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Analysis of mercury (Hg) content and chlorophyll content of seagrass *Enhalus acoroides* in Cidaun and Sindangbarang waters, Cianjur regency, West Java Province, Indonesia

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Abstract

Seagrass is a type of vegetation that can live and develop well in coastal areas and is a habitat for several marine animals, such as sea cucumbers, sea urchins, starfish, and sea urchins. Seagrass is an organism that is susceptible to pollution of the coastal environment, such as mercury (Hg), so it impacts the chlorophyll content of seagrass leaves. This study aims to determine the content of heavy metal Hg and its relationship with the chlorophyll content of seagrass *Enhalus acoroides* in the waters of Cidaun and Sindangbarang in Cianjur District, West Java Province. The study was conducted in May 2020, and laboratory analysis was conducted in June 2020. Samples were taken at two stations, namely Sindangbarang Beach and Cidaun Beach. The results showed the heavy metal Hg content in roots> rhizoma> seagrass leaves. The content of heavy metals and seagrass chlorophyll has a negative correlation where the Hg metal correlates (r = - 0.97) and indicates that the higher the content of heavy metals will reduce the seagrass chlorophyll content.

Keywords: Chlorophyll; *Enhalus acoroides*; Pollution; Hg; Vegetation

1 Introduction

Seagrass is a type of vegetation that can live and develop well in coastal areas and a habitat for several marine animals, such as sea cucumbers, sea urchins, starfish, and sea urchins. Seagrass is an aquatic organism susceptible to the entry of pollutants into the sea, such as household waste anthropogenic and heavy metals. This plant is a living organism that can be used as a bioindicator of heavy metal pollution in the aquatic environment. Heavy metal waste is the most dangerous waste because it has toxic effects on humans [1, 2]. Heavy metal pollution that enters the aquatic environment will dissolve and accumulate in sediments or biota and can increase over time, depending on the conditions of the aquatic environment [2].

Bioaccumulation of metals in a marine organism is the first step before it shows its response to pollutants or contaminants and the geochemical cycle [2,3]. Research on the ability of aquatic vegetation, both seagrass and macroalgae, to accumulate heavy metals has been previously studied [3-7], who found that seagrass is one way to determine the level of pollution in marine waters. Seagrass is a marker of metal accumulation capacity because it interacts directly with water bodies and groundwater (substrate) through the leaves and roots to uptake ions so that seagrass can reflect the overall health status of the waters [4-7].

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Enhalus acoroides (Hydrocharitaceae) was widespread in the Indo-Pacific, Indian Ocean, and it ranges from western to eastern Indonesia [1]. Heavy metals in *E. acoroides* have been reported in several studies. Most studies used the whole plant (consisting of leaves, rhizomes, and roots) as a bioindicator for heavy metals [4-7]. In addition, above-ground (leaves) and below-ground materials (including rhizomes and roots) showed significant differences in their accumulation of specific heavy metals [1-7]. *E. acoroides* can easily be divided into three main organs: leaves, rhizomes, and roots. This raises the question of whether there are significant differences in the accumulation of different heavy metals in the three different organs of the tropical seagrass species *E. acoroides*.

Cidaun and Sindangbarang are waters located in Cianjur Regency, West Java Province, and are located along the southern coastal waters of West Java. Due to iron sand mining activities operating in the Sidangbarang coastal area, these waters are prone to degradation. Uncontrolled mining of iron sand in this area will threaten environmental sustainability. A damaged environment will impact coastal and marine ecosystems and, in the long term, threaten the sustainability of the fishery ecosystem on the coast of Sindangbarang. [8] reported that iron sand mining is one of the sources of degradation of the aquatic environment.

Based on the observations that have been made, the growth of seagrass in this area has decreased in population with only the presence of *Enhalus acoroides* seagrass in two waters, namely Cidaun and Sindangbarang. This growth is caused by disrupting the photosynthetic process in seagrass, resulting in seagrass growth. There is a green pigment needed in this photosynthesis process, namely chlorophyll. Chlorophyll is the main component of chloroplasts, and chlorophyll content is relatively positively correlated with the rate of photosynthesis [9-10]. Chlorophyll is synthesized in leaves and has a role in capturing sunlight, and the amount is different for each species. The function of chlorophyll will be disrupted by exposure to pollutants that plants absorb through the roots. This process was thought to cause a decrease in chlorophyll content in seagrass leaves. The absorbed pollutants will be involved in the metabolic process.

The effectiveness of plants in absorbing pollutants will decrease with increasing concentrations of pollutants. The subsequent impact is disruption of plant function in the environment [9, 12]. [5, 6] stated that the photosynthetic process in transport vessel plants is sensitive to biotic (pathogenic) and abiotic (drought, temperature, nutrient deficiency, pollutant) stresses. This paper aims to examine the relationship between the accumulation of heavy metal mercury (Hg) and its relationship with chlorophyll content in *Enhalus acoroides* in Cidaun and Sindangbarang waters, Cianjur Regency, West Java Province.

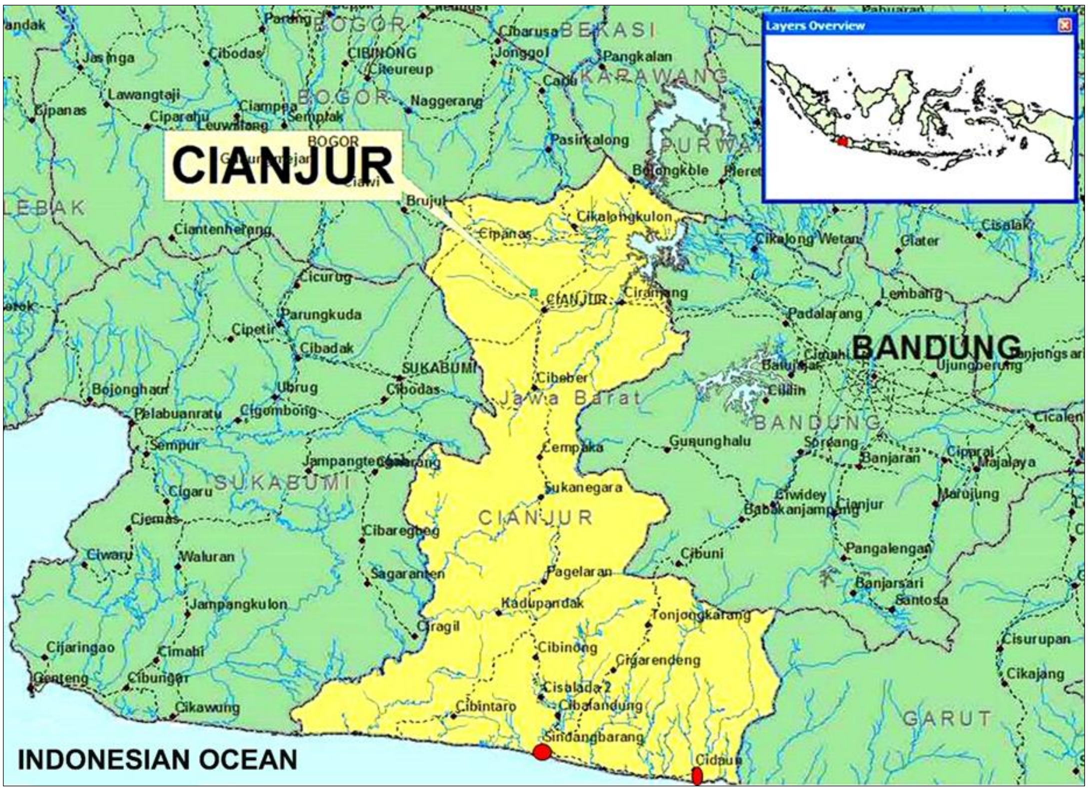

Figure 1 Map of Cianjur Regency, West Java Province

2 Material and methods

2.1 Determination of Sampling Stations

The sampling station was determined using the Garmin Geographic Positioning System (GPS). The sampling station was determined deliberately by considering the needs and sources of pollutant input into the waters from land. Sampling stations were carried out at two stations along the waters of Cidaun and Sindangbarang Beach because seagrass is only found in these two waters. There are activities on land such as mining (drum), settlements, and docks in this area. Seagrass samples were collected using a *tropol* tool and then slowly removed so that the seagrass would not be damaged. Seagrass *Enhalus acoroides* samples were taken in Cidaun waters, where seagrass is present. Seagrass samples were put in a plastic bag, then stored in a cool box and taken to the laboratory for preparation before being analyzed for the content of heavy metal Hg in root organs, rhizomes, and seagrass.

2.2 Analysis of Heavy Metal Content of Hg

Samples of *Enhalus acoroides* were taken at two stations (Cidaun and Sindangbarang) where there were seagrasses by removing them slowly so as not to damage the roots, rhizomes, and leaves. Then the *Enhalus acoroides* samples that have been taken are separated from the roots, rhizomes, and leaves to be used as test samples. The parts of *Enhalus acoroides* seagrass are mashed using a blender, then dried. A fine sample was taken and weighed as much as 30 g with analytical scales. The sample is put in a plastic that has been labeled. Then do the extraction: Put the sample into the digestion tube. Added 5 ml of HNO3 p.a. and 0.5 ml of HClO4 p.a. and left it one night. The next day it is heated in block digestions with 100 ^{0}C for one hour, then the temperature is increased to 150 ^{0}C. After the yellow steam is used up, the digestion block temperature is increased to 200 ^{0}C. The digestion is complete after white smoke comes out and the remaining extract is approximately 0.5 ml. The tube is removed and allowed to cool. Dilute the extract with ion-free water to an exact volume of 50 ml and shake with a tube shaker until homogeneous.

This extract can be used for the measurement of macro and microelements. For standards without going through the ashes process. Samples were analyzed using Cold Vapor Atomic Absorption Spectrophotometer (CV-AAS). Chlorophyll levels were measured by weighing 1 gram of leaves, then mashed with mortal and given 25 ml of 85% alcohol. The solution was filtered with filter paper, and the filtrate was put into a cuvet as much as 3 ml using a micropipette. The cuvette was inserted into the spectrophotometer by adjusting the wavelength of 640 nm and observing the absorbance of the chlorophyll content shown on the spectrophotometer screen. The Hg heavy metal content data obtained are displayed in tables, graphs, and histograms and then discussed descriptively. The content of heavy metal Hg in seagrass organs is compared with the quality standard of SNI 7387: 2009. The closeness of the relationship between the heavy metal mercury content and chlorophyll content was analyzed using the Pearson correlation [13-20].

3 Results and discussion

3.1 Content of Heavy Metal Hg in Seagrass Root of *Enhalus acoroides*

The results showed that the Hg content in seagrass roots at the Cidaun and Sindangbarang was 16.71 ± 0.21 mg/kg and 5.26 ± 0.16 mg/kg (Figure 2). The highest Hg content was obtained at ST 8 and the lowest at ST 10. The quality standard values of mercury in seagrass were 0.5 mg / kg (SNI 7387: 2009) and 1.0 mg / kg [15-20].

The Hg content found in the seagrass organs (roots) shows exceeded the quality standard of 0.5 mg / Kg based on SNI 7387: 2009, both in Cidaun and Sindangbarang. This process was considered dangerous for plants because mercury may be transported to other plants' parts through the xylem and phloem transport networks. The high Hg content at ST 8 is caused by iron sand mining activities located nearby. This activity has previously polluted the surrounding soil and river water. Plants will respond first to the roots. Several physiological processes play a role in metal accumulation throughout the plant life cycle. The first process is rhizosphere interaction in the root zone, where there is a process of processing elements in the soil from non-absorbable forms to absorbable forms by involving several exudates produced by roots. Hyperaccumulator plants have a higher ability to convert metals in the root zone into available forms. Hyperaccumulator plant roots have high selectivity to certain metal elements. The absorption of metal by roots is determined, among others, by permeability, transpiration, root pressure, and the presence of an enhanced metal uptake system, which is thought to be only owned by hyperaccumulator plants.

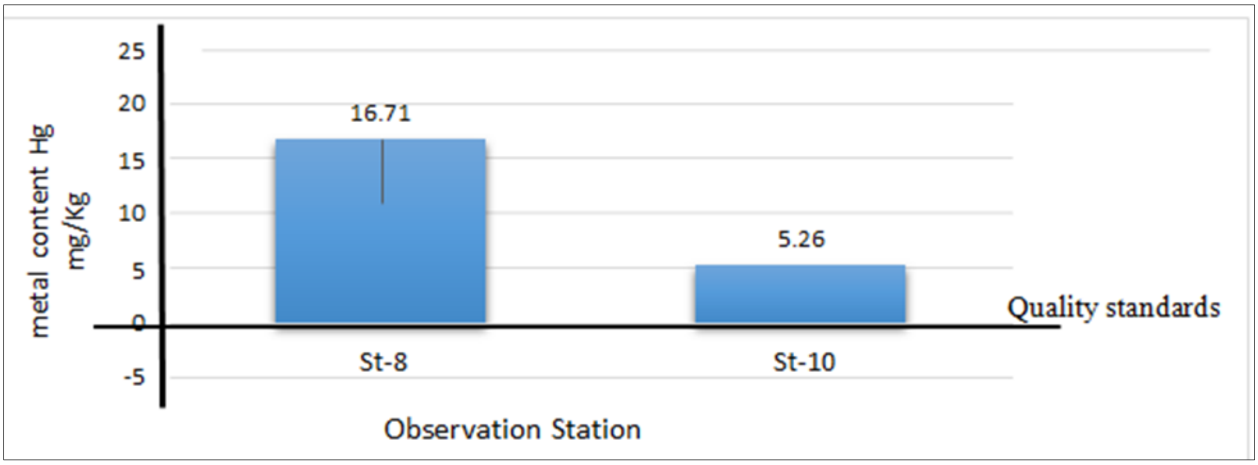

Figure 2 Mercury (Hg) Content in Roots

The analysis results based on the T-test showed that the Hg content at ST 8 was different (P <0.05) from ST 10. The Hg content in the roots in Cidaun waters was higher than in Sindangbarang. It is suspected that sand and mud particulates containing heavy metals are carried away by the current to form sediment and settle in the seagrass growing media. The response of plants to heavy metals for each plant species varies significantly in the ability of plants to be tolerant or intolerant of metal poisoning [13-19]. Plants have three essential strategies to grow on media contaminated with heavy metals: 1. Metal excluder plants prevent the entry of metal from the aerial parts or keep metal concentrations low in the soil; 2. Metal indicator, plants tolerate metal concentrations by producing metal-binding compounds or changing metal composition by storing metal in an insensitive part; 3. Metal accumulators, plants that concentrate high metal concentrations in plants' aerial parts, absorb high levels of contaminants and are deposited in roots, stems, leaves, or shoots [6-10].

3.2 Hg Heavy Metal Content in Rhizoma Seagrass *Enhalus acoroides*

Based on the analysis conducted in the laboratory, the content of heavy metal Hg in the rhizomes of the seagrass *Enhalus acoroides* was 3.97 ± 0.31 mg / Kg at ST 8 ST 10 was 11.93 ± 0.24 mg / Kg (Figure 3).

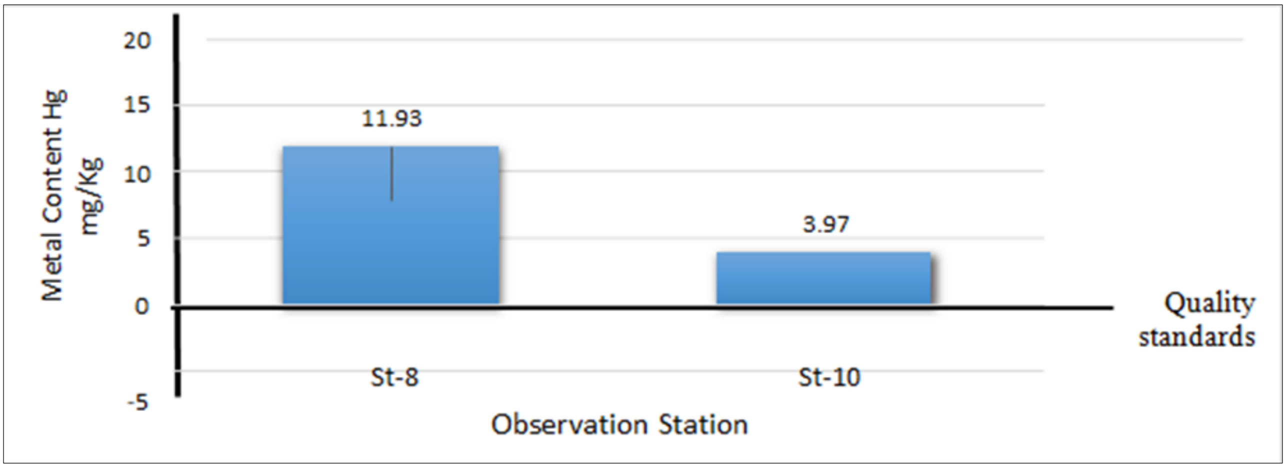

Figure 3 Mercury (Hg) Content in Rhizoma

The picture above shows that the Hg content at ST 8 is higher (11.93 ± 0.24 mg / Kg) than ST 10 (3.97 ± 0.31 mg / Kg). The standard quality value of the heavy metal mercury in seagrass is 0.5 mg/kg (SNI 7387: 2009) and 1.0 mg/kg (BPOM). The average laboratory analysis results of mercury metal on rhizomes obtained from the research location have exceeded the quality standards in both Cidaun and Sindangbarang. The content of heavy metal mercury in rhizome samples is smaller than in roots. However, the increase in metal content needs to be watched out for due to the role of plants as the primary producers in the food chain system. The concentration of Hg may be significant (accumulate) in biota with higher trophic levels. The analysis results based on the T-test showed that the Hg content at ST 8 was different (P <0.05) from ST 10. The Hg content in rhizomes in Cidaun waters was higher than in Sindangbarang.

The high content of heavy metal Hg in seagrass rhizomes, both ST 8 and ST 10, is thought to have undergone a physiological process for seagrass plants as an initial response to metal concentrations absorbed by metal translocation roots to rhizomes. The plants at this point have a rate far exceeding typical plants. Two main processes control this translocation: the movement of ions to the xylem and the volume of flux in the xylem, which is mediated by root pressure and transpiration. This process also indicates an efficient metal translocation system from the root to the canopy. Sequestration and complexation are the processes that are followed to determine the form of metal bonds accumulated and wherein the tissue will be deposited. Compartmentalization and metal accumulation occur more efficiently in hyperaccumulator plants. Besides, seagrass rhizomes as hyperaccumulators have a high degree of selection for metals.

3.3 Content of Heavy Metal Hg in *Enhalus acoroides* Seagrass Leaves

The research results on the content of heavy metal mercury in the leaves that were taken showed different results at the two stations. The mercury content in leaves was 6.79 ± 0.26 mg / kg for ST 8 and 2.02 ± 0.21 mg / kg at ST 10.The content of heavy metal mercury in plants with normal limits was between 0.01 - 0.3 mg/kg and critical in the range of 0.3 - 0.5 mg/kg (Mirdat et al., 2013). The content of heavy metal Hg in the leaves of the seagrass *Enhalus acoroides* is presented in Figure 4 below.

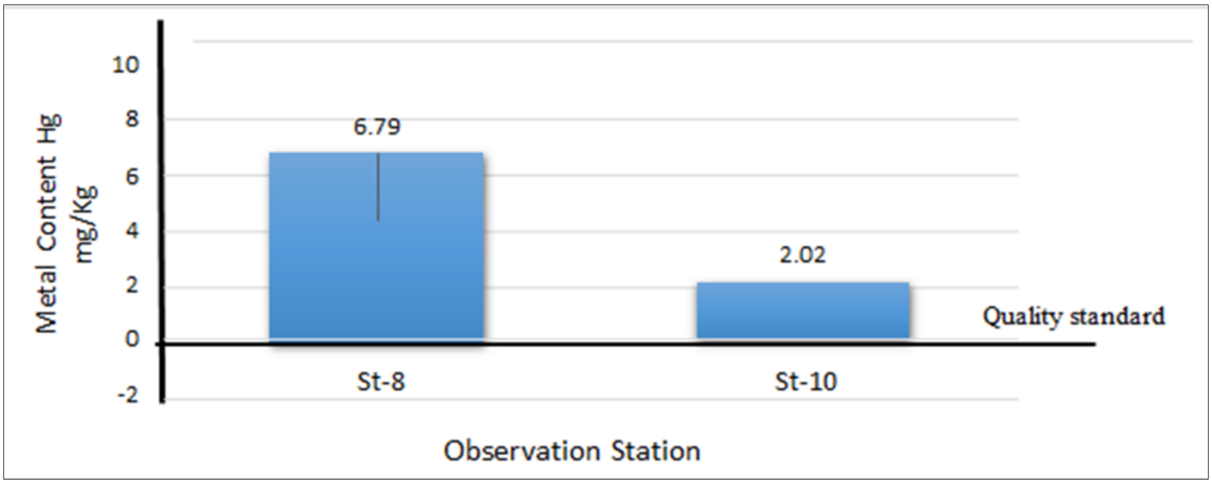

Figure 4 Mercury (Hg) Content in Leaf

Mercury is one of the most dangerous contaminants when it pollutes the aquatic environment and is widely considered one of the leading causes of highest environmental pollution by the European Water Framework Directive (WFD) and globally [4-15]. Heavy metal pollution from any source allows accumulating these metals in the environment, including seagrass habitat. The mercury content in the leaves found in the ST 8 (Cidaun) and ST 10 (Sindangbarang) research locations has exceeded the standard threshold of 0.5 mg / Kg based on SNI 7387: 2009. The Hg content at this location has entered a very worrying condition, and this has increased in line with the pollutants that regularly enter from activities around the water. The increase in metal content in that location should have started to be watched out for and realized because the danger of mercury contamination in this area has started to be confirmed. The waste of iron sand mining activities that flows along the river will increase the mercury content in this area. The analysis results based on the T-test showed that the Hg content at ST 8 was significantly different ($P < 0.05$) from ST 10. The Hg content in the leaves in Cidaun waters was higher than in Sindangbarang.

3.4 Chlorophyll content of *Enhalus acoroides* leaves

The results showed that the chlorophyll content in *Enhalus acoroides* seagrass leaves at ST 8 at the Cidaun location showed an average of 4.55 ± 5.55 mg/gr and at ST 10 Sindangbarang location showed an average chlorophyll content of 26.79 ± 6.02 mg/gr. Chlorophyll content for station eight and station ten is presented in Figure 5.

The ST 8 area shows a low chlorophyll content compared to ST 10. Field observations show that the Cidaun area is an area with water conditions that are becoming cloudy. The low level of water brightness will affect the penetration of sunlight entering the water. Brightness determines the thickness of the productive layer. Reduced water transparency will reduce aquatic plants' photosynthetic ability and physiological activities. The Cidaun area's turbulence is also caused by the suspension of sediment, domestic waste, and sewage from iron sand mining processing around the waters. Pollutants from iron sand mining waste play an essential role as a supplier of waste that causes water pollution, causing sedimentation. This process will adversely affect the growth of seagrass, which acts as a producer. [4-19] states that the most common environmental impact of excess nutrients or sediment is the subsidence of seagrass beds. The condition of *Enhalus acoroides* seagrass in the field shows the morphology of stunted plants, small leaves, not green, and tends to be yellowish due to chlorosis, known as Yellow Disease. The absorption of heavy metals that enter with nutrients affects the adequate quantity of plant nutrients so that the leaves are still young. Looks wilted, and the growth of seagrass is disturbed.

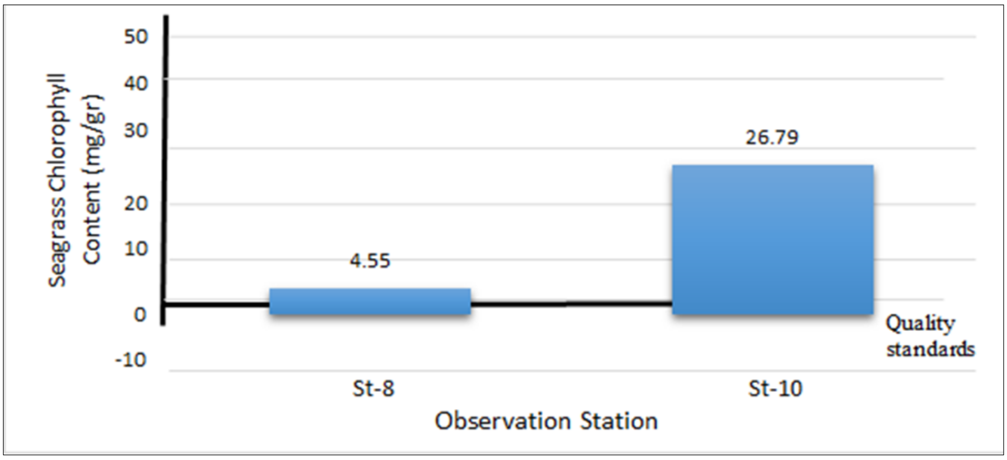

Figure 5 Chlorophyll Levels in Seagrass Leaves at Two Different Locations

At ST 10, the Sindangbarang area has water conditions that are still natural and clear. The intensity of sunlight entering the waters is more optimal. This process allows seagrass in this area to experience an increased process of photosynthesis. The rate of metabolism by plants will increase in line with the increase in temperature in this area. Chlorophyll will attract electrons from sunlight so that photosynthesis occurs and acts as an absorbent of energy from sunlight to turn into high-energy molecules and release electrons from water molecules and protons from oxygen. Chlorophyll-a concentration is the leading indicator for estimating primary productivity and is an essential variable in photosynthesis [5-15]. The Physico-chemical parameters that control and influence the distribution of chlorophyll-a are light intensity and nutrients.

In general, the distribution of high chlorophyll concentrations in coastal waters results from the high nutrient input from the land through river water runoff, but there are some offshore places where chlorophyll concentrations are still high. Like the Sindangbarang area, this condition is thought to be due to a water mass circulation process that allows the transport of several nutrients from other places (land). This process followed [4-9] that nutrient input from the land through river water runoff can transport nutrients that enter coastal waters. In addition, Canion et al. (2017) explained that the distribution of chlorophyll-a in the water column is highly dependent on nutrient concentrations. Using correlation, testing the relationship between chlorophyll content and heavy metals in seagrass leaves was carried out. The hypothesis test shows that H0 is rejected, which means a significant correlation to the chlorophyll content of *Enhalus acoroides* seagrass.

The significant correlation between heavy metal Hg and chlorophyll is -0.97, with a significance of 0.00 for both waters at ST 10 and ST 8. The results of the correlation analysis between heavy metal and chlorophyll *Enhalus acoroides* showed that the higher the heavy metal content that accumulates in the leaves, the more chlorophyll content is reduced. The entry and increase in heavy metal content into the seagrass *Enhalus acoroides* will change the chlorophyll content if the content exceeds the threshold because Hg is a non-essential heavy metal that is toxic. The decrease in chlorophyll in *Enhalus acoroides* was caused by the excessive entry of heavy metals, which affected the production of pigments in chlorophyll and was reduced.

The heavy metal content in the leaves in this area will inhibit leaf growth and physiologically result in the epidermis' thickening. The content of heavy metal Hg in leaves in the Cidaun area shows higher levels than Sindangbarang. Heavy metals absorbed through the leaf cuticles will attack the sulfide bonds in cell protein molecules, causing damage to the associated protein structure, blocking the work of enzymes, and causing metabolic imbalances in the body. Pollutant stress in the environment causes an increase in free radicals, which destroys various enzymes that can reduce protein in plant organs [4, 13-18, 24-32].

Figure 6 *Enhalus acoroides* (seagrass)

In conditions of excess metal content than the threshold, according to [4-10], will cause cell death. Cell death due to poisoning begins with the breakdown of chloroplasts. Chloroplast damage causes inhibition of the photosynthesis process. The disruption of photosynthetic activity causes the ability of cells to reproduce to be reduced. This causes the growth and increases in the number of cells to be inhibited. The toxic effect of heavy metals will reduce the chlorophyll pigment in *Enhalus acoroides*. The higher the Hg heavy metal content will affect the chlorophyll content, and this condition can result in a decrease in absorbed light energy, thus inhibiting photosynthesis.

Chlorophyll content will determine the speed of photosynthesis. The greater the chlorophyll content, the faster the photosynthesis rate is. The Hg content in the leaves at both stations indicated that Cidaun and Sindangbarang had exceeded the established threshold. The content of heavy metal Hg in leaves in Cidaun and Sindangbarang has passed the quality standard, namely 6.79 ± 0.26 for the Cidaun area and 2.02 ± 0.25 for the Sindangbarang area. The toxic effect of heavy metal Hg is thought to affect the formation of chlorophyll. The analysis results show a negative correlation which states that the higher the concentration of heavy metal Hg in the water, the lower the chlorophyll content.

4 Conclusion

The results showed that the content of the heavy metal Hg in the roots was more significant (>) than the content in rhizomes. There are primary and significant differences among the accumulation levels of heavy metals in the different organs. To conclude, the differentiation into different organs and their analysis is highly recommendable for use. acoroidesasbioindicator. Compared to the published data, the second significant progress and difference are that the present study was carried out at six different seagrass beds containing significantly different heavy metal levels.

The content of heavy metal Hg in rhizomes is greater (>) than the content in seagrass leaves. Heavy metal content and chlorophyll content of seagrass have a negative correlation where Hg metal correlates (r = - 0.97) and shows that the higher the heavy metal content will reduce the chlorophyll content of seagrass. Leaves are one of the organs where photosynthesis occurs, accumulating heavy metals in each of its tissues. In general, all tissue or parts of seagrass morphology could be used as bio accumulators and bioindicators of heavy metal pollution from waters and sediments.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declare that No conflict of interest in this work.

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