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## Lead Accumulation and Its Histological Impact on *Cymodocea serrulata* Seagrass in the Laboratory

(Pengumpulan Plumbum dan Kesan Histologinya pada Rumpai Laut *Cymodocea serrulata* dalam Makmal)

DWI ROSALINA\*, ENDANG YULI HERAWATI, MUHAMMAD MUSA, DINI SOFARINI,

MOHAMMAD AMIN & YENNY RISJANI

### ABSTRACT

The purpose of this study was to determine the concentration of lead (Pb) in *Cymodocea serrulata* tissues (roots, rhizomes, and leaves) using the AAS method, also to figure out Pb's impact on seagrass histology and elements using the SEM-EDX Mapping method. The results showed that the higher the concentration and the length of the planting period, the higher the accumulation of heavy metals in the seagrass tissues. In this study, Pb was largely accumulated in the leaves, roots, and rhizomes tissue. Moreover, the seagrass histology in the epidermis and endodermis underwent shape and structure changes; it also went through damage or thickening at 15 ppm concentration, compared to control. This study strengthens the usefulness and relationship of *Cymodocea serrulata* seagrass as a biological indicator of metal contamination in the waters.

Keywords: *Cymodocea serrulata*; lead; mapping; seagrass; SEM-EDX

### ABSTRAK

Tujuan kajian ini adalah untuk menentukan kepekatan plumbum (Pb) di dalam tisu *Cymodocea serrulata* (akar, rizom dan daun) menggunakan kaedah AAS, serta mengkaji kesan Pb pada histologi dan unsur rumpai laut menggunakan kaedah pemetaan SEM-EDX. Keputusan menunjukkan bahawa semakin tinggi kepekatan dan tempoh penanaman yang lama, semakin tinggi pengumpulan logam berat dalam tisu rumpai laut. Dalam kajian ini, Pb banyak terkumpul di bahagian tisu daun, akar dan rizom. Selain itu, histologi rumpai laut di epidermis dan endodermis mengalami perubahan bentuk dan struktur; ia juga mengalami kerosakan atau penebalan pada kepekatan 15 ppm, berbanding dengan kawalan. Kajian ini mengukuhkan lagi kegunaan rumpai laut jenis *Cymodocea serrulata* sebagai penanda biologi untuk mengesan pencemaran logam dalam laut.

Kata kunci: *Cymodocea serrulata*; pemetaan; plumbum; rumpai laut; SEM-EDX

### INTRODUCTION

Lead or Pb found in waters could be free metal ions in the form of  $Pb^{2+}$  and pairs of inorganic ions in the form of  $Pb(CO_3)_2$ . According to Connel and Miller (1995), metal solubility in water was naturally regulated by pH, metal type, oxidation state of mineral components and redox environment, and dynamic interactions of solid-liquid phases. Pb in waters was found in dissolved and suspended form. The levels and toxicity of lead were influenced by hardness, pH, alkalinity and oxygen content, salinity, biochemical transformation and the presence of complex forming substances (Connel & Miller 2006; Effendi 2003; Palar 2004; Wood et al. 2012). Marine plants were also able to absorb metals, such as phytoplankton, macroalgae and seagrass. Seagrass could reduce heavy metal pollution in a waters. Seagrass could be used as an indicator to determine the presence of heavy metals in the waters (Prange & Dennison 2000).

Based on previous research, Sari et al. (2017), stated that Cd and Pb in water showed that the Cd concentration

in water was higher than Pb 0.29-0.39 mg/L. The highest concentration of heavy metals in sediments was Pb 4,74-7,68 mg/kg. The highest Cd concentration in seagrass leaves was 1.76-2.44 mg/kg, while the highest Pb was in seagrass roots 1.94-6.52 mg/kg. Bidayani et al. (2017), stated that Pb heavy metals in seagrass roots were  $0.34 \pm 0.08 - 3.04 \pm 0.11$  ppm, rhizoma was  $0.11 \pm 0.00 - 3.01 \pm 0.08$  ppm, and leaves were  $0.26 \pm 0.03 - 0.94 \pm 0.07$  ppm. The Pb content in water was  $0.02 \pm 0.01 - 0.07 \pm 0.01$  ppm and sediments were  $1.55 \pm 0.10 - 19.58 \pm 0.03$  ppm. Rosalina et al. (2018) stated that Pb heavy metals on Ketawai Island were 1,896-2,792 mg/L in seagrass leaves and South Bangka 0.001-1,536 mg/L in seagrass roots. While Pb in water was 0.001-0.079 mg/L and Pb in sediment was 3.87-8.364 ppm in South Bangka. Pb in water was 0.001-0,735 mg/L and Pb was in sediment 1,536-1,896 ppm on Ketawai Island.

Seagrass was sea angiosperm which was also suitable for metal biomonitoring (Ferrat et al. 2003; Pergent-Martini & Pergent 2000; Ralph et al. 2006). In addition, seagrasses

had a high bioaccumulation capacity of metals to interact directly with water columns (through leaves) and sediments (through roots), thus becoming a good bioindicator. The purpose of this study was to analyze the concentration of Pb and its impact on tissue histology of seagrass *Cymodocea serrulata*.

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#### MATERIALS AND METHODS

The *Cymodocea serrulata* seagrass sampling was performed in the waters of South Bangka Regency in Bangka Belitung Province and used the transect method (Short & Coles 2006). The samples were those with 3-4 leaves on each plant of which the width was 0.5-0.8 cm and length was 6.2 - 22.5 cm; additionally, the plants had to appear healthy and fresh. Afterwards, the samples were put into 12 aquariums of which the dimensions were 30 cm long, 30 cm wide and 25 cm high; they were filled with 30 seagrasses for 4 weeks. The plants were exposed to lead from Pb (NO<sub>3</sub>)<sub>2</sub> solutions at 0, 5, 10 and 15 ppm concentrations for 4 weeks, with 3 repetitions on each treatment. They were observed at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> week. After that, the seagrass samples were analyzed for their heavy metals concentrations using the AAS (Atomic Absorption Spectrophotometer); while the histology analysis utilized the SEM (Scanning Electron Microscope), EDX (Energy Dispersive X-ray) and Mapping.

#### ANALYSIS OF METAL CONTENT

Analysis of metals in water and sediment was based on standard method procedures (APHA 2005) while analysis of heavy metals in biota networks was based on SNI 2354.5: 2011. The sample preparation procedure was a modification of Sarong et al. (2015). The Pb heavy metal test and analysis were conducted in the Soil, Land and Environmental Laboratory, University of Trunojoyo, Madura.

#### METAL ON WATER

A total of 100 mL of sample  $\pm$  1 mL of HNO<sub>3</sub> was boiled and evaporated on a hot plate until the sample volume of 10-20 mL, added HNO<sub>3</sub> which was intended as a preservative and solvent, if needed until the destruction was complete (clear solution), made as a 100 mL sample volume with distilled water. Measure with ICE 3000 type AAS thermo scientific.

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#### METAL ON SEDIMENT

Sediment samples that had been dried in an oven at 105°C for 12 h were weighed as much as 0.5 g then added 100 mL of distilled water, 1 mL of HNO<sub>3</sub> and 10 mL of HCl. After that, destruction was done by arranging the microwave program. The results of destruction were transferred to 50 mL measuring flask. The results of the treatment were then analyzed by AAS thermoscientific type ICE 3000 (Buccolieri et al. 2006).

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#### METALS IN THE SEAGRASS

Samples (roots, rhizomes and leaves) seagrass *Cymodocea serrulata* was dried and weighed, then the seagrass sample was blended until smooth. The sample was then weighed as much as 1 g, put in 25 mL beaker glass and added 5 mL of 5M HNO<sub>3</sub>, then stirred and heated with a hot plate until dissolved. Next it was lifted and cooled for 15 min. The cold filtrate was filtered with the whatman filter paper into a 50 mL measuring cup until filtered out. Next, it was added with distilled water up to the boundary line, and was ready to be analyzed. To see the concentration of heavy metals, the researcher took 2 mL of the prepared sample and analyzed it using the Atomic Absorption Spectrophotometer (AAS).

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#### THE ANALYSIS OF *Cymodocea serrulata* SEAGRASS HISTOLOGY TISSUE USING THE SEM-EDX MAPPING

Histology observation in *Cymodocea serrulata* seagrass 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, i.e. samples that had been placed on the glass cover were then cut according to the SEM holder, then the holder was given a carbon tip to attach the glass cover to the holder. The next step was to coat the sample with AUPD (gold palladium) to make it conductive. After the preparation was finished, the sample was put into a sputtering coating tool. It was left to be vacuumed for around 60 min; it was coated around 3 min until turning purple.

The seagrass tissue samples (from roots, rhizomes, and leaves) were taken for the SEM-EDX analysis of 5 mm (Sridhar et al. 2005). The preparation technique was to put the specimen on the SEM-EDX to perform tissue fixation using 1-3% buffer glutaraldehyde with pH level of 7.2-7.4. The next steps were washing and post-fixation in 1-2% buffer osmium tetroxide with pH level of 7.2-7.4. The researcher washed it with distilled water and dehydrated it in acetone. Next, it was washed using liquid CO<sub>2</sub>. Then, it was dried with the critical point at 31°C until the CO<sub>2</sub> disappeared. Following this step, the specimens were fixed on the holder using the colloid silver solution, and put on the sputter-coating machine to make the cathode ray splashes gold. Then, the sample was ready to be observed using the JEOL-JSM-6390 SEM model LV; the researcher attached a dispersive X-ray energy unit, with an acceleration voltage of 20 kV.

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#### THE STATISTICAL ANALYSIS

The researcher used ANOVA analysis at 5% significance ( $p < 0.05$ ) to determine Pb metal accumulation differences in roots, rhizomes, and leaves based on different concentration treatments and exposure time. As the results show a significant difference, the next step was to proceed using the smallest real difference test (BNT) at the 5% significance level. After that, the data were analyzed using the SPSS ver. 21; the data obtained before analysis were

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first tested for normality, homogeneity, and non-additives as the ANOVA test conditions.

## RESULTS AND DISCUSSION

### Pb CONCENTRATION IN *Cymodocea serrulata*'S ROOTS, RHIZOMES AND LEAVES IN DIFFERENT Pb CONCENTRATION TREATMENTS

Seagrass *Cymodocea serrulata* accumulated Pb metal varies depending on the concentration and length of time of exposure and plant tissue analyzed (Table 1). Pb heavy metal content in roots, rhizomes and seagrass leaves during exposure increased with increasing concentration of Pb treatment. This proved that *Cymodocea serrulata* roots, rhizomes and seagrass leaves had the ability to absorb Pb heavy metals.

The average concentration of Pb was 4.02 ppm in the plants leaves, 0.71 ppm in the rhizomes, and 1.27 ppm in the root. These results display that the rhizome had the lowest Pb average, while the leaves had the highest one.

Based on Table 2, the leaves had the highest Pb metal concentration and were significantly different from all seagrass parts including the rhizomes and roots. Furthermore, the rhizomes Pb metal concentrations were the lowest and significantly different from the leaves; but not from the roots. Since aquatic plants bodies (including

seagrasses) are submerged in the water, they can absorb water and water-soluble materials (such as metals) through their entire body parts; this includes the leaves, whereas the roots and rhizomes absorb less. The Pb particles entered the leaf tissues through its large stomata, which was about 10  $\mu\text{m}$  long and around 2-7  $\mu\text{m}$  wide; the Pb particle size was smaller so that it easily entered the seagrass' leaf tissues through a passive absorption process. This absorption process can be translocated to the plant's rhizoma and roots through the phloem tissue (Kuo 1983; Tomlinson 1980).

Leaves, rhizomes and roots in seagrass plants which were aquatic plants were able to absorb water and nutrients including heavy metals from the surrounding environment, and leaves had a greater absorption rate than roots and rhizomes, therefore, it was indicated that high Pb concentrations in the leaves were due to large absorption of leaves against Pb metal from water. In accordance with the opinion of Brix and Lyngby (1984), Capone et al. (1983) and Catsiki et al. (1987), asserted that *Posidonia oceanica*, *Z. Marina* and *Ruppia* have higher Pb concentrations in their leaves than in their roots and rhizomes.

The average Pb concentration is based on the concentration factor. This study showed that 0 ppm concentration produced 0.29 ppm; 5 ppm produced 1.82 ppm; 10 ppm produced 2.15 ppm; and 15 ppm produced

TABLE 1. Pb metal accumulation of *Cymodocea serrulata* roots, rhizomes and leaves during exposure to Pb metal

Tissues	Pb Concentration (ppm)	Pb accumulation (ppm) at week to			
		1	2	3	4
Leaves	0	0,0001±0,00	0,0087±0,01	1,245±0,01	1,7603±3,04
	5	0,9654±0,23	2,9214±2,90	3,7513±2,20	4,2887±2,11
	10	1,1434±0,40	2,6466±4,33	6,1807±4,52	8,2230±2,98
	15	2,4505±0,96	9,2888±1,50	9,5329±3,12	9,8411±3,46
Rhizome	0	0,0017±0,00	0,0074±0,00	0,0001±0,00	0,2103±0,01
	5	0,5211±0,33	0,4263±0,11	0,5079±0,01	0,9277±0,01
	10	0,3188±0,32	0,9950±0,17	0,0486±0,00	1,0830±0,05
Root	15	0,6058±0,54	0,8996±0,36	1,6147±0,07	3,1528±0,01
	0	0,0001±0,00	0,0041±0,00	0,0097±0,01	0,2915±0,50
	5	0,1422±0,24	1,0188±1,04	2,2266±1,18	4,1592±3,11
	10	0,3321±0,48	0,6180±0,22	1,3681±0,66	2,8414±1,01
	15	0,7946±0,34	0,9928±0,91	1,6169±2,07	3,9642±2,31

TABLE 2. Results from ANOVA analysis of Pb accumulation based on *Cymodocea serrulata* seagrass tissue

Tissues	Means	Probability			Notation
		Rhizome	Root	Leaves	
Rhizome	0.708		0.321	0.000	a
Root	1.274	0.321		0.000	a
Leaves	4.015	0.000	0.000		b

3.73 ppm. These results showed that the concentration of 0 ppm produced the lowest Pb, while the concentration of 15 ppm produced the highest one.

Based on Table 3, 15 ppm concentration produced the highest Pb and was significantly different from 0 ppm, 5 ppm, and 10 ppm concentrations (0 ppm concentration produced the lowest Pb). This indicates that the longer the Pb exposure time and the greater the concentration, the higher the accumulation of Pb metal absorption. A research by Catsiki and Panayotidis (1993) showed that the translocation of heavy metals indeed occurs from the *Cymodocea nodosa* and *Posidonia oceanica* seagrass leaves to the roots (Lafabrie et al. 2008).

Pb HEAVY METAL CONTENT IN WATER AND SEDIMENT ON DIFFERENT Pb CONCENTRATION TREATMENTS

The results of the measurement of Pb metal content in water and sediment during exposure to Pb heavy metals, could be seen in Table 4.

The ANOVA analysis, based on the tests of different influences of sample factors on Pb metal accumulation, resulted in the F statistical test in the amount of 23.374 with a 0.000 probability. Therefore, it can be stated that there are significant differences of Pb metal accumulation in water and sediment samples.

Water samples showed an average Pb metal of 0.004 ppm, while the sediment's had 0.633 ppm. Table 4 presents that the water samples had the lowest Pb compared to the sediment. Furthermore, the concentration of 0 ppm was 0.19 ppm, 5 ppm was 0.38 ppm, 10 ppm was 0.42 ppm and 15 ppm was 0.29 ppm. Thus, the concentration of 0

ppm had the lowest Pb concentration compared to the concentration of 10 ppm.

The tests on different influences of concentration factor on Pb resulted in the F statistical test in the amount of 0.601 with a 0.620 probability. Therefore, it appears that there is no significant difference in the concentration factor on Pb. The 4th week's test was the highest and was significantly different from the 1st, 2nd, and 3rd week. Moreover, the accumulation of Pb metal at the 1st week was the lowest compared to the 4th week. This indicates that the longer the time of exposure to seagrass, the higher the concentration of Pb in the sediments; it suggests that Pb transferred from water to sediment. This is supported by Wilson (1988)'s opinion stating that heavy metals dissolved in water will move into sediments if they bind to organic matters that are free or are coating the sediment; additionally, there will be direct absorption by the surface of sedimentary particles.

THE HISTOLOGY TISSUE ANALYSIS OF *Cymodocea serrulata* SEAGRASS (ROOTS, RHIZOMES, AND LEAVES) USING THE SEM-EDX MAPPING ON DIFFERENT Pb CONCENTRATION TREATMENTS IN THE LABORATORY

The SEM Results of *Cymodocea Serrulata* Seagrass Root The analysis using the SEM on seagrass roots was conducted to evaluate the root's surface morphology through 1500x and 2000x magnifications (Figure 1). Figure 1 shows the seagrass root structure at 0 ppm of which the exodermis part has regular air space and is circular to hexagonal; while at 15 ppm, the structure and air space are irregular; the endodermis was thickened due to Pb entering the seagrass.

TABLE 3. Results from ANOVA analysis of Pb accumulation based on the concentration of seagrass treatment *Cymodocea serrulata*

Concentration	Means	Probability				Notation
		0 ppm	5 ppm	10 ppm	15 ppm	
0 ppm	0.295		0.024	0.007	0.000	a
5 ppm	1.821	0.024		0.617	0.006	b
10 ppm	2.150	0.007	0.617		0.020	b
15 ppm	3.730	0.000	0.006	0.020		c

TABLE 4. Accumulation of Pb heavy metals in water and sediment

Sample	Pb Concentration (ppm)	Pb accumulation (ppm) at week to			
		1	2	3	4
Water	0	0,0005±0,00	0,0007±0,00	0,0004±0,00	0,0025±0,00
	5	0,0023±0,00	0,0025±0,00	0,0023±0,00	0,0048±0,00
	10	0,0044±0,00	0,0054±0,00	0,0056±0,00	0,0111±0,00
	15	0,0055±0,00	0,0059±0,00	0,0064±0,00	0,0086±0,00
Sediment	0	0,0583±0,09	0,1661±0,17	0,5157±0,68	0,7937±1,37
	5	0,1792±0,20	0,1613±0,21	0,4222±0,29	2,2505±1,68
	10	0,4281±0,31	0,5945±0,35	0,5452±0,48	1,7546±2,10
	15	0,4184±0,32	0,4400±0,16	0,6739±0,51	0,7332±0,25

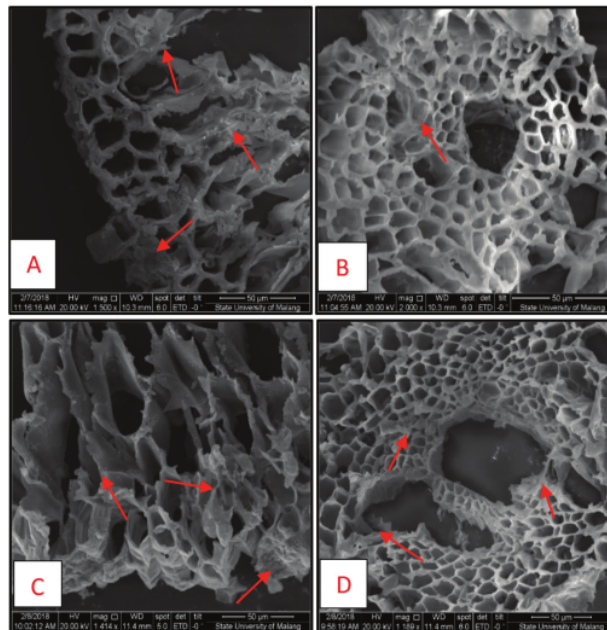


FIGURE 1. SEM root seagrass control; A = exodermis seagrass; B = seagrass endodermis, damage (arrow) and SEM at 15 ppm treatment; C = exodermis seagrass; D = seagrass endodermis, damage (arrow)

The exodermic and endodermic underwent cells damage based on the concentration treatment and exposure time. When the Pb concentration was higher and the Pb exposure was longer, the cells were damaged and the surface structure was thickened. According to Tupan (2016), the accumulation and contamination of Pb metal can accelerate the maturation of exodermic and endodermic cell walls in *Thalassia hemprichii* seagrass. Furthermore, Barcelo et al. (1990) and Sandalio et al. (2001) asserted that in addition to the induction of cell degeneration, changes in cell form and organization showed that heavy metal disrupted the level of root maturity; this occurs due to the ability of heavy metals in disrupting hormone balance. Enstone et al. (2003) explained that apoplast, exodermis, and endodermis have an important part in protecting against various types of stress on plants. According to Gomes et al. (2011), *Brachiaria decumbens* exodermis and endodermis have a good ability in limiting metal flow through the apoplastic method. The thickening of roots cell walls provides an area for retention of heavy metals; and thereby reduces metal translocation in the leaves. The endodermis and exodermis cell thickening is one of *Cymodocea serrulata* strategies to minimize the translocation of Pb metal into other cell tissues. Lux et al. (2004) asserted that exodermis and endodermis cell tissue in plants' roots is immensely characterized by high tolerance to heavy metals.

#### THE RESULTS OF EDX AND SEAGRASS ROOT MAPPING TREATMENT ON 0 PPM AND 15 PPM

EDX devices that were integrated with SEM enabled qualitative and semi-quantitative microanalysis of elements. EDX could be used to analyze quantitatively the percentage of content of each element. The results of EDX and Mapping analysis on seagrass root (Figure 2). The elemental content of seagrass roots in the control consisted of C 40.79%, O 28.68%, Na 06.36%, Al 00.62%, S 00.75%, Cl 12.43%, K 03.75%, Zn 01.59%, Hg 04.20%, Se 00.88%. At 15 ppm treatment consisted of C 28.57%, O 39.57%, Na 04.68%, Al 01.79%, Cl 03.91%, Cd 01.97%, K 03.83%, Sn 01.93%, Ca 02.92%, Cr 00.83%, Cu 01.37%, Zn 03.86% and Hg 04.77%. In the seagrass root sample, Pb is not detected; this is presumably because Pb was compounded with ions or compounds in the experimental unit, therefore, Pb activity at the root was invisible. Kuo and den Hartog (2000) explained that in all types of seagrass, endodermic cell walls can be thin or thick, with or without lignin, and have a Casparian band. The endodermis in seagrass is similar to land-flowering plants having a role in limiting the movement of dissolved substances and water between the cortex to stele.

#### THE SEM RESULTS OF *Cymodocea serrulata* RHIZOMES

The analysis using SEM on seagrass' rhizoma was performed to evaluate its surface morphology through 1500x and 2000x magnification (Figure 3). Figure 3

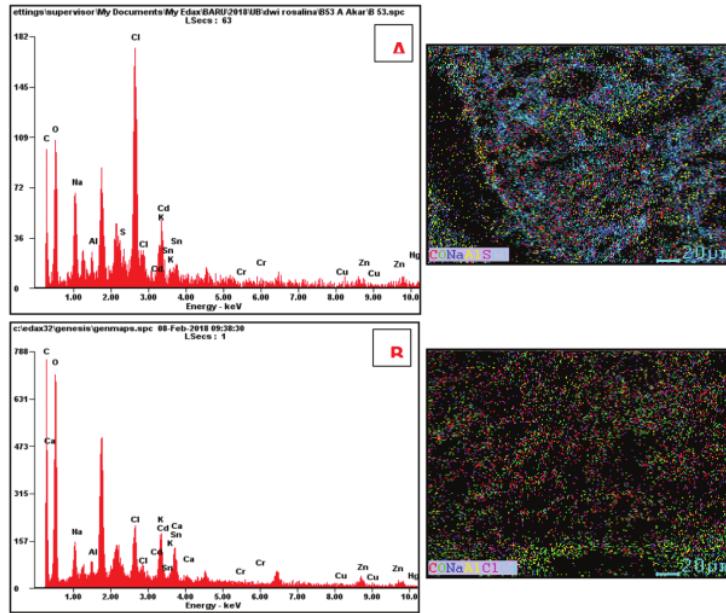


FIGURE 2. EDX spectrum and seagrass root mapping; A = control; B = 15 ppm treatment

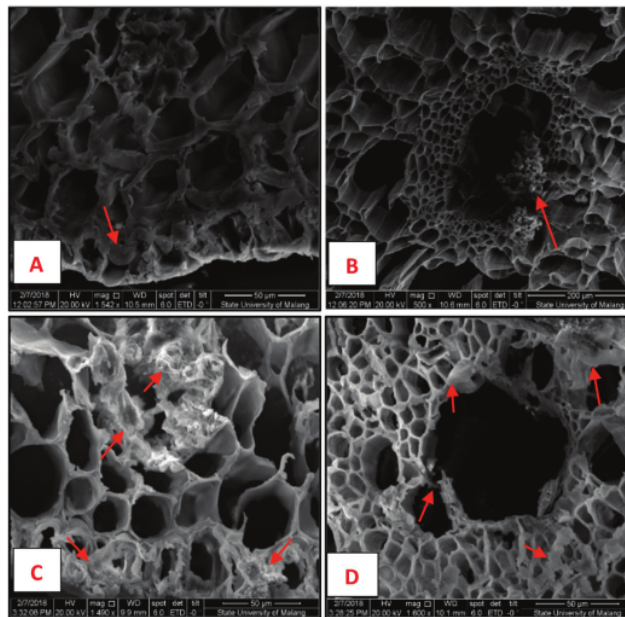


FIGURE 3. SEM control seagrass rhizome; A = seagrass epidermis; B = seagrass endodermis, damage (arrow) and SEM at 15 ppm treatment; C = epidermis of seagrass; D = seagrass endodermis, damage (arrow)

displays the structure of seagrass rhizomes at 0 ppm, of which the seagrass epidermis had regular airspace and were circular to hexagonal; whereas at 15 ppm, the air

space and seagrass structures were irregular and there was thickening in the epidermis, cortex, and endodermis. This is due to damage at the epidermal layer in the rhizome as a

result of Pb metal absorption coming in with the nutrients; therefore, it resulted in cells maturation. Additionally, Pb metal deposition on the cell wall also causes thickening of the epidermal tissue. The thickening of epidermal tissue in seagrass rhizome is a form of adaptation to prevent Pb metal translocation to the central stele. The rhizomes cortex cells were seen to experience changes in the inner air chambers. Gomes et al. (2011) stated that changes in the size, shape, and arrangement of cortical parenchymal cells are due to the ability of heavy metals to disrupt the balance of the hormonal system. The research conducted by Al-Saadi et al. (2013) found parenchyma and cortex air space changes in the *Potamogeton* aquatic plant stem and concluded that the change occurred due to metal binding.

#### THE RESULTS OF EDX AND RHIZOMA SEAGRASS MAPPING ON 0 PPM AND 15 PPM

EDX devices that were integrated with SEM enabled qualitative and semi-quantitative microanalysis of elements. EDX could be used to analyze quantitatively the percentage of content of each element. The results of analysis of EDX and Mapping in seagrass rhizomes are shown in Figure 4. The elemental content in seagrass rhizomes in the control consisted of C 20.49%, O 31.88%, Na 07.93%, Mg 01.77%, Cl 14.80%, Cd 02.04%, K 06.44%, Sn 01.93%, Ca 01.39%, Cr 00.93%, Cu 01.00%, Zn 03.65%, Hg 05.55%. At 15 ppm treatment consisted of C 23.22%, O 36.37%, Na 05.21%, Cl 07.92%, Cd 02.03%, K 06.50%, Sn 02.13%, Ca 00.96%, Ti 02.21%, Cr 00.92%, Cu 01.25%, Zn 04.68% and Hg 06.59%. Pb was undetected

in the seagrass rhizome. It was possible that the Pb was compounded with ions or compounds in the experimental unit; thus, Pb activity in the rhizome could not be seen. The stem epidermis in hydrophytic plants does not function to protect; instead, it is to absorb gases and nutrients directly from the water column. Gomes et al. (2011) asserted that changes in the size, shape, and arrangement of cortical parenchymal cells are caused by the ability of heavy metals to disrupt hormone balance.

#### THE SEM RESULTS OF *Cymodocea serrulata* LEAVES

The analysis using SEM on the seagrass leaves was performed to evaluate its surface morphology through 1500x and 2000x magnification (Figure 5). Figure 5 presents the structure of seagrass leaves; at 0 ppm, the seagrasses cuticle and epidermis have regular airspace and are circular to hexagonal; as to the 15 ppm, the air space and seagrass structure are irregular and there is thickening in the epidermis, cuticle and air space.

According to Kuo and Den Hartog (2006), the presence of porous cuticles facilitated ion movement and carbon diffusion so that the leaves could absorb nutrients directly from seawater because sea water was a source of bicarbonate for plants in the process of photosynthesis. The leaves of the cuticle and epidermis of *Cymodocea serrulata* leaves were thickened as the accumulation of Pb increases (Kuo 1983; Tomlinson 1980). This cuticle served as the entry point of Pb metal through the leaves into the body of seagrass, therefore, this study indicated a thickening of the cuticle caused by trapping Pb metal in the cuticle

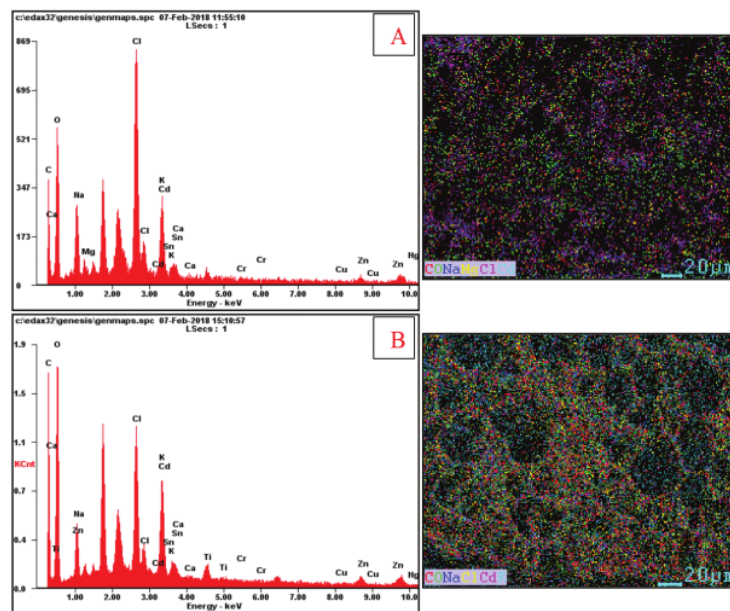


FIGURE 4. EDX spectrum and Mapping of seagrass rhizomes; A = control; B = 15 ppm treatment



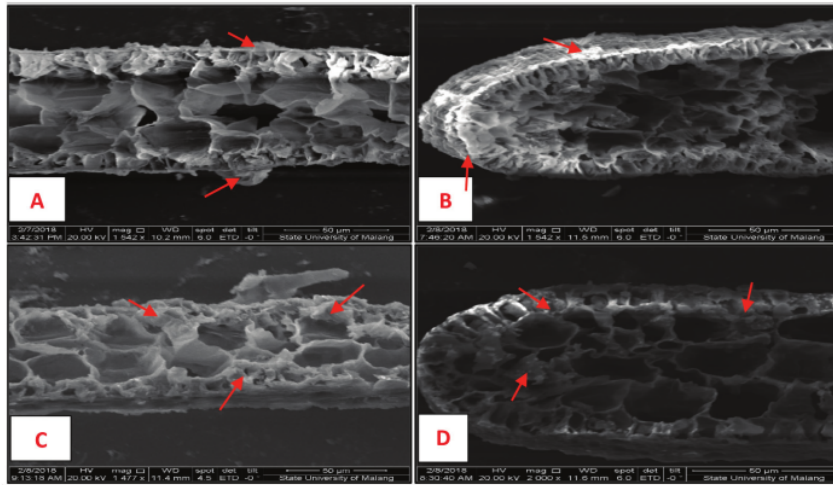


FIGURE 5. SEM leaves of seagrass control; A = cuticle seagrass; B = seagrass epidermis, damage (arrow) and SEM at 15 ppm treatment; C = cuticle seagrass; D = seagrass epidermis, damage (arrow)

cavity (Larkum et al. 2006), thickening that occurred in epidermal tissue was possible as a strategy to prevent further Pb metal transported into chloroplasts cells which could disrupt the CO<sub>2</sub> fixation system in photosynthesis. Al-Saadi et al. (2013) in their research on *Potamogeton* sp. swamp which was exposed to Ag metal resulted that thick abdominal and adaxial epidermis thickening in the leaf tissue of the swamp plant was related to metal binding to the cell wall, which was an alternative pathway for ion

allocation thus preventing translocation to photosynthetic tissue.

THE RESULTS OF EDX AND MAPPING OF SEAGRASS' LEAVES TREATMENT OF 0 PPM AND 15 PPM

The results of EDX and Mapping analysis on seagrass leaves (Figure 6). The elemental content of seagrass leaves in the control consisted of C 23.94%, O 34.76%, Na 09.71%, Al 02.14%, Cl 08.60%, Cd 01.79%, K 05.18%, Sn 01.83%, Cr

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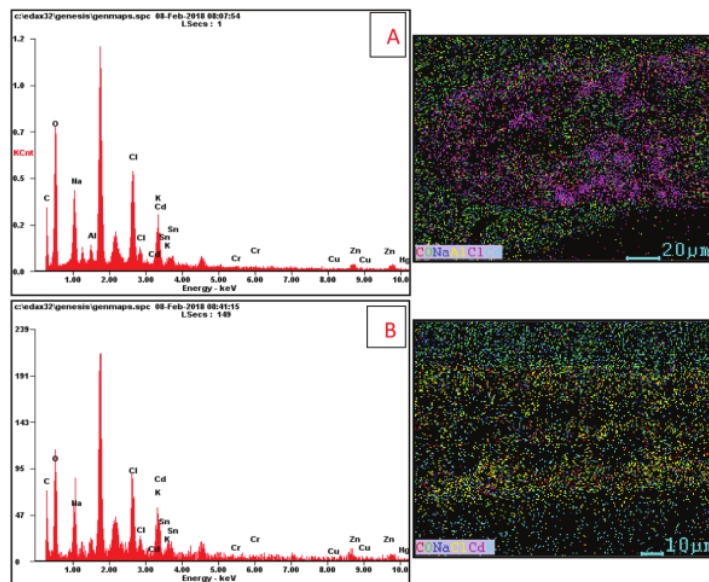


FIGURE 6. EDX and Mapping spectrum of seagrass leaves; A = control; B = 15 ppm treatment

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00.81%, Cu 01.01%, Zn 04.24%, Hg 05.98%. At 15 ppm treatment consisted of C 32.90%, O 35.08%, Na 09.27%, Cl 07.90%, K 06.94%, Cu 00.60%, Zn 06.10% and Se 01, 22%. In the samples of seagrass leaves, there was no Pb metal detected, this was allegedly because Pb metal was compounded with ions or compounds in the experimental unit so that it did not show Pb heavy metal activity in the leaf sample.

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#### CONCLUSION

*Cymodocea serrulata* seagrass can accumulate Pb; the higher the concentration and duration of exposure to Pb, the higher the Pb in seagrass. The SEM-EDX Mapping spectrum shows a thickening in seagrass and it is one of its strategies in minimizing the translocation of Pb metal into other cell tissues.

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Dini Sofarini  
Water Resources Management  
Faculty of Fisheries and Marine Sciences  
University of Lambung Mangkurat, South Kalimantan  
Indonesia

Mohammad Amin  
Biology  
Faculty of Mathematic and Sciences  
University of Malang, Malang 65145  
Indonesia

\*Corresponding author; email: myrafirifky@gmail.com

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Dwi Rosalina, Endang Yuli Herawati, Muhammad Musa &  
Yenny Risjani  
Water Resources Management  
Faculty of Fisheries and Marine Sciences  
University of Brawijaya Malang, Malang 65145  
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