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[IK.IJMS] Submission Acknowledgement

1 pesan

Ambariyanto <ijms@live.undip.ac.id> 28 September 2024 pukul 05.33
Balas Ke: "Dr Rozirwan M.Sc. Rozirwan" <rozirwan@unsri.ac.id>
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Dr Rozirwan M.Sc. Rozirwan:

Thank you for submitting the manuscript, "Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia" to ILMU KELAUTAN: Indonesian Journal of Marine Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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[IK.IJMS] Editor Decision

2 pesan

Indonesian Journal of Marine Science <ejournal@rumahjurnalundip.id> 29 Januari 2025 pukul 17.04
Balas Ke: Indonesian Journal of Marine Science <ijms.undip@gmail.com>
Kepada: "Dr Rozirwan M.Sc. Rozirwan" <rozirwan@unsri.ac.id>
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Dr Rozirwan M.Sc. Rozirwan:

We have reached a decision regarding your submission to ILMU KELAUTAN: Indonesian Journal of Marine Sciences, "Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia".

Our decision is to:
Revison Required

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INDONESIAN JOURNAL OF MARINE SCIENCE
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436K

Rozirwan ROZIRWAN <rozirwan@unsri.ac.id> 30 Januari 2025 pukul 09.43
Kepada: Indonesian Journal of Marine Science <ijms.undip@gmail.com>

Dear Editor,

Thank you for your email and for the opportunity to revise our manuscript, "Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia."

We appreciate the feedback provided by the reviewers and will carefully address all comments to improve the quality of our paper. We will work on the necessary revisions and submit the revised manuscript.

Best regards,
Author
[Kutipan teks disembunyikan]
--
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Scientific Article Review Form

Article Title **:** Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia

Manuscript ID: 67097-219495-1-RV 2025

Section I: Overall Evaluation

Please rate the overall quality of the manuscript based on the following criteria:

1. **Originality and Novelty**
    - Does the manuscript contribute new knowledge to the field?
      ☐ Highly Original
      ×☐ Moderately Original
      ☐ Somewhat Original
      ☐ Not Original

Comments:

- *The article does not highlight why this specific location was chosen and how it adds unique insights compared to other regions in Indonesia. Does Pahawang Island serve as a critical biodiversity hotspot, subject to specific anthropogenic pressures, or it has unique seagrass dynamics. A brief discussion on the ecological or conservation significance of Pahawang Island could be added to establish the originality of the study.*

- *It would be beneficial to state if there any previous research investigated heavy metal accumulation in these species specifically, especially in the Indonesian context. Mention if this is the first study analyzing heavy metals in Cymodocea and Thalassia in Pahawang Island or whether the findings address a knowledge gap in the literature.*

- *The finding that seagrass ecosystems are currently at low ecological risk is valuable, but it could be discussed more for broader implications. How do these findings contribute to understanding heavy metal dynamics in tropical seagrass ecosystems or guide future management practices? Discuss how these results compare to heavy metal accumulation and ecological risks in other seagrass ecosystems, especially within Southeast Asia. Also discuss why does Thalassia have a higher Cu concentration in leaves compared to Cymodocea?*

- *The study appears to be a snapshot in time, how its findings might inform future temporal or spatial studies. Additional statements may also be included on how the findings could guide policymakers or stakeholders in managing coastal pollution in the area.*

- *The article does not adequately address potential sources of heavy metals (e.g., agricultural runoff, industrial activities, or natural processes). A brief discussion on potential heavy metal sources affecting Pahawang Island is recommended. While various indices are presented, there is no discussion on how the detected metal concentrations compare with established environmental or regulatory thresholds (e.g., WHO or Indonesian standards). Please include a comparison of the detected values with international or national standards to contextualize the results.*

2. **Relevance to the Journal's Scope**

- Does the manuscript align with the scope of the journal?
  ×☐ Highly Relevant
  ☐ Relevant
  ☐ Somewhat Relevant
  ☐ Not Relevant

Comments:

Yes, this article is considered to be aligns well with the scope of the IJMS which is dedicated to publishing research on various aspects of marine sciences, including marine biology, marine ecology, marine conservation, and marine pollution. This study fits within these thematic areas, making it a suitable for the journal.

3. **Clarity of Research Objectives and Hypothesis**

- Are the research objectives and hypothesis clearly stated?
  ☐ Very Clear
  ×☐ Clear
  ☐ Somewhat Clear
  ☐ Unclear

Comments:

- *Research objective is already explicitly stated which helps focus the study. However, the introduction does not articulate a specific hypothesis. For instance, it does not propose expected patterns of heavy metal accumulation in different seagrass parts (roots, rhizomes, leaves) or sediments. A* hypothesis based on previous studies could be introduced, for example: *"It is hypothesized that heavy metal concentrations will be higher in seagrass roots and sediments compared to leaves, reflecting limited mobility and high sediment deposition rates".*

- *The use of BCF, TF, Igeo, Cf, and PLI indices in assessing ecological risks specific to seagrass ecosystems in tropical coastal areas could be briefly justified. Highlight how the study advances current knowledge on heavy metal pollution in seagrass ecosystems, particularly in Indonesia.*

Section II: Methodology and Data Analysis

1. **Adequacy of Research Design and Methodology**

- Is the methodology suitable for addressing the research objectives?
  ☐ Highly Suitable
  ×☐ Suitable
  ☐ Somewhat Suitable
  ☐ Inappropriate

Comments:

- *The methodology is well-detailed, and the use of purposive sampling is appropriate for this study, as the two locations have distinct tourism activities, providing a meaningful comparison of anthropogenic impacts on seagrass ecosystems. Justification for why certain indices (e.g., PLI, Igeo) were chosen over others is required. Are these indices more suitable for tropical coastal ecosystems, or were they selected based on prior studies in similar environments? Specify the source of 'background concentrations' or clarify how they were determined.*

- *Discuss the reliability of the wet digestion method used, and if possible, consider cross-validation with CRM (certified reference materials). Also clearly state the number of replicates used for each sample type to improve transparency.*

2. **Appropriateness of Data Analysis and Statistical Methods**

   - Are the data analysis and statistical methods correctly applied and appropriate?

     ☐ Highly Appropriate

     ×☐ Appropriate

     ☐ Somewhat Appropriate

     ☐ Inappropriate

Comments:

- *The inclusion of multiple metrics (BCF, TF, Igeo, Cf, and PLI) ensures a multidimensional approach to assessing heavy metal contamination and its ecological risks. Please include statistical methods such as t-tests, ANOVA, or non-parametric tests to assess differences between the two sites or between seagrass and sediment samples. Regression analysis could also explore relationships between heavy metal concentrations and ecological indices.*

- *Individual metals are analyzed, but there is still no clear methodology for interpreting the cumulative effect of multiple metals. Try to consider using multivariate analysis techniques (e.g., Principal Component Analysis, PCA) to assess overall contamination patterns and identify dominant sources of pollution.*

- *If possible, please also discuss how findings from the two sampling sites might extrapolate to other areas and acknowledge limitations in spatial coverage.*

3. **Reproducibility of Results**

   - Are the results reproducible, and is there sufficient detail for replication?

     ☐ Highly Reproducible

     ×☐ Reproducible

     ☐ Somewhat Reproducible

     ☐ Not Reproducible

Comments:

- *The methodology already describes the sampling procedures, sample preparation, and analytical techniques in detail. But the methodology does not clearly specify the number of replicates for sampling or analysis. This is because replication is critical for assessing variability and ensuring reproducibility.*

- *The sampling locations are broadly described, but exact GPS coordinates, dates, and times of sampling are not provided. Please include precise geographic coordinates and temporal details to account for spatial and seasonal variability. Also describe the calibration process for the AAS (e.g., use of standard solutions) and specify quality control measures, such as the inclusion of blanks, spiked samples, or certified reference materials. Specify the grade of reagents, digestion temperature, and duration to standardize the process.*

Section III: Results and Discussion

1. **Clarity and Accuracy of Results**

   - Are the results clearly presented and accurate?
     ☐ Very Clear and Accurate
     ×☐ Clear and Accurate
     ☐ Somewhat Clear and Accurate
     ☐ Unclear and Inaccurate

Comments:

- *The morphological description of the seagrass species (Cymodocea* and *Thalassia) is clear and supported by references. However, the connection between morphology and the study's ecological risk focus could be emphasized further. For example, how does the root structure of Thalassia specifically affect heavy metal uptake?*

- *The comparisons (e.g., Pb levels in Cymodocea* vs. *Thalassia) could be made more concise to improve flow. Consider using tables for summarizing key findings to avoid repetition in text and improve clarity.*

- *Please ensure that units (e.g., mg/kg) are consistently stated throughout the section to avoid confusion. The author claims that finer sediments in Station 1 result in higher metal concentrations is reasonable and consistent with literature. However, by providing sediment texture data, if any, (e.g., percentage of clay, silt, or sand) would substantiate this conclusion.*

2. **Interpretation and Discussion of Results**

   - Are the results interpreted and discussed correctly in the context of existing literature?
     ☐ Excellent Interpretation
     ×☐ Good Interpretation
     ☐ Adequate Interpretation
     ☐ Inaccurate Interpretation

Comments:

- *The interpretation of heavy metal concentrations in seagrass and sediments is well-linked to ecological concepts, including bioaccumulation and translocation strategies. The study concludes low ecological risk, which is based on data from specific sites. Author may acknowledge this limitation and the potential variability in nearby regions which would enhance the robustness of the conclusion.*

- *The discussion of Pb concentrations linked to human activities (e.g., boat traffic) is good, but it lacks sufficient detail on the specific anthropogenic sources and mitigation recommendations. The transition between sections (e.g., from* *heavy metal discussion to ecological risk assessment) could be smoother with brief summary sentences to bridge ideas. A brief acknowledgment of study limitations (e.g., spatial scope, temporal variability) and suggestions for future research would also enhance the credibility of the discussion.*

3. **Contribution to the Field**

   - Does the article make a significant contribution to the current body of knowledge in marine science?

     ☐ Highly Significant

     ×☐ Significant

     ☐ Somewhat Significant

     ☐ Not Significant

Comments:

This article presents valuable findings on heavy metal accumulation and ecological risk in seagrass ecosystems and therefore it gives valuable contribution to better understanding the marine sciences.

Section IV: Structure and Presentation

1. **Clarity and Coherence of Writing**

   - Is the manuscript well-written, clear, and logically organized?

     ☐ Excellent

     ×☐ Good

     ☐ Fair

     ☐ Poor

Comments:

- *The data is systematically presented, with clear references to figures and tables, and the use of thresholds and indices makes the explanation more concrete and relatable to the readers. However, some points such as the low contamination levels and the healthy status of the ecosystem, are repeated across paragraphs, reducing conciseness. Also, certain sentences are long and complex, which could affect readability. Breaking them into shorter sentences with focused ideas would improve clarity.*

- *Logical progression from seagrass descriptions to heavy metal concentrations, followed by ecological risk assessments, ensures a cohesive narrative. The use of terms like "threshold" and "legal limit" could be standardized. For instance, always specify the regulatory framework (e.g., ANZECC) when mentioning thresholds. A brief acknowledgment of study limitations (e.g., spatial scope, temporal variability) and suggestions for future research would enhance the credibility of the discussion.*

2. **Figures, Tables, and Graphs**

   - Are the figures, tables, and graphs clear, relevant, and appropriately used?

     ☐ Excellent

     ×☐ Good

☐ Fair
☐ Poor

Comments:

- *Ensure that each figure, table, or graph directly supports or illustrates a specific point discussed in the text. Avoid redundant visualizations or overlap in information. Ensure all visuals have clear, descriptive titles, legends, and axis labels. Labels should specify units (e.g., mg/kg) and be easily interpretable without needing to refer back to the text. Use consistent styles, colors, and formatting across all figures and tables to maintain a professional appearance and improve readability.*

- *Ensure the graph accurately distinguishes heavy metal concentrations in sediments, roots, and leaves for each station. Use different colors or patterns for sediment, roots, and leaves, and provide a clear legend.*

- *Where possible, integrate figures and tables to reduce redundancy. For example, a combined table and graph could present heavy metal concentrations and exceedance of regulatory thresholds.*

3. **References and Citations**

   - Are the references up-to-date, relevant, and correctly cited?
     ☐ Excellent
     ×☐ Good
     ☐ Fair
     ☐ Poor

Comments:

- *The current references display inconsistencies in formatting, particularly in the presentation of journal names, volume and issue numbers, page ranges, and DOI links. Some journal names are abbreviated (e.g., Mar. Pollut. Bull.), while others are spelled out (e.g., Marine Pollution Bulletin). The use of capitalization varies across references. DOI links are sometimes included and other times not, and their formatting is inconsistent. Publisher information is included for some books but not others.*

- *Adopt a uniform referencing style throughout the References section. Common styles include APA, MLA, Chicago, or the specific style mandated by the Indonesian Journal of Marine Sciences (IJMS). IJMS has specific guidelines, ensure to comply with those.*

- *Ensure that every reference is complete and free from typographical errors. Verify each reference against the original source to confirm accuracy.*

- *Arrange references by the same author(s) in chronological order, starting with the earliest publication. Cross-check the References list against in-text citations to ensure completeness and accuracy.*

Section V: Ethical Considerations

1. **Ethical Approval and Research Integrity**

- Does the manuscript adhere to ethical guidelines for research?
  ☐ Fully Adheres
  ×☐ Mostly Adheres
  ☐ Somewhat Adheres
  ☐ Does Not Adhere

Comments:

There is no mention of ethical approval or permits for field sampling in the manuscript. The research does not mention any cultural or legal considerations, such as compliance with local regulations regarding natural resource use. Ensure the study explicitly states adherence to local and national laws governing environmental sampling and biodiversity research.

2. **Conflict of Interest**

   - Are any potential conflicts of interest disclosed?
     ☐ Fully Disclosed
     ☐ Disclosed
     ×☐ Not Disclosed

Comments:

There is no explicit mention of conflicts of interest. Add a standard conflict of interest statement, such as: *"The authors declare no conflict of interest."*

Section VI: Recommendation

1. **Recommendation for Article Acceptance**
   Please indicate your recommendation for the manuscript:
   ☐ Accept Without Revisions
   ×☐ Accept with Minor Revisions
   ☐ Accept with Major Revisions
   ☐ Reject

2. **If Revisions are Required:**

   - Please specify the major revisions required:

There are areas where the clarity of the overall article presentation can be improved as suggested in each section given earlier.

3. **Final Comments or Suggestions for the Authors:**

The article presents valuable findings on heavy metal accumulation and ecological risk in seagrass ecosystems. However, to enhance the clarity of the originality and novelty, refer to those comments and suggestions given in each section.

Reviewer Signature (optional): ___________________________
Date: 13 January 2025

This form is designed to help reviewers evaluate all key aspects of the article while providing detailed, structured feedback. The questions are tailored to ensure clarity, scientific rigor, and relevance, making it easier for the reviewer to assess and provide useful comments.

Heavy metal accumulation and ecological risk on seagrass *Cymodocea* and *Thalassia* in Pahawang Island, Indonesia

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Abstract

Seagrass species thrive in coastal ecosystems and known for their ability to accumulate heavy metals from their surrounding environment. This study aims to evaluate the ecological risks related to the accumulation of heavy metals in seagrass roots, rhizomes, leaves, and sediments. The seagrass examined belong to the genera *Cymodocea* and *Thalassia*, collected from two sites: Jeralangan and Cukuh Nyai on Pahawang Island, Lampung, Indonesia. The heavy metals analyzed included Pb, Cu, Ni, and Zn, which were measured using the wet desctruction method and quantified with a SHIMADZU AA-7000 Atomic Absorption Spectrophotometer (AAS). The ecological risk was evaluated through various indices, such as the Bioconcentration Factor (BCF), Translocation Factor

(TF), Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI). The highest concentrations of heavy metals in sediment were detected at station 1 was Zn (15.486 mg/kg). In the roots-rhizomes of *Cymodocea* was Zn (8.772 mg/kg), while the highest concentration in leaves was Cu in *Thalassia* (10.541 mg/kg). The ecological risk assessment revealed that BCF < 1 categorize an excluder, while TF > 1 for Pb and Zn indicate effective translocation from roots to leaves. Additionally, I_{geo} < 0 signify no contamination, Cf < 1 indicate low pollution levels, and PLI < 0 confirm a non-polluted status. In conclusion, the results show that the seagrass ecosystems at the study sites currently have low levels of heavy metal pollution and minimal ecological risk, suggesting they remain in a relatively safe condition.

Keywords: Accumulation, *Cymodocea*, Ecological risk assessment, Heavy metal, *Thalassia*

Introduction

Heavy metals are elements with low molecular weight that can lead to serious environmental issues when accumulated at certain concentrations (Wang, C. *et al*., 2022). Pollution from heavy metals due to tourism activities can create new problems, such as bioaccumulation and the transfer of pollutants through the food chain, a process known as biomagnification (Wang, Z. *et al*., 2022). Biomagnification can occur in various organism components, including seagrass. Seagrass can absorb and accumulate pollutants like heavy metals in its tissues, which enables it to serve effectively as a bioindicator (Nel *et al*., 2023). Seagrass acts as a biomonitor and bioaccumulator of heavy metals because of its limited mobility, making it possible to estimate the concentrations and sources of heavy metals for analysis (Bonanno and Lo Giudice, 2010). Seagrass, which is found along the coast and in subtidal areas of Pahawang, is susceptible to direct impacts from the presence of heavy metals (Noor *et al*., 2021).

Seagrass plays a crucial ecological role as an important habitat, providing abundant ecosystem services such as breeding grounds and food sources (Sun *et al*., 2020). In recent decades, seagrass has faced ecological challenges due to significant contamination, particularly from heavy metals entering coastal areas (Tu *et al*., 2023). Several studies have revealed the presence of heavy metals such as Pb, Cu, Zn, Cr, and Cd in various environments and organisms, including those in port areas, rivers, seagrass beds, and fish populations (Liu *et al*., 2019; Hosokawa *et al*., 2020; Fang *et al*., 2022; Souza-Araujo *et al*., 2022). Research on heavy metals in seagrass in other coastal regions has also shown that seagrass is vulnerable to accumulating all types of heavy metals, as observed in seagrass along the South China Sea (Zhang *et al*., 2024). Additionally, seagrass can accumulate heavy metals in its roots and leaves (Jeong *et al*., 2021). A study conducted in the central Gulf of Gabes (Southeast Tunisia) indicated that seagrass not only serves as a bioindicator but can also be an effective bioremediation tool (El Zrelli *et al*., 2023).

The occurrence of heavy metals and their negative effects on the ecological balance of seagrass, as previously discussed, serve as a primary motivation for this research. The seagrass species *Cymodocea* and *Thalassia* were chosen due to their dominance in the research sites. Previous studies have shown that these seagrasses have been investigated for heavy metal presence in their tissues, exhibiting relatively high levels of accumulation in various regions, including Italy, South China, India, and Florida (Bonanno *et al*., 2020; Gopi *et al*., 2020; Zhang *et al*., 2021). However, there has been no investigation using these seagrasses to monitor heavy metal pollution on Pahawang Island. This island is known as a popular tourist destination in Indonesia, hosting significant populations of *Cymodocea* and *Thalassia*. Therefore, this study aims to provide valuable insights into the bioaccumulation patterns of heavy metals in the roots and leaves of seagrass, while also identifying the ecological risks involved. These insights can assist in developing management strategies for coastal areas and ecosystems in the region. The primary objective of this research is to analyze the ecological risks associated with the accumulation of heavy metals (Pb, Cu, Ni, and Zn) in the roots, rhizomes, and leaves of *Cymodocea* and *Thalassia*, as well as in the sediments of Pahawang Island.

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Materials and Methods

Study area and sampling

This research was carried out on the coast of Pahawang Island in Lampung, Indonesia, at two distinct sampling locations. The sites were selected using purposive sampling within the seagrass ecosystem in Jeralangan and Cukuh Nyai (Figure 1). The first location, Jeralangan, is characterized by a high volume of tourism activities, while the second location, Cukuh Nyai, experiences relatively low tourism activity.

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Commented [A3]: Perbandingan kedua kondisi ini sangat menarik diteliti. Hal ini dapat ditambahkan mengapa kegiatan wisata bisa bertanggung jawab terhadap kontribusi logam berat sehingga memperkuat studi Anda

Two types of samples were collected at each station, consisting of seagrass and sediment. At Station One, samples of *Cymodocea* seagrass and sediment were collected, while Station Two involved the collection of *Thalassia* seagrass and sediment. The identification of seagrass species was conducted using a field guide for seagrass and dugong monitoring (Herandarudewi *et al*., 2019). he collected seagrass samples included both leaves and roots, gathered from shallow water habitats or intertidal zones through random sampling (Trevathan-Tackett *et al*., 2015; Singh *et al*., 2021). Sediment samples were taken using a grab sampler, with a total weight of 1 kg from the surface layer (0-10 cm). The collected samples were placed in clip-lock plastic bags and stored in containers (Xiao *et al*., 2022).

Heavy metals analysis

The seagrass leaf and root samples, along with sediment, were thoroughly cleaned and subsequently dried. The dried samples were then ground to a fine powder and stored in sealed containers (Stewart *et al*., 2021). Sample digestion was performed using a wet destruction method, where 0.5 g of the powdered seagrass was mixed with 5 ml of a reagent in a ratio of 5:2:1 (nitric acid: perchloric acid: sulfuric acid), along with an additional 5 ml of 2N hydrochloric acid (FAO/SIDA, 1983; European Commission, 2011). For sediment samples, the wet destruction process involved adding 5 ml of concentrated HNO_3 to each sample, which included 50 ml of water sample and approximately 3 g of sediment sample (Gao *et al*., 2021). The heavy metals analyzed in this study included Pb, Cu, Ni, and Zn. The concentration of heavy metals in the samples was measured using an Atomic Absorption Spectrophotometer (AAS) SHIMADZU AA-7000.

Ecological risk assessment

Ecological risk assessment can be conducted by calculating the bioconcentration factor (BCF), translocation factors (TF), geoaccumulation index (I_{geo}), contamination factor (Cf), and pollution load index (PLI) (Hakanson, 1980; Rozirwan *et al*., 2023).

Bioconcentration factor (BCF)

he accumulation of heavy metals in the roots and leaves of seagrass from the surrounding sediment can be assessed using the Bioconcentration Factor (BCF) as described below (Steingräber *et al*., 2022).

$$BCF = \frac{concentration\ of\ organs}{concentration\ of\ sediment} \quad (1)$$

where: BCF < 1 is an excluder; BCF = 1 is an indicator, and BCF = 1 is a hyperaccumulator (Almahasheer, 2019).

Commented [A4]: Please kindly clarify this category

Translocation Factors (TF)

The Translocation Factor (TF) is used to measure the movement of heavy metals from the roots to the leaves (Usman *et al*., 2019).

$$TF = \frac{BCF\ leaf}{BCF\ of\ root{-}rhizome} \quad (2)$$

Commented [A5]: Bagaimana penulis menentukan BCF pada rhizome, karena bagian ini tidak dianalisis

TF Value > 1 indicates that the sample functions as a phytoextractor, effectively translocating metals from the roots to the leaves, whereas TF value < 1 indicates that the sample functions as a phytostabilizer (Dinu *et al*., 2020).

Geoaccumulation index (I_{geo})

The Geoaccumulation Index (I_{geo}) is used to evaluate the degree of heavy metal contamination and classify pollution levels according to established criteria (Rozirwan *et al*., 2023).

$$Igeo = Log\ 2\left(\frac{concentration\ of\ sediment}{1{,}5\ background}\right) \quad (3)$$

where: the I_{geo} value classification consists of not contaminated (I_{geo}≤0), not contaminated to moderately contaminated (I_{geo}0–1), moderately contaminated (I_{geo}1–2), moderate to highly contaminated (I_{geo}2–3), highly contaminated (I_{geo}3–4), highly contaminated to very high (I_{geo} 4–5), and highly contaminated (I_{geo}≥5).

Contamination factor (Cf)

The Contamination Factor (CF) value is used as a basis for determining the degree of heavy metal contamination (Rozirwan *et al*., 2024).

$$CF = \frac{concentration\ of\ sediment}{Background} \quad (4)$$

where: contamination factor criteria according to (Shaheen *et al*., 2019); Cf < 1 = low level of contamination; 1 < Cf < 3 = moderate level of contamination; 3 < Cf < 6 = moderate level of contamination; Cf > 6 = very high level of contamination.

Pollution load index (PLI)

The Pollution Load Index (PLI) is used to evaluate the pollution quality (Mosa *et al*., 2022).

$$PLI = [Cf1\ x\ Cf2\ x\ Cf3 \ldots .\ x\ Cfn]^{1/n} \ldots \quad (5)$$

where: pollution load index criteria: PLI 2 = not polluted to lightly polluted; PLI 24 = moderately polluted; PLI 4–6 = heavily polluted; PLI 6–8 = heavily polluted; PLI 8–10 = heavily polluted

Result and Discussion

Description of seagrass

The seagrass samples were collected based on the dominant species found at the research site, specifically *Cymodocea* and *Thalassia* (Figure 2). *Cymodocea* was predominantly observed at Station One. This species is characterized by long, ribbon-like leaves that taper to a rounded tip, white to slightly yellowish rhizomes, and thin, brown fibrous roots. Both *Cymodocea* and *Thalassia* are commonly found in substrates ranging from muddy to sandy (Hartati *et al*., 2018; Thangaradjou and Bhatt, 2018), which explains their prevalence in the research area where the majority of locations feature such substrates. *Cymodocea* exhibits a strong tolerance to various environmental stresses and can thrive in diverse habitats, categorizing it as a species of least concern according to the IUCN (Terrados *et al*., 1998; Short *et al*., 2011). *Thalassia* is mainly found at Station Two and is recognized by its crescent-shaped leaves with neatly arranged margins, brown rhizomes, and thick, sheathed roots. This species allocates a greater proportion of its biomass to underground structures, such as roots and rhizomes, rather than to its leaves. This morphological variation can be seen as an adaptive response to environmental physical stressors, such as burial by sand to prevent displacement during tidal fluctuations and storms (Westlake *et al*., 2022). According to Dilipan and Arulbalachandran (2022), both *Cymodocea* and *Thalassia* are promising candidates for bioremediation of contaminated sites and are frequently utilized as biological indicators of heavy metal accumulation.

Heavy metals concentration in sediment and seagrass

The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments, roots, and leaves of seagrasses at both stations are summarized in Figure 3. The results indicate that Station 1 (*Cymodocea*) had the highest concentrations of Pb and Zn in the sediments, roots, and leaves. Specifically, the maximum concentrations of Cu in sediments and roots were also recorded at Station 1 (*Cymodocea*), at 0.896 mg/kg and 0.537 mg/kg, respectively, while the highest concentration in leaves was observed at Station 2 (*Thalassia*), measuring 0.654 mg/kg. The highest levels of Ni were found in the leaves and roots of *Cymodocea*, with concentrations of 2.331 mg/kg and 1.968 mg/kg, respectively. In contrast, the sediment at Station 2 exhibited the highest concentration of Ni, recorded at 7.730 mg/kg. A comparative analysis of heavy metal concentrations across different seagrass species and locations is summarized in Figure 3.

Based on Figure 3, the concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments of this study are still below the threshold values established by ANZECC and ARMCANZ (2000) as referenced *in* (Ogundele *et al*., 2015), and by the Gazzetta Ufficiale della Repubblica Italiana as noted *in* (Bonanno *et al*., 2020), which are set at 50 mg/kg, 65 mg/kg, 50 mg/kg, and 30 mg/kg, respectively. Station 1 is characterized by a predominant presence of *Cymodocea*, which is directly influenced by tidal dynamics. Tidal-affected areas experience dynamic water movement, enabling heavy metals carried by the water to adhere to sediment particles (Maulana *et al*., 2023). The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments are higher compared to those in the roots and leaves, corroborating the findings of Khotimah *et al*., (2024) which indicate that sediment concentrations of heavy metals exceed those in the roots, implying that the roots may serve as a barrier preventing heavy metals from translocating to the upper plant structures. As noted by Flefel *et al*., (2020), sediments act as primary reservoirs for heavy metal absorption in aquatic environments. Furthermore, the higher concentrations of heavy metals in the sediments at Station 1 compared to Station 2 could be attributed to the finer sediment texture found at Station 1. Heavy metals tend to bind more effectively to sediment types with higher clay content or finer particle sizes (Borah *et al*., 2018).

The concentrations of heavy metal Pb in the sediments, leaves, and roots of *Cymodocea* and *Thalassia* differ between the two research locations. At Station 1, the concentration of Pb in the roots of *Cymodocea* is recorded at 0.761 mg/kg, while the leaves show a concentration of 0.846 mg/kg. The higher concentration of Pb in *Cymodocea* at Station 1 may be attributed to the significant human activities in this area, including increased boat traffic and residential developments. Heavy metal Pb levels can be exacerbated by factors such as maritime traffic, fishing activities, paint on boats, and sedimentation (Irandoost *et al*., 2021). Notably, the highest concentration of Pb is found in the leaves of *Cymodocea* when compared to its roots, with values of 0.846 mg/kg for leaves and 0.761 mg/kg for root-rhizome. Similar findings were reported by El Zrelli *et al*., (2023), which indicated that the Pb concentration in the leaves of *Cymodocea* is greater than that in its rhizomes. According to the WHO (1996) as cited in Ogundele *et al*., (2015), the concentration of Pb in *Cymodocea* and *Thalassia* remains below the quality standard of 2 mg/kg (Pb), suggesting that these seagrasses are not currently subjected to contamination.

In this study, the highest concentration of the heavy metal Cu was observed in the leaves of *Thalassia* at Station 2, measuring 10.541 mg/kg. This concentration is still below the quality standard of 20 mg/kg (Kabata-Pendias, 2011) indicating that the accumulation of Cu in seagrass does not pose any toxic effects. Copper is considered an essential metal, as it is required by organisms; however, at significantly high concentrations, Cu can be more toxic than non-essential metals such as Pb and Cd due to the active absorption mechanisms and tolerance strategies that plants employ A similar study conducted on the leaves and roots of *Thalassia* in Xincun Bay, China, also reported the highest concentration in leaves at 11.2 mg/kg (Zhang *et al*., 2021). Previous research on various seagrass species has demonstrated that aboveground leaves are more efficient at accumulating Cu compared to belowground tissues (roots and rhizomes) (Lin *et al*., 2018; Hu *et al*., 2019).

The concentration of Ni based on the research findings indicates that the highest level was found in the sediment, followed by the roots of *Cymodocea*, measuring 2.331 mg/kg. This concentration is significantly lower than the legal limit allowed in marine waters, which is set at 20 mg/kg. Furthermore, increases in Ni concentrations of 3 to 5 times are generally observed in environments impacted by human activities (Bonanno *et al*., 2020). The Ni concentrations in this study are consistent with previous research that reported low Ni levels in seagrasses, such as 3.20–6.65 mg/kg in Italy (Bonanno *et al*., 2017) and 1.8–3.1 mg/kg in South Africa (Nel *et al*., 2023), , particularly in locations with minimal human activities. This suggests that the study site is not yet contaminated with heavy metals, specifically Ni.

According to Figure 3, the highest concentration of Zn in seagrass was found in the leaves of *Cymodocea* at station 1, reaching 9.309 mg/kg. This concentration remains below the threshold established by the Australia and New Zealand Food Authority (ANZFA), which is 14 mg/kg (Smitha *et al*., 2010). Furthermore, the measured Zn levels are significantly lower than the concentrations that can cause phytotoxicity, which range from 500-1500 mg/kg (Chaney, 1989). Thus, the concentration of Zn detected in this study does not indicate any toxic effects. The results demonstrate that the concentrations of Zn in both the leaves and roots of the two seagrass species are higher than those of Pb and Cu (Figure 3). This suggests that Zn plays a crucial role in plant metabolism (Bonanno and Orlando-Bonaca, 2017). These findings are consistent with previous research, which reported that Zn concentrations are highest in the roots and leaves of various seagrass species, including *Cymodocea* and *Thalassia*, in locations such as Sicily, Italy, China, and India (Bonanno *et al*., 2017; Hu *et al*., 2019; Gopi *et al*., 2020).

Bioaccumulation and Ecological risk assessments

Based on the research results presented in Table 2, the bioconcentration factors (BCF) for the roots of *Cymodocea* and *Thalassia*, which accumulate heavy metals from the sediment biologically, were determined to be 0.0828 and 0.0502 for Pb, respectively. The BCF for Cu in *Cymodocea* is 0.5991, while in *Thalassia* it is 0.0082. For Ni, the BCF values are 0.3140 for *Cymodocea* and 0.0894 for *Thalassia*, and for Zn, the BCF values are 0.5665 and 0.1670, respectively. The geoaccumulation index indicates an unpolluted status for Pb with values of -1.9696 and -1.9344, for Cu with values of -5.0658 and -4.5659, for Ni with values of -2.2167 and -2.1581, and for Zn with values of -2.6982 and -2.8687. The contamination factor (CF) suggests low contamination levels for Pb at 0.3830 and 0.3924, for Cu at 0.0448 and not detected (nd), for Ni at 0.3227 and 0.3361, and for Zn at 0.2311 and 0.2055. The pollution load index (PLI) was calculated, yielding results of 0.0013 and 0.0051, indicating that both stations are not polluted.

There are differences in the translocation factor (TF) values of metals (Table 3) between *Cymodocea* and *Thalassia*. In *Thalassia*, the TF for Cu indicates a translocation value of < 1, suggesting minimal transfer of this metal to the above-ground biomass or leaves. This can also be attributed to the very low concentrations of Cu in the roots and leaves of the sampled seagrass at the study site. The metal with the highest TF value is Zn in *Thalassia*, followed by Ni and Pb. Similarly, *Cymodocea* shows the highest TF values for Zn and Pb, which are both > 1. The differences in translocation values may arise from the biological characteristics of seagrasses, which have developed two distinct adaptive strategies to cope with the levels of contamination in their environment. The first strategy involves the storage of accumulated metals from the sediment in the root-rhizome system, while the second strategy entails the translocation of some contaminants from the root-rhizome to the above-ground parts of the seagrass, such as the leaves (El Zrelli *et al*., 2023). According to the TF values presented in Table 2, *Thalassia* employs the second adaptive strategy for Zn, Ni, and Pb by translocating these contaminants to the leaves, whereas for Cu, it utilizes the first adaptive strategy. In contrast, *Cymodocea* adopts the second adaptive strategy for Pb and Zn. Previous research has also indicated that Zn undergoes significant translocation in *Cymodocea*, with a value > 1 (Bonanno and Borg, 2018; Nel *et al*., 2023).

The bioconcentration factor (BCF) of heavy metals from sediment to leaves and roots is summarized in Table 2. The BCF of zinc (Zn) in the leaves is higher than that of lead (Pb), nickel (Ni), and copper (Cu). This indicates that the accumulation level of Zn in the leaves is greater compared to the other metals, which may be influenced by several factors, including the adaptive strategies of seagrass in absorbing heavy metals and the chemical properties of the metals as well as the selectivity of the plants (Khotimah *et al*., 2024). Zinc is an essential metal for seagrass, as it is utilized in photosynthesis and growth (Kabata-Pendias and Pendias, 2011). This leads to higher Zn accumulation since it is more readily absorbed by both *Cymodocea* and *Thalassia*. According to Table 2, *Cymodocea* exhibits the highest BCF value for Zn. Similarly, high BCF values for Zn have also been found in the leaves of *Zostera noltei*, as well as in the roots and leaves of *Thalassia hemprichii* and in the roots and leaves of *Enhalus acoroides* (Li and Huang, 2012; Boutahar *et al*., 2019; Jeong *et al*., 2021).

The values of the geoaccumulation index (I_{geo}) from this study are presented in Table 4, indicating that the sediments at both research locations are unpolluted (I_{geo}< 0). This suggests that the sources of heavy metal contamination at these sites are low, attributed to factors such as small residential areas, the absence of industrial and mining activities, and relatively low shipping traffic. Additionally, the physical and chemical conditions of the aquatic environment, including current patterns and tidal movements, also play a significant role (Siregar *et al*., 2020). The contamination factor (CF) in this study indicates that each metal contributes to low or negligible contamination, as evidenced by CF values < 1. These findings align with research conducted along the southern coast of Sumatra, Indonesia, which showed low CF values for each metal, except for lead (Pb), due to significant anthropogenic sources in the vicinity (Rozirwan *et al*., 2023; Khotimah *et al*., 2024). The pollution status of the seagrass ecosystem at the research locations can be assessed using the pollution load index (PLI). The sediment PLI analyzed at the study sites is classified as unpolluted, with PLI values ranging from 0 to 2. The PLI values at both locations are not significantly different, indicating that both ecosystems are healthy and safe for living organisms. In contrast, other studies on seagrass ecosystems in the Southern Mediterranean Sea reported PLI values categorized as moderately contaminated and polluted, posing risks to the seagrass ecosystems, primarily due to the discharge of various industrial wastes, particularly phosphogypsum (El Zrelli *et al*., 2023). Therefore, based on the analyses from this study, it can be concluded that all sampling locations exhibit low levels of metal pollution and ecological risk.

Conclusion

The highest concentrations of Pb, Cu, Ni, and Zn in seagrass are found in the leaves, while the sediments at Station 1 show the highest levels. Overall, the concentrations of heavy metals in the roots and leaves of *Cymodocea* and *Thalassia* indicate relatively high levels; however, the ecological risk assessment shows that the bioaccumulation factor (BCF) is categorized as low. In contrast, the translocation factors (TF) for Pb and Zn demonstrate values greater than 1, indicating that the seagrass functions effectively in phytoremediation or translocating metals from the roots to the leaves. In summary, the ecological risk assessment of the research locations, which includes the geoaccumulation index (Igeo), contamination factor (CF), and pollution load index (PLI), indicates that the environment within the seagrass ecosystems at these sites has low levels of heavy metal pollution and ecological risk.

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Figure 1. A map of seagrass sampling location

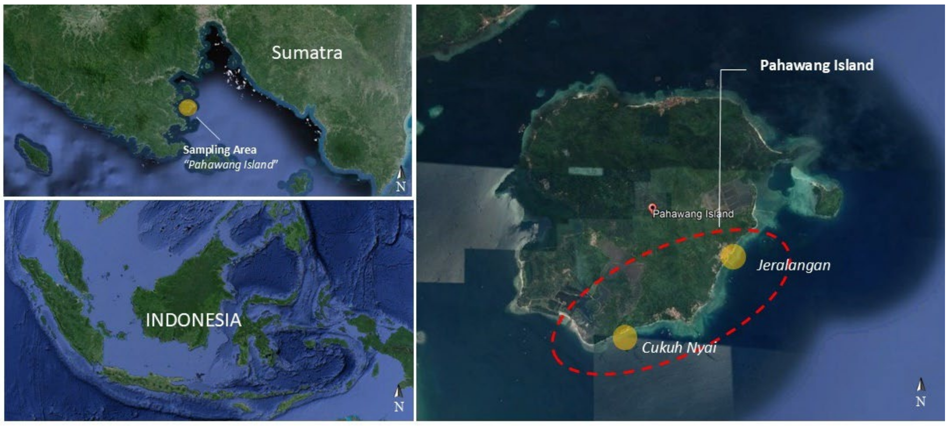

Figure 2. Seagrass, (a) *Cymodocea*, (b) *Thalassia*

Figure 3. Concentrations of Pb, Cu, Ni and Zn in organs of *Cymodocea* and *Thalassia* (leaf and root-rhizome; mg/kg), and associated sediments (mg/kg)

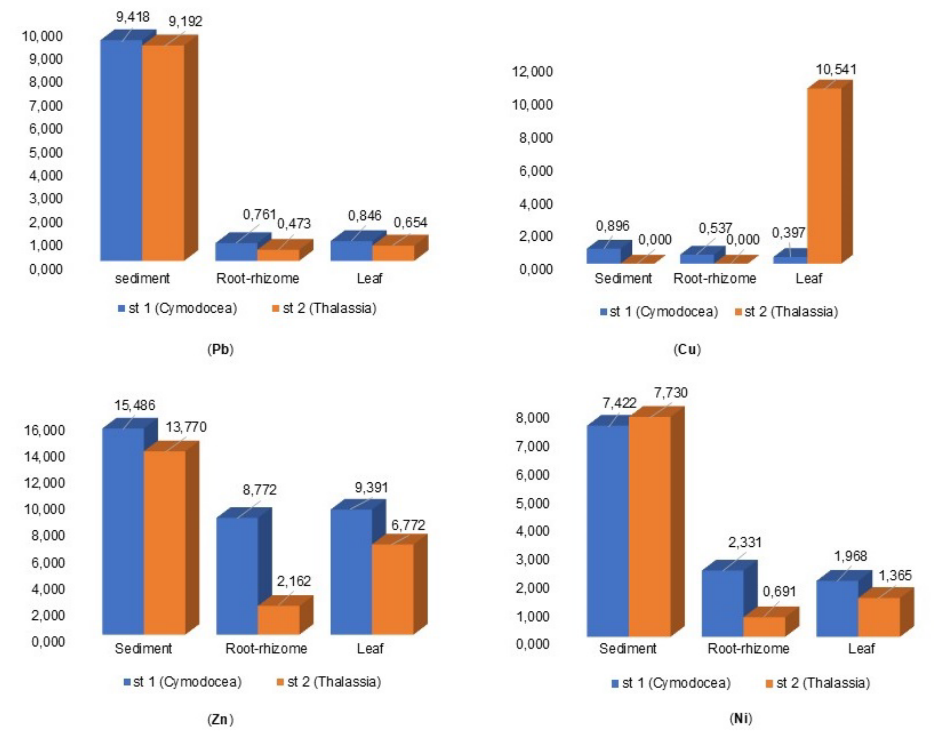

Table 1. Comparison of heavy metal concentrations in *Cymodocea* and *Thalassia* with those from the open literatures

Location	Seagrass	Tissue	Heavy metals (mg/kg)	References
Sicily (Italy)	*Cymodocea*	Leaf	3.61–28.8 (Cu) 3.20–6.65 (Ni) 1.42–3.85 (Pb) 42.6–65.8 (Zn)	(Bonanno *et al*., 2017)
Sicily (Italy)	*Cymodocea*	Leaf	3.90–19.5 (Cu) 2.40–22.5 (Ni) 1.52–6.10 (Pb) 4.21–13.9 (Zn)	(Bonanno *et al*., 2020)
Sicily (Italy)	*Cymodocea*	Rhizome	10,6 (Cu 1,15 (Ni) 0,73 (Pb) 21,7 (Zn)	(Bonanno and Borg, 2018)
		Root	8,83 (Cu) 4,79 (Ni) 4,81 (Pb) 38,7 (Zn)	
Florida, USA	*Thalassia*	Rhizome+root+leaf	20,23 (Pb) 9,55 (Cu) 86,68 (Zn) 1,56 (Cd)	(Smith *et al*., 2019)
Palk Bay, South Eastern India	*Cymodocea*	Rhizome+root +leaf	1,23 (Cd) 18,59 (Cu) 1,74 (Pb) 34,63 (Zn)	(Gopi *et al*., 2020)
Xincun Bay,China	*Thalassia*	Rhizome+root +leaf	11,2 (Cu) 4,05 (Ni) 2,1 (Pb) 39,1 (Zn)	(Zhang *et al*., 2021)
Southern Mediterranean Sea	*Cymodocea*	Rhizome	0,99 (Cu) 0,26 (Pb) 55 (Zn) 0,45 (Cd)	(El Zrelli *et al*., 2023)
		Root	3,51 (Cu) 3,76 (Pb) 31 (Zn) 1,33 (Cd)	
		Leaf	3,95 (Cu) 1,74 (Pb) 131,33 (Zn) 2,40 (Cd)	

Table 2. Values of the Bioconcentration factor (BCF)

	BCF			
	Pb	Cu	Ni	Zn
Seagrass roots				
St 1 (*Cymodoceae*)	0,0804	0,5991	0,3140	0,5665
St 2 (*Thalassia*)	0,0518	0,0082	0,0894	0,1570
Seagrass leaves				
St 1 (*Cymodoceae*)	0,0893	0,4436	0,2652	0,6064
St 2 (*Thalassia*)	0,0716	-8,3217	0,1766	0,4918

Table 3. The translocation factor (TF) from the roots to the leaves of seagrass

	TF			
	Pb	Cu	Ni	Zn
St 1 (*Cymodoceae*)	1,1112	0,7405	0,8445	1,0705
St 2 (*Thalassia*)	1,3834	-1011,6867	1,9764	3,1328

Table 4. Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI)

	Igeo				CF				PLI
	Pb	Cu	Ni	Zn	Pb	Cu	Ni	Zn	
St 1	-1,9696	-5,0658	-2,2167	-2,6982	0,3830	0,0448	0,3227	0,2311	0,0013
St 2	-1,9344	-4,5659	-2,1581	-2,8676	0,3924	nd	0,3361	0,2055	0,0051

Table of Responses

No.	Report	Answears
Section I: Overall Evaluation		
1.	- The article does not highlight why this specific location was chosen and how it adds unique insights compared to other regions in Indonesia. Does Pahawang Island serve as a critical biodiversity hotspot, subject to specific anthropogenic pressures, or it has unique seagrass dynamics. A brief discussion on the ecological or conservation significance of Pahawang Island could be added to establish the originality of the study. - It would be beneficial to state if there any previous research investigated heavy metal accumulation in these species specifically, especially in the Indonesian context. Mention if this is the first study analyzing heavy metals in Cymodocea and Thalassia in Pahawang Island or whether the findings address a knowledge gap in the literature. - The finding that seagrass ecosystems are currently at low ecological risk is valuable, but it could be discussed more for broader implications. How do these findings contribute to understanding heavy metal dynamics in tropical seagrass ecosystems or guide future management practices? Discuss how these results compare to heavy metal accumulation and ecological risks in other seagrass ecosystems, especially within Southeast Asia. Also discuss why does Thalassia have a higher Cu concentration in leaves compared to Cymodocea?	- I have added to the script that Pahawang Island is an important biodiversity hotspot with extensive seagrass meadows that support various marine organisms. Additionally, the island is experiencing increasing anthropogenic pressures, including tourism, which may influence heavy metal accumulation in the seagrass ecosystem. These factors make Pahawang Island a critical area for assessing ecological risk and understanding seagrass dynamics in response to environmental stressors. - I have added previous studies that specifically investigated heavy metal accumulation in these species, particularly in the context of Indonesia. This study is the first to analyze heavy metals in *Cymodocea* and *Thalassia* on Pahawang Island, and the discussion on whether these findings address knowledge gaps in the literature has been included in the script. - I have added explanations in the script as requested

	- The study appears to be a snapshot in time, how its findings might inform future temporal or spatial studies. Additional statements may also be included on how the findings could guide policymakers or stakeholders in managing coastal pollution in the area. - The article does not adequately address potential sources of heavy metals (e.g., agricultural runoff, industrial activities, or natural processes). A brief discussion on potential heavy metal sources affecting Pahawang Island is recommended. While various indices are presented, there is no discussion on how the detected metal concentrations compare with established environmental or regulatory thresholds (e.g., WHO or Indonesian standards). Please include a comparison of the detected values with international or national standards to contextualize the results.	- I have added explanations in the script as requested - I have added a discussion on the potential sources of heavy metals affecting Pahawang Island in the manuscript. Additionally, we have already compared the detected metal concentrations with relevant environmental and regulatory thresholds, including WHO, ANZECC and ARMCANZ , the Gazzetta Ufficiale della Repubblica Italiana, and the Australia and New Zealand Food Authority (ANZFA), to provide context for the results.
3.	- Research objective is already explicitly stated which helps focus the study. However, the introduction does not articulate a specific hypothesis. For instance, it does not propose expected patterns of heavy metal accumulation in different seagrass parts (roots, rhizomes, leaves) or sediments. A hypothesis based on previous studies could be introduced, for example: "It is hypothesized that heavy metal concentrations will be higher in seagrass roots and sediments compared to leaves, reflecting limited mobility and high sediment deposition rates". - The use of BCF, TF, Igeo, Cf, and PLI indices in assessing ecological risks specific to seagrass ecosystems in	- I have added the hypothesis regarding the concentration of heavy metals in seagrass roots, leaves, and sediments. - I have included how this study advances current knowledge on heavy metal pollution in seagrass ecosystems.

	tropical coastal areas could be briefly justified. Highlight how the study advances current knowledge on heavy metal pollution in seagrass ecosystems, particularly in Indonesia.	
Section II: Methodology and Data Analysis		
1.	- The methodology is well-detailed, and the use of purposive sampling is appropriate for this study, as the two locations have distinct tourism activities, providing a meaningful comparison of anthropogenic impacts on seagrass ecosystems. Justification for why certain indices (e.g., PLI, Igeo) were chosen over others is required. Are these indices more suitable for tropical coastal ecosystems, or were they selected based on prior studies in similar environments? Specify the source of 'background concentrations' or clarify how they were determined. - Discuss the reliability of the wet digestion method used, and if possible, consider cross-validation with CRM (certified reference materials). Also clearly state the number of replicates used for each sample type to improve transparency.	- Ecological risk indices such as BCF, TF, CF, Igeo and PLI were chosen based on previous studies in similar environments, i.e. in tropical coastal ecosystems. I have added the source of "background concentration" in the script - I have added an explanation of wet digestion and written the number of replicates used for each sample type. I did not validate the CRM, instead to control the quality of the data was carried out by other methods, namely replicate analysis, use of blanks and comparison with previous studies have been carried out to ensure the accuracy of the results.
2.	- The inclusion of multiple metrics (BCF, TF, Igeo, Cf, and PLI) ensures a multidimensional approach to assessing heavy metal contamination and its ecological risks. Please include statistical methods such as t-tests, ANOVA, or non-parametric tests to assess differences between the two sites or between seagrass and sediment samples. Regression analysis could also explore relationships between heavy	- I have added statistical analysis to determine the differences in heavy metal concentrations between the two locations.

	metal concentrations and ecological indices. - Individual metals are analyzed, but there is still no clear methodology for interpreting the cumulative effect of multiple metals. Try to consider using multivariate analysis techniques (e.g., Principal Component Analysis, PCA) to assess overall contamination patterns and identify dominant sources of pollution. - If possible, please also discuss how findings from the two sampling sites might extrapolate to other areas and acknowledge limitations in spatial coverage.	 - To achieve the main objective of this study, I have used an established ecological risk assessment index, which provides a clear and direct evaluation of the level of contamination and potential environmental impacts. Although multivariate techniques can be useful for identifying pollution sources and contamination patterns, our focus is more on assessing the ecological risk posed by heavy metal accumulation rather than source apportionment. In addition, the limited number of samples in this study makes the application of PCA less than optimal. - I have added explanations in the script as requested
3.	- The methodology already describes the sampling procedures, sample preparation, and analytical techniques in detail. But the methodology does not clearly specify the number of replicates for sampling or analysis. This is because replication is critical for assessing variability and ensuring reproducibility. - The sampling locations are broadly described, but exact GPS coordinates, dates, and times of sampling are not provided. Please include precise geographic coordinates and temporal details to account for spatial and seasonal variability. Also describe the calibration process for the AAS (e.g., use of standard solutions) and specify quality control measures, such as the	- I have written the number of replications used for each type of sample analyzed for heavy metals - I have added GPS coordinates, date and time of sampling. I have also explained the calibration process for AAS and quality control steps, as well as reagent levels, digestion temperatures and durations to standardize the process.

	inclusion of blanks, spiked samples, or certified reference materials. Specify the grade of reagents, digestion temperature, and duration to standardize the process.	
Section III: Results and Discussion		
1.	- The morphological description of the seagrass species (Cymodocea and Thalassia) is clear and supported by references. However, the connection between morphology and the study's ecological risk focus could be emphasized further. For example, how does the root structure of Thalassia specifically affect heavy metal uptake? - The comparisons (e.g., Pb levels in Cymodocea vs. Thalassia) could be made more concise to improve flow. Consider using tables for summarizing key findings to avoid repetition in text and improve clarity. - Please ensure that units (e.g., mg/kg) are consistently stated throughout the section to avoid confusion. The author claims that finer sediments in Station 1 result in higher metal concentrations is reasonable and consistent with literature. However, by providing sediment texture data, if any, (e.g., percentage of clay, silt, or sand) would substantiate this conclusion.	- I have added the relationship between seagrass morphology and ecological risk focus to this study. - To enhance clarity and avoid redundancy, we have already presented the comparisons, including heavy metals concentration in Cymodocea and Thalassia, in graphical form. The visual representation allows for a more concise and effective comparison. However, we will review the text to ensure better flow and minimize any unnecessary repetition. - This study did not include sediment analysis, and therefore, sediment texture data (e.g., clay, silt, or sand composition) were not available. We acknowledge that such data would provide a stronger basis for discussing metal accumulation patterns and could be an important aspect for future research. Therefore, I have corrected by removing the statement regarding the type of sediment at the study site. In addition, I have corrected the units to maintain consistency.
2.	- The interpretation of heavy metal concentrations in seagrass and sediments is well-linked to ecological concepts, including bioaccumulation and translocation strategies. The study	- I have added about the limitations of this study and the potential for variability in the surrounding area.

	concludes low ecological risk, which is based on data from specific sites. Author may acknowledge this limitation and the potential variability in nearby regions which would enhance the robustness of the conclusion. - The discussion of Pb concentrations linked to human activities (e.g., boat traffic) is good, but it lacks sufficient detail on the specific anthropogenic sources and mitigation recommendations. The transition between sections (e.g., from heavy metal discussion to ecological risk assessment) could be smoother with brief summary sentences to bridge ideas. A brief acknowledgment of study limitations (e.g., spatial scope, temporal variability) and suggestions for future research would also enhance the credibility of the discussion.	 - I have added mitigation recommendations in the form of improving regulations and waste management systems around coastal areas and long-term monitoring to identify pollution trends and their impacts on seagrass ecosystems. I have also added transitions between the sections on heavy metals and ecological risk assessment, and written study limitations and suggestions for future research.
Section IV: Structure and Presentation		
1.	- The data is systematically presented, with clear references to figures and tables, and the use of thresholds and indices makes the explanation more concrete and relatable to the readers. However, some points such as the low contamination levels and the healthy status of the ecosystem, are repeated across paragraphs, reducing conciseness. Also, certain sentences are long and complex, which could affect readability. Breaking them into shorter sentences with focused ideas would improve clarity. - Logical progression from seagrass descriptions to heavy metal concentrations, followed by ecological risk assessments, ensures a cohesive	- The manuscript has been revised to increase conciseness by reducing repetition. Additionally, long and complicated sentences have been simplified - The terms related to thresholds and legal limits have been standardized. Additionally, a brief acknowledgment of the study's limitations, including spatial

	narrative. The use of terms like "threshold" and "legal limit" could be standardized. For instance, always specify the regulatory framework (e.g., ANZECC) when mentioning thresholds. A brief acknowledgment of study limitations (e.g., spatial scope, temporal variability) and suggestions for future research would enhance the credibility of the discussion.	scope and temporal variability, has been included, along with suggestions for future research to strengthen the discussion
2.	- Ensure that each figure, table, or graph directly supports or illustrates a specific point discussed in the text. Avoid redundant visualizations or overlap in information. Ensure all visuals have clear, descriptive titles, legends, and axis labels. Labels should specify units (e.g., mg/kg) and be easily interpretable without needing to refer back to the text. Use consistent styles, colors, and formatting across all figures and tables to maintain a professional appearance and improve readability. - Ensure the graph accurately distinguishes heavy metal concentrations in sediments, roots, and leaves for each station. Use different colors or patterns for sediment, roots, and leaves, and provide a clear legend. - Where possible, integrate figures and tables to reduce redundancy. For example, a combined table and graph could present heavy metal concentrations and exceedance of regulatory thresholds.	I have revised the visuals to clearly support the text, with standard formatting, clear labels (including units), and adjusted colors. Redundancy has been minimized by integrating relevant images and tables where necessary.
3.	- The current references display inconsistencies in formatting, particularly in the presentation of journal names, volume and issue numbers, page ranges, and DOI links. Some journal names are abbreviated (e.g., Mar. Pollut.	I have revised the references according to the specific guidelines from IJMS

	Bull.), while others are spelled out (e.g., Marine Pollution Bulletin). The use of capitalization varies across references. DOI links are sometimes included and other times not, and their formatting is inconsistent. Publisher information is included for some books but not others. - Adopt a uniform referencing style throughout the References section. Common styles include APA, MLA, Chicago, or the specific style mandated by the Indonesian Journal of Marine Sciences (IJMS). IJMS has specific guidelines, ensure to comply with those. - Ensure that every reference is complete and free from typographical errors. Verify each reference against the original source to confirm accuracy. - Arrange references by the same author(s) in chronological order, starting with the earliest publication. Cross-check the References list against in-text citations to ensure completeness and accuracy.	
Section V: Ethical Considerations		
1.	There is no mention of ethical approval or permits for field sampling in the manuscript. The research does not mention any cultural or legal considerations, such as compliance with local regulations regarding natural resource use. Ensure the study explicitly states adherence to local and national laws governing environmental sampling and biodiversity research.	I confirm that all field sampling was conducted in accordance with local and national regulations. This study complies with ethical guidelines for environmental research. I will include a statement in the manuscript to clarify this.
2.	There is no explicit mention of conflicts of interest. Add a standard conflict of interest statement, such as: *"The authors declare no conflict of interest."*	A conflict of interest statement has been included in the manuscript to ensure transparency
Section VI: Recommendation		
1.	- There are areas where the clarity of the overall article presentation can be	The clarity of the article has been improved based on the suggested revisions in each

	improved as suggested in each section given earlier. - The article presents valuable findings on heavy metal accumulation and ecological risk in seagrass ecosystems. However, to enhance the clarity of the originality and novelty, refer to those comments and suggestions given in each section.	section. I have revised the text for better readability and coherence.

Rozirwan ROZIRWAN <rozirwan@unsri.ac.id>

[IK.IJMS] [ID-67097] Revised Version Acknowledgement

1 pesan

Ambariyanto <ijms@live.undip.ac.id> 17 Maret 2025 pukul 13.02
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Dr Rozirwan M.Sc. Rozirwan:

Thank you for submitting the revision of manuscript, "Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia" to ILMU KELAUTAN: Indonesian Journal of Marine Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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[IK.IJMS] Editor Decision

1 pesan

Indonesian Journal of Marine Science <ejournal@rumahjurnalundip.id> 3 April 2025 pukul 18.08
Balas Ke: Indonesian Journal of Marine Science <ijms.undip@gmail.com>
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Dear
Dr Rozirwan M.Sc. Rozirwan:

We have reached a decision regarding your submission to ILMU KELAUTAN: Indonesian Journal of Marine Sciences, "Heavy metal accumulation and ecological risk on seagrass Cymodocea and Thalassia in Pahawang Island, Indonesia".

Our decision is to:
Revision

Check your submission to have an updated revision,
Please make the necessary revisions to your manuscript and submit the revised version to us before April 18, 2025.
We will be awaiting your submission by this deadline.
Thank you for your attention to this matter.

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Heavy metal accumulation and ecological risk on seagrass *Cymodocea* and *Thalassia* in Pahawang Island, Indonesia

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Abstract

Seagrass species thrive in coastal ecosystems and known for their ability to accumulate heavy metals from their surrounding environment. This study aims to evaluate the ecological risks related to the accumulation of heavy metals in seagrass roots, leaves, and sediments. The seagrass examined belong to the genera *Cymodocea* and *Thalassia*, collected from two sites: Jeralangan and Cukuh Nyai on Pahawang Island, Lampung, Indonesia. The heavy metals analyzed included Pb, Cu, Ni, and Zn, which were measured using the wet desctruction method and quantified with a SHIMADZU AA-7000 Atomic Absorption Spectrophotometer (AAS). The ecological risk was evaluated through various indices, such as the Bioconcentration Factor (BCF), Translocation Factor

(TF), Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI). The highest concentrations of heavy metals in sediment were detected at station 1 was Zn (15.486 mg/kg). In the roots of *Cymodocea* was Zn (8.772 mg/kg), while the highest concentration in leaves was Cu in *Thalassia* (10.541 mg/kg). The ecological risk assessment revealed that BCF < 1 categorize an excluder, while TF > 1 for Pb and Zn indicate effective translocation from roots to leaves. Additionally, I_{geo} < 0 signify no contamination, Cf < 1 indicate low pollution levels, and PLI < 0 confirm a non-polluted status. In conclusion, the results show that the seagrass ecosystems at the study sites currently have low levels of heavy metal pollution and minimal ecological risk, suggesting they remain in a relatively safe condition.

Commented [A1]: $mg.kg^{-1}$

Keywords: Accumulation, *Cymodocea*, Ecological risk assessment, Heavy metal, *Thalassia*

Introduction

Heavy metals are elements with low molecular weight that can lead to serious environmental issues when accumulated at certain concentrations (Wang *et al.*, 2022a). Pollution from heavy metals due to tourism activities can create new problems, such as bioaccumulation and the transfer of pollutants through the food chain, a process known as biomagnification (Wang *et al.*, 2022b). Biomagnification can occur in various organism components, including seagrass. Seagrass can absorb and accumulate pollutants like heavy metals in its tissues, which enables it to serve effectively as a bioindicator (Nel *et al.*, 2023). Seagrass acts as a biomonitor and bioaccumulator of heavy metals because of its limited mobility, making it possible to estimate the concentrations and sources of heavy metals for analysis (Bonanno and Lo Giudice, 2010). Seagrass, which is found along the coast and in subtidal areas of Pahawang, is susceptible to direct impacts from the presence of heavy metals (Noor *et al.*, 2021).

Seagrass plays a crucial ecological role as an important habitat, providing abundant ecosystem services such as breeding grounds and food sources (Sun *et al.*, 2020). In recent decades, seagrass has faced ecological challenges due to significant contamination, particularly from heavy metals entering coastal areas (Tu *et al.*, 2023). Several studies have revealed the presence of heavy metals such as Pb, Cu, Zn, Cr, and Cd in various environments and organisms, including those in port areas, rivers, seagrass beds, and fish populations (Liu *et al*., 2019; Hosokawa *et al*., 2020; Fang *et al*., 2022; Souza-Araujo *et al*., 2022). Research on heavy metals in seagrass in other coastal regions has also shown that seagrass is vulnerable to accumulating all types of heavy metals, as observed in seagrass along the South China Sea (Zhang *et al*., 2024). Additionally, seagrass can accumulate heavy metals in its roots and leaves (Jeong *et al*., 2021). A study conducted in the central Gulf of Gabes (Southeast Tunisia) indicated that seagrass not only serves as a bioindicator but can also be an effective bioremediation tool (El Zrelli *et al*., 2023).

Seagrass can accumulate heavy metals in its roots and leaves (Jeong *et al*., 2021). Several previous studies have reported that heavy metal concentrations in seagrass roots and sediments are generally higher than in leaves, as observed in studies conducted in the Sydney estuaries, Australia, and Sicily, Italy (Birch *et al*., 2018; Bonanno and Borg, 2018; Bonanno *et al*., 2020). However, other studies have shown different results, such as research on seagrasses in the Southern Mediterranean Sea (El Zrelli *et al*., 2023). From these findings, a hypothesis can be formulated that heavy metals are generally concentrated in the lower parts of seagrasses, such as roots and sediments, reflecting limited mobility and high sediment deposition rates. Therefore, measuring heavy metal concentrations in various seagrass organs is necessary to test this hypothesis.

The occurrence of heavy metals and their negative effects on the ecological balance of seagrass, as previously discussed, serve as a primary motivation for this research. The seagrass genus *Cymodocea* and *Thalassia* were chosen due to their dominance in the research sites. Previous studies have shown that these seagrasses have been investigated for heavy metal presence in their tissues, exhibiting relatively high levels of accumulation in various regions, including Italy, South China, India, and Florida (Bonanno *et al*., 2020; Gopi *et al*., 2020; Zhang *et al*., 2021). Indonesia, research on heavy metal accumulation in *Thalassia* and *Cymodocea* has been conducted in the Seribu Islands, showing that these two seagrass species can accumulate heavy metals such as Pb, Cd, and Hg (Bowo, 2013; Sofhia, 2019; Triyanto *et al.,* 2024). However, there has been no investigation using these seagrasses to monitor heavy metal pollution on Pahawang Island.

Research on the presence of heavy metals on Pahawang Island has never been conducted, but research in the bay area adjacent to Pahawang Island (Pesawaran Chain Bay Coast) has been conducted, where the research results found heavy metal content above the quality standard in Cr of 415.86 $mg.kg^{-1}$, Cu of 163.312 $mg.kg^{-1}$, Fe of 3,339.89 $mg.kg^{-1}$ in marine organism (Fitrianingsih and Widiastuti, 2021).

Pahawang Island in Lampung Province is a popular marine tourism destination with diverse ecosystems, including coral reefs, seagrass beds, and mangrove forests especially significant populations of *Cymodocea* and *Thalassia* (Mardani *et al*., 2018; Novita *et al*., 2022). According to the Central Bureau of Statistics of Pesawaran Regency, tourist visits reached 448,008 in 2019 and decreased to approximately 165,342 in 2020 (BPS Statistic, 2023). The high boat activity, as the only means of transportation to the island, potentially contributes to heavy metal pollution in the (Moelyaningrum, 2017; Saraswati and Rachmadiarti, 2021; Swain *et al*. 2021).

Therefore, this study aims to provide valuable insights into the bioaccumulation patterns of heavy metals in the roots and leaves of seagrass, while also identifying the ecological risks involved. These insights can assist in developing management strategies for coastal areas and ecosystems in the region. The primary objective of this research is to analyze the ecological risks associated with the accumulation of heavy metals (Pb, Cu, Ni, and Zn) in the roots and leaves of *Cymodocea* and *Thalassia*, as well as in the sediments of Pahawang Island.

Materials and Methods

Study area and sampling

This research conducted in March 2024 off the coast of Pahawang Island in Lampung, Indonesia, at two distinct sampling locations. The sites were selected using purposive sampling within the seagrass ecosystem in Jeralangan and Cukuh Nyai (Figure 1). The first location, Jeralangan at coordinates 5°40'50.53"S and 105°13'51.15"E, is characterized by a high volume of tourism activities. The second location, Cukuh Nyai at coordinates 5°41'19.76"S and 105°13'9.28"E, has relatively low tourism activity. In addition, this location has a different residential area where the first location has a denser settlement than the second location. Tourism and residential activities can cause heavy metal pollutants to enter the waters (Sunaryo *et al*., 2013; Purwiyanto *et al*., 2020). Sampling was carried out during low tide, namely in the morning and evening.

Two types of samples were collected at each station, consisting of seagrass and sediment. At Station One, samples of *Cymodocea* seagrass and sediment were collected, while Station Two involved the collection of *Thalassia* seagrass and sediment. The identification of seagrass species was conducted using a field guide for seagrass and dugong monitoring (Herandarudewi *et al*., 2019). he collected seagrass samples included both leaves and roots, gathered from shallow water habitats or intertidal zones through random sampling (Trevathan-Tackett *et al*., 2015; Singh *et al*., 2021). Sediment samples were taken using a grab sampler, with a total weight of 1 kg from the surface layer (0-10 cm). The collected samples were placed in clip-lock plastic bags and stored in containers (Xiao *et al*., 2022).

The results of this study indicate a pattern of heavy metal accumulation in seagrass at two locations with different anthropogenic pressures. While these findings provide valuable insights, it is important to note that the spatial coverage of this study is limited. Therefore, further investigation in other seagrass ecosystems is needed to determine the extent to which the observed patterns can be extrapolated to other areas with similar conditions. Additional studies with broader spatial coverage, encompassing various levels of environmental pressure and different oceanographic characteristics, will help strengthen the generalization of these findings.

Heavy metals analysis

The seagrass leaf and root samples, along with sediment, were thoroughly cleaned and subsequently dried. The dried samples were then ground to a fine powder and stored in sealed containers (Stewart *et al*., 2021). Before the destruction, AAS calibration is carried out based on the Indonesian National Standard to ensure the accuracy and precision of heavy metal concentration measurements. The calibration process consists of the preparation of heavy metal standard solutions made by diluting certified stock solutions to various concentrations that cover the expected measurement range in the sample. These standards are used to create a calibration curve (Badan Standarisasi Nasional, 2004, 2009).

The sample digestion was carried out using the wet digestion method, where 0.5 g of powdered seagrass was mixed with 5 ml of a reagent in a ratio of 5:2:1 (65% nitric acid: concentrated perchloric acid: sulfuric acid) on a hot plate for 10 minutes, along with an additional 5 ml of 2N hydrochloric acid, and heated on the hot plate for another 10 minutes. After reaching acid digestion, the sample was cooled and then filtered using Whatman filter paper (0.45 μm). The filtrate was then transferred into a 25 ml volumetric flask and diluted with deionized water up to the mark (FAO/SIDA, 1983; European Commission, 2011). For sediment samples, the wet digestion process involved adding 5 ml of concentrated HNO_3 to each sample, which included 50 ml of water sample and approximately 3 g of sediment sample. The sample was heated on a hot plate at a temperature of 105°C – 120°C until the volume was reduced to approximately 10 ml. After cooling, 5 ml of HNO_3 and 1-3 ml of perchloric acid were added, and the sample was reheated until white fumes appeared, followed by further heating for approximately 30 minutes. The sample was then filtered using quantitative filter paper with a pore size of 8.0 μm. The filtrate was transferred into a 100 ml volumetric flask and diluted with distilled water up to the mark (Badan Standarisasi Nasional, 2009; Gao *et al* ., 2021). The heavy metal analysis for each sample was performed in three replicates. The heavy metals analyzed in this study included Pb, Cu, Ni, and Zn.

The concentration of heavy metals in the samples was measured using an Atomic Absorption Spectrophotometer (AAS) SHIMADZU AA-7000The wet digestion method is used because it is more effective in extracting heavy metals and preventing the loss of volatile elements such as Pb (Alfaro *et al* ., 2015). However, if not performed carefully, there is a possibility of contamination from reagents or laboratory equipment that may affect the results. Therefore, quality assurance and quality control are carried out by ensuring the use of contaminant-free glassware, pure quality chemicals (p.a), and calibrated and verified atomic absorption spectrophotometers, operated by competent analysts. Quality control includes linearity of the calibration curve ($r^2 \geq 0.99$), blank analysis to control contamination with lead levels below the detection limit, and triplicate preparation to ensure accuracy with a difference in results of ≤ 20% (Badan Standarisasi Nasional, 2004, 2009).

Ecological risk assessment

Ecological risk assessment can be conducted by calculating the bioconcentration factor (BCF), translocation factors (TF), geoaccumulation index (I_{geo}), contamination factor (Cf), and pollution load index (PLI) (Hakanson, 1980; Rozirwan *et al* ., 2023).

Bioconcentration factor (BCF)

he accumulation of heavy metals in the roots and leaves of seagrass from the surrounding sediment can be assessed using the Bioconcentration Factor (BCF) as described below (Steingräber *et al* ., 2022).

$$BCF = \frac{concentration\ of\ organs}{concentration\ of\ sediment} \quad (1)$$

where:

BCF < 1 is an excluder; The organism (usually a plant) does not accumulate the substance efficiently.

BCF = 1 is an indicator; The organism takes up the substance at the same rate as it is found in the environment.

BCF = 1 is a hyperaccumulator; The organism accumulates the substance at a much higher concentration than in the environment (Almahasheer, 2019).

Translocation Factors (TF)

The Translocation Factor (TF) is used to measure the movement of heavy metals from the roots to the leaves (Usman *et al* ., 2019).

$$TF = \frac{BCF\ leaf}{BCF\ of\ root} \quad (2)$$

TF Value > 1 indicates that the sample functions as a phytoextractor, effectively translocating metals from the roots to the leaves, whereas TF value < 1 indicates that the sample functions as a phytostabilizer (Dinu *et al* ., 2020).

Geoaccumulation index (I_{geo})

The Geoaccumulation Index (I_{geo}) is used to evaluate the degree of heavy metal contamination and classify pollution levels according to established criteria (Rozirwan *et al* ., 2023).

$$Igeo = Log\ 2\left(\frac{concentration\ of\ sediment}{1{,}5\ background}\right) \quad (3)$$

where: the I_{geo} value classification consists of not contaminated ($I_{geo} \leq 0$), not contaminated to moderately contaminated (I_{geo}0–1), moderately contaminated (I_{geo}1–2), moderate to highly contaminated (I_{geo}2–3), highly contaminated (I_{geo}3–4), highly contaminated to very high (I_{geo} 4–5), and highly contaminated (I_{geo}≥5).

Background concentration (Alfaro *et al* ., 2015).

Contamination factor (Cf)

The Contamination Factor (CF) value is used as a basis for determining the degree of heavy metal contamination (Rozirwan *et al* ., 2024).

$$CF = \frac{concentration\ of\ sediment}{Background} \quad (4)$$

where: contamination factor criteria according to (Shaheen *et al* ., 2019); Cf < 1 = low level of contamination; 1 < Cf < 3 = moderate level of contamination; 3 < Cf < 6 = moderate level of contamination; Cf > 6 = very high level of contamination.

Pollution load index (PLI)

The Pollution Load Index (PLI) is used to evaluate the pollution quality (Mosa *et al* ., 2022).

$$PLI = [Cf1\ x\ Cf2\ x\ Cf3 \ldots .\ x\ Cfn]^{1/n} \quad (5)$$

where: pollution load index criteria: PLI 2 = not polluted to lightly polluted; PLI 24 = moderately polluted; PLI 4–6 = heavily polluted; PLI 6–8 = heavily polluted; PLI 8–10 = heavily polluted

Statistical Analysis

The concentrations of heavy metals were analyzed using the Mann-Whitney test in SPSS, as the data were not normally distributed. This statistical test was applied to compare the concentrations of these metals between the two study sites based on the mean differences in the samples. The significance level (α) was set at 0.05 to determine statistical differences. If the 2-tailed significance value is greater than 0.05, it indicates no significant difference in the mean concentration between the two sites (H_0 is accepted). Conversely, if the 2-tailed significance value is less than 0.05, it suggests a significant difference in the mean concentrations (H_1 is rejected).

Result and Discussion

Description of seagrass

The seagrass samples were collected based on the dominant species found at the research site, specifically *Cymodocea* and *Thalassia* (Figure 2). *Cymodocea* was predominantly observed at Station One. This species is characterized by long, ribbon-like leaves that taper to a rounded tip, white to slightly yellowish rhizomes, and thin, brown fibrous roots. Both *Cymodocea* and *Thalassia* are commonly found in substrates ranging from muddy to sandy (Hartati *et al* ., 2018; Thangaradjou and Bhatt, 2018), which explains their prevalence in the research area where the majority of locations feature such substrates. *Cymodocea* exhibits a strong tolerance to various environmental stresses and can thrive in diverse habitats, categorizing it as a species of least concern according to the IUCN (Terrados *et al* ., 1998; Short *et al* ., 2011). *Thalassia* is mainly found at Station Two and is recognized by its crescent-shaped leaves with neatly arranged margins, brown rhizomes, and thick, sheathed roots. This species allocates a greater proportion of its biomass to underground structures, such as roots and rhizomes, rather than to its leaves. This morphological variation can be seen as an adaptive response to environmental physical stressors, such as burial by sand to prevent displacement during tidal fluctuations and storms (Westlake *et al* ., 2022).

The root structure of Thalassia plays an important role in the absorption of heavy metals in the marine environment. Thalassia has an extensive root and rhizome system, allowing greater contact with sediments containing heavy metals (Rosalina *et al* ., 2022). Exposure to heavy metals can cause changes in the structure of root tissue, such as thickening or damage to the epidermis and endodermis in both seagrass roots. These changes can affect the ability of roots to absorb and translocate heavy metals (Rosalina *et al* ., 2019). Thalassia hemprichii and Cymodocea serrulata leaves play an important role in the absorption of heavy metals from the water column. The broad and thin leaf structure increases the surface area for direct contact with water, facilitating the absorption of heavy metals (Nugraha, 2016). Additionally, the presence of aerenchyma tissue in the roots and leaves enables efficient gas exchange, which can influence the process of heavy metal absorption (Chedadi *et al* ., 2024). According to Dilipan and Arulbalachandran (2022), both *Cymodocea* and *Thalassia* are promising candidates for bioremediation of contaminated sites and are frequently utilized as biological indicators of heavy metal accumulation.

Heavy metals concentration in sediment and seagrass

The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments, roots, and leaves of seagrasses at both stations are summarized in Figure 3. The results indicate that Station 1 (*Cymodocea*) had the highest concentrations of Pb and Zn in the sediments, roots, and leaves. Specifically, the maximum concentrations of Cu in sediments and roots were also recorded at Station 1 (*Cymodocea*), while the highest concentration in leaves was observed at Station 2 (*Thalassia*). The highest levels of Ni were found in the leaves and roots of *Cymodocea*. In contrast, the sediment at Station 2 exhibited the highest concentration of Ni. A comparative analysis of heavy metal concentrations across different seagrass species and locations is summarized in Figure 3. Based on the research results, the concentration of each sample between the two stations was analyzed using the Mann-Whitney test (as the data were not normally distributed) and showed a 2-tailed significance value of > 0.05, indicating no significant difference between the two stations.

Based on Figure 3, the concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments of this study are still below the threshold values established by ANZECC and ARMCANZ (2000) as referenced *in* (Ogundele *et al* ., 2015), and by the Gazzetta Ufficiale della Repubblica Italiana as noted *in* (Bonanno *et al* ., 2020), which are set at 50 mg/kg, 65 mg/kg, 50 mg/kg, and 30 mg/kg, respectively. Station 1 is characterized by a predominant presence of *Cymodocea*, which is directly influenced by tidal dynamics. Tidal-affected areas experience dynamic water movement, enabling heavy metals carried by the water to adhere to sediment particles (Maulana *et al* ., 2023). The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments are higher compared to those in the roots and leaves, corroborating the findings of Khotimah *et al* ., (2024) which indicate that sediment concentrations of heavy metals exceed those in the roots, implying that the roots may serve as a barrier preventing heavy metals from translocating to the upper plant structures. As noted by Flefel *et al* ., (2020), sediments act as primary reservoirs for heavy metal absorption in aquatic environments. In addition, the higher concentration of heavy metals in the sediment at Station 1 compared to Station 2 can be assumed to be due to the finer sediment texture. Heavy metals tend to bind more effectively to sediment types with higher clay content or finer particle sizes (Borah *et al* ., 2018).

The concentrations of heavy metal Pb in the sediments, leaves, and roots of *Cymodocea* and *Thalassia* differ between the two research locations (figure 3). The higher concentration of Pb in *Cymodocea* at Station 1 may be attributed to the significant human activities in this area, including increased boat traffic and residential developments. Heavy metal Pb levels can be exacerbated by factors such as maritime traffic, fishing activities, paint on boats, and sedimentation (Irandoost *et al* ., 2021). Mitigation measures that can be implemented include strengthening regulations and waste treatment systems around coastal areas, as well as conducting long-term monitoring to identify pollution trends and their impacts on the seagrass ecosystem (Jeong *et al* ., 2021). Notably, the highest concentration of Pb is found in the leaves of *Cymodocea* when compared to its roots (figure 3). Similar findings were reported by El Zrelli *et al* ., (2023), which indicated that the Pb concentration in the leaves of *Cymodocea* is greater than that in its roots. According to the WHO (1996) as cited in Ogundele *et al* ., (2015), the concentration of Pb in *Cymodocea* and *Thalassia* remains below the quality standard of 2 mg/kg (Pb), suggesting that these seagrasses are not currently subjected to contamination.

In this study, the highest concentration of the heavy metal Cu was observed in the leaves of *Thalassia* at Station 2. This concentration is still below the quality standard of 20 mg/kg (Kabata-Pendias, 2011) indicating that the accumulation of Cu in seagrass does not pose any toxic effects. Copper is considered an essential metal, as it is required by organisms; however, at significantly high concentrations, Cu can be more toxic than non-essential metals such as Pb and Cd due to the active absorption mechanisms and tolerance strategies that plants employ A similar study conducted on the leaves and roots of *Thalassia* in Xincun Bay, China, also reported the highest concentration in leaves at 11.2 mg/kg (Zhang *et al*., 2021). Previous research on various seagrass species has demonstrated that aboveground leaves are more efficient at accumulating Cu compared to belowground tissues (roots and rhizomes) (Lin *et al*., 2018; Hu *et al*., 2019). The concentration of heavy metal Cu in the leaves of *Thalassia* is higher than in *Cymodocea*. This may be due to *Thalassia* having broader leaf morphology and often exhibiting a slower growth rate compared to *Cymodocea*, allowing more heavy metals to accumulate in leaf tissues over a longer period (Nugraha, 2016). Additionally, different physiological mechanisms in each seagrass species may influence the uptake of heavy metals through the leaf surface (El Zrelli *et al*., 2023). Previous studies on these two seagrass species, particularly in Indonesia, have shown that Cu concentrations in *Thalassia* leaves are higher than in *Cymodocea* (Bidayani *et al*., 2017; Kholil *et al*., 2019).

The concentration of Ni based on the research findings indicates that the highest level was found in the sediment, followed by the roots of *Cymodocea*, measuring 2.331 $mg.kg^{-1}$. This concentration is significantly lower than the legal limit allowed in marine waters, which is set at 20 $mg.kg^{-1}$. Furthermore, increases in Ni concentrations of 3 to 5 times are generally observed in environments impacted by human activities (Bonanno *et al*., 2020). The Ni concentrations in this study are consistent with previous research that reported low Ni levels in seagrasses, such as 3.20–6.65 $mg.kg^{-1}$ in Italy (Bonanno *et al*., 2017) and 1.8–3.1 $mg.kg^{-1}$ in South Africa (Nel *et al*., 2023), , particularly in locations with minimal human activities. This suggests that the study site is not yet contaminated with heavy metals, specifically Ni.

According to Figure 3, the highest concentration of Zn in seagrass was found in the leaves of *Cymodocea* at station 1. This concentration remains below the threshold established by the Australia and New Zealand Food Authority (ANZFA), which is 14 $mg.kg^{-1}$ (Smitha *et al*., 2010). Furthermore, the measured Zn levels are significantly lower than the concentrations that can cause phytotoxicity, which range from 500-1500 $mg.kg^{-1}$(Elrashidi, 1990). Thus, the concentration of Zn detected in this study does not indicate any toxic effects. The results demonstrate that the concentrations of Zn in both the leaves and roots of the two seagrass species are higher than those of Pb and Cu (Figure 3). This suggests that Zn plays a crucial role in plant metabolism (Bonanno and Orlando-Bonaca, 2017). These findings are consistent with previous research, which reported that Zn concentrations are highest in the roots and leaves of various seagrass species, including *Cymodocea* and *Thalassia*, in locations such as Sicily, Italy, China, and India (Bonanno *et al*., 2017; Hu *et al*., 2019; Gopi *et al*., 2020). After knowing the concentration of heavy metals in seagrass and sediment, the next step is to evaluate its ecological impact. Therefore, an ecological risk assessment is needed to assess the potential threat to seagrass ecosystems and organisms that depend on them.

Bioaccumulation and Ecological risk assessments

Based on the research results presented in Table 2, the bioconcentration factors (BCF) for the roots of *Cymodocea* and *Thalassia*, which accumulate heavy metals from the sediment biologically, were determined to be 0.0828 and 0.0502 for Pb, respectively. The BCF for Cu in *Cymodocea* is 0.5991, while in *Thalassia* it is 0.0082. For Ni, the BCF values are 0.3140 for *Cymodocea* and 0.0894 for *Thalassia*, and for Zn, the BCF values are 0.5665 and 0.1670, respectively. The geoaccumulation index indicates an unpolluted status for Pb with values of -1.9696 and -1.9344, for Cu with values of -5.0658 and -4.5659, for Ni with values of -2.2167 and -2.1581, and for Zn with values of -2.6982 and -2.8687. The contamination factor (CF) suggests low contamination levels for Pb at 0.3830 and 0.3924, for Cu at 0.0448 and not detected (nd), for Ni at 0.3227 and 0.3361, and for Zn at 0.2311 and 0.2055. The pollution load index (PLI) was calculated, yielding results of 0.0013 and 0.0051, indicating that both stations are not polluted.

There are differences in the translocation factor (TF) values of metals (Table 3) between *Cymodocea* and *Thalassia*. In *Thalassia*, the TF for Cu indicates a translocation value of <1, suggesting minimal transfer of this metal to the above-ground biomass or leaves. This can also be attributed to the very low concentrations of Cu in the roots and leaves of the sampled seagrass at the study site. The metal with the highest TF value is Zn in *Thalassia*, followed by Ni and Pb. Similarly, *Cymodocea* shows the highest TF values for Zn and Pb, which are both >1. The differences in translocation values may arise from the biological characteristics of seagrasses, which have developed two distinct adaptive strategies to cope with the levels of contamination in their environment. The first strategy involves the storage of accumulated metals from the sediment in the root-rhizome system, while the second strategy entails the translocation of some contaminants from the root-rhizome to the above-ground parts of the seagrass, such as the leaves (El Zrelli *et al*., 2023). According to the TF values presented in Table 2, *Thalassia* employs the second adaptive strategy for Zn, Ni, and Pb by translocating these contaminants to the leaves, whereas for Cu, it utilizes the first adaptive strategy. In contrast, *Cymodocea* adopts the second adaptive strategy for Pb and Zn. Previous research has also indicated that Zn undergoes significant translocation in *Cymodocea*, with a value > 1 (Bonanno and Borg, 2018; Nel *et al*., 2023).

The bioconcentration factor (BCF) of heavy metals from sediment to leaves and roots is summarized in Table 2. The BCF of zinc (Zn) in the leaves is higher than that of lead (Pb), nickel (Ni), and copper (Cu). This indicates that the accumulation level of Zn in the leaves is greater compared to the other metals, which may be influenced by several factors, including the adaptive strategies of seagrass in absorbing heavy metals and the chemical properties of the metals as well as the selectivity of the plants (Khotimah *et al*., 2024). Zinc is an essential metal for seagrass, as it is utilized in photosynthesis and growth (Kabata-Pendias and Pendias, 2000). This leads to higher Zn accumulation since it is more readily absorbed by both *Cymodocea* and *Thalassia*. According to Table 2, *Cymodocea* exhibits the highest BCF value for Zn. Similarly, high BCF values for Zn have also been found in the leaves of *Zostera noltei*, as well as in the roots and leaves of *Thalassia hemprichii* and in the roots and leaves of *Enhalus acoroides* (Li and Huang, 2012; Boutahar *et al*., 2019; Jeong *et al*., 2021). Compared to previous studies, particularly in Southeast Asia, such as research conducted in the Johor Strait, Malaysia, the accumulation of heavy metals in seagrasses and their ecological risks show a similar pattern in the accumulation of Pb and Cu, with varying levels of pollution depending on proximity to pollution sources (Sidi *et al*., 2018). A comparison of heavy metal accumulation in seagrasses from different locations worldwide is presented in Table 1.

The values of the geoaccumulation index (I_{geo}) from this study are presented in Table 4, indicating that the sediments at both research locations are unpolluted (I_{geo}< 0). This suggests that the sources of heavy metal contamination at these sites are low, attributed to factors such as small residential areas, the absence of industrial and mining activities, and relatively low shipping traffic. Additionally, the physical and chemical conditions of the aquatic environment, including current patterns and tidal movements, also play a significant role (Siregar *et al*., 2020). The contamination factor (CF) in this study indicates that each metal contributes to low or negligible contamination, as evidenced by CF values < 1. These findings align with research conducted along the southern coast of Sumatra, Indonesia, which showed low CF values for each metal, except for lead (Pb), due to significant anthropogenic sources in the vicinity (Rozirwan *et al*., 2023; Khotimah *et al*., 2024).

The pollution status of the seagrass ecosystem at the research locations can be assessed using the pollution load index (PLI). The sediment PLI analyzed at the study sites is classified as unpolluted, with PLI values ranging from 0 to 2. The PLI values at both locations are not significantly different, indicating that both ecosystems are healthy and safe for living organisms. In contrast, other studies on seagrass ecosystems in the Southern Mediterranean Sea reported PLI values categorized as moderately contaminated and polluted, posing risks to the seagrass ecosystems, primarily due to the discharge of various industrial wastes, particularly phosphogypsum (El Zrelli *et al*., 2023). Therefore, based on the analyses from this study, it can be concluded that all sampling locations exhibit low levels of metal pollution and ecological risk. The results of this study are limited to the surveyed location and may not fully reflect conditions in other areas.

The results of this study provide new insights into how tropical seagrasses, particularly *Cymodocea* and *Thalassia*, play a role in accumulating heavy metals in the marine environment. These findings contribute to understanding the distribution patterns of heavy metals in seagrass ecosystems, which can be used to monitor water quality. They also help identify seagrass species that are more effective as bioindicators of heavy metal pollution. Furthermore, this study provides a scientific basis for environmental management policies, such as the protection of seagrass beds in areas with high anthropogenic activity. However, this study is limited in its spatial coverage and does not account for broader temporal variability. Therefore, further research with a wider study area and long-term monitoring is necessary to gain a more comprehensive understanding of heavy metal accumulation patterns and to identify potential changes over time.

Conclusion

The highest concentrations of Pb, Cu, Ni, and Zn in seagrass are found in the leaves, while the sediments at Station 1 show the highest levels. Overall, the concentrations of heavy metals in the roots and leaves of *Cymodocea* and *Thalassia* indicate relatively high levels; however, the ecological risk assessment shows that the bioaccumulation factor (BCF) is categorized as low. In contrast, the translocation factors (TF) for Pb and Zn demonstrate values greater than 1, indicating that the seagrass functions effectively in phytoremediation or translocating metals from the roots to the leaves. In summary, the ecological risk assessment of the research locations, which includes the geoaccumulation index (Igeo), contamination factor (CF), and pollution load index (PLI), indicates that the environment within the seagrass ecosystems at these sites has low levels of heavy metal pollution and ecological risk. The findings of this study are specific to the examined site and may not be entirely representative of conditions in other regions.

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~~Ethical Approval and Research Integrity~~

~~All field sampling activities were conducted in accordance with local and national regulations governing environmental research and biodiversity conservation. This study also adhered to ethical guidelines to ensure responsible and sustainable sampling practices.~~

~~Conflict of interest~~

~~The authors declare that they have no conflicts of interest.~~

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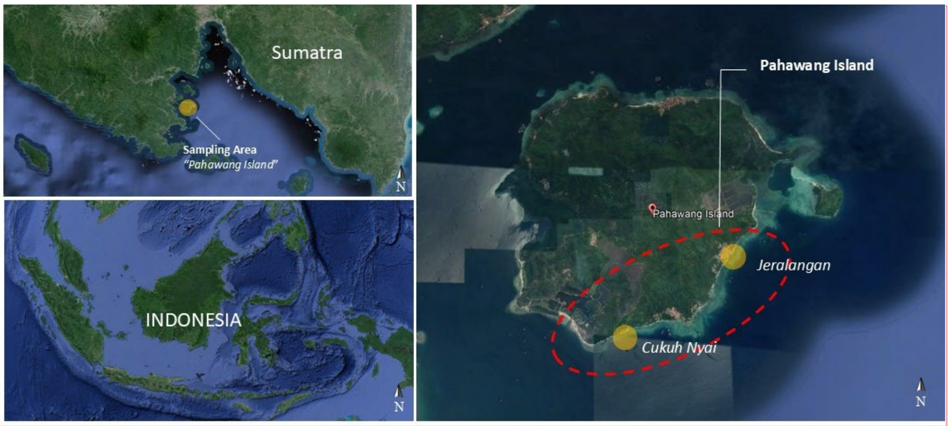
Figure 1. A map of seagrass sampling location



Commented [A3]: You need to put sign of sampling location on Indonesian map

You should also put all elements required in a map like this.

Figure 2. Seagrass, (a) *Cymodocea*, (b) *Thalassia*

(a) (b)

Commented [A4]: Where these seagrasses taken from? Put in the legend

Figure 3. Concentrations of Pb, Cu, Ni and Zn in organs of *Cymodocea* and *Thalassia* (leaf and root mg/kg), and associated sediments (mg/kg)

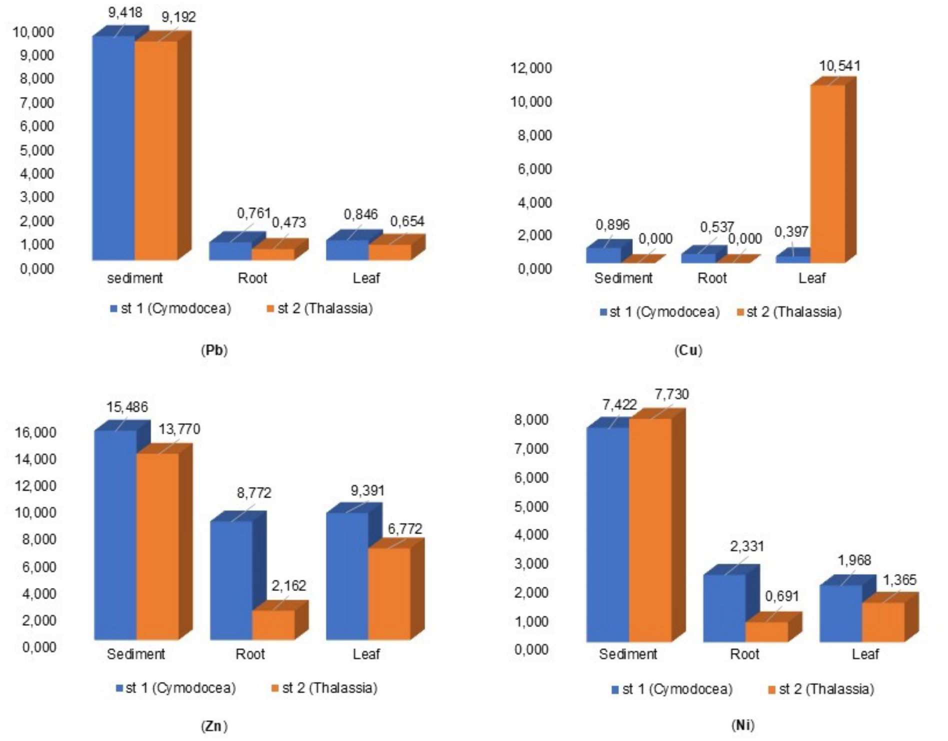


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Table 1. Comparison of heavy metal concentrations in *Cymodocea* and *Thalassia* with those from the open literatures

Location	Seagrass	Tissue	Heavy metals (mg/kg)	References
Sicily (Italy)	*Cymodocea*	Leaf	3.61–28.8 (Cu) 3.20–6.65 (Ni) 1.42–3.85 (Pb) 42.6–65.8 (Zn)	(Bonanno *et al*., 2017)
Sicily (Italy)	*Cymodocea*	Leaf	3.90–19.5 (Cu) 2.40–22.5 (Ni) 1.52–6.10 (Pb) 4.21–13.9 (Zn)	(Bonanno *et al*., 2020)
Sicily (Italy)	*Cymodocea*	Rhizome	10.6 (Cu 1.15 (Ni) 0.73 (Pb) 21.7 (Zn)	(Bonanno and Borg, 2018)
		Root	8.83 (Cu) 4.79 (Ni) 4.81 (Pb) 38.7 (Zn)	
Florida, USA	*Thalassia*	Rhizome+root+leaf	20.23 (Pb) 9.55 (Cu) 86.68 (Zn) 1.56 (Cd)	(Smith *et al*., 2019)
Palk Bay, South Eastern India	*Cymodocea*	Rhizome+root +leaf	1.23 (Cd) 18.59 (Cu) 1.74 (Pb) 34.63 (Zn)	(Gopi *et al*., 2020)
Xincun Bay,China	*Thalassia*	Rhizome+root +leaf	11.2 (Cu) 4.05 (Ni) 2.1 (Pb) 39.1 (Zn)	(Zhang *et al*., 2021)
Southern Mediterranean Sea	*Cymodocea*	Rhizome	0.99 (Cu) 0.26 (Pb) 55 (Zn) 0.45 (Cd)	(El Zrelli *et al*., 2023)
		Root	3.51 (Cu) 3.76 (Pb) 31 (Zn) 1.33 (Cd)	
		Leaf	3.95 (Cu) 1.74 (Pb) 131.33 (Zn) 2.40 (Cd)	

Commented [A6]: The results of this study should be put in the table as well.

Table 2. Values of the Bioconcentration factor (BCF)

	BCF			
	Pb	Cu	Ni	Zn
Seagrass roots				
St 1 (*Cymodoceae*)	0.0804	0.5991	0.3140	0.5665
St 2 (*Thalassia*)	0.0518	0.0082	0.0894	0.1570
Seagrass leaves				
St 1 (*Cymodoceae*)	0.0893	0.4436	0.2652	0.6064
St 2 (*Thalassia*)	0.0716	-8.3217	0.1766	0.4918

Table 3. The translocation factor (TF) from the roots to the leaves of seagrass

	TF			
	Pb	Cu	Ni	Zn
St 1 (*Cymodoceae*)	1.1112	0.7405	0.8445	1.0705
St 2 (*Thalassia*)	1.3834	-1011.6867	1.9764	3.1328

Table 4. Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI)

	Igeo				CF				PLI
	Pb	Cu	Ni	Zn	Pb	Cu	Ni	Zn	
St 1	-1.9696	-5.0658	-2.2167	-2.6982	0.3830	0.0448	0.3227	0.2311	0.0013
St 2	-1.9344	-4.5659	-2.1581	-2.8676	0.3924	nd	0.3361	0.2055	0.0051

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Heavy Metal Accumulation and Ecological Risk on Seagrass *Cymodocea* and *Thalassia* in Pahawang Island, Indonesia

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Abstract

Seagrass species thrive in coastal ecosystems and known for their ability to accumulate heavy metals from their surrounding environment. This study aims to evaluate the ecological risks related to the accumulation of heavy metals in seagrass roots, leaves, and sediments. The seagrass examined belong to the genera Cymodocea and Thalassia, collected from two sites: Jeralangan and Cukuh Nyai on Pahawang Island, Lampung, Indonesia. The heavy metals analyzed included Pb, Cu, Ni, and Zn, which were measured using the wet destruction method and quantified with a SHIMADZU AA-7000 Atomic Absorption Spectrophotometer (AAS). The ecological risk was evaluated through various indices, such as the Bioconcentration Factor (BCF), Translocation Factor (TF), Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI). The highest concentrations of heavy metals in sediment were detected at station 1 was Zn (15.486 $mg.kg^{-1}$). In the roots of Cymodocea was Zn (8.772 $mg.kg^{-1}$), while the highest concentration in leaves was Cu in Thalassia (10.541 $mg.kg^{-1}$). The ecological risk assessment revealed that BCF < 1 categorize an excluder, while TF > 1 for Pb and Zn indicate effective translocation from roots to leaves. Additionally, I_{geo} < 0 signify no contamination, Cf < 1 indicate low pollution levels, and PLI < 0 confirm a non-polluted status. In conclusion, the results show that the seagrass ecosystems at the study sites currently have low levels of heavy metal pollution and minimal ecological risk, suggesting they remain in a relatively safe condition.

Keywords: *Accumulation, Cymodocea, Ecological risk assessment, Heavy metal, Thalassia*

Introduction

Heavy metals are elements with low molecular weight that can lead to serious environmental issues when accumulated at certain concentrations (Wang *et al*., 2022a). Pollution from heavy metals due to tourism activities can create new problems, such as bioaccumulation and the transfer of pollutants through the food chain, a process known as biomagnification (Wang *et al*., 2022b). Biomagnification can occur in various organism components, including seagrass. Seagrass can absorb and accumulate pollutants like heavy metals in its tissues, which enables it to serve effectively as a bioindicator (Nel *et al*., 2023). Seagrass acts as a biomonitor and bioaccumulator of heavy metals because of its limited mobility, making it possible to estimate the concentrations and sources of heavy metals for analysis (Bonanno and Lo Giudice, 2010). Seagrass, which is found along the coast and in subtidal areas of Pahawang, is susceptible to direct impacts from the presence of heavy metals (Noor *et al*., 2021).

Seagrass plays a crucial ecological role as an important habitat, providing abundant ecosystem services such as breeding grounds and food sources (Sun *et al*., 2020; Rozirwan *et al*., 2025a). In recent decades, seagrass has faced ecological challenges due to significant contamination, particularly from heavy metals entering coastal areas (Tu *et al*., 2023). Several studies have revealed the presence of heavy metals such as Pb, Cu, Zn, Cr, and Cd in various environments and organisms, including those in port areas, rivers, seagrass beds, and fish populations (Liu *et al*., 2019; Hosokawa *et al*., 2020; Fang *et al*., 2022; Souza-Araujo *et al*., 2022). Research on heavy metals in seagrass in other coastal regions has also shown that seagrass is vulnerable to accumulating all types of heavy metals, as observed in seagrass along the South China Sea (Zhang *et al*., 2024). Additionally, seagrass can accumulate heavy metals in its roots and leaves (Jeong *et al*., 2021). A study

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conducted in the central Gulf of Gabes (Southeast Tunisia) indicated that seagrass not only serves as a bioindicator but can also be an effective bioremediation tool (El Zrelli *et al*., 2023).

Seagrass and other plants can accumulate heavy metals in its roots and leaves (Jeong *et al*., 2021; Rozirwan *et al*., 2025b). Several previous studies have reported that heavy metal concentrations in seagrass roots and sediments are generally higher than in leaves, as observed in studies conducted in the Sydney estuaries, Australia, and Sicily, Italy (Birch *et al*., 2018; Bonanno and Borg, 2018; Bonanno *et al*., 2020). However, other studies have shown different results, such as research on seagrasses in the Southern Mediterranean Sea (El Zrelli *et al*., 2023). From these findings, a hypothesis can be formulated that heavy metals are generally concentrated in the lower parts of seagrasses, such as roots and sediments, reflecting limited mobility and high sediment deposition rates. Therefore, measuring heavy metal concentrations in various seagrass organs is necessary to test this hypothesis.

The occurrence of heavy metals and their negative effects on the ecological balance of seagrass, as previously discussed, serve as a primary motivation for this research. The seagrass genus *Cymodocea* and *Thalassia* were chosen due to their dominance in the research sites. Previous studies have shown that these seagrasses have been investigated for heavy metal presence in their tissues, exhibiting relatively high levels of accumulation in various regions, including Italy, South China, India, and Florida (Bonanno *et al*., 2020; Gopi *et al*., 2020; Zhang *et al*., 2021). Indonesia, research on heavy metal accumulation in *Thalassia* and *Cymodocea* has been conducted in the Seribu Islands, showing that these two seagrass species can accumulate heavy metals such as Pb, Cd, and Hg (Triyanto *et al.,* 2024). However, there has been no investigation using these seagrasses to monitor heavy metal pollution on Pahawang Island. Research on the presence of heavy metals on Pahawang Island has never been conducted, but research in the bay area adjacent to Pahawang Island (Pesawaran Chain Bay Coast) has been conducted, where the research results found heavy metal content above the quality standard in Cr of 415.86 $mg.kg^{-1}$, Cu of 163.312 $mg.kg^{-1}$, Fe of 3,339.89 $mg.kg^{-1}$ in marine organism (Fitrianingsih and Widiastuti, 2021).

Pahawang Island in Lampung Province is a popular marine tourism destination with diverse ecosystems, including coral reefs, seagrass beds, and mangrove forests especially significant populations of *Cymodocea* and *Thalassia* (Mardani *et al*., 2018; Novita *et al*., 2022). According to the Central Bureau of Statistics of Pesawaran Regency, tourist visits reached 448,008 in 2019 and decreased to approximately 165,342 in 2020 (BPS Statistic, 2023). The high boat activity, as the only means of transportation to the island, potentially contributes to heavy metal pollution in the (Saraswati and Rachmadiarti, 2021; Swain *et al*., 2021; Rozirwan *et al*., 2024).

Therefore, this study aims to provide valuable insights into the bioaccumulation patterns of heavy metals in the roots and leaves of seagrass, while also identifying the ecological risks involved. These insights can assist in developing management strategies for coastal areas and ecosystems in the region. The primary objective of this research is to analyze the ecological risks associated with the accumulation of heavy metals (Pb, Cu, Ni, and Zn) in the roots and leaves of *Cymodocea* and *Thalassia*, as well as in the sediments of Pahawang Island.

Materials and Methods

This research conducted in March 2024 off the coast of Pahawang Island in Lampung, Indonesia, at two distinct sampling locations. The sites were selected using purposive sampling within the seagrass ecosystem in Jeralangan and Cukuh Nyai (Figure 1). The first location, Jeralangan at coordinates 5°40'50.53"S and 105°13'51.15"E, is characterized by a high volume of tourism activities. The second location, Cukuh Nyai at coordinates 5°41'19.76"S and 105°13'9.28"E, has relatively low tourism activity. In addition, this location has a different residential area where the first location has a denser settlement than the second location. Tourism and residential activities can cause heavy metal pollutants to enter the waters (Purwiyanto, 2013; Purwiyanto *et al*., 2020). Sampling was carried out during low tide, namely in morning and evening.

Two types of samples were collected at each station, consisting of seagrass and sediment. At Station One, samples of *Cymodocea* seagrass and sediment were collected, while Station Two involved the collection of *Thalassia* seagrass and sediment. The identification of seagrass species was conducted using a field guide for seagrass and dugong monitoring (Herandarudewi *et al*., 2019). The collected of seagrass samples included both leaves and roots, gathered from shallow water habitats or intertidal zones through random sampling (Trevathan-Tackett *et al*., 2015; Singh *et al*., 2021). Sediment samples were taken using a grab sampler, with a total weight of 1 kg from the surface layer (0-10 cm). The collected samples were placed in plastic bags and stored in containers (Xiao *et al*., 2022).

Figure 1. A map of seagrass sampling location

The results of this study indicate a pattern of heavy metal accumulation in seagrass at two locations with different anthropogenic pressures. While these findings provide valuable insights, it is important to note that the spatial coverage of this study is limited. Therefore, further investigation in other seagrass ecosystems is needed to determine the extent to which the observed patterns can be extrapolated to other areas with similar conditions. Additional studies with broader spatial coverage, encompassing various levels of environmental pressure and different oceanographic characteristics, will help strengthen the generalization of these findings.

Heavy metals analysis

The seagrass leaf and root samples, along with sediment, were thoroughly cleaned and subsequently dried. The dried samples were then ground to a fine powder and stored in sealed containers (Stewart *et al*., 2021). Before the destruction, AAS calibration is carried out based on the Indonesian National Standard to ensure the accuracy and precision of heavy metal concentration measurements. The calibration process consists of the preparation of heavy metal standard solutions made by diluting certified stock solutions to various concentrations that cover the expected measurement range in the sample. These standards are used to create a calibration curve (Badan Standarisasi Nasional, 2004, 2009).

The sample digestion was carried out using the wet digestion method, where 0.5 g of powdered seagrass was mixed with 5 ml of a reagent in a ratio of 5:2:1 (65% nitric acid: concentrated perchloric acid: sulfuric acid) on a hot plate for 10 min, along with an additional 5 ml of 2N hydrochloric acid, and heated on the hot plate for another 10 min. After reaching acid digestion, the sample was cooled and then filtered using Whatman filter paper (0.45 μm). The filtrate was then transferred into a 25 ml volumetric flask and diluted with deionized water up to the mark (FAO/SIDA, 1983; European Commission, 2011). For sediment samples, the wet digestion process involved adding 5 ml of concentrated HNO_3 to each sample, which included 50 ml of water sample and approximately 3 g of sediment sample. The sample was heated on a hot plate at a temperature of 105°C – 120°C until the volume was reduced to approximately 10 ml. After cooling, 5 ml of HNO_3 and 1-3 ml of perchloric acid were added, and the sample was reheated until white fumes appeared, followed by further heating for approximately 30 minutes. The sample was then filtered using quantitative filter paper with a pore size of 8.0 μm. The filtrate was transferred into a 100 ml volumetric flask and diluted with distilled water up to the mark (Badan Standarisasi Nasional, 2009; Gao *et al.,* 2021). The heavy metal analysis for each sample was performed in three replicates. The heavy metals analyzed in this study included Pb, Cu, Ni, and Zn.

The concentration of heavy metals in the samples was measured using an Atomic Absorption Spectrophotometer (AAS) SHIMADZU AA-7000The wet digestion method is used because it is more effective in extracting heavy metals and preventing the loss of volatile elements such as Pb (Alfaro *et al.,* 2015). However, if not performed carefully, there is a possibility of contamination from reagents or laboratory equipment that may affect the results. Therefore, quality assurance and quality control are carried out by ensuring the use of contaminant-free glassware, pure quality chemicals (p.a), and calibrated and verified atomic absorption spectrophotometers, operated by competent analysts. Quality control includes linearity of the calibration curve ($r^2 \geq 0.99$), blank analysis to control contamination with lead levels below the detection limit, and triplicate preparation to ensure accuracy with a difference in results of ≤20% (Badan Standarisasi Nasional, 2004, 2009).

Ecological risk assessment

Ecological risk assessment can be conducted by calculating the bioconcentration factor (BCF), translocation factors (TF), geoaccumulation index (I_{geo}), contamination factor (Cf), and pollution load index (PLI) (Hakanson, 1980; Rozirwan *et al.,* 2023).

Bioconcentration Factor (BCF)

Accumulation of heavy metals in the roots and leaves of seagrass from the surrounding sediment can be assessed using the Bioconcentration Factor (BCF) as described below (Steingräber *et al.,* 2022).

$$BCF = \frac{concentration\ of\ organs}{concentration\ of\ sediment} \qquad (1)$$

where: BCF< 1 is an excluder; The organism (usually a plant) does not accumulate the substance efficiently; BCF= 1 is an indicator; The organism takes up the substance at the same rate as it is found in the environment; BCF= 1 is a hyperaccumulator; The organism accumulates the substance at a much higher concentration than in the environment (Almahasheer, 2019).

Translocation Factors (TF)

The Translocation Factor (TF) is used to measure the movement of heavy metals from the roots to the leaves (Usman *et al.,* 2019):

$$TF = \frac{BCF\ leaf}{BCF\ of\ root} \qquad (2)$$

TF Value > 1 indicates that the sample functions as a phytoextractor, effectively translocating metals from the roots to the leaves, whereas TF value < 1 indicates that the sample functions as a phytostabilizer (Dinu *et al.,* 2020).

Geoaccumulation Index (I_{geo})

The Geoaccumulation Index (I_{geo}) is used to evaluate the degree of heavy metal contamination and classify pollution levels according to established criteria (Rozirwan *et al.,* 2023).

$$Igeo = Log\ 2\left(\frac{concentration\ of\ sediment}{1{,}5\ background}\right) \qquad (3)$$

Note the I_{geo} value classification consists of not contaminated (I_{geo}≤0), not contaminated to moderately contaminated (I_{geo}0–1), moderately contaminated (I_{geo}1–2), moderate to highly contaminated (I_{geo}2–3), highly contaminated (I_{geo}3–4), highly contaminated to very high (I_{geo} 4–5), and highly contaminated (I_{geo}≥5).

Background concentration (Alfaro *et al.,* 2015).

Contamination Factor (Cf)

The Contamination Factor (CF) value is used as a basis for determining the degree of heavy metal contamination (Rozirwan, Az-Zahrah et al., 2024).

$$CF = \frac{concentration\ of\ sediment}{Background} \qquad (4)$$

where: contamination factor criteria according to (Shaheen *et al.,* 2019); Cf<1= low level of contamination; 1<Cf<3= moderate level of contamination; 3<Cf<6= moderate level of contamination; Cf>6= very high level of contamination.

Pollution Load Index (PLI)

The Pollution Load Index (PLI) is used to evaluate the pollution quality (Mosa *et al.,* 2022).

$$PLI = [Cf1\ x\ Cf2\ x\ Cf3\ ....\ x\ Cfn]^{1/n} \qquad (5)$$

where: pollution load index criteria: PLI 2= not polluted to lightly polluted; PLI 24= moderately polluted; PLI 4–6= heavily polluted; PLI 6–8= heavily polluted; PLI 8–10= heavily polluted

Statistical analysis

The concentrations of heavy metals were analyzed using the Mann-Whitney test in SPSS, as the data were not normally distributed. This statistical test was applied to compare the concentrations of these metals between the two study sites based on the mean differences in the samples. The significance level (α) was set at 0.05 to determine statistical differences. If the 2-tailed significance value is greater than 0.05, it indicates no significant difference in the mean concentration between the two sites (H_0 is accepted). Conversely, if the 2-tailed significance value is less than 0.05, it suggests a significant difference in the mean concentrations (H_1 is rejected).

Result and Discussion

The seagrass samples were collected based on the dominant species found at the research site, specifically *Cymodocea* and *Thalassia* (Figure 2). *Cymodocea* was predominantly observed at Station One. This species is characterized by long, ribbon-like leaves that taper to a rounded tip, white to slightly yellowish rhizomes, and thin, brown fibrous roots. Both *Cymodocea* and *Thalassia* are commonly found in substrates ranging from muddy to sandy (Hartati *et al*., 2018; Thangaradjou and Bhatt, 2018), which explains their prevalence in the research area where the majority of locations feature such substrates. *Cymodocea* exhibits a strong tolerance to various environmental stresses and can thrive in diverse habitats, categorizing it as a species of least concern according to the IUCN (Terrados *et al*., 1998; Short *et al*., 2011). *Thalassia* is mainly found at Station Two and is recognized by its crescent-shaped leaves with neatly arranged margins, brown rhizomes, and thick, sheathed roots. This species allocates a greater proportion of its biomass to underground structures, such as roots and rhizomes, rather than to its leaves. This morphological variation can be seen as an adaptive response to environmental physical stressors, such as burial by sand to prevent displacement during tidal fluctuations and storms (Westlake *et al.,* 2022).

The root structure of *Thalassia* plays an essential role in the absorption of heavy metals in the marine environment. *Thalassia* has an extensive root and rhizome system, allowing greater contact with sediments containing heavy metals (Rosalina *et al.,* 2022). Exposure to heavy metals can cause changes in the structure of root tissue, such as thickening or damage to the epidermis and endodermis in both seagrass roots. These changes can affect the ability of roots to absorb and translocate heavy metals (Rosalina *et al.,* 2019). *Thalassia hemprichii* and *Cymodocea serrulata* leaves play an important role in the absorption of heavy metals from the water column. The broad and thin leaf structure increases the surface area for direct contact with water, facilitating the absorption of heavy metals (Nugraha, 2016). Additionally, the presence of aerenchyma tissue in the roots and leaves enables efficient gas exchange, which can influence the process of heavy metal absorption (Chedadi *et al.,* 2024). According to Dilipan and Arulbalachandran (2022), both *Cymodocea* and *Thalassia* are promising candidates for bioremediation of contaminated sites and are frequently utilized as biological indicators of heavy metal accumulation.

Figure 2. Seagrass, (a) *Cymodocea*, (b) *Thalassia*

Heavy metals concentration in sediment and seagrass

The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments, roots, and leaves of seagrasses at both stations are summarized in Figure 3. The results indicate that Station 1 (*Cymodocea*) had the highest concentrations of Pb and Zn in the sediments, roots, and leaves. Specifically, the maximum concentrations of Cu in sediments and roots were also recorded at Station 1 (*Cymodocea*), while the highest concentration in leaves was observed at Station 2 (*Thalassia*). The highest levels of Ni were found in the leaves and roots of *Cymodocea*. In contrast, the sediment at Station 2 exhibited the highest concentration of Ni. A comparative analysis of heavy metal concentrations across different seagrass species and locations is summarized in Figure 3. Based on the research results, the concentration of each sample between the two stations was analyzed using the Mann-Whitney test (as the data were not normally distributed) and showed a 2-tailed significance value of >0.05, indicating no significant difference between the two stations.

Based on Figure 3, the concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments of this study are still below the threshold values established by ANZECC and ARMCANZ (2000) as referenced *in* (Ogundele *et al.,* 2015), and by the Gazzetta Ufficiale della Repubblica Italiana as noted *in* (Bonanno *et al.,* 2020), which are set at 50 $mg.kg^{-1}$, 65 $mg.kg^{-1}$, 50 $mg.kg^{-1}$, and 30 $mg.kg^{-1}$, respectively. Station 1 is characterized by a predominant presence of *Cymodocea*, which is directly influenced by tidal dynamics. Tidal-affected areas experience dynamic water movement, enabling heavy metals carried by the water to adhere to sediment particles (Maulana *et al.,* 2023). The concentrations of heavy metals Pb, Cu, Zn, and Ni in the sediments are higher compared to those in the roots and leaves, corroborating the findings of Khotimah *et al.* (2024) which indicate that sediment concentrations of heavy metals exceed those in the roots, implying that the roots may serve as a barrier preventing heavy metals from translocating to the upper plant structures. As noted by Flefel *et al.* (2020), sediments act as primary reservoirs for heavy metal absorption in aquatic environments. In addition, the higher concentration of heavy metals in the sediment at Station 1 compared to Station 2 can be assumed to be due to the finer sediment texture. Heavy metals tend to bind more effectively to sediment types with higher clay content or finer particle sizes (Borah *et al*., 2018; Fitria *et al*., 2023).

The concentrations of heavy metal Pb in the sediments, leaves, and roots of *Cymodocea* and *Thalassia* differ between the two research locations (Figure 3). The higher concentration of Pb in *Cymodocea* at Station 1 may be attributed to the significant human activities in this area, including increased boat traffic and residential developments. Heavy metal Pb levels can be exacerbated by factors such as maritime traffic, fishing activities, paint on boats, and sedimentation (Irandoost *et al*., 2021). Mitigation measures that can be implemented include strengthening regulations and waste treatment systems around coastal areas, as well as conducting long-term monitoring to identify pollution trends and their impacts on the seagrass ecosystem (Jeong *et al.,* 2021). Notably, the highest concentration of Pb is found in the leaves of *Cymodocea* when compared to its roots (figure 3). Similar findings were reported by El Zrelli *et al.* (2023), which indicated that the Pb concentration in the leaves of *Cymodocea* is greater than that in its roots. According to the WHO (1996) as cited in Ogundele *et al.* (2015), the concentration of Pb in *Cymodocea* and *Thalassia* remains below the quality standard of 2 $mg.kg^{-1}$(Pb), suggesting that these seagrasses are not currently subjected to contamination.

In this study, the highest concentration of the heavy metal Cu was observed in the leaves of *Thalassia* at Station 2. This concentration is still below the quality standard of 20 $mg.kg^{-1}$ (Kabata-Pendias, 2011) indicating that the accumulation of Cu in seagrass does not pose any toxic effects. Copper is considered an essential metal, as it is required by organisms; however, at significantly high concentrations, Cu can be more toxic than non-essential metals such as Pb and Cd due to the active absorption mechanisms that plants employ A similar study conducted on the leaves and roots of *Thalassia* in Xincun Bay, China, also reported the highest concentration in leaves at 11.2 $mg.kg^{-1}$ (Zhang *et al*., 2021). Previous research on various seagrass species has demonstrated that aboveground leaves are more efficient at accumulating Cu compared to belowground tissues (roots and rhizomes) (Lin *et al*., 2018; Hu *et al*., 2019). The concentration of heavy metal Cu in the leaves of *Thalassia* is higher than in *Cymodocea*. This may be due to *Thalassia* having broader leaf morphology and often exhibiting a slower growth rate compared to *Cymodocea*, allowing more heavy metals to accumulate in leaf tissues over a longer period (Nugraha, 2016). Additionally, different physiological mechanisms in each seagrass species may influence the uptake of heavy metals through the leaf surface (El Zrelli *et al*., 2023). Previous studies on these two seagrass species, particularly in Indonesia, have shown that Cu concentrations in *Thalassia* leaves are higher than in *Cymodocea* (Bidayani *et al*., 2017; Kholil *et al*., 2019).

The concentration of Ni based on the research findings indicates that the highest level was found in the sediment, followed by the roots of *Cymodocea*, measuring 2.331 mg.kg^{-1}. This concentration is significantly lower than the legal limit allowed in marine waters, which is set at 20 mg.kg^{-1}. Furthermore, increases in Ni concentrations of 3 to 5 times are generally observed in environments impacted by human activities (Bonanno *et al*., 2020). The Ni concentrations in this study are consistent with previous research that reported low Ni levels in seagrasses, such as 3.20–6.65 mg.kg^{-1} in Italy (Bonanno *et al*., 2017) and 1.8–3.1 mg.kg^{-1} in South Africa (Nel *et al*., 2023), , particularly in locations with minimal human activities. This suggests that the study site is not yet contaminated with heavy metals, specifically Ni.

According to Figure 3, the highest concentration of Zn in seagrass was found in the leaves of *Cymodocea* at station 1. This concentration remains below the threshold established by the Australia and New Zealand Food Authority (ANZFA), which is 14 mg.kg^{-1} (Smitha *et al*., 2010). Furthermore, the measured Zn levels are significantly lower than the concentrations that can cause phytotoxicity, which range from 500-1500 mg.kg^{-1} (Elrashidi, 1990). Thus, the concentration of Zn detected in this study does not indicate any toxic effects. The results demonstrate that the concentrations of Zn in both the leaves and roots of the two seagrass species are higher than those of Pb and Cu (Figure 3). This suggests that Zn plays a crucial role in plant metabolism (Bonanno and Orlando-Bonaca, 2017). These findings are consistent with previous research, which reported that Zn concentrations are highest in the roots and leaves of various seagrass species, including *Cymodocea* and *Thalassia*, in locations such as Sicily, Italy, China, and India (Bonanno *et al*., 2017; Hu *et al*., 2019; Gopi *et al*., 2020). After knowing the concentration of heavy metals in seagrass and sediment, the next step is to evaluate its ecological impact. Therefore, an ecological risk assessment is needed to assess the potential threat to seagrass ecosystems and organisms that depend on them.

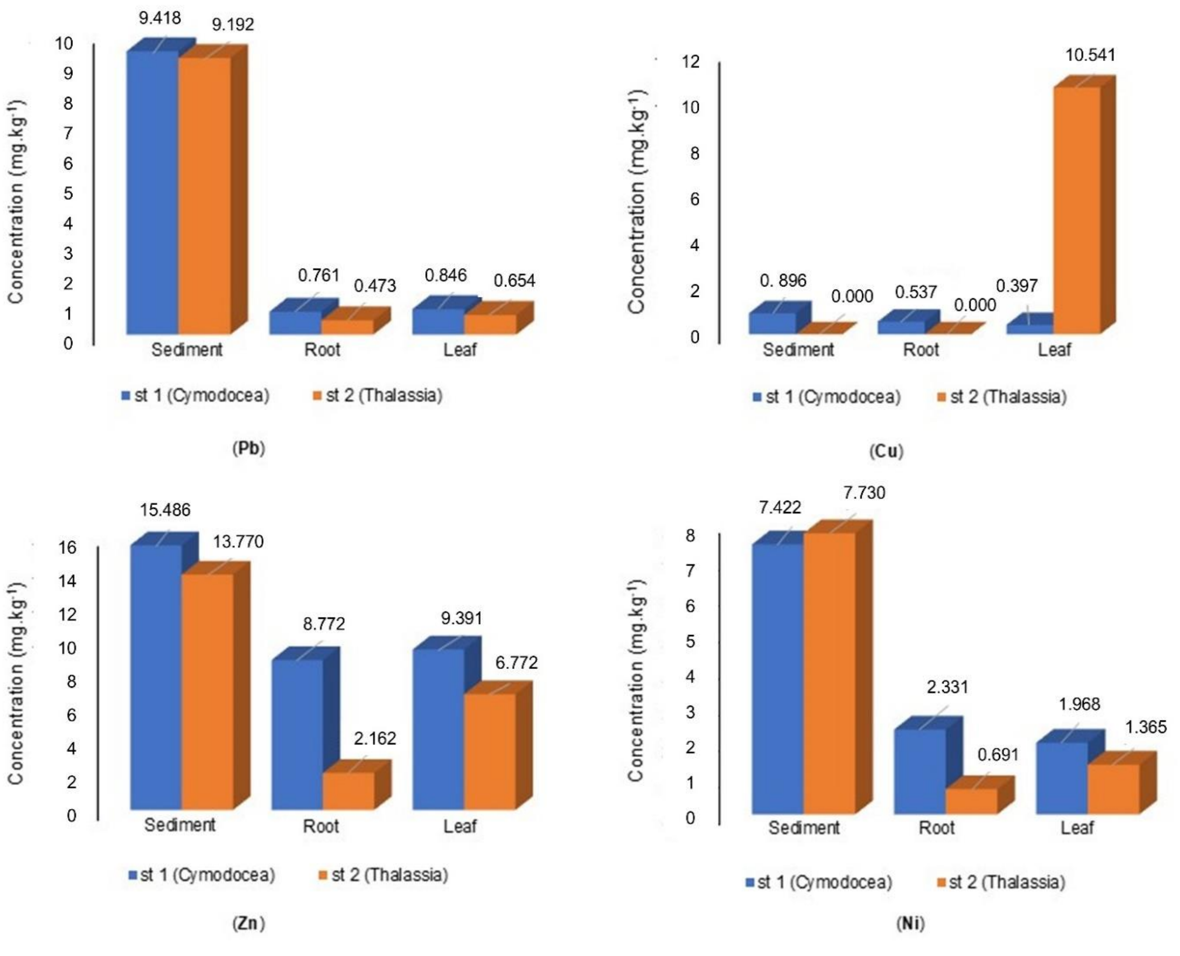

Figure 3. Concentration of Pb, Cu, Ni and Zn in organs of *Cymodocea* and *Thalassia* (leaf and root mg.kg^{-1}), and associated sediments (mg.kg^{-1})

Table 1. Comparison of heavy metal concentrations in *Cymodocea* and *Thalassia* with those from the open literatures

Location	Seagrass	Tissue	Heavy metals (mg.kg^{-1})	References
Sicily (Italy)	*Cymodocea*	Leaf	3.61–28.8 (Cu) 3.20–6.65 (Ni) 1.42–3.85 (Pb) 42.6–65.8 (Zn)	(Bonanno *et al*., 2017)
Sicily (Italy)	*Cymodocea*	Leaf	3.90–19.5 (Cu) 2.40–22.5 (Ni) 1.52–6.10 (Pb) 4.21–13.9 (Zn)	(Bonanno *et al*., 2020)
Sicily (Italy)	*Cymodocea*	Rhizome	10.6 (Cu 1.15 (Ni) 0.73 (Pb) 21.7 (Zn)	(Bonanno and Borg, 2018)
		Root	8.83 (Cu) 4.79 (Ni) 4.81 (Pb) 38.7 (Zn)	
Florida, USA	*Thalassia*	Rhizome+root+leaf	20.23 (Pb) 9.55 (Cu) 86.68 (Zn) 1.56 (Cd)	(Smith *et al*., 2019)
Palk Bay, South Eastern India	*Cymodocea*	Rhizome+root +leaf	1.23 (Cd) 18.59 (Cu) 1.74 (Pb) 34.63 (Zn)	(Gopi *et al*., 2020)
Xincun Bay,China	*Thalassia*	Rhizome+root +leaf	11.2 (Cu) 4.05 (Ni) 2.1 (Pb) 39.1 (Zn)	(Zhang *et al*., 2021)
Southern Mediterranean Sea	*Cymodocea*	Rhizome	0.99 (Cu) 0.26 (Pb) 55 (Zn) 0.45 (Cd)	(El Zrelli *et al*., 2023)
		Root	3.51 (Cu) 3.76 (Pb) 31 (Zn) 1.33 (Cd)	
		Leaf	3.95 (Cu) 1.74 (Pb) 131.33 (Zn) 2.40 (Cd)	
Pahawang Island, Indonesia	*Cymodocea*	Root	0.76 (Pb) 0.54 (Cu) 8.77 (Zn) 2.33 (Ni)	This study
		Leaf	0.85 (Pb) 0.39 (Cu) 9.39 (Zn) 1.97 (Ni)	
	Thalassia	Root	0.47 (Pb) nd (Cu) 2.16 (Zn) 0.69 (Ni)	
		Leaf	0.65 (Pb) 10.54 (Cu) 6.77 (Zn) 1.36 (Ni)	

Note: nd (not detection)

Bioaccumulation and ecological risk assessments

Based on the research results presented in Table 2, the bioconcentration factors (BCF) for the roots of *Cymodocea* and *Thalassia*, which accumulate heavy metals from the sediment biologically, were determined to be 0.0828 and 0.0502 for Pb, respectively. The BCF for Cu in *Cymodocea* is 0.5991, while in *Thalassia* it is 0.0082. For Ni, the BCF values are 0.3140 for *Cymodocea* and 0.0894 for *Thalassia*, and for Zn, the BCF values are 0.5665 and 0.1670, respectively. The geoaccumulation index indicates an unpolluted status for Pb with values of -1.9696 and -1.9344, for Cu with values of -5.0658 and -4.5659, for Ni with values of -2.2167 and -2.1581, and for Zn with values of -2.6982 and -2.8687. The contamination factor (CF) suggests low contamination levels for Pb at 0.3830 and 0.3924, for Cu at 0.0448 and not detected (nd), for Ni at 0.3227 and 0.3361, and for Zn at 0.2311 and 0.2055. The pollution load index (PLI) was calculated, yielding results of 0.0013 and 0.0051, indicating that both stations are not polluted.

There are differences in the translocation factor (TF) values of metals (Table 3) between *Cymodocea* and *Thalassia*. In *Thalassia*, the TF for Cu indicates a translocation value of <1, suggesting minimal transfer of this metal to the above-ground biomass or leaves. This can also be attributed to the very low concentrations of Cu in the roots and leaves of the sampled seagrass at the study site. The metal with the highest TF value is Zn in *Thalassia*, followed by Ni and Pb. Similarly, *Cymodocea* shows the highest TF values for Zn and Pb, which are both >1. The differences in translocation values may arise from the biological characteristics of seagrasses, which have developed two distinct adaptive strategies to cope with the levels of contamination in their environment. The first strategy involves the storage of accumulated metals from the sediment in the root-rhizome system, while the second strategy entails the translocation of some contaminants from the root-rhizome to the above-ground parts of the seagrass, such as the leaves (El Zrelli *et al*., 2023). According to the TF values presented in Table 2, *Thalassia* employs the second adaptive strategy for Zn, Ni, and Pb by translocating these contaminants to the leaves, whereas for Cu, it utilizes the first adaptive strategy. In contrast, *Cymodocea* adopts the second adaptive strategy for Pb and Zn. Previous research has also indicated that Zn undergoes significant translocation in *Cymodocea*, with a value >1 (Bonanno and Borg, 2018; Nel *et al*., 2023).

The bioconcentration factor (BCF) of heavy metals from sediment to leaves and roots is summarized in Table 2. The BCF of zinc (Zn) in the leaves is higher than that of lead (Pb), nickel (Ni), and copper (Cu). This indicates that the accumulation level of Zn in the leaves is greater compared to the other metals, which may be influenced by several factors, including the adaptive strategies of seagrass in absorbing heavy metals and the chemical properties of the metals as well as the selectivity of the plants (Khotimah *et al*., 2024). Zinc is an essential metal for seagrass, as it is utilized in photosynthesis and growth (Kabata-Pendias and Pendias, 2000). This leads to higher Zn accumulation since it is more readily absorbed by both *Cymodocea* and *Thalassia*. According to Table 2, *Cymodocea* exhibits the highest BCF value for Zn. Similarly, high BCF values for Zn have also been found in the leaves of *Zostera noltei*, as well as in the roots and leaves of *Thalassia hemprichii* and in the roots and leaves of *Enhalus acoroides* (Li and Huang, 2012; Boutahar *et al*., 2019; Jeong *et al*., 2021). Compared to previous studies, particularly in Southeast Asia, such as research conducted in the Johor Strait, Malaysia, the accumulation of heavy metals in seagrasses and their ecological risks show a similar pattern in the accumulation of Pb and Cu, with varying levels of pollution depending on proximity to pollution sources (Sidi *et al*., 2018). A comparison of heavy metal accumulation in seagrasses from different locations worldwide is presented in Table 1.

The values of the geoaccumulation index (I_{geo}) from this study are presented in Table 4, indicating that the sediments at both research locations are unpolluted ($I_{geo}< 0$). This suggests that the sources of heavy metal contamination at these sites are low, attributed to factors such as small residential areas, the absence of industrial and mining activities, and relatively low shipping traffic. Additionally, the physical and chemical conditions of the aquatic environment, including current patterns and tidal movements, also play a significant role (Siregar *et al*., 2020). The contamination factor (CF) in this study indicates that each metal contributes to low or negligible contamination, as evidenced by CF values <1. These findings align with research conducted along the southern coast of Sumatra, Indonesia, which showed low CF values for each metal, except for lead (Pb), due to significant anthropogenic sources in the vicinity (Rozirwan *et al*., 2023; Khotimah *et al*., 2024).

The pollution status of the seagrass ecosystem at the research locations can be assessed using the pollution load index (PLI). The sediment PLI analyzed at the study sites is classified as unpolluted, with PLI values ranging from 0 to 2. The PLI values at both locations are not significantly different, indicating that both ecosystems are healthy and safe for living organisms. In contrast, other studies on seagrass ecosystems in the Southern Mediterranean Sea reported PLI values categorized as moderately contaminated and polluted, posing risks to the seagrass ecosystems, primarily due to the discharge of various industrial wastes, particularly phosphogypsum (El Zrelli *et al*., 2023). Therefore, based on the analyses from this study, it can be concluded that all sampling locations exhibit low levels of metal pollution and ecological risk. The results of this study are limited to the surveyed location and may not fully reflect conditions in other areas.

Table 2. Values of the Bioconcentration factor (BCF)

	BCF			
	Pb	Cu	Ni	Zn
Seagrass roots				
St 1 (*Cymodoceae*)	0.0804	0.5991	0.3140	0.5665
St 2 (*Thalassia*)	0.0518	0.0082	0.0894	0.1570
Seagrass leaves				
St 1 (*Cymodoceae*)	0.0893	0.4436	0.2652	0.6064
St 2 (*Thalassia*)	0.0716	-8.3217	0.1766	0.4918

Table 3. The translocation factor (TF) from the roots to the leaves of seagrass

	TF			
	Pb	Cu	Ni	Zn
St 1 (*Cymodoceae*)	1.1112	0.7405	0.8445	1.0705
St 2 (*Thalassia*)	1.3834	-1011.6867	1.9764	3.1328

Table 4. Geoaccumulation Index (I_{geo}), Contamination Factor (Cf), and Pollution Load Index (PLI)

	Igeo				CF				PLI
	Pb	Cu	Ni	Zn	Pb	Cu	Ni	Zn	
St 1	-1.9696	-5.0658	-2.2167	-2.6982	0.3830	0.0448	0.3227	0.2311	0.0013
St 2	-1.9344	-4.5659	-2.1581	-2.8676	0.3924	nd	0.3361	0.2055	0.0051

The results of this study provide new insights into how tropical seagrasses, particularly *Cymodocea* and *Thalassia*, play a role in accumulating heavy metals in the marine environment. These findings contribute to understanding the distribution patterns of heavy metals in seagrass ecosystems, which can be used to monitor water quality. They also help identify seagrass species that are more effective as bioindicators of heavy metal pollution. Furthermore, this study provides a scientific basis for environmental management policies, such as the protection of seagrass beds in areas with high anthropogenic activity. However, this study is limited in its spatial coverage and does not account for broader temporal variability. Therefore, further research with a wider study area and long-term monitoring is necessary to gain a more comprehensive understanding of heavy metal accumulation patterns and to identify potential changes over time.

Conclusion

The highest concentrations of Pb, Cu, Ni, and Zn in seagrass were found in the leaves, while the sediments at Station 1 show the highest levels. Overall, the concentrations of heavy metals in the roots and leaves of *Cymodocea* and *Thalassia* indicate relatively high levels; however, the ecological risk assessment shows that the bioaccumulation factor (BCF) is categorized as low. In contrast, the translocation factors (TF) for Pb and Zn demonstrate values greater than 1, indicating that the seagrass functions effectively in phytoremediation or translocating metals from the roots to the leaves. In summary, the ecological risk assessment of the research locations, which includes the geoaccumulation index (Igeo), contamination factor (CF), and pollution load index (PLI), indicates that the environment within the seagrass ecosystems at these sites has low levels of heavy metal pollution and ecological risk. The findings of this study are specific to the examined site and may not be entirely representative of conditions in other regions.

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