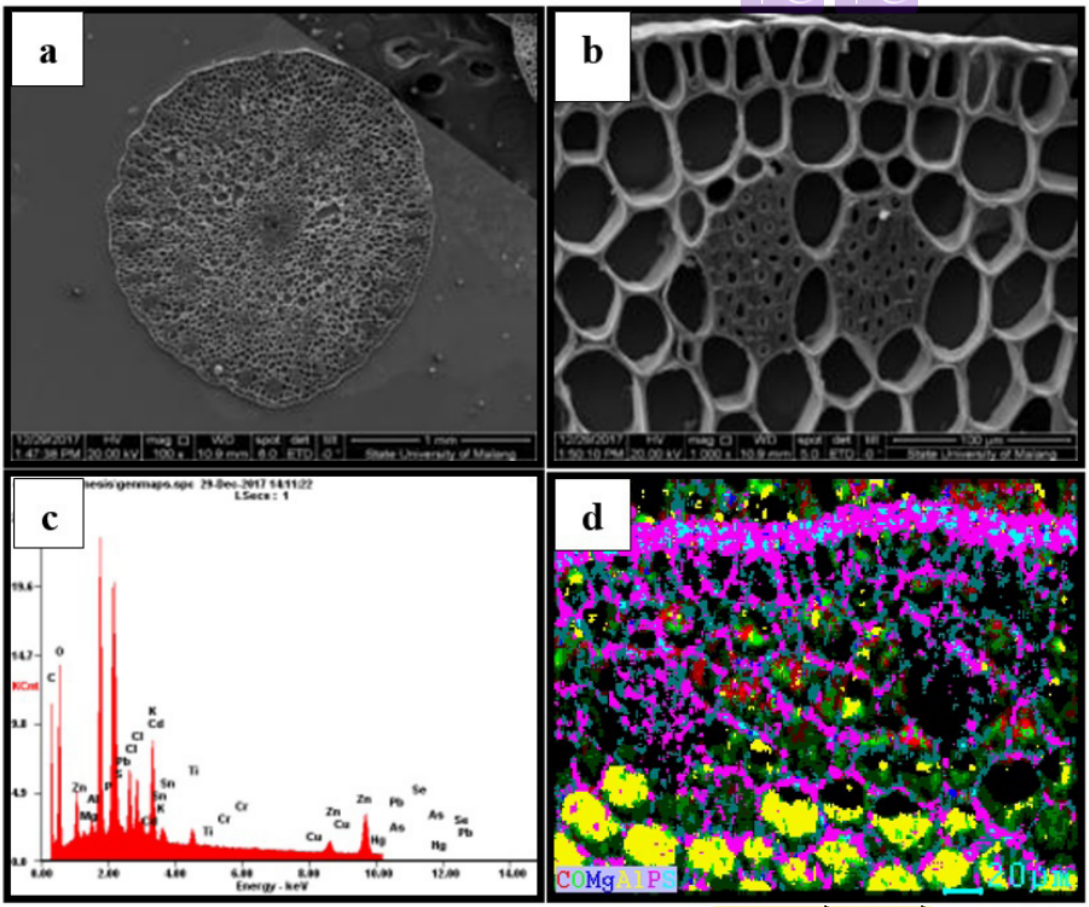

Figure 4. SEM showed the transversal part of the seagrass rhizoma of *Cymodocea serrulata*, cross-section of seagrass rhizoma as a whole in South Bangka (a), epidermis of seagrass rhizoma in South Bangka (b), graph of SEM-EDX (c) and mapping (d) on *Cymodocea serrulata* seagrass roots in South Bangka

Analysis results with 100x magnification (Figure 4a) showed that seagrass rhizoma formed cavities (not homogeneous). To clarify the SEM analysis on roots, 1000x magnification (Fig. 4b) was able to show two important informations, namely particle shape and size in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of seagrass rhizome and to identify the elements found on the surface of the rhizome sample. The results of EDX analysis showed that the most elements found were C, O, Na, Mg, Al, and S, with the relative mass percentage of O element of 32.19%, C element of 23.00%, Na element of 6.46%, Mg element of 1.66%, Al element of 2.64%, and S element of 1.21% (Figure 4c and Figure 4d).

Analysis results with 1000x magnification (Figure 5b) showed that seagrass rhizome formed cavities (not homogeneous). In addition, it showed two important informations, namely particle shape and size that were in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of seagrass rhizome and to identify the elements found on the surface of the rhizome sample. The results of EDX analysis showed that the most elements found were C, O, Mg, Al, P, and S, with the relative mass percentage of O element of 24.48%, C element of 28.31%, Mg element of 1.45%, Al element of 1.87%, P element of 1.94%, and S element of 2.01% (Figure 5c and Figure 5d).

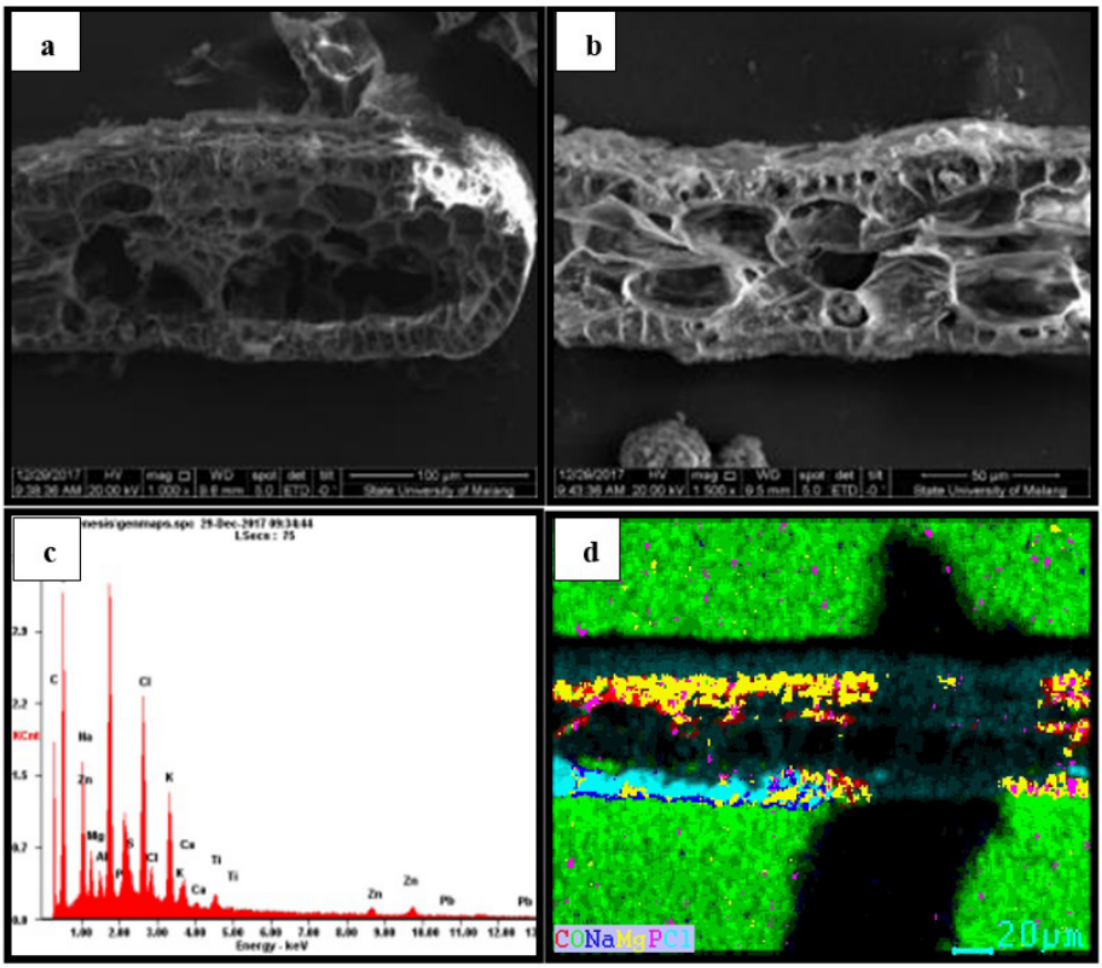

Figure 5. SEM showed the transversal part of seagrass leaves of *Cymodocea serrulata* in overall leaf crossings in Ketawai Island (a), epidermis of seaweed leaves of Ketawai Island (b); graph of SEM-EDX (c) and mapping (d) on seagrass roots of *Cymodocea serrulata* on Ketawai Island.

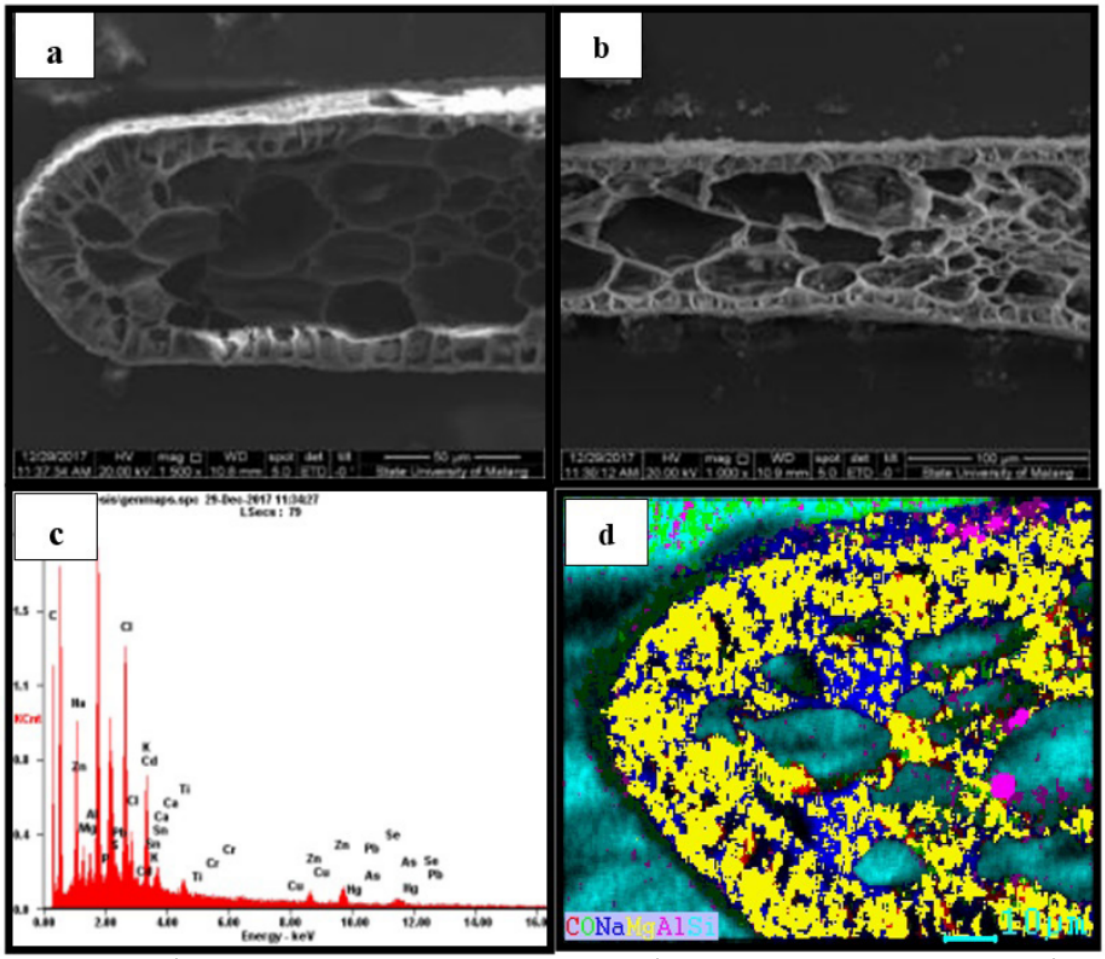

Figure 6. Overall cross section of seagrass leaves in South Bangka (a), seagrass epidermis in South Bangka (b); graph of SEM-EDX (c) and mapping (d) on *Cymodocea serrulata* seagrass roots in South Bangka

3.4.3 Cymodocea serrulata seagrass leaves

Analysis results with 1000x magnification showed that seagrass leaves formed cavities (not homogeneous) (Figure 6a). To clarify the SEM analysis on leaves 1500x magnification was carried out (Figure 6b), and it was able to show two important informations, namely particle shape and size in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of seagrass leaf surface and to identify the elements found on the surface of leaf samples.

EDX analysis results obtained the most elements, namely C, O, Na, Mg, P, and Cl, with relative mass percentage of O element of 38.37%, C element of 10.99%, Na element of 8.62%, Mg element of 1.96%, P element of 1.23%, and Cl element of 4.73% (Figure 6c and Figure 6d).Changes in the leaf epidermis occurred due to heavy metals, which affected the structure, shape, and size of the cells of the *Cymodocea serrulata* seagrass. The metal-induced changes in the leaf epidermal structure involved reduction in cell size, more wax coating and an increase in the number of stomata and trichomes per unit area with a simultaneous reduction in guard cell size (Rafia *et al*., 2006; Ozyigit and Akinci, 2009; Rai *et al*., 2010; Weryszko-Chmielewska and Chwil, 2005).

Analysis results with 1500x magnification

(Figure 6b) indicated that seagrass leaves formed cavities (not homogeneous). In addition, it also showed two important informations, namely: particle shape and size that were in the micron range. EDX and Mapping analysis were conducted to determine the composition of the elements of seagrass leaf surface and to identify the elements found on the surface of leaf samples. The results of EDX analysis obtained the most elements, namely C, O, Na, Mg, Al, and Si, with relative mass percentage of O element of 14.25%, C element of 23.08%, Mg element of 1.91%, A1 element of 1.50%, Na element of 12.20% and Si element of 8.42% (Figure 6c and Figure 6d). In the sample of seagrass leaves in South Bangka, there was also Pb element of 0.60%.

Plants experienced rapid morphological, anatomical and metabolic changes in facing metal stress. A visible symptom of metal toxicity in plants was the expression of changes induced by metals at the structural and ultrastructural levels. These changes in cells, tissues and organ levels, were the result of direct interactions of toxic metals with the structural components of the tissue (Singh and Sinha, 2004). Leaves were more sensitive but more flexible to environmental stresses (Shi and Cai, 2009).

4. Conclusion

Expression of metallothionein in seagrass *Cymodocea serrulata* was abundant in the body tissues namely leaves,> roots,> and rhizomes. This is due to the higher the metal contained in the seagrass body tissue, the higher the metallothionein levels, because metallothionein function to bind metals so as not to damage other body tissues and SEM-EDX showed changes in seagrass tissue and detecting Pb in the roots and leaves of seagrass in South Bangka because the plant undergoes rapid morphological, anatomical, and metabolic changes in facing metal stress.

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Author's Contributions

The authors have contributed very well, from designing research to checking the draft final of the manuscript before it is sent to journal managers. Dwi Rosalina designs and carries out surveys, monitors data processing and analysis activities, also writes research reports and scientific publication manuscripts. Dini Sofarini compiled, tabulated, and separated the data, especially the seagrass sampling results. Ruly Isfatul Khasanah and Misbakhul Munir carried out quantitative data processing, and write down the resume. Firman Farid Muhsoni analyzed the results of data processing.

Conflict of Interest

The authors state that they do not have competing interests and conflicts of interest between them.

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Prep. You may be using the wrong preposition.

Dup. Did you mean to repeat this word?

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Missing "," Review the rules for using punctuation marks.

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Article Error You may need to remove this article.

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Proofread This part of the sentence contains an error or misspelling that makes your meaning unclear.

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Garbled This sentence contains several grammatical or spelling errors that make your meaning unclear. Proofread the sentence to identify and fix the mistakes.

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Proper Nouns You may need to use a capital letter for this proper noun.

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P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.

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