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ANATOMICAL AND PHYSIOLOGICAL APPEARANCES OF SEAGRASS (*Enhalus acoroides*) IN THE POLLUTED ENVIRONMENT OF KAYELI BAY, MALUKU PROVINCE

TAMPILAN ANATOMI FISIOLOGI LAMUN (*Enhalus acoroides*) DI LINGKUNGAN TERCEMAR TELUK KAYELI PROVINSI MALUKU

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Abstract

Pollution in Kayeli Bay is from the Marlosso and Nametek rivers. This area forces full adaptation of biota, including the seagrass *Enhalus acoroides*. This study aimed to compare changes in the anatomical structure and physiological response of the seagrass *Enhalus acoroides*. The preparation method uses a modification of the Ruzin method. Measurements are made using a microscope equipped with a micrometer and a spectrophotometer. The results showed that the thickness of the root epidermis and endodermis in the Marlosso area was 5.77 µm and 2.3 µm; and in Nametek, epidermis 5.58 µm and endodermis 1.54 µm. The epidermis and endodermis of the Marlosso rhizomes are 9.98 µm and 3.5 µm. Nametek rhizomes are 6.62 µm and 3 µm. The leaf tissue in Marlosso, namely the adaxial and abaxial cuticle, is 0.57 µm, the adaxial and abaxial epidermis is 2.84 µm and 2.78 µm, the thickness of the epidermis and cuticle is 10.2 µm and 1.81 µm. Nametek Waters are 0.45 µm and 0.54 µm, and the adaxial and abaxial epidermis are 1.62 µm and 2.29 µm. It was concluded that there was thickening of the cell walls in all tissues in the Marlosso River, and showed lower chlorophyll content compared to Nametek.

Keywords: Anatomy; *Enhalus acoroides*; Pollution; Chlorophyll

Abstrak

Pencemaran di Teluk Kayeli yakni Sungai Marlosso dan Nametek memaksa biota untuk beradaptasi secara penuh, termasuk lamun Enhalus acoroides. Penelitian ini bertujuan untuk membandingkan perubahan struktur anatomi dan respons fisiologis lamun Enhalus acoroides. Metode preparasi menggunakan modifikasi metode Ruzin. Pengukuran menggunakan mikroskop yang dilengkapi mikrometer dan spektrofotometer. Hasil penelitian menunjukkan bahwa ketebalan epidermis dan endodermis akar di daerah Marlosso adalah 5.77 µm dan 2.3 µm, sedangkan di daerah Nametek, epidermis 5.58 µm dan endodermis 1.54 µm. Epidermis dan endodermis rimpang Marlosso masing-masing berukuran 9.98 µm dan 3.5 µm. Rimpang Nametek masing-masing berukuran 6.62 µm dan 3 µm. Jaringan daun di Sungai Marlosso, yaitu kutikula adaksial dan abaksial, berukuran 0.57 µm, epidermis adaksial dan abaksial berukuran 2.84 µm dan 2.78 µm, dan ketebalan epidermis serta kutikula masing-masing 10.2 µm dan 1.81 µm. Ketebalan epidermis dan kutikula di Sungai Nametek adalah 0.45 µm dan 0.54 µm, sedangkan ketebalan epidermis adaksial dan abaksial berukuran 1.62 µm dan 2.29 µm. Disimpulkan bahwa terdapat penebalan dinding sel pada semua jaringan di Sungai Marlosso dan menunjukkan kandungan klorofil yang lebih rendah dibandingkan dengan Sungai Nametek.

***Kata Kunci**: Anatomi; Enhalus acoroides; Pencemaran; Klorofil*

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INTRODUCTION

Kayeli Bay is a body of water that has experienced a decline in quality. Gold mining activities and anthropogenic waste by the community have contributed significant pollutants since it was launched in 2011. It is known that these waters have diverse biota, including seagrass (*Enhalus acoroides*). Seagrass is an aquatic biota that can live in polluted locations, including those that have been polluted by heavy metals. Seagrass tolerance to environmental pollution is carried out by fully adapting to environmental fluctuations that do not support their lives. Metal deposits accumulated by seagrass are usually higher in the organism than in the water, which can adversely affect biota inhabiting the seagrass ecosystem and result in the mortality of biota associated with it (Zurba, 2018). Seagrass can function as a hyperaccumulator because it has a detoxification mechanism in its tissue. This plant possesses potential in coastal areas because it acts as a primary producer, biota habitat, sediment catcher, and nutrient recycler (Rochmady, 2010). Seagrass also has ecological and economic potential (Hartini & Lestarini, 2019).

Kayeli Bay is the estuary of several rivers in the surrounding area. Among them are the Marlosso and Nametek Rivers. Marlosso and Nametek are water areas that are categorized as polluted. Natsir et al. (2019) reported that the content of heavy metals Pb and Hg had exceeded the threshold in the Marlosso and Nametek areas, so they were included in the polluted category. Gold extraction activities, residential areas, and sea transportation by the community have increased the accumulation of pollutants flowing into Kayeli Bay. The level of contamination that occurs is influenced by the intensity of activities between Marlosso and Nametek, so it has an impact on the biota. These waters are also known to have seagrass that is able to adapt to the pressures of the surrounding environment. However, the population and macroscopic appearance experienced abnormal growth.

Seagrass populations are crucial as regulators of aquatic systems and biotic habitats. Therefore, their sustainability requires significant attention. Environmental stress conditions force these plants to adapt to every organ of their body, be it roots, rhizomes, or leaves. Macroscopically, the reduction in seagrass growth rate is thought to be related to excessive absorption of pollutants in each of its tissues. Basically, plants have multiple defense mechanisms, including: 1) the synthesis of phytochelatin peptides and metallothioneins, which function as metal chelators, 2) immobilization, and 3) compartmentalization of metal ions that occur in vacuoles (Cobbett, 2000). Changes in the aquatic ecosystem in the Kayeli Bay area have been extensively documented. For example, Manullang (2021) reported that marine sediments at all sampling points in Kayeli Bay contained mercury ranging from 0.035 to 4.802 mg/kg^{-1}DW, surpassing the National Oceanic and Atmospheric Administration (NOAA) metal contamination threshold of 0.15 mg/kg^{1}DW. The quality of Kayeli Bay waters has deteriorated due to mercury pollution (Sehol et al., 2023). Seagrass resilience to an adverse environment in Kayeli Bay waters inspired us to identify microscopically the changes in its anatomical structure, namely the epidermis, endodermis, and cuticle in the roots, rhizomes, and leaves. In addition, we were also interested in investigating the physiological response of seagrass in the form of leaf chlorophyll levels, which play a role in the process of photosynthesis. Microscopically, the methods employed to determine the tissue response to the presence of pollutants include the tissue slicing method (Tistama et al., 2012). This study focused on comparing the physiological responses and the form of seagrass adaptation to two polluted locations, Marlosso and Nametek Rivers by microscopically observing changes in the anatomical structure of its tissue, including roots, rhizomes, and leaves.

MATERIALS AND METHODS

The current research was conducted from April to June 2024 in the Marlosso and Nametek Waters that flow into Kayeli Bay. The Marlosso Waters are seagrass-covered waters situated at 127° 01'47.57"E and 3°16'30.66" S. Human activities in the Marlosso Waters include gold extraction using drum tools whose liquid waste flows into the waters, settlements, and transportation. The Nametek Waters are located at 127° 06'22.57"E and 3°16'54.08"S. In the Nametek Waters, the existence of seagrass is relatively sustainable, with minimal gold extraction activity, even though there are settlements and docks. Testing of the anatomical structure of *Enhalus acoroides* seagrass was carried out at the Laboratory of Taxonomy, Structure and Plant Development, Brawijaya University in Malang. The physiological response of seagrass (*Enhalus acoroides*) using chlorophyll levels as an indicator in its leaves was analyzed in the Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. Preparations of roots, rhizomes, and seagrass leaves were made using the Ruzin method (Ruzin, 2000). The first stage of the Ruzin method involved cleansing the sample with distilled water, followed by immersion in a formaldehyde, acetic acid alcohol (FAA) solution which, served as a fixative for 24 hours. Fixation sought to preserve the original tissue. Next, a semi-permanent preparation was made. The part of the plant that had been fixed with FAA was then cut as thin as possible (2 µm) using a sliding microtome that had been fitted with a razor blade (clamp-on hand microtome). Then, the sample was inserted into the hole/split of the cork. The cork cylinder and sample were inserted into the sliding microtome holder. The sample was sliced using a microtome. The slices were taken with a brush and placed in a petri dish filled with water/distilled water. The thinnest plant slices were then transferred to a petri-dish containing distilled water, 1% safranin dye was added, and left for 5–10 minutes. The slices were then placed on a glass object and dipped with glycerin solution to coat the preparation to prevent safranin from fading. The finished preparation was observed under a light microscope.

The observed anatomical parameters of seagrass included the thickness of the root exodermis and endodermis, the thickness of the rhizome epidermis and endodermis, and the thickness of the abaxial and adaxial epidermis and cuticles of the leaves. Physiological assessments were conducted by quantifying the total chlorophyll content of seagrass leaves collected from the research sites (Marlosso and Nametek). The collected seagrass leaves were subsequently cleaned with distilled water and placed into a labeled plastic zipper bag. During the extraction procedure, the samples were measured using an analytical balance with a precision of 0.3 g. Each leaf sample was pulverized in a cold mortar until homogenous, after which 2 mL of methanol was incorporated into the crushed leaves (Porra, 1989). The ground samples were transferred into a 1.5 mL Eppendorf tube and vortexed for 25 minutes to achieve homogeneity in the solution. Subsequent to the acquisition of the filtrate, 1 mL was extracted and dissolved in 4 mL of methanol, and its absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-Vis 1800) at wavelengths of 665 nm (chlorophyll a) and 652 nm (chlorophyll b). Figure 1 illustrates the map of the research locations.

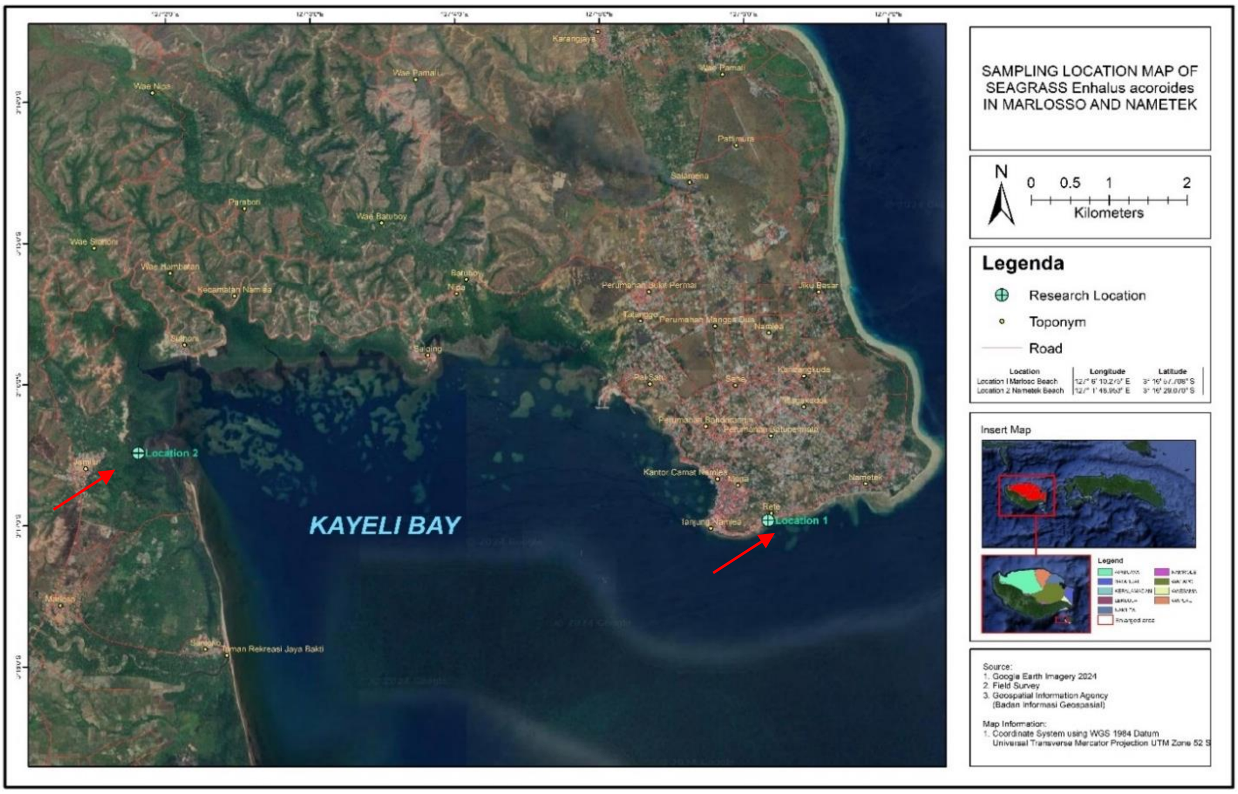

Figure 1. Research locations (Location 1 (Marlosso Waters) and Location 2 (Nametek Waters))

RESULTS

The anatomical transformation of seagrass in the Kayeli Bay area was observed in this study. The anatomical dimensions of seagrass roots of *Enhalus acorroides* in the Kayeli Bay region are illustrated in Table 1.

Table 1. Changes in the structure of the epidermis and endodermis of seagrass roots

Tissue observation	Thickness of epidermis (μm)	Thickness of endodermis (μm)
Marlosso waters	5.77 ± 0.02	2.3 ± 0.05
Nametek waters	5.58 ± 0.11	1.54 ± 0.10

Note: *there are differences in the means of the two locations being compared (T-test; P <0.05)

Figures 2 and 3 show the appearance of the epidermis and endodermis of Enhalus acoroides roots obtained under a microscope at 40x magnification. Information was obtained that there was thickening of the epidermal and endodermal cell walls in the Marlosso River compared to Nametek.

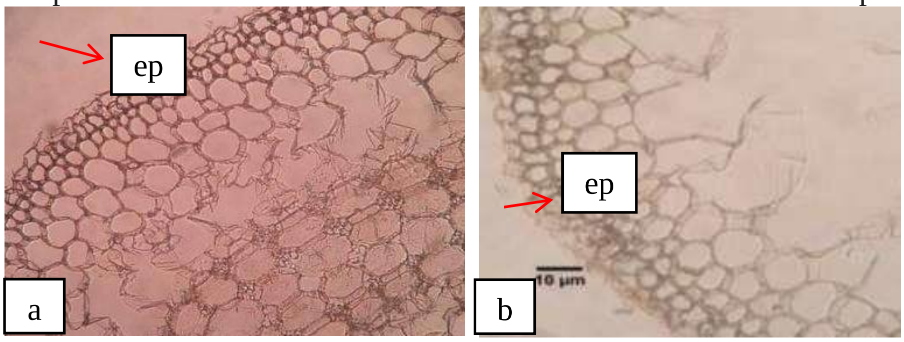

Figure 2. Comparison of the thickness of the seagrass root epidermis. Seagrass root epidermis of Marlosso Waters (a) and Nametek Waters (b) (ep= epidermis)

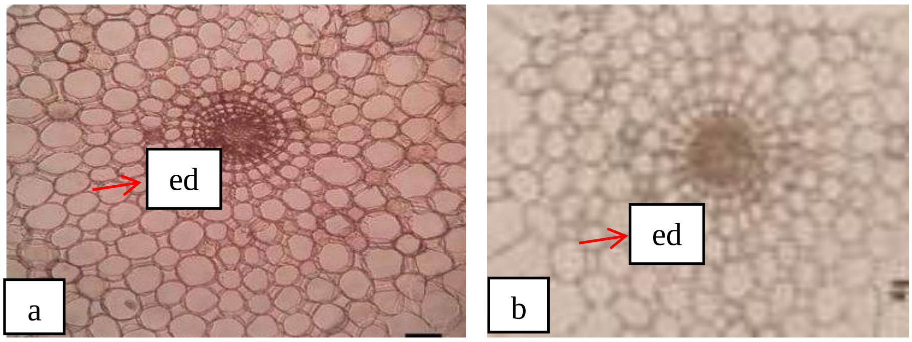

Figure 3. Comparison of the thickness of the seagrass root endodermis. Seagrass root endodermis of Marlosso Waters (a) and Nametek Waters (b) (ed= endodermis)

Table 2 presents data on variations in the size of the rhizome of the seagrass *Enhalus acoroides* as observed via a micrometer microscope. The size differences indicate that Marlosso is thicker than Nametek, indicating a cellular response to rhizome size.

Table 2. Changes in the structure of the seagrass rhizome epidermis and endodermis

Tissue observation	Thickness of epidermis (μm)	Thickness of endodermis (μm)
Marlosso waters	9.98 ± 0.01	3.5 ± 0.02
Nametek waters	6.62 ± 0.30	3 ± 0.09

Note: *there are differences in the means of the two locations being compared (T-test; P <0.05)

Figure 4 shows a cross-section of the epidermal tissue of the *Enhalus acoroides* rhizome. The endodermis of seagrass rhizomes at both locations also showed changes, both in size and appearance. The endodermis of Marlosso showed thickening compared to Nametek (Figure 5).

Table 3 delineates the variations in the size of the components of *Enhalus acoroides* seagrass leaves as observed by a micrometer microscope at the two research sites. The results of the study provide information that overall tissue observations indicate that Marlosso is thicker than Nametek.

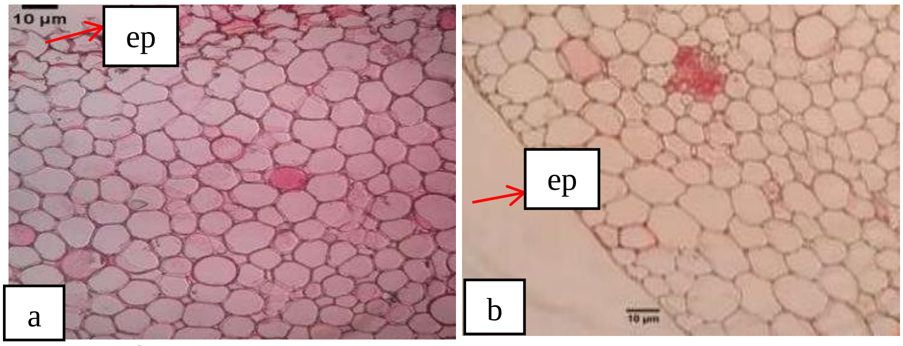

Figure 4. Comparison of the thickness seagrass rhizome epidermis. Seagrass rhizome epidermis of Marlosso Waters (a) and Nametek Waters (b) (ep epidermis)

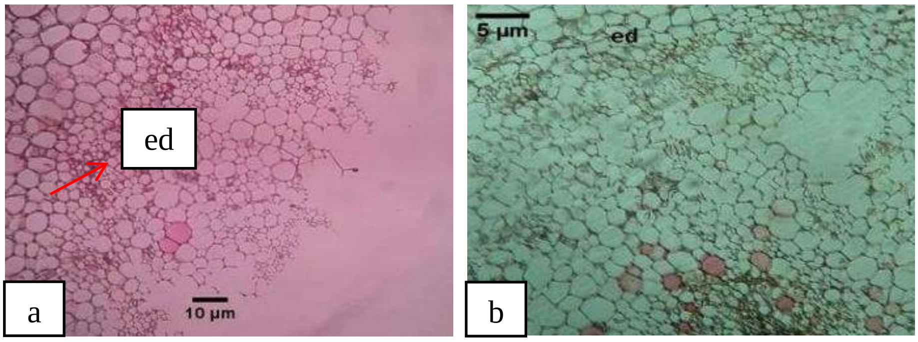

Figure 5. Comparison of seagrass rhizome endodermis. Seagrass rhizome endodermis of Marlosso Waters (a) and Nametek Waters (b) (ed= endodermis)

Table 3. Seagrass leaves anatomical structure

Tissue observation (µm)	Marlosso waters	Nametek waters
Adaxial cuticle	0.57 ± 0.01	0.45 ± 0.01
Abaxial cuticle	0.57 ± 0.02	0.54 ± 0.01
Adaxial epidermis	2.84 ± 0.04	1.62 ± 0.01
Abaxial epidermis	2.78 ± 0.03	2.29 ± 0.02
Epidermis thickness	10.20 ± 0.04	8.75 ± 0.02
Cuticle thickness	1.81 ± 0.04	1.47 ± 0.03

Note: *there are differences in the means of the two locations being compared (T-test; P <0.05)

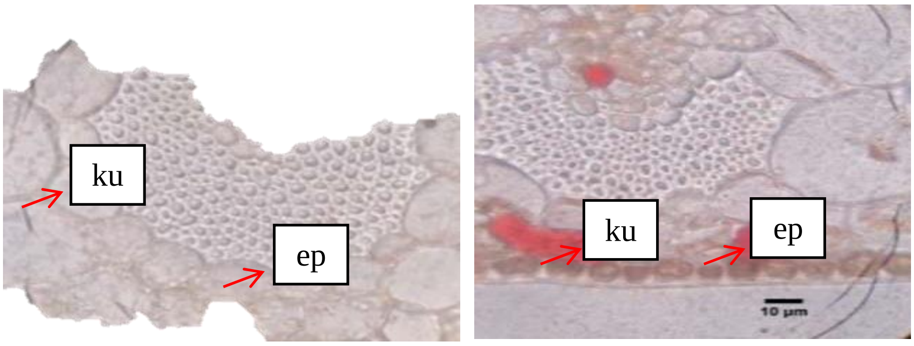

Figure 6. Cuticle and leaf epidermis thickness. Cuticle and leaf epidermis of Marlosso Waters (a) and Nametek Waters (b) (ku cuticle; ep epidermis)

Cross-sectional view of the cuticle and epidermis of the *Enhalus acoroides* leaf. The image shows that thickening has occurred in the cuticle and epidermis tissue of the leaves (Figure 6). Comparison of the epidermal thickness (adaxial and abaxial) and cuticle (adaxial and abaxial) of seagrass leaves at both locations. The image shows that thickening has occurred in the cuticle and epidermis tissue of the leaves. Tissue thickening is a form of plant cellular response to environmental changes. Marlosso exhibits thicker leaf tissue than Nametek, as shown in Figure 7.

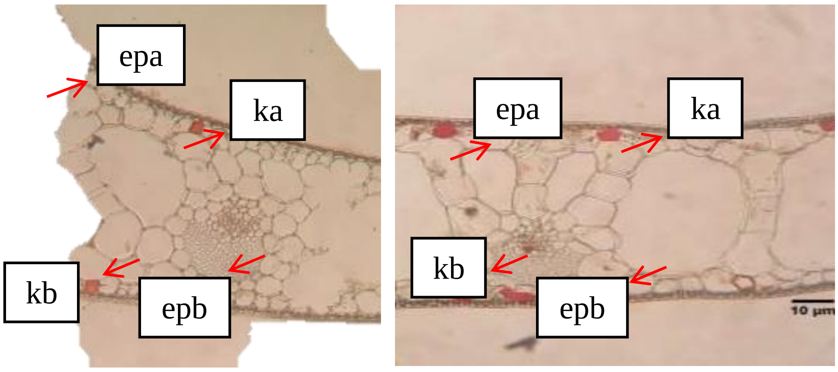

Figure 7. Thickness of adaxial-abaxial cuticle and epidermis of the seagrass leaves. Adaxial-abaxial cuticle and epidermis of seagrass leaves from Marlosso Waters (a) and Nametek Waters (b) (epa= adaxial epidermis; epb= abaxial epidermis; ka= adaxial cuticle; kb= abaxial cuticle)

The study's results indicated that chlorophyll content in seagrass was lower in Marlosso Waters than in Nametek Waters. This indicates a problem with the plants, resulting in environmental stress. Pollution results in unhealthy leaf appearance and reduced chlorophyll production (Table 4).

Table 4. Chlorophyll content in Marlosso and Nametek Waters

Research location	Chlorophyll content (mg/g)
Marlosso waters	4.55 ± 0.52
Nametek waters	26.79 ± 0.02

Note: *there are differences in the means of the two locations being compared (T-test; P <0.05)

Table 4 indicates that the chlorophyll content in the *Enhalus acoroides* leaves from Marlosso Waters averaged 4.55 ± 0,52 mg/g, while in Nametek Waters it was 26.79 ± 0,02 mg/g. Figure 8 illustrates the chlorophyll content of both water samples.

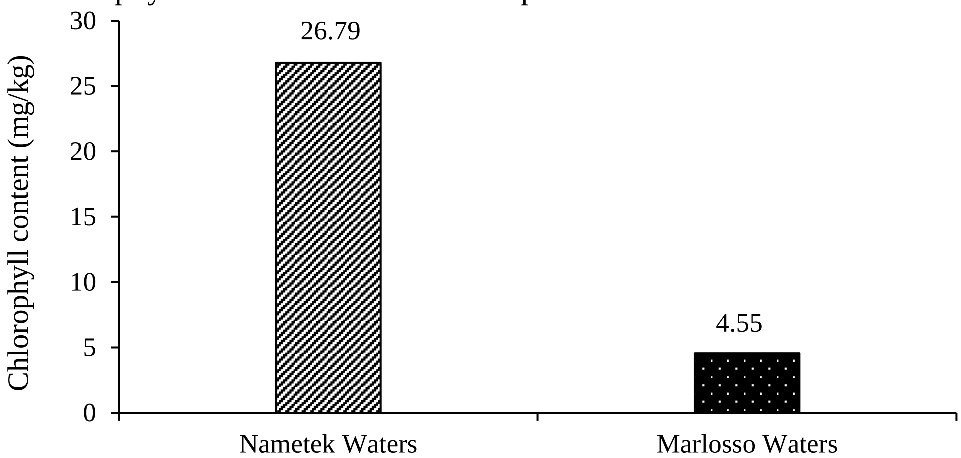

Figure 8. Average chlorophyll content in seagrass leaves in Nametek and Marlosso Waters

DISCUSSION

The results of the study showed that there had been changes in the anatomy of seagrass in the roots, rhizomes, and leaves. This finding thus indicates an adaptive response of seagrass to the increasing amount of pollutants absorbed into the tissue. The roots are the initial component of the plant to react to environmental changes. The successful role of seagrass roots in mitigating ocean wave impact will diminish if root strength is compromised by alterations in morphology. The absorption of nutrients by the roots is believed to be impaired, thereby altering the entire metabolic processes of the plant. The epidermis and endodermis of seagrass roots seen in the Nametek area were thinner than in the Marlosso area (Table 1). The different tissue size conditions in seagrass roots in these waters suggest different pollutant absorption. Based on research by Natsir et al. (2019), the uptake of Pb and Hg pollutants in the Marlosso Water is higher compared to Nametek in the roots.

The accumulation of various contaminants will result in variations in the thickness of the cell walls of both the epidermis and endodermis of the roots. Pollutant concentrations are typically higher in sediments near the water's bottom than on the top, from which they are subsequently absorbed by the roots. Wang et al. (2007) reported that roots commonly accumulate contaminants around plants, followed by the leaves. Diffusion takes place during the access of contaminants to the roots which eventually find their way into the xylem flow. Another study by Aprilya et al. (2020) showed that the histological structure of seagrass *Halodule uninervis* showed thickening in both the epidermis and endodermis. Increasing thickness of tissue indicates a high pollutant concentration. This is a seagrass strategy in preventing the spread of metal so that it does not move to other tissues. In addition, thickening is caused by seagrass response factors to their habitat and the synthesis of specific metallothionein proteins in plant cells to overcome environmental stress.

Pollutants have thus far caused impaired development of plant organs, damage to cell walls, and disruption of cellular division. Pollutant-induced stress in aquatic environments leads to gradual alterations in the metabolic system of seagrass, particularly affecting its cellular structure (Figure 1). The increased lignin content resulting from variations in lignin concentration and other secondary metabolites will directly affect seagrass growth (Aprilya et al., 2020). Lignin is recognized as a complex cell wall structure of covalently bonded phenolic heteropolymers, polysaccharides, and proteins. Lignin imparts structural integrity to the cell walls of higher plants and serves as mechanical support for the plant's vascular system. This situation facilitates the transfer of water and nutrients while safeguarding plants from external disturbances, such as excessive heavy metals.

The seagrass root cells of *Enhalus acoroides* in this study were seen to experience several changes, one of which was a change in the thickness of the root epidermis at both locations (Figure 1). This is thought to be due to the interaction between pollutants such as heavy metal ions and the seagrass cell walls. Root cell walls are known to have the ability to combine heavy metal ion bonds as an effort to prevent heavy metal ions from entering the cell and being transported to other cell parts and tissues, but the consequence of this is the loss of cell wall elasticity which causes cell deformities to cell damage (Irawanto & Mangkoedihardjo, 2015; Manurung et al., 2019). The degradation of cellular integrity resulting from morphological alterations and cellular shrinkage is attributable to the diminished elasticity of the cell wall and membrane, induced by pollutants like heavy metals (Barceló & Poschenrieder, 2014). The root epidermis in Malosso waters is thicker than Nametek, signifying the presence of oxidative stress due to contaminants, including heavy metals, in the vicinity. According to Del Río et al. (2002) and Siahaan et al. (2013), pollutant stress such as heavy metals may affect the thickening of cell walls, organelles, and cell nuclei. This cell damage might be caused by increased free radicals attributed to heightened metal ion concentrations in plant tissue.

In addition, the thickened epidermis layer of the *Enhalus acoroides* roots in Marlosso Waters is a response to the flow of pollutants such as heavy metals into the cells. The thickening of the root epidermis strengthens the role of the epidermis to protect the underlying tissue. Plants withstand environmental stress by storing pollutants in the root epidermis layer and not releasing pollutants, such as metals moving towards the aerial part. Tupan and Azrianingsih (2016) showed that increased exposure to Pb metal to the roots of seagrass *Thalassia hemprichii* resulted in thickening of the epidermis of the roots as an effort and strategy for plant defense against metals. Plant tolerance and resistance to heavy metal stress are closely related to one mechanism. In other words, plants accumulate metals in the roots so that there is a process of inhibiting metals towards the aerial parts and the movement of metals in cell walls that contain a lot of cellulose and lignin. The ability of seagrass plants to absorb these metals is due to increased transpiration and is strengthened by their root system, which is evenly distributed in all directions of the growing medium.

Exodermis is a layer located under the epidermis. It has a polyhedral shape and is large in size. The exodermis is also thought to thicken along with the absorption of pollutants through the epidermis. In addition, it has dense and regular cells and consists of parenchymal cells that have thick walls (Larkum et al., 2018). The exodermis appears in root tissue as a form of plant tolerance to pollutants, including heavy metals (Lux et al., 2004). The exodermis functions to protect root cells from the infiltration of hazardous substances that surpass environmental thresholds. Thickening of the exodermis is carried out by plants to reduce the absorption of toxic elements by root cells (Atabayeva et al., 2016). The exodermis is known to be a special apoplastic barrier in plants to protect them from environmental stress (Enstone et al., 2003 *in* Raharja et al., 2020).

The roots have a cortex section wherein the cells will experience lignification and structural transformation into periderm. Periderm is known to act as one of the plant's defense mechanisms against heavy metal toxicity to reduce the absorption of pollutants such as heavy metals by the roots. Xylem is also thought to experience thickening of the cell walls, namely in the trachea section. Xylem is a water conductor in vascular plants, which will undergo an increase in the quantity of tracheae as contaminants infiltrate the roots An increase in the number of tracheae elements in the xylem will increase the hydraulic capacity and risk damaging the tissue. Changes in the anatomical shape of a plant are an adaptive response so that the plant becomes tolerant to heavy metals. Zou et al. (2012) in their report stated that heavy metals also cause abnormal conditions such as chromosome fractures, which will have an impact on the cell division process. The results of the study showed disruption of cell structure in the root epidermis in Marlosso Waters with a thicker shape compared to Nametek Waters.

Endodermis is a layer of tightly closed cylindrical cells. It acts as a selective layer for plants against minerals and nutrients that enter the central cylinder (stele) so that plant metabolism remains safe. Figure 2 depicts the results of micrometer microscope observations, which showed that there were differences in the thickness of the endodermis layer in the roots of *Enhalus acoroides* seagrass. The results of these observations indicated that the endodermis layer of the seagrass roots in Marlosso Waters was thicker than the endodermis layer of the seagrass roots in Nametek Waters. The endodermis structure has one layer of cells that are tightly patterned without an inter-cellular space under the cortex. The endodermis has many functions, including regulating water absorption into vessels, storing food reserves, supporting plants, and selecting minerals that move towards the central cylinder (stele). Seagrass is a plant that has special successor cells or endodermis cells that do not experience thickening of their cell walls. The thickening of the endodermis that occurred in this study is thought to be a form of seagrass self-defense, through its root layer, against heavy metals that are absorbed with water. Root endodermis tolerance is carried out as a form of plant selectivity towards water absorption towardsinto the stele. Enstone et al. (2003) , Tupan and Azrianingsih (2016) argue that apoplast, exodermis, and endodermis play a special role in protecting the plant from various environmental stresses. Root exodermis and endodermis tissues play a major role in forming a tolerance system to heavy metals (Lux et al., 2004). Hilmi et al. (2018) reported a significant increase in exodermis and endodermis ($P < 0.05$) compared to controls in weed species that had been induced by heavy metals Cu, Cd, Zn, and Pb.

A rhizome is a cylindrical stem of seagrass that extends horizontally beneath the soil or substrate (Rahman et al., 2022). A rhizome is a component of seagrass that is substantially embedded in a broad substrate, enabling the formation of seagrass meadows. *Enhalus acoroides* possesses a more rigid and inflexible rhizome, with some specimens containing lignin and resembling wood. The rise in water contaminants affects the thickness of the rhizome epidermis. The morphology and dimensions of the epidermal tissue result from pollutant accumulation, accelerated cellular maturation, and enhanced tissue integrity (Aprilya et al., 2020). The disparity in epidermal tissue size at the two research sites is believed to reflect various causes, specifically the differing responses of seagrass to pollutants, including heavy metals such as lead present in both seagrass regions. Pollutant-laden tissue results in the thickening of seagrass tissue structure, leading to tissue stress. The absorption and buildup of pollutants at elevated concentrations will be cytotoxic to seagrass organ tissue, leading to alterations in form and structure that impact plant growth and physiology (Alkorta et al., 2004; Rosalina et al., 2019; Aprilya et al., 2020). Exposure to contaminants, particularly metals, will augment cell wall thickness and lead to diminished seagrass growth by interfering with the cell division process (Steinhagen et al., 2004).

The second factor contributing to variations in the size of the epidermal tissue at the two research sites is the makeup of specialized proteins known as Metallothioneins. This synthesis arises from the physiological reaction of plants to pollutant contamination, such as metals, resulting in cellular stress. Metallothioneins (MTs) constitute a super-family of cysteine-rich, low molecular weight metalloproteins capable of binding heavy metals. Cytosolic metallopeptides are extensively present in living organisms and are believed to have a role in metal homeostasis, metal detoxification, and defense against oxidative stress. Plants synthesize these metal chelators to mitigate the toxic effects of heavy metals, and they are prevalent in plant organs such as roots, leaves, and calluses. Plants possess a specialized mechanism involving phytochelatins (PC) that enables them to safeguard against metal toxicity by forming complexes with high-affinity ligands in the vacuole, thus sequestering released toxins away from the metal-sensitive metabolic center in the cytoplasm. Moreover, seagrass has distinct physiological characteristics. The accumulation of contaminants can induce various physiological alterations in the tissues of different species (Joshi, 2022).

This study's results showed that the epidermal cells of seagrass rhizomes underwent thickening due to persistent pollution exposure. The increased thickness of seagrass rhizomes in Marlosso Waters relative to Nametek Waters indicated that contaminants, including heavy metals, might induce oxidative stress in plants (Sandalio et al., 2001). Physiological responses of plants, including impairment of cellular metabolic processes, are evidenced by alterations in mesophyll cell area, reduction of intercellular space, and disruption of chloroplast structure. The adaptation exhibited by these plants likewise seeks to impede the transfer of heavy metals toward the central stele. Metal pollution ingested with nutrition induces fast cellular differentiation. Plants utilize specific systems inside their tissues to absorb gases and nutrients, enabling them to manage harmful amounts in their environment (Ningsih et al., 2024). Zhang (2013) asserted that plants residing in polluted environments would thicken their tissues as a mechanism to reduce metal transfer. The epidermal structure of seagrass rhizomes in Marlosso and Nametek Waters exhibited variations in morphology as observed under a micrometer microscope. The rhizome in Marlosso Waters was thicker than that in Nametek, as indicated by the tissue size. The technique for inhibiting heavy metal translocation to the central stele is executed by plants as a manifestation of tolerance and adaptation. The absorption of metals, along with water and nutrients, facilitates rapid cell differentiation (Figure 3). Plants employ specific mechanisms to respond to harmful environmental exposures to ensure their survival and development (Firmansyah, 2010). The epidermis functions to safeguard against mechanical tissue injury by regulating the quick transpiration process in plants (Tobing et al., 2021).

Figure 4 illustrates that the endodermal tissue of the seagrass rhizome has undergone changes as determined by the micrometer microscope analysis. Furthermore, the study's results indicated a greater thickness of the endodermis in Marlosso Waters compared to Nametek Waters. The endodermis serves as a physical barrier that inhibits the translocation of metals from the roots to the crown. According to Pourrut et al. (2011), metal contaminants taken by the roots via the apoplastic pathway are maintained in the endodermis and subsequently expelled by the plant's detoxifying system. Prior research by Lux et al. (2004), Zhang (2013), and Atabayeva et al. (2016) demonstrated that the thickening of the endodermis in the rhizome of Marlosso Waters might signify the capacity of the plants to endure environmental stress. In an inhospitable environment, plants exhibit thickening of the endodermis, which occludes intercellular spaces due to the build up of contaminants within their tissues. The cross-sectional view of the seagrass endodermis in Marlosso Waters is thinner than that in Nametek, likely due to the reduction of endodermis cell walls in seagrass resulting from direct absorption of heavy metals through the vascular tissue in the stele. The absorption of gas and nutrients can transpire directly due to the thin cellulose walls and cuticles of seagrass (Irawanto & Mangkoedihardjo, 2015). The absorption of metal occurs concurrently with the uptake of gas and nutrients through the tissue.

Leaves serve as the site for evaporation and transpiration processes in plants. Leaves exposed to polluted environmental conditions will exhibit an adaptive process as a form of tolerance to certain mechanisms. This study examined the adaxial cuticle, abaxial cuticle, adaxial epidermis, abaxial epidermis, leaf epidermal thickness, and cuticular thickness of *Enhalus acoroides* seagrass. The cuticle is a part of the leaf that consists of a thick wax layer on the outside of the epidermis. The cuticle layer is a hydrophobic layer on the air surface of all plants. The plant cuticle protects plants from dehydration or uncontrolled water absorption (Eigenbrode & Espelie, 2019), pathogen infection

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(Xu et al., 2016), insect attacks (Eigenbrode & Espelie, 2019), UV radiation (Chen, 2021), and organ fusion (Ingram & Nawrath, 2017). The cuticle plays a role in maintaining and preventing excessive transpiration in plants growing in high environmental temperatures such as the coast. This condition is exhibited by plants in response to adverse environmental stress. Figure 5 illustrates a cross-section of the cuticle and epidermis of *Enhalus acoroides* seagrass leaves.

The epidermis is the outermost cellular layer of the leaf that serves as a protective barrier for plant tissue. This layer has densely packed cells, creating a compact structure devoid of intercellular space. The plant epidermis comprises a waxy cuticle, comprised of upper and lower epidermal layers that serve to retain water. The leaf epidermis and cuticle in the Marlosso area are 10.2 µm and 1.81 µm in thickness, respectively, surpassing those of Nametek, which are 8.75 µm and 1.47 µm. The leaf cuticle's surface is typically smooth (Surya & Hari, 2018), although plants may adjust to environmental stress by augmenting the cuticle's thickness. The waters of Marlosso are believed to possess higher levels of pollution contamination, particularly heavy metals that leach into Kayeli Bay. The thickness of the cuticle is a plant strategy in controlling transpiration rates and maintaining stable water levels. This part has a shiny leaf surface and is able to reflect sunlight that hits the leaf surface, thereby reducing evaporation (Panawala, 2017). In line with the opinion of Kim et al. (2017), the cuticle can facilitate the transmission of sunlight to the thylakoid membrane, hence regulating the rate of carbon assimilation. The thickening response of the cuticle and epidermis of *Enhalus acoroides* leaves in both aquatic environments is anticipated to mitigate excessive water loss beyond the threshold.

Seagrass has special characteristics compared to flowering plants on land, notably the absence of stomata. However, seagrass leaves possess a thin cuticle layer that can substitute for the function of stomata in regulating water and gas exchange. The cuticle allows gases and nutrients to diffuse directly into and out of the leaves from the water. Seagrass is a plant devoid of stomata yet capable of assimilating carbon for photosynthesis due to its thin layer of hollow cuticle (Susetiono, 2004; Ahmad et al., 2017). Elevated pollutant concentrations in water will enhance the thickness of the cuticle and leaf epidermis. The absorption of contaminants into plant tissue is influenced by the structure and permeability of the cuticle layer. Tupan et al. (2014) reported that plants could accumulate Pb in their leaves and bark. Metal translocation to leaf tissue passes through a passive mechanism. Metals enter the plant body along with the water absorption process. Sandalio et al. (2001) indicated that the physiological reaction of plants to heavy metals might result in an increase in mesophyll cell size, a reduction in intercellular space, and disruption of chloroplast structure. Larkum et al. (2018) found that the epidermis of seagrass leaves *Enhalus acoroides* was rich in chloroplasts, and its epidermal cells could connect with the epidermis of neighboring cells.

Furthermore, Colombo (2015) *in* Larkum et al. (2018) indicated that *Enhalus acoroides* comprised phenolics in the form of tannins, which play a role in the physiological adaption to aquatic settings. Larkum et al. (2018) posited that the absorption of heavy metals could diminish the density of grana in chloroplasts, hence disrupting the photosynthesis process in seagrass. The epidermis constitutes the outermost layer of the leaf. The epidermis is segmented between the upper epidermis (adaxial) and the lower epidermis (abaxial). The adaxial epidermis is located behind the cuticle and above the hypodermis, whereas the abaxial epidermis is situated beneath the parenchymal sponge tissue. The adaxial and abaxial epidermal layers include a single layer of densely packed cells covered by a cuticle. The adaxial surface of the leaf features a robust cuticle made of cutin and wax. The abaxial surface is thinner than the adaxial surface (Crang et al., 2018).

Figure 6 illustrates a cross-section of a seagrass leaf exhibiting an intercellular space between the upper and lower epidermis, which is believed to serve as a reservoir for metal contaminants that may impact plant photosynthesis. Seagrass response to contaminated settings is believed to enhance phloem thickness. In the waters of Marlosso, there is an enhancement in the protective role and function of leaf cells. In Nametek Waters, the epidermis and cuticle tissue of seagrass are less robust than those in Marlosso. This state facilitates enhanced ion mobility and carbon diffusion, enabling the absorption of heavy metals into the leaves alongside water and nutrients. The accumulation of heavy metals on leaves will influence the thickness of leaf tissue in the Nametek region. Sandalio et al. (2001), Tupan and Azrianingsih (2016) describe that metal deposition in cells diminishes vascular bundles, resulting in a reduction of xylem elements. This is a plant adaptation to sustain water flow. The epidermis and the adaxial and abaxial leaf cuticles regulate gas exchange between plant leaves and their surroundings. This condition can influence plant metabolism and general growth and development. Jacoeb et al. (2011) assert that aquatic plants possess two layers of epidermal tissue: the upper epidermis and the lower epidermis. The adaxial and abaxial epidermis exhibit some distinctions in their derivatives. Tobing et al. (2021) claim that the top epidermis produces glandular trichomes, while the lower epidermis has two types of derivatives: glandular and nonglandular trichomes.

Physiologically, the photosynthesis ability of seagrass is assisted by green leaf pigments known as chlorophyll. This green substance is found in plant leaves. This substance is able to utilize solar energy, trigger CO_2 fixation and provide energy for the ecosystem as a whole (Bayang et al., 2021). In response to an adverse environment, seagrass develops a self-defense mechanism to allow its physiological processes to occur normally. Rosidah et al. (2014) explained that the physiological response of plants to pollutant stress might be characterized by root growth and leaf color. The results of the current study showed that the chlorophyll content in seagrass in Marlosso Waters decreased significantly when compared to that in seagrass in Nametek Waters (Table 4). In line with the results obtained, the decrease in chlorophyll levels in these leaves can occur simultaneously with increasing levels of pollutants in waters such as metals (Sembiring & Sulistyawati, 2006). This phenomenon may be caused by damage to the chloroplast structure. In line with Göthberg (2008), Pb pollutants contained in plant tissue will reduce the amount of chlorophyll in the plant and disrupt the photosynthesis process.

Seagrass *Enhalus acoroides* is recognized as a hyperaccumulator plant, capable of accumulating metals at levels exceeding 100 times those of typical plants. This aquatic plant is able to store many heavy metal ions in its tissues, such as cell walls, vacuoles, and cytoplasm and bind to other organic materials (Puspita et al., 2011). This occurs because of the physiological processes and gene expression that control the absorption, accumulation, and tolerance of the plants to metals (Hidayati, 2013). Nonetheless, knowledge of pollutant discharge from gold mine waste and anthropogenic activities must remain a significant concern for both the government and society to ensure the sustainability of the seagrass environment.

CONCLUSION

This study has shown that there have been changes in the anatomy of *Enhalus acoroides* as a whole in all organs, including roots, rhizomes, and leaves. Thickening of the epidermis and endodermis in the roots, rhizomes, and leaf cuticles appears to be more dominant in Marlosso Waters compared to Nametek Waters. Likewise, the chlorophyll content of seagrass leaves in Marlosso Waters is lower than that in Nametek Waters. Further research is recommended with other aquatic biota indicators. The research data can be used as a reference for similar research.

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