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Deciphering the Environmental Code: SEM-EDX Analysis of Lead and Mercury Distribution in Enhalus Acoroides from Kayeli Bay, Indonesia

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Abstract

Background: Illegal gold mining activities around Kayeli Bay have led to increased heavy metal contamination that has the potential to impair water quality and growth of Enhalus acoroides, an important seaweed species. This study aimed to investigate the distribution and concentration of heavy metals Pb and Hg in Enhalus acoroides organs (roots, rhizomes, and leaves) using SEM-EDX techniques and evaluate the impact of mining activities on the surface microstructure of these organs.

Methods: Enhalus acoroides samples were collected from ten sampling points in Kayeli Bay, which were selected based on potential heavy metal contamination. SEM-EDX analysis was performed to assess the surface microstructure and distribution of heavy metals in the seaweed samples.

Results: Results showed significant differences in Pb and Hg concentrations among samples from different locations, with Hg content consistently higher than Pb. Surface microstructure analysis showed morphological changes in roots, rhizomes, and leaves exposed to heavy metals, indicating environmental stress in Enhalus acoroides.

Conclusion: This study confirmed the negative impact of heavy metal contamination on Enhalus acoroides in Kayeli Bay due to illegal gold mining activities. High concentrations of heavy metals, especially Hg, in seaweed organs indicate potential risks to marine ecosystem health and the sustainability of fisheries resources. These findings emphasize the need for stricter environmental monitoring and management to protect this important marine habitat from heavy metal pollution.

Keywords: *Enhalus acoroides, heavy metals, SEM-EDX, contamination, illegal gold mining.*

Introduction

Nestled within the dynamic interplay of land and sea, Kayeli Bay presents an exemplary model of an estuarine environment intricately influenced by terrestrial runoff that deposits into its waters. This unique geographical setting not only enriches the bay with continuous influxes of nutrients, thereby augmenting water fertility but also plays a crucial role in bolstering the phytoplankton populations. Such enhancements in phytoplankton density are pivotal for the sustenance and prosperity of commercial fisheries encircling the bay, as indicated by pivotal studies (Nugraha, 2016; Male, 2014). Furthermore, Kayeli Bay extends its nurturing capacity beyond its immediate confines, enriching adjacent waters with vital nutrients and elevated chlorophyll levels, thereby acting as a cradle of marine life (Pentury & Waas, 2009).

However, the bay's ecological equilibrium faces threats from anthropogenic activities, notably from unregulated gold mining operations in the vicinity of Mount Botak. These activities, primarily characterized by the rudimentary amalgamation method that employs Mercury for ore processing, pose a significant risk of heavy-metal contamination, notably with Mercury and lead, thereby jeopardizing the aquatic life and water quality within the bay (Kautsar, 2017; Zhang et al, 2014; Nugraha, 2016). The disposal of mining waste, a direct consequence of these unlicensed operations, has been a persistent issue since 2012, with considerable volumes of pollutants being discharged into rivers and the sea, thereby adversely affecting the

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bay's marine ecosystem, including the seagrass populations which serve as bioindicators of pollution (Male et al., 2016; Rijal & Rosmawati, 2020; Mercado-Santana, 2017).

The degradation of coastal ecosystems due to heavy metal contamination is a significant environmental issue, posing threats to both marine life and human health. Heavy metals such as lead (Pb) and mercury (Hg) are particularly concerning due to their toxicity, persistence in the environment, and ability to bioaccumulate in marine organisms. Understanding the distribution and concentration of these metals in coastal flora, such as seagrasses, is crucial for assessing the health of marine ecosystems and formulating appropriate remediation strategies.

Seagrasses are key components of coastal ecosystems, providing numerous ecological services, including habitat provision, carbon sequestration, and sediment stabilization. Enhalus acoroides, a prevalent seagrass species in the Indo-Pacific region, has been extensively studied for its ecological importance and sensitivity to environmental changes. The bioindicator potential of E. acoroides for monitoring heavy metal pollution is of particular interest due to its widespread distribution and ability to accumulate metals from the surrounding environment.

Kayeli Bay, located in Indonesia, is an area of ecological and economic importance. The bay supports diverse marine life and sustains local fisheries and tourism. However, rapid industrialization and increased anthropogenic activities have raised concerns about the contamination of this coastal region with heavy metals. Previous studies have reported elevated levels of Pb and Hg in various components of the bay's ecosystem, highlighting the need for comprehensive monitoring and analysis.

Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX) analysis is a powerful tool for investigating the distribution and concentration of heavy metals in environmental samples. This technique combines high-resolution imaging with elemental analysis, allowing for precise localization of metal contaminants at the microscale. SEM-EDX has been successfully employed in various environmental studies to elucidate the mechanisms of metal uptake and accumulation in plants and other organisms.

This study aims to decipher the environmental code of Pb and Hg distribution in E. acoroides from Kayeli Bay using SEM-EDX analysis. By mapping the spatial distribution of these metals within seagrass tissues, we seek to gain insights into their uptake pathways, accumulation patterns, and potential impacts on the health of E. acoroides and the broader marine ecosystem. The findings of this research will contribute to a better understanding of heavy metal dynamics in coastal environments and support the development of effective environmental management strategies for Kayeli Bay and similar regions.

In summary, the objectives of this study are to:

Determine the concentrations of Pb and Hg in E. acoroides samples from Kayeli Bay.

Analyze the spatial distribution of these metals within the seagrass tissues using SEM-EDX.

Assess the implications of heavy metal accumulation for the health of E. acoroides and the surrounding marine ecosystem.

Provide recommendations for environmental monitoring and management based on the findings.

By addressing these objectives, this research aims to enhance our knowledge of heavy metal contamination in coastal ecosystems and inform efforts to mitigate the impacts of environmental pollution on marine life and human communities.

Material and Method

Samples were taken at ten sampling points: Station 1 (Kayeli estuary), Station 2 (Suket estuary); Station 3 (Anahoni estuary); Station 4 and 5 (Waelata I and Waelata II rivers); Station 6 (Waeapo estuary) and Station

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7, 8 and 9 (Sanleko river, Marlosso river, and Bara beach), and Station 10 (Nametek beach). The determination of the research sample locations was based on the potential of seagrass contaminated by heavy metals. Seagrass organs observed included roots, rhizomes, and leaves.

Prior to analysis, the seagrass samples were mashed using a mortar or mill grinding. Seagrass that had been mashed was then attached to the sample place containing carbon tape. The remaining sample that was not attached was then cleaned. 10 mm of sample was inserted into the SEM sample holder. The type of SEM used was SEM Brand FEI Type Inspect S50, which is integrated with the Energy Dispersive X-ray Spectroscopy test.

The SEM-EDX device has two monitors. Since electrons are very small and light, they are easily scattered by collisions with air molecules before hitting the target. Therefore, the tool works with a vacuum system. Before the analysis process took place, air molecules in the device were removed by closing the gas. The removal of the air molecules is very important in a vacuum system because air molecules can deviate electrons from their target (Setyaningsih et al, 2017). Inside this device is an electron gun that produces electron beams and is accelerated with anodes. The tool's magnetic lens focused the electrons toward the sample, and the focused electron beam scanned the entire sample directed by the scanning coil. The adjusted camera would show the surface of the sample, then the brightness and magnification were adjusted to focus on the sample. Spot size was set on the SEM monitor and collected on the EDX monitor. When the electron beam hits the sample, the sample issues new electrons, which are received by the detector and read on the monitor (Prasetyo, 2011). The result was obtained in the form of the sample surface drawing on SEM and graphs or diagrams on EDX that showed the percentage of elements contained in the analyzed sample. The data obtained were then presented in the form of tables, graphs, and histograms and discussed descriptively (Srivastava & Vinod, 2012)

Result and Discussion

Heavy metals are natural ingredients that make up the earth's soil layer (Yudatomo, 2009). Heavy metals cannot be broken down or destroyed. The physical properties of metals commonly used are high melting and boiling points and good electrical and thermal conductivity (Salatutin et al., 2015). Metals can be forged, bent, drawn, and shined. However, this characterization has not provided detailed metal content information, so other characterization tools are needed to provide simple/uncomplicated information regarding the physical and chemical properties of metals (Julinawati et al, 2015).

The concentration of metals can be observed using SEM-EDX - EDX (Martinez, 2010; Heriani et al., 2014). This type of analysis will provide information on the morphology of metals, such as their shape and size. The composition of metal elements in a sample can be quantitatively and qualitatively measured by firing at the desired position so that certain peaks appear to represent an element contained in the sample.

Identification using SEM-EDX can provide faster, simpler, and more accurate results. In SEM-EDX measurements, each sample is analyzed using area analysis. The electron beam produced by the gun area is diverted to hit the sample. This electron beam flow is then focused using the columb optical electron before the electron beam forms or hits the sample. After the electron beam hits the sample, several interactions will occur with the irradiated samples. These interactions will then be detected and converted into an image by SEM analysis and graphically by EDX analysis (Sudratjat & Bayuseno, 2014).

SEM produces two signals, including secondary electron signals produced from inelastic reflections and backscattered electron signals produced from elastic reflections. Secondary electrons produce the topography of objects being analyzed. High surfaces produce brighter colors than low surfaces. On the other hand, backscattered electron signals show the differences in the molecular weight of the atoms that make up the surface. Atoms with high molecular weight will be brighter in color than atoms with low molecular weight (Prasetyo, 2011).

The discussion of this study began with the SEM analysis of the samples that had been crushed using a mortar or mill grinding. The samples were tested using SEM and, after that, using the EDX test. Both tests

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were used to determine the surface microstructure of seagrass organs (roots, rhizomes, leaves) and the distribution of Pb and Hg metals in the seagrass (*Enhalus acoroides*) organs.

SEM Analysis

SEM analysis was performed to examine the surface microstructure of *Enhalus acoroides* organs (roots, rhizomes, and leaves), including the shape and size of the seagrass sample. SEM analysis is used because it has a high degree of accuracy in examining the surface of a sample up to 1,000,000 times. Following are the results of the SEM analysis conducted at the Central Laboratory of the Faculty of Mathematics and Science, Universitas Negeri Malang.

SEM-EDX Analysis on the Seagrass Root Samples from Marlosso and Nametek

SEM analysis was conducted at 500x, 1000x, and 2000x magnification to evaluate the morphology of the root surface samples. The micrographs of the Marlosso's root samples were presented in Figure 1

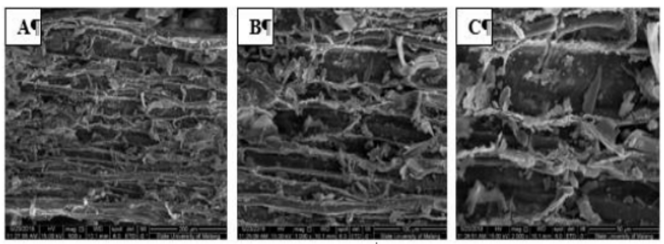

Figure 1. The surface microstructure of the Marlosso's root samples at 500x (A), 1000x (B), and 2000x (C) magnification

Figure 1 shows SEM images at 500X, 1000x, and 2000x magnification. The morphology of the root samples A, B, and C from Marlosso shows irregular stringy surface structures with varying shapes of size. The surface structure becomes quite irregular after 2000x magnification. Bright colors appear more dominant on each surface of the root sample. High surfaces release more electrons than low surfaces, resulting in brighter and clearer colors. Figure C shows brighter colors compared to A and B after 2000x magnification. The micrographs of the Nametek's root samples were presented in Figure 2

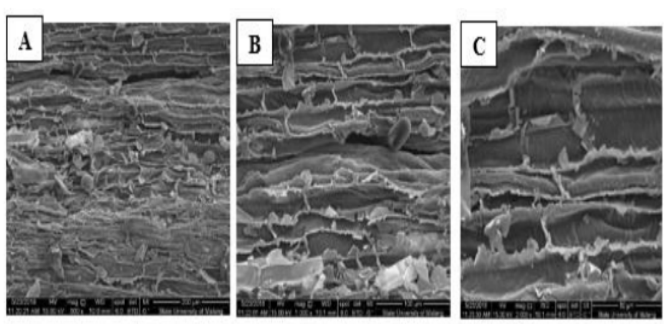

Figure 2. The surface microstructure of Nametek's root samples at 500x (A), 1000x (B), and 2000x (C) magnification

EDX Analysis on the Seagrass Root Samples from Marlosso and Nametek

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EDX analysis was carried out to find out phenomena that occurred at the root surface and metal composition of the root surface (Figure 3, 4, 5). The EDX analysis was conducted at the Central Laboratory of Universitas Negeri Malang

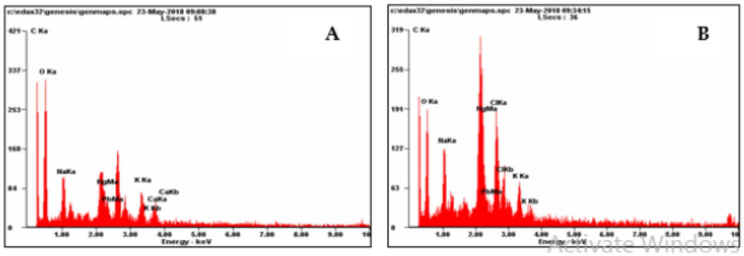

Figure 3. EDX Graphs of Marlosso (A) and Nametek (B) Root Sample 1

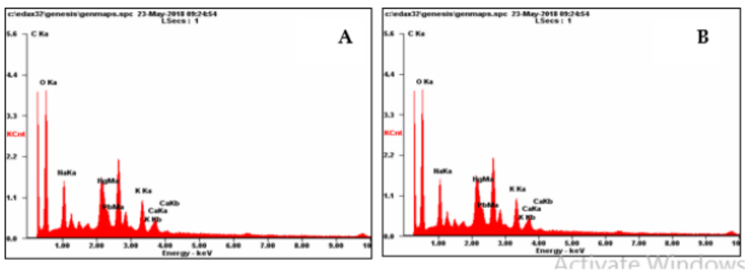

Figure 4. EDX Graphs of Marlosso (A) and Nametek (B) Root Sample 2

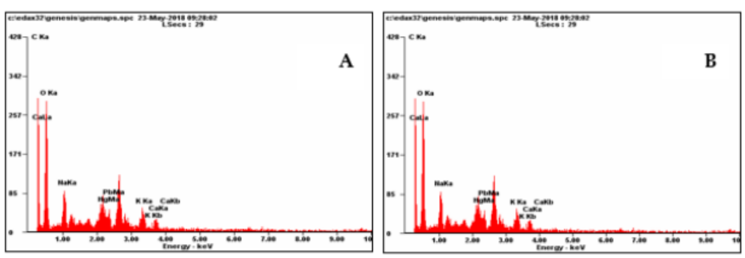

Figure 5. EDX Graphs of Marlosso (A) and Nametek (B) Root Sample 3

The comparison between SEM-EDX analyses on seagrass roots collected from two locations (Marlosso and Nametek) was presented in Table 1

Table 1. The Average Percentage of Lead and Mercury Concentrations in Marlosso and Nametek Root Samples Based on SEM-EDX

No	Element	Marlosso		Nametek	
		Wt (%)	At (%)	Wt (%)	At (%)
1	Pb	3,39	0,31	0,00	0,00
2	Hg	15,16	1,40	29,33	3,28

Notes: Wt = Percentage of Weight At = Percentage of Atoms

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Table 1 shows that there are differences in the percentage (%) of Lead and Mercury contents in the two locations. Descriptively presented that Mercury is more dominant with Wt (%) and At (%) greater than Lead. This is presumably because Mercury is a natural element that has a strong bond so that it can be easily combined with other elements and is rarely found in a separate element form. The stable chemical properties of mercury in the sedimentary environment allow for absorption by the roots. High solubility will cause mercury to accumulate both through bioaccumulation and biomagnification through food chains (Tupan & Azrianingsih, 2016; Rijal et al., 2014; Nugraha, 2016).

SEM-EDX Analysis on the Seagrass Rhizome Samples from Marlosso and Nametek

The result of the SEM analysis on the rhizome samples was presented at 500x, 1000x, and 2000x magnification. The micrographs of Marlosso's samples were presented in Figure 6

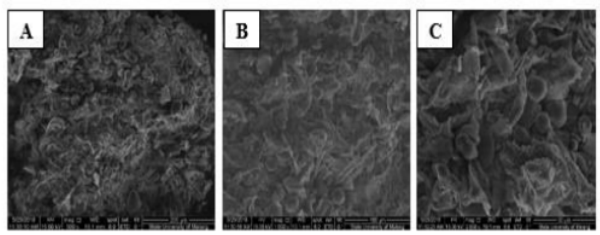

Figure 6. The surface microstructure of the Marlosso's rhizome samples at 500x (A), 1000x (B), and 2000x (C) magnification

Figure 6 shows SEM images at 500X, 1000x, and 2000x magnification. The morphology of Marloso's rhizome samples shows rounded rhizoma protrusions with a large number of irregular structures. The micrographs of Nametek's root samples are presented in Figure 6.

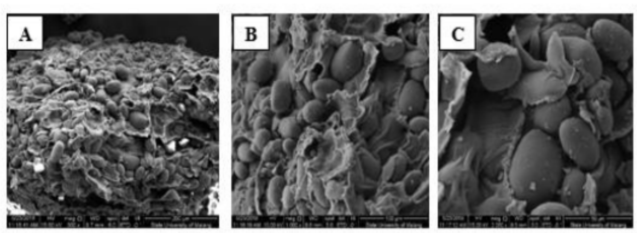

Figure 7. The surface microstructure of the Nametek's rhizome samples at 500x (A), 1000x (B), and 2000x (C) magnification

EDX Analysis on the Seagrass Rhizome Samples from Marlosso and Nametek

EDX analysis was carried out to find out phenomena that occurred at the rhizome surface and the metal composition of the rhizome surface (Figure 8, 9, 10). The EDX analysis was conducted at the Central Laboratory of Universitas Negeri Malang

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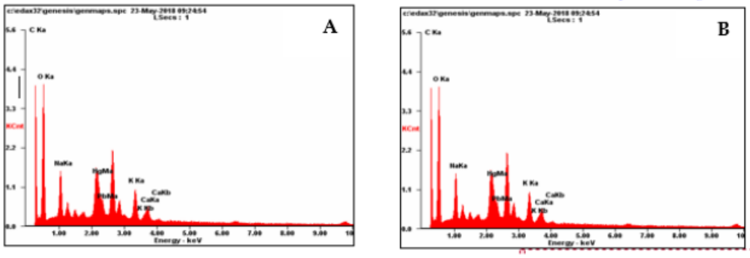

Figure 8. EDX Graphs of Marlosso (A) and Nametek (B) Rhizome Sample 1

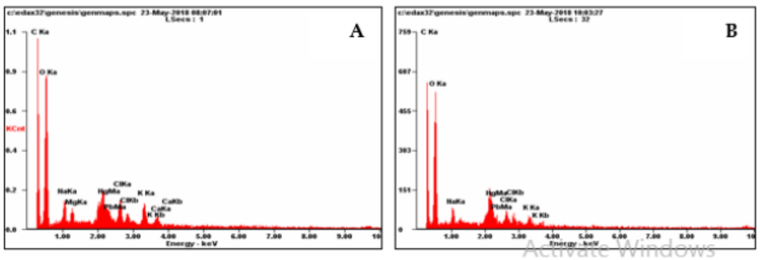

Figure 9. EDX Graphs of Marlosso (A) and Nametek (B) Rhizome Sample 2

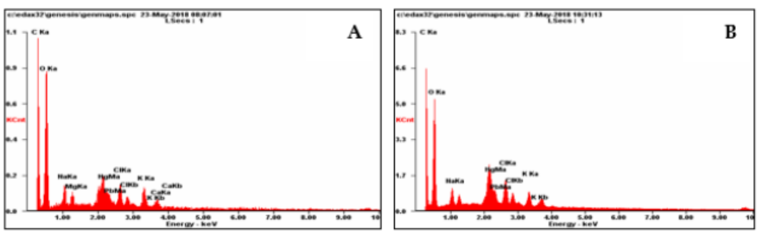

Figure 10. EDX Graphs of Marlosso (A) and Nametek (B) Rhizome Sample 3

The comparison between SEM-EDX analyses on seagrass rhizomes collected from two locations (Marlosso and Nametek) was presented in Table 2

Table 2. The Average Percentage of Lead (Pb) and Mercury (Hg) Concentrations in Marlosso and Nametek Rhizome Samples Based on SEM- EDX

No	Element	Marlosso		Nametek	
		Wt (%)	At (%)	Wt (%)	At (%)
1	Pb	2,19	0,19	2,49	0,22
2	Hg	7,66	0,63	14,48	1,32

Note : Wt = Percentage of Weight At = Percentage of Atoms

SEM-EDX on the Seagrass Leaf Samples from Marlosso and Nametek

The result of the SEM analysis on the leaf samples was presented at 500x, 1000x, and 2000x magnification. The micrographs of Marlosso's leaf samples are presented in Figure 11.

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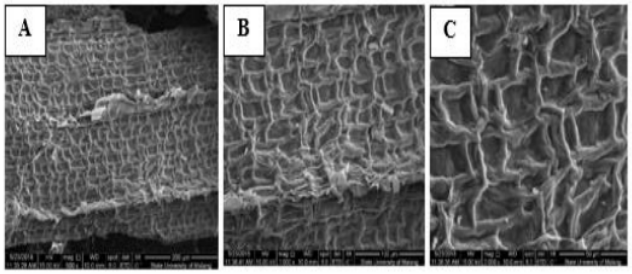

Figure 11. The surface microstructure of the Marlosso's leaf samples at 500x (A), 1000x (B), and 2000x (C) magnification

Figure 11 shows SEM images at 500X, 1000x, and 2000x magnification. The morphology of the leaf samples from Marlosso shows the thickening of epidermal cell walls or inter-space bulkhead walls in various structured shapes and sizes. The space between cells experiences widening, and it is suspected that there is an increase in size so that some parts of the screen or cell wall can undergo lysis. This is thought to be the result of the buildup of heavy metals in the cell. The micrographs of the nametek's leaf samples are presented in Figure 12.

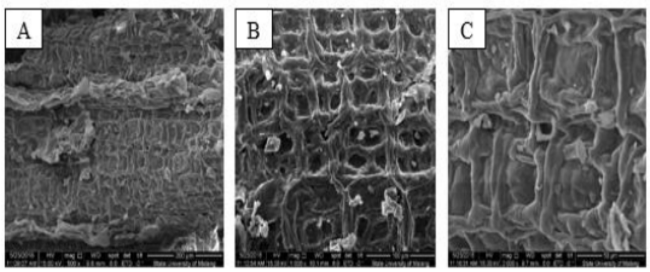

Figure 12. The surface microstructure of the Nametek's leaf samples at 500x (A), 1000x (B), and 2000x (C) magnification

EDX Analysis on the Seagrass Leaf Samples from Marlosso and Nametek

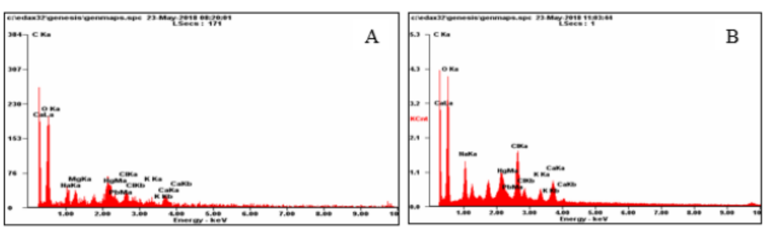

Figure 13. EDX Graphs of Marlosso (A) and Nametek (B) Leaf Sample 1

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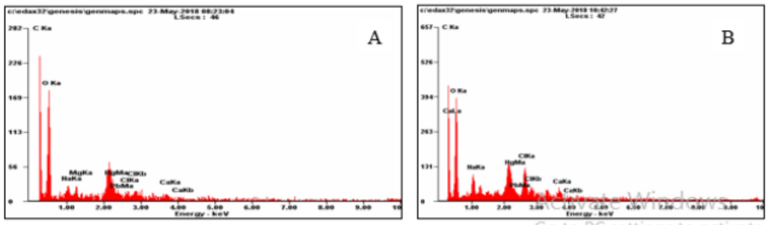

Figure 14. EDX Graphs of Marlosso (A) and Nametek (B) Leaf Sample 2

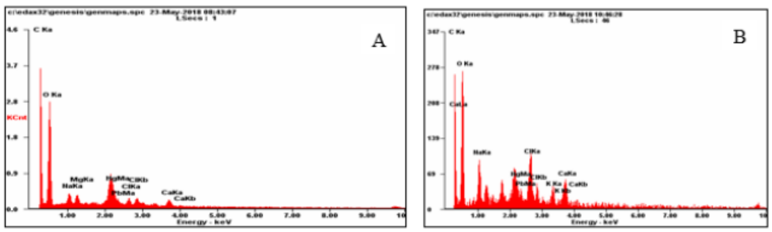

Figure 15. EDX Graphs of Marlosso (A) and Nametek (B) Leaf Sample 3

The comparison between SEM-EDX analyses on seagrass rhizomes collected from two locations is presented in Table 3.

Table 3. The Average Percentage of Lead (Pb) and Mercury (Hg) Concentrations in Marlosso and Nametek Leaf Samples Based on SEM- EDX

No	Element	Marlosso		Nametek	
		Wt (%)	At (%)	Wt (%)	At (%)
1	Pb	3,42	0,52	1,03	0,09
2	Hg	4,39	9,20	13,38	1,22

Note: Wt = Percentage of Weight At = Percentage of Atoms

Table 3 shows that there are differences in the percentage (%) of lead and mercury contents found at the two locations. Descriptively presented that Mercury is more dominant with Wt (%) and At (%) greater than lead.

Conclusion

This study investigated the impact of unauthorized gold mining activities around Kayeli Bay, which contribute to heavy metal waste pollution, particularly Pb (Lead) and Hg (Mercury), on the marine ecosystem, particularly the growth of Enhalus acoroides (seaweed). The use of SEM-EDX (Scanning Electron Microscope - Energy Dispersive X-ray Spectroscopy) enabled qualitative and quantitative analysis of elements in the samples, providing a detailed picture of the surface morphology and distribution of heavy metals in the seaweed organs (roots, rhizomes, and leaves).

From this study, it was found that:

Unauthorized gold mining activities increase heavy metal contamination in Kayeli Bay, potentially affecting water quality and growth of Enhalus acoroides.

SEM-EDX was effective in observing the surface microstructure of seaweed organs and the distribution of Pb and Hg.

There were differences in Pb and Hg concentrations among samples from different locations, indicating variations in the level of heavy metal pollution in the environment.

Mercury (Hg) was more dominant than Lead (Pb) in the samples, indicating significant accumulation rates and potential negative impacts on marine ecosystems through bioaccumulation and biomagnification processes.

This research highlights the importance of monitoring and controlling mining activities to protect marine ecosystems from heavy metal contamination, as well as using SEM-EDX technology to monitor and analyze the environmental impacts of such pollution.

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We would like to thank the chairman (Fuad) and laboratory staff (Arisandy) of the Central Laboratory of Universitas Negeri Malang for their assistance in analyzing the seaweed organ surface microstructure and distribution of Pb and Hg in Kayeli Bay waters, Buru, Maluku.

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