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Evaluation of Lead (Pb) Bioaccumulation Levels by sea grass (*Enhalus acoroides*) at Tulehu Village Port

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Abstract

This research aims to study the level of heavy metal Pb bioaccumulation by sea grass (*Enhalus acoroides*) in the waters of Tulehu Village, Central Maluku Regency, where the type of particles and Pb content in sediment, roots and leaves are important variables for studying the accumulation and translocation of Pb metal ions by sea grass. The type of sediment particles was analyzed using a sieve shaker. Lead (Pb) content in sediment, roots and leaves of sea grass was measured using an atomic absorption spectrophotometer. The ability to accumulate and translocate lead (Pb) in sea grass (*Enhalus acoroides*) is known from the BCF (bioconcentration factor) and TF (translocation factor) values. The results of the study showed that the type of sediment particle grains at point I was 2.92% gravel, 86.67% sand, 26.67% mud, the size of sediment grains at point II was 16.67% gravel, 82.94% sand, 2.35% mud, and the size of sediment grains at point III was 19.77%, 64.58% sand, 1.04% mud. The Pb metal content produced at point I ranged from 4.80 mg/kg, point II ranged from 4.85 mg/kg and point III ranged from 4.44 mg/kg. The BCF values obtained at points I, II, and III were respectively 0.55; 2.20; 2.12. The TF values at stations I, II, and III were respectively 2.13, 1.80, and 1.99.

Keywords: Sea grass, Lead, Accumulation, sieve shaker, AAS

INTRODUCTION

The waters of Tulehu Village cover an area of approximately 9.15 km², with a coastline stretching around 4.7 km. This area serves as a gateway and maritime transportation route connecting the city of Ambon with the surrounding islands. The port, located in Salahutu District, Central Maluku Regency, not only functions as a hub for sea transportation activities but also plays a significant role in supporting the livelihoods of the local community, particularly in the fisheries sector (Natsir et al., 2021).

A port is a highly complex system and is often confronted with various environmental issues, including waste disposal into the sea or onto land, air pollution, noise, and dredging activities. In addition, various operations within the port—such as fishing, industrial activities, and the storage of hazardous materials—pose further risks of environmental impacts (Muninggar et al., 2016).

One of the main factors disrupting environmental balance is the presence of waste containing heavy metals. This type of pollution can appear both in water and in solid forms, such as sediments in aquatic areas. The presence of heavy metals in aquatic ecosystems is typically caused by the release of toxic substances from household waste, industrial discharge, and other anthropogenic activities (Budiastuti et al., 2016).

Pollutants such as heavy metals (e.g., lead/Pb) in coastal areas pose serious threats to human health and aquatic biota. Lead can enter living organisms through the gastrointestinal tract, the respiratory tract (inhalation), and dermal penetration. Once it enters the human body, lead cannot be excreted. In aquatic environments, heavy metals not only contaminate the water but can also settle into the seabed, where they may persist for thousands of years. These metals can accumulate within organisms through bioaccumulation and further magnify through the food chain (biomagnification) (Mariwy et al., 2024).

Numerous studies have explored the reduction of heavy metal concentrations through the use of plants to mitigate the adverse effects of lead contamination. The accumulation of heavy metals can occur in living plants; one such plant capable of this is seagrass. Seagrasses are flowering plants (Angiosperms) that can live fully submerged in high-salinity marine environments and possess structures such as roots, leaves, and rhizomes similar to terrestrial plants (Sarinawaty et al., 2020).

Morphologically, the roots of seagrasses play a role in absorbing nutrients from the substrate where they grow. These nutrients and absorbed metals are then transported to the rhizome and leaves, where they are stored as energy reserves (Fakaubun et al., 2020). One seagrass species with this capability is *Enhalus acoroides*. Male et al., (2014) conducted a study analyzing the lead (Pb) content in *Enhalus acoroides* seagrass found in the waters of Tulehu Village. Their findings showed that the lead content in the seagrass roots reached 23.377 ppm.

The objective of this study is to investigate the capacity of *Enhalus acoroides* to bioaccumulate lead (Pb) from the waters of Tulehu Village. The novelty of this research lies in the use of seagrass as a phytoremediation agent to reduce the elevated lead levels in the Tulehu marine environment, thereby preventing potential poisoning of the local population through the food chain via biomagnification.

METHODOLOGY

Materials and Instrumentals

The materials used in this study included concentrated nitric acid (HNO_3), concentrated hydrochloric acid (HCl), roots and leaves of seagrass (*Enhalus acoroides*), sediment samples, Whatman No. 42 filter paper, and distilled water.

The instruments employed in this research were as follows: a Global Positioning System (GPS, Garmin), a refractometer (ATC), a pH meter (ATC), a digital thermometer (Omron Digital Thermometer MC-341a), an Atomic Absorption Spectrophotometer (AAS, SHIMADZU 7000), a sieve shaker (Sieving Machine AS 200 basic), an analytical balance (Cyberscan CON 1110), an oven (Memmert), a mortar and pestle, glassware (Pyrex), and a hot plate (Cimarec).

Methods

Measurement of Temperature, Salinity, and pH

Seawater samples were placed in a 100 mL beaker, and the temperature was measured using a thermometer. The recorded values were then noted. Salinity was measured using a refractometer. Prior to use, the refractometer glass was rinsed with distilled water and dried. A few drops of the seawater sample were then applied to the lower surface of the refractometer. The salinity level was determined by observing the boundary line between the white and blue areas on the scale. To measure pH, the seawater sample was poured into a 100 mL beaker, and the pH meter was immersed in the solution for 5 minutes. The pH value was then recorded.

Sediment Sample Collection

Sediment sampling was carried out using a PVC pipe to a depth of approximately ±10 cm. Samples were collected from three different locations. The first location was situated 20 meters from the shoreline and 25 meters from the speedboat dock bridge. The second location was 25 meters from the shoreline and 25 meters away from the first location. The third location was 15 meters from the shoreline and 25 meters from the second location. After collection, samples were placed in labeled plastic bags.

Collection of Seagrass Root and Leaf Samples

The samples collected included both roots and leaves of the seagrass, totaling approximately 50 blades (200 grams). The samples were collected during low tide at three sampling points. Point 1 was located 20 meters from the shoreline and 25 meters from the speedboat dock bridge. Point 2 was located 25 meters from the shoreline and 25 meters from Point 1. Point 3 was located 15 meters from the shoreline and 25 meters from Point 2. The collected samples were then placed into labeled sample bags.

Determination of Sediment Particle Size

The analysis of sediment grain size was carried out using the sieving method (wet or dry) with a sieve shaker. Sediment samples were first dried in an oven at 70–80 °C for 24 hours, then weighed, and the dry weight recorded. The dried sample was soaked in distilled water for 5 hours to separate the particles. The sieves were arranged in descending order of mesh size: 4.00, 2.00, 1.00, 0.500, 0.250, 0.125, 0.090, 0.063, and 0.032 mm. The soaked sample was placed on the top sieve (4.00 mm), rinsed with running water, and brushed to ensure full disaggregation of the particles. Each sediment fraction retained on the sieves was collected in a 100 mL aluminum pan and dried again in the oven at 70–80 °C for 2 hours. After drying, each fraction was weighed, and the result was recorded as the grain size fraction weight.

Preparation of Sediment Samples

Sediment samples were placed in Petri dishes and dried in an oven at 105 °C until completely dry. The dried samples were then packaged in labeled sample bags for further analysis.

Preparation of Seagrass Samples

Seagrass samples (roots and leaves) were dried in an oven at 105 °C. After drying, the samples were stored in labeled sample bags for subsequent analysis.

Digestion of Sediment and Seagrass Samples

Three grams of each sample (sediment, root, and leaf) were weighed and placed into 100 mL beakers. The samples were then digested with 15 mL of concentrated HNO_3 and 5 mL of concentrated $HClO_4$. The mixture was left to stand for 24 hours to allow the solution to become clear. The samples were then heated on a hotplate until nearly dry. After cooling, 10 mL of distilled water was added. The resulting solution was filtered into a 25 mL volumetric flask and diluted to the mark with distilled water. The final solution was then ready for analysis using Atomic Absorption Spectrophotometry (AAS).

Preparation of Pb Standard Solution

A 1 mL aliquot of a 1000 ppm Pb stock solution was pipetted and diluted to 100 mL with distilled water in a volumetric flask to prepare an intermediate solution. Subsequently, a series of Pb standard solutions with concentrations of 0.05, 0.1, 1, 1.5, 2, 3, and 4 ppm were prepared by pipetting 0.5, 1, 10, 15, 20, 30, and 40 mL of the 10 ppm solution into separate volumetric flasks and diluting each to 100 mL with distilled water. The absorbance of each standard solution was measured using Atomic Absorption Spectrophotometry (AAS) at a wavelength of 217 nm. These measurements were used to construct a calibration curve by plotting absorbance (A) versus concentration (C), resulting in a linear standard curve.

Calculation of Bioconcentration Factor (BCF)

The purpose of this calculation is to determine the level of Pb accumulation in the seagrass. The BCF is calculated by comparing the concentration of Pb in the roots to that in the sediment using the following formula:

$$\mathrm{BCF} = \frac{\text{Pb concentration in roots}}{\text{Pb concentration in sediment}}$$

According to Baker 1981, as cited in Mariwy et al., (2024), the Bioconcentration Factor (BCF) can be classified into three categories. A plant with a BCF value greater than one (BCF > 1) is classified as an accumulator, indicating its ability to absorb and store high levels of heavy metals from the environment. When the BCF value equals one (BCF = 1), the plant functions as an indicator, reflecting the environmental conditions. Conversely, a BCF value of less than one (BCF < 1) characterizes the plant as an excluder, meaning it has mechanisms to limit the uptake of heavy metals into its tissues.

Calculation of Translocation Factor (TF)

The purpose of calculating TF is to determine the extent of Pb translocation from the roots to the leaves of the seagrass. TF is calculated using the following formula:

$$\mathrm{BCF} = \frac{\text{Pb concentration in leaves}}{\text{Pb concentration in roots}}$$

According to Yoon et al. (2006), as cited in Mariwy et al. (2021), the TF values are categorized as follows:

TF > 1: Phytoextraction mechanism
TF < 1: Phytostabilization mechanism

RESULTS AND DISCUSSION

Description of Sampling Process

Sampling was conducted between 16:00 and 18:30 Eastern Indonesian Time (WIT) during low tide, which facilitated easier access to the sampling sites. The samples collected included sediment, roots, and leaves of seagrass (*Enhalus acoroides*), and were taken from three different points, namely Point I, Point II, and Point III. The sampling locations are shown in Figure 1.

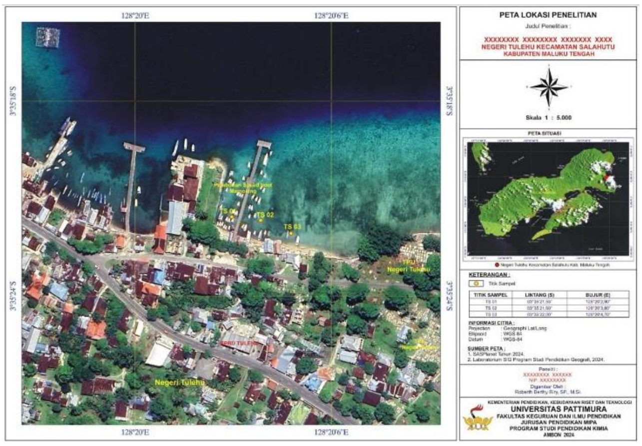

Figure 1. Sampling Locations

Seawater Quality Measurement

The results of seawater quality measurements are presented in Table 1.

Table 1. Results of Physical and Chemical Parameter Measurements

Parameter	Unit	Location		
		I	II	II
pH	–	7.3	7.6	7.7
Salinity	%	21	22	22
Temperature	°C	29	30	30

The data in Table 1 show that the water temperature in Tulehu Village ranged from 29°C to 30°C across all three locations. The pH values were recorded as 7.3 at Location I, 7.6 at Location II, and 7.7 at Location III, indicating a gradual increase in pH from the first to the third location. According to the Government Regulation of the Republic of Indonesia Number 22 of 2021, the ideal pH range for aquatic environments is between 7.0 and 8.5 (Wahyuningsih et al., 2021). A decrease in pH can increase the toxicity of metals in water (Sari et al., 2022). Based on this regulation, the pH levels recorded in Tulehu Village waters are still within the safe range for marine biota.

The salinity levels in the waters of Tulehu Village were in the range of 21–22%. According to Government Regulation No. 22 of 2021, the standard salinity for seawater is between 33–34%. Seagrasses are known to tolerate a wide range of salinity, typically from 10 to 40%, with optimal growth occurring at around 35% (Leslida et al., 2024). Despite the suboptimal salinity levels observed, seagrass species such as *Enhalus acoroides* may still persist due to their adaptive capacity.

The data in Table 1 also shows that the measured water temperature in the waters of Tulehu Village ranged between 29°C and 30°C. The highest temperatures were recorded at locations II and III, while the lowest was at location I. The variations in temperature at each measurement point were influenced by light intensity and the amount of seagrass vegetation present at each site (Jupriyati et al., 2014). The temperatures at the three locations in Tulehu Village waters are still tolerable for marine biota, as they fall within the quality standard limits set by the Government Regulation of the Republic of Indonesia No. 22 Year 2021, which stipulates a range of 28–32°C.

Determination of Sediment Particle Size

The classification of sediment particle size from the three research stations measured using a sieve shaker is presented in Figure 2.

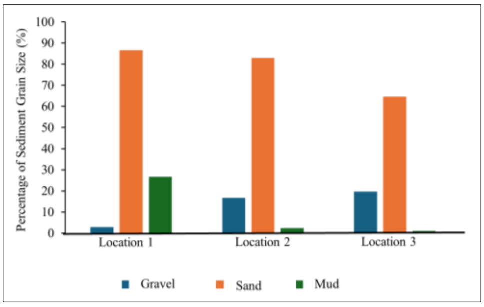

Figure 2. Sediment Grain Size Classification

Figure 2 shows a graph of sediment particle size proportions, indicating varying compositions of sand, silt, and gravel. At all three locations (I, II, and III), the sand fraction predominates over silt and gravel, categorizing the sediments at these sites as sandy substrates. Sandy substrates have a low capacity to bind heavy metals due to their coarser particle size. Fine sediments tend to have a larger surface area and more stable ion density, which enhances their ability to bind heavy metals compared to coarse-grained sediments. Moreover, fine sediments are better at retaining dissolved nutrients, increasing their heavy metal absorption capacity.

Destruction of Sediment, Root, and Leaf Samples

Sediment, root, and leaf *(Enhalus acoroides*) samples were dried at 105°C. The samples were then digested using concentrated HNO_3 and HCl (1:3), commonly known as aqua regia, a strong oxidizing mixture widely used to expedite sample destruction. The addition of acids serves a specific purpose—HNO_3 acts as a powerful oxidizer and is effective for dissolving metals.

Preparation of the Standard Curve

The standard curve serves as a reference for determining the concentration of a sample based on specific measurements (Sahumena et al., 2020). The calibration curve was constructed by preparing standard lead (Pb) solutions at various concentrations: 0.05, 0.1, 1, 1.5, 2, 3, and 4 ppm. The absorbance of each solution was measured using an Atomic Absorption Spectrophotometer (AAS). The absorbance data of the standard solutions are presented in Table 2.

Table 2. Absorbance of Lead (Pb) Standard Solutions

Concentration (ppm)	Absorbance
0.05	0.00158
0.1	0.00365
1	0.03329
1.5	0.04966
2	0.06186
3	0.09439
4	0.11641

The absorbance values were plotted against the standard solution concentrations to create the standard curve. The results show that higher concentrations correspond to higher absorbance values, indicating a linear relationship between concentration and absorbance (Mariwy et al., 2023).

From the standard curve (Figure 3), a slope value of 0.0295, an intercept of 0.0024, and a correlation coefficient of 0.9958 were obtained. The coefficient of determination (R^2), which is close to 1, indicates a strong relationship between the variables and meets the requirements of SNI 6989.84:2019, which stipulates a minimum linear regression correlation coefficient of ≥ 0.9.

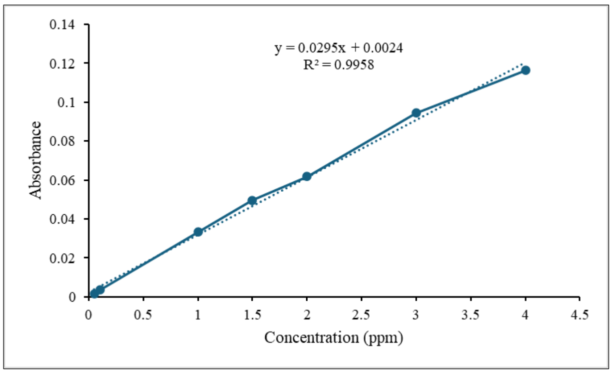

Figure 3. Standard Curve of Pb

Pb Concentration in Sediment, Root, and Leaf Samples

After establishing the standard curve for Pb, the absorbance of the sample solutions was measured using AAS, yielding the Pb concentrations at each station. The data for station 1 are shown in Table 4.

Table 4. Pb Concentration in Samples from Location I

Sample Type	Mass (gr)	Volume (mL)	Concentration of Pb (mg/kg)		
			Detected	Total	Average
Sediment	3.2718	25	0.3888	2.971	3.06
Root	3.1648	25	0.072	0.57	
Leaf	3.3745	25	0.1586	1.17	

The analysis shows that Pb levels at station I were high, likely due to its location being the main docking area for speedboats, resulting in frequent oil spills into the water. This condition causes heavy metal Pb to settle and undergo sedimentation, contributing to the high Pb concentration. Regarding sediment particle size at station I (see Figure 2), the sediments are dominated by sand and silt. Although sandy substrates have limited capacity to bind metals, fine particles like silt enhance the retention of heavy metals and organic materials (Male et al., 2017).

Table 4 also shows that Pb levels in sediments at station I are below the limits set by the National Sediment Quality Survey (US EPA), which range from 47.82 to 161.06 ppm. Pb concentrations in root and leaf tissues are very low, possibly due to efficient translocation from root to leaf or incomplete Pb absorption by the root. Accumulation in leaves reflects the translocation mechanism in seagrass plants (Mariwy et al., 2024).

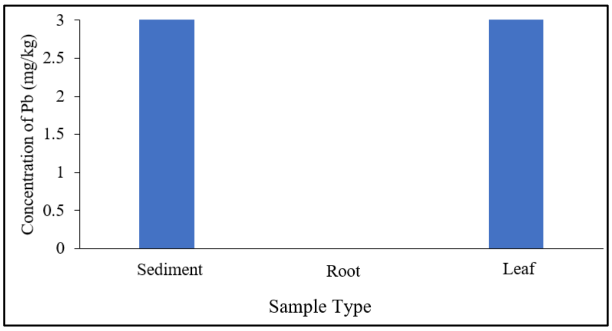

Figure 4. Pb Concentration at Station 1

Pb Concentration in Samples from Station 2

Pb concentrations at station II were lower than at station I, likely because station II is farther from the boat docking area. Sediment at station II (Figure 2) consists mostly of sand and gravel, which are less effective in binding heavy metals due to their coarse texture (Male et al., 2017). The Pb levels remain below US EPA standards (47.82–161.06 ppm).

Table 5. Pb Concentration in Samples from Location II

Sample Type	Mass (gr)	Volume (mL)	Concentration of Pb (mg/kg)		
			Detected	Total	Average
Sediment	3.3599	25	0.2036	1.515	1.51
Root	3.0781	25	0.148	1.20	
Leaf	3.1864	25	0.2727	2.14	

Pb levels in roots at station II were higher than at stations I and III, possibly due to the endodermal cells that act as filters during metal absorption (Selanno et al., 2014). Pb accumulation in leaves was also the highest at this site, indicating active translocation by seagrass plants.

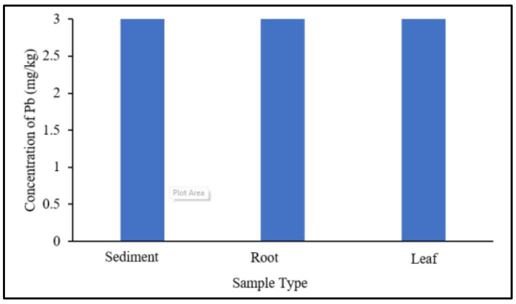

Figure 5. Pb Concentration at Station 2

Pb Concentration in Samples from Station 3

The analysis of lead (Pb) concentration at station III (Table 6) shows that Pb levels in the sediment are lower compared to those at Locations I and II. This lower concentration is attributed to the considerable distance of Location III from the speedboat harbor, resulting in reduced activities that could potentially cause contamination. Another contributing factor is the grain size characteristics of the sediment at this location, as shown in Figure 2, which is dominated by sand and gravel. Compared to muddy substrates that have a high capacity to absorb heavy metals, sediments with sandy and gravel textures have a lower adsorption capacity due to their coarser particle sizes, making it difficult for organic matter and heavy metals to settle (Male et al., 2017). Moreover, data in Table 4.6 indicate that Pb concentration in the sediment at Point III remains below the standard threshold set by the National Sediment Quality Survey (US EPA), which is 47.82–161.06 ppm.

Table 6. Pb Concentration in Samples from Location III

Sample Type	Mass (gr)	Volume (mL)	Concentration of Pb (mg/kg)		
			Detected	Total	Average
Sediment	3.2742	25	0.1862	1.422	1.42
Root	3.1421	25	0.1278	1.02	
Leaf	3.3308	25	0.266	2.00	

Pb concentrations in roots and leaves at station III also show relatively low results. This condition is likely due to two main factors: first, the translocation process functions effectively, allowing the absorbed Pb in the roots to be quickly transported to the shoot or leaves; second, Pb may not have yet been absorbed by the root tissues, resulting in low metal concentrations in the roots.

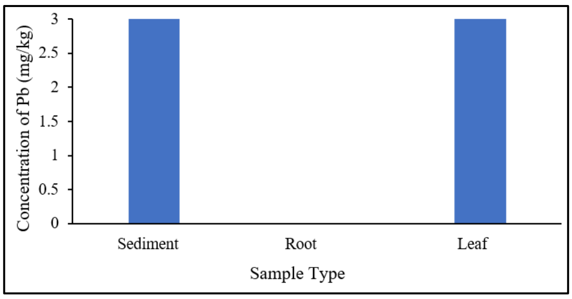

Figure 6. Pb Concentration at Station 3

The accumulation of Pb in the leaves reflects the translocation process, which is one of the natural mechanisms found in seagrass plants. The graphical results of Pb concentration measurements in each sample at Station 3 are presented in Figure 6.

Bioconcentration Factor (BCF) and Translocation Factor (TF) of Pb in Seagrass (*Enhalus acoroides*)

BCF and TF values were calculated to assess the plant's ability to absorb Pb. The results are shown in Table 7.

Table 7. BCF and TF Values

Station	BCF Root	BCF Leaf	BCF Total	TF
1	0.177	0.377	0.554	2.134
2	0.787	1.419	2.206	1.803
3	0.707	1.414	2.121	1.997

The BCF values at stations II and III were greater than 1 (>1), indicating that *E. acoroides* functions as an accumulator plant. At station I, the BCF value was less than 1 (<1), classifying *E. acoroides* as an excluder plant—plants that effectively prevent heavy metals from entering aboveground tissues.

TF values at all stations were greater than 1 (>1), showing that *E. acoroides* performs phytoremediation via phytoextraction, where absorbed pollutants are translocated from roots to shoots and leaves. Higher TF values may result from plant age, which affects leaf absorption capacity (Muninggar et al., 2016). Phytoextraction involves uptake and translocation of pollutants, which can be further processed or eliminated through leaf shedding.

CONCLUSION

Based on the research results, it can be concluded that: The Pb concentrations in sediments at stations 1, 2, and 3 in the waters of Tulehu Village, Salahutu District, are below the limits set by the National Sediment Quality Survey (US EPA, 2022). Sediments with fine silt substrates have higher heavy metal adsorption capacity compared to those with coarser substrates. BCF values at stations II and III were greater than 1, indicating that *E. acoroides* acts as an accumulator. In contrast, at station I, the BCF was less than 1, suggesting *E. acoroides* acts as an excluder. TF values at all three stations were greater than 1, which demonstrates that *E. acoroides* functions in phytoextraction of Pb, aided by efficient translocation mechanisms in the plant.

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