



[biodiv] Submission Acknowledgement

1 pesan

Ahmad Dwi Setyawan via SMUJO <support@smujo.com>

30 September 2024 pukul 11.13

Balas Ke: Ahmad Dwi Setyawan <editors@smujo.id>

Kepada: Rozirwan Rozirwan <rozirwan@unsri.ac.id>

Rozirwan Rozirwan:

Thank you for submitting the manuscript, "Implication of Microplastics Presence in Sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia" to Biodiversitas Journal of Biological Diversity. 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:

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/19666>

Username: rozirwanb

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

[Biodiversitas Journal of Biological Diversity](#)

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**[biodiv] Editor Decision**

3 pesan

Smujo Editors via SMUJO <support@smujo.com>
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Rozirwan Rozirwan <rozirwan@unsri.ac.id>

2 Oktober 2024 pukul 05.56

Rozirwan Rozirwan:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of Microplastics Presence in Sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.**

Our decision is: Revisions Required

Reviewer A:

Dear Author(s),

Thank you very much for your submission. Here is my review.

- Abstract is too brief. An abstract is required (about 200-300 words).
- Keywords are about five words, covering scientific and local names (if any), research themes, and special methods used; and sorted from A to Z.
- Running title is about 5-7 words.
- The introduction is too brief. An introduction is required (about 600-700 words).
- Materials and Methods should emphasize the procedures and data analysis.
- Figures and tables must be mentioned in the body text
- For non mother tongue, a Certificate of Proofreading from USA, UK, Canada or Australia is needed.
- All manuscripts must be written in clear and grammatically correct English (U.S.).
- Please write all the citations and references based on the author's guidelines (<https://smujo.id/biodiv/guidance-for-author>), include DOI. Kindly see the example below:
e.g.,
Mukkun L, Kleden YL, Simamora AV. 2021. Detection of Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. Intl J Trop Drylands 5: 20-26. DOI: 10.13057/tropdrylands/t050104.
- The usage of "et al." in long author lists will also be accepted. For example, if the number of authors is more than 20, then it is permissible to use "et al."; if there are less than 20, writing all the authors' names is recommended.

Kindly check and correct accordingly
Thank youRecommendation: Revisions Required

5/10/25, 10:28 AM

Email Sriwijaya University - [biodiv] Editor Decision

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Rozirwan ROZIRWAN <rozirwan@unsri.ac.id>
Kepada: Smujo Editors <editors@smujo.id>

8 Oktober 2024 pukul 10.12

Dear Editor,

We hereby submit the revised manuscript titled "Implications of Microplastic Presence in Sediments and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". The manuscript has been revised following the Reviewer's suggestions, and we have attached the updated version for your consideration.

Thank you,
Regards

[Kutipan teks disembunyikan]

--

Dr. Rozirwan
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2 lampiran

 **Author(s) Answers to Reviewer.doc**
37K

 **Manuscript-Revision1.doc**
4525K

Smujo Editors <editors@smujo.id>
Kepada: Rozirwan ROZIRWAN <rozirwan@unsri.ac.id>

8 Oktober 2024 pukul 18.19

Pls, send it through ojs.

[Kutipan teks disembunyikan]

Author(s)' Comments to Reviewer A:

Dear Reviewer,

We sincerely thank you for taking the time to thoroughly review our manuscript and for providing such insightful feedback, which will help improve the quality of our work. In response to your comments, we have made the necessary revisions to the manuscript. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Authors

We greatly appreciate the reviewer's thoughtful comments. Based on the valuable feedback provided, we have revised the manuscript accordingly to address all the points raised.

Comments	Resposns
Abstract is too brief. An abstract is required (about 200-300 words).	We have carefully considered the reviewer's suggestions and have made improvements to the abstract section of the manuscript, which now contains 215 words (Page, lines 12-23).
Keywords are about five words, covering scientific and local names (if any), research themes, and special methods used; and sorted from A to Z.	We have respectfully reordered the keywords in alphabetical order (Page 1, line 2) for clarity and consistency.
Running title is about 5-7 words.	In response to the reviewer's suggestion, we have included the running title on Page 1, line 25.
The introduction is too brief. An introduction is required (about 600-700 words).	We have respectfully expanded the introduction section by approximately 600 to 700 words (Page 2, lines 49-50) to further enrich the context and background of the study.
Materials and Methods should emphasize the procedures and data analysis	We have revised the "Materials and Methods" section to improve clarity and facilitate better reader understanding (Page 2, lines 65-117).
Figures and tables must be mentioned in the body text	We have revised the manuscript to ensure that "figures and tables" are appropriately referenced within the body text.
For non mother tongue, a Certificate	We sincerely appreciate your valuable

<p>of Proofreading from USA, UK, Canada or Australia is needed.</p>	<p>feedback regarding the necessity of a Certificate of Proofreading from an English-speaking country. While we acknowledge the importance of ensuring linguistic accuracy and clarity in our manuscript, we have made every effort to comply with American English guidelines and have conducted comprehensive internal reviews to enhance the quality of the text. We trust that these measures sufficiently support the clarity and comprehensibility of our work.</p>
<p>All manuscripts must be written in clear and grammatically correct English (U.S.).</p>	<p>We have made every effort to compose the English narration following American English guidelines. Additionally, we have diligently reviewed the sentences and grammar to ensure clarity and accuracy.</p>
<p>Please write all the citations and references based on the author's guidelines (https://smujo.id/biodiv/guidance-for-author), include DOI. Kindly see the example below: e.g., Mukkun L, Kleden YL, Simamora AV. 2021. Detection of <i>Spodoptera frugiperda</i> (J.E. Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. <i>Intl J Trop Drylands</i> 5: 20-26. DOI: 10.13057/tropdrylands/t050104.</p> <p>The usage of "et al." in long author lists will also be accepted. For example, if the number of authors is more than 20, then it is permissible to use "et al."; if there are less than 20, writing all the authors' names is recommended.</p>	<p>We have revised the manuscript to improve the overall referencing format (last page)</p>

**[biodiv] Editor Decision**

1 pesan

Smujo Editors via SMUJO <support@smujo.com>
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Rozirwan Rozirwan <rozirwan@unsri.ac.id>

27 Oktober 2024 pukul 16.43

Rozirwan Rozirwan:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of microplastics presence in sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.** We are waiting for your revision in the system (<https://smujo.id/biodiv>), do not send it via email.

Our decision is: Revisions Required

Reviewer L:

Reviewer Comments

Here is a detailed review of the manuscript:

The study's focus on the bioaccumulation of microplastics in blood clams, particularly in a specific location like the Musi River Estuary, contributes to a growing body of research that is both timely and relevant. This localized approach can help fill a gap in current knowledge.

Introduction: Highlighting the economic significance of blood clams adds an important angle that underscores the study's relevance to local communities and ecosystems.

Suggestions for Improvement:

Briefly summarize the potential implications of your research findings earlier in the text; Simplify complex sentences where possible to ensure the information is easily digestible; Avoid redundancy in discussing microplastic accumulation, which may detract from the impact of your findings.

- 1) Lines 22 – 23: I suggest that the authors expand on the origins of microplastics by including other significant pathways, such as the direct release of microplastics from products like cosmetics, clothing, and industrial processes. This addition would provide a more comprehensive overview of the various sources of microplastics, enhancing the depth of the discussion on their environmental impact.
- 2) Lines 34 – 35: In "which leads to significant microplastic uptake," you might specify "which can lead to" to indicate that this is a possibility rather than a certainty.
- 3) Lines 41 – 42: The phrase "microplastics is a significant factor in the accumulation of microplastics" is repetitive. You might rephrase it for clarity, e.g., "microplastics is a significant factor contributing to their accumulation in benthic animals."
- 4) Lines 45 – 46: The statement "Due to high levels of anthropogenic activity, a variety of pollutants have contaminated both the watershed and the estuary" could be simplified to improve clarity. For instance: "High levels of human activity have led to various pollutants contaminating both the watershed and the estuary."
- 5) Line 78: The authors do not specify whether the salt used for the saline solution was filtered to remove impurities. Clarifying this detail would be beneficial, as it ensures that the solution used for density separation was free from contaminants that could affect the results. Including this information would enhance the reproducibility and accuracy of the methodology.
- 6) " Procedures for quality assurance and contamination prevention" Section – It is essential to include a dedicated quality control section in every stage of the study. In addition to adopting widely accepted methods for contamination prevention, it is crucial to analyze samples alongside appropriate controls. Subtracting the particles identified in the controls from the sample dataset is an indispensable measure to avoid inaccurate assessments and ensure the integrity of the results.
- 7) Please, pay attention to the spacing between topics and figures.
- 8) Images of equations can be problematic for people with visual impairments who use screen readers. You can provide a textual description or an alternative accessible format. There are tools that allow you to create equations in formats that are both visually appealing and accessible. LaTeX, for example, is a widely used language for writing scientific documents that allows you to create equations in an efficient and accessible way.
- 9) Suggested reorganization: To improve the flow and clarity of your paper, I recommend combining the Results and Discussion statements into a single section, titled "Results and Discussion." This approach allows the results to be immediately contextualized and interpreted, making them easier for the reader to understand. Despite having this section in the paper, the authors separate the discussion from the results by creating a new section called "Discussion". This information can be confusing to the reader.
- 10) Terminology Consistency: Be consistent with the terms used to describe microplastics (e.g., "items," "particles," "fragments"). This will prevent confusion and improve clarity.
- 11) While you mention human activities contributing to microplastic pollution, a deeper exploration of local sources and their specific impacts on the estuarine ecosystem would enhance the environmental perception of your work.
- 12) While you compare your findings to other regions, it would be beneficial to draw more explicit connections regarding why the Musi River Estuary exhibits different levels of microplastic contamination. Discuss potential socio-economic or environmental factors influencing these differences.
- 13) Expand on the ecological implications of microplastic pollution, particularly how it affects local species and ecosystems. Discuss potential long-term impacts on biodiversity, food webs, and human health.

5/10/25, 10:30 AM

Email Sriwijaya University - [biodiv] Editor Decision

- 14) The risk assessment section should more clearly articulate what the identified risk values mean for local ecosystems and communities. Consider discussing the potential health risks for consumers of blood clams and the broader implications for local fisheries.
- 15) While you mention hydrodynamic conditions, consider elaborating on how seasonal variations or climatic events might influence microplastic distribution and accumulation in the estuary.
- 16) I suggest that the authors consider including recommendations for mitigating microplastic pollution based on their findings. Providing actionable insights for local stakeholders could enhance the practical relevance of the study and contribute to more effective environmental protection efforts in the region.
- 17) Conclude with a succinct summary of your key findings and their significance. Emphasize the urgency for monitoring and management strategies to address microplastic pollution in the Musi River Estuary.

Recommendation: Revisions Required

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Author(s) Response to Reviewer L:

Dear Reviewer,

We sincerely thank you for taking the time to thoroughly review our manuscript and for providing such insightful feedback, which will help improve the quality of our work. In response to your comments, we have made the necessary revisions to the manuscript. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Authors

Table of Author(s) Response

No	Comments	Respos
1.	Lines 22 – 23: I suggest that the authors expand on the origins of microplastics by including other significant pathways, such as the direct release of microplastics from products like cosmetics, clothing, and industrial processes. This addition would provide a more comprehensive overview of the various sources of microplastics, enhancing the depth of the discussion on their environmental impact.	Thank you for your valuable feedback and insightful suggestion. In response to your recommendation, we have revised the manuscript on Lines 22–23 to include additional significant pathways for microplastic origins, such as direct releases from products like cosmetics, clothing, and industrial processes. (Now: Lines 27-40, P1) We would like to thank you for your guidance in enhancing the clarity and depth of our study.
2.	Lines 34 – 35: In "which leads to significant microplastic uptake," you might specify "which can lead to" to indicate that this is a possibility rather than a certainty	We have revised the phrase on lines 34–35 as per your recommendation. The wording now reads "which can lead to significant microplastic uptake" to better reflect the possibility rather than a certainty.
3.	Lines 41 – 42: The phrase "microplastics is a significant factor in the accumulation of microplastics" is repetitive. You might rephrase it for clarity, e.g., "microplastics is a significant factor contributing to their accumulation in benthic animals."	The suggested revision has been made to improve clarity. The phrase in lines 41–42 has been rephrased to read, "microplastics is a significant factor contributing to their accumulation in benthic animals," as per your recommendation
4.	Lines 45 – 46: The statement "Due to high levels of anthropogenic activity, a variety of pollutants have contaminated both the watershed and the estuary" could be simplified to improve clarity. For instance: "High levels of human activity have led to various pollutants contaminating both the watershed and the estuary."	We have carefully revised the manuscript to incorporate the suggested changes. Specifically, we have simplified the statement Lines 45-46 for clarity as recommended.
5.	Line 78: The authors do not specify whether the salt used for the saline solution was filtered to remove impurities. Clarifying this detail would be beneficial, as it ensures that the solution used for density separation was free from contaminants that could affect the results. Including this information would enhance the reproducibility and accuracy of the methodology.	We have carefully revised the manuscript to address your concern. We have clarified that the salt used for the saline solution was indeed filtered to remove impurities. This detail has been included to ensure that the solution used for density separation was free from contaminants, thereby enhancing the reproducibility and accuracy of our methodology. (Now: "Microplastic extraction from sediment"(Lines 89-

- | | | |
|-----|---|--|
| 6. | Procedures for quality assurance and contamination prevention" Section – It is essential to include a dedicated quality control section in every stage of the study. In addition to adopting widely accepted methods for contamination prevention, it is crucial to analyze samples alongside appropriate controls. Subtracting the particles identified in the controls from the sample dataset is an indispensable measure to avoid inaccurate assessments and ensure the integrity of the results. | In the “Procedures for Quality Assurance and Prevention of Contamination” section, we have now included a dedicated quality control section, as recommended, to detail the steps taken at each stage of the study to ensure data integrity. |
| 7. | Please, pay attention to the spacing between topics and figures. | We have carefully revised the spacing between topics and figures in accordance with your recommendations to ensure clarity and readability. |
| 8. | Images of equations can be problematic for people with visual impairments who use screen readers. You can provide a textual description or an alternative accessible format. There are tools that allow you to create equations in formats that are both visually appealing and accessible. LaTeX, for example, is a widely used language for writing scientific documents that allows you to create equations in an efficient and accessible way. | <p>Thank you very much for your very useful feedback on the accessibility of the equations in our manuscript. We have taken into consideration the reviewer's suggestions to ensure that the document is fully accessible, especially for readers with visual impairments. However, since the submitted file is (.doc), we are unable to enter the formulas accurately as the equation feature is not supported in this instrument. As a result, the formula is still in the form of an image.</p> <p>In response, we described the formula in a text statement in the manuscript. In addition, we added LaTeX-based equations to the comment feature when needed for publication.</p> |
| 9. | Suggested reorganization: To improve the flow and clarity of your paper, I recommend combining the Results and Discussion statements into a single section, titled “Results and Discussion.” This approach allows the results to be immediately contextualized and interpreted, making them easier for the reader to understand. Despite having this section in the paper, the authors separate the discussion from the results by creating a new section called "Discussion". This information can be confusing to the reader. | We have carefully revised the manuscript according to your suggestion to combine the Results and Discussion sections into a single, integrated section titled “Results and Discussion”. |
| 10. | Terminology Consistency: Be consistent with the terms used to describe microplastics (e.g., “items,” “particles,” “fragments”). This will prevent confusion and improve clarity. | We have revised the manuscript to ensure consistency in the terminology used to describe microplastics. Specifically, we have standardized terms (e.g., “object,” “particle,” and “fragment”) throughout the manuscript, with the term “particle”. We are conforming to our previous publications, for consistency throughout our work on microplastics. |

- | | | |
|-----|---|---|
| 11. | While you mention human activities contributing to microplastic pollution, a deeper exploration of local sources and their specific impacts on the estuarine ecosystem would enhance the environmental perception of your work. | we have revised the manuscript to include a more detailed analysis of the local sources contributing to microplastic contamination. We believe this additional information enhances the environmental context of the study and provides a clearer understanding of the specific impacts on the estuarine ecosystem. (Lines 205-223) |
| 12. | While you compare your findings to other regions, it would be beneficial to draw more explicit connections regarding why the Musi River Estuary exhibits different levels of microplastic contamination. Discuss potential socio-economic or environmental factors influencing these differences. | We have revised the manuscript to address your suggestions. In the updated version, we have expanded the discussion on potential socio-economic and environmental factors that could contribute to differences in microplastic contamination levels in the Musi River Estuary compared to other areas. (Lines 244-271) |
| 13. | Expand on the ecological implications of microplastic pollution, particularly how it affects local species and ecosystems. Discuss potential long-term impacts on biodiversity, food webs, and human health. | We have carefully revised the manuscript to expand on the ecological implications of microplastic pollution, particularly its effects on local species and ecosystems. We have included a more detailed discussion on the potential long-term impacts on biodiversity, food webs, and human health, as you recommended. (Lines 430-448) |
| 14. | The risk assessment section should more clearly articulate what the identified risk values mean for local ecosystems and communities. Consider discussing the potential health risks for consumers of blood clams and the broader implications for local fisheries. | We have carefully revised the risk assessment section in accordance with your recommendation. The revised version now provides a clearer explanation of what the identified risk values mean for local ecosystems and communities. Additionally, we have included a discussion on the potential health risks for consumers of blood clams and elaborated on the broader implications for local fisheries. (Lines 449 - 461) |
| 15. | While you mention hydrodynamic conditions, consider elaborating on how seasonal variations or climatic events might influence microplastic distribution and accumulation in the estuary. | We have elaborated on this aspect in the revised manuscript, discussing how these factors may influence the behavior of microplastics in estuarine environments, including the influence of seasonality. (Lines 302-321) |
| 16. | I suggest that the authors consider including recommendations for mitigating microplastic pollution based on their findings. Providing actionable insights for local stakeholders could enhance the practical relevance of the study and contribute to more effective environmental protection efforts in the region. | As per your suggestion, we have revised the manuscript to incorporate actionable insights for local stakeholders. We believe these additions will enhance the practical relevance of the study and contribute to more effective environmental protection efforts in the region. (Lines 462-482) |
| 17. | Conclude with a succinct summary of your key findings and their significance. Emphasize the urgency for monitoring and management strategies to address microplastic pollution in the Musi River Estuary. | A concise summary of our key findings has been provided, which emphasizes the significant presence of microplastics in sediments and blood clams in the Musi River Estuary. We also highlighted |

the urgency of implementing effective monitoring and management strategies to address the growing problem of microplastic pollution in the region. (Lines 483-493).



[biodiv] Editor Decision

1 pesan

Smujo Editors via SMUJO <support@smujo.com>
Balas Ke: Smujo Editors <editors@smujo.id>
Kepada: Rozirwan Rozirwan <rozirwan@unsri.ac.id>

18 November 2024 pukul 06.38

Rozirwan Rozirwan:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of microplastics presence in sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.** We are waiting for your revision in the system (<https://smujo.id/biodiv>), do not send it via email.

Our decision is: Revisions Required

Reviewer A:

Dear author

This article needs some work. All comments are available in the text. Send the revision file along with a rebuttal letter in a separate file

Recommendation: Revisions Required

[Biodiversitas Journal of Biological Diversity](#)

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 **A-19666 1.doc**
4704K

Author(s) Response to Reviewer :

Dear Reviewer,

We sincerely thank you for taking the time to thoroughly review our manuscript and for providing such insightful feedback, which will help improve the quality of our work. In response to your comments, we have made the necessary revisions to the manuscript. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Authors

Table of Author(s) Response

No	Comments	Response
1.	Title in Lines 1; Title change from “Implication of microplastics presence in sediment and blood clams <i>Anadara granosa</i> (Linnaeus, 1758) (Molusca: Bivalvia) in the Musi Estuary, Indonesia” to: “Implication of microplastics presence in sediment and blood clams <i>Anadara granosa</i> (Linnaeus, 1758) (Molusca: Bivalvia) in the Musi Estuary, Indonesia	We agree with your suggested title revision and have updated it to: Implication of microplastic presence in sediment and blood clams (<i>Anadara granosa</i> , Linnaeus 1758) (Mollusca: Bivalvia) in the Musi Estuary, Indonesia.”
2.	The introduction starts from: 1. General condition of the estuary ecosystem 2. The importance of macrozoobenthos as a bioindicator 3. Then go into general pollution before getting into the topic of microplastics	We have revised the introduction to follow the structure as outlined: 1. General condition of the estuary ecosystem 2. The importance of macrozoobenthos as a bioindicator 3. A discussion on general pollution, leading into the topic of microplastics. The order of the introduction has been revised significantly, as suggested by the reviewer.
3.	Lines 44; Add reference for: Blood clams <i>Anadara granosa</i> are benthic bivalves commonly consumed as seafood	Due to the change in the order of the introduction, we have rearranged the sentences to eliminate any ambiguity Lines 49: Among these, <i>A. granosa</i> is a prominent bivalve species that plays a crucial role in maintaining ecosystem stability and generating substantial economic benefits for coastal communities (Yulinda et al. 2020; Prasetyono et al. 2022; Mahary et al. 2023). G
4.	Delete ‘blood clams’ for the next text. Only use <i>Anadara granosa</i> or <i>A. granosa</i>	We have removed the term "blood clam" as requested and have only used it at the beginning as an introductory term. From that point onward, we refer to the species as <i>A. granosa</i> .
5.	Mention as <i>A. granosa</i> for the next text	We have removed the term "blood clam" as requested and have only used it at the beginning as an introductory term. From

6.	Provide all detailed location in the Table, such as coordinates dan description of the sites	that point onward, we refer to the species as <i>A. granosa</i> . We have added the coordinates and descriptions to the table as requested. Additionally, we have adjusted the numbering of the table on entry.
7.	Method Chapter: Provide all detailed references for the extraction	We have revised the manuscript to include all detailed references related to the extraction process as requested.
8.	Data analysis; Pollution Load Index; See guideline of cites	We have revised the reference writing and refer to the guidelines. Lines 203
9.	Provide the figures of sample	We have included the sample figures as requested and have updated the manuscript accordingly (Line 400).
10.	is the reference font size appropriate?	We have reviewed the font size of the references and confirmed that it complies with the journal's guidelines. If any adjustments are needed, we will be happy to make the necessary changes.

Implication of microplastics presence in sediment and blood clams (*Anadara granosa* (Linnaeus, 1758) (Molusca: Bivalvia)) in the Musi Estuary, Indonesia

Abstract. Microplastic pollution poses a serious risk to estuarine ecosystems, affecting sediments and benthic species. Blood clams (*Anadara granosa*) represent a significant commercial seafood product in Indonesia, including those from the Musi River Estuary. This study investigated the risks associated with microplastic contamination in sediments and blood clams (*Anadara granosa*) in the Musi River Estuary, located in South Sumatra, Indonesia. Sediment samples were extracted using sodium chloride (NaCl, 1.2 g cm⁻³) and hydrogen peroxide (30% H₂O₂). In contrast, blood clam samples were digested with 10% KOH for microplastic extraction. The abundance and shape of microplastics were identified from both samples. Pollution risk assessment was conducted through the calculation of Pollution Load Index (PLI), Nemerow Pollution Index (NPI), and Bioconcentration Factor (BCF). The results showed the presence of microplastics in sediments, with a mean abundance of 1.31±0.41 particles/g dw, while in blood clams, it was 21.05±10.31 particles/ind. Both samples exhibited high microplastic bioaccumulation (NPI > 2), although the pollution load remained relatively low (minor category, PLI < 10). The bioconcentration factor between blood clams and sediment was determined to be 23.28, indicating that microplastics present in the sediment were absorbed by the blood clams. These findings highlight the significant bioaccumulation potential of microplastics in blood clams within the Musi River Estuary. It is important for the community and local government to establish mitigations for future microplastic management efforts.

Keywords: Bioaccumulation, blood clams, microplastics, Musi Estuary, sediment

Running title: Microplastics in Sediment and Blood Clams

INTRODUCTION

Plastic waste contamination in aquatic ecosystems has become a major global issue in recent decades (Borrelle et al. 2020; Hecker et al. 2023). Over the past five decades, the widespread use of single-use plastic products has skyrocketed, leading to their fragmentation in the environment (Zhang et al. 2021; Walker and Fequet 2023). As plastics degrade, they form microplastics that range in size from 1 µm to 5 mm (Frias and Nash 2019). Various sources, including products like facial cleansers, scrubs, and toothpaste that intentionally contain these particles, introduce these microplastics into the environment (Napper et al. 2015; Praveena et al. 2018). Washing synthetic clothing releases fine fibers into water systems, significantly contributing to microplastic pollution (Belzagui and Gutiérrez-Bouzán 2022). Moreover, washing synthetic clothing releases fine fibers into water systems, significantly contributing to microplastic pollution (Gan et al. 2023). Microplastics are particularly concerning because they release toxic additives and serve as vectors for organic pollutants and heavy metals (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). As a consequence, microplastics have the potential to disrupt food chains, alter nutrient cycling, change habitats, and affect the genetic composition of organisms (Thacharodi et al. 2024). Given these characteristics, microplastics pose a growing threat to ecosystems worldwide, particularly aquatic ecosystems (Patria et al. 2023).

The presence of microplastics has become pervasive in almost all global environments, including sediments (C. Wang et al. 2021). It is estimated that approximately 3.05 million tons of microplastics have been deposited in marine sediments to date (Harris et al. 2023). Estuaries are particularly vulnerable to microplastic deposition, ranking as the second largest sink for microplastics after fjords (Harris 2020). Estuaries serve as major conduits for plastic waste entering the ocean, with approximately 70% of microplastics transported through estuaries ending up in marine sediments (Frias et al. 2016). High levels of microplastics in estuarine sediments can threaten aquatic ecosystems, especially benthic organisms that depend on sediments for their habitat. Various studies around the world have shown that benthic organisms, especially bivalves, are filter feeders, which can lead to significant microplastic uptake (Ward et al. 2019; Theerachat et al. 2020; Ding et al. 2021; Bonifacio et al. 2022). Blood clams (*Anadara granosa* (Linnaeus, 1758)) are benthic bivalves commonly consumed as seafood (Theerachat et al. 2020). In Indonesia, these clams hold substantial economic value and contribute significantly to coastal area economies (Yulinda et al. 2020; Prasetyono et al. 2022; Mahary et al. 2023). Due to their filter-feeding mechanism, blood clams are prone to accumulating microplastics from sediments in their digestive tracts (Fitri and Patria 2019; Saleh et al. 2023; Rahmatin et al. 2024). Consequently, the presence of microplastics in both sediment and clams raises ecological concerns and poses health risks for humans who consume these clams.

Blood clams are commonly found in coastal areas and estuaries across Indonesia, including the Musi River Estuary. This estuary serves as a fishing ground and a habitat for various benthic species (Rozirwan et al. 2021; Rozirwan et al. 2022). However, intense human activity has resulted in the contamination of both the watershed and the estuary with various pollutants (Rahutami et al. 2022; Rozirwan et al. 2024). Plastic contamination is one of the biggest ecosystem

Commented [VH1]: The introduction starts from:
1. General condition of the estuary ecosystem
2. The importance of macrozoobenthos as a bioindicator
3. Then go into general pollution before getting into the topic of microplastics

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53 threats in these areas. Previous research has shown that the Musi River Estuary waters are polluted by microplastics, with
 54 concentrations ranging from 467.67 ± 127.84 particles/m³ during flood tide to 723.67 ± 112.05 particles/m³ during ebb tide
 55 (Diansyah et al. 2024). Additionally, harmful heavy metals like lead (Pb) and copper (Cu) have been detected on the
 56 surfaces of these microplastics (Purwiyanto et al. 2022). However, research on the presence of microplastics in sediments
 57 and their bioaccumulation in benthic species used as bioindicators remains limited. This study aims to investigate the
 58 bioaccumulation and risks of microplastic pollution in sediments and blood clams (*A. nodosa-granosa*) in the Musi River
 59 Estuary, Indonesia. It is expected to provide deeper insights into microplastic bioaccumulation in benthic organisms,
 60 especially blood clams, and its potential health risks for local seafood consumers.

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61 **MATERIALS AND METHODS**

62 **Study Area and Sample Collection**

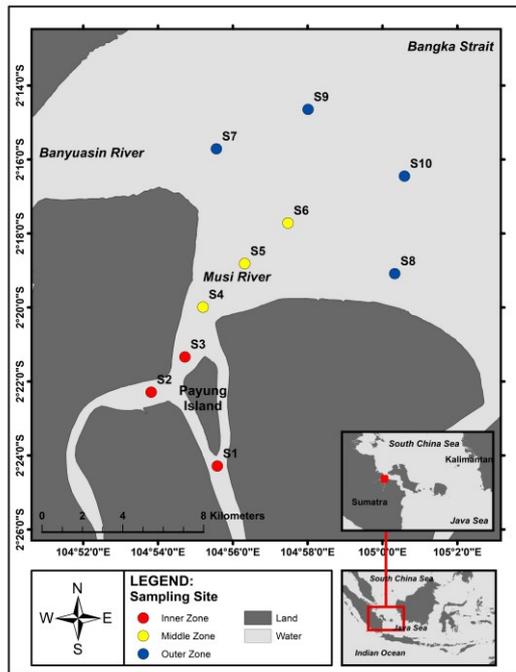
63 The Musi River estuary, located on the east coast of South Sumatra, is where seawater from the Bangka Strait mixes
 64 with freshwater from the Musi River. The Musi River is an important area for fisheries and benthic habitats in South
 65 Sumatra, Indonesia (Rozirwan et al. 2021; Rozirwan et al. 2022). Sample sites were selected based on the flow direction
 66 and the possibility of sediment deposition in the estuary. Sediment and blood clams were collected during low tide from
 67 the Musi River Estuary, South Sumatra, Indonesia. Locations of the sampling sites are shown in Figure 1. Sediment
 68 samples were collected from ten sampling points, and a Peterson Grab was used at each station (Dwiyitno et al. 2024).
 69 Samples were placed in glass jars that had been rinsed with pure distilled water and covered using aluminum foil to avoid
 70 contamination. Samples were placed in a cool box ($\pm 4^\circ\text{C}$) for further analysis in the laboratory.

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71 Blood clam samples (*Anadara granosa*) were identified based on their morphological traits according to the World
 72 Register of Marine Species (WoRMS) (Abelouah et al. 2024). Twenty adult blood clams (7.48–11.44 cm) were collected
 73 from fishermen's catch in the Musi River Estuary. Blood clam samples (*Anadara granosa*) were identified based on their
 74 morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). The mussels
 75 were rinsed with pure distilled water to remove any dirt. Blood clams were wrapped in aluminum foil and stored in a
 76 cooler at approximately 4 °C to preserve their freshness. After that, they were transported to the laboratory and frozen at -
 77 20°C before further analysis (Ding et al. 2021).

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78 **Figure 1.** Study site at the Musi River Estuary, Indonesia
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Microplastic extraction from sediment

Microplastics in sediments were extracted using methods of density separation and organic matter removal. A total of 500 g of wet sediment samples were placed in a beaker glass covered with aluminum foil. The samples were dried in an oven at 60 °C for 48 hours, or until they reached a constant mass. The sediment samples were pulverized and sieved through a 5 mm steel sieve. A portion of 100 g of dried sediment was taken from the sieve, placed into a glass cup, and covered with aluminum foil to prevent external contamination. The sample was suspended in 400 mL of saturated NaCl solution (1.2 g cm⁻³), which is four times the sample weight, using a magnetic stirrer. This NaCl solution was prepared by dissolving pure NaCl crystals (Merck Millipore EMSURE®) in filtered distilled water that was free from contaminants. The selection of a NaCl solution for the separation of microplastics from sediments was based on the premise that this method is cost-effective and environmentally friendly (Perumal and Muthuramalingam 2022). Stirring was carried out for 5 minutes until completely dissolved, and the mixture was allowed to stand for 1 hour. After one hour, 10 mL of 30% H₂O₂ was added to assist in the breakdown of organic matter, followed by stirring for an additional five minutes. Hydrogen peroxide (H₂O₂) is commonly used in similar studies and is regarded as highly effective for organic matter removal (Lee et al. 2023). The sample was allowed to stand for 24 hours, covered with aluminum foil. The samples were then filtered using the Whatman No. 42 filter paper (mesh size 0.45 µm, Φ = 90 mm), assisted by a vacuum pump at 17 kPa to isolate the microplastics. The collected samples were deposited into petri dishes for identification.

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Microplastic extraction from blood clams

The previously frozen blood clam samples were defrosted under controlled conditions at room temperature (± 25°C) for 1 hour. After the ice melted, the blood clams were washed with pure distilled water to eliminate contamination from other objects, such as sediment. The length and wet weight of the blood clams were measured using an analytical balance (0.01 g). The digestive tract was carefully removed from the blood clams using sterile stainless steel utensils that had been rinsed with distilled water. The extraction of microplastics from the digestive tract of the blood clams was adapted from previous studies (Ding et al. 2018; Ding et al. 2021). The digestive tract was transferred into an Erlenmeyer flask, and 100 mL of KOH solution (10%) was added. The use of KOH is regarded as a more efficacious method for the digestion of biological material, and it has no impact on the integrity of the plastic polymer (Karami et al. 2017). The sample was covered with aluminum foil and stirred for 5 minutes using a 150 rpm magnetic stirrer. The supernatant was incubated at room temperature (±25°C) until complete digestion of organic matter occurred. The sample was then filtered through Whatman 42 filter paper (mesh size 0.45 µm, Φ = 90 mm) to isolate microplastic. The filter paper was dried in an oven at 40°C for 5 hours and stored in petri dishes for subsequent identification of microplastic content.

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Microplastic identification and quantification

Identification and quantification of microplastics from sediments and blood clam were performed using the same method. Microplastic identification was performed using an Olympus CX23 microscope with 10 x 10 or 4 x 10 magnification to visually detect microplastics. The filter paper was observed closely and carefully to avoid contaminants that could enter the filter paper. Microplastics were identified based on the number and form of microplastics (fragment, film, fiber, and foam). The data obtained were recorded by sample type and station/individual for statistical purposes. To prevent contamination, personnel wear cotton lab coats, latex gloves, and cotton masks. Furthermore, access to the detection room is restricted to prevent outside contact during observations.

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Procedures for quality assurance and contamination prevention

The quality and contamination control measures were implemented to prevent any alterations to the microplastics in the samples. Before sampling, glass jars and aluminum foil were rinsed with filtered distilled water. Although the samples were in a cool box, they were stored in a place that was not exposed to direct sunlight to keep the samples in good condition. Personnel are also required to wear latex gloves during sampling. During the study, from sampling to laboratory analysis, the use of plastic equipment was minimized to avoid unintentional fragmentation of the microplastics. The distilled water used was filtered with filter paper (0.45 µm) to prevent microplastics from entering our materials. All equipment was washed with filtered distilled water to remove potential contamination from external particles. We prepared two controls by filtering each solution we used to avoid contaminants from materials, equipment, and air. No microplastics were found contaminating the instrument. The sterilized equipment was wrapped in aluminum foil to prevent any input of contaminants from outside. Laboratory personnel were required to wear latex gloves, lab coats, and masks throughout the analysis. Access to the laboratory was restricted during the analysis to minimize external interference that could lead to protocol errors.

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Data analysis

Pollution Load Index (PLI)

PLI was calculated using a pollutant load approach, which assesses the total concentration of contaminants relative to a baseline level, to evaluate its significance. Equations were utilized to quantify the level of microplastic pollution in both sediments and the blood clam population in the Musi River Estuary (G. Wang et al. 2021).

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Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 27. Descriptive statistics, including total, mean, standard deviation, percentage, minimum, and maximum, were utilized to assess data variation. A one-way ANOVA was applied to analyze differences in microplastic abundance between estuarine zones when the data met normality assumptions. For non-normal data, the Kruskal-Wallis test was employed to assess the significance of differences between zones. All tests were carried out with a significance level of $\alpha = 0.05$.

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RESULTS AND DISCUSSION

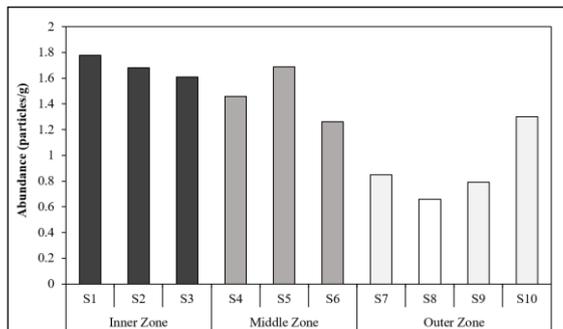
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Microplastic presence in sediment from Musi River Estuary

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The results of this study indicate that microplastics were detected at all sampling stations, as shown in Figure 2. A total of 1,308 microplastic particles were identified, with concentrations ranging from 66 to 178 particles per 100 g of dry sediment (dry weight, dw). The average abundance of microplastics was 1.31 ± 0.41 particles/g dw, with the highest concentration found at station S1 (1.78 particles/g dw) and the lowest at station S8 (0.66 particles/g dw). High levels of human activity in the upstream area of Muara Musi, which flows downstream to the estuary and ultimately into the ocean, are responsible for the presence of microplastics (Rozirwan et al. 2021; Diansyah et al. 2024). Urban activities in close proximity to the Musi River Watershed, particularly in Palembang City, significantly contribute to the generation of plastic waste. Increased population density near riverbanks enhances the potential for plastic pollution. Furthermore, population density is a crucial factor in the entry of microplastics into rivers (Eo et al. 2023; Dwiyoitno et al. 2024). As a result, plastic pollutants from domestic activities, such as the disposal of food packaging and plastic bags, can be transported through stormwater drains and eventually end up in rivers (Kunz et al. 2023). Fishing activities around Muara Musi can contribute to microplastic pollution, primarily through fibers released from fishing nets (Z. Li et al. 2022; Fauziyah et al. 2023). This increases anthropogenic pressure at river mouths, which can lead to greater accumulation of microplastics (Castro-Jiménez et al. 2024). Additionally, tidal fluctuation influences water flow, which facilitates the deposition of microplastics in sediment at these locations (Harris 2020). The findings of this study indicate that anthropogenic activities are responsible for high levels of microplastic deposition in the sediments of Musi Estuary. Microplastic particles that accumulate in sediments serve as a habitat for benthic organisms, which can have long-term implications for ecosystem health and balance (Rahmatin et al. 2024). As vectors for pollutants, microplastics can exacerbate toxicity, potentially endangering various populations (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). Furthermore, the processes of bioaccumulation and biomagnification in benthic organisms present heightened risks to these animals, potentially leading to human consumption (Unuofin and Igwaran 2023). Therefore, it is crucial for local governments to address the handling and prevention of further microplastic accumulation.



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Figure 2. Distribution of microplastics from sediments in Musi Estuary, Indonesia.

483 stricter waste management practices, particularly for plastic disposal, to reduce further contamination. A comprehensive
484 strategy is essential to controlling plastic waste throughout the river system—from source to estuary. Effective measures
485 should include coordinated waste management practices, public education on the impact of pollution, and cross-sector
486 collaboration to preserve estuarine health. Such initiatives are crucial to protecting ecosystem health and sustaining local
487 fisheries, ensuring these resources continue to support community well-being.

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[biodiv] Editor Decision

1 pesan

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Balas Ke: Smujo Editors <editors@smujo.id>
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Rozirwan Rozirwan:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of microplastics presence in sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.** We are waiting for your revision in the system (<https://smujo.id/biodiv>), do not send it via email.

Our decision is: Revisions Required

Reviewer A:

This article has interesting and structured content but needs some revision. All suggestions are available in the text. Send revised files accompanied by track changes and a letter of objection in a separate file

Recommendation: Revisions Required

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Author(s) Response to Reviewer :

Dear Reviewer,

We sincerely appreciate your time and effort in reviewing our manuscript and providing valuable feedback. Your insightful comments have been instrumental in improving the quality of our work. In response, we have carefully revised the manuscript accordingly. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Author (s)

Table of Author(s) Response

No	Comments	Respons
1.	Add references in Introduction	Thank you for your valuable suggestion. We have added relevant references in the Introduction section to support our statements and provide a stronger foundation for our study (Lines 29-41) .
2.	Need justification and reference that this species is also a food source for local communities	We have provided justification and added relevant references to support that this species is a food source for local communities. The revised manuscript now includes citations highlighting its consumption and nutritional importance (Lines 49-50) .
3.	Full name for the start of the sentence	Thank you for your comment. We have revised the manuscript to ensure that the full scientific name, <i>Anadara granosa</i> , is used at the beginning of sentences, while the abbreviated form (<i>A. granosa</i>) is used appropriately thereafter (Every occurrence of <i>Anadara granosa</i> at the beginning of a sentence)
4.	Add a brief description of the conditions of each site	We have added a brief description of the environmental conditions in each sampling zone in the revised manuscript (Lines 103-104) .
5.	Provide the figure of general condition of the sampling site	We have included a figure illustrating the general environmental conditions of the sampling site in the revised manuscript. (Lines 247-250) .
6.	Add references in last result	We have added relevant references in result section to support our statements and provide a stronger foundation for our study (Lines 495-514)
7.	See guideline of references	We have revised the references to ensure they comply with the journal's guidelines (Lines 523-Last)

COVERING LETTER

Dear **Editor-in-Chief**,

I herewith enclosed a research article,

- The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
- The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.
- Most of the references come from current scientific journals (c. 80% published in the last 10 years), except for taxonomic papers.
- Where available, DOIs for the references have been provided.
- When available, a certificate for proofreading is included.

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

- E-mail address
- Full postal address (incl street name and number (location), city, postal code, state/province, country)
- Phone and facsimile numbers (incl country phone code)

All necessary files have been uploaded, and contain:

- Keywords
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Further considerations

- Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
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- Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate

Title:

Implication of microplastic presence in sediment and blood clams *Anadara granosa* (Linnaeus, 1758) (Mollusca: Bivalvia) in the Musi Estuary, Indonesia

Author(s) name:

M. AKBAR RAHMAN¹, ICA DELYA², ROZIRWAN^{2*}, WIKE EKA AYU PUTRI², GUSTI DIANSYAH², MELKI²

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This research is groundbreaking because there is currently no information available on the abundance of microplastics in sediments of the Musi Estuary, Indonesia. This article provides a comprehensive review of recent research on microplastics in the sediments of the Musi Estuary and their impact on benthic animals (*Anadara granosa*), one of the seafood from the Musi Estuary.

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Rozirwan

49 and Patria 2019; Rahmatin et al. 2024). *Anadara granosa* is widely consumed by the general public as a nutrient-rich
50 seafood, particularly among local communities (Rozirwan, Nugroho, et al. 2022; Rozirwan, Ramadani, et al. 2023) Among
51 these, *A. granosa* is a prominent bivalve species that plays a crucial role in maintaining ecosystem stability and generating
52 substantial economic benefits for coastal communities (Prasetyono et al. 2022; Yulinda et al. 2020; Mahary et al. 2023).
53 Given its ecological importance and economic value within coastal and estuarine systems, it is imperative to investigate
54 pollutant contamination in *A. granosa* to comprehensively understand the broader impacts of environmental pollution.

55 Various pollutants, including heavy metals, hydrocarbons, pesticides, and microplastics, have been identified as
56 significant contaminants of estuarine ecosystems, with many of these pollutants accumulating in sediments (Kılıç et al.
57 2023; Han et al. 2024; Jacq et al. 2024; Zhao J et al. 2015). Among these, plastic pollution has emerged as a critical
58 concern due to its pervasive impact on aquatic environments (Hecker et al. 2023; Borrelle et al. 2020). Over the last five
59 decades, the widespread reliance on single-use plastics has led to extensive accumulation and fragmentation of plastic
60 materials across diverse ecosystems (Walker and Fequet 2023; Zhang K et al. 2021). As plastics degrade, they break down
61 into microplastics, defined as particles ranging in size from 1 μm to 5 mm (Frias and Nash 2019). These microplastics
62 enter aquatic ecosystems through various pathways, such as the fragmentation of discarded plastic products, the release of
63 synthetic fibers during washing, and the intentional addition of microbeads in personal care products (Belzagui and
64 Gutiérrez-Bouzán 2022; Gan et al. 2023; Napper et al. 2015; Praveena et al. 2018). Microplastics pose multiple threats by
65 releasing toxic additives and acting as carriers of hazardous organic pollutants and heavy metals (Caruso 2019; Issac and
66 Kandasubramanian 2021; Fu et al. 2021; Ta and Babel 2023). These impacts are multifaceted, disrupting food webs,
67 altering nutrient cycles, modifying habitats, and potentially inducing genetic changes in aquatic organisms (Thacharodi et
68 al. 2024).

69 Prior research has quantified microplastic concentrations in the waters of the Musi Estuary, with reported levels of
70 467.67 ± 127.84 particles/ m^3 during flood tide and 723.67 ± 112.05 particles/ m^3 at ebb tide (Diansyah et al. 2024). These
71 microplastics are frequently associated with toxic heavy metals, such as lead (Pb) and copper (Cu), which exacerbate their
72 harmful environmental effects (Purwiyanto et al. 2022). In the water column, microplastics tend to settle into estuarine
73 sediments, which have been identified as the second-largest global reservoir for microplastics after fjords (Harris 2020).
74 The accumulation of microplastics in estuarine sediments poses significant ecological challenges, particularly due to their
75 ingestion by benthic organisms, including *Anadara granosa* (Mohan et al. 2024; Saleh et al. 2023; Fitri and Patria 2019;
76 Rahmatin et al. 2024). While numerous studies have examined microplastic concentrations in the water column, research
77 on their distribution in sediments and bioaccumulation in benthic species, particularly *Anadara granosa*, remains limited.

78 This study aims to investigate the bioaccumulation and risks of microplastic pollution in sediments and *A. granosa* in
79 the Musi Estuary, Indonesia. The findings are expected to enhance understanding of benthic organisms, especially *A.*
80 *granosa*, and its potential health risks for local seafood consumers.

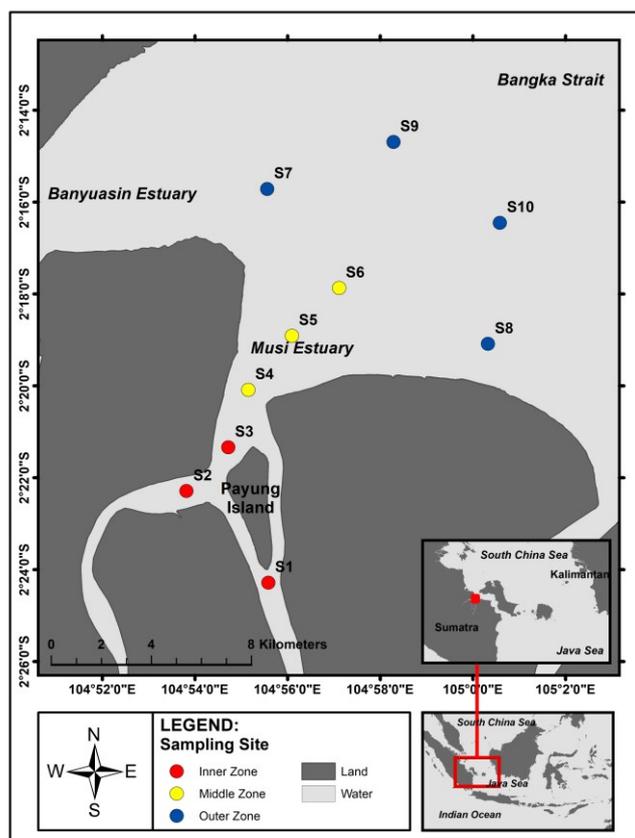
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MATERIALS AND METHODS

82 Study Area and Sample Collection

83 The Musi Estuary, located on the east coast of South Sumatra, is where seawater from the Bangka Strait mixes with
84 freshwater from the Musi River. The Musi Estuary is an important area for fisheries and benthic habitats in South Sumatra,
85 Indonesia (Rozirwan et al. 2021; Rozirwan, Fauziah, Wulandari, et al. 2022). Sampling locations were selected based on
86 hydrodynamic conditions influencing microplastic transport and deposition, considering salinity variations and water mass
87 interactions (Cheng et al. 2024; Diansyah et al. 2024). Locations of the sampling sites are shown in Figure 1 and Table 1.
88 Sediment and *A. granosa* were collected during low tide from the Musi Estuary, South Sumatra, Indonesia. Sediment
89 samples were collected from ten sampling points using a Peterson Grab at each station (Dwiyitno et al. 2024). Samples
90 were placed in glass jars that had been rinsed with pure distilled water and covered using aluminum foil to avoid
91 contamination. Samples were placed in a cool box ($\pm 4^\circ\text{C}$) for further analysis in the laboratory.

92 *Anadara granosa* samples were identified based on their morphological traits according to the World Register of
93 Marine Species (WoRMS) (Abelouah et al. 2024). Twenty adult *A. granosa* (7.48–11.44 cm) were collected from
94 fishermen's catch in the Musi Estuary. *Anadara granosa* samples were identified based on their morphological traits
95 according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). The mussels were rinsed with pure
96 distilled water to remove any dirt. *A. granosa* were wrapped in aluminum foil and stored in a cooler at approximately 4°C
97 to preserve their freshness. After that, they were transported to the laboratory and frozen at -20°C before further analysis
98 (Ding J et al. 2021).



99

100 **Figure 1.** Study site at the Musi Estuary, Indonesia.

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Table 1. Coordinates of the sampling location.

Estuary Zone	Description	Station	Latitude (°S)	Longitude (°E)
Inner Zone	Predominantly influenced by freshwater, with low salinity (<10 PSU)	S1	2.40470	104.92643
		S2	2.37144	104.89695
		S3	2.35558	104.91200
Middle Zone	A mixing zone of freshwater and seawater, with salinity ranging from 10 to 20 PSU	S4	2.33316	104.92003
		S5	2.31353	104.93862
		S6	2.29524	104.95794
Outer Zone	Primarily dominated by seawater, with high salinity (>20 PSU)	S7	2.26183	104.92603
		S8	2.31807	105.00547
		S9	2.24403	104.96686
		S10	2.27413	105.00978

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Microplastic extraction from sediment

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Microplastics in sediments were extracted using the standard method of density separation and organic matter removal with minor modifications (Thompson et al. 2004; Liebezeit and Dubaish 2012). A total of 500 g of wet sediment samples were placed in a beaker glass covered with aluminum foil. The samples were dried in an oven at 60 °C for 48 hours, or until they reached a constant mass (Patria et al. 2023). The sediment samples were pulverized and sieved through a 5 mm steel sieve. A portion of 100 g of dried sediment was taken from the sieve, placed into a beaker glass, and covered with aluminum foil to prevent external contamination. The sample was suspended in 400 mL of saturated NaCl solution (1.2 g cm⁻³), which is four times the sample weight, using a magnetic stirrer. This NaCl solution was prepared by dissolving pure NaCl crystals (Merck Millipore EMSURE®) in filtered distilled water that was free from contaminants. The selection of a NaCl solution for the separation of microplastics from sediments was based on the premise that this method is cost-effective and environmentally friendly (Perumal and Muthuramalingam 2022). Stirring was carried out for 5 minutes until completely dissolved, and the mixture was allowed to stand for 1 hour. After one hour, 10 mL of 30% H₂O₂ was added to assist in the breakdown of organic matter, followed by stirring for an additional five minutes. Hydrogen peroxide (H₂O₂) is

119 commonly used in similar studies and is regarded as highly effective for organic matter removal (Lee et al. 2023). The
120 sample was allowed to stand for 24 hours, covered with aluminum foil. The samples were then filtered using the Whatman
121 No. 42 filter paper (mesh size 0.45 μm , $\Phi = 90$ mm), assisted by a vacuum pump at 17 kPa to isolate the microplastics.
122 The collected samples were deposited into petri dishes for identification.
123

124 **Microplastic extraction from *Anadara granosa***

125 The previously frozen *A. granosa* samples were defrosted under controlled conditions at room temperature ($\pm 25^\circ\text{C}$) for
126 1 hour. After the ice melted, the *A. granosa* were washed with pure distilled water to eliminate contamination from other
127 objects, such as sediment. The length and wet weight of the *A. granosa* were measured using an analytical balance (0.01
128 g). The digestive tract was carefully removed from the *A. granosa* using sterile stainless steel utensils that had been rinsed
129 with distilled water. The extraction of microplastics from the digestive tract of the *A. granosa* was adapted from previous
130 studies with minor modifications (Ding J-F et al. 2018; Ding J et al. 2021). The digestive tract was transferred into an
131 Erlenmeyer flask, and 100 mL of KOH solution (10%) was added. The use of KOH is regarded as a more efficacious
132 method for the digestion of biological material, and it has no impact on the integrity of the plastic polymer (Karami et al.
133 2017). The sample was covered with aluminum foil and stirred for 5 minutes using a 150 rpm magnetic stirrer. The
134 supernatant was incubated at room temperature ($\pm 25^\circ\text{C}$) until complete digestion of organic matter occurred. The sample
135 was then filtered through Whatman 42 filter paper (mesh size 0.45 μm , $\Phi = 90$ mm) to isolate microplastic. The filter
136 paper was dried in an oven at 40°C for 5 hours and stored in petri dishes for subsequent identification of microplastic
137 content.
138

139 **Microplastic identification and quantification**

140 Identification and quantification of microplastics from sediments and *A. granosa* were performed using the same
141 method. Microplastic identification was performed using an Olympus CX23 microscope with 10 x 10 or 4 x 10
142 magnification to visually detect microplastics (Diansyah et al. 2024). The filter paper was observed closely and carefully
143 to avoid contaminants that could enter the filter paper. Microplastics were identified based on the number and form of
144 microplastics (fragment, film, fiber, and foam). The data obtained were recorded by sample type and station/individual for
145 statistical purposes. To prevent contamination, personnel wear cotton lab coats, latex gloves, and cotton masks.
146 Furthermore, access to the detection room is restricted to prevent outside contact during observations.
147

148 **Procedures for quality assurance and contamination prevention**

149 The quality and contamination control measures were implemented to prevent any alterations to the microplastics in
150 the samples. Before sampling, glass jars and aluminum foil were rinsed with filtered distilled water. Although the samples
151 were in a cool box, they were stored in a place that was not exposed to direct sunlight to keep the samples in good
152 condition. Personnel are also required to wear latex gloves during sampling. During the study, from sampling to laboratory
153 analysis, the use of plastic equipment was minimized to avoid unintentional fragmentation of the microplastics. The
154 distilled water used was filtered with filter paper (0.45 μm) to prevent microplastics from entering our materials. All
155 equipment was washed with filtered distilled water to remove potential contamination from external particles (Ding J et al.
156 2021). We prepared two controls by filtering each solution we used to avoid contaminants from materials, equipment, and
157 air. No microplastics were found contaminating the instrument. The sterilized equipment was wrapped in aluminum foil to
158 prevent any input of contaminants from outside. Laboratory personnel were required to wear latex gloves, lab coats, and
159 masks throughout the analysis. Access to the laboratory was restricted during the analysis to minimize external
160 interference that could lead to protocol errors.
161

162 **Data analysis**

163 **Pollution Load Index (PLI)**

164 PLI was calculated using a pollutant load approach, which assesses the total concentration of contaminants relative to a
165 baseline level, to evaluate its significance. Equations were utilized to quantify the level of microplastic pollution in both
166 sediments and *Anadara granosa* in the Musi Estuary, with the method adapted accordingly (Tomlinson et al. 1980; Xu P et
167 al. 2018; Wang G et al. 2021).

$$CF_i = \frac{C_i}{C_{0i}}$$

168
169 This equation shows that the concentration factor (CF_i) at a particular sample is obtained by dividing the current
170 concentration (C_i) by the initial concentration (C_{0i}). C_i indicates the quantified presence of microplastics at each sampling
171 location or within each clam (individual), while C_{0i} denotes the background concentration, defined as the lowest value
172 recorded across all sampling locations. Given the lack of previously published research on microplastics in sediments or *A.*
173 *granosa* in the Musi Estuary, we determined C_{0i} values from minimum concentrations across sampling stations.
174
175

$$PLI = \sqrt{CF_i}$$

176

177 This equation shows that the Pollution Load Index (PLI) is generated by calculating the square root of the Concentration
 178 Factor (CF). The PLI value of microplastics is derived from the contaminant factor (CF_i) calculated for each
 179 station/individual.
 180
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$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \dots \times PLI_n}$$

182
 183
 184 Pollution Load Index for a Zone (PLI_{zone}) is determined by taking the square root of the product of individual PLI values
 185 from multiple sites within the zone. This method integrates data from several measurement points, providing a more
 186 comprehensive assessment of microplastic contamination within the analyzed. A PLI_{zone} is used to assess pollution based
 187 on specific estuary zones as well as the overall estuary area. PLI values are categorized into four predefined categories of
 188 pollution load, as shown in Table 2.
 189

190 **Table 2.** Microplastic pollution load categories

Definition	Value			
Value of the pollution load index (PLI)	<10	10-20	20-30	>30
Risk category	I	II	III	IV
Keterangan	Minor	Middle	High	Extreme

191
 192
 193 Nemerow Pollution Index (NPI)

194 NPI is designed to assess the bioavailability of microplastics in sediments and *A. granosa*. This index was adapted
 195 from equations used in prior studies evaluating heavy metal contamination (Alam et al. 2023).
 196

$$NPI = \sqrt{\frac{\left(\frac{C_i}{S_i}\right)_{max}^2 + \left(\frac{C_i}{S_i}\right)_{ave}^2}{2}}$$

197
 198 The NPI value is calculated using the square root of the mean squared deviation, incorporating both the maximum and
 199 average concentration ratios relative to the background concentration (S_i). The formula includes the sum of the squares of
 200 the maximum concentration ratio (C_{i max}/S_i) and the average concentration ratio (C_{i ave}). The C_i value represents the
 201 concentration of microplastics found in the sediment or clams at a specific station or individual, while the S_i value denotes
 202 the background level of microplastics, determined as the lowest concentration recorded across all sample types. The NPI
 203 provides a measure of microplastic bioavailability in sediments and *A. granosa*, with values below 2 indicating low
 204 bioavailability and values above 2 indicating high bioavailability.
 205

206 Bioconcentration Factor (BCF)

207 The Bioconcentration Factor (BCF) quantifies the extent to which microplastics accumulate in biota relative to their
 208 environmental concentration and is calculated using the following equation (Li Z et al. 2022).
 209

$$BCF = \frac{C_{biota\ ave}}{C_{sediment\ ave}}$$

210
 211
 212 In this equation, BCF represents the bioconcentration factor, which is derived from the calculation of the average
 213 concentration of microplastic in biota (*A. granosa*) divided by the average concentration of microplastic in its environment
 214 (sediment). This value is intended to assess how much microplastic is concentrated in the biota.
 215

216 Statistical analysis

217 Statistical analyses were conducted using IBM SPSS Statistics 27. Descriptive statistics, including total, mean,
 218 standard deviation, percentage, minimum, and maximum, were utilized to assess data variation. A one-way ANOVA was
 219 applied to analyze differences in microplastic abundance between estuarine zones when the data met normality
 220 assumptions. For non-normal data, the Kruskal-Wallis test was employed to assess the significance of differences between
 221 zones. All tests were carried out with a significance level of $\alpha = 0.05$.

Microplastic presence in sediment from Musi Estuary

224 The results of this study indicate that microplastics were detected at all sampling stations, as shown in Figure 3. A total
225 of 1,308 microplastic particles were identified, with concentrations ranging from 66 to 178 particles per 100 g of dry
226 sediment (dry weight, dw). The average abundance of microplastics was 1.31 ± 0.41 particles/g dw, with the highest
227 concentration found at station S1 (1.78 particles/g dw) and the lowest at station S8 (0.66 particles/g dw). High levels of
228 human activity in the upstream area of Muara Musi, which flows downstream to the estuary and ultimately into the ocean,
229 are responsible for the presence of microplastics (Diansyah et al. 2024; Rozirwan et al. 2021). Urban activities in close
230 proximity to the Musi River Watershed, particularly in Palembang City, significantly contribute to the generation of plastic
231 waste. Increased population density near riverbanks enhances the potential for plastic pollution. Furthermore, population
232 density is a crucial factor in the entry of microplastics into rivers (Dwiyitno et al. 2024; Eo et al. 2023). As a result, plastic
233 pollutants from domestic activities, such as the disposal of food packaging and plastic bags, can be transported through
234 stormwater drains and eventually end up in rivers (Kunz et al. 2023). Fishing activities around Muara Musi can contribute
235 to microplastic pollution, primarily through fibers released from fishing nets (Li Z et al. 2022; Fauziyah et al. 2023). This
236 increases anthropogenic pressure at river mouths, which can lead to greater accumulation of microplastics (Castro-Jiménez
237 et al. 2024). Additionally, tidal fluctuation influences water flow, which facilitates the deposition of microplastics in
238 sediment at these locations (Harris 2020). The findings of this study indicate that anthropogenic activities are responsible
239 for high levels of microplastic deposition in the sediments of Musi Estuary. Microplastic particles that accumulate in
240 sediments serve as a habitat for benthic organisms, which can have long-term implications for ecosystem health and
241 balance (Rahmatin et al. 2024). As vectors for pollutants, microplastics can exacerbate toxicity, potentially endangering
242 various populations (Caruso 2019; Issac and Kandasubramanian 2021; Fu et al. 2021; Ta and Babel 2023). Furthermore,
243 the processes of bioaccumulation and biomagnification in benthic organisms present heightened risks to these animals,
244 potentially leading to human consumption (Unuofin and Igwaran 2023). Therefore, it is crucial for local governments to
245 address the handling and prevention of further microplastic accumulation.
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Figure 2. General conditions in the Musi estuary. A. Coastal Settlements; B. Shipping Activities; C. Fisheries; D. Mangrove Ecosystem.

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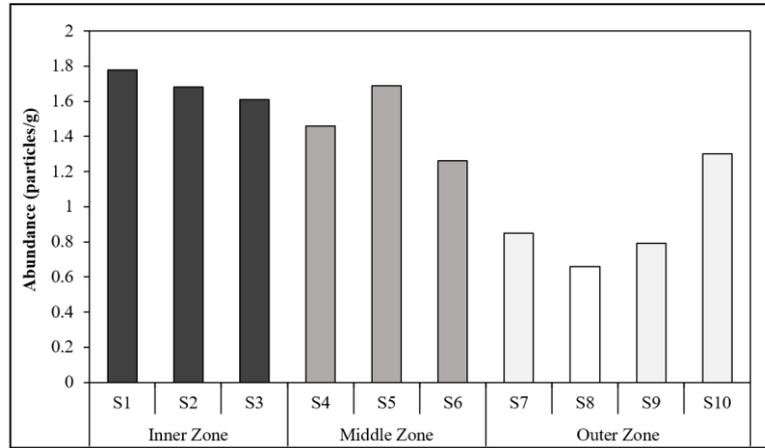


Figure 3. Distribution of microplastics from sediments in Musi Estuary, Indonesia.

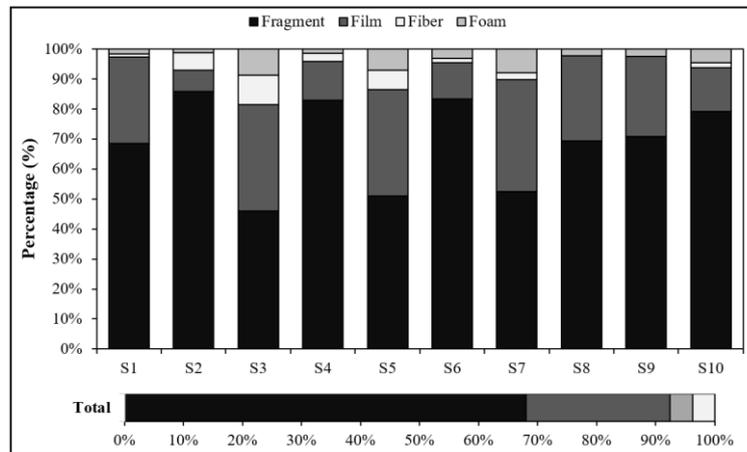


Figure 4. Percentage abundance of microplastic shapes form sediments in Musi Estuary, Indonesia

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The microplastics identified in the sediments were predominantly fragments (68.92%), followed by films (23.86%), fibers (4.05%), and foams (3.17%) (Figure 4). The dominance of fragments, which are a common form of secondary microplastics, occurs as a result of macroplastic degradation (Barnes et al. 2009). Fragments represented the most common type across all sediment samples analyzed. This dominance of fragments in estuarine sediments aligns with findings from various studies conducted in other estuarine environments. Research indicates that estuarine sediments globally tend to accumulate diverse microplastic forms, particularly fibers (Samuels et al. 2024; Firdaus et al. 2020; Santucci et al. 2024; Alam et al. 2023), with many studies also reporting a predominance of fragment types (Zhou et al. 2021; Suteja et al. 2024). These observations corroborate the literature, which consistently shows that fibers and fragments constitute the majority of microplastic pollution in estuarine ecosystems (Feng, An, et al. 2023).

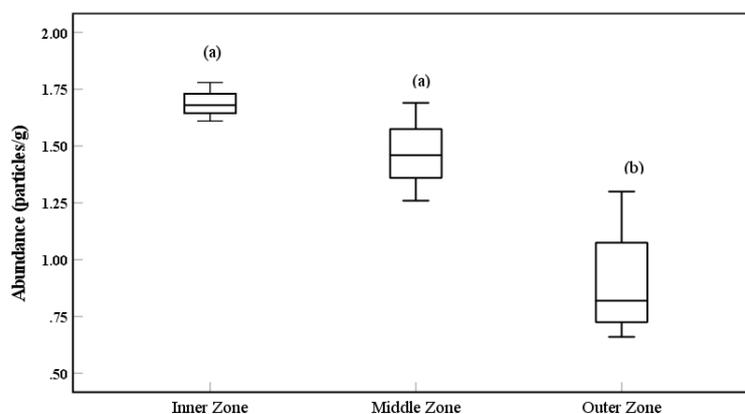
This study reveals notable variations in microplastic concentrations compared to similar research in estuaries globally. Our findings indicate that the Musi Estuary has lower microplastic concentrations than those reported in several other Indonesian and international estuaries. For example, the Jakarta Bay Estuary exhibits concentrations nearly ten times higher, ranging from 1,184 to 1,337 particles per 100 grams of river sediment and 804 to 1,055 particles per 100 grams of beach sediment (Dwiyitno et al. 2024). Such elevated levels are likely due to increased anthropogenic activities along Jakarta's rivers, which substantially contribute to plastic waste flow. In another comparison, the Pearl River Estuary in China also shows higher microplastic densities, with concentrations between 2.05×10^3 and 7.75×10^3 particles per kilogram of dry weight sediment (Xu K et al. 2024). This estuary's coastal regions experience high human activity, enhancing the potential for microplastic deposition. Similarly, the Meghna Estuary in Bangladesh, which receives sediment from the Ganges River Basin, reports even higher microplastic levels at $4,014.66 \pm 1,717.59$ particles per kilogram dry weight (Alam et al. 2023). These findings underscore the significant role of anthropogenic influences on microplastic accumulation in global estuarine sediments, including the Musi Estuary. Other environmental factors, like location, sampling period, sediment depth, sediment composition, and local hydrodynamic conditions, may also affect these changes in the amount of microplastics found (Feng, Chen, et al. 2023; Zheng et al. 2020; Yuan B et al. 2023).

In contrast, the abundance of microplastics in the sediments of the Musi Estuary surpasses levels reported in other regions, both within Indonesia and globally. Concentrations in the Musi Estuary are notably higher than those in the

286 Zandvlei River Watershed and estuarine areas of South Africa (70.23 ± 7.36 particles/kg dw) (Samuels et al. 2024), the
287 Claromecó Estuary in Argentina (299 ± 114 particles/kg dw) (Truchet et al. 2021), and the upper sediment layer (0-5 cm)
288 of the Fuhe River Estuary in Northern China (1049 ± 462 particles/kg dw) (Zhou et al. 2021). These numbers are also
289 higher than those found in coastal Río de la Plata (547.83 ± 620.06 particles/kg dw) (Santucci et al. 2024), Benoa Bay,
290 Bali (31.08 ± 21.53 particles/kg dw) (Suteja et al. 2024), the Jagir Estuary in Surabaya (up to 590 particles/kg dw) (Firdaus
291 et al. 2020), and the Pekalongan River Estuary in Java (0.77 to 1.01 particles/g dw) (Ismanto et al. 2023). The high
292 microplastic concentration in the Musi Estuary is likely due to extensive plastic waste disposal in the river basin by the
293 surrounding community. Research supports that plastic debris forms a substantial part of the macro-waste in the Musi
294 River (Maherlsa et al. 2019). Additionally, local communities frequently establish settlements along the river, relying on it
295 for water access and transportation. Consequently, domestic waste, including plastic, often enters the river directly. This
296 local waste management issue reflects a broader trend, with Indonesia identified as the world's second-largest contributor
297 to oceanic plastic pollution, following China (Jambeck et al. 2015).
298

299 Spatial distribution microplastic in Sediments

300 The study revealed that the abundance of microplastics was significantly higher in the inner and middle zones when
301 compared to the outer zone. The mean abundances were 1.69 ± 0.08 particles/g dw in the inner zone, 1.47 ± 0.22 particles/g
302 dw in the middle zone, and 0.9 ± 0.28 particles/g dw in the outer zone. Significant differences in mean microplastic
303 abundance between zones were revealed by the one-way ANOVA ($p < 0.05$), as illustrated in Figure 5. The Tukey HSD
304 test revealed no significant difference in mean microplastic abundance between the inner and middle zones. However, we
305 observed a significant difference in the outer zone compared to both the middle and outer zones. Our results indicate that
306 the inner and middle estuary regions exhibit greater mean concentrations of microplastics compared to the outer estuary.
307 Natural factors, particularly the effects of currents and wave action in coastal and estuarine environments, significantly
308 influence this phenomenon by altering microplastic distribution. The influx of freshwater promotes the sedimentation and
309 prolonged retention of high-density microplastics within the system (Li G et al. 2024). Furthermore, the sedimentation
310 process is affected by the density and buoyancy of microplastics, with increased salinity in estuarine waters enhancing
311 buoyancy forces and influencing distribution patterns (Cheng et al. 2024). Our findings align with research from the
312 Liaohe Estuary in China, revealing a higher accumulation of microplastics in inner river sediments compared to the outer
313 estuary (Xu Q et al. 2020). Additionally, sampling depth plays a critical role in accurately measuring microplastics in
314 surface waters, bottom layers, and sediments (Feng, An, et al. 2023).



315
316 **Figure 5.** The difference in microplastic abundance between zones in the sediments
317

318 The spatial analysis revealed distinct patterns of microplastic accumulation between the two sides of the coast adjacent
319 to the estuary. Notably, Site S7 exhibited higher concentrations compared to Site S8, likely due to its proximity to other
320 pollution sources. Specifically, S7 is located near the Banyuasin Estuary, which is believed to significantly contribute to
321 the deposition of microplastics in the surrounding sediments. Costa's (2023), research confirms that nearby sources of
322 pollution heavily influence microplastic deposition in coastal regions. The accumulation of microplastics at the mouth of
323 the adjacent river further exacerbates this phenomenon, facilitating the settlement of microplastics within the sediment.
324 Additionally, studies suggest that the depth of the sampling locations significantly influences microplastic deposition,
325 revealing a greater abundance of microplastics at deeper water levels (Bayo et al. 2022). The substantial accumulation of
326 microplastics in sediments is attributed to their persistent characteristics as well as the protective conditions offered by
327 deeper environments, which are shielded from UV light, maintain lower temperatures, and exhibit lower oxygen levels,
328 thus slowing biodegradation processes (Zhang K et al. 2021).

329 Our findings confirm the presence of microplastics in the sediments of the Musi Estuary, likely originating from
330 suspended microplastics that eventually settle from the water's surface. Previous research has found that there are $467.67 \pm$
331 127.84 particles/m³ and 723.67 ± 112.05 particles/m³ of microplastic in surface waters during ebb and flow conditions,

332 respectively (Diansyah et al. 2024). Hydrodynamic conditions in the estuary and processes like aggregation and
333 biofouling, which increase microplastic density, influence the deposition of these particles (Malli et al. 2022). The specific
334 properties of the microplastics and the water dynamics of the estuary strongly influence microplastic deposition. For
335 instance, biofouling can make microplastics denser, accelerating their descent into sediments (Lin et al. 2023). Since
336 microplastics often have higher densities than water, they can remain suspended in the water column before eventually
337 settling (Dai et al. 2022). High Total Suspended Solids (TSS) in the Musi Estuary have the potential to cause aggregation
338 of microplastics (Rahutami et al. 2022). High TSS levels make it easier for sediments and other suspended particles to
339 stick together on microplastic surfaces, which speeds up their deposition (Yang et al. 2022). However, microplastics can
340 also become resuspended in the water column (Tang et al. 2020). Stirring forces in estuarine environments can lift
341 microplastics back into the water, where turbulence and bioturbation may redistribute them (Malli et al. 2022). Therefore,
342 additional studies are required to fully characterize the mechanisms of microplastic deposition in the Musi Estuary.
343 Climate factors also influence microplastic distribution in Musi Estuary sediments. In Indonesia, where regions such as the
344 Musi Estuary experience distinct rainy and dry seasons, seasonal changes affect water dynamics and microplastic
345 transport. During the rainy season, increased river velocity may carry microplastics upward from sediments, while in the
346 dry season, slower flows facilitate microplastic accumulation at the water's surface (Zhao X et al. 2020). Currently, there
347 are no studies specifically examining the seasonal impact on microplastic presence in the Musi Estuary, underscoring the
348 need for further research to address this gap.

349 350 **Bioaccumulation of microplastics in *Anadara granosa***

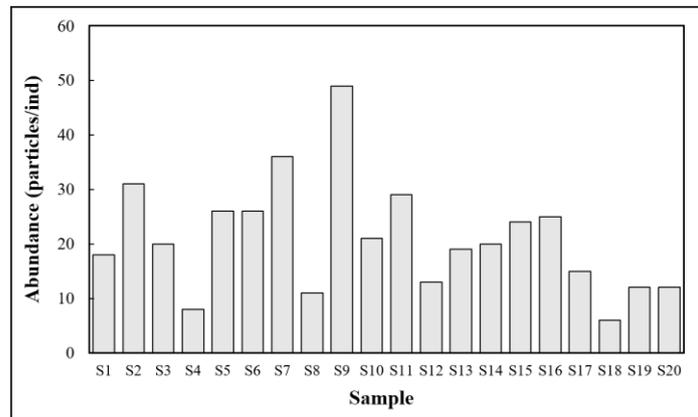
351 We extracted 133 microplastic particles from the digestive tracts of twenty samples of *A. granosa*. Each sample from
352 the Musi Estuary had accumulated microplastics, ranging from 6 to 49 particles/ind (ind) or 8 to 69.01 particles/g ww, as
353 shown in Figure 7 and Figure 8. The mean abundance of microplastics found in the digestive tracts was 21.05 ± 10.31
354 particles/ind or 30.46 ± 16.7 particles/g ww. Several factors contribute to the increased microplastic inputs in bivalve
355 species, including human activities, tourism, fishing equipment, freshwater runoff, and wastewater discharge, combined
356 with the area's hydrodynamic conditions (Vital et al. 2021). The elevated levels of microplastics found in *A. granosa*
357 from the Musi Estuary suggest a significant degree of contamination in their habitat, including both water and sediment, which
358 may adversely affect the health of these organisms and disrupt local ecological balances. This observation aligns with
359 existing literature, which indicates a strong correlation between the abundance of microplastics in bivalves, such as
360 mussels, and their sediment environments (Narmatha Sathish et al. 2020), reinforcing the need for monitoring these
361 pollutants in aquatic ecosystems.



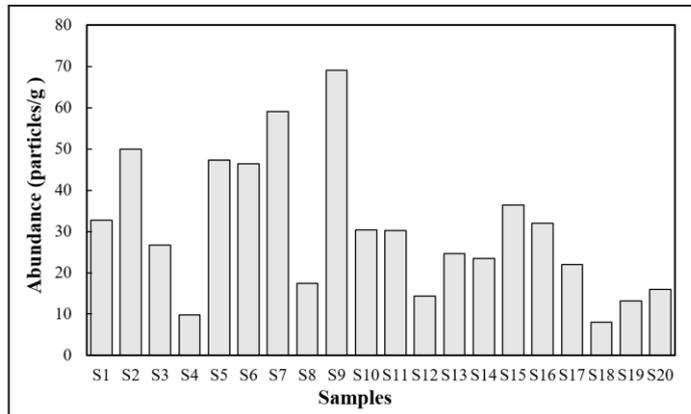
362
363 **Figure 6.** Morphology of *Anadara granosa* from Musi Estuary, Indonesia.
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365 Microplastics were observed in three morphological forms: film, fiber, and fragment. Film accounted for the majority
366 of microplastics (59.38%), followed by fiber (31.59%) and fragments (9.03%), as shown in Figure 9. The digestive tracts
367 of the *A. granosa* studied did not contain any pellets or foam. Statistical analysis using the Kruskal-Wallis test revealed
368 significant differences in the abundance of the three microplastic forms ($p < 0.05$). This predominance of film
369 microplastics in *A. granosa* is likely attributable to the extensive use of disposable plastic packaging within the
370 community, which subsequently degrades into microplastics. Although fragments dominate the sediment substrate, *A.*
371 *granosa* predominantly absorb film microplastics. The lower density of film microplastics may facilitate their
372 accumulation in the upper sediment layer, making them susceptible to resuspension in the overlying water column. This
373 phenomenon is consistent with the feeding behavior of *A. granosa*, which frequently filter water from the sediment
374 surface, resulting in the accumulation of film microplastics within their digestive tracts.

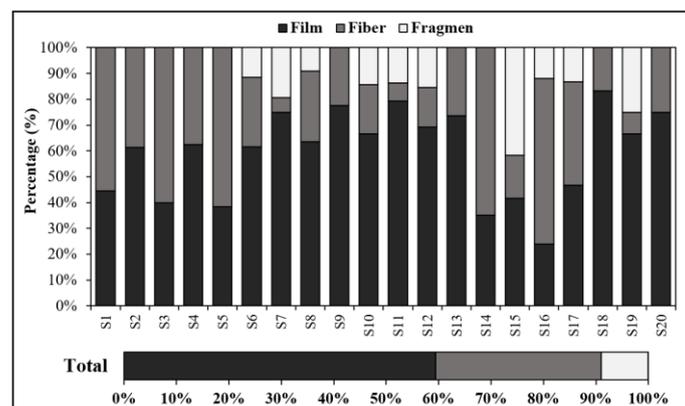
375 Our findings indicate a higher abundance of microplastics in *A. granosa* from the Musi Estuary compared to certain
 376 other regions globally. For instance, *A. granosa* from the Chanthaburi estuarine ecosystem in eastern Thailand contained
 377 0.40 ± 0.16 particles/g ww (Potipat et al. 2024), while samples from Peninsular Malaysia showed concentrations of $0.20 \pm$
 378 0.08 particles/g ww and 1.54 ± 0.30 particles/ind ww (Mohan et al. 2024). Additionally, microplastic levels in *A. granosa*
 379 from Pao Village, Tarowang District, Jeneponto Regency, Indonesia, were measured at 0.0144 particles/g ww (Namira et
 380 al. 2023), and those from Likas Bay Beach in North Borneo, Malaysia, at 24.4 ± 0.6 particles/g ww (Abd Rahman et al.
 381 2024). In contrast, the *A. granosa* analyzed in this study accumulated lower microplastic concentrations than those
 382 reported in several other studies. For instance, *A. granosa* from the Pangkal Babu mangrove forest area in Tanjung Jabung
 383 Barat district, Jambi, exhibited 434 ± 97.05 particles/ind (Fitri and Patria 2019), while those from Lada Bay in Pandeglang,
 384 Banten, had concentrations of 618.8 ± 121.4 particles/ind (Ukhrowi et al. 2021). Overall, this study shows that
 385 microplastics have contaminated the sediment environment of the Musi Estuary, South Sumatra, indicating that *A. granosa*
 386 can serve as effective bioindicators of this pollution.
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 390 **Figure 7.** Microplastic abundance of *Anadara granosa* from the Musi Estuary
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 394 **Figure 8.** Microplastic abundance (in particles/g) of *Anadara granosa* from the Musi Estuary
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 397 **Figure 9.** Shape of microplastics in *Anadara granosa* from the Musi Estuary

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Risk assessment of microplastics in sediments and *Anadara granosa*

A risk assessment of microplastic contamination in the Musi River Estuary was carried out by calculating the Pollution Load Index (PLI), the Nemerow Pollution Index (NPI), and the Bioconcentration Factor (BCF). The PLI values across all sediment samples indicate that the estuarine sediment is subject to minor pollution, with values ranging between 1.00 and 1.64, as shown in Table 3. In the three zones of the estuary (inner, middle, and outer), the PLI values were 1.60, 1.49, and 1.20, respectively. We calculated the overall average PLI for the Musi Estuary to be 1.37. All zones reported PLI values below 3.00, which suggests that the area experiences only minor microplastic pollution. The analysis of microplastic presence in sediment samples revealed a significantly high concentration of microplastics, with an NPI value of 2.37. This suggests a substantial bioavailability of microplastics in the estuarine environment. This elevated NPI underscores the ecological risk posed by microplastic contamination, despite the relatively low PLI observed across the study area.

Table 3. The microplastic risk factor in sediments of Musi Estuary

Sediment Station	CF	PLI	PLI Zone	PLI Sediment	NPI
S1	2.70	1.64	Inner Zone 1.60	1.37	2.37
S2	2.55	1.60			
S3	2.44	1.56			
S4	2.21	1.49	Middle Zone 1.49		
S5	2.56	1.60			
S6	1.91	1.38			
S7	1.29	1.13	Outer Zone 1.20		
S8	1.00	1.00			
S9	1.20	1.09			
S10	1.97	1.40			

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Anadara granosa analyzed in this study exhibited significant risk values associated with microplastic contamination. The PLI for these clams ranged from 1 to 2.86, with an overall mean PLI value of 1.77 for the entire sample, as shown in Table 4. This indicates that the pollution load in *A. granosa* falls within the minor category (PLI < 3). Furthermore, the bioavailability of microplastics in *A. granosa* was significantly higher, with an NPI value of 6.29, indicating a high bioavailability. The bioconcentration factor of microplastics from sediment to *A. granosa* was measured at 23.28, suggesting a significant potential for the transfer of microplastics from sediment to *A. granosa* in the Musi Estuary.

Our findings demonstrated that the bioavailability of microplastics in sediments and *A. granosa* was categorized as high (NPI = 2.37; NPI > 2). In comparison, the bioavailability of microplastics in *A. granosa* was even higher, with an NPI value of 9.89. The elevated bioavailability in sediments suggests a greater potential for absorption by aquatic organisms, particularly benthic animals. Furthermore, the bioconcentration factor (BCF) calculated for *A. granosa* in sediment was 23.28, indicating a significant transfer of microplastics from sediments to these organisms in the Musi Estuary. This high BCF value is attributed to the non-discriminatory feeding process of *A. granosa*, allowing them to ingest microplastics along with other particles. When compared to several benthic species from the Yangtze River Estuary, which exhibited a BCF of 29.48 ± 6.52 (Li Z et al. 2022), the BCF of *A. granosa* in the Musi Estuary remains lower. Nonetheless, both sediments and *A. granosa* from the Musi Estuary are classified within the minor pollution risk index category (PLI < 10). The bioaccumulation of microplastics by benthic animals like *A. granosa* poses a significant risk to human health for those consuming these clams (Winiarska et al. 2024; Wang Y et al. 2023). Therefore, periodic assessments are crucial to evaluate the risk of microplastic contamination in sediments and *A. granosa*, providing essential information to understand the ecological and health implications in the Musi Estuary.

The impact of high bioaccumulation extends beyond the toxicity of microplastic materials; it also facilitates the accumulation of other pollutants. Previous research has detected heavy metals, such as lead (Pb) and copper (Cu), adhering to the surfaces of microplastics in the Musi Estuary (Purwiyanto et al. 2020). Additionally, various hazardous heavy metals, including iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), and zinc (Zn), were identified with high bioavailability in the aquatic environment of the Musi Estuary (Rahutami et al. 2022). This accumulation allows for the deposition of significant quantities of heavy metals on microplastic surfaces, thereby increasing the risk of microplastic toxicity for estuarine ecosystems. Moreover, the involvement of microorganism vectors, misidentification in aquatic organisms' diets, and their harmful toxicological effects on benthic animals heighten the risk associated with microplastics (Gong and Xie 2020). Consequently, the high output of microplastics from the Musi Estuary has the potential to jeopardize the condition of the surrounding aquatic environment, including fisheries and migratory birds, which are critical to the ecosystem (Rozirwan et al. 2019; Rozirwan, Fauziyah, Nugroho, et al. 2022).

Table 4. Risk factors for microplastics in *Anadara granosa* from the Musi Estuary

<i>A. granosa</i> sample	CF	PLI	PLI <i>A. granosa</i>	NPI	BCF
S1	3.00	1.73			
S2	5.17	2.27			
S3	3.33	1.83			
S4	1.33	1.15			
S5	4.33	2.08			
S6	4.33	2.08			
S7	6.00	2.45			
S8	1.83	1.35			
S9	8.17	2.86			
S10	3.50	1.87			
S11	4.83	2.20	1.77	6.29	23.28
S12	2.17	1.47			
S13	3.17	1.78			
S14	3.33	1.83			
S15	4.00	2.00			
S16	4.17	2.04			
S17	2.50	1.58			
S18	1.00	1.00			
S19	2.00	1.41			
S20	2.00	1.41			

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This study holds significant ecological implications for the Musi Estuary ecosystem, as the presence of microplastics poses a long-term threat to ecosystem stability. Previous studies have shown that microplastics can affect the physicochemical properties of sediments, such as electrical conductivity, organic matter content, and nutrients, and well as impact enzymatic activity in sediment bacteria (Yuan B et al. 2022; Rillig 2012; Li W et al. 2022). These changes can lead to structural and functional alterations in aquatic habitats (Li W et al. 2022). Microplastics can also enter various levels of the food chain through bioaccumulation and biomagnification processes. For example, microplastics in plankton can cause a decline in zooplankton populations, which impacts food availability for small fish and predatory species at higher trophic levels (Malinowski et al. 2023). The accumulation of microplastics in *A. granosa* in this study indicates that the pressure of plastic waste in the Musi Estuary is already quite high. Microplastics can act as vectors for other pollutants, such as microorganisms, heavy metals, and other inorganic pollutants can increase the risk of toxicity for aquatic organisms, including *A. granosa* (Rafa et al. 2024; Purwiyanto et al. 2020). Research indicates that exposure to microplastics can lead to behavioral and physiological changes in aquatic organisms, including changes in diet and reproductive success (Guo et al. 2020; Liang et al. 2023). Moreover, microplastics disrupt endocrine functions and metabolic pathways, causing oxidative stress, cell necrosis, and apoptosis, which can ultimately lead to mortality (Pannetier et al. 2020; Jeyavani et al. 2021). Disruption of any component of the food chain can trigger a domino effect, threatening the balance of the Musi Estuary ecosystem. However, this study only examines microplastic pollution in sediment and *A. granosa* as environmental indicators. Additional research is needed to understand microplastic buildup in other species, such as fish, shrimp, and benthic organisms, for a more complete picture of microplastic pollution in the Musi Estuary and its impact on the global marine environment.

Microplastic contamination of *A. granosa* in the Musi Estuary poses a significant threat to local communities, particularly clam fishers. Microplastics that accumulate in *A. granosa* may pose health risks to community members who consume them regularly as a primary source of protein. Long-term exposure to microplastics can potentially disrupt various human body systems, including the digestive, respiratory, reproductive, nervous, and cardiovascular systems (Li Y et al. 2024). Furthermore, microplastics may cause both acute and subchronic toxicity and are considered potentially carcinogenic and disruptive to human development (Yuan Z et al. 2022). Once in the bloodstream, microplastics can be transported to the liver, the primary organ responsible for detoxification. Accumulation in the liver may trigger adverse physiological reactions, including increased oxidative stress, liver fibrosis, and impaired lipid metabolism. In addition to health risks, microplastic contamination in *A. granosa* in the Musi Estuary may reduce consumer confidence and decrease market demand due to food safety concerns (Unuofin and Igwaran 2023). The long-term effect could weaken local economic resilience as marine products in the region are perceived to be contaminated with microplastics (Barrientos et al.

477 2024). Therefore, it is crucial for governments and communities to recognize these risks and collaborate on mitigation
478 efforts to protect ecosystems, public health, and local economies.

479 The presence of microplastics in sediments and *A. granosa* in the Musi Estuary poses a significant threat to the
480 ecosystem's sustainability. To address microplastic pollution effectively, collaboration between governmental bodies and
481 local communities is essential. Several strategies can be implemented to reduce microplastic pollution in aquatic
482 environments, including the following measures:

- 483 1. Educating the public on the importance of controlling plastic waste (Evode et al. 2021).
- 484 2. Reducing single-use plastic consumption and encouraging the use of environmentally friendly alternatives.
- 485 3. Banning plastic microbeads in personal care products and cosmetics (Zhang Z et al. 2022).
- 486 4. Promoting sustainable plastic recycling at individual, community, national, and international levels (Babaremu et al.
487 2022; Thu Nguyen 2024)
- 488 5. Strengthening waste management policies for product packaging and disposal practices within the industrial sector
489 (Wu et al. 2023).
- 490 6. Enforcing stricter regulations on wastewater treatment facilities and implementing comprehensive plastic waste
491 management through mechanical, physical, chemical, and biological methods to reduce microplastics (Meiyerani et
492 al. 2024; Ragaert et al. 2017).
- 493 7. Prioritizing microplastic control by evaluating land-use contributions in urban areas, focusing on those with the
494 highest pollution impact (Jin et al. 2022).

495 Furthermore, seafood hygiene practices in local communities and fishing industries can help reduce microplastic
496 contamination (Kandeyaya et al. 2023). For example, *Anadara granosa* and other seafood, when thoroughly cleaned by
497 repeated washing and clam removal before cooking, may reduce microplastic intake (Li J et al. 2022). The concentration
498 of microplastics in shellfish is significantly lower after boiling and steaming compared to frying, suggesting that cooking
499 methods affect microplastic retention in seafood (Evode et al. 2021). This method can also be applied to shrimp, fish, and
500 other shellfish to lower the risk of microplastic exposure. Implementing these recommendations will help reduce
501 microplastic pollution and provide long-term benefits for public health, ecosystem stability, and the economic resilience of
502 local fisheries.

503 In conclusion, this study demonstrates the significant presence of microplastics in the sediment and *A. granosa* of the
504 Musi Estuary, Indonesia. These findings indicate a serious threat to the aquatic ecosystem and to local public health, as
505 microplastics can enter the food chain and potentially harm organisms across trophic levels, including humans (Ningrum
506 and Patria 2022; Edwin et al. 2023; Patria et al. 2023). This pollution heightens environmental risks, particularly for
507 species integral to both the community's diet and economy (Jeong et al. 2024). The microplastics likely originate from
508 human activities, especially urban waste from upstream areas that flows downstream and accumulates in the estuary
509 (Diansyah et al. 2024). These results highlight the urgent need for routine monitoring and stricter waste management
510 practices, particularly for plastic disposal, to reduce further contamination. A comprehensive strategy is essential to
511 controlling plastic waste throughout the river system—from source to estuary. Effective measures should include
512 coordinated waste management practices, public education on the impact of pollution, and cross-sector collaboration to
513 preserve estuarine health. Such initiatives are crucial to protecting ecosystem health and sustaining local fisheries, ensuring
514 these resources continue to support community well-being.

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802



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We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of microplastics presence in sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.** We are waiting for your revision in the system (<https://smujo.id/biodiv>), do not send it via email.

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This article has interesting and structured content but needs some revision. all suggestions are available in the text. Send revised files accompanied by track changes and a letter of objection in a separate file

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Dear Reviewer,

We sincerely appreciate your time and effort in reviewing our manuscript and providing valuable feedback. Your insightful comments have been instrumental in improving the quality of our work. In response, we have carefully revised the manuscript accordingly. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Author (s)

Table of Author(s) Response

No	Comments	Respon
1.	Add references in Introduction	Thank you for your valuable suggestion. We have added relevant references in the Introduction section to support our statements and provide a stronger foundation for our study (Lines 29-32)
2.	More detailed condition of each site, such as vegetation, substrate, near industry etc	We have provided more specific description to sampling location Table (Table 1)
3.	Provide the figure of microplastics	We have added the Various forms of microplastics as Figure 3 (Line 262)
4.	Specimens	We have revised Morphology to Specimens (Lines 375)
5.	Delete this section Lines 481 - 496	We have deleted this section
6.	See guideline of References	We have revised the references

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I herewith enclosed a research article,

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Implication of microplastic presence in sediment and blood clams (*Anadara granosa*, Linnaeus 1758) (Mollusca: Bivalvia) in the Musi Estuary, Indonesia

Author(s) name:

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This research is groundbreaking because there is currently no information available on the abundance of microplastics in sediments of the Musi Estuary, Indonesia. This article provides a comprehensive review of recent research on microplastics in the sediments of the Musi Estuary and their impact on benthic animals (*Anadara granosa*), one of the seafood from the Musi Estuary.

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1 **Implication of microplastic presence in sediment and blood clams**
2 ***Anadara granosa* (Linnaeus 1758) (Mollusca: Bivalvia) in the Musi**
3 **Estuary, Indonesia**

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11
12
13 **Abstract.** Microplastic pollution poses a serious risk to estuarine ecosystems, affecting sediments and benthic species. Blood clams
14 (*Anadara granosa*, Linnaeus 1758; ~~Mollusca: Bivalvia~~) represent a significant commercial seafood product in Indonesia, including those
15 from the Musi Estuary. This study investigated the risks associated with microplastic contamination in sediments and *A. granosa* in the
16 Musi Estuary, located in South Sumatra, Indonesia. Sediment samples were extracted using sodium chloride (NaCl, 1.2 g cm⁻³) and
17 hydrogen peroxide (30% H₂O₂). In contrast, *A. granosa* samples were digested with 10% KOH for microplastic extraction. The
18 abundance and shape of microplastics were analyzed from both samples. Pollution risk assessment was conducted through the
19 calculation of the Pollution Load Index (PLI), Nemerow Pollution Index (NPI), and Bioconcentration Factor (BCF). The results
20 revealed the presence of microplastics in sediments, with a mean abundance of 1.31±0.41 particles/g dw, while in *A. granosa*, it was
21 21.05±10.31 particles/ind. Both samples exhibited high microplastic bioaccumulation (NPI > 2), although the pollution load remained
22 relatively low (minor category, PLI < 10). The bioconcentration factor between *A. granosa* and sediment was determined to be 23.28,
23 indicating that ~~the *A. granosa* absorbed microplastics present in the sediment~~ *microplastics present in the sediment were absorbed by the*
24 *A. granosa*. These findings highlight the significant bioaccumulation potential of microplastics in blood clams within the Musi Estuary.
25 It is important for the local community and government to establish mitigations for future microplastic management efforts.

26 **Keywords:** Bioaccumulation, blood clams, microplastics, Musi Estuary, sediment

27 **Running title:** Microplastics in sediment and blood clams

28 **INTRODUCTION**

29 River estuaries are intricate and dynamic ecosystems that play a vital role in ecological sustainability and support a
30 variety of socio-economic activities (Boerema and Meire 2017; Hasan et al. 2022; Retnaningdyah et al. 2022; Hasan et al.
31 2023). These ecosystems provide essential habitats for diverse species and act as focal points for human activities,
32 including urbanization, recreation, and commercial enterprises (Islamy and Hasan 2020; Lonsdale et al. 2022; Natsir et al.
33 2022; Anurrahman et al. 2023). Among the major estuaries in Sumatra, the Musi Estuary holds significant ecological and
34 economic importance (Surbakti et al. 2023). It serves as a critical habitat for various fish species and benthic organisms,
35 which in turn sustain the livelihoods of local communities through the utilization of water resources (Rozirwan et al. 2021;
36 Fauziyah et al. 2022; Rozirwan et al. 2022; Fauziyah et al. 2023). Nevertheless, the intensification of human activities
37 within and surrounding the Musi Estuary has resulted in significant environmental deterioration (Maherlsa et al. 2019).
38 Major contributors to this issue include heavy metal and microplastic contamination, which pose a substantial threat to
39 ecosystem health (Purwiyanto et al. 2022; Rahutami et al. 2022; Fitria et al. 2023; R. Rozirwan et al. 2023; Diansyah et al.
40 2024; Rozirwan et al. 2024). These pollutants endanger the ecological equilibrium of the estuary, with the potential to
41 affect its biodiversity and the livelihoods dependent upon it.

42 Macrozoobenthos are considered pivotal components in preserving the equilibrium of estuarine ecosystems (Sari et al.
43 2022; Isoni et al. 2023). These organisms play a crucial role in the food web and nutrient cycling, thereby supporting a
44 variety of marine species (Griffiths et al. 2017). Moreover, macrozoobenthos are highly sensitive to environmental
45 changes, making them reliable bioindicators of ecological degradation (Sahidin et al. 2018). Research worldwide
46 highlights that macrozoobenthos, including bivalves such as blood clams *Anadara granosa* (Linnaeus 1758) are often used
47 as bioindicators because they can take in and store pollutants from their environment through filter-feeding (Fitri and
48 Patria 2019; Ward et al. 2019; Ding et al. 2021; Bonifacio et al. 2022; Saleh et al. 2023; Rahmatin et al. 2024). *Anadara*
49 *granosa* is widely consumed by the general public as a nutrient-rich seafood, particularly among local communities

(Rozirwan, et al. 2023; Rozirwan, et al. 2023). Among these, *A. granosa* is a prominent bivalve species that plays a crucial role in maintaining ecosystem stability and generating substantial economic benefits for coastal communities (Yulinda et al. 2020; Prasetyono et al. 2022; Mahary et al. 2023). Given its ecological importance and economic value within coastal and estuarine systems, it is imperative to investigate pollutant contamination in *A. granosa* to [understand the broader impacts of environmental pollution comprehensively](#). ~~comprehensively understand the broader impacts of environmental pollution.~~

Various pollutants, including heavy metals, hydrocarbons, pesticides, and microplastics, have been identified as significant contaminants of estuarine ecosystems, with many of these pollutants accumulating in sediments (Zhao et al. 2015; Kılıç et al. 2023; Han et al. 2024; Jacq et al. 2024). Among these, plastic pollution has emerged as a critical concern due to its pervasive impact on aquatic environments (Borrelle et al. 2020; Hecker et al. 2023). Over the last five decades, the widespread reliance on single-use plastics has led to extensive accumulation and fragmentation of plastic materials across diverse ecosystems (Zhang et al. 2021; Walker and Fequet 2023). As plastics degrade, they break down into microplastics, defined as particles ranging in size from 1 µm to 5 mm (Frias and Nash 2019). These microplastics enter aquatic ecosystems through various pathways, such as the fragmentation of discarded plastic products, the release of synthetic fibers during washing, and the intentional addition of microbeads in personal care products (Napper et al. 2015; Praveena et al. 2018; Belzagui and Gutiérrez-Bouzán 2022; Gan et al. 2023). Microplastics pose multiple threats by releasing toxic additives and acting as carriers of hazardous organic pollutants and heavy metals (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). These impacts are multifaceted, disrupting food webs, altering nutrient cycles, modifying habitats, and potentially inducing genetic changes in aquatic organisms (Thacharodi et al. 2024).

Prior research has quantified microplastic concentrations in the waters of the Musi Estuary, with reported levels of 467.67 ± 127.84 particles/m³ during flood tide and 723.67 ± 112.05 particles/m³ at ebb tide (Diansyah et al. 2024). These microplastics are frequently associated with toxic heavy metals, such as lead (Pb) and copper (Cu), which exacerbate their harmful environmental effects (Purwiyanto et al. 2022). In the water column, microplastics tend to settle into estuarine sediments, which have been identified as the second-largest global reservoir for microplastics after fjords (Harris 2020). The accumulation of microplastics in estuarine sediments poses significant ecological challenges, particularly due to their ingestion by benthic organisms, including *A. granosa* (Fitri and Patria 2019; Saleh et al. 2023; Rahmatin et al. 2024; Mohan et al. 2024). While numerous studies have examined microplastic concentrations in the water column, research on their distribution in sediments and bioaccumulation in benthic species, particularly *A. granosa*, remains limited.

This study aims to investigate the bioaccumulation and risks of microplastic pollution in sediments and *A. granosa* in the Musi Estuary, Indonesia. The findings are expected to enhance understanding of benthic organisms, especially *A. granosa*, and its potential health risks for local seafood consumers.

MATERIALS AND METHODS

Study Area and Sample Collection

The Musi Estuary, located on the east coast of South Sumatra, is where seawater from the Bangka Strait mixes with freshwater from the Musi River. The Musi Estuary is an important area for fisheries and benthic habitats in South Sumatra, Indonesia (Rozirwan et al. 2021; Rozirwan et al. 2022). Sampling locations were selected based on hydrodynamic conditions influencing microplastic transport and deposition, considering salinity variations and water mass interactions (Cheng et al. 2024; Diansyah et al. 2024). Locations of the sampling sites are shown in Figure 1 and Table 1. Sediment and *A. granosa* were collected during low tide from the Musi Estuary, South Sumatra, Indonesia. Sediment samples were collected from ten sampling points using a Peterson Grab at each station (Dwiyitno et al. 2024). Samples were placed in glass jars that had been rinsed with pure distilled water and covered using aluminum foil to avoid contamination. Samples were placed in a cool box (± 4 °C) for further analysis in the laboratory.

Anadara granosa samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). Twenty adult *A. granosa* (7.48–11.44 cm) were collected from fishermen's catch in the Musi Estuary. *Anadara granosa* samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). The mussels were rinsed with pure distilled water to remove any dirt. *Anadara granosa* were wrapped in aluminum foil and stored in a cooler at approximately 4 °C to preserve their freshness. After that, they were transported to the laboratory and frozen at -20°C before further analysis (Ding et al. 2021).

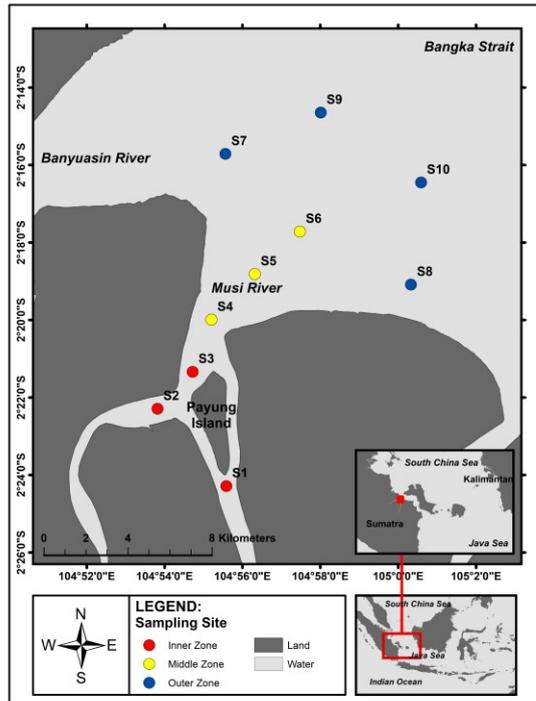


Figure 1. Study site at the Musi Estuary, Indonesia.

Table 1. Coordinates of the sampling location.

Estuary Zone	Description	Station	Latitude (°S)	Longitude (°E)
Inner Zone	(closest to the river body and, redominantly influenced by freshwater	S1	2.40470	104.92643
	mangrove vegetation and muddy substrate near densely populated coastal village activities, shipping and fishing activities)	S2	2.37144	104.89695
Middle Zone	(between the inner zone and the outer zone, a mixing-zone of freshwater and seawater	S3	2.35558	104.91200
	mangrove vegetation and muddy substrate shipping and fishing activities)	S4	2.33316	104.92003
		S5	2.31353	104.93862
Outer Zone	(closest to the ocean (at the sea), dominated by seawater, no vegetation, muddy substrate shipping and fishing activities)	S6	2.29524	104.95794
		S7	2.26183	104.92603
		S8	2.31807	105.00547
		S9	2.24403	104.96686
		S10	2.27413	105.00978

Microplastic extraction from sediment

Microplastics in sediments were extracted using the standard method of density separation and organic matter removal with minor modifications (Thompson et al. 2004; Liebezeit and Dubaish 2012). A total of 500 g of wet sediment samples were placed in a beaker glass covered with aluminum foil. The samples were dried in an oven at 60 °C for 48 hours, or until they reached a constant mass (Patria et al. 2023). The sediment samples were pulverized and sieved through a 5 mm steel sieve. A portion of 100 g of dried sediment was taken from the sieve, placed into a beaker glass, and covered with aluminum foil to prevent external contamination. The sample was suspended in 400 mL of saturated NaCl solution (1.2 g

114 cm³), which is four times the sample weight, using a magnetic stirrer. This NaCl solution was prepared by dissolving pure
115 NaCl crystals (Merck Millipore EMSURE®) in filtered distilled water that was free from contaminants. The selection of a
116 NaCl solution for the separation of microplastics from sediments was based on the premise that this method is cost-
117 effective and environmentally friendly (Perumal and Muthuramalingam 2022). Stirring was carried out for 5 minutes until
118 completely dissolved, and the mixture was allowed to stand for 1 hour. After one hour, 10 mL of 30% H₂O₂ was added to
119 assist in the breakdown of organic matter, followed by stirring for an additional five minutes. Hydrogen peroxide (H₂O₂) is
120 commonly used in similar studies and is regarded as highly effective for organic matter removal (Lee et al. 2023). The
121 sample was allowed to stand for 24 hours, covered with aluminum foil. The samples were then filtered using the Whatman
122 No. 42 filter paper (mesh size 0.45 µm, Φ = 90 mm), assisted by a vacuum pump at 17 kPa to isolate the microplastics.
123 The collected samples were deposited into petri dishes for identification.

124 125 **Microplastic extraction from *Anadara granosa***

126 The previously frozen *A. granosa* samples were defrosted under controlled conditions at room temperature (± 25°C) for
127 1 hour. After the ice melted, the *A. granosa* were washed with pure distilled water to eliminate contamination from other
128 objects, such as sediment. The length and wet weight of the *A. granosa* were measured using an analytical balance (0.01
129 g). The digestive tract was carefully removed from the *A. granosa* using sterile stainless steel utensils that had been rinsed
130 with distilled water. The extraction of microplastics from the digestive tract of the *A. granosa* was adapted from previous
131 studies with minor modifications (Ding et al. 2018; Ding et al. 2021). The digestive tract was transferred into an
132 Erlenmeyer flask, and 100 mL of KOH solution (10%) was added. The use of KOH is regarded as a more efficacious
133 method for the digestion of biological material, and it has no impact on the integrity of the plastic polymer (Karami et al.
134 2017). The sample was covered with aluminum foil and stirred for 5 minutes using a 150 rpm magnetic stirrer. The
135 supernatant was incubated at room temperature (±25°C) until complete digestion of organic matter occurred. The sample
136 was then filtered through Whatman 42 filter paper (mesh size 0.45 µm, Φ = 90 mm) to isolate microplastic. The filter
137 paper was dried in an oven at 40°C for 5 hours and stored in petri dishes for subsequent identification of microplastic
138 content.

139 140 **Microplastic identification and quantification**

141 Identification and quantification of microplastics from sediments and *A. granosa* were performed using the same
142 method. Microplastic identification was performed using an Olympus CX23 microscope with 10 x 10 or 4 x 10
143 magnification to visually detect microplastics (Diansyah et al. 2024). The filter paper was observed closely and carefully
144 to avoid contaminants that could enter the filter paper. Microplastics were identified based on the number and form of
145 microplastics (fragment, film, fiber, and foam). The data obtained were recorded by sample type and station/individual for
146 statistical purposes. To prevent contamination, personnel wear cotton lab coats, latex gloves, and cotton masks.
147 Furthermore, access to the detection room is restricted to prevent outside contact during observations.

148 149 **Procedures for quality assurance and contamination prevention**

150 The quality and contamination control measures were implemented to prevent any alterations to the microplastics in
151 the samples. Before sampling, glass jars and aluminum foil were rinsed with filtered distilled water. Although the samples
152 were in a cool box, they were stored in a place that was not exposed to direct sunlight to keep the samples in good
153 condition. Personnel are also required to wear latex gloves during sampling. During the study, from sampling to laboratory
154 analysis, the use of plastic equipment was minimized to avoid unintentional fragmentation of the microplastics. The
155 distilled water used was filtered with filter paper (0.45 µm) to prevent microplastics from entering our materials. All
156 equipment was washed with filtered distilled water to remove potential contamination from external particles (Ding et al.
157 2021). We prepared two controls by filtering each solution we used to avoid contaminants from materials, equipment, and
158 air. No microplastics were found contaminating the instrument. The sterilized equipment was wrapped in aluminum foil to
159 prevent any input of contaminants from outside. Laboratory personnel were required to wear latex gloves, lab coats, and
160 masks throughout the analysis. Access to the laboratory was restricted during the analysis to minimize external
161 interference that could lead to protocol errors.

162 163 **Data analysis**

164 **Pollution Load Index (PLI)**

165 PLI was calculated using a pollutant load approach, which assesses the total concentration of contaminants relative to a
166 baseline level, to evaluate its significance. Equations were utilized to quantify the level of microplastic pollution in both
167 sediments and *Anadara-A. granosa* in the Musi Estuary, with the method adapted accordingly (Tomlinson et al. 1980; Xu
168 et al. 2018; Wang et al. 2021)

$$CF_i = \frac{C_i}{C_{0i}}$$

This equation shows that the concentration factor (CF_i) at a particular sample is obtained by dividing the current concentration (C_i) by the initial concentration (C_{0i}). C_i indicates the quantified presence of microplastics at each sampling location or within each clam (ind), while C_{0i} denotes the background concentration, defined as the lowest value recorded across all sampling locations. Given the lack of previously published research on microplastics in sediments or *A. granosa* in the Musi River Estuary, we determined C_{0i} values from minimum concentrations across sampling stations.

$$PLI = \sqrt{CF_i}$$

This equation shows that the Pollution Load Index (PLI) is generated by calculating the square root of the Concentration Factor (CF). The PLI value of microplastics is derived from the contaminant factor (CF_i) calculated for each station/individual.

$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \dots \times PLI_n}$$

Pollution Load Index of Zone (PLI_{zone}) is determined by taking the square root of the product of individual PLI values from multiple sites within the zone. This method integrates data from several measurement points, providing a more comprehensive assessment of microplastic contamination within the analyzed. A PLI_{zone} is used to assess pollution based on specific estuary zones as well as the overall estuary area. PLI values are categorized into four predefined categories of pollution load, as shown in Table 2.

Table 2. Microplastic pollution load categories

Definition	Value			
Value of the pollution load index (PLI)	<10	10-20	20-30	>30
Risk category	I	II	III	IV
Keterangan	Minor	Middle	High	Extreme

Nemerow Pollution Index (NPI)

NPI is designed to assess the bioavailability of microplastics in sediments and *A. granosa*. This index was adapted from equations used in prior studies evaluating heavy metal contamination (Alam et al. 2023).

$$NPI = \sqrt{\frac{\left(\frac{C_i}{S_i}\right)_{max}^2 + \left(\frac{C_i}{S_i}\right)_{ave}^2}{2}}$$

The NPI value is calculated using the square root of the mean squared deviation, incorporating both the maximum and average concentration ratios relative to the background concentration (S_i). The formula includes the sum of the squares of the maximum concentration ratio (C_{i max}/S_i) and the average concentration ratio (C_{i ave}). The C_i value represents the concentration of microplastics found in the sediment or clams at a specific station or individual, while the S_i value denotes the background level of microplastics, determined as the lowest concentration recorded across all sample types. The NPI provides a measure of microplastic bioavailability in sediments and *A. granosa*, with values below 2 indicating low bioavailability and values above 2 indicating high bioavailability.

Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) quantifies the extent to which microplastics accumulate in biota relative to their environmental concentration and is calculated using the following equation (Li et al. 2022).

$$BCF = \frac{C_{biota\ ave}}{C_{sediment\ ave}}$$

In this equation, BCF represents the bioconcentration factor, which is derived from the calculation of the average concentration of microplastic in biota (*A. granosa*) divided by the average concentration of microplastic in its environment (sediment). This value is intended to assess how much microplastic is concentrated in the biota.

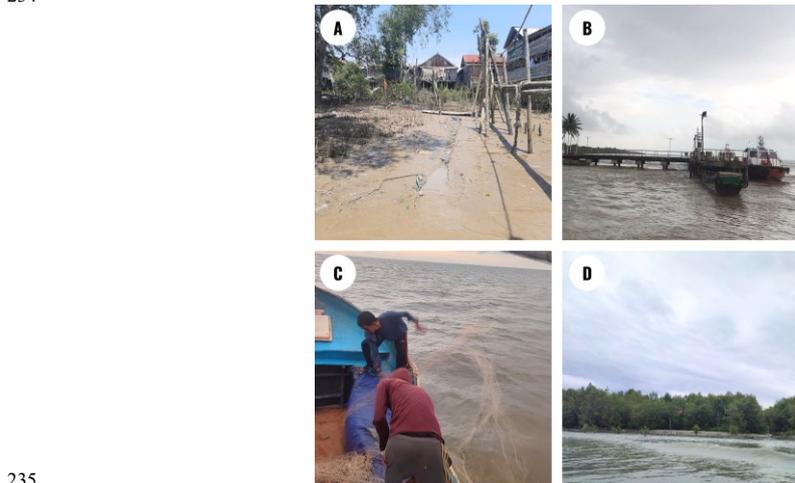
Statistical analysis

219 Statistical analyses were conducted using IBM SPSS Statistics 27. Descriptive statistics, including total, mean,
220 standard deviation, percentage, minimum, and maximum, were utilized to assess data variation. A one-way ANOVA was
221 applied to analyze differences in microplastic abundance between estuarine zones when the data met normality
222 assumptions. For non-normal data, the Kruskal-Wallis test was employed to assess the significance of differences between
223 zones. All tests were carried out with a significance level of $\alpha = 0.05$.

224 RESULTS AND DISCUSSION

225 Microplastic presence in sediment from Musi Estuary

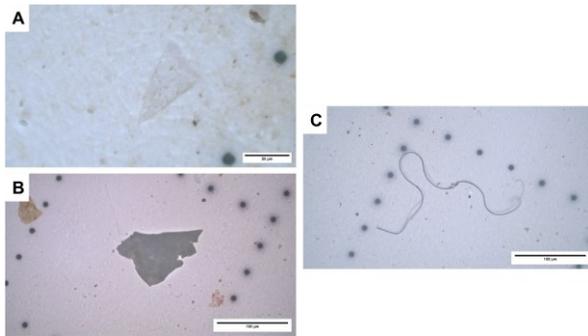
226 The Musi Estuary in South Sumatra, Indonesia, is a dynamic brackish water environment where freshwater from the
227 Musi River meets the saltwater of the Bangka Strait. Characterized by turbid waters and muddy sediments rich in organic
228 matter, the estuary is fringed by mangrove forests that provide habitat for aquatic species, including the blood clam
229 (*Anadara granosa*), a key species in local aquaculture (Rozirwan et al. 2023). Surrounded by urban and industrial areas,
230 including Palembang, the estuary faces pollution from domestic, agricultural, and industrial waste, making it susceptible to
231 microplastic accumulation in sediments and ingestion by benthic organisms like blood clams (Almiza and Patria 2021;
232 Diansyah et al. 2024).



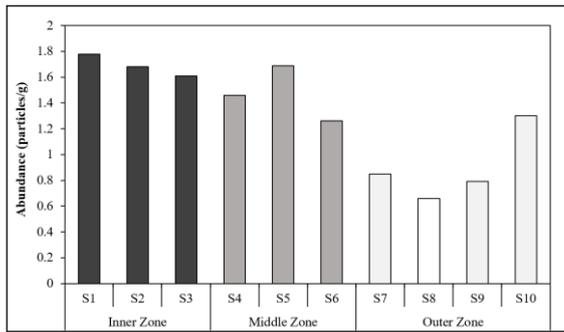
235 **Figure 2.** General conditions in the Musi estuary. A. Coastal Settlements; B. Shipping Activities; C. Fisheries; D.
236 Mangrove Ecosystem
237
238

239 The results of this study indicate that microplastics were detected at all sampling stations, as shown in Figure 4. A total
240 of 1,308 microplastic particles were identified, with concentrations ranging from 66 to 178 particles per 100 g of dry
241 sediment (dry weight, dw). The average abundance of microplastics was 1.31 ± 0.41 particles/g dw, with the highest
242 concentration found at station S1 (1.78 particles/g dw) and the lowest at station S8 (0.66 particles/g dw). High levels of
243 human activity in the upstream area of Musi Estuary, which flows downstream to the estuary and ultimately into the ocean,
244 are responsible for the presence of microplastics (Rozirwan et al. 2021; Diansyah et al. 2024). Urban activities in close
245 proximity to the Musi River Watershed, particularly in Palembang City, significantly contribute to the generation of plastic
246 waste. Increased population density near riverbanks enhances the potential for plastic pollution. Furthermore, population
247 density is a crucial factor in the entry of microplastics into rivers (Eo et al. 2023; Dwiyitno et al. 2024). As a result, plastic
248 pollutants from domestic activities, such as the disposal of food packaging and plastic bags, can be transported through
249 stormwater drains and eventually end up in rivers (Kunz et al. 2023). Fishing activities around Musi Estuary can contribute
250 to microplastic pollution, primarily through fibers released from fishing nets (Li et al. 2022; Fauziyah et al. 2023). This
251 increases anthropogenic pressure at river mouths, which can lead to greater accumulation of microplastics (Castro-Jiménez
252 et al. 2024). Additionally, tidal fluctuation influences water flow, which facilitates the deposition of microplastics in
253 sediment at these locations (Harris 2020). The findings of this study indicate that anthropogenic activities are responsible
254 for high levels of microplastic deposition in the sediments of Musi Estuary. Microplastic particles that accumulate in
255 sediments serve as a habitat for benthic organisms, which can have long-term implications for ecosystem health and

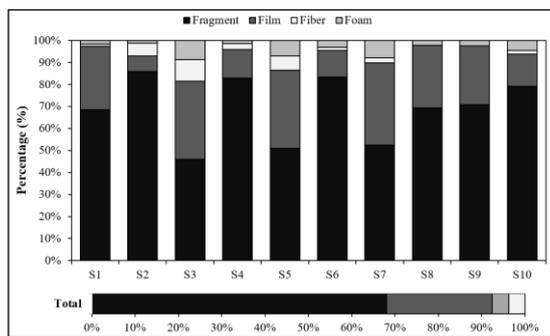
256 balance (Rahmatin et al. 2024). As vectors for pollutants, microplastics can exacerbate toxicity, potentially endangering
 257 various populations (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). Furthermore,
 258 the processes of bioaccumulation and biomagnification in benthic organisms present heightened risks to these animals,
 259 potentially leading to human consumption (Unuofin and Igwaran 2023). Therefore, it is crucial for local governments to
 260 address the handling and prevention of further microplastic accumulation.
 261



262 **Figure 3.** Various forms of microplastics, A. Film; B. Fragment; C. Fiber
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 264



265 **Figure 4.** Distribution of microplastics from sediments in Musi Estuary, Indonesia.
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 268



269 **Figure 5.** Percentage abundance of microplastic shapes form sediments in Musi Estuary, Indonesia
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The microplastics identified in the sediments were predominantly fragments (68.92%), followed by films (23.86%), fibers (4.05%), and foams (3.17%) (Figure 3 and Figure 5). The dominance of fragments, which are a common form of

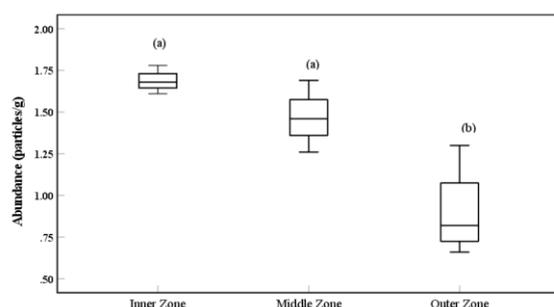
secondary microplastics, occurs as a result of macroplastic degradation (Barnes et al. 2009). Fragments represented the most common type across all sediment samples analyzed. This dominance of fragments in estuarine sediments aligns with findings from various studies conducted in other estuarine environments. Research indicates that estuarine sediments globally tend to accumulate diverse microplastic forms, particularly fibers (Firdaus et al. 2020; Alam et al. 2023; Samuels et al. 2024; Santucci et al. 2024), with many studies also reporting a predominance of fragment types (Zhou et al. 2021; Suteja et al. 2024). These observations corroborate the literature, which consistently shows that fibers and fragments constitute the majority of microplastic pollution in estuarine ecosystems (Feng, An, et al. 2023).

This study reveals notable variations in microplastic concentrations compared to similar research in estuaries globally. Our findings indicate that the Musi Estuary has lower microplastic concentrations than those reported in several other Indonesian and international estuaries. For example, the Jakarta Bay Estuary exhibits concentrations nearly ten times higher, ranging from 1,184 to 1,337 particles per 100 grams of river sediment and 804 to 1,055 particles per 100 grams of beach sediment (Dwiyitno et al. 2024). Such elevated levels are likely due to increased anthropogenic activities along Jakarta's rivers, which substantially contribute to plastic waste flow. In another comparison, the Pearl River Estuary in China also shows higher microplastic densities, with concentrations between 2.05×10^3 and 7.75×10^3 particles per kilogram of dry weight sediment (Xu et al. 2024). This estuary's coastal regions experience high human activity, enhancing the potential for microplastic deposition. Similarly, the Meghna Estuary in Bangladesh, which receives sediment from the Ganges River Basin, reports even higher microplastic levels at $4,014.66 \pm 1,717.59$ particles per kilogram dry weight (Alam et al. 2023). These findings underscore the significant role of anthropogenic influences on microplastic accumulation in global estuarine sediments, including the Musi Estuary. Other environmental factors, like location, sampling period, sediment depth, sediment composition, and local hydrodynamic conditions, may also affect these changes in the amount of microplastics found (Zheng et al. 2020; Feng, Chen, et al. 2023; Yuan et al. 2023).

In contrast, the abundance of microplastics in the sediments of the Musi Estuary surpasses levels reported in other regions, both within Indonesia and globally. Concentrations in the Musi Estuary are notably higher than those in the Zandvlei River Watershed and estuarine areas of South Africa (70.23 ± 7.36 particles/kg dw) (Samuels et al. 2024), the Claromecó Estuary in Argentina (299 ± 114 particles/kg dw) (Truchet et al. 2021), and the upper sediment layer (0-5 cm) of the Fuhe River Estuary in Northern China (1049 ± 462 particles/kg dw) (Zhou et al. 2021). These numbers are also higher than those found in coastal Río de la Plata (547.83 ± 620.06 particles/kg dw) (Santucci et al. 2024), Benoa Bay, Bali (31.08 ± 21.53 particles/kg dw) (Suteja et al. 2024), the Jagir Estuary in Surabaya (up to 590 particles/kg dw) (Firdaus et al. 2020), and the Pekalongan River Estuary in Java (0.77 to 1.01 particles/g dw) (Ismanto et al. 2023). The high microplastic concentration in the Musi Estuary is likely due to extensive plastic waste disposal in the river basin by the surrounding community. Research supports that plastic debris forms a substantial part of the macro-waste in the Musi River (Maherlsa et al. 2019). Additionally, local communities frequently establish settlements along the river, relying on it for water access and transportation. Consequently, domestic waste, including plastic, often enters the river directly. This local waste management issue reflects a broader trend, with Indonesia identified as the world's second-largest contributor to oceanic plastic pollution, following China (Jambeck et al. 2015).

Spatial distribution microplastic in Sediments

The study revealed that the abundance of microplastics was significantly higher in the inner and middle zones when compared to the outer zone. The mean abundances were 1.69 ± 0.08 particles/g dw in the inner zone, 1.47 ± 0.22 particles/g dw in the middle zone, and 0.9 ± 0.28 particles/g dw in the outer zone. Significant differences in mean microplastic abundance between zones were revealed by the one-way ANOVA ($p < 0.05$), as illustrated in Figure 56. The Tukey HSD test revealed no significant difference in mean microplastic abundance between the inner and middle zones. However, we observed a significant difference in the outer zone compared to both the middle and outer zones. Our results indicate that the inner and middle estuary regions exhibit greater mean concentrations of microplastics compared to the outer estuary. Natural factors, particularly the effects of currents and wave action in coastal and estuarine environments, significantly influence this phenomenon by altering microplastic distribution. The influx of freshwater promotes the sedimentation and prolonged retention of high-density microplastics within the system (G. Li et al. 2024). Furthermore, the sedimentation process is affected by the density and buoyancy of microplastics, with increased salinity in estuarine waters enhancing buoyancy forces and influencing distribution patterns (Cheng et al. 2024). Our findings align with research from the Liaohu Estuary in China, revealing a higher accumulation of microplastics in inner river sediments compared to the outer estuary (Xu et al. 2020). Additionally, sampling depth plays a critical role in accurately measuring microplastics in surface waters, bottom layers, and sediments (Feng, An, et al. 2023).



327
328 **Figure 56.** The difference in microplastic abundance between zones in the sediments
329

330 The spatial analysis revealed distinct patterns of microplastic accumulation between the two sides of the coast adjacent
331 to the estuary. Notably, Site S7 exhibited higher concentrations compared to Site S8, likely due to its proximity to other
332 pollution sources. Specifically, S7 is located near the Banyuasin Estuary, which is believed to significantly contribute to
333 the deposition of microplastics in the surrounding sediments. Based on Costa et al. (2023), confirmed that nearby sources
334 of pollution heavily influence microplastic deposition in coastal regions. The accumulation of microplastics at the mouth
335 of the adjacent river further exacerbates this phenomenon, facilitating the settlement of microplastics within the sediment.
336 Additionally, studies suggest that the depth of the sampling locations significantly influences microplastic deposition,
337 revealing a greater abundance of microplastics at deeper water levels (Bayo et al. 2022). The substantial accumulation of
338 microplastics in sediments is attributed to their persistent characteristics as well as the protective conditions offered by
339 deeper environments, which are shielded from UV light, maintain lower temperatures, and exhibit lower oxygen levels,
340 thus slowing biodegradation processes (Zhang et al. 2021).

341 Our findings confirm the presence of microplastics in the sediments of the Musi Estuary, likely originating from
342 suspended microplastics that eventually settle from the water's surface. Previous research has found that there are $467.67 \pm$
343 127.84 particles/ m^3 and 723.67 ± 112.05 particles/ m^3 of microplastic in surface waters during ebb and flow conditions,
344 respectively (Diansyah et al. 2024). Hydrodynamic conditions in the estuary and processes like aggregation and
345 biofouling, which increase microplastic density, influence the deposition of these particles (Malli et al. 2022). The specific
346 properties of the microplastics and the water dynamics of the estuary strongly influence microplastic deposition. For
347 instance, biofouling can make microplastics denser, accelerating their descent into sediments (Lin et al. 2023). Since
348 microplastics often have higher densities than water, they can remain suspended in the water column before eventually
349 settling (Dai et al. 2022). High Total Suspended Solids (TSS) in the Musi Estuary have the potential to cause aggregation
350 of microplastics (Rahutami et al. 2022). High TSS levels make it easier for sediments and other suspended particles to
351 stick together on microplastic surfaces, which speeds up their deposition (Yang et al. 2022). However, microplastics can
352 also become resuspended in the water column (Tang et al. 2020). Stirring forces in estuarine environments can lift
353 microplastics back into the water, where turbulence and bioturbation may redistribute them (Malli et al. 2022). Therefore,
354 additional studies are required to fully characterize the mechanisms of microplastic deposition in the Musi Estuary.
355 Climate factors also influence microplastic distribution in Musi Estuary sediments. In Indonesia, where regions such as the
356 Musi Estuary experience distinct rainy and dry seasons, seasonal changes affect water dynamics and microplastic
357 transport. During the rainy season, increased river velocity may carry microplastics upward from sediments, while in the
358 dry season, slower flows facilitate microplastic accumulation at the water's surface (Zhao et al. 2020). Currently, there are
359 no studies specifically examining the seasonal impact on microplastic presence in the Musi Estuary, underscoring the need
360 for further research to address this gap.

361 **Bioaccumulation of microplastics in *Anadara granosa***

362 We extracted 133 microplastic particles from the digestive tracts of twenty samples of *A. granosa* species presented in
363 Figure 76. Each sample from the Musi Estuary had accumulated microplastics, ranging from 6 to 49 particles/ind or 8 to
364 69.01 particles/g ww, as shown in Figure 78 and Figure 89. The mean abundance of microplastics found in the digestive
365 tracts was 21.05 ± 10.31 particles/ind or 30.46 ± 16.7 particles/g ww. Several factors contribute to the increased microplastic
366 inputs in bivalve species, including human activities, tourism, fishing equipment, freshwater runoff, and wastewater
367 discharge, combined with the area's hydrodynamic conditions (Vital et al. 2021). The elevated levels of microplastics
368 found in *A. granosa* from the Musi River Estuary suggest a significant degree of contamination in their habitat, including
369 both water and sediment, which may adversely affect the health of these organisms and disrupt local ecological balances.
370 This observation aligns with existing literature, which indicates a strong correlation between the abundance of
371

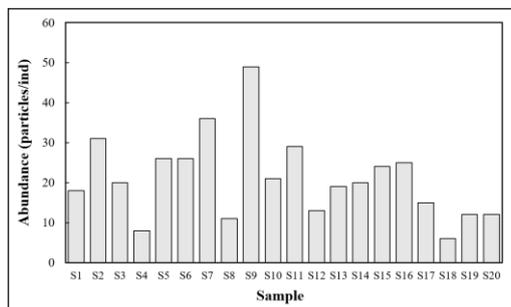
372 microplastics in bivalves, such as mussels, and their sediment environments (Narmatha Sathish et al. 2020), reinforcing the
373 need for monitoring these pollutants in aquatic ecosystems.



374 **Figure 67. Morphology-Specimens** of *Anadara granosa* from Musi Estuary, Indonesia.

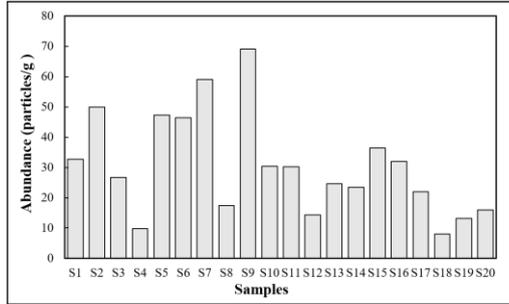
375
376
377 Microplastics were observed in three morphological forms: film, fiber, and fragment. Film accounted for the majority
378 of microplastics (59.38%), followed by fiber (31.59%) and fragments (9.03%), as shown in Figure 109. The digestive
379 tracts of the *A. granosa* studied did not contain any pellets or foam. Statistical analysis using the Kruskal-Wallis test
380 revealed significant differences in the abundance of the three microplastic forms ($p < 0.05$). This predominance of film
381 microplastics in *A. granosa* is likely attributable to the extensive use of disposable plastic packaging within the
382 community, which subsequently degrades into microplastics. Although fragments dominate the sediment substrate, *A.*
383 *granosa* predominantly absorb film microplastics. The lower density of film microplastics may facilitate their
384 accumulation in the upper sediment layer, making them susceptible to resuspension in the overlying water column. This
385 phenomenon is consistent with the feeding behavior of *A. granosa*, which frequently filter water from the sediment
386 surface, resulting in the accumulation of film microplastics within their digestive tracts.

387 Our findings indicate a higher abundance of microplastics in *A. granosa* from the Musi Estuary compared to certain
388 other regions globally. For instance, *A. granosa* from the Chanthaburi estuarine ecosystem in eastern Thailand contained
389 0.40 ± 0.16 particles/g ww (Potipat et al. 2024), while samples from Peninsular Malaysia showed concentrations of $0.20 \pm$
390 0.08 particles/g ww and 1.54 ± 0.30 particles/ind ww (Mohan et al. 2024). Additionally, microplastic levels in *A. granosa*
391 from Pao Village, Tarawang District, Jeneponto Regency, Indonesia, were measured at 0.0144 particles/g ww (Namira et
392 al. 2023), and those from Likas Bay Beach in North Borneo, Malaysia, at 24.4 ± 0.6 particles/g ww (Abd Rahman et al.
393 2024). In contrast, the *A. granosa* analyzed in this study accumulated lower microplastic concentrations than those
394 reported in several other studies. For instance, *A. granosa* from the Pangkal Babu mangrove forest area in Tanjung Jabung
395 Barat district, Jambi, exhibited 434 ± 97.05 particles/ind (Fitri and Patria 2019), while those from Lada Bay in Pandeglang,
396 Banten, had concentrations of 618.8 ± 121.4 particles/ind (Ukhrowi et al. 2021). Overall, this study shows that
397 microplastics have contaminated the sediment environment of the Musi Estuary, South Sumatra, indicating that *A. granosa*
398 can serve as effective bioindicators of this pollution.



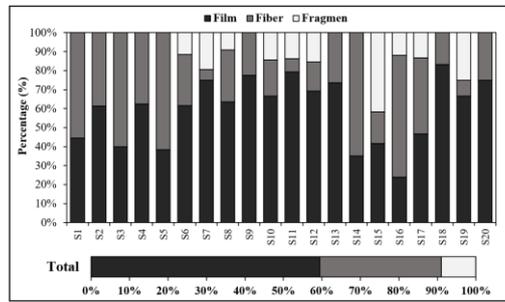
401
402
403 **Figure 87.** Microplastic abundance of *Anadara granosa* from the Musi Estuary

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405
406
407

Figure 89. Microplastic abundance (in particles/g) of *Anadara granosa* from the Musi Estuary



408
409
410

Figure 910. Shape of microplastics in *Anadara granosa* from the Musi Estuary

Risk assessment of microplastics in sediments and *Anadara granosa*

A risk assessment of microplastic contamination in the Musi River Estuary was carried out by calculating the Pollution Load Index (PLI), the Nemerow Pollution Index (NPI), and the Bioconcentration Factor (BCF). The PLI values across all sediment samples indicate that the estuarine sediment is subject to minor pollution, with values ranging between 1.00 and 1.64, as shown in Table 3. In the three zones of the estuary (inner, middle, and outer), the PLI values were 1.60, 1.49, and 1.20, respectively. We calculated the overall average PLI for the Musi Estuary to be 1.37. All zones reported PLI values below 3.00, which suggests that the area experiences only minor microplastic pollution. The analysis of microplastic presence in sediment samples revealed a significantly high concentration of microplastics, with an NPI value of 2.37. This suggests a substantial bioavailability of microplastics in the estuarine environment. This elevated NPI underscores the ecological risk posed by microplastic contamination, despite the relatively low PLI observed across the study area.

421

Table 3. The microplastic risk factor in sediments of Musi Estuary

Sediment Station	CF	PLI	PLI Zone	PLI Sediment	NPI
S1	2.70	1.64	Inner Zone 1.60	1.37	2.37
S2	2.55	1.60			
S3	2.44	1.56			
S4	2.21	1.49	Middle Zone 1.49		
S5	2.56	1.60			
S6	1.91	1.38			
S7	1.29	1.13	Outer Zone 1.20		
S8	1.00	1.00			
S9	1.20	1.09			
S10	1.97	1.40			

423

424 *Anadara granosa* analyzed in this study exhibited significant risk values associated with microplastic contamination.
 425 The PLI for these clams ranged from 1 to 2.86, with an overall mean PLI value of 1.77 for the entire sample, as shown in
 426 Table 4. This indicates that the pollution load in *A. granosa* falls within the minor category (PLI < 3). Furthermore, the
 427 bioavailability of microplastics in *A. granosa* was significantly higher, with an NPI value of 6.29, indicating a high
 428 bioavailability. The bioconcentration factor of microplastics from sediment to *A. granosa* was measured at 23.28,
 429 suggesting a significant potential for the transfer of microplastics from sediment to *A. granosa* in the Musi Estuary.

430 Our findings demonstrated that the bioavailability of microplastics in sediments and *A. granosa* was categorized as
 431 high (NPI = 2.37; NPI > 2). In comparison, the bioavailability of microplastics in *A. granosa* was even higher, with an NPI
 432 value of 9.89. The elevated bioavailability in sediments suggests a greater potential for absorption by aquatic organisms,
 433 particularly benthic animals. Furthermore, the bioconcentration factor (BCF) calculated for *A. granosa* in sediment was
 434 23.28, indicating a significant transfer of microplastics from sediments to these organisms in the Musi Estuary. This high
 435 BCF value is attributed to the non-discriminatory feeding process of *A. granosa*, allowing them to ingest microplastics
 436 along with other particles. When compared to several benthic species from the Yangtze River Estuary, which exhibited a
 437 BCF of 29.48 ± 6.52 (Z. Li et al. 2022), the BCF of *A. granosa* in the Musi Estuary remains lower. Nonetheless, both
 438 sediments and *A. granosa* from the Musi Estuary are classified within the minor pollution risk index category (PLI < 10).
 439 The bioaccumulation of microplastics by benthic animals like *A. granosa* poses a significant risk to human health for those
 440 consuming these clams (Wang et al. 2023; Winiarska et al. 2024). Therefore, periodic assessments are crucial to evaluate
 441 the risk of microplastic contamination in sediments and *A. granosa*, providing essential information to understand the
 442 ecological and health implications in the Musi Estuary.

443 The impact of high bioaccumulation extends beyond the toxicity of microplastic materials; it also facilitates the
 444 accumulation of other pollutants. Previous research has detected heavy metals, such as lead (Pb) and copper (Cu), adhering
 445 to the surfaces of microplastics in the Musi Estuary (Purwiyanto et al. 2020). Additionally, various hazardous heavy
 446 metals, including iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), and zinc (Zn), were identified with high
 447 bioavailability in the aquatic environment of the Musi Estuary (Rahutami et al. 2022). This accumulation allows for the
 448 deposition of significant quantities of heavy metals on microplastic surfaces, thereby increasing the risk of microplastic
 449 toxicity for estuarine ecosystems. Moreover, the involvement of microorganism vectors, misidentification in aquatic
 450 organisms' diets, and their harmful toxicological effects on benthic animals heighten the risk associated with
 451 microplastics (Gong and Xie 2020). Consequently, the high output of microplastics from the Musi Estuary has the
 452 potential to jeopardize the condition of the surrounding aquatic environment, including fisheries and migratory birds,
 453 which are critical to the ecosystem (Rozirwan et al. 2019; Rozirwan et al. 2022).

454
 455 **Table 4.** Risk factors for microplastics in *Anadara granosa* from the Musi Estuary

<i>A. granosa</i> sample	CF	PLI	PLI <i>A. granosa</i>	NPI	BCF
S1	3.00	1.73			
S2	5.17	2.27			
S3	3.33	1.83			
S4	1.33	1.15			
S5	4.33	2.08			
S6	4.33	2.08			
S7	6.00	2.45			
S8	1.83	1.35			
S9	8.17	2.86			
S10	3.50	1.87	1.77	6.29	23.28
S11	4.83	2.20			
S12	2.17	1.47			
S13	3.17	1.78			
S14	3.33	1.83			
S15	4.00	2.00			
S16	4.17	2.04			
S17	2.50	1.58			
S18	1.00	1.00			
S19	2.00	1.41			

This study holds significant ecological implications for the Musi Estuary ecosystem, as the presence of microplastics poses a long-term threat to ecosystem stability. Previous studies have shown that microplastics can affect the physicochemical properties of sediments, such as electrical conductivity, organic matter content, and nutrients, and well as impact enzymatic activity in sediment bacteria (Rillig 2012; B. Yuan et al. 2022; W. Li et al. 2022). These changes can lead to structural and functional alterations in aquatic habitats (W. Li et al. 2022). Microplastics can also enter various levels of the food chain through bioaccumulation and biomagnification processes. For example, microplastics in plankton can cause a decline in zooplankton populations, which impacts food availability for small fish and predatory species at higher trophic levels (Malinowski et al. 2023). The accumulation of microplastics in *A. granosa* in this study indicates that the pressure of plastic waste in the Musi Estuary is already quite high. Microplastics can act as vectors for other pollutants, such as microorganisms, heavy metals, and other inorganic pollutants can increase the risk of toxicity for aquatic organisms, including *A. granosa* (Purwiyanto et al. 2020; Rafa et al. 2024). Research indicates that exposure to microplastics can lead to behavioral and physiological changes in aquatic organisms, including changes in diet and reproductive success (Guo et al. 2020; Liang et al. 2023). Moreover, microplastics disrupt endocrine functions and metabolic pathways, causing oxidative stress, cell necrosis, and apoptosis, which can ultimately lead to mortality (Pannetier et al. 2020; Jeyavani et al. 2021). Disruption of any component of the food chain can trigger a domino effect, threatening the balance of the Musi Estuary ecosystem. However, this study only examines microplastic pollution in sediment and *A. granosa* as environmental indicators. Additional research is needed to understand microplastic buildup in other species, such as fish, shrimp, and benthic organisms, for a more complete picture of microplastic pollution in the Musi Estuary and its impact on the global marine environment.

Microplastic contamination of *A. granosa* in the Musi Estuary poses a significant threat to local communities, particularly clam fishers. Microplastics that accumulate in *A. granosa* may pose health risks to community members who consume them regularly as a primary source of protein. Long-term exposure to microplastics can potentially disrupt various human body systems, including the digestive, respiratory, reproductive, nervous, and cardiovascular systems (Y. Li et al. 2024). Furthermore, microplastics may cause both acute and subchronic toxicity and are considered potentially carcinogenic and disruptive to human development (Z. Yuan et al. 2022). Once in the bloodstream, microplastics can be transported to the liver, the primary organ responsible for detoxification. Accumulation in the liver may trigger adverse physiological reactions, including increased oxidative stress, liver fibrosis, and impaired lipid metabolism. In addition to health risks, microplastic contamination in *A. granosa* in the Musi Estuary may reduce consumer confidence and decrease market demand due to food safety concerns (Unuofin and Igwaran 2023). The long-term effect could weaken local economic resilience as marine products in the region are perceived to be contaminated with microplastics (Barrientos et al. 2024). Therefore, it is crucial for governments and communities to recognize these risks and collaborate on mitigation efforts to protect ecosystems, public health, and local economies.

~~The presence of microplastics in sediments and *A. granosa* in the Musi Estuary poses a significant threat to the ecosystem's sustainability. To address microplastic pollution effectively, collaboration between governmental bodies and local communities is essential. Several strategies can be implemented to reduce microplastic pollution in aquatic environments, including the following measures:~~

- ~~1. Educating the public on the importance of controlling plastic waste (Evode et al. 2021).~~
- ~~2. Reducing single-use plastic consumption and encouraging the use of environmentally friendly alternatives.~~
- ~~3. Banning plastic microbeads in personal care products and cosmetics (Zhang et al. 2022).~~
- ~~4. Promoting sustainable plastic recycling at individual, community, national, and international levels (Babaremu et al. 2022; Thu Nguyen 2024).~~
- ~~5. Strengthening waste management policies for product packaging and disposal practices within the industrial sector (Wu et al. 2023).~~
- ~~6. Enforcing stricter regulations on wastewater treatment facilities and implementing comprehensive plastic waste management through mechanical, physical, chemical, and biological methods to reduce microplastics (Ragaert et al. 2017; Meijerani et al. 2024).~~
- ~~7. Prioritizing microplastic control by evaluating land-use contributions in urban areas, focusing on those with the highest pollution impact (Jin et al. 2022).~~

Furthermore, seafood hygiene practices in local communities and fishing industries can help reduce microplastic contamination (Lusher et al. 2017; Smith et al. 2018). For example, *Anadara-A. granosa* and other seafood, when thoroughly cleaned by repeated washing and clam removal before cooking, may reduce microplastic intake (J. Li et al. 2022). The concentration of microplastics in shellfish is significantly lower after boiling and steaming compared to frying, suggesting that cooking methods affect microplastic retention in seafood (Evode et al. 2021). This method can also be applied to shrimp, fish, and other shellfish to lower the risk of microplastic exposure. Implementing these recommendations will help reduce microplastic pollution and provide long-term benefits for public health, ecosystem stability, and the economic resilience of local fisheries (Prata et al. 2019).

In conclusion, this study demonstrates the significant presence of microplastics in the sediment and *A. granosa* of the Musi Estuary, Indonesia. These findings indicate a serious threat to the aquatic ecosystem and to local public health, as

515 microplastics can enter the food chain and potentially harm organisms across trophic levels, including humans (Ningrum
516 and Patria 2022; Edwin et al. 2023; Patria et al. 2023). This pollution heightens environmental risks, particularly for
517 species integral to both the community's diet and economy (Jeong et al. 2024). The microplastics likely originate from
518 human activities, especially urban waste from upstream areas that flows downstream and accumulates in the estuary
519 (Diansyah et al. 2024). These results highlight the urgent need for routine monitoring and stricter waste management
520 practices, particularly for plastic disposal, to reduce further contamination (Barletta et al. 2019). A comprehensive strategy
521 is essential to controlling plastic waste throughout the river system—from source to estuary. Effective measures should
522 include coordinated waste management practices, public education on the impact of pollution, and cross-sector
523 collaboration to preserve estuarine health (Wakwella et al. 2023; Ihenetu et al. 2024). Such initiatives are crucial to
524 protecting ecosystem health and sustaining local fisheries, ensuring these resources continue to support community well-
525 being.

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We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Implication of microplastics presence in sediment and Blood Clams (*Anadara granosa*) in the Musi Estuary Indonesia". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes.** We are waiting for your revision in the system (<https://smujo.id/biodiv>), do not send it via email.

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Dear Reviewer,

We sincerely appreciate your time and effort in reviewing our manuscript and providing valuable feedback. Your insightful comments have been instrumental in improving the quality of our work. In response, we have carefully revised the manuscript accordingly. Please find our detailed responses to each of your suggestions below.

With kind regards,
The Author (s)

Table of Author(s)' Response

No	Comments	Response
1.	Change "Blood clams" into the species name " <i>A. granosa</i> "	We have changed into the species name
2.	Are there water quality information in this research? Such as salinity, DO, turbidity, temperature, etc	Yes, we have measured the water quality such as temperature, pH, DO, salinity, water brightness, current speed. We have added the information in paragraph 1 in Study Area Section (Line 87 – 89)
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Implication of microplastic presence in sediment and blood clams (*Anadara granosa*, Linnaeus 1758) (Mollusca: Bivalvia) in the Musi Estuary, Indonesia

Author(s) name:

M. AKBAR RAHMAN¹, ROZIRWAN^{2*}, WIKE EKA AYU PUTRI², GUSTI DIANSYAH², MELKI², ICA DELYA², REDHO YOGA NUGROHO²

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This research is groundbreaking because there is currently no information available on the abundance of microplastics in sediments of the Musi Estuary, Indonesia. This article provides a comprehensive review of recent research on microplastics in the sediments of the Musi Estuary and their impact on benthic animals (*Anadara granosa*), one of the seafood from the Musi Estuary.

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Implication of microplastic presence in sediment and blood clams *Anadara granosa* (Linnaeus 1758) (Mollusca: Bivalvia) in the Musi Estuary, Indonesia

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Manuscript received: 30 September 2024. Revision accepted: 2024.

Abstract. Microplastic pollution poses a serious risk to estuarine ecosystems, affecting sediments and benthic species. Blood clams (*Anadara granosa*, (Linnaeus 1758) represent a significant commercial seafood product in Indonesia, including those from the Musi Estuary. This study investigated the risks associated with microplastic contamination in sediments and *A. granosa* in the Musi Estuary, located in South Sumatra, Indonesia. Sediment samples were extracted using sodium chloride (NaCl, 1.2 g cm⁻³) and hydrogen peroxide (30% H₂O₂). In contrast, *A. granosa* samples were digested with 10% KOH for microplastic extraction. The abundance and shape of microplastics were analyzed from both samples. Pollution risk assessment was conducted through the calculation of the Pollution Load Index (PLI), Nemerow Pollution Index (NPI), and Bioconcentration Factor (BCF). The results revealed the presence of microplastics in sediments, with a mean abundance of 1.31±0.41 particles/g dw, while in *A. granosa*, it was 21.05±10.31 particles/ind. Both samples exhibited high microplastic bioaccumulation (NPI > 2), although the pollution load remained relatively low (minor category, PLI < 10). The bioconcentration factor between *A. granosa* and sediment was determined to be 23.28, indicating that the *A. granosa* absorbed microplastics present in the sediment. These findings highlight the significant bioaccumulation potential of microplastics in *A. granosa* blood clams within the Musi Estuary. It is important for the local community and government need to establish mitigations for future microplastic management efforts.

Keywords: Bioaccumulation, blood clams, microplastics, Musi Estuary, sediment

Running title: Microplastics in sediment and blood clams

INTRODUCTION

River estuaries are intricate and dynamic ecosystems that play a vital role in ecological sustainability and support a variety of socio-economic activities (Boerema and Meire 2017; Hasan et al. 2022; Retnaningdyah et al. 2022; Hasan et al. 2023). These ecosystems provide essential habitats for diverse species and act as focal points for human activities, including urbanization, recreation, and commercial enterprises (Islamy and Hasan 2020; Lonsdale et al. 2022; Natsir et al. 2022; Anurrahman et al. 2023). Among the major estuaries in Sumatra, the Musi Estuary holds significant ecological and economic importance (Surbakti et al. 2023). It serves as a critical habitat for various fish species and benthic organisms, which in turn sustain the livelihoods of local communities through the utilization of water resources (Rozirwan et al. 2021; Fauziyah et al. 2022; Rozirwan et al. 2022; Fauziyah et al. 2023). Nevertheless, the intensification of human activities within and surrounding the Musi Estuary has resulted in significant environmental deterioration (Maherlsa et al. 2019). Major contributors to this issue include heavy metal and microplastic contamination, which pose a substantial threat to ecosystem health (Purwiyanto et al. 2022; Rahutami et al. 2022; Fitria et al. 2023; R. Rozirwan et al. 2023; Diansyah et al. 2024; Rozirwan et al. 2024). These pollutants endanger the ecological equilibrium of the estuary, with the potential to affect its biodiversity and the livelihoods dependent upon it.

Macrozoobenthos are considered pivotal components in preserving the equilibrium of estuarine ecosystems (Sari et al. 2022; Isoni et al. 2023). These organisms play a crucial role in the food web and nutrient cycling, thereby supporting a variety of marine species (Griffiths et al. 2017). Moreover, macrozoobenthos are highly sensitive to environmental changes, making them reliable bioindicators of ecological degradation (Sahidin et al. 2018). Research worldwide highlights that macrozoobenthos, including bivalves such as blood clams *Anadara granosa* (Linnaeus 1758) are often used as bioindicators because they can take in and store pollutants from their environment through filter-feeding (Fitri and Patria 2019; Ward et al. 2019; Ding et al. 2021; Bonifacio et al. 2022; Saleh et al. 2023; Rahmatin et al. 2024). *Anadara granosa* is widely consumed by the general public as a nutrient-rich seafood, particularly among local communities

(Rozirwan; et al. 2023; Rozirwan; et al. 2023). Among these, *A. granosa* is a prominent bivalve species that plays a crucial role in maintaining ecosystem stability and generating substantial economic benefits for coastal communities (Yulinda et al. 2020; Prasetyono et al. 2022; Mahary et al. 2023). Given its ecological importance and economic value within coastal and estuarine systems, it is imperative to investigate pollutant contamination in *A. granosa* to understand the broader impacts of environmental pollution comprehensively.

Various pollutants, including heavy metals, hydrocarbons, pesticides, and microplastics, have been identified as significant contaminants of estuarine ecosystems, with many of these pollutants accumulating in sediments (Zhao et al. 2015; Kılıç et al. 2023; Han et al. 2024; Jacq et al. 2024). Among these, plastic pollution has emerged as a critical concern due to its pervasive impact on aquatic environments (Borrelle et al. 2020; Hecker et al. 2023). Over the last five decades, the widespread reliance on single-use plastics has led to extensive accumulation and fragmentation of plastic materials across diverse ecosystems (Zhang et al. 2021; Walker and Fequet 2023). As plastics degrade, they break down into microplastics, defined as particles ranging in size from 1 μm to 5 mm (Frias and Nash 2019). These microplastics enter aquatic ecosystems through various pathways, such as the fragmentation of discarded plastic products, the release of synthetic fibers during washing, and the intentional addition of microbeads in personal care products (Napper et al. 2015; Praveena et al. 2018; Belzagui and Gutiérrez-Bouzán 2022; Gan et al. 2023). Microplastics pose multiple threats by releasing toxic additives and acting as carriers of hazardous organic pollutants and heavy metals (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). These impacts are multifaceted, disrupting food webs, altering nutrient cycles, modifying habitats, and potentially inducing genetic changes in aquatic organisms (Thacharodi et al. 2024).

Prior research has quantified microplastic concentrations in the waters of the Musi Estuary, with reported levels of 467.67 ± 127.84 particles/ m^3 during flood tide and 723.67 ± 112.05 particles/ m^3 at ebb tide (Diansyah et al. 2024). These microplastics are frequently associated with toxic heavy metals, such as lead (Pb) and copper (Cu), which exacerbate their harmful environmental effects (Purwiyanto et al. 2022). In the water column, microplastics tend to settle into estuarine sediments, which have been identified as the second-largest global reservoir for microplastics after fjords (Harris 2020). The accumulation of microplastics in estuarine sediments poses significant ecological challenges, particularly due to their ingestion by benthic organisms, including *A. granosa* (Fitri and Patria 2019; Saleh et al. 2023; Rahmatin et al. 2024; Mohan et al. 2024). While numerous studies have examined microplastic concentrations in the water column, research on their distribution in sediments and bioaccumulation in benthic species, particularly *A. granosa*, remains limited.

This study aims to investigate the bioaccumulation and risks of microplastic pollution in sediments and *A. granosa* in the Musi Estuary, Indonesia. The findings are expected to enhance understanding of benthic organisms, especially *A. granosa*, and its potential health risks for local seafood consumers.

MATERIALS AND METHODS

Study Area and Sample Collection

The Musi Estuary, located on the east coast of South Sumatra, is where seawater from the Bangka Strait mixes with freshwater from the Musi River. The Musi Estuary is an important area for fisheries and benthic habitats in South Sumatra, Indonesia (Rozirwan et al. 2021; Rozirwan et al. 2022). Sampling locations were selected based on hydrodynamic conditions influencing microplastic transport and deposition, considering salinity variations and water mass interactions (Cheng et al. 2024; Diansyah et al. 2024). Locations of the sampling sites are shown in Figure 1 and Table 1. [Based on in situ measurements, water parameters include temperature \(27.31 – 31.6 °C\), pH \(6.95 – 8\), dissolved oxygen \(3.62 – 5.21 mg/L\), salinity \(6 – 30 ‰\), water brightness \(0.1 – 0.9 m\), water current speed \(0.1 – 1.8 m/s\).](#) Sediment and *A. granosa* were collected during low tide from the Musi Estuary, South Sumatra, Indonesia. Sediment samples were collected from ten sampling points using a Peterson Grab at each station (Dwiyitno et al. 2024). Samples were placed in glass jars that had been rinsed with pure distilled water and covered using aluminum foil to avoid contamination. Samples were placed in a cool box (± 4 °C) for further analysis in the laboratory.

Anadara granosa samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). Twenty adult *A. granosa* (7.48–11.44 cm) were collected from fishermen's catch in the Musi Estuary. *Anadara granosa* samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). The mussels were rinsed with pure distilled water to remove any dirt. *Anadara granosa* were wrapped in aluminum foil and stored in a cooler at approximately 4 °C to preserve their freshness. After that, they were transported to the laboratory and frozen at -20°C before further analysis (Ding et al. 2021).

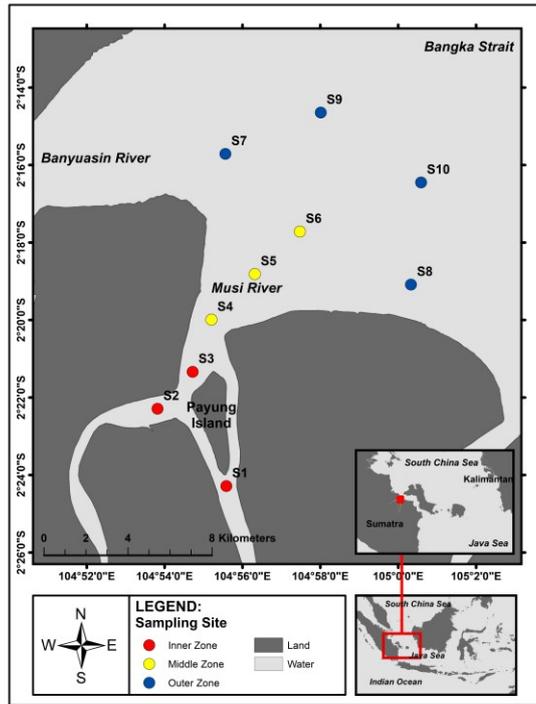


Figure 1. Study site at the Musi Estuary, Indonesia.

Table 1. Coordinates of the sampling location.

Estuary Zone	Description	Station	Latitude (°S)	Longitude (°E)
Inner Zone	closest to the river body and dominated by freshwater mangrove vegetation and muddy substrate near densely populated coastal village activities, shipping and fishing activities	S1	2.40470	104.92643
		S2	2.37144	104.89695
		S3	2.35558	104.91200
Middle Zone	between the inner zone and the outer zone mangrove vegetation and muddy substrate shipping and fishing activities	S4	2.33316	104.92003
		S5	2.31353	104.93862
		S6	2.29524	104.95794
Outer Zone	closest to the ocean (at the sea), dominated by seawater, no vegetation, muddy substrate shipping and fishing activities	S7	2.26183	104.92603
		S8	2.31807	105.00547
		S9	2.24403	104.96686
		S10	2.27413	105.00978

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Microplastic extraction from sediment

Microplastics in sediments were extracted using the standard method of density separation and organic matter removal with minor modifications (Thompson et al. 2004; Liebezeit and Dubaish 2012). A total of 500 g of wet sediment samples were placed in a beaker glass covered with aluminum foil. The samples were dried in an oven at 60 °C for 48 hours, or until they reached a constant mass (Patria et al. 2023). The sediment samples were pulverized and sieved through a 5 mm steel sieve. A portion of 100 g of dried sediment was taken from the sieve, placed into a beaker glass, and covered with aluminum foil to prevent external contamination. The sample was suspended in 400 mL of saturated NaCl solution (1.2 g cm⁻³), which is four times the sample weight, using a magnetic stirrer. This NaCl solution was prepared by dissolving pure

116 NaCl crystals (Merck Millipore EMSURE®) in filtered distilled water that was free from contaminants. The selection of a
117 NaCl solution for the separation of microplastics from sediments was based on the premise that this method is cost-
118 effective and environmentally friendly (Perumal and Muthuramalingam 2022). Stirring was carried out for 5 minutes until
119 completely dissolved, and the mixture was allowed to stand for 1 hour. After one hour, 10 mL of 30% H₂O₂ was added to
120 assist in the breakdown of organic matter, followed by stirring for an additional five minutes. Hydrogen peroxide (H₂O₂) is
121 commonly used in similar studies and is regarded as highly effective for organic matter removal (Lee et al. 2023). The
122 sample was allowed to stand for 24 hours, covered with aluminum foil. The samples were then filtered using the Whatman
123 No. 42 filter paper (mesh size 0.45 µm, Φ = 90 mm), assisted by a vacuum pump at 17 kPa to isolate the microplastics.
124 The collected samples were deposited into petri dishes for identification.
125

126 **Microplastic extraction from *Anadara granosa***

127 The previously frozen *A. granosa* samples were defrosted under controlled conditions at room temperature (± 25°C) for
128 1 hour. After the ice melted, the *A. granosa* were washed with pure distilled water to eliminate contamination from other
129 objects, such as sediment. The length and wet weight of the *A. granosa* were measured using an analytical balance (0.01
130 g). The digestive tract was carefully removed from the *A. granosa* using sterile stainless steel utensils that had been rinsed
131 with distilled water. The extraction of microplastics from the digestive tract of the *A. granosa* was adapted from previous
132 studies with minor modifications (Ding et al. 2018; Ding et al. 2021). The digestive tract was transferred into an
133 Erlenmeyer flask, and 100 mL of KOH solution (10%) was added. The use of KOH is regarded as a more efficacious
134 method for the digestion of biological material, and it has no impact on the integrity of the plastic polymer (Karami et al.
135 2017). The sample was covered with aluminum foil and stirred for 5 minutes using a 150 rpm magnetic stirrer. The
136 supernatant was incubated at room temperature (±25°C) until complete digestion of organic matter occurred. The sample
137 was then filtered through Whatman 42 filter paper (mesh size 0.45 µm, Φ = 90 mm) to isolate microplastic. The filter
138 paper was dried in an oven at 40°C for 5 hours and stored in petri dishes for subsequent identification of microplastic
139 content.
140

141 **Microplastic identification and quantification**

142 Identification and quantification of microplastics from sediments and *A. granosa* were performed using the same
143 method. Microplastic identification was performed using an Olympus CX23 microscope with 10 x 10 or 4 x 10
144 magnification to visually detect microplastics (Diansyah et al. 2024). The filter paper was observed closely and carefully
145 to avoid contaminants that could enter the filter paper. Microplastics were identified based on the number and form of
146 microplastics (fragment, film, fiber, and foam). The data obtained were recorded by sample type and station/individual for
147 statistical purposes. To prevent contamination, personnel wear cotton lab coats, latex gloves, and cotton masks.
148 Furthermore, access to the detection room is restricted to prevent outside contact during observations.
149

150 **Procedures for quality assurance and contamination prevention**

151 The quality and contamination control measures were implemented to prevent any alterations to the microplastics in
152 the samples. Before sampling, glass jars and aluminum foil were rinsed with filtered distilled water. Although the samples
153 were in a cool box, they were stored in a place that was not exposed to direct sunlight to keep the samples in good
154 condition. Personnel are also required to wear latex gloves during sampling. During the study, from sampling to laboratory
155 analysis, the use of plastic equipment was minimized to avoid unintentional fragmentation of the microplastics. The
156 distilled water used was filtered with filter paper (0.45 µm,) to prevent microplastics from entering our materials. All
157 equipment was washed with filtered distilled water to remove potential contamination from external particles (Ding et al.
158 2021). We prepared two controls by filtering each solution we used to avoid contaminants from materials, equipment, and
159 air. No microplastics were found contaminating the instrument. The sterilized equipment was wrapped in aluminum foil to
160 prevent any input of contaminants from outside. Laboratory personnel were required to wear latex gloves, lab coats, and
161 masks throughout the analysis. Access to the laboratory was restricted during the analysis to minimize external
162 interference that could lead to protocol errors.
163

164 **Data analysis**

165 **Pollution Load Index (PLI)**

166 PLI was calculated using a pollutant load approach, which assesses the total concentration of contaminants relative to a
167 baseline level, to evaluate its significance. Equations were utilized to quantify the level of microplastic pollution in both
168 sediments and *A. granosa* in the Musi Estuary, with the method adapted accordingly (Tomlinson et al. 1980; Xu et al.
169 2018; Wang et al. 2021)
170

$$CF_i = \frac{C_i}{C_{0i}}$$

171
172 This equation shows that the concentration factor (CF_i) at a particular sample is obtained by dividing the current
173 concentration (C_i) by the initial concentration (C_{0i}). C_i indicates the quantified presence of microplastics at each sampling
174

location or within each clam (ind), while C_{0i} denotes the background concentration, defined as the lowest value recorded across all sampling locations. Given the lack of previously published research on microplastics in sediments or *A. granosa* in the Musi River Estuary, we determined C_{0i} values from minimum concentrations across sampling stations.

$$PLI = \sqrt{CF_i}$$

This equation shows that the Pollution Load Index (PLI) is generated by calculating the square root of the Concentration Factor (CF). The PLI value of microplastics is derived from the contaminant factor (CF_i) calculated for each station/individual.

$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \dots \times PLI_n}$$

Pollution Load Index of Zone (PLI_{zone}) is determined by taking the square root of the product of individual PLI values from multiple sites within the zone. This method integrates data from several measurement points, providing a more comprehensive assessment of microplastic contamination within the analyzed. A PLI_{zone} is used to assess pollution based on specific estuary zones as well as the overall estuary area. PLI values are categorized into four predefined categories of pollution load, as shown in Table 2.

Table 2. Microplastic pollution load categories

Definition	Value			
Value of the pollution load index (PLI)	<10	10-20	20-30	>30
Risk category	I	II	III	IV
Keterangan	Minor	Middle	High	Extreme

Nemerow Pollution Index (NPI)

NPI is designed to assess the bioavailability of microplastics in sediments and *A. granosa*. This index was adapted from equations used in prior studies evaluating heavy metal contamination (Alam et al. 2023).

$$NPI = \sqrt{\frac{\left(\frac{C_i}{S_i}\right)_{max}^2 + \left(\frac{C_i}{S_i}\right)_{ave}^2}{2}}$$

The NPI value is calculated using the square root of the mean squared deviation, incorporating both the maximum and average concentration ratios relative to the background concentration (S_i). The formula includes the sum of the squares of the maximum concentration ratio (C_i_{max}/S_i) and the average concentration ratio (C_i_{ave}). The C_i value represents the concentration of microplastics found in the sediment or clams at a specific station or individual, while the S_i value denotes the background level of microplastics, determined as the lowest concentration recorded across all sample types. The NPI provides a measure of microplastic bioavailability in sediments and *A. granosa*, with values below 2 indicating low bioavailability and values above 2 indicating high bioavailability.

Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) quantifies the extent to which microplastics accumulate in biota relative to their environmental concentration and is calculated using the following equation (Li et al. 2022).

$$BCF = \frac{C_{biota\ ave}}{C_{sediment\ ave}}$$

In this equation, BCF represents the bioconcentration factor, which is derived from the calculation of the average concentration of microplastic in biota (*A. granosa*) divided by the average concentration of microplastic in its environment (sediment). This value is intended to assess how much microplastic is concentrated in the biota.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 27. Descriptive statistics, including total, mean, standard deviation, percentage, minimum, and maximum, were utilized to assess data variation. A one-way ANOVA was applied to analyze differences in microplastic abundance between estuarine zones when the data met normality

223 assumptions. For non-normal data, the Kruskal-Wallis test was employed to assess the significance of differences between
224 zones. All tests were carried out with a significance level of $\alpha = 0.05$.

225 RESULTS AND DISCUSSION

226 Microplastic presence in sediment from Musi Estuary

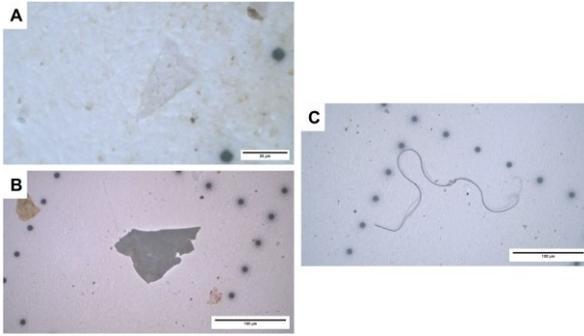
227
228 The Musi Estuary in South Sumatra, Indonesia, is a dynamic brackish water environment where freshwater from the
229 Musi River meets the saltwater of the Bangka Strait. Characterized by turbid waters and muddy sediments rich in organic
230 matter, the estuary is fringed by mangrove forests that provide habitat for aquatic species, including the **blood-clam**
231 (*Anadara A. granosa*), a key species in local aquaculture (Rozirwan et al. 2023). Surrounded by urban and industrial areas,
232 including Palembang, the estuary faces pollution from domestic, agricultural, and industrial waste, making it susceptible to
233 microplastic accumulation in sediments and ingestion by benthic organisms like *A. granosa* **blood-clams** (Almiza and
234 Patria 2021; Diansyah et al. 2024).
235



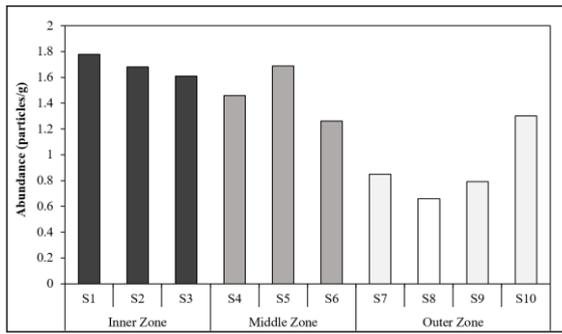
236
237 **Figure 2.** General conditions in the Musi estuary. A. Coastal Settlements; B. Shipping Activities; C. Fisheries; D.
238 Mangrove Ecosystem
239

240 The results of this study indicate that microplastics were detected at all sampling stations, as shown in Figure 4. A total
241 of 1,308 microplastic particles were identified, with concentrations ranging from 66 to 178 particles per 100 g of dry
242 sediment (dry weight, dw). The average abundance of microplastics was 1.31 ± 0.41 particles/g dw, with the highest
243 concentration found at station S1 (1.78 particles/g dw) and the lowest at station S8 (0.66 particles/g dw). High levels of
244 human activity in the upstream area of Musi Estuary, which flows downstream to the estuary and ultimately into the ocean,
245 are responsible for the presence of microplastics (Rozirwan et al. 2021; Diansyah et al. 2024). Urban activities in close
246 proximity to the Musi River Watershed, particularly in Palembang City, significantly contribute to the generation of plastic
247 waste. Increased population density near riverbanks enhances the potential for plastic pollution. Furthermore, population
248 density is a crucial factor in the entry of microplastics into rivers (Eo et al. 2023; Dwiyitno et al. 2024). As a result, plastic
249 pollutants from domestic activities, such as the disposal of food packaging and plastic bags, can be transported through
250 stormwater drains and eventually end up in rivers (Kunz et al. 2023). Fishing activities around Musi Estuary can contribute
251 to microplastic pollution, primarily through fibers released from fishing nets (Li et al. 2022; Fauziyah et al. 2023). This
252 increases anthropogenic pressure at river mouths, which can lead to greater accumulation of microplastics (Castro-Jiménez
253 et al. 2024). Additionally, tidal fluctuation influences water flow, which facilitates the deposition of microplastics in
254 sediment at these locations (Harris 2020). The findings of this study indicate that anthropogenic activities are responsible
255 for high levels of microplastic deposition in the sediments of Musi Estuary. Microplastic particles that accumulate in
256 sediments serve as a habitat for benthic organisms, which can have long-term implications for ecosystem health and
257 balance (Rahmatin et al. 2024). As vectors for pollutants, microplastics can exacerbate toxicity, potentially endangering
258 various populations (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). Furthermore,
259 the processes of bioaccumulation and biomagnification in benthic organisms present heightened risks to these animals,

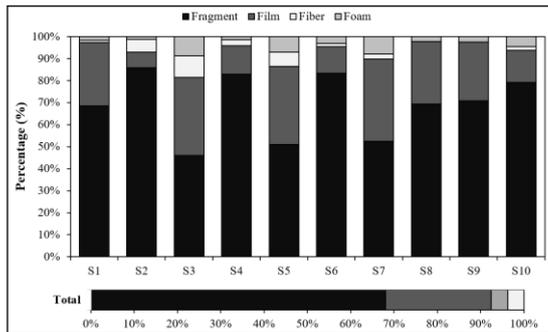
260 potentially leading to human consumption (Unuofin and Igwaran 2023). Therefore, it is crucial for local governments to
 261 address the handling and prevention of further microplastic accumulation.
 262



263
 264 **Figure 3.** Various forms of microplastics, A. Film; B. Fragment; C. Fiber
 265



266
 267 **Figure 4.** Distribution of microplastics from sediments in Musi Estuary, Indonesia.
 268
 269



270
 271 **Figure 5.** Percentage abundance of microplastic shapes form sediments in Musi Estuary, Indonesia
 272
 273

274 The microplastics identified in the sediments were predominantly fragments (68.92%), followed by films (23.86%),
 275 fibers (4.05%), and foams (3.17%) (Figure 3 and Figure 5). The dominance of fragments, which are a common form of
 276 secondary microplastics, occurs as a result of macroplastic degradation (Barnes et al. 2009). Fragments represented the
 277 most common type across all sediment samples analyzed. This dominance of fragments in estuarine sediments aligns with
 278 findings from various studies conducted in other estuarine environments. Research indicates that estuarine sediments

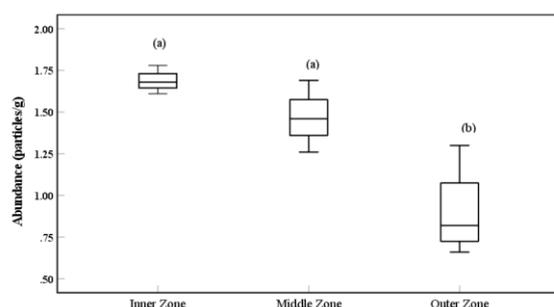
279 globally tend to accumulate diverse microplastic forms, particularly fibers (Firdaus et al. 2020; Alam et al. 2023; Samuels
280 et al. 2024; Santucci et al. 2024), with many studies also reporting a predominance of fragment types (Zhou et al. 2021;
281 Suteja et al. 2024). These observations corroborate the literature, which consistently shows that fibers and fragments
282 constitute the majority of microplastic pollution in estuarine ecosystems (Feng, An, et al. 2023).

283 This study reveals notable variations in microplastic concentrations compared to similar research in estuaries globally.
284 Our findings indicate that the Musi Estuary has lower microplastic concentrations than those reported in several other
285 Indonesian and international estuaries. For example, the Jakarta Bay Estuary exhibits concentrations nearly ten times
286 higher, ranging from 1,184 to 1,337 particles per 100 grams of river sediment and 804 to 1,055 particles per 100 grams of
287 beach sediment (Dwiyitno et al. 2024). Such elevated levels are likely due to increased anthropogenic activities along
288 Jakarta's rivers, which substantially contribute to plastic waste flow. In another comparison, the Pearl River Estuary in
289 China also shows higher microplastic densities, with concentrations between 2.05×10^3 and 7.75×10^3 particles per
290 kilogram of dry weight sediment (Xu et al. 2024). This estuary's coastal regions experience high human activity,
291 enhancing the potential for microplastic deposition. Similarly, the Meghna Estuary in Bangladesh, which receives
292 sediment from the Ganges River Basin, reports even higher microplastic levels at $4,014.66 \pm 1,717.59$ particles per
293 kilogram dry weight (Alam et al. 2023). These findings underscore the significant role of anthropogenic influences on
294 microplastic accumulation in global estuarine sediments, including the Musi Estuary. Other environmental factors, like
295 location, sampling period, sediment depth, sediment composition, and local hydrodynamic conditions, may also affect
296 these changes in the amount of microplastics found (Zheng et al. 2020; Feng, Chen, et al. 2023; Yuan et al. 2023).

297 In contrast, the abundance of microplastics in the sediments of the Musi Estuary surpasses levels reported in other
298 regions, both within Indonesia and globally. Concentrations in the Musi Estuary are notably higher than those in the
299 Zandvlei River Watershed and estuarine areas of South Africa (70.23 ± 7.36 particles/kg dw) (Samuels et al. 2024), the
300 Claromec6 Estuary in Argentina (299 ± 114 particles/kg dw) (Truchet et al. 2021), and the upper sediment layer (0-5 cm)
301 of the Fuhe River Estuary in Northern China (1049 ± 462 particles/kg dw) (Zhou et al. 2021). These numbers are also
302 higher than those found in coastal Rio de la Plata (547.83 ± 620.06 particles/kg dw) (Santucci et al. 2024), Benoa Bay,
303 Bali (31.08 ± 21.53 particles/kg dw) (Suteja et al. 2024), the Jagir Estuary in Surabaya (up to 590 particles/kg dw) (Firdaus
304 et al. 2020), and the Pekalongan River Estuary in Java (0.77 to 1.01 particles/g dw) (Ismanto et al. 2023). The high
305 microplastic concentration in the Musi Estuary is likely due to extensive plastic waste disposal in the river basin by the
306 surrounding community. Research supports that plastic debris forms a substantial part of the macro-waste in the Musi
307 River (Maherlsa et al. 2019). Additionally, local communities frequently establish settlements along the river, relying on it
308 for water access and transportation. Consequently, domestic waste, including plastic, often enters the river directly. This
309 local waste management issue reflects a broader trend, with Indonesia identified as the world's second-largest contributor
310 to oceanic plastic pollution, following China (Jambeck et al. 2015).

311 **Spatial distribution microplastic in Sediments**

312 The study revealed that the abundance of microplastics was significantly higher in the inner and middle zones when
313 compared to the outer zone. The mean abundances were 1.69 ± 0.08 particles/g dw in the inner zone, 1.47 ± 0.22 particles/g
314 dw in the middle zone, and 0.9 ± 0.28 particles/g dw in the outer zone. Significant differences in mean microplastic
315 abundance between zones were revealed by the one-way ANOVA ($p < 0.05$), as illustrated in Figure 6. The Tukey HSD
316 test revealed no significant difference in mean microplastic abundance between the inner and middle zones. However, we
317 observed a significant difference in the outer zone compared to both the middle and outer zones. Our results indicate that
318 the inner and middle estuary regions exhibit greater mean concentrations of microplastics compared to the outer estuary.
319 Natural factors, particularly the effects of currents and wave action in coastal and estuarine environments, significantly
320 influence this phenomenon by altering microplastic distribution. The influx of freshwater promotes the sedimentation and
321 prolonged retention of high-density microplastics within the system (G. Li et al. 2024). Furthermore, the sedimentation
322 process is affected by the density and buoyancy of microplastics, with increased salinity in estuarine waters enhancing
323 buoyancy forces and influencing distribution patterns (Cheng et al. 2024). Our findings align with research from the
324 Liaohe Estuary in China, revealing a higher accumulation of microplastics in inner river sediments compared to the outer
325 estuary (Xu et al. 2020). Additionally, sampling depth plays a critical role in accurately measuring microplastics in surface
326 waters, bottom layers, and sediments (Feng, An, et al. 2023).



328
329 **Figure 6.** The difference in microplastic abundance between zones in the sediments
330

331 The spatial analysis revealed distinct patterns of microplastic accumulation between the two sides of the coast adjacent
332 to the estuary. Notably, Site S7 exhibited higher concentrations compared to Site S8, likely due to its proximity to other
333 pollution sources. Specifically, S7 is located near the Banyuasin Estuary, which is believed to significantly contribute to
334 the deposition of microplastics in the surrounding sediments. Based on Costa et al. (2023), confirmed that nearby sources
335 of pollution heavily influence microplastic deposition in coastal regions. The accumulation of microplastics at the mouth
336 of the adjacent river further exacerbates this phenomenon, facilitating the settlement of microplastics within the sediment.
337 Additionally, studies suggest that the depth of the sampling locations significantly influences microplastic deposition,
338 revealing a greater abundance of microplastics at deeper water levels (Bayo et al. 2022). The substantial accumulation of
339 microplastics in sediments is attributed to their persistent characteristics as well as the protective conditions offered by
340 deeper environments, which are shielded from UV light, maintain lower temperatures, and exhibit lower oxygen levels,
341 thus slowing biodegradation processes (Zhang et al. 2021).

342 Our findings confirm the presence of microplastics in the sediments of the Musi Estuary, likely originating from
343 suspended microplastics that eventually settle from the water's surface. Previous research has found that there are $467.67 \pm$
344 127.84 particles/ m^3 and 723.67 ± 112.05 particles/ m^3 of microplastic in surface waters during ebb and flow conditions,
345 respectively (Diansyah et al. 2024). Hydrodynamic conditions in the estuary and processes like aggregation and
346 biofouling, which increase microplastic density, influence the deposition of these particles (Malli et al. 2022). The specific
347 properties of the microplastics and the water dynamics of the estuary strongly influence microplastic deposition. For
348 instance, biofouling can make microplastics denser, accelerating their descent into sediments (Lin et al. 2023). Since
349 microplastics often have higher densities than water, they can remain suspended in the water column before eventually
350 settling (Dai et al. 2022). High Total Suspended Solids (TSS) in the Musi Estuary have the potential to cause aggregation
351 of microplastics (Rahutami et al. 2022). High TSS levels make it easier for sediments and other suspended particles to
352 stick together on microplastic surfaces, which speeds up their deposition (Yang et al. 2022). However, microplastics can
353 also become resuspended in the water column (Tang et al. 2020). Stirring forces in estuarine environments can lift
354 microplastics back into the water, where turbulence and bioturbation may redistribute them (Malli et al. 2022). Therefore,
355 additional studies are required to fully characterize the mechanisms of microplastic deposition in the Musi Estuary.
356 Climate factors also influence microplastic distribution in Musi Estuary sediments. In Indonesia, where regions such as the
357 Musi Estuary experience distinct rainy and dry seasons, seasonal changes affect water dynamics and microplastic
358 transport. During the rainy season, increased river velocity may carry microplastics upward from sediments, while in the
359 dry season, slower flows facilitate microplastic accumulation at the water's surface (Zhao et al. 2020). Currently, there are
360 no studies specifically examining the seasonal impact on microplastic presence in the Musi Estuary, underscoring the need
361 for further research to address this gap.

362 **Bioaccumulation of microplastics in *Anadara granosa***

363 We extracted 133 microplastic particles from the digestive tracts of twenty samples of *A. granosa* species presented in
364 Figure 7. Each sample from the Musi Estuary had accumulated microplastics, ranging from 6 to 49 particles/ind or 8 to
365 69.01 particles/g ww, as shown in Figure 8 and Figure 9. The mean abundance of microplastics found in the digestive
366 tracts was 21.05 ± 10.31 particles/ind or 30.46 ± 16.7 particles/g ww. Several factors contribute to the increased microplastic
367 inputs in bivalve species, including human activities, tourism, fishing equipment, freshwater runoff, and wastewater
368 discharge, combined with the area's hydrodynamic conditions (Vital et al. 2021). The elevated levels of microplastics
369 found in *A. granosa* from the Musi River Estuary suggest a significant degree of contamination in their habitat, including
370 both water and sediment, which may adversely affect the health of these organisms and disrupt local ecological balances.
371 This observation aligns with existing literature, which indicates a strong correlation between the abundance of
372

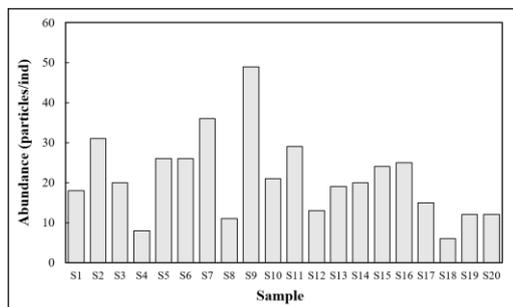
373 microplastics in bivalves, such as mussels, and their sediment environments (Narmatha Sathish et al. 2020), reinforcing the
374 need for monitoring these pollutants in aquatic ecosystems.



375 **Figure 7.** Specimens of *Anadara granosa* from Musi Estuary, Indonesia.

376
377
378 Microplastics were observed in three morphological forms: film, fiber, and fragment. Film accounted for the majority
379 of microplastics (59.38%), followed by fiber (31.59%) and fragments (9.03%), as shown in Figure 10. The digestive tracts
380 of the *A. granosa* studied did not contain any pellets or foam. Statistical analysis using the Kruskal-Wallis test revealed
381 significant differences in the abundance of the three microplastic forms ($p < 0.05$). This predominance of film
382 microplastics in *A. granosa* is likely attributable to the extensive use of disposable plastic packaging within the
383 community, which subsequently degrades into microplastics. Although fragments dominate the sediment substrate, *A.*
384 *granosa* predominantly absorb film microplastics. The lower density of film microplastics may facilitate their
385 accumulation in the upper sediment layer, making them susceptible to resuspension in the overlying water column. This
386 phenomenon is consistent with the feeding behavior of *A. granosa*, which frequently filter water from the sediment
387 surface, resulting in the accumulation of film microplastics within their digestive tracts.

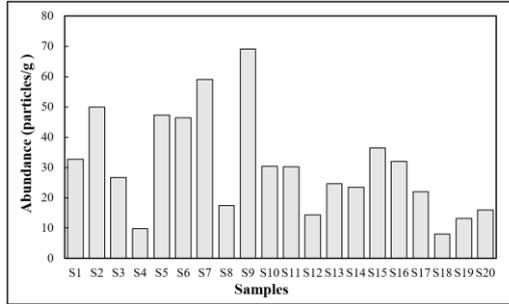
388 Our findings indicate a higher abundance of microplastics in *A. granosa* from the Musi Estuary compared to certain
389 other regions globally. For instance, *A. granosa* from the Chanthaburi estuarine ecosystem in eastern Thailand contained
390 0.40 ± 0.16 particles/g ww (Potipat et al. 2024), while samples from Peninsular Malaysia showed concentrations of $0.20 \pm$
391 0.08 particles/g ww and 1.54 ± 0.30 particles/ind ww (Mohan et al. 2024). Additionally, microplastic levels in *A. granosa*
392 from Pao Village, Tarawang District, Jeneponto Regency, Indonesia, were measured at 0.0144 particles/g ww (Namira et
393 al. 2023), and those from Likas Bay Beach in North Borneo, Malaysia, at 24.4 ± 0.6 particles/g ww (Abd Rahman et al.
394 2024). In contrast, the *A. granosa* analyzed in this study accumulated lower microplastic concentrations than those
395 reported in several other studies. For instance, *A. granosa* from the Pangkal Babu mangrove forest area in Tanjung Jabung
396 Barat district, Jambi, exhibited 434 ± 97.05 particles/ind (Fitri and Patria 2019), while those from Lada Bay in Pandeglang,
397 Banten, had concentrations of 618.8 ± 121.4 particles/ind (Ukhrowi et al. 2021). Overall, this study shows that
398 microplastics have contaminated the sediment environment of the Musi Estuary, South Sumatra, indicating that *A. granosa*
399 can serve as effective bioindicators of this pollution.



402 **Figure 8.** Microplastic abundance of *Anadara granosa* from the Musi Estuary

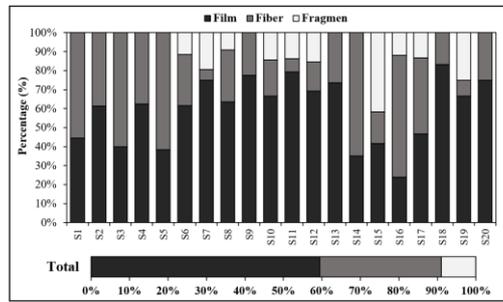
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Figure 9. Microplastic abundance (in particles/g) of *Anadara granosa* from the Musi Estuary



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Figure 10. Shape of microplastics in *Anadara granosa* from the Musi Estuary

Risk assessment of microplastics in sediments and *Anadara granosa*

A risk assessment of microplastic contamination in the Musi River Estuary was carried out by calculating the Pollution Load Index (PLI), the Nemerow Pollution Index (NPI), and the Bioconcentration Factor (BCF). The PLI values across all sediment samples indicate that the estuarine sediment is subject to minor pollution, with values ranging between 1.00 and 1.64, as shown in Table 3. In the three zones of the estuary (inner, middle, and outer), the PLI values were 1.60, 1.49, and 1.20, respectively. We calculated the overall average PLI for the Musi Estuary to be 1.37. All zones reported PLI values below 3.00, which suggests that the area experiences only minor microplastic pollution. The analysis of microplastic presence in sediment samples revealed a significantly high concentration of microplastics, with an NPI value of 2.37. This suggests a substantial bioavailability of microplastics in the estuarine environment. This elevated NPI underscores the ecological risk posed by microplastic contamination, despite the relatively low PLI observed across the study area.

422

423 **Table 3.** The microplastic risk factor in sediments of Musi Estuary

Sediment Station	CF	PLI	PLI Zone	PLI Sediment	NPI
S1	2.70	1.64	Inner Zone 1.60	1.37	2.37
S2	2.55	1.60			
S3	2.44	1.56			
S4	2.21	1.49	Middle Zone 1.49		
S5	2.56	1.60			
S6	1.91	1.38			
S7	1.29	1.13	Outer Zone 1.20		
S8	1.00	1.00			
S9	1.20	1.09			
S10	1.97	1.40			

424

425 *Anadara granosa* analyzed in this study exhibited significant risk values associated with microplastic contamination.
 426 The PLI for these clams ranged from 1 to 2.86, with an overall mean PLI value of 1.77 for the entire sample, as shown in
 427 Table 4. This indicates that the pollution load in *A. granosa* falls within the minor category (PLI < 3). Furthermore, the
 428 bioavailability of microplastics in *A. granosa* was significantly higher, with an NPI value of 6.29, indicating a high
 429 bioavailability. The bioconcentration factor of microplastics from sediment to *A. granosa* was measured at 23.28,
 430 suggesting a significant potential for the transfer of microplastics from sediment to *A. granosa* in the Musi Estuary.

431 Our findings demonstrated that the bioavailability of microplastics in sediments and *A. granosa* was categorized as
 432 high (NPI = 2.37; NPI > 2). In comparison, the bioavailability of microplastics in *A. granosa* was even higher, with an NPI
 433 value of 9.89. The elevated bioavailability in sediments suggests a greater potential for absorption by aquatic organisms,
 434 particularly benthic animals. Furthermore, the bioconcentration factor (BCF) calculated for *A. granosa* in sediment was
 435 23.28, indicating a significant transfer of microplastics from sediments to these organisms in the Musi Estuary. This high
 436 BCF value is attributed to the non-discriminatory feeding process of *A. granosa*, allowing them to ingest microplastics
 437 along with other particles. When compared to several benthic species from the Yangtze River Estuary, which exhibited a
 438 BCF of 29.48 ± 6.52 (Z. Li et al. 2022), the BCF of *A. granosa* in the Musi Estuary remains lower. Nonetheless, both
 439 sediments and *A. granosa* from the Musi Estuary are classified within the minor pollution risk index category (PLI < 10).
 440 The bioaccumulation of microplastics by benthic animals like *A. granosa* poses a significant risk to human health for those
 441 consuming these clams (Wang et al. 2023; Winiarska et al. 2024). Therefore, periodic assessments are crucial to evaluate
 442 the risk of microplastic contamination in sediments and *A. granosa*, providing essential information to understand the
 443 ecological and health implications in the Musi Estuary.

444 The impact of high bioaccumulation extends beyond the toxicity of microplastic materials; it also facilitates the
 445 accumulation of other pollutants. Previous research has detected heavy metals, such as lead (Pb) and copper (Cu), adhering
 446 to the surfaces of microplastics in the Musi Estuary (Purwiyanto et al. 2020). Additionally, various hazardous heavy
 447 metals, including iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), and zinc (Zn), were identified with high
 448 bioavailability in the aquatic environment of the Musi Estuary (Rahutami et al. 2022). This accumulation allows for the
 449 deposition of significant quantities of heavy metals on microplastic surfaces, thereby increasing the risk of microplastic
 450 toxicity for estuarine ecosystems. Moreover, the involvement of microorganism vectors, misidentification in aquatic
 451 organisms' diets, and their harmful toxicological effects on benthic animals heighten the risk associated with
 452 microplastics (Gong and Xie 2020). Consequently, the high output of microplastics from the Musi Estuary has the
 453 potential to jeopardize the condition of the surrounding aquatic environment, including fisheries and migratory birds,
 454 which are critical to the ecosystem (Rozirwan et al. 2019; Rozirwan et al. 2022).

455
 456 **Table 4.** Risk factors for microplastics in *Anadara granosa* from the Musi Estuary

<i>A. granosa</i> sample	CF	PLI	PLI <i>A. granosa</i>	NPI	BCF
S1	3.00	1.73			
S2	5.17	2.27			
S3	3.33	1.83			
S4	1.33	1.15			
S5	4.33	2.08			
S6	4.33	2.08			
S7	6.00	2.45			
S8	1.83	1.35			
S9	8.17	2.86			
S10	3.50	1.87	1.77	6.29	23.28
S11	4.83	2.20			
S12	2.17	1.47			
S13	3.17	1.78			
S14	3.33	1.83			
S15	4.00	2.00			
S16	4.17	2.04			
S17	2.50	1.58			
S18	1.00	1.00			
S19	2.00	1.41			

This study holds significant ecological implications for the Musi Estuary ecosystem, as the presence of microplastics poses a long-term threat to ecosystem stability. Previous studies have shown that microplastics can affect the physicochemical properties of sediments, such as electrical conductivity, organic matter content, and nutrients, and well as impact enzymatic activity in sediment bacteria (Rillig 2012; B. Yuan et al. 2022; W. Li et al. 2022). These changes can lead to structural and functional alterations in aquatic habitats (W. Li et al. 2022). Microplastics can also enter various levels of the food chain through bioaccumulation and biomagnification processes. For example, microplastics in plankton can cause a decline in zooplankton populations, which impacts food availability for small fish and predatory species at higher trophic levels (Malinowski et al. 2023). The accumulation of microplastics in *A. granosa* in this study indicates that the pressure of plastic waste in the Musi Estuary is already quite high. Microplastics can act as vectors for other pollutants, such as microorganisms, heavy metals, and other inorganic pollutants can increase the risk of toxicity for aquatic organisms, including *A. granosa* (Purwiyanto et al. 2020; Rafa et al. 2024). Research indicates that exposure to microplastics can lead to behavioral and physiological changes in aquatic organisms, including changes in diet and reproductive success (Guo et al. 2020; Liang et al. 2023). Moreover, microplastics disrupt endocrine functions and metabolic pathways, causing oxidative stress, cell necrosis, and apoptosis, which can ultimately lead to mortality (Pannetier et al. 2020; Jeyavani et al. 2021). Disruption of any component of the food chain can trigger a domino effect, threatening the balance of the Musi Estuary ecosystem. However, this study only examines microplastic pollution in sediment and *A. granosa* as environmental indicators. Additional research is needed to understand microplastic buildup in other species, such as fish, shrimp, and benthic organisms, for a more complete picture of microplastic pollution in the Musi Estuary and its impact on the global marine environment.

Microplastic contamination of *A. granosa* in the Musi Estuary poses a significant threat to local communities, particularly clam fishers. Microplastics that accumulate in *A. granosa* may pose health risks to community members who consume them regularly as a primary source of protein. Long-term exposure to microplastics can potentially disrupt various human body systems, including the digestive, respiratory, reproductive, nervous, and cardiovascular systems (Y. Li et al. 2024). Furthermore, microplastics may cause both acute and subchronic toxicity and are considered potentially carcinogenic and disruptive to human development (Z. Yuan et al. 2022). Once in the bloodstream, microplastics can be transported to the liver, the primary organ responsible for detoxification. Accumulation in the liver may trigger adverse physiological reactions, including increased oxidative stress, liver fibrosis, and impaired lipid metabolism. In addition to health risks, microplastic contamination in *A. granosa* in the Musi Estuary may reduce consumer confidence and decrease market demand due to food safety concerns (Unuofin and Igwaran 2023). The long-term effect could weaken local economic resilience as marine products in the region are perceived to be contaminated with microplastics (Barrientos et al. 2024). Therefore, it is crucial for governments and communities to recognize these risks and collaborate on mitigation efforts to protect ecosystems, public health, and local economies.

Furthermore, seafood hygiene practices in local communities and fishing industries can help reduce microplastic contamination (Lusher et al. 2017; Smith et al. 2018). For example, *A. granosa* and other seafood, when thoroughly cleaned by repeated washing and clam removal before cooking, may reduce microplastic intake (J. Li et al. 2022). The concentration of microplastics in shellfish is significantly lower after boiling and steaming compared to frying, suggesting that cooking methods affect microplastic retention in seafood (Evode et al. 2021). This method can also be applied to shrimp, fish, and other shellfish to lower the risk of microplastic exposure. Implementing these recommendations will help reduce microplastic pollution and provide long-term benefits for public health, ecosystem stability, and the economic resilience of local fisheries (Prata et al. 2019).

In conclusion, this study demonstrates the significant presence of microplastics in the sediment and *A. granosa* of the Musi Estuary, Indonesia. These findings indicate a serious threat to the aquatic ecosystem and to local public health, as microplastics can enter the food chain and potentially harm organisms across trophic levels, including humans (Ningrum and Patria 2022; Edwin et al. 2023; Patria et al. 2023). This pollution heightens environmental risks, particularly for species integral to both the community's diet and economy (Jeong et al. 2024). The microplastics likely originate from human activities, especially urban waste from upstream areas that flows downstream and accumulates in the estuary (Diansyah et al. 2024). These results highlight the urgent need for routine monitoring and stricter waste management practices, particularly for plastic disposal, to reduce further contamination (Barletta et al. 2019). A comprehensive strategy is essential to controlling plastic waste throughout the river system—from source to estuary. Effective measures should include coordinated waste management practices, public education on the impact of pollution, and cross-sector collaboration to preserve estuarine health (Wakwella et al. 2023; Ihenetu et al. 2024). Such initiatives are crucial to protecting ecosystem health and sustaining local fisheries, ensuring these resources continue to support community well-being.

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Implication of microplastic presence in sediment and blood clams *Anadara granosa* (Mollusca: Bivalvia) in the Musi Estuary, Indonesia

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Abstract. Rahman MA, Rozirwan, Putri WEA, Diansyah G, Melki, Delya I, Nugroho RY. 2025. Implication of microplastic presence in sediment and blood clams *Anadara granosa* (Mollusca: Bivalvia) in the Musi Estuary, Indonesia. *Biodiversitas* 26: 1720-1733. Microplastic pollution poses a serious risk to estuarine ecosystems, affecting sediments and benthic species. Blood clams *Anadara granosa* represent a significant commercial seafood product in Indonesia, including those from the Musi Estuary. This study investigated the risks associated with microplastic contamination in sediments and *A. granosa* in the Musi Estuary, located in South Sumatra, Indonesia. Sediment samples were extracted using sodium chloride (NaCl, 1.2 g cm⁻³) and hydrogen peroxide (30% H₂O₂). In contrast, *A. granosa* samples were digested with 10% KOH for microplastic extraction. The abundance and shape of microplastics were analyzed from both samples. Pollution risk assessment was conducted through the calculation of the Pollution Load Index (PLI), Nemerow Pollution Index (NPI), and Bioconcentration Factor (BCF). The results revealed the presence of microplastics in sediments, with a mean abundance of 1.31±0.41 particles/g dw, while in *A. granosa*, it was 21.05±10.31 particles/ind. Both samples exhibited high microplastic bioaccumulation (NPI>2), although the pollution load remained relatively low (minor category, PLI<10). The bioconcentration factor between *A. granosa* and sediment was determined to be 23.28, indicating that the *A. granosa* absorbed microplastics present in the sediment. These findings highlight the significant bioaccumulation potential of microplastics in *A. granosa* within the Musi Estuary. The local community and government need to establish mitigations for future microplastic management efforts.

Keywords: Bioaccumulation, blood clams, microplastics, Musi Estuary, sediment

INTRODUCTION

River estuaries are dynamic ecosystems crucial for ecological balance and socio-economic functions (Boerema and Meire 2017; Hasan et al. 2022, et al. 2023; Retnaningdyah et al. 2022). They support diverse species and serve as hubs for urban, recreational, and commercial use (Islamy and Hasan 2020; Lonsdale et al. 2022; Natsir et al. 2022; Aunurrahman et al. 2023). Among the major estuaries in Sumatra, Indonesia, the Musi Estuary holds significant ecological and economic importance (Surbakti et al. 2023). It provides essential habitat for fish and benthic species that support local livelihoods (Rozirwan et al. 2021, et al. 2022a; Fauziyah et al. 2022, 2023). However, Intensified human activity around the Musi River has caused severe environmental degradation, mainly due to heavy metal and microplastic pollution (Maherlsa et al. 2019; Purwiyanto et al. 2022; Rahutami et al. 2022; Fitriah et al. 2023; Rozirwan et al. 2023a, et al. 2024; Diansyah et al. 2024). These pollutants may compromise estuarine stability, with potential impacts on biodiversity and dependent communities.

Macrozoobenthos are considered pivotal components in preserving the equilibrium of estuarine ecosystems (Sari et al. 2022; Isoni et al. 2023). These organisms play a crucial role in the food web and nutrient cycling (Griffiths et al.

2017). Moreover, macrozoobenthos are highly sensitive to environmental changes, making them reliable bioindicators of ecological degradation (Sahidin et al. 2018). Bivalves such as *Anadara granosa* (Linnaeus, 1758) are commonly used as bioindicators due to their ability to accumulate pollutants through filter-feeding (Fitri and Patria 2019; Ward et al. 2019; Ding et al. 2021; Bonifacio et al. 2022; Saleh et al. 2023; Rahmatin et al. 2024). *Anadara granosa* is widely consumed by the general public as a nutrient-rich seafood, particularly among local communities (Rozirwan et al. 2023b, c). Among these, *A. granosa* is an important bivalve that helps maintain ecosystem balance and supports the livelihoods of coastal communities (Yulinda et al. 2020; Prasetyono et al. 2022; Mahary et al. 2023). *Anadara granosa's* ecological and economic roles make assessing its pollutant contamination essential to understanding environmental pollution impacts.

Pollutants such as heavy metals, hydrocarbons, pesticides, and microplastics frequently accumulate in estuarine sediments (Zhao et al. 2015; Kılıç et al. 2023; Han et al. 2024; Jacq et al. 2024). Among these, plastic pollution has emerged as a critical concern due to its pervasive impact on aquatic environments (Borrelle et al. 2020; Hecker et al. 2023). Over the last five decades, single-use plastics have accumulated and fragmented widely across ecosystems (Zhang et al. 2021; Walker and

Fequet 2023). As plastics degrade, they break down into microplastics, defined as particles ranging in size from 1 μm to 5 mm (Frias and Nash 2019). Microplastics in aquatic ecosystems originate from plastic fragmentation, synthetic fiber release during washing, and microbeads in personal care products (Napper et al. 2015; Praveena et al. 2018; Belzagui and Gutiérrez-Bouzán 2022; Gan et al. 2023). Microplastics threaten aquatic ecosystems by releasing toxic additives and transporting organic pollutants and heavy metals (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). These impacts may disrupt food webs, alter nutrient cycles, modify habitats, and potentially cause genetic changes in aquatic organisms (Thacharodi et al. 2024).

Previous studies reported surface water microplastic concentrations in the Musi Estuary at 467.67 ± 127.84 particles/ m^3 during flood tide and 723.67 ± 112.05 particles/ m^3 at ebb tide (Diansyah et al. 2024). These microplastics often bind with toxic heavy metals, such as lead (Pb) and copper (Cu), amplifying their environmental harm (Purwiyanto et al. 2022). Microplastics in the water column tend to settle in estuarine sediments, the second-largest global reservoir after fjords (Harris 2020). Microplastic accumulation in estuarine sediments raises ecological concerns, especially due to ingestion by benthic organisms like *A. granosa* (Fitri and Patria 2019; Saleh et al. 2023; Mohan et al. 2024; Rahmatin et al. 2024). While numerous studies have examined microplastic concentrations in the water column, research on their distribution in sediments and bioaccumulation in benthic species, particularly *A. granosa*, remains limited.

This study aims to investigate the bioaccumulation and risks of microplastic pollution in sediments and *A. granosa*

in the Musi Estuary, Indonesia. The findings are expected to enhance understanding of benthic organisms, especially *A. granosa*, and its potential health risks for local seafood consumers.

MATERIALS AND METHODS

Study area and sample collection

The Musi Estuary, located on the east coast of South Sumatra, Indonesia is where seawater from the Bangka Strait mixes with freshwater from the Musi River. The Musi Estuary is an important area for fisheries and benthic habitats in South Sumatra, Indonesia (Rozirwan et al. 2021, et al. 2022b). Sampling locations were selected based on hydrodynamic conditions influencing microplastic transport and deposition, considering salinity variations and water mass interactions (Cheng et al. 2024; Diansyah et al. 2024). Locations of the sampling sites are shown in Figure 1 and Table 1. Based on in situ measurements, water parameters include temperature (27.31-31.6°C), pH (6.95-8), dissolved oxygen (3.62-5.21 mg/L), salinity (6-30‰), water brightness (0.1-0.9 m), water current speed (0.1-1.8 m/s). Sediment and *A. granosa* were collected during low tide from the Musi Estuary, South Sumatra, Indonesia. Sediment samples were collected from ten sampling points using a Peterson Grab at each station (Dwiyitno et al. 2024). Samples were placed in glass jars that had been rinsed with pure distilled water and covered using aluminum foil to avoid contamination. Samples were placed in a cool box ($\pm 4^\circ\text{C}$) for further analysis in the laboratory.

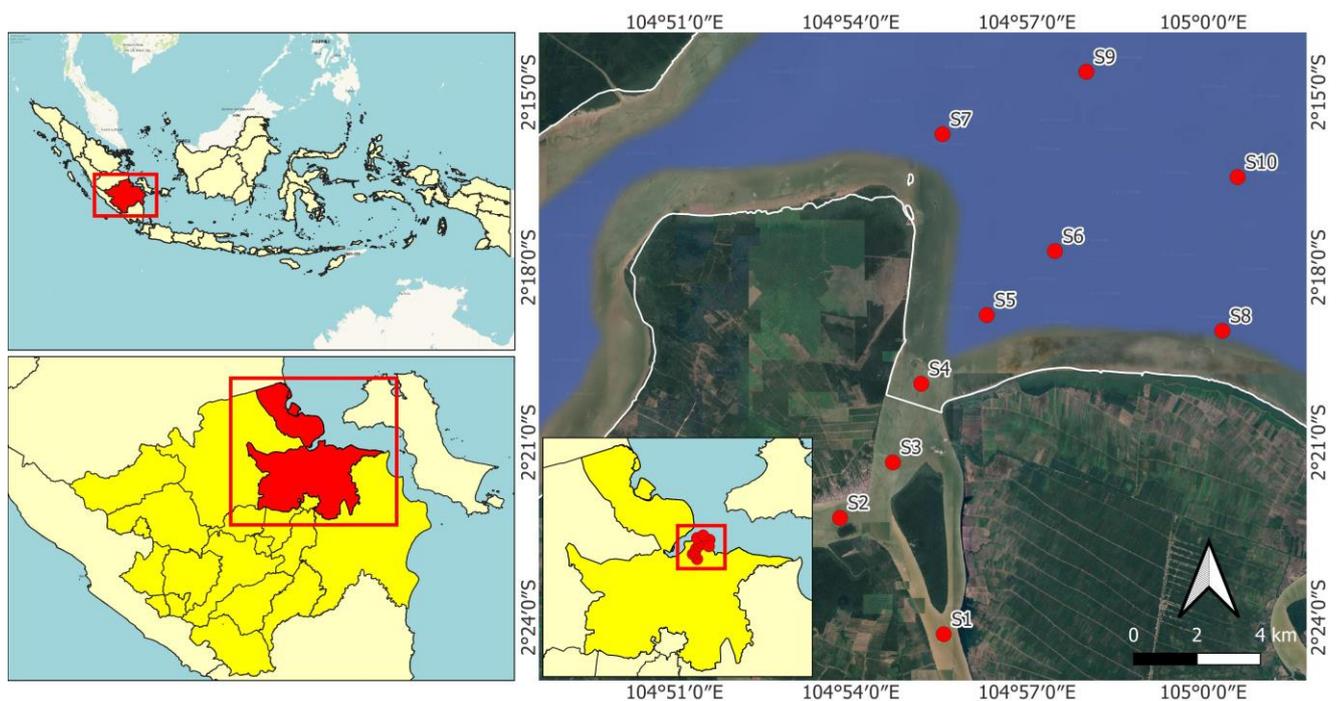


Figure 1. Study site at the Musi Estuary, South Sumatra, Indonesia

Table 1. Coordinates of the sampling location

Estuary zone	Description	Station	Latitude (°S)	Longitude (°E)
Inner zone	closest to the river body and dominated by freshwater mangrove vegetation and muddy substrate near densely populated coastal village activities, shipping and fishing activities	S1	2.40470°S	104.92643°E
		S2	2.37144°S	104.89695°E
		S3	2.35558°S	104.91200°E
Middle zone	between the inner zone and the outer zone mangrove vegetation and muddy substrate shipping and fishing activities	S4	2.33316°S	104.92003°E
		S5	2.31353°S	104.93862°E
		S6	2.29524°S	104.95794°E
Outer zone	closest to the ocean (at the sea), dominated by seawater, no vegetation, muddy substrate shipping and fishing activities	S7	2.26183°S	104.92603°E
		S8	2.31807°S	105.00547°E
		S9	2.24403°S	104.96686°E
		S10	2.27413°S	105.00978°E

Anadara granosa samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). Twenty adult *A. granosa* (7.48-11.44 cm) were collected from fishermen's catch in the Musi Estuary. *Anadara granosa* samples were identified based on their morphological traits according to the World Register of Marine Species (WoRMS) (Abelouah et al. 2024). The mussels were rinsed with pure distilled water to remove any dirt. *Anadara granosa* were wrapped in aluminum foil and stored in a cooler at approximately 4°C to preserve their freshness. After that, they were transported to the laboratory and frozen at -20°C before further analysis (Ding et al. 2021).

Microplastic extraction from sediment

Microplastics in sediments were extracted using the standard method of density separation and organic matter removal with minor modifications (Thompson et al. 2004; Liebezeit and Dubaish 2012). A total of 500 g of wet sediment samples were placed in a beaker glass covered with aluminum foil. The samples were dried in an oven at 60°C for 48 hours, or until they reached a constant mass (Patria et al. 2023). The sediment samples were pulverized and sieved through a 5 mm steel sieve. A portion of 100 g of dried sediment was taken from the sieve, placed into a beaker glass, and covered with aluminum foil to prevent external contamination.

The sample was suspended in 400 mL of saturated NaCl solution (1.2 g cm⁻³), which is four times the sample weight, using a magnetic stirrer. This NaCl solution was prepared by dissolving pure NaCl crystals (Merck Millipore EMSURE®) in filtered distilled water that was free from contaminants. The selection of a NaCl solution for the separation of microplastics from sediments was based on the premise that this method is cost-effective and environmentally friendly (Perumal and Muthuramalingam 2022).

Stirring was carried out for 5 minutes until completely dissolved, and the mixture was allowed to stand for 1 hour. After one hour, 10 mL of 30% H₂O₂ was added to assist in the breakdown of organic matter, followed by stirring for an additional five minutes. Hydrogen peroxide (H₂O₂) is commonly used in similar studies and is regarded as highly effective for organic matter removal (Lee et al. 2023). The sample was allowed to stand for 24 hours, covered with

aluminum foil. The samples were then filtered using the Whatman No. 42 filter paper (mesh size 0.45 µm, Φ = 90 mm), assisted by a vacuum pump at 17 kPa to isolate the microplastics. The collected samples were deposited into petri dishes for identification.

Microplastic extraction from *Anadara granosa*

The previously frozen *A. granosa* samples were defrosted under controlled conditions at room temperature (±25°C) for 1 hour. After the ice melted, the *A. granosa* were washed with pure distilled water to eliminate contamination from other objects, such as sediment. The length and wet weight of the *A. granosa* were measured using an analytical balance (0.01 g). The digestive tract was carefully removed from the *A. granosa* using sterile stainless steel utensils that had been rinsed with distilled water. The extraction of microplastics from the digestive tract of the *A. granosa* was adapted from previous studies with minor modifications (Ding et al. 2018, et al. 2021). The digestive tract was transferred into an Erlenmeyer flask, and 100 mL of KOH solution (10%) was added. The use of KOH is regarded as a more efficacious method for the digestion of biological material, and it has no impact on the integrity of the plastic polymer (Karami et al. 2017). The sample was covered with aluminum foil and stirred for 5 minutes using a 150 rpm magnetic stirrer. The supernatant was incubated at room temperature (±25°C) until complete digestion of organic matter occurred. The sample was then filtered through Whatman 42 filter paper (mesh size 0.45 µm, Φ = 90 mm) to isolate microplastic. The filter paper was dried in an oven at 40°C for 5 hours and stored in petri dishes for subsequent identification of microplastic content.

Microplastic identification and quantification

Identification and quantification of microplastics from sediments and *A. granosa* were performed using the same method. Microplastic identification was performed using an Olympus CX23 microscope with 10 × 10 or 4 × 10 magnification to visually detect microplastics (Diansyah et al. 2024). The filter paper was observed closely and carefully to avoid contaminants that could enter the filter paper. Microplastics were identified based on the number and form of microplastics (fragment, film, fiber, and foam).

The data obtained were recorded by sample type and station/individual for statistical purposes. To prevent contamination, personnel wear cotton lab coats, latex gloves, and cotton masks. Furthermore, access to the detection room is restricted to prevent outside contact during observations.

Procedures for quality assurance and contamination prevention

The quality and contamination control measures were implemented to prevent any alterations to the microplastics in the samples. Before sampling, glass jars and aluminum foil were rinsed with filtered distilled water. Although the samples were in a cool box, they were stored in a place that was not exposed to direct sunlight to keep the samples in good condition. Personnel are also required to wear latex gloves during sampling. During the study, from sampling to laboratory analysis, the use of plastic equipment was minimized to avoid unintentional fragmentation of the microplastics. The distilled water used was filtered with filter paper (0.45 μm,) to prevent microplastics from entering our materials. All equipment was washed with filtered distilled water to remove potential contamination from external particles (Ding et al. 2021). We prepared two controls by filtering each solution we used to avoid contaminants from materials, equipment, and air. No microplastics were found contaminating the instrument. The sterilized equipment was wrapped in aluminum foil to prevent any input of contaminants from outside. Laboratory personnel were required to wear latex gloves, lab coats, and masks throughout the analysis. Access to the laboratory was restricted during the analysis to minimize external interference that could lead to protocol errors.

Data analysis

Pollution Load Index (PLI)

PLI was calculated using a pollutant load approach, which assesses the total concentration of contaminants relative to a baseline level, to evaluate its significance. Equations were utilized to quantify the level of microplastic pollution in both sediments and *A. granosa* in the Musi Estuary, with the method adapted accordingly (Tomlinson et al. 1980; Xu et al. 2018; Wang et al. 2021):

$$CF_i = \frac{C_i}{C_{0i}}$$

This equation shows that the Concentration Factor (CF_i) at a particular sample is obtained by dividing the current Concentration (C_i) by the Initial Concentration (C_{0i}). C_i indicates the quantified presence of microplastics at each sampling location or within each clam (ind), while C_{0i} denotes the background concentration, defined as the lowest value recorded across all sampling locations. Given the lack of previously published research on microplastics in sediments or *A. granosa* in the Musi River Estuary, we determined C_{0i} values from minimum concentrations across sampling stations.

$$PLI = \sqrt{CF_i}$$

This equation shows that the Pollution Load Index (PLI) is generated by calculating the square root of the Concentration Factor (CF). The PLI value of microplastics is derived from the Contaminant Factor (CF_i) calculated for each station/individual.

$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \dots \times PLI_n}$$

Pollution Load Index of Zone (PLI_{zone}) is determined by taking the square root of the product of individual PLI values from multiple sites within the zone. This method integrates data from several measurement points, providing a more comprehensive assessment of microplastic contamination within the analyzed. A PLI_{zone} is used to assess pollution based on specific estuary zones as well as the overall estuary area. PLI values are categorized into four predefined categories of pollution load, as shown in Table 2.

Nemerow Pollution Index (NPI)

NPI is designed to assess the bioavailability of microplastics in sediments and *A. granosa*. This index was adapted from equations used in prior studies evaluating heavy metal contamination (Alam et al. 2023).

$$NPI = \sqrt{\frac{\left(\frac{C_i}{S_i}\right)_{max}^2 + \left(\frac{C_i}{S_i}\right)_{ave}^2}{2}}$$

The NPI value is calculated using the square root of the mean squared deviation, incorporating both the maximum and average concentration ratios relative to the background concentration (S_i). The formula includes the sum of the squares of the maximum concentration ratio (C_{i max}/S_i) and the average concentration ratio (C_{i ave}). The C_i value represents the concentration of microplastics found in the sediment or clams at a specific station or individual, while the S_i value denotes the background level of microplastics, determined as the lowest concentration recorded across all sample types. The NPI provides a measure of microplastic bioavailability in sediments and *A. granosa*, with values below 2 indicating low bioavailability and values above 2 indicating high bioavailability.

Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) quantifies the extent to which microplastics accumulate in biota relative to their environmental concentration and is calculated using the following equation (Li et al. 2022a).

Table 2. Microplastic pollution load categories

Definition	Value			
	<10	10-20	20-30	>30
Value of the Pollution Load Index (PLI)				
Risk category	I	II	III	IV
Information	Minor	Middle	High	Extreme

$$BCF = \frac{C_{biota\ ave}}{C_{sediment\ ave}}$$

In this equation, BCF represents the bioconcentration factor, which is derived from the calculation of the average concentration of microplastic in biota (*A. granosa*) divided by the average concentration of microplastic in its environment (sediment). This value is intended to assess how much microplastic is concentrated in the biota.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 27. Descriptive statistics, including total, mean, standard deviation, percentage, minimum, and maximum, were utilized to assess data variation. A One-Way ANOVA was applied to analyze differences in microplastic abundance between estuarine zones when the data met normality assumptions. For non-normal data, the Kruskal-Wallis test was employed to assess the significance of differences between zones. All tests were carried out with a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Microplastic presence in sediment from Musi Estuary

The Musi Estuary in South Sumatra, Indonesia, is a dynamic brackish water environment where freshwater from the Musi River meets the saltwater of the Bangka Strait (Figure 2). Characterized by turbid waters and muddy sediments rich in organic matter, the estuary is fringed by mangrove forests that provide habitat for aquatic species, including the *A. granosa*, a key species in local aquaculture (Rozirwan et al. 2023c). Surrounded by urban and industrial areas, including Palembang, the estuary faces pollution from domestic, agricultural, and industrial waste, making it susceptible to microplastic accumulation in sediments and ingestion by benthic organisms like *A. granosa* (Almiza and Patria 2021; Diansyah et al. 2024).

The results of this study indicate that microplastics were detected at all sampling stations, as shown in Figure 4. A total of 1,308 microplastic particles were identified, with concentrations ranging from 66 to 178 particles per

100 g of dry sediment (dry weight, dw). The average abundance of microplastics was 1.31 ± 0.41 particles/g dw, with the highest concentration found at station S1 (1.78 particles/g dw) and the lowest at station S8 (0.66 particles/g dw). High levels of human activity in the upstream area of Musi Estuary, which flows downstream to the estuary and ultimately into the ocean, are responsible for the presence of microplastics (Rozirwan et al. 2021; Diansyah et al. 2024). Urban activities in close proximity to the Musi River Watershed, particularly in Palembang City, significantly contribute to the generation of plastic waste. Increased population density near riverbanks enhances the potential for plastic pollution. Furthermore, population density is a crucial factor in the entry of microplastics into rivers (Eo et al. 2023; Dwiyitno et al. 2024). As a result, plastic pollutants from domestic activities, such as the disposal of food packaging and plastic bags, can be transported through stormwater drains and eventually end up in rivers (Kunz et al. 2023). Fishing activities around Musi Estuary can contribute to microplastic pollution, primarily through fibers released from fishing nets (Li et al. 2022a; Fauziyah et al. 2023). This increases anthropogenic pressure at river mouths, which can lead to a greater accumulation of microplastics (Castro-Jiménez et al. 2024). Additionally, tidal fluctuation influences water flow, which facilitates the deposition of microplastics in sediment at these locations (Harris 2020). The findings of this study indicate that anthropogenic activities are responsible for high levels of microplastic deposition in the sediments of Musi Estuary. Microplastic particles that accumulate in sediments serve as a habitat for benthic organisms, which can have long-term implications for ecosystem health and balance (Rahmatin et al. 2024). As vectors for pollutants, microplastics can exacerbate toxicity, potentially endangering various populations (Caruso 2019; Fu et al. 2021; Issac and Kandasubramanian 2021; Ta and Babel 2023). Furthermore, the processes of bioaccumulation and biomagnification in benthic organisms present heightened risks to these animals, potentially leading to human consumption (Unuofin and Igwaran 2023). Therefore, it is crucial for local governments to address the handling and prevention of further microplastic accumulation.



Figure 2. General conditions in the Musi Estuary, South Sumatra, Indonesia: A. Coastal settlements; B. Shipping activities; C. Fisheries; D. Mangrove ecosystem

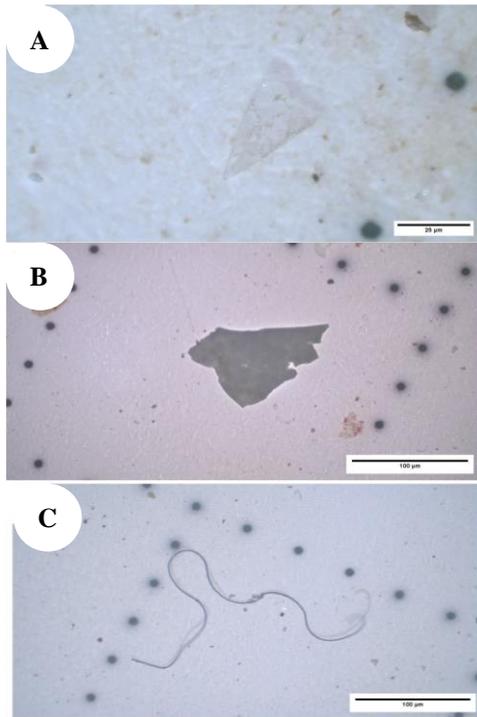


Figure 3. Various forms of microplastics from Musi Estuary, South Sumatra, Indonesia: A. Film; B. Fragment; C. Fiber

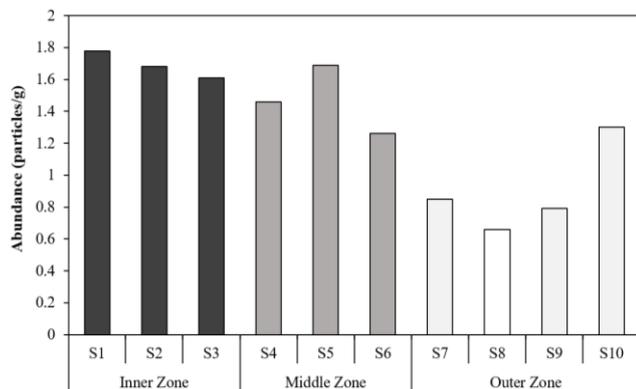


Figure 4. Distribution of microplastics from sediments in Musi Estuary, South Sumatra, Indonesia

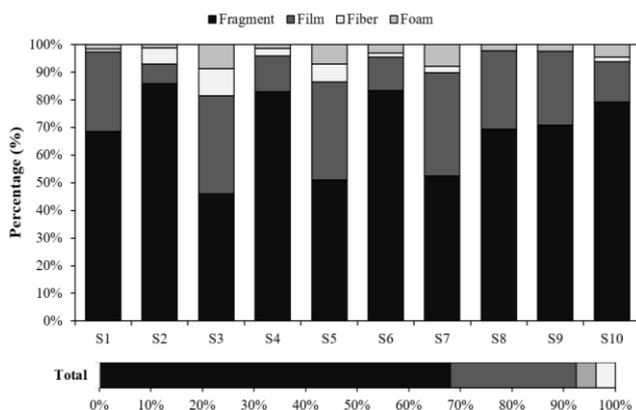


Figure 5. Percentage abundance of microplastic shapes form sediments in Musi Estuary, South Sumatra, Indonesia

The microplastics identified in the sediments were predominantly fragments (68.92%), followed by films (23.86%), fibers (4.05%), and foams (3.17%) (Figure 3 and Figure 5). The dominance of fragments, which are a common form of secondary microplastics, occurs as a result of macroplastic degradation (Barnes et al. 2009). Fragments represented the most common type across all sediment samples analyzed. This dominance of fragments in estuarine sediments aligns with findings from various studies conducted in other estuarine environments. Research indicates that estuarine sediments globally tend to accumulate diverse microplastic forms, particularly fibers (Firdaus et al. 2020; Alam et al. 2023; Samuels et al. 2024; Santucci et al. 2024), with many studies also reporting a predominance of fragment types (Zhou et al. 2021; Suteja et al. 2024). These observations corroborate the literature, which consistently shows that fibers and fragments constitute the majority of microplastic pollution in estuarine ecosystems (Feng et al. 2023a).

This study reveals notable variations in microplastic concentrations compared to similar research in estuaries globally. Our findings indicate that the Musi Estuary has lower microplastic concentrations than those reported in several other Indonesian and international estuaries. For example, the Jakarta Bay Estuary exhibits concentrations nearly ten times higher, ranging from 1,184 to 1,337 particles per 100 grams of river sediment and 804 to 1,055 particles per 100 grams of beach sediment (Dwiyoitno et al. 2024). Such elevated levels are likely due to increased anthropogenic activities along Jakarta’s rivers, which substantially contribute to plastic waste flow. In another comparison, the Pearl River Estuary in China also shows higher microplastic densities, with concentrations between 2.05×10^3 and 7.75×10^3 particles per kilogram of dry weight sediment (Xu et al. 2024). This estuary's coastal regions experience high human activity, enhancing the potential for microplastic deposition. Similarly, the Meghna Estuary in Bangladesh, which receives sediment from the Ganges River Basin, reports even higher microplastic levels at $4,014.66 \pm 1,717.59$ particles per kilogram dry weight (Alam et al. 2023). These findings underscore the significant role of anthropogenic influences on microplastic accumulation in global estuarine sediments, including the Musi Estuary. Other environmental factors, like location, sampling period, sediment depth, sediment composition, and local hydrodynamic conditions, may also affect these changes in the amount of microplastics found (Zheng et al. 2020; Feng et al. 2023b; Yuan et al. 2023).

In contrast, the abundance of microplastics in the sediments of the Musi Estuary surpasses levels reported in other regions, both within Indonesia and globally. Concentrations in the Musi Estuary are notably higher than those in the Zandvlei River Watershed and estuarine areas of South Africa (70.23 ± 7.36 particles/kg dw) (Samuels et al. 2024), the Claromecó Estuary in Argentina (299 ± 114 particles/kg dw) (Truchet et al. 2021), and the upper sediment layer (0-5 cm) of the Fuhe River Estuary in Northern China (1049 ± 462 particles/kg dw) (Zhou et al. 2021). These numbers are also higher than those found in

coastal Río de la Plata (547.83 ± 620.06 particles/kg dw) (Santucci et al. 2024), Benoa Bay, Bali (31.08 ± 21.53 particles/kg dw) (Suteja et al. 2024), the Jagir Estuary in Surabaya (up to 590 particles/kg dw) (Firdaus et al. 2020), and the Pekalongan River Estuary in Java (0.77 to 1.01 particles/g dw) (Ismanto et al. 2023). The high microplastic concentration in the Musi Estuary is likely due to extensive plastic waste disposal in the river basin by the surrounding community. Research supports that plastic debris forms a substantial part of the macro-waste in the Musi River (Maherlsa et al. 2019). Additionally, local communities frequently establish settlements along the river, relying on it for water access and transportation. Consequently, domestic waste, including plastic, often enters the river directly. This local waste management issue reflects a broader trend, with Indonesia identified as the world's second-largest contributor to oceanic plastic pollution, following China (Jambeck et al. 2015).

Spatial distribution microplastic in sediments

The study revealed that the abundance of microplastics was significantly higher in the inner and middle zones when compared to the outer zone. The mean abundances were 1.69 ± 0.08 particles/g dw in the inner zone, 1.47 ± 0.22 particles/g dw in the middle zone, and 0.9 ± 0.28 particles/g dw in the outer zone. Significant differences in mean microplastic abundance between zones were revealed by the one-way ANOVA ($p < 0.05$), as illustrated in Figure 6. The Tukey HSD test revealed no significant difference in mean microplastic abundance between the inner and middle zones. However, we observed a significant difference in the outer zone compared to both the middle and outer zones. Our results indicate that the inner and middle estuary regions exhibit greater mean concentrations of microplastics compared to the outer estuary. Natural factors, particularly the effects of currents and wave action in coastal and estuarine environments, significantly influence this phenomenon by altering microplastic distribution. The influx of freshwater promotes the sedimentation and prolonged retention of high-density microplastics within the system (Li et al. 2024a). Furthermore, the sedimentation process is affected by the density and buoyancy of microplastics, with increased salinity in estuarine waters enhancing buoyancy forces and influencing distribution patterns (Cheng et al. 2024). Our findings align with research from the Liaohu Estuary in China, revealing a higher accumulation of microplastics in inner river sediments compared to the outer estuary (Xu et al. 2020). Additionally, sampling depth plays a critical role in accurately measuring microplastics in surface waters, bottom layers, and sediments (Feng et al. 2023a).

The spatial analysis revealed distinct patterns of microplastic accumulation between the two sides of the coast adjacent to the estuary. Notably, Site S7 exhibited higher concentrations compared to Site S8, likely due to its proximity to other pollution sources. Specifically, S7 is located near the Banyuasin Estuary, which is believed to significantly contribute to the deposition of microplastics in the surrounding sediments. Based on da Costa et al. (2023), confirmed that nearby sources of pollution heavily

influence microplastic deposition in coastal regions. The accumulation of microplastics at the mouth of the adjacent river further exacerbates this phenomenon, facilitating the settlement of microplastics within the sediment. Additionally, studies suggest that the depth of the sampling locations significantly influences microplastic deposition, revealing a greater abundance of microplastics at deeper water levels (Bayo et al. 2022). The substantial accumulation of microplastics in sediments is attributed to their persistent characteristics as well as the protective conditions offered by deeper environments, which are shielded from UV light, maintain lower temperatures, and exhibit lower oxygen levels, thus slowing biodegradation processes (Zhang et al. 2021).

Our findings confirm the presence of microplastics in the sediments of the Musi Estuary, likely originating from suspended microplastics that eventually settle from the water's surface. Previous research has found that there are 467.67 ± 127.84 particles/ m^3 and 723.67 ± 112.05 particles/ m^3 of microplastic in surface waters during ebb and flow conditions, respectively (Diansyah et al. 2024). Hydrodynamic conditions in the estuary and processes like aggregation and biofouling, which increase microplastic density, influence the deposition of these particles (Malli et al. 2022). The specific properties of the microplastics and the water dynamics of the estuary strongly influence microplastic deposition. For instance, biofouling can make microplastics denser, accelerating their descent into sediments (Lin et al. 2023). Since microplastics often have higher densities than water, they can remain suspended in the water column before eventually settling (Dai et al. 2022). High Total Suspended Solids (TSS) in the Musi Estuary have the potential to cause aggregation of microplastics (Rahutami et al. 2022). High TSS levels make it easier for sediments and other suspended particles to stick together on microplastic surfaces, which speeds up their deposition (Yang et al. 2022). However, microplastics can also become resuspended in the water column (Tang et al. 2020). Stirring forces in estuarine environments can lift microplastics back into the water, where turbulence and bioturbation may redistribute them (Malli et al. 2022). Therefore, additional studies are required to fully characterize the mechanisms of microplastic deposition in the Musi Estuary. Climate factors also influence microplastic distribution in Musi Estuary sediments. In Indonesia, where regions such as the Musi Estuary experience distinct rainy and dry seasons, seasonal changes affect water dynamics and microplastic transport. During the rainy season, increased river velocity may carry microplastics upward from sediments, while in the dry season, slower flows facilitate microplastic accumulation at the water's surface (Zhao et al. 2020). Currently, there are no studies specifically examining the seasonal impact on microplastic presence in the Musi Estuary, underscoring the need for further research to address this gap.

Bioaccumulation of microplastics in *Anadara granosa*

We extracted 133 microplastic particles from the digestive tracts of twenty samples of *A. granosa* species presented in Figure 7. Each sample from the Musi Estuary

had accumulated microplastics, ranging from 6 to 49 particles/ind or 8 to 69.01 particles/g ww, as shown in Figures 8 and 9. The mean abundance of microplastics found in the digestive tracts was 21.05 ± 10.31 particles/ind or 30.46 ± 16.7 particles/g ww. Several factors contribute to the increased microplastic inputs in bivalve species, including human activities, tourism, fishing equipment, freshwater runoff, and wastewater discharge, combined with the area's hydrodynamic conditions (Vital et al. 2021). The elevated levels of microplastics found in *A. granosa* from the Musi River Estuary suggest a significant degree of contamination in their habitat, including both water and sediment, which may adversely affect the health of these organisms and disrupt local ecological balances. This observation aligns with existing literature, which indicates a strong correlation between the abundance of microplastics in bivalves, such as mussels, and their sediment environments (Sathish et al. 2020), reinforcing the need for monitoring these pollutants in aquatic ecosystems.

Microplastics were observed in three morphological forms: film, fiber, and fragment. Film accounted for the majority of microplastics (59.38%), followed by fiber (31.59%) and fragments (9.03%), as shown in Figure 10. The digestive tracts of the *A. granosa* studied did not contain any pellets or foam. Statistical analysis using the Kruskal-Wallis test revealed significant differences in the abundance of the three microplastic forms ($p < 0.05$). This predominance of film microplastics in *A. granosa* is likely attributable to the extensive use of disposable plastic packaging within the community, which subsequently degrades into microplastics. Although fragments dominate the sediment substrate, *A. granosa* predominantly absorbs film microplastics. The lower density of film microplastics may facilitate their accumulation in the upper sediment layer, making them susceptible to resuspension in the overlying water column. This phenomenon is consistent with the feeding behavior of *A. granosa*, which frequently filters water from the sediment surface, resulting in the accumulation of film microplastics within their digestive tracts.

Our findings indicate a higher abundance of microplastics in *A. granosa* from the Musi Estuary compared to certain other regions globally. For instance, *A. granosa* from the Chanthaburi estuarine ecosystem in eastern Thailand contained 0.40 ± 0.16 particles/g ww (Potipat et al. 2024), while samples from Peninsular Malaysia showed concentrations of 0.20 ± 0.08 particles/g ww and 1.54 ± 0.30 particles/ind ww (Mohan et al. 2024). Additionally, microplastic levels in *A. granosa* from Pao Village, Tarawang Sub-district, Jeneponto District, Indonesia, were measured at 0.0144 particles/g ww (Namira et al. 2023), and those from Likas Bay Beach in North Borneo, Malaysia, at 24.4 ± 0.6 particles/g ww (Abd Rahman et al. 2024). In contrast, the *A. granosa* analyzed in this study accumulated lower microplastic concentrations than those reported in several other studies. For instance, *A. granosa* from the Pangkal Babu mangrove forest area in Tanjung Jabung Barat District, Jambi, exhibited 434 ± 97.05 particles/ind (Fitri and Patria 2019),

while those from Lada Bay in Pandeglang, Banten, had concentrations of 618.8 ± 121.4 particles/ind (Ukhrowi et al. 2021). Overall, this study shows that microplastics have contaminated the sediment environment of the Musi Estuary, South Sumatra, indicating that *A. granosa* can serve as effective bioindicators of this pollution.

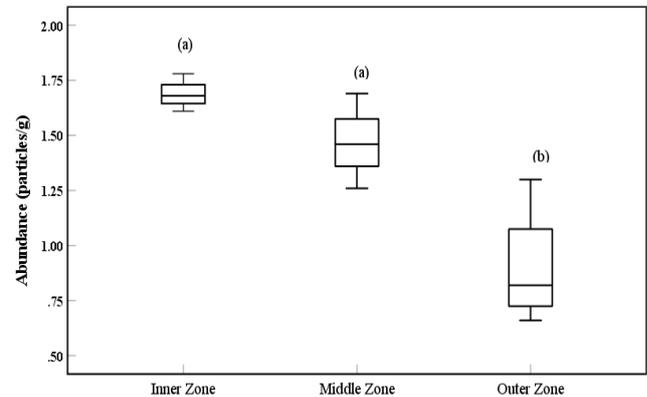


Figure 6. The difference in microplastic abundance between zones in the sediments from Musi Estuary, South Sumatra, Indonesia



Figure 7. Specimens of *Anadara granosa* from Musi Estuary, South Sumatra, Indonesia

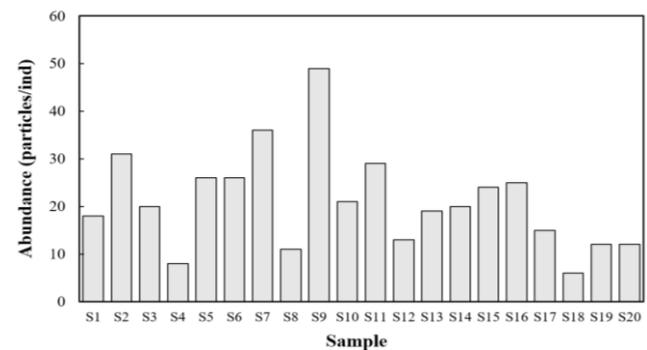


Figure 8. Microplastic abundance of *Anadara granosa* from the Musi Estuary, South Sumatra, Indonesia

Table 3. The microplastic risk factor in sediments of Musi Estuary, South Sumatra, Indonesia

Sediment Station	CF	PLI	PLI zone	PLI sediment	NPI
S1	2.70	1.64	Inner zone	1.37	2.37
S2	2.55	1.60			
S3	2.44	1.56	Middle zone		
S4	2.21	1.49			
S5	2.56	1.60			
S6	1.91	1.38	Outer zone		
S7	1.29	1.13			
S8	1.00	1.00			
S9	1.20	1.09			
S10	1.97	1.40			

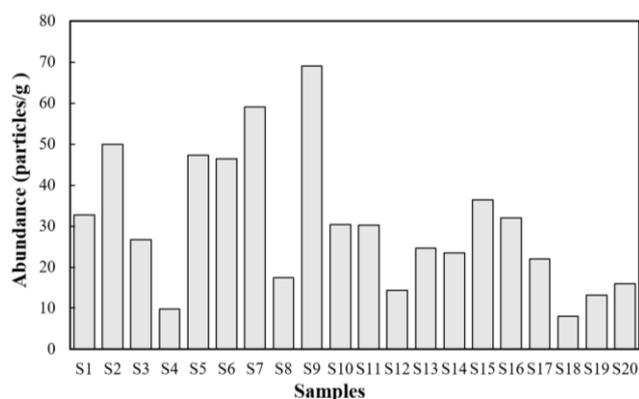


Figure 9. Microplastic abundance (in particles/g) of *Anadara granosa* from the Musi Estuary, South Sumatra, Indonesia

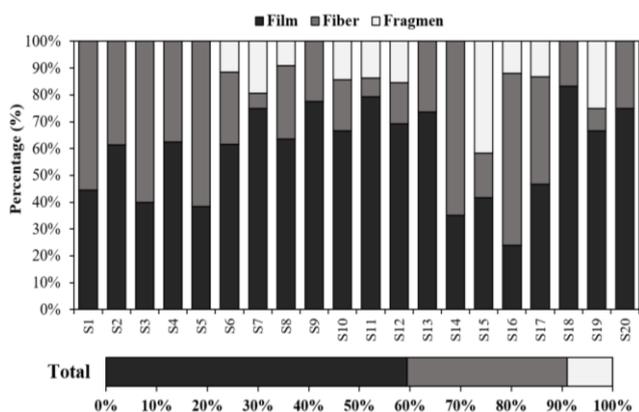


Figure 10. Shape of microplastics in *Anadara granosa* from the Musi Estuary, South Sumatra, Indonesia

Risk assessment of microplastics in sediments and *Anadara granosa*

A risk assessment of microplastic contamination in the Musi River Estuary was carried out by calculating the Pollution Load Index (PLI), the Nemerow Pollution Index (NPI), and the Bioconcentration Factor (BCF). The PLI values across all sediment samples indicate that the estuarine sediment is subject to minor pollution, with

values ranging between 1.00 and 1.64, as shown in Table 3. In the three zones of the estuary (inner, middle, and outer), the PLI values were 1.60, 1.49, and 1.20, respectively. We calculated the overall average PLI for the Musi Estuary to be 1.37. All zones reported PLI values below 3.00, which suggests that the area experiences only minor microplastic pollution. The analysis of microplastic presence in sediment samples revealed a significantly high concentration of microplastics, with an NPI value of 2.37. This suggests a substantial bioavailability of microplastics in the estuarine environment. This elevated NPI underscores the ecological risk posed by microplastic contamination, despite the relatively low PLI observed across the study area.

Anadara granosa analyzed in this study exhibited significant risk values associated with microplastic contamination. The PLI for these clams ranged from 1 to 2.86, with an overall mean PLI value of 1.77 for the entire sample, as shown in Table 4. This indicates that the pollution load in *A. granosa* falls within the minor category (PLI<3). Furthermore, the bioavailability of microplastics in *A. granosa* was significantly higher, with an NPI value of 6.29, indicating a high bioavailability. The bioconcentration factor of microplastics from sediment to *A. granosa* was measured at 23.28, suggesting a significant potential for the transfer of microplastics from sediment to *A. granosa* in the Musi Estuary.

Our findings demonstrated that the bioavailability of microplastics in sediments and *A. granosa* was categorized as high (NPI = 2.37; NPI>2). In comparison, the bioavailability of microplastics in *A. granosa* was even higher, with an NPI value of 9.89. The elevated bioavailability in sediments suggests a greater potential for absorption by aquatic organisms, particularly benthic animals. Furthermore, the Bioconcentration Factor (BCF) calculated for *A. granosa* in sediment was 23.28, indicating a significant transfer of microplastics from sediments to these organisms in the Musi Estuary. This high BCF value is attributed to the non-discriminatory feeding process of *A. granosa*, allowing them to ingest microplastics along with other particles. When compared to several benthic species from the Yangtze River Estuary, which exhibited a BCF of 29.48 ± 6.52 (Li et al. 2022c), the BCF of *A. granosa* in the Musi Estuary remains lower. Nonetheless, both sediments and *A. granosa* from the Musi Estuary are classified within the minor pollution risk index category (PLI<10). The bioaccumulation of microplastics by benthic animals like *A. granosa* poses a significant risk to human health for those consuming these clams (Wang et al. 2023; Winiarska et al. 2024). Therefore, periodic assessments are crucial to evaluate the risk of microplastic contamination in sediments and *A. granosa*, providing essential information to understand the ecological and health implications in the Musi Estuary.

The impact of high bioaccumulation extends beyond the toxicity of microplastic materials; it also facilitates the accumulation of other pollutants. Previous research has detected heavy metals, such as lead (Pb) and copper (Cu), adhering to the surfaces of microplastics in the Musi Estuary (Purwiyanto et al. 2020). Additionally, various hazardous heavy metals, including iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), and zinc (Zn), were

identified with high bioavailability in the aquatic environment of the Musi Estuary (Rahutami et al. 2022). This accumulation allows for the deposition of significant quantities of heavy metals on microplastic surfaces, thereby increasing the risk of microplastic toxicity for estuarine ecosystems. Moreover, the involvement of microorganism vectors, misidentification in aquatic organisms' diets, and their harmful toxicological effects on benthic animals heighten the risk associated with microplastics (Gong and Xie 2020). Consequently, the high output of microplastics from the Musi Estuary has the potential to jeopardize the condition of the surrounding aquatic environment, including fisheries and migratory birds, which are critical to the ecosystem (Rozirwan et al. 2019, 2022a).

This study holds significant ecological implications for the Musi Estuary ecosystem, as the presence of microplastics poses a long-term threat to ecosystem stability. Previous studies have shown that microplastics can affect the physicochemical properties of sediments, such as electrical conductivity, organic matter content, and nutrients, and well as impact enzymatic activity in sediment bacteria (Rillig 2012; Li et al. 2022b; Yuan et al. 2023). These changes can lead to structural and functional alterations in aquatic habitats (Li et al. 2022b). Microplastics can also enter various levels of the food chain through bioaccumulation and biomagnification processes. For example, microplastics in plankton can cause a decline in zooplankton populations, which impacts food availability for small fish and predatory species at higher trophic levels (Malinowski et al. 2023). The accumulation of microplastics in *A. granosa* in this study indicates that the pressure of plastic waste in the Musi Estuary is already quite high. Microplastics can act as vectors for other pollutants, such as microorganisms, heavy metals, and other inorganic pollutants can increase the risk of toxicity for aquatic organisms, including *A. granosa* (Purwiyanto et al. 2020; Rafa et al. 2024). Research indicates that exposure to microplastics can lead to behavioral and physiological changes in aquatic organisms, including changes in diet and reproductive success (Guo et al. 2020; Liang et al. 2023). Moreover, microplastics disrupt endocrine functions and metabolic pathways, causing oxidative stress, cell necrosis, and apoptosis, which can ultimately lead to mortality (Pannetier et al. 2020; Jeyavani et al. 2021). Disruption of any component of the food chain can trigger a domino effect, threatening the balance of the Musi Estuary ecosystem. However, this study only examines microplastic pollution in sediment and *A. granosa* as environmental indicators. Additional research is needed to understand microplastic buildup in other species, such as fish, shrimp, and benthic organisms, for a more complete picture of microplastic pollution in the Musi Estuary and its impact on the global marine environment.

Microplastic contamination of *A. granosa* in the Musi Estuary poses a significant threat to local communities, particularly clam fishers. Microplastics that accumulate in *A. granosa* may pose health risks to community members who consume them regularly as a primary source of protein. Long-term exposure to microplastics can potentially disrupt various human body systems, including the digestive, respiratory, reproductive, nervous, and

cardiovascular systems (Li et al. 2024b). Furthermore, microplastics may cause both acute and subchronic toxicity and are considered potentially carcinogenic and disruptive to human development (Yuan et al. 2022). Once in the bloodstream, microplastics can be transported to the liver, the primary organ responsible for detoxification. Accumulation in the liver may trigger adverse physiological reactions, including increased oxidative stress, liver fibrosis, and impaired lipid metabolism. In addition to health risks, microplastic contamination in *A. granosa* in the Musi Estuary may reduce consumer confidence and decrease market demand due to food safety concerns (Unuofin and Igwaran 2023). The long-term effect could weaken local economic resilience as marine products in the region are perceived to be contaminated with microplastics (Barrientos et al. 2024). Therefore, it is crucial for governments and communities to recognize these risks and collaborate on mitigation efforts to protect ecosystems, public health, and local economies.

Furthermore, seafood hygiene practices in local communities and fishing industries can help reduce microplastic contamination (Lusher et al. 2017; Smith et al. 2018). For example, *A. granosa* and other seafood, when thoroughly cleaned by repeated washing and clam removal before cooking, may reduce microplastic intake (Li et al. 2022a). The concentration of microplastics in shellfish is significantly lower after boiling and steaming compared to frying, suggesting that cooking methods affect microplastic retention in seafood (Evide et al. 2021). This method can also be applied to shrimp, fish, and other shellfish to lower the risk of microplastic exposure. Implementing these recommendations will help reduce microplastic pollution and provide long-term benefits for public health, ecosystem stability, and the economic resilience of local fisheries (Prata et al. 2019).

Table 4. Risk factors for microplastics in *Anadara granosa* from the Musi Estuary, South Sumatra, Indonesia

<i>A. granosa</i> sample	CF	PLI	PLI <i>A. granosa</i>	NPI	BCF
S1	3.00	1.73	1.77	6.29	23.28
S2	5.17	2.27			
S3	3.33	1.83			
S4	1.33	1.15			
S5	4.33	2.08			
S6	4.33	2.08			
S7	6.00	2.45			
S8	1.83	1.35			
S9	8.17	2.86			
S10	3.50	1.87			
S11	4.83	2.20			
S12	2.17	1.47			
S13	3.17	1.78			
S14	3.33	1.83			
S15	4.00	2.00			
S16	4.17	2.04			
S17	2.50	1.58			
S18	1.00	1.00			
S19	2.00	1.41			
S20	2.00	1.41			

In conclusion, this study demonstrates the significant presence of microplastics in the sediment and *A. granosa* of the Musi Estuary, Indonesia. These findings indicate a serious threat to the aquatic ecosystem and to local public health, as microplastics can enter the food chain and potentially harm organisms across trophic levels, including humans (Ningrum and Patria 2022; Edwin et al. 2023; Patria et al. 2023). This pollution heightens environmental risks, particularly for species integral to both the community's diet and economy (Jeong et al. 2024). The microplastics likely originate from human activities, especially urban waste from upstream areas that flows downstream and accumulates in the estuary (Diansyah et al. 2024). These results highlight the urgent need for routine monitoring and stricter waste management practices, particularly for plastic disposal, to reduce further contamination (Barletta et al. 2019). A comprehensive strategy is essential to controlling plastic waste throughout the river system—from source to estuary. Effective measures should include coordinated waste management practices, public education on the impact of pollution, and cross-sector collaboration to preserve estuarine health (Wakwella et al. 2023; Ihenetu et al. 2024). Such initiatives are crucial to protecting ecosystem health and sustaining local fisheries, ensuring these resources continue to support community well-being.

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