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2 November 2024 pukul 20.13

Manuscript Number: TOXREP-D-24-00875 Manuscript Title: Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones Journal: Toxicology Reports

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Rozirwan ROZIRWAN <rozirwan@unsri.ac.id> Kepada: Toxicology Reports <support@elsevier.com> 2 Januari 2025 pukul 12.54

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After a few months ago we submitted our article, we would like to inquire about the progress of my submitted manuscript entitled " Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones".

Manuscript No: TOXREP-D-24-00875

Thank you Best regards [Kutipan teks disembunyikan]

Prof. Dr. Rozirwan

Head of Marine Bioecology Laboratory Department of Marine Science Faculty of Mathematics and Natural Sciences Sriwijaya University Jalan Raya Palembang-Prabumulih KM 32, Indralaya Ogan Ilir, Sumatera Selatan, Indonesia, Pos Code: 30862 Email: rozirwan@unsri.ac.id, rozirwan@gmail.com



Re: TOXREP-D-24-00875 - Confirming your submission to Toxicology Reports [250102-006855]

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I understand the importance of a swift editorial decision, and work hard to ensure articles are reviewed quickly.

From checking, your paper is under review. Currently, we have received review comments from 1 reviewer. We are waiting for the required reviews to be completed before a decision can be made.

In line with this, I have contacted the Editor regarding on your manuscript to expedite the process.

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CC: I.h.lash@wayne.edu, nbasaran@hacettepe.edu.tr, anbasaran@baskent.edu.tr

Manuscript Number: TOXREP-D-24-00875

Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones

Dear Dr Rozirwan,

Thank you for submitting your manuscript to Toxicology Reports.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by **Mar 02, 2025**.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

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Lawrence Lash Editor-in-Chief Toxicology Reports

Editor and Reviewer comments:

Reviewer #1: Research highlights need improvement, and the graphical abstract should be of high quality.

How does this study differ from previous research on mangrove species, metals, and biomarkers? What is the necessity of conducting research in this specific location, and how extensively has this area been investigated in terms of metal contamination?

The sampling map should indicate the sampling stations (Station 1 and Station 2).

The methods section should detail the sampling procedures, including how the mangrove samples and sediment were collected. Please specify how many mangrove samples were taken. Additionally, the author should clarify whether roots or leaves were collected for sampling. The author previously mentioned roots but later referred to leaves.

How long were the sediments air-dried at room temperature?

The author must clearly describe how the mangrove leaves or roots were dried in indirect sunlight. Furthermore, the author needs to include a section on heavy metals analysis, as this part is currently missing from the methods.

The use of "inhibition" should be revised in Equation 5, and the column name in Table 8 on page 30, line 51, should be adjusted to proper English.

The word of "inhibition" in eq 5, column name Table 8, and section title line 51 page 30, please kindly adjust to English

How does the dataset in this study fulfill the criteria for parametric analysis?

In the figure depicting mangrove leaves, the author should include a scale for better visualization.

What is the role of industrial activities in the accumulation of fine particles, specifically clay particles?

How do unmanaged anthropogenic activities contribute to the increase of copper (Cu)?

How do mangroves regulate copper in their system?

In the discussion, the author states that metal concentration shows a negative correlation with antioxidant activity, yet the abstract claims that heavy metal concentration increases antioxidant activity. This discrepancy needs clarification.

Reviewer #2: Review report for: Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones

The study addresses a significant environmental concern—heavy metal pollution in mangrove ecosystems—and its biological response through antioxidant activity. The study aims to analyze the correlation between Pb and Cu concentration and antioxidant activity in Avicennia alba and Excoecaria agallocha. The results provide useful insights into bioaccumulation, pollution indices, and biochemical responses of mangroves to heavy metal stress.

Areas for Improvement and Suggested Revisions

The writing should be more fluid and concise. Some sentences are lengthy or unclear.

In the abstract section

"Mangroves can mitigate the impact of free radicals by producing antioxidant compounds" can be written as "Mangroves produce antioxidant compounds to mitigate the impact of free radicals."

"The correlation analysis between heavy metal concentrations and antioxidant activity indicates that as heavy metal concentrations increase, antioxidant activity and total phenol content also increase." can be written as "Correlation analysis shows that higher heavy metal concentrations correspond to increased antioxidant activity and total phenol content."

Rearrange some details for logical flow: The sentence about Pearson correlation analysis should come after mentioning heavy metal concentration and antioxidant activity measurements.

The concentration values for Pb and Cu should be clearly formatted:

Instead of Pb values of $0.67 \pm 0.16 - 18.70 \pm 0.48 \text{ mg}$ / kg and Cu values of $3.39 \pm 0.20 - 6.07 \pm 0.37 \text{ mg}$ / kg, write Pb values of 0.67 ± 0.16 to $18.70 \pm 0.48 \text{ mg/kg}$ and Cu values of $3.39 \pm 0.20 - 6.07 \pm 0.37 \text{ mg/kg}$ for clarity.

In the sentence:"The results of sediment pollution assessment for heavy metals Pb and Cu at Igeo < 0, 1 < Cf < 3, and PLI 0-2." Define Igeo, Cf, and PLI for readers unfamiliar with these indices. "BCF < 1" should be clarified: Does this mean bioaccumulation is low?

The Pearson correlation analysis result should be explicitly stated (e.g., r = X, p < 0.05).

Introduction

Some sentences are long and could be more concise for better readability. Example: "Several previous studies reported that human activities that occur in coastal areas involve various industrial sectors such as fertilizer processing, oil and gas, fiberboard, and crude palm oil." Suggestion: "Previous studies report that industrial activities like fertilizer processing, oil and gas, and crude palm oil production contribute to coastal pollution."

Some references are inconsistently formatted, e.g., [8,[9][10] instead of [8,9,10]. Some citations lack integration into the sentence structure. Instead of "According to [15]...", it should be "According to Smith et al. (2015)..."

"environmental stresse" should be "environmental stress"

"making them valuable indicators for assessing pollution levels in coastal waters [18,19]. Their ability to absorb and store these pollutants..." (Consider merging these two sentences for smoother flow.)

The introduction lacks a clear research gap. What specific knowledge gap does this study address that hasn't been explored before?

Materials and methods

"Leaves sampling" should be "Leaf Sampling" for consistency with "Sediment Sampling."

"Sediment grain size measurement" should be "Sediment Grain Size Analysis" to match scientific terminology.

"This area was selected due to the significant accumulation of heavy metals resulting from industrial activities along the Musi River." could be written as "This area was chosen due to the high accumulation of heavy metals from industrial activities along the Musi River."

"Additionally, sediment substrate types (gravel, sand, silt, and clay) were identified using Shepard's triangle analysis, which was processed with Microsoft Excel V.2021." could be written as "Sediment types (gravel, sand, silt, and clay) were classified using Shepard's triangle analysis and processed with Microsoft Excel V.2021."

Ensure all formulas are formatted correctly. For example, Igeo's formula should be written properly in LaTeX or inline notation.

Ensure units are consistently reported (e.g., ppm, µM, µg/ml).

Results and discussion

Figures and tables should be directly referenced within the text. Example., "Figure 2 shows the morphological differences between A. alba and E. agallocha" instead of "These differences are particularly evident in their leaf types (Figure 2) "

Table 2 should be described with more interpretation rather than just restating numbers.

In Table 3, use "Station" instead of "St." for clarity.

Strengthen the discussion by comparing findings with similar studies. This will help validate your results and provide broader ecological relevance.

The increase in Cu levels is mentioned but not fully explained. What anthropogenic activities specifically contribute to this? Providing more context will enhance the scientific argument.

The bioaccumulation factor (BCF), geoaccumulation index (Igeo), contamination factor (Cf), and pollution load index (PLI) are mentioned, but their ecological implications are unclear. Briefly explain what these values indicate in relation to contamination risk. Compare these index values with other mangrove ecosystems to provide context on pollution levels.

The study states that the area is "not polluted" based on the PLI but also suggests potential bioaccumulation risks. This needs clarification.

Recommend future monitoring frequency and how this data could inform environmental policies.

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Lawrence Lash <em@editorialmanager.com> Balas Ke: Lawrence Lash <l.h.lash@wayne.edu> Kepada: Rozirwan Rozirwan <rozirwan@unsri.ac.id> 23 Februari 2025 pukul 13.23

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Manuscript Number: TOXREP-D-24-00875

Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones

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Rozirwan ROZIRWAN <rozirwan@unsri.ac.id> Kepada: Lawrence Lash <l.h.lash@wayne.edu> 23 Februari 2025 pukul 22.10

Dear Editor,

Thank you for the reminder regarding the revision of manuscript TOXREP-D-24-00875. We appreciate your continued support.

I would like to inform you that we are currently in the process of revising the manuscript, and we are working diligently to address the reviewers' comments. We will ensure that the revised manuscript is submitted before the deadline of March 2, 2025.

29/03/25, 05.05 Email Sriwijaya University - Revision of "Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessme...

Thank you once again for your patience and for the opportunity to revise our work. We look forward to submitting the updated manuscript shortly.

Best regards, [Kutipan teks disembunyikan]

Prof. Dr. Rozirwan

Head of Marine Bioecology Laboratory Department of Marine Science Faculty of Mathematics and Natural Sciences Sriwijaya University Jalan Raya Palembang-Prabumulih KM 32, Indralaya Ogan Ilir, Sumatera Selatan, Indonesia, Pos Code: 30862 Email: rozirwan@unsri.ac.id, rozirwan@gmail.com



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	Reviewer #1	[
1.	Research highlights need improvement, and the graphical abstract should be of high quality.	We have revised and improved the research highlights as per your recommendation. Additionally, we have enhanced the graphical abstract to ensure high quality and clarity.
2.	How does this study differ from previous research on mangrove species, metals, and biomarkers? What is the necessity of conducting research in this specific location, and how extensively has this area been investigated in terms of metal contamination?	This study differs from previous research on mangrove species, metals, and biomarkers by examining two mangrove species with distinct morphological and habitat characteristics. It focuses on the bioaccumulation of Pb and Cu, metals that are commonly found due to anthropogenic activities. While previous studies primarily focused on metal concentrations, this research also considers the physiological responses of mangroves to these contaminants, filling a gap in the literature. The study is conducted in two different locations to assess whether areas with high anthropogenic influence exhibit higher contamination and physiological responses, providing a more comprehensive understanding of how environmental stressors impact mangrove ecosystems.
3.	The sampling map should indicate the sampling stations (Station 1 and Station 2).	We have updated the sampling map to show the sampling stations (Station 1 and Station 2) (Page 6).
4.	The methods section should detail the sampling procedures, including how the mangrove samples and sediment were collected. Please specify how many mangrove samples were taken. Additionally, the author should clarify whether roots or leaves were collected for sampling. The author previously mentioned roots but later referred to leaves.	We have now provided a more detailed description of the sampling procedures in the Methods section, including how the mangrove and sediment samples were collected. Additionally, we have specified the number of mangrove samples taken. We sincerely apologize for the previous inconsistency regarding the

		sampled plant parts. To clarify, our
		study focused solely on mangrove
		leaves, not roots (Page 7).
5.	How long were the sediments air-dried at	We have now specified the duration of
	room temperature?	air-drying the sediments at room
		temperature (72 hours) to clarify the
		methodology (Page 8).
6.	The author must clearly describe how the	We have added a clearer description of
	mangrove leaves or roots were dried in	the drying process for mangrove
	indirect sunlight. Furthermore, the author	leaves using indirect sunlight, ensuring
	needs to include a section on heavy metals	that the procedure is more detailed and
	analysis, as this part is currently missing	understandable. Additionally, we have
	from the methods.	included a section on heavy metals
		analysis in the methodology, which
		was previously missing (Page 8).
7.	The use of "inhibition" should be revised in	We have revised (Page 10, 29)
	Equation 5, and the column name in Table 8	(ruge 10, 2))
	on page 30, line 51, should be adjusted to	
	proper English.	
8.	How does the dataset in this study fulfill the	The detect in this study meets the
0.		The dataset in this study meets the
	criteria for parametric analysis?	criteria for parametric analysis, proven
		by the results of the assumption test,
		which show a normal distribution. This
		ensures that the statistical parameters
		used in the correlation analysis are
		accurate. In addition, the Pearson
		correlation test (r) and coefficient of
		determination (Kd) further validate the
		analyzed relationship.
9.	In the figure depicting mangrove leaves, the	We have now included a scale in the
	author should include a scale for better	figure to enhance visualization and
	visualization.	provide a clearer reference for the leaf
		sizes (Page 13).
10.	What is the role of industrial activities in the	We have carefully revised the
	accumulation of fine particles, specifically	manuscript according to the reviewers'
	clay particles?	comments. Specifically, in response to
		the concern regarding the role of
		industrial activities in fine particle
		accumulation, we have clarified that
		the dominant contributors in our study
		area are fertilizer processing, oil and
		gas industries, crude palm oil
		5 ⁴⁵ mausures, crude pann on

		production, port operations, and
		related activities (Page 13).
11.	How do unmanaged anthropogenic activities contribute to the increase of copper (Cu)?	We have revised the paragraph to clarify how unmanaged anthropogenic activities contribute to increasing Cu concentrations in the study area. Specifically, unregulated industrial waste disposal, improper wastewater treatment, and uncontrolled agricultural runoff serve as major sources of Cu pollution. Industrial activities discharge Cu-containing effluents directly into water bodies, while antifouling paints from ship maintenance release Cu into the environment. Additionally, agricultural runoff carrying Cu-based pesticides exacerbates contamination. Once introduced, Cu binds to suspended particles and accumulates in sediments, leading to long-term
12.	How do mangroves regulate copper in their system?	environmental pollution (Page 19). We have incorporated the requested explanation regarding how mangroves regulate Pb and Cu in their system. The revised discussion now elaborates on Pb exclusion mechanisms, including its limited uptake and accumulation in roots, as well as Cu regulation through controlled absorption, detoxification, and antioxidant responses (Page 19).
13.	In the discussion, the author states that metal concentration shows a negative correlation with antioxidant activity, yet the abstract claims that heavy metal concentration increases antioxidant activity. This discrepancy needs clarification.	The negative correlation mentioned in the discussion refers specifically to the DPPH assay values, where a lower DPPH value indicates stronger antioxidant activity. This means that although the numerical correlation appears negative, the actual antioxidant strength remains high. In other words, as heavy metal concentrations increase, antioxidant activity also intensifies, aligning with the statement in the abstract.

	Reviewer #2	2
1.	"Mangroves can mitigate the impact of free radicals by producing antioxidant compounds" can be written as "Mangroves produce antioxidant compounds to mitigate impact of free radicals."	We have revised (Page 1).
2.	"The correlation analysis between heavy metal concentrations and antioxidant activity indicates that as heavy metal concentrations increase, antioxidant activity and total phenol content also increase." can be written as "Correlation analysis shows that higher heavy metal concentrations correspond to increased antioxidant activity and total phenol content."	We have revised (Page 1).
3.	Instead of Pb values of $0.67 \pm 0.16 - 18.70 \pm 0.48 \text{ mg} / \text{kg}$ and Cu values of $3.39 \pm 0.20 - 6.07 \pm 0.37 \text{ mg} / \text{kg}$, write Pb values of 0.67 ± 0.16 to $18.70 \pm 0.48 \text{ mg/kg}$ and Cu values of $3.39 \pm 0.20 - 6.07 \pm 0.37 \text{ mg/kg}$ for clarity.	We have revised (Page 1).
4.	In the sentence:"The results of sediment pollution assessment for heavy metals Pb and Cu at Igeo $< 0, 1 < Cf < 3$, and PLI 0- 2." Define Igeo, Cf, and PLI for readers unfamiliar with these indices. "BCF < 1 " should be clarified: Does this mean bioaccumulation is low?	We have revised the sentence to define Igeo, Cf, and PLI for better clarity, as well as to explain that BCF < 1 indicates low bioaccumulation, meaning that the mangrove species studied function more as excluders rather than accumulators of Pb and Cu (Page 1).
5.	The Pearson correlation analysis result should be explicitly stated (e.g., $r = X$, $p < 0.05$).	We acknowledge the importance of providing statistical details for clarity. However, given that all correlation values (r) in our analysis are nonzero (r \neq 0), we initially summarized this in the abstract to avoid excessive numerical data. To ensure transparency while maintaining conciseness, we have now revised the abstract to include a representative correlation value (e.g., r \neq 0, p < 0.05) while keeping the full

		statistical details within the results
		section (Page 1).
6.	Some sentences are long and could be more concise for better readability. Example: "Several previous studies reported that human activities that occur in coastal areas involve various industrial sectors such as fertilizer processing, oil and gas, fiberboard, and crude palm oil." Suggestion: "Previous studies report that industrial activities like fertilizer processing, oil and gas, and crude palm oil production contribute to coastal pollution."	We have revised (Page 1)
7.	Some references are inconsistently formatted, e.g., [8,[9][10] instead of [8,9,10].	We have revised (Page 3).
8.	Some citations lack integration into the sentence structure. Instead of "According to [15]", it should be "According to Smith et al. (2015)"	We have revised
9.	"making them valuable indicators for assessing pollution levels in coastal waters [18,19]. Their ability to absorb and store these pollutants" (Consider merging these two sentences for smoother flow.)	We have revised (Page 3).
10.	The introduction lacks a clear research gap. What specific knowledge gap does this study address that hasn't been explored before?	We have added aspects to the gaps that existed in previous studies which only studied the heavy metal content in mangroves without considering their physiological responses. Apart from that, this research also uses two types of mangroves and two different regional conditions, which is innovation and novelty in this research (Page 4).
11.	"Leaves sampling" should be "Leaf Sampling" for consistency with "Sediment Sampling."	We have revised (Page 5).
12.	"Sediment grain size measurement" should be "Sediment Grain Size Analysis" to match scientific terminology.	We have revised (Page 7).

13.	"This area was selected due to the	We have revised (Page 5).
	significant accumulation of heavy metals	
	resulting from industrial activities along the	
	Musi River." could be written as "This area	
	was chosen due to the high accumulation of	
	heavy metals from industrial activities	
	along the Musi River."	
14.	"Additionally, sediment substrate types	We have revised (Page 7).
17.	(gravel, sand, silt, and clay) were identified	we have revised (rage 7).
	using Shepard's triangle analysis, which	
	was processed with Microsoft Excel	
	V.2021." could be written as "Sediment	
	types (gravel, sand, silt, and clay) were	
	classified using Shepard's triangle analysis	
	and processed with Microsoft Excel	
	V.2021."	
15.	Ensure all formulas are formatted correctly.	We have revised the text to ensure that
10.	For example, Igeo's formula should be	all formulas are now written correctly
	written properly in LaTeX or inline	using inline notation (Page 8,9,10).
	notation.	
16.	Ensure units are consistently reported (e.g.,	We have revised
100	ppm, μ M, μ g/ml).	
17.	Figures and tables should be directly	We have revised (Page 12).
	referenced within the text. Example.,	(2 mg 2 12)
	"Figure 2 shows the morphological	
	differences between A. alba and E.	
	agallocha" instead of "These differences are	
	particularly evident in their leaf types	
	(Figure 2) "	
18.	Table 2 should be described with more	We have revised Table 2 as per your
	interpretation rather than just restating	suggestion by adding more
	numbers.	interpretation and contextualizing the
		data. Rather than merely restating the
		numbers, we have now provided an
		analysis that discusses the implications
		of the sediment fractions and grain size
		in both the industrial area and
		conservation zone (Pag 16).
19.	In Table 3, use "Station" instead of "St." for	We have revised (Page 18).
	clarity.	
20.	Strengthen the discussion by comparing	We have incorporated similar studies
	findings with similar studies. This will help	in the discussion to strengthen the

	validate your results and provide broader	findings (In page results and
	ecological relevance.	discussion).
21.	The increase in Cu levels is mentioned but not fully explained. What anthropogenic activities specifically contribute to this? Providing more context will enhance the scientific argument.	We have revised the sentence to provide clearer context on the anthropogenic activities contributing to Cu level increases. Specifically, we have included references to ship hull cleaning and the use of Cu-coated nets in fisheries, which are known to release Cu into the marine
22.	The bioaccumulation factor (BCF), geoaccumulation index (Igeo), contamination factor (Cf), and pollution load index (PLI) are mentioned, but their ecological implications are unclear. Briefly explain what these values indicate in relation to contamination risk. Compare these index values with other mangrove ecosystems to provide context on pollution levels.	environment (Page 19). We have clarified the ecological implications of the bioaccumulation factor (BCF), geoaccumulation index (Igeo), contamination factor (Cf), and pollution load index (PLI) in our manuscript. The higher BCF values suggesting greater ecological risk, particularly for toxic metals like Cu. The geoaccumulation index (Igeo) reflects sediment contamination levels, with high values indicating significant anthropogenic input and potential long-term ecological risks, particularly for Pb. The contamination factor (Cf) highlights the relative pollution risk, with higher values for Pb in industrial areas suggesting greater susceptibility to pollution compared to Cu. The pollution load index (PLI) provides an overall assessment of pollution, with higher values in industrial areas indicating elevated contamination. Comparisons with other mangrove ecosystems have also been presented
23.	The study states that the area is "not	(Page 22-23). We have revised the paragraph to
	polluted" based on the PLI but also suggests potential bioaccumulation risks. This needs clarification.	clarify the distinction between the PLI classification and the potential bioaccumulation risks. While the PLI indicates that the area is "not polluted" based on sediment contamination

		levels, it does not fully account for the
		bioavailability and potential uptake of
		heavy metals by aquatic organisms.
		Even at relatively low sediment
		concentrations, metals like Cu and Pb
		can accumulate in biota over time,
		posing ecological risks (Page 23).
24.	Recommend future monitoring frequency	We have added the recommended
	and how this data could inform	section regarding future monitoring
	environmental policies.	and its implications for environmental
		policies in the final paragraph of the
		discussion (Page 36).

Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones

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Abstract

Heavy metal contamination from industrial activities in coastal regions can lead to pollution in mangrove ecosystems. <u>Mangroves produce antioxidant compounds to mitigate the impact of free radicals.</u> <u>Mangroves can mitigate the impact of free radicals by producing antioxidant compounds</u> This study aimed to analyze the correlation between the concentration of heavy metals Pb and Cu and antioxidant activity in *Avicennia alba* and *Excoecaria agallocha* mangroves from areas affected by industrial activities and conservation areas, Banyuasin, South Sumatra, Indonesia. This study was conducted in September 2023 with sampling locations in the Payung Island area and the Barong River conservation area, Berbak Sembilang National Park. The samples taken included sediment and mangrove leaves. The concentration of heavy metals Pb and Cu was measured by atomic absorption spectrometry. Antioxidant activity test using the DPPH test, total phenol using the Folin-Ciocalteu method, and phytochemical profile screening using GCMS. Statistical analysis of the correlation between

antioxidant activity and heavy metal concentration using the Pearson correlation. The results showed that the highest concentration of heavy metals in sediment and mangrove leaves was found in the area affected by industrial activity, with a range of Pb values of 0.67 ± 0.16 to 18.70 ± 0.48 mg/kg and Cu values of 3.39 ± 0.20 to 6.07 ± 0.37 mg / kg. The results of sediment pollution assessment for heavy metals Pb and Cu at Igeo < 0 indicates uncontaminated, 1 < Cf < 3 indicates low contamination, and PLI 0-2 indicates not polluted. While the results of heavy metal bioaccumulation in leaves were BCF < 1, indicates low bioaccumulation. E. agallocha leaves from the Pulau Payung area showed very strong antioxidant activity of 21.63 µg/ml, and the highest total phenol content reached 398.80 mg GAE/g. Analysis of compounds with the highest antioxidant activity identified the presence of esters, aldehydes, alcohols, fatty acids, glycosides, flavonoids, terpenoids, and steroids. Correlation analysis shows that higher heavy metal concentrations correspond to increased antioxidant activity and total phenol content (r \neq 0)The correlation analysis between heavy metal concentrations and antioxidant activity indicates that as heavy metal concentrations increase, antioxidant activity and total phenol content also increase. These findings are expected to contribute to scientific knowledge that enhances environmental sustainability, supporting effective management of coastal natural resources.

Keywords: Biomarkers, conservation zones, heavy metals, industrial activities, mangrove

Introduction

Coastal areas are transitional areas between land and sea that have abundant biodiversity and unique ecosystems [1,2]. Coastal areas face great pressure from various anthropogenic activities that can cause pollution [3,4]. <u>Previous studies report that industrial activities like</u> <u>fertilizer processing, oil and gas, and crude palm oil production contribute to coastal pollution.</u> Several previous studies reported that human activities that occur in coastal areas involve various industrial sectors such as fertilizer processing, oil and gas, fiberboard, and erude palm oil [3,5,6]. In addition, there are also agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households [7]. Continuous anthropogenic activities in coastal areas can produce pollutants, such as microplastics, heavy metals, as well as various organic and inorganic contaminants [8, 9, 10]. Among various pollutant types, heavy metals are categorized as persistent pollutants due to their resistance to decomposition [11]. Heavy metals initially present in the water column gradually settle to the sediment and eventually accumulate in aquatic organisms [12]. This condition may have adverse impacts, particularly if it exceeds environmental quality standards. These adverse impacts can affect aquatic ecosystems, including mangroves [13,14]. According to Xu et al. (2024), as the largest plant community in coastal areas, mangroves are also directly affected by pollution.

Mangrove ecosystems play a vital role in coastal protection, supporting biological diversity, and contributing to the socio-economic development of local communities [16,17]. Additionally, their capacity to accumulate pollutants makes them valuable indicators for assessing pollution levels in coastal waters, as they can absorb and store these pollutants in their tissues, enhancing their role in monitoring environmental health [18,19, 20]. Additionally, mangroves possess the capacity to accumulate pollutants, making them valuable indicators for assessing pollution levels in coastal waters [18,19]. Their ability to absorb and store these pollutants in their tissues enhances their role in monitoring environmental health [20]. Roots and leaves are important parts of mangroves in the absorption, accumulation, and response to pollutants [21]. Roots are the first part exposed to pollutants from their growth media. Furthermore, roots also have the ability to translocate pollutants to the leaves. Leaves are the primary site for photosynthesis in plants, supplying the energy essential for cell development,

and overall plant function [22]. High concentrations of pollutants in roots and leaves can potentially increase excessive reactive oxygen species (ROS), resulting in oxidative stress in mangroves [23,24]. Oxidative stress arises from an imbalance between ROS production and detoxification, potentially leading to harmful cellular damage [25,26]. Although oxidative stress can be detrimental, plants also have a resistance response mechanism against free radicals [27]. This process involves producing antioxidant enzymes and molecules to counteract the harmful effects of free radicals. In response to environmental changes, plants enhance the activity of antioxidant defenses, including both enzymatic and non-enzymatic components such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPx), and phenolic compounds. These antioxidants serve as protective mechanisms against various environmental <u>stress</u> [28,29].

Research on the specific physiological adaptations of various mangrove species to pollutants is still limited. Most previous studies only focused on the accumulation of heavy metals in mangroves without exploring in depth the biochemical defense mechanisms they employ [30, 31, 32]. However, studies on how different mangrove species respond to industrial pollution in environments with varying levels of pollution have not yet been conducted. In addition, most studies only examine one mangrove species without comparing the adaptability of different species in the face of heavy metal contamination [33, 34].

This study aimed to evaluate the accumulation of heavy metals (Pb and Cu) in two mangrove species (*Avicennia alba* and *Excoecaria agallocha*) and assess their antioxidant activity in industrial and conservation zones. The selection of these two species was based on their prevalence in the research location as well as differences in habitat zones and morphological characteristics [35, 36]. This study was carried out in the mangrove ecosystem, which includes areas influenced by industrial activities such as Payung Island as well as conservation areas in the Berbak Sembilang National Park [37, 38]. Field Code Changed

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By assessing biomarkers, new insights are provided into how mangrove species adapt to environmental stress caused by heavy metal pollution. Additionally, the research explores the impact of heavy metal contamination on the physiological responses of mangroves, focusing on their biochemical defense mechanisms. The findings aim to enhance understanding of mangrove adaptation strategies in response to pollution and offer valuable implications for coastal ecosystem conservation and environmental pollution management.

Materials and Method

Leaves Leaf sampling

This study was conducted in September 2023. The samples included *Avicennia alba*, *Excoecaria agallocha*, and sediments collected from industrial and conservation zones in Banyuasin, South Sumatra, Indonesia (Figure 1). The first area is the mangrove ecosystem on Payung Island. <u>This area was chosen due to the high accumulation of heavy metals from</u> <u>industrial activities along the Musi River</u>. <u>This area was selected due to the significant</u> <u>accumulation of heavy metals resulting from industrial activities along the Musi River</u>. Additionally, the area includes agricultural activities, ports, fish ponds, and settlements [39, 40]. The second area is the conservation forest in Sungai Barong, Sembilang National Park, which represents a natural area and protects flora and fauna from the threat of damage, scarcity, or deforestation [41, 42, 43]. Formatted: Indent: First line: 0 cm

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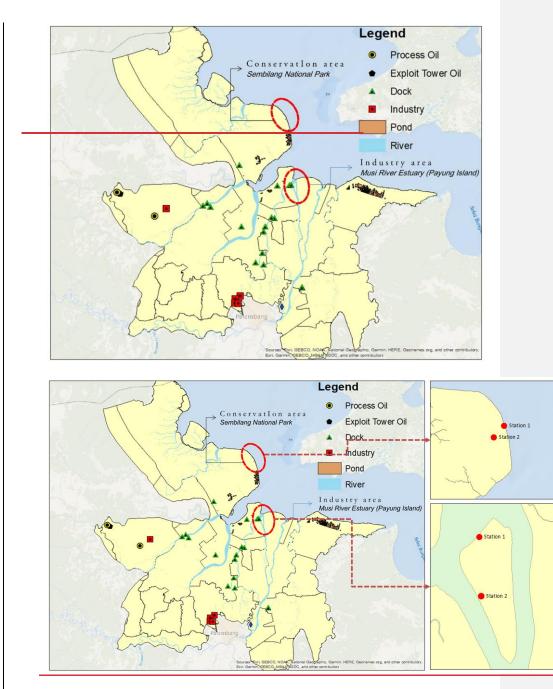


Fig.1. Map of sample collection

The sampling stages include collecting sediment samples and mangrove leaves. Sediment samples were taken as supporting data to determine the concentration of heavy metals in the mangrove growth media. The availability of heavy metals in sediments has a direct effect on the bioaccumulation and biomagnification processes in aquatic organisms. Sediment data helps understand the level of risk and potential impacts to organisms in mangrove ecosystems. Sediment samples were taken using a grab pipe at a depth of \pm 10 cm from the surface [44]. Sediment depth shows a very significant impact on heavy metal content, with a greater decrease in heavy metal content as sediment depth increases [45], amples were taken at three location points for each station, which were considered as replications. Samples were taken compositely together (taken as needed, 500 g) and placed into a polyethylene plastic container and stored in a cool box for analysis in the laboratory.

The method for collecting mangrove leaves taken from the field uses a random sampling method [46]. The random sampling method can be used if the sample studied is homogeneous. The mangrove species taken were \underline{A} . *alba* and \underline{E} . *agallocha*. The samples taken consisted of \pm 1 kg of leaves and were put in polyethylene plastic.

Sediment grain size measurement analysis

Grain size analysis was conducted using the sieving and pipetting methods as outlined by [47]. <u>Sediment types (gravel, sand, silt, and clay) were classified using Shepard's triangle</u> <u>analysis and processed with Microsoft Excel V.2021</u> <u>Additionally, sediment substrate types</u> (gravel, sand, silt, and clay) were identified using Shepard's triangle analysis, which was processed with Microsoft Excel V.2021, following the protocols established by [48, 49]. The sediment fraction type was determined by identifying the most dominant composition from the analysis results. Formatted: Font: Italic
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Sample preparation

Sediment sample preparation involved removing foreign objects such as plastic fragments and leaves. The sediment was then air dried at room temperature for 72 hours until fully dry, ground to a homogeneous consistency, and stored in a tightly sealed polyethylene bottle. They were then air-dried in a shaded, well-ventilated area for five days, ensuring indirect exposure to sunlight to prevent the degradation of bioactive compounds. The drying process was conducted at ambient temperature with sufficient airflow to facilitate moisture evaporation. Once dried, the samples were ground into a fine powder and stored in sealed containers for further analysis. The mangrove leaves were first cleaned and then dried in indirect sunlight before grinding. The extraction of heavy metals (Pb and Cu) from the sediment samples and mangrove leaves was performed using the wet destruction method, following the procedures outlined by [8, 50].

Atomic absorption spectroscopic measurement

Measuring the concentration of heavy metals Pb and Cu using an atomic absorption spectrophotometer with a wavelength of 283.3 nm for Pb and 324.7 nm for Cu.

Determination of heavy metals in leaves and sediments

Determination of sediment pollution

Geoacumulation index (Igeo)

The Igeo (geo-accumulation index) quantitatively evaluates the degree of heavy metal

contamination and classifies the level of pollution based on detailed categorization [51].

$Igeo = log_2 (Cn/1.5 Bn)$

 $F_{geo} = rac{Concentration heavy metals in sediment}{1.5.Backaround}$

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The classification of Igeo values includes the following categories: uncontaminated (Igeo ≤ 0), uncontaminated to moderately contaminated (Igeo 0–1), moderately contaminated (Igeo 1–2), moderately to highly contaminated (Igeo 2–3), highly contaminated (Igeo 3–4), highly contaminated to very highly contaminated (Igeo 4–5), and very highly contaminated (Igeo ≥ 5) [52].

Contamination factor (Cf)

The contamination factor is determined experimentally as the ratio of the element concentration in the sample to its background concentration [53].

$\underline{Cf} = (\underline{Cn}/\underline{Bn})$	(2)
Contamination factor (Cf) = $\frac{Concentration of heavy metals in sediment}{Contamination}$	(2)
Background	(2)

The contamination factor (Cf) classifications are as follows: [54]: Cf < 1 = low contamination; 1 < Cf < 3 = moderate contamination; 3 < Cf < 6 = sufficient contamination; Cf > 6 = very high contamination.

Pollution load index (PLI)

The pollution load index (PLI) is utilized to assess pollution quality in a given area. The pollution load index value uses the formula [55].

$PLI = \left[Cf1 \ x \ Cf2 \ x \ Cf3 \dots \ x \ Cfn \right]^{1/n}$	(3)
Pollution load index (PLI) = [$Cf1 \times Cf2 \times Cf3 \dots \times Cfn$] ^{1/n}	(3)

Pollution load index (PLI) criteria: PLI 8-10 = severely polluted; PLI 4-8 = heavily polluted; PLI 2-4 = moderately polluted; PLI 0-2 = not polluted to lightly polluted; PLI < 0 = not polluted.

Bioaccumulation of metal in leaves

Bioconcentration factor (BCF)

The absorption of metals by leaf from sediment occurs through a process known as bioaccumulation. The Bioconcentration Factor (BCF) values are utilized to assess the extent of metal bioaccumulation in mangrove <u>leaf</u> originating from sediment [56].

BCF = (Cn.leaf/Cn. sediment)	(4)
BCF= Concentration of leaf	(4)

BCF > 1 hyperaccumulator; BCF = 1 indicator; BCF < 1 is an excluder [57].

Analysis of antioxidant non-enzymes in leaves

Antioxidant activity evaluated by DPPH assay

Antioxidant activity analysis was carried out using ethanol solvent based on a method adapted from [58]. A 50 ml 0.1 µM DPPH solution was prepared, followed by the preparation of a sample stock solution and a 10 ml pure ascorbic acid stock solution of 2000 ppm, which was homogenized. Furthermore, a series of solutions were made with concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. At each concentration, 1 ml of 0.1 µM DPPH solution was added to the mixture, which was then homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the extract is expressed as IC₅₀, which quantifies the strength of its antioxidant capacity (Table 1). The IC_{50} value is calculated using the following formula:

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% inhibition inhibisi = $\frac{blank \ abs-sample \ abs}{blank \ abs} \times 100\%$

(5)

The IC₅₀ value was derived by inputting the data into a linear regression equation, where the sample concentration was plotted on the X-axis and the percentage of inhibition of antioxidant activity on the Y-axis. The regression equation used is represented as y=ax + b [59].

Table 1

Characteristic value of IC50

Concentration (µg/ml)	Characteristic
<50	Very strong
50-100	Strong
100-150	Moderate
150-200	Low

Determination of phenol content

The analysis of total phenol content in the samples was conducted using the Folin-Ciocalteu method, as outlined in the literature [60, 61, 62]. A standard solution of 1000 ppm gallic acid as much as 50 ml was prepared, then variations in concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were made, each as much as 5 ml. For each concentration variation, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were pipetted into a 10 ml measuring flask containing a standard solution of 100 ppm gallic acid. A total of 50 mg of sample was weighed, then 2 ml of methanol and 5 ml of distilled water were added, then homogenized in a 10 ml measuring flask. In both the standard series and sample variations, 0.5 ml of 50% Folin-Ciocalteu reagent was added, followed by the addition of distilled water up to the mark. The mixture was then allowed to stand for 5 minutes. Next, one ml of a 5% Na2CO3 solution was added and incubated in a dark place for one hour. After incubation, the absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 750 nm.

Pearson correlation analysis (correlation bivariate)

The use of Pearson correlation analysis (bivariate correlation) is a method used to evaluate the relationship between two variables [63, 64], in this case to see the relationship between antioxidant activity and heavy metal concentrations. This analysis was carried out using SPSS software version 28.

Result and Discussion

Description of mangrove leaves

The mangrove species *A. alba* and *E. agallocha* found at the sampling location exhibit distinct characteristics. Figure 2 shows the morphological differences between <u>A. alba</u> and <u>E. agallocha</u>. These differences are particularly evident in their leaf types (Figure 2).

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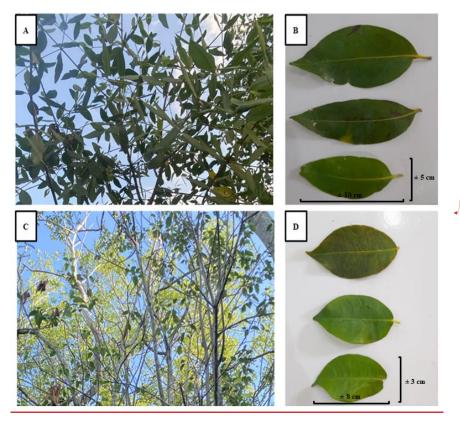


Fig.2. Leaves description. A-B). A. alba, C-D). E. agallocha

Leaves are the part that characterizes a mangrove species. When identifying each type of mangrove, observation of the morphology of the leaf shape is very important to understand the characteristics and differences in each type of leaf [31, 65]. *A. alba* leaves have a green surface with a smooth and slippery texture, while the underside is yellowish green with a rough texture. The morphology of the leaves is elliptical, almost oval, with a tapered tip. Based on observations, the length of the leaves ranges from 10 to 13 cm, and the width of the leaves ranges from 4 to 5 cm. *E. agallocha* leaves are elliptical and dark green in color, with finely serrated edges and tapered tips. The observed leaf sizes ranged from 8 to 10 cm in length and

3 to 4.5 cm in width. Old leaves were selected as samples for the study of heavy metal content and bioactive compounds due to several considerations related to their maturity and potential accumulation of pollutants and compounds of interest. According to [66], plants tend to produce bioactive compounds in higher amounts in older parts. This could be a plant strategy to protect itself from pests, diseases, or the external environment [67, 68]. Older leaves may have more stable chemical conditions, thus facilitating analysis and minimizing variability in results.

Sediment grain size

The determination of substrate types in the sampling was conducted using the Shepard triangle method (Figure 3). In the mangrove ecosystem of both industrial and conservation areas, sediment substrates were categorized into four types: gravel, sand, mud, and clay. The results indicated that the predominant substrate type in both areas was clay.

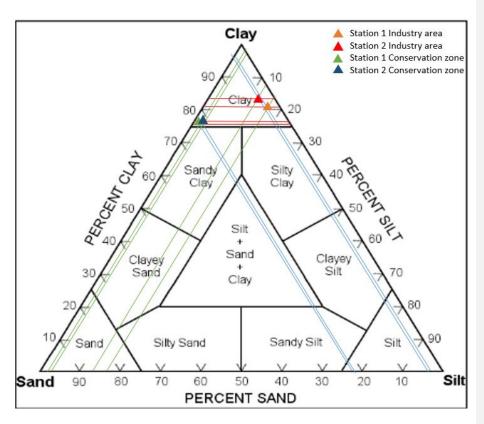


Fig.3. Classifications of sediment type with shepard triangle method

The sediment substrate surrounding the mangrove ecosystem in both areas is predominantly clay, with clay percentages ranging from 80,5% to 84,03%. The highest clay content was observed at station 1 in the industrial area (Table 2).

Tabel 2

Sediment grain size in each station

		Sediment fraction (%)					
Location	Station		N 1	CI	Grain size		
		Gravel Sand	Mud	Clay			

Industry area	St. 1 (<i>A. alba</i>)	0,00	3,6	12,37	84,03	Clay
	St.2 (E. agallocha)	0,00	3,36	16,14	80,5	Clay
Conservation zone	<mark>St.</mark> 1 (<i>A. alba</i>)	0,00	22,5	1,95	75,55	Clay
	St.2 (E. agallocha)	0,00	21,91	2,02	76,07	Clay

Based on the results of Table 2, <u>distribution of sediment fractions and grain sizes at two</u> different locations, which represents two stations with different mangrove species (*A*, *alba*, and *E*, *agallocha*). In the industrial area, most of the sediments consist of clay with a very low sand content (3.6% for Station 1 and 3.36% for Station 2), which indicates the predominance of fine materials that can influence the mobility of heavy metals and nutrients in the sediments. In contrast, in the conservation zone, although the sediment composition is still dominated by clay, the sand content is higher (22.5% for Station 1 and 21.91% for Station 2), indicating differences in sedimentation processes and a higher potential for water infiltration.

In the industrial area, both stations (*A. alba* and *E. agallocha*) showed a dominance of clay fractions with a very high percentage. The dominant clay fractions indicate that the sediment in this area consists of fine particles, which may be caused by the accumulation of fine particles from industrial activities around this location. <u>Industries such as fertilizer processing, oil and gas, crude palm oil production, agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households contribute to the presence of fine particles in sediments [3, 5, 6, 7]. Port activities involve frequent vessel movement, dredging, and cargo handling, all of which can resuspend fine particles and increase sedimentation rates [69, 70]. Crude oil processing and petroleum industries may contribute to fine particle deposition through air emissions, which settle via atmospheric deposition [71]. Additionally, agricultural activities, particularly palm oil plantations, can contribute to</u>

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increased fine particle accumulation through soil erosion and runoff carrying clay-rich sediments into adjacent water systems, particularly during heavy rainfall [72, 73].

Fine particles such as clay are usually carried by water and can accumulate in areas with slow water movement, such as near mangrove roots [74, 75]. In the conservation zone, the clay fraction also dominates, although with a slightly lower percentage than the industrial area. The conservation zone may also have less influence from human activities, so the sediment pattern is more natural than the industrial area. Clay is a sediment particle with a very fine grain size and a large surface area [76, 77]. Due to its small size and its tendency to be negatively charged, clay has a high adsorption capacity, which allows clay particles to attract and bind heavy metal ions such as Hg, Pb, Cd, Cu, and others [78, 79, 80]. Consequently, sediments dominated by clay fractions tend to accumulate more heavy metals than larger sediment fractions [81, 82].

Determination of heavy metals

The results of the heavy metal concentration analysis for Pb and Cu in sediments and mangrove leaves from both areas are summarized in Table 3. The concentrations of heavy metals, specifically Pb and Cu, in sediments from both the industrial area and the conservation zone exhibit variability; however, they generally remain below hazardous thresholds (ERL, ERM, TEL, and PEL). In the industrial area, the highest Pb concentration was found at Station 2 (18.70 \pm 0.48 mg/kg), while in the conservation zone, the highest concentrations of Pb and Cu were each at Station 2 (Pb 14.22 \pm 0.16 mg/kg; Cu 5.17 \pm 0.17 mg/kg). For metal accumulation in mangrove leaves, Cu was recorded higher than Pb at all stations. In the industrial area, *A. alba* (Station 1) had Pb 0.67 \pm 0.17 mg/kg and Cu 3.39 \pm 0.20 mg/kg, while *E. agallocha* (Station 2) showed Pb 1.27 \pm 0.31 mg/kg and Cu 3.73 \pm 0.16 mg/kg. In the conservation zone, the highest accumulation of Cu in mangrove leaves was 3.69 \pm 0.23 mg/kg at Station 2.

Tabel 3

Average concentrations of heavy metals (mg/kg) in mangrove sediments and leaves

	D1		
	Pb	Cu	Formatted Table
Sediments			
St <u>ation</u> .1 Industry area	12.63±0.01	5.58±0.05	Formatted Table
Station.2 Industry area	18.70 ± 0.48	6.07±0.37	
-			
Station.1 Conservation zone	12.61±0.32	4.21±0.03	
Station.2 Conservation zone	14.22±0.16	5.17±0.17	
ERL	46.7	34	
ERM	218	270	
	20.2	10.5	
TEL	30.2	18.7	
PEL	112	108.2	
1			
Mangrove leaves			
Station.1 Industry area (A. alba)	0.67±0.17	3.39±0.20	Formatted Table
Station 2 Induction and (E. no alloch.)	1 27 0 21	2 72 0 16	
St <u>ation</u> .2 Industry area (<i>E. agallocha</i>)	1.27±0.31	3.73±0.16	
Station.1 Conservation zone (A. alba)	$0.84{\pm}0.12$	3.50±0.35	
Station 2 Concernation game $(E_{1}, agg^{H}_{1}, b_{2})$	0.00 ± 0.27	2 60 10 22	
Station.2 Conservation zone (E. agallocha)	0.99±0.37	3.69±0.23	

The industrially impacted area in the Musi River Estuary is affected by highanthropogenic activities, making it susceptible to accumulating pollutants, especially heavy metals such as Pb and Cu. Sediments in this area tend to contain higher pollutants than water and biota, influenced by domestic, industrial, and river transportation activities that pollute the environment [83, 84]. Ship and coastal building maintenance activities, including the use of anti-rust materials, electronic waste, and pipe corrosion, are the main sources of Pb, while

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sources of Cu in the aquatic environment come from antifouling paint, agricultural pesticides, and industrial waste [85, 86]. <u>In addition, previous studies report that cleaning ship hulls can</u> release Cu into the marine environment [87, 88]. Fisheries sector that uses Cu-coated nets to prevent biofouling can also contribute to increasing Cu levels in waters [89, 90].

The conservation area in the Barong River is also exposed to heavy metal pollution, although at a lower level, considering that some human activities such as fishing are still ongoing [91]. Unmanaged anthropogenic activities, including unregulated industrial waste disposal, improper wastewater treatment, and uncontrolled agricultural runoff, have contributed to the increasing Cu concentrations observed in both locations, as indicated by the findings of this study and previous research [7, 50]. These activities introduce Cu into the aquatic system, where it binds to suspended particles and accumulates in sediments, further exacerbating environmental pollution.

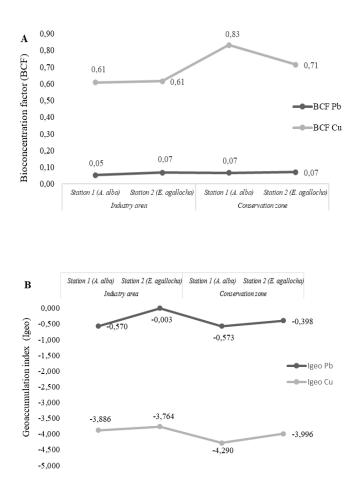
In mangrove leaves, Pb was detected at low concentrations. Plants regulate Pb primarily by limiting its uptake and translocation. Because Pb is a non-essential and highly toxic metal, most of it accumulates in the roots rather than being transported to the leaves [92]. In contrast, Cu an important micronutrient for plants, is regulated through controlled absorption and detoxification mechanisms [93, 94]. Plants manage excess Cu by binding it to metallothionein and phytochelatin, storing it in vacuoles, and activating the antioxidant defense system to fight oxidative stress [95]. Although heavy metal concentrations vary between locations, they are still below the threshold, indicating a relatively low risk of contamination. However, long-term monitoring is essential to track bioaccumulation trends in mangrove ecosystems.

Sediment quality indices

The results of the sediment quality index assessment are summarized in Figure 4. The results of the leaf bioconcentration factor (BCF) in bioaccumulating Pb and Cu metals from

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sediment with a BCF value <1 indicating low bioaccumulation. The geoaccumulation index shows uncontaminated properties for Pb and Cu with an Igeo value <0 indicating uncontaminated. The contamination factor (Cf) shows that contamination is low and moderate in Pb and Cu with a value of 1 < Cf <3 indicating low contamination. The PLI ranges from 0 to 2 indicating that both areas are not polluted.



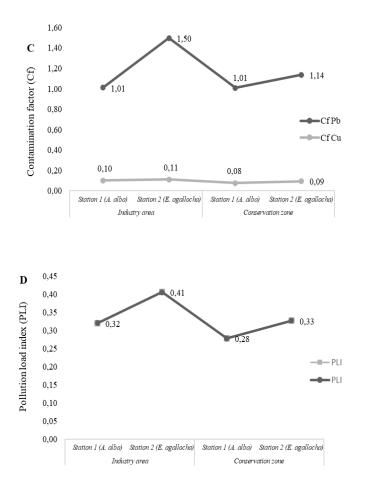


Fig.4. Sediment quality indices. A). Bioconcentration factor (BCF), B). Geoaccumulation index (Igeo), C). Contamination factor (Cf), and D). Pollution load index (PLI).

The difference in bioconcentration factor (BCF) between Cu and Pb can be explained by the chemical properties of each metal. Cu accumulates more easily in biota tissues than Pb. Cu is an essential element for organisms, although at higher concentrations it can be toxic [96]. In contrast, Pb is a non-essential heavy metal that tends not to accumulate much in biota tissues [97]. The previous studies stated that essential heavy metals are more easily absorbed by organisms because they have physiological mechanisms to regulate the concentration of these elements [98, 99]. Analuddin et al. (2023) have also examined BCF in mangrove ecosystems, with results showing that the BCF values for Hg, Cu, Mn, Pb, and Zn > 1. This finding is thought to be related to the impact of anthropogenic activities in Kendari City, which has a high population density.

The Igeo index shows higher Pb contamination than Cu in industrial areas. Pb is thought to originate from human activities such as ports, agriculture, ship transportation, and household waste that tends to settle in sediments [8, 32]. <u>A high Igeo value indicate an anthropogenic contamination and show sediments contaminated heavy metal [101]. In addition, long-term exposure to these heavy metals can change community structure and disrupt ecosystem function through bioaccumulation and biomagnification in the food chain [102, 103].</u>

The high value of the Pb contamination factor (Cf) in industrial areas indicates that this environment is more susceptible to Pb pollution than Cu. <u>Relevan study by</u> Hasan et al. (2023) that the CF value of Pb (0.76) > Cu (0.68) in core sediment from a mangrove at the Pasur River. Cu is more likely to be bound to organic particles and accumulate in the tissues of benthic organisms, which may explain the lower Cf Cu value. The areas suspected of being polluted tend to have higher anthropogenic activity than conservation zones, which causes significant differences in the levels of contamination and accumulation of heavy metals [105]. Industries around mangrove areas may contribute to elevated levels of heavy metals. Meanwhile, the conservation zone which is relatively protected from industrial activities, shows lower contamination values, although there are still traces of pollution due to remote pollution sources [106, 107].

The PLI value in the industrial area is higher than the conservation zone. This indicates that industrial activities play a role in elevating heavy metal pollution in the area. Industrial

areas are usually exposed to pollution sources such as factory waste, air pollution, and surface runoff that carry heavy metals into the sediment [108, 109]. Although both stations are in the same area, there is a difference in the PLI value between Station 1 and Station 2 at both locations. Local factors, including water movement, sediment composition, and proximity to pollution sources, significantly influence the distribution of heavy metals. [110]. The PLI in the conservation zone still shows heavy metal pollution. This could be due to atmospheric deposition from industrial activities in the surrounding area or pollutants carried by water currents from more contaminated areas [111, 112]. This suggests that although the conservation zone has better protection, it is not completely protected from the impacts of nearby industrial pollution.

The study indicate that both areas are classified as not polluted. In line with these findings by Karmakar et al. (2025), the PLI value in mangrove planting areas due to heavy metals from ship demolition activities is still below 1. Even though the PLI reflects low levels of pollution over time, it can increase the potential for absorption by aquatic organisms and pose ecological risks, therefore, continuous monitoring is required to identify dynamic changes in heavy metal concentrations

Antioxidant non-enzyme activities

The results of percentage of depreciation data for the *A. alba* species taken from the industrial area were 66%, and the conservation zone was 65.8%. While for the *E. agallocha* species from the industrial area it was 68.5% and the conservation zone was 67.9% in the conservation zone. Conversely, the findings of the percentage of dry weight of *A. alba* in the industrial area were 34%, and the conservation zone was 34.3%. In the *E. agallocha*, the percentage of dry weight in the industrial area was recorded at 31.5% and the zone was 32.1% (Table 4).

Table 4

zone

	G 1	C 1	. 1.()	D : /:	XX7 · 14	
Location	Sample	Sample weight (g)		Depreciation	Weight	
Location	leaves	Wet	Dry	percentage (%)	percentage (%)	
Industry area	A. alba	800	272	66	3	
	E. agallocha	800	252	68.5	31.	
Conservation	A. alba	800	274	65.8	34.	

800

34

31.5

34.3

32.1

Depreciation percentage of weight

E. agallocha

The removal of water content from the sample can be achieved by drying it until all moisture is eliminated, as the presence of water can influence the stability of bioactive compounds during extraction. Certain compounds may remain more stable or be less prone to chemical degradation or oxidation in dry conditions. The extraction of leaf samples from A. alba and E. agallocha was performed using ethanol as the solvent. The results indicated that the extract yield from the A. alba leaves was the highest at 8.80%, which was obtained from the conservation area (Table 5).

257

67.9

Table 5

Percentage of etanol extract

	Sample	Extract weight (g)		Depreciation	Extract	
Location	leaves	Dry	Crude	percentage (%)	percentage (%)	
		powder	extract			
Industry area	A. alba	250	22.01	91.20	8.80	
	E. agallocha	250	17.33	93.07	6.93	

Conservation	A. alba	250	13.17	94.73	5.27
zone	E. agallocha	250	21.42	91.43	8.57

Based on Table 5, these results indicate that environmental conditions, both in industrial areas and conservation zones, have the potential to affect the weight of crude extracts and the percentage of depreciation of *A. alba* and *E. agallocha* leaves, with the possibility of differences in the composition of bioactive compounds in each location. The maceration and extraction processes are important steps in testing the content of bioactive compounds in samples, especially in separating compound components from mangrove extracts [114]. The use of solvents such as ethanol, which are amphipathic, allows the dissolution of both polar and nonpolar compounds, so that it is optimal for obtaining various bioactive compounds from mangroves, which contain various types of compounds with these properties [115, 116, 117]. A high percentage of extraction weight indicates the effectiveness of the extraction method, indicating the method's ability to obtain active compounds from the sample optimally [118]. High extraction results also indicate a high content of active compounds in the sample, which possess the capability to have biological value and other practical applications [119].

The potential antioxidant content is illustrated by the percentage value of free radical scavenging inhibition along with the IC₅₀ value. The results of the antioxidant test on mangrove leaves using the DPPH radical scavenging method using ethanol solvent (Table 6). The IC₅₀ value content in the industrial area for *A. alba* of 137.8 µg/ml is classified as a moderate and *E. agallocha* of 21.63 µg/ml is classified as a very strong. While in the conservation area, *A. alba* of 64.32 µg/ml is classified as a strong and *E. agallocha* of 41.43 µg/ml is also classified as a very strong.

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Table 6

Classification of IC50

.	Sample	Linear r	egression		IC ₅₀	Category	Formatted: Subs
Location	leaves	a	b	R ²	(µg/ml)		
T	A. alba	36,277	128,7	0,9429	137,8	Moderate	-
Industry area	E. agallocha	30,953	45,165	0,9419	21,63	Very strong	
Conservation	A. alba	28,726	69,611	0,8905	64,32	Strong	
zone	E. agallocha	18,425	18,661	0,904	41,43	Very strong	

The IC_{$\xi0$} classification results indicate that *A. alba* leaves from both areas fall into the strong-moderate category, while *E. agallocha* is classified as very strong. According to Kodikara et al. (2020), the difference in the strength of antioxidant activity in each species is thought to be because mangroves have different tolerances to certain environmental conditions, and this can affect the extent to which they can overcome heavy metal toxicity. Previous research explained that the genus Avicennia is a mangrove found in the front zone and directly facing the waters [121]. *Avicennia spp.* has strong and dense aerial roots so that it is able to efficiently capture and bind mud and various pollutants carried by water [122, 123]. As a type of plant that is periodically submerged in water, the roots of mangroves are able to take, absorb, or reduce contaminants through the dilution process [124, 125]. Therefore, it is hypothesized that contaminants absorbed by roots do not induce excessive oxidative stress and do not increase the production of secondary metabolites.

Another study in the Island of Weno area, Chuuk State of Micronesia, for the antioxidant activity of *Rhizophora stylosa* roots was 41.3% and *Sonneratia alba* 40.7% [60]. While the IC_{50} value of the *E. agallocha* in both areas is included in the high category. *E. agallocha* in this study was found in the ladward zone. This zone is rarely submerged by seawater and is more often affected by lower tides. This is thought to be the cause of the low water content in

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the leaves of *E. agallocha* as presented in Table 4, so that the pollutants absorbed are greater and last longer in the leaves. Therefore, the roots act to mitigate stress effectively by producing antioxidant activity [126]. The concentration of antioxidant activity (IC₅₀) in the leaves showed different values in the two areas. The differences that occur in the ability to produce antioxidant activity in each mangrove as a form of self-defense against oxidative stress are due to differences in morphology, habitat, tides, sediment substrates, and environmental conditions [127, 128]. Differences in IC₅₀ classification results can reflect differences in the level of heavy metal exposure in the two locations.

In addition to testing antioxidant activity using the DPPH method, this activity can also be analyzed by calculating total phenol. Measuring the total phenol content is done by adding Folin-ciocalteu reagent to the solution sample being tested (Table 7). Phenols possess antioxidant properties that play a role in protecting plant tissues from damage induced by free radicals. Therefore, the total phenol test can provide information about the potential antioxidant activity of mangrove leaf extracts. In this study, the highest quantitative phenol value was found in *E. agallocha* at 398.80 mg GAE/gr from the industrial area and the smallest in *A. alba* at 21.85 mg GAE/gr from the conservation forest area.

Table 7

Total phenol of mangrove leaves extract

Location	Sample leaves	Phenol (mg GAE/g)	
Industry	A. alba		36.68
maastry	E. agallocha		398.80
	A. alba		21.85
Conservation	E. agallocha		320.44

The total phenol obtained in this study has a positive relationship with antioxidant activity, as indicated by the IC_{50} value in Table 7. The antioxidant activity of this mangrove is influenced by its total phenol content. The total phenol content is positively correlated with antioxidant activity, where the higher the total phenol content, the higher the antioxidant activity in the sample [129]. Based on this study, A. alba has a lower total phenol content than E. agallocha, which is strongly suspected due to differences in environmental factors. Mangroves in the pioneer zone more pressure from pollutants and the physicochemical conditions of the habitat. This is in line with previous findings, where the total phenol content in the roots of A. marina in the pioneer zone was 26.11 mg GAE/g, lower than B. gymnorrhiza in the landward zone with 344.02 mg GAE/g [130]. Mangrove ecosystems located in the pioneer zone tend to have special adaptations to survive in coastal environments that are often inundated by sea tides [131, 132]. Mangroves mitigate pollutants by reducing their concentration and toxicity through internal water content regulation, preventing excessive accumulation of absorbed contaminants [133]. According to Laoué et al. (2022)], nonenzymatic antioxidant activity is not produced exclusively because there is a certain limit for excess free radicals. However, the non-enzymatic antioxidant system is usually activated when free radical levels or oxidative stress exceed normal defense capacity [26].

GC-MS analysis using *E. agallocha* mangrove leaf samples from industrial areas because they are included in the IC₅₀ classification is very strong among others. The graph revealed 15 peak points identifying compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids (Figure 5). The identified compounds, based on chromatogram peak heights and mass spectra from the analysis, match those in the WILEY 7 database library (Table 8).

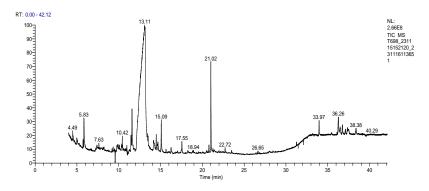


Figure 5. GC-MS chromatogram of bioactive compounds in mangrove leaves E. agallocha (Industry area)

Table 8

Retetion time, peak area, -compound name, formula, and compound group (Daun E. agallocha)

Ret.	Peak	Compound name	Formula	Compound group	
time	Area %				
5.84	2.45	4H-Pyran-4-one, 2,3	- C ₆ H ₈ O ₄	Flavonoid	-
		dihydro-3,5-dihydroxy-			
		6-methyl			
9.49	1.68	2-Myristynoyl	$C_{23}H_{45}N_2O_4S$	Lipid	
		pantetheine			
9.77	1.65	Paromomycin	C ₂₃ H ₄₅ N ₅ O ₁₄	Glikosida	
9.87	1.17	2-Myristynoyl	$C_{23}H_{45}N_2O_4S$	Lipid	
		pantetheine			
11.46	1.16	Desulphosinigrin	$C_{11}H_{21}NO_9S_2$	Glukosinolat	
11.59	3.31	2-O-Methyl-D-	<u>C7H14O6</u>	Glikosida	Formatted: Font colour:
		mannopyranosa			

13.10	73.97	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Glukosa	
14.16	1.84	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Glukosa	
14.48	0.99	7-Methyl-Z-tetradecen-	C ₁₇ H ₃₄ O ₂	Ester	
		1-ol acetate			
14.69	1.05	9-Octadecenoic acid, (2-	<u>C₂₈H₄₄O₄</u>	Ester	Formatted: Font colour: Auto
		phenyl-1,3-dioxolan-4-			
		yl)methyl ester, trans-			
15.09	2.29	2,6,8-	$C_{11}H_{18}O_2$	Terpenoid	Formatted: Font colour: Auto
		Trimethylbicyclo[4.2.0]			
		oct-2-ene -1,8-diol			
17.55	0.98	Hexadecanoic acid,	$C_{17}H_{34}O_2$	Asam lemak	
		methyl ester			
21.01	4.87	Phytol	C ₂₀ H ₄₀ O	Terpenoid	
33.97	0.94	9-Desoxo-9-x-acetoxy-	$C_{21}H_{30}O_9$	Glikosida	
		3,8,12-tri-O-ac			
		etylingol			
36.27	1.65	1-	$C_{21}H_{44}O_4Si$	Ester	
		Monolinoleoylglycerol			
		trimethylsilyl ether			

Based on Table 8, 8 groups of compounds were found. The groups of compounds that are thought to be formed in response to the environment that increases antioxidant activity, such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl found in these leaves is classified as a flavonoid. Flavonoids are specialized metabolites commonly found in plants, serving multiple functions such as defense and signaling, particularly under stress conditions [135]. Flavonoids are categorized into several groups, including chalcones, aurones, flavanonols, flavones, isoflavones, flavanols, flavonols, anthocyanins, proanthocyanidins, and leucoanthocyanidins. They can exist as aglycones, glycosides, and methylated derivatives. The compounds 2-Myristynoyl pantetheine and 2-O-Methyl-D-mannopyranose are classified as lipids. Lipid compounds can exhibit antioxidant activity, especially through mechanisms involving phenols and other structures that modulate oxidative stress and lipid peroxidation processes [136].

The compounds Paromomycin, 2-O-Methyl-D-mannopyranose, and 9-Desoxo-9-xacetoxy-3,8,12-tri-O-ac etylingol are classified as glycoside compounds. Based on the results of the study of Yang et al. (2018), flavonoid glycosides are widely distributed in plants, where they function as phytoalexins to combat biotic stress. Desulphosinigrin is a glucosinolate known to exhibit anticancer and antimicrobial properties [138]. 4-methylsulfinylbutyl glucosinolate is a glucosinolate derived from the amino acid methionine, which has antioxidant, antifungal, and antimicrobial activities [139]. The compounds 7-Methyl-Ztetradecen-1-ol acetate, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, and 1-Monolinoleoylglycerol trimethylsilyl ether are classified as esters. Clearly show that ester groups with different aromatic and alkyl chains will increase antioxidant capacity. e compound 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1 carboxaldehyde is categorized as an aldehyde. This type of compound is commonly found in various essential oils and contributes a distinctive aroma to certain plants. Several phenolic aldehydes and derivatives have antioxidant activity [140].

Compounds 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene -1,8-diol and phytol belong to the terpenoid compound group. Terpenoids are promising lead compounds for further structural modification and optimization because of their potent anti-inflammatory effects [141, 142].

Terpenoids (such as monoterpenes and carotenoids) and polyphenols (such as quercetin and other flavonoids) are important phytochemicals with various antioxidant effects [143]. Hexadecanoic acid, methyl ester compounds are classified as fatty acid compounds. Fatty acids have been found to be associated with various biological activities such as anti-inflammatory, antioxidant, antifeedant, antimicrobial, and neuroprotective [144]. While compounds that have no relationship with antioxidant activity are the glucose compound group found in leaf extracts. Glucose produced through photosynthesis and other carbohydrate processes can be used as an energy source to maintain cell vitality [145].

Correlation of heavy metal concentrations and biomarkers

The relationship between heavy metal concentrations and antioxidant activities in mangrove leaves in both areas using Pearson correlation analysis, which begins with assumption testing (Table 9). The test results were obtained for all variables with significance > 0.05, and if the skewness and quasi-sequence ratios are in the range of -1.96 and +1.96, it can be concluded that the data distribution is normal.

Table 9

Assumption test results

Sample	Variable	Mean	St.Dev	Sig.2 tailed	Skewness Kurtosis	Values
	Pb	0.94	0.12	0.927	0.55 dan 0.55	Normal
	Cu	3.57	0.080	0.498	0.33 dan 1.35	Normal
Leaves	IC50	66.35	25.19	0.457	1.31 dan 0.69	Normal
	Total Phenol	194.44	193.48	0.182	0.13 dan 1.93	Normal

Based on the results of the assumption test, the normal distribution of the data can explain that the statistical parameters used in the correlation analysis provide an accurate picture of the center and distribution of the data. Furthermore, the results of the Pearson correlation test (r) and the coefficient of determination (Kd) are summarized in Table 10.

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Table 10

Results of the Pearson correlation test (r) and coefficient of determination (Kd)

Sample	Variable (X-Y)	r	Kd (%)	Interpretation
Leaves	$Pb - IC_{50}$	-0.906	82.08	Strong correlation
	$Cu - IC_{50}$	-0.937	87.79	Strong correlation
	Pb – Total Phenol	0.904	81.72	Strong correlation
	Cu – Total Phenol	0.949	90.06	Strong correlation
-				

The results of the correlation test is a significant correlation or relationship between heavy metals and physiological responses ($r \neq 0$). The relationship between Pb and Cu to antioxidant activity in mangrove leaves produced from both areas has a very high negative correlation direction of -0.906 and -0.937. The relationship between Pb and Cu to total phenol in leaf samples is also very strong, with a very high positive correlation value of 0.904 and 0.949. In addition, the percentage of the determination coefficient (Kd) indicates that variables X and Y have a strong relationship. The Kd value of mangrove leaf samples ranges from 81.72% to 90.06%. This indicates that most of the variations in IC₅₀ and total phenol can be explained by the Pb and Cu variables in both types of samples.

A high correlation indicates a strong relationship between the variables concerned and significantly supports the hypothesis. A negative relationship with IC_{50} indicates that the higher the concentration of Pb or Cu, the lower the IC_{50} value (higher antioxidant potential). A positive

relationship with total phenol indicates that the higher the concentration of Pb or Cu, the total phenol content also increases. Furthermore, the results of GCMS screening also showed the presence of compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Previous studies have shown that some of these compounds, especially the flavonoid and terpenoid groups, have significant antioxidant activity [146]. Therefore, increasing concentrations of heavy metals can indirectly affect the profile of secondary metabolite compounds in mangrove plants, which in turn can affect antioxidant activity and response to oxidative stress. Excessive concentrations of heavy metals cause the formation of ROS and affect the activity of antioxidants involved in plant metabolism [147]. According to Georgiadou et al. (2018), detoxification of ROS due to heavy metal contamination by producing antioxidant enzymes plays a central and vital role in protection in mangrove species.

In line with the research by [149], that under abiotic stress conditions, such as heavy metal contamination, the production of reactive oxygen species (ROS) increases in plants, resulting in the induction of oxidative stress, and plants initiate antioxidant production that significantly delays or prevents oxidative stress. According to Angon et al. (2024), secondary metabolite compounds are involved in plant responses to biotic and abiotic stresses and contribute significantly to the antioxidant activity of plant tissues. Antioxidant activity is a common approach used to increase heavy metal tolerance, strengthening the defense system against oxidative stress [151, 152]. Several previous studies have found a relationship between heavy metal pollution and the physiological response of plants, especially mangroves. The decline in sediment quality due to heavy metal pollution in a gradual pattern that has the potential to have a negative impact on the biogeochemical cycle, with potentially fatal consequences for the survival of biodiversity (*A. marina*) [153]. Furthermore, the results of the study by Ghosh et al. (2021) also stated that there was a statistically significant relationship

between the activity of antioxidant enzymes, photosynthetic pigments, and heavy metal contamination, resulting in the biotic response of riparian mangroves characterized by reduced photosynthetic pigments (chlorophyll a and b) and increased activity of antioxidant stress enzymes (POD, CAT, and SOD). The response of two tropical medicinal plant species to heavy metal accumulation can increase hydrogen peroxide (H₂O₂) activity, malondialdehyde content, enzymatic activity, and nonenzymatic antioxidants [154].

Mangroves cause trigger antioxidant defenses to overcome heavy metal absorption and normalize excessive production of oxidative stress mediated by reactive oxygen species (ROS) [155]. However, antioxidant responses in mangroves vary depending on the concentration and type of heavy metals, plant species, and duration of exposure [156]. Previous findings related to plant reactions to higher concentrations of heavy metals in the soil. For example, Kulbat-Warycha et al. (2020) observed that an increase in the concentration of heavy metals (Ni, Cu, Zn) caused a decrease in the concentration of phenols in oregano, which was associated with the induction of severe oxidative stress. According to Mansoor et al. (2023), excessive ROS production due to severe oxidative stress can cause damage to the mitochondrial respiratory chain, uncoupling of oxidative phosphorylation, and mitochondrial death in plants. However, this can also experience a decrease in the antioxidant activity defense system of the mangrove itself if the contamination of absorbed pollutants exceeds the threshold and severe oxidative stress occurs, which can cause damage and death to the mangrove ecosystem [159, 160].

The correlation between heavy metals and antioxidant activity in mangroves illustrates the complex relationship between heavy metal pollution and plant responses to oxidative stress. In this context, high concentrations of heavy metals can trigger ROS production, which in turn affects plant antioxidant activity. Excessive ROS can induce oxidative stress that activates the plant defense system to increase the production of antioxidant compounds. Thus, the relationship between heavy metals and antioxidant activity, total phenols, and secondary metabolite compound profiles in mangroves provides a deeper understanding of the mechanism of the plant's response to heavy metal pollution and oxidative stress. Therefore, if there is an indication that pollutant contamination exceeds the threshold and causes severe oxidative stress, some coastal environmental management policies can be expected in response to these findings.

To ensure the sustainability of mangrove ecosystems and mitigate the impact of heavy metal pollution, routine monitoring is recommended every 3–6 months to capture seasonal variations in heavy metal concentrations and antioxidant responses. Additionally, long-term monitoring (≥5 years) is necessary to identify trends in heavy metal accumulation and its effects on coastal ecosystems. Supplemental monitoring is also advised following specific events, such as industrial waste spills or land-use changes, to assess their immediate environmental impact. The data from this study can serve as a basis for environmental policy development, including updating regulations on heavy metal thresholds in sediments and coastal biota, strengthening conservation and mangrove rehabilitation policies, and improving industrial zone management in coastal areas. Furthermore, these findings can be utilized to raise public awareness about the importance of protecting coastal ecosystems and promoting sustainable resource management practices.

Conclusion

Heavy metal pollution of Pb and Cu resulting from areas affecting industrial and conservation activities has a significant effect on antioxidant activity in mangroves, especially *A. alba* and *E. agallocha*. Sediment pollution assessment showed that the Igeo value was at a low level, while the concentration factor (Cf) and Pollution Load Index (PLI) showed a relatively moderate level of pollution (Cf between 1 and 3, and PLI between 0 and 2). The bioaccumulation value of heavy metals in mangrove leaves was low (BCF < 1), indicating

moderate accumulation of heavy metals in leaf tissue. The antioxidant activity of *E. agallocha* leaves from the industrial area was very strong and had the highest total phenol content. The compounds identified as having high antioxidant activity included flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Correlation analysis showed that increasing heavy metal concentrations were directly proportional to increasing antioxidant activity and total phenol content in mangrove leaves. This study contributes to our understanding of the potential of mangroves to respond to heavy metal exposure through increased antioxidant activity, which can support conservation efforts and sustainable management of coastal natural resources.

Author statement

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

CRediT authorship contribution statement

Rozirwan: Writing – review & editing, Supervision, Project administration, Conceptualization. Redho Yoga Nugroho: Resources, Formal analysis, Data curation. Nadila Nur Khotimah: Validation, Resources, Data curation. Fauziyah: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. Wike Ayu Eka Putri: Writing – original draft, Software, Investigation. Riris Aryawati: Methodology, Data curation. Gusti Diansyah: Software, Investigation.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The data supporting the findings of this study can be obtained from the corresponding author upon a reasonable request.

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		graphical abstract.							
2.	Regarding to previous study on biomarker	We have now incorporated the							
	which involve sediment and mangrove this	reference into our manuscript,							
	reference	particularly in the Results and							
	((<u>https://doi.org/10.1016/j.ecss.2019.106403</u>)	Discussion section related to antioxidant activity and total phenol							
	may strengthen the manuscript								
		content.							
3.	Please kindly correct this word (ample) Line	We have revised.							
	57 - Leaf sampling section								
4.	Please kindly reorganize Line 1 - 10 - Atomic	We have revised.							
	absorption and determination of heavy metals								
5.	Please kindly mention the detail in AAS	We have made specifications for the							
	instrument (e.g. Shimidzu or etc)	equipment used, namely the Atomic							
		Absorption Spectrophotometer							
		(Shimadzu AA-7000).							
6.	Please kindly added detail in measurement	We have added details in the							
	procedure on using AAS	measurement procedure using AAS							
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Biomarkers of heavy metals pollution in mangrove ecosystems: comparative assessment in industrial impact and conservation zones

Rozirwan^{1*}, Nadila Nur Khotimah², Wike Ayu Eka Putri¹, Fauziyah¹, Riris Aryawati¹, Gusti Diansyah¹, Redho Yoga Nugroho¹

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Abstract

Heavy metal contamination from industrial activities in coastal regions can lead to pollution in mangrove ecosystems. Mangroves produce antioxidant compounds to mitigate the impact of free radicals. This study aimed to analyze the correlation between the concentration of heavy metals Pb and Cu and antioxidant activity in *Avicennia alba* and *Excoecaria agallocha* mangroves from areas affected by industrial activities and conservation areas, Banyuasin, South Sumatra, Indonesia. This study was conducted in September 2023 with sampling locations in the Payung Island area and the Barong River conservation area, Berbak Sembilang National Park. The samples taken included sediment and mangrove leaves. The concentration of heavy metals Pb and Cu was measured by atomic absorption spectrometry. Antioxidant activity test using the DPPH test, total phenol using the Folin-Ciocalteu method, and phytochemical profile screening using GCMS. Statistical analysis of the correlation between antioxidant activity and heavy metal concentration using the Pearson correlation. The results

showed that the highest concentration of heavy metals in sediment and mangrove leaves was found in the area affected by industrial activity, with a range of Pb values of 0.67 ± 0.16 to 18.70 ± 0.48 mg/kg and Cu values of 3.39 ± 0.20 to 6.07 ± 0.37 mg/kg. The results of sediment pollution assessment for heavy metals Pb and Cu at Igeo < 0 indicates uncontaminated, 1 < Cf < 3 indicates low contamination, and PLI 0-2 indicates not polluted. While the results of heavy metal bioaccumulation in leaves were BCF < 1, indicates low bioaccumulation. *E. agallocha* leaves from the Pulau Payung area showed very strong antioxidant activity of 21.63 µg/ml, and the highest total phenol content reached 398.80 mg GAE/g. Analysis of compounds with the highest antioxidant activity identified the presence of esters, aldehydes, alcohols, fatty acids, glycosides, flavonoids, terpenoids, and steroids. Correlation analysis shows that higher heavy metal concentrations correspond to increased antioxidant activity and total phenol content (r \neq 0). These findings are expected to contribute to scientific knowledge that enhances environmental sustainability, supporting effective management of coastal natural resources. **Keywords:** Biomarkers, conservation zones, heavy metals, industrial activities, mangrove

Introduction

Coastal areas are transitional areas between land and sea that have abundant biodiversity and unique ecosystems [1,2]. Coastal areas face great pressure from various anthropogenic activities that can cause pollution [3,4]. Previous studies report that industrial activities like fertilizer processing, oil and gas, and crude palm oil production contribute to coastal pollution. [3,5,6]. In addition, there are also agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households [7]. Continuous anthropogenic activities in coastal areas can produce pollutants, such as microplastics, heavy metals, as well as various organic and inorganic contaminants [8, 9, 10]. Among various pollutant types, heavy metals are categorized as persistent pollutants due to their resistance to decomposition [11]. Heavy metals initially present in the water column gradually settle to the sediment and eventually accumulate in aquatic organisms [12]. This condition may have adverse impacts, particularly if it exceeds environmental quality standards. These adverse impacts can affect aquatic ecosystems, including mangroves [13,14]. According to Xu et al. (2024), as the largest plant community in coastal areas, mangroves are also directly affected by pollution.

Mangrove ecosystems play a vital role in coastal protection, supporting biological diversity, and contributing to the socio-economic development of local communities [16,17]. Additionally, their capacity to accumulate pollutants makes them valuable indicators for assessing pollution levels in coastal waters, as they can absorb and store these pollutants in their tissues, enhancing their role in monitoring environmental health [18,19, 20]. Roots and leaves are important parts of mangroves in the absorption, accumulation, and response to pollutants [21]. Roots are the first part exposed to pollutants from their growth media. Furthermore, roots also have the ability to translocate pollutants to the leaves. Leaves are the primary site for photosynthesis in plants, supplying the energy essential for cell development, and overall plant function [22]. High concentrations of pollutants in roots and leaves can potentially increase excessive reactive oxygen species (ROS), resulting in oxidative stress in mangroves [23,24]. Oxidative stress arises from an imbalance between ROS production and detoxification, potentially leading to harmful cellular damage [25,26]. Although oxidative stress can be detrimental, plants also have a resistance response mechanism against free radicals [27]. This process involves producing antioxidant enzymes and molecules to counteract the harmful effects of free radicals. In response to environmental changes, plants enhance the activity of antioxidant defenses, including both enzymatic and non-enzymatic components such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPx), and phenolic compounds. These antioxidants serve as protective mechanisms against various environmental stress [28,29].

Research on the specific physiological adaptations of various mangrove species to pollutants is still limited. Most previous studies only focused on the accumulation of heavy metals in mangroves without exploring in depth the biochemical defense mechanisms they employ [30, 31, 32]. However, studies on how different mangrove species respond to industrial pollution in environments with varying levels of pollution have not yet been conducted. In addition, most studies only examine one mangrove species without comparing the adaptability of different species in the face of heavy metal contamination [33, 34].

This study aimed to evaluate the accumulation of heavy metals (Pb and Cu) in two mangrove species (*Avicennia alba* and *Excoecaria agallocha*) and assess their antioxidant activity in industrial and conservation zones. The selection of these two species was based on their prevalence in the research location as well as differences in habitat zones and morphological characteristics [35, 36]. This study was carried out in the mangrove ecosystem, which includes areas influenced by industrial activities such as Payung Island as well as conservation areas in the Berbak Sembilang National Park [37, 38].

By assessing biomarkers, new insights are provided into how mangrove species adapt to environmental stress caused by heavy metal pollution. Additionally, the research explores the impact of heavy metal contamination on the physiological responses of mangroves, focusing on their biochemical defense mechanisms. The findings aim to enhance understanding of mangrove adaptation strategies in response to pollution and offer valuable implications for coastal ecosystem conservation and environmental pollution management.

Materials and Method

Leaf sampling

This study was conducted in September 2023. The samples included Avicennia alba, Excoecaria agallocha, and sediments collected from industrial and conservation zones in Banyuasin, South Sumatra, Indonesia (Figure 1). The first area is the mangrove ecosystem on Payung Island. This area was chosen due to the high accumulation of heavy metals from industrial activities along the Musi River. Additionally, the area includes agricultural activities, ports, fish ponds, and settlements [39, 40]. The second area is the conservation forest in Sungai Barong, Sembilang National Park, which represents a natural area and protects flora and fauna from the threat of damage, scarcity, or deforestation [41, 42, 43].

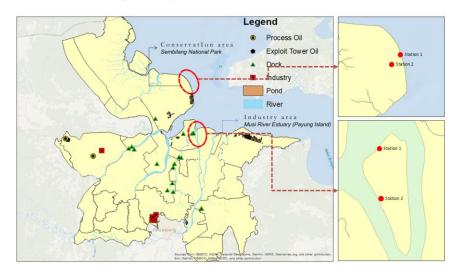


Fig.1. Map of sample collection

The sampling stages include collecting sediment samples and mangrove leaves. Sediment samples were taken as supporting data to determine the concentration of heavy metals in the mangrove growth media. The availability of heavy metals in sediments has a direct effect on the bioaccumulation and biomagnification processes in aquatic organisms. Sediment data helps understand the level of risk and potential impacts to organisms in mangrove ecosystems. Sediment samples were taken using a grab pipe at a depth of \pm 10 cm from the surface [44]. Sediment depth shows a very significant impact on heavy metal content, with a greater decrease in heavy metal content as sediment depth increases [45]. <u>Samples</u> were taken at three location points for each station, which were considered as replications. Samples were taken compositely

together (taken as needed, 500 g) and placed into a polyethylene plastic container and stored in a cool box for analysis in the laboratory.

The method for collecting mangrove leaves taken from the field uses a random sampling method [46]. The random sampling method can be used if the sample studied is homogeneous. The mangrove species taken were *A. alba* and *E. agallocha*. The samples taken consisted of \pm 1 kg of leaves and were put in polyethylene plastic.

Sediment grain size analysis

Grain size analysis was conducted using the sieving and pipetting methods as outlined by [47]. Sediment types (gravel, sand, silt, and clay) were classified using Shepard's triangle analysis and processed with Microsoft Excel V.2021, following the protocols established by [48, 49]. The sediment fraction type was determined by identifying the most dominant composition from the analysis results.

Sample preparation

Sediment sample preparation involved removing foreign objects such as plastic fragments and leaves. The sediment was then air dried at room temperature for 72 hours until fully dry, ground to a homogeneous consistency, and stored in a tightly sealed polyethylene bottle. They were then air-dried in a shaded, well-ventilated area for five days, ensuring indirect exposure to sunlight to prevent the degradation of bioactive compounds. The drying process was conducted at ambient temperature with sufficient airflow to facilitate moisture evaporation. Once dried, the samples were ground into a fine powder and stored in sealed containers for further analysis. The extraction of heavy metals (Pb and Cu) from the sediment samples and mangrove leaves was performed using the wet destruction method, following the procedures outlined by [8, 50].

Atomic absorption spectroscopic measurement

Measuring the concentration of heavy metals Pb and Cu using an atomic absorption spectrophotometer with a wavelength of 283.3 nm for Pb and 324.7 nm for Cu-Measuring the concentration of heavy metals Pb and Cu using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Operational parameters: Pb (283.3 nm, 5 mA lamp current) and Cu (324.7 nm, 4 mA), slit width 0.5 nm, air-acetylene flame (2.0 L/min air; 1.5 L/min acetylene), burner height 5–7 mm. After 15–20 min warm-up, calibration was performed using blank and standard solutions (0.1–2.0 ppm Pb; 0.05–1.0 ppm Cu), achieving R² ≥0.995. Samples were aspirated in triplicate with 15-sec distilled water rinsing between measurements and acid blank checks every 5 samples. Quality control included spike recovery (85–115%), duplicate analyses (RSD <5%), and certified reference materials (NIST SRM 1640a/2711a). LODs: 0.02 ppm Pb; 0.01 ppm Cu (3×SD blank) [51].

Determination of heavy metals in leaves and sediments

Determination of sediment pollution

Geoacumulation index (Igeo)

The Igeo (geo-accumulation index) quantitatively evaluates the degree of heavy metal contamination and classifies the level of pollution based on detailed categorization [52]. Igeo = $\log_2 (Cn/1.5 Bn)$ (1)

The classification of Igeo values includes the following categories: uncontaminated (Igeo ≤ 0), uncontaminated to moderately contaminated (Igeo 0–1), moderately contaminated (Igeo 1–2), moderately to highly contaminated (Igeo 2–3), highly contaminated (Igeo 3–4), highly contaminated to very highly contaminated (Igeo 4–5), and very highly contaminated (Igeo ≥ 5) [53].

Contamination factor (Cf)

The contamination factor is determined experimentally as the ratio of the element concentration in the sample to its background concentration [54].

$$Cf = (Cn/Bn)$$
(2)

The contamination factor (Cf) classifications are as follows: [55]: Cf < 1 = low contamination; 1 < Cf < 3 = moderate contamination; 3 < Cf < 6 = sufficient contamination; Cf > 6 = very high contamination.

Pollution load index (PLI)

The pollution load index (PLI) is utilized to assess pollution quality in a given area. The pollution load index value uses the formula [56].

$$PLI = [Cfl x Cf2 x Cf3 \dots x Cfn]^{1/n}$$
(3)

Pollution load index (PLI) criteria: PLI 8-10 = severely polluted; PLI 4-8 = heavily polluted; PLI 2-4 = moderately polluted; PLI 0-2 = not polluted to lightly polluted; PLI < 0 = not polluted.

Bioaccumulation of metal in leaves

Bioconcentration factor (BCF)

The absorption of metals by leaf from sediment occurs through a process known as bioaccumulation. The Bioconcentration Factor (BCF) values are utilized to assess the extent of metal bioaccumulation in mangrove leaf originating from sediment [57].

BCF = (Cn.leaf/Cn. sediment) (4)

BCF > 1 hyperaccumulator; BCF = 1 indicator; BCF < 1 is an excluder [58].

Analysis of antioxidant non-enzymes in leaves

Antioxidant activity evaluated by DPPH assay

Antioxidant activity analysis was carried out using ethanol solvent based on a method adapted from [59]. A 50 ml 0.1 μ M DPPH solution was prepared, followed by the preparation of a sample stock solution and a 10 ml pure ascorbic acid stock solution of 2000 ppm, which was homogenized. Furthermore, a series of solutions were made with concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. At each concentration, 1 ml of 0.1 μ M DPPH solution was added to the mixture, which was then homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) at a wavelength of 517 nm. The antioxidant activity of the extract is expressed as IC₅₀, which quantifies the strength of its antioxidant capacity (Table 1). The IC₅₀ value is calculated using the following formula:

$$\% inhibition = \frac{blank \ abs-sample \ abs.}{blank \ abs} \ge 100\%$$
(5)

The IC₅₀ value was derived by inputting the data into a linear regression equation, where the sample concentration was plotted on the X-axis and the percentage of inhibition of antioxidant activity on the Y-axis. The regression equation used is represented as y=ax + b [60].

Table 1

Characteristic value of IC50

Concentration (µg/ml)

Characteristic

<50	Very strong
50-100	Strong
100-150	Moderate
100 120	modeluie
150-200	Low

Determination of phenol content

The analysis of total phenol content in the samples was conducted using the Folin-Ciocalteu method, as outlined in the literature [60, 61, 62]. A standard solution of 1000 ppm gallic acid as much as 50 ml was prepared, then variations in concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were made, each as much as 5 ml. For each concentration variation, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were pipetted into a 10 ml measuring flask containing a standard solution of 100 ppm gallic acid. A total of 50 mg of sample was weighed, then 2 ml of methanol and 5 ml of distilled water were added, then homogenized in a 10 ml measuring flask. In both the standard series and sample variations, 0.5 ml of 50% Folin-Ciocalteu reagent was added, followed by the addition of distilled water up to the mark. The mixture was then allowed to stand for 5 minutes. Next, one ml of a 5% Na2CO3 solution was added and incubated in a dark place for one hour. After incubation, the absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 750 nm.

Pearson correlation analysis (correlation bivariate)

The use of Pearson correlation analysis (bivariate correlation) is a method used to evaluate the relationship between two variables [63, 64], in this case to see the relationship between antioxidant activity and heavy metal concentrations. This analysis was carried out using SPSS software version 28.

Result and Discussion

Description of mangrove leaves

The mangrove species *A. alba* and *E. agallocha* found at the sampling location exhibit distinct characteristics. Figure 2 shows the morphological differences between *A. alba* and *E. agallocha*.

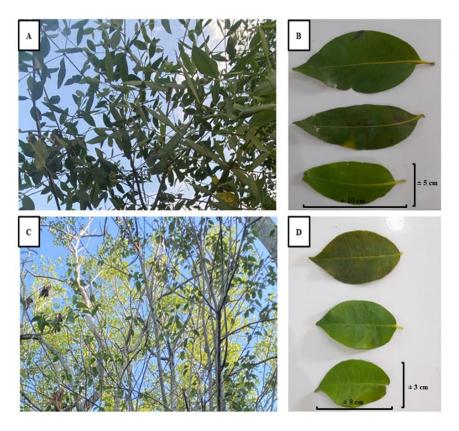


Fig.2. Leaves description. A-B). A. alba, C-D). E. agallocha

Leaves are the part that characterizes a mangrove species. When identifying each type of mangrove, observation of the morphology of the leaf shape is very important to understand the

characteristics and differences in each type of leaf [31, 65]. *A. alba* leaves have a green surface with a smooth and slippery texture, while the underside is yellowish green with a rough texture. The morphology of the leaves is elliptical, almost oval, with a tapered tip. Based on observations, the length of the leaves ranges from 10 to 13 cm, and the width of the leaves ranges from 4 to 5 cm. *E. agallocha* leaves are elliptical and dark green in color, with finely serrated edges and tapered tips. The observed leaf sizes ranged from 8 to 10 cm in length and 3 to 4.5 cm in width. Old leaves were selected as samples for the study of heavy metal content and bioactive compounds due to several considerations related to their maturity and potential accumulation of pollutants and compounds of interest. According to [67], plants tend to produce bioactive compounds in higher amounts in older parts. This could be a plant strategy to protect itself from pests, diseases, or the external environment [67, 68]. Older leaves may have more stable chemical conditions, thus facilitating analysis and minimizing variability in results.

Sediment grain size

The determination of substrate types in the sampling was conducted using the Shepard triangle method (Figure 3). In the mangrove ecosystem of both industrial and conservation areas, sediment substrates were categorized into four types: gravel, sand, mud, and clay. The results indicated that the predominant substrate type in both areas was clay.

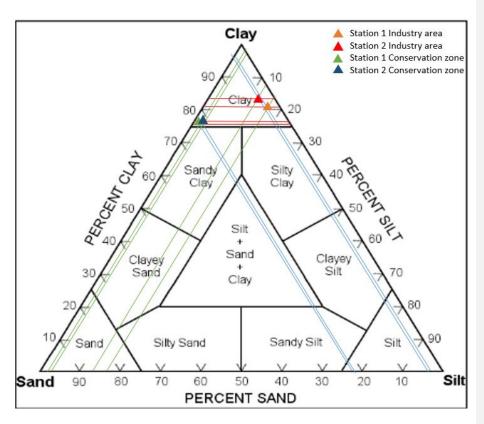


Fig.3. Classifications of sediment type with shepard triangle method

The sediment substrate surrounding the mangrove ecosystem in both areas is predominantly clay, with clay percentages ranging from 80,5% to 84,03%. The highest clay content was observed at station 1 in the industrial area (Table 2).

Tabel 2

Sediment grain size in each station

		Sediment			
Location	Station		N 1	CI	Grain size
		Gravel Sand	Mud	Clay	

Industry area	1 (A. alba)	0.00	3,6	12,37	84,03	Clay
ý		0.00		,		2
	2 (E. agallocha)	0,00	3,36	16,14	80,5	Clay
Conservation zone	1 (A. alba)	0,00	22,5	1,95	75,55	Clay
	2 (E. agallocha)	0,00	21,91	2,02	76,07	Clay

Based on the results of Table 2, distribution of sediment fractions and grain sizes at two different locations, which represents two stations with different mangrove species (*A. alba* and *E. agallocha*). In the industrial area, most of the sediments consist of clay with a very low sand content (3.6% for Station 1 and 3.36% for Station 2), which indicates the predominance of fine materials that can influence the mobility of heavy metals and nutrients in the sediments. In contrast, in the conservation zone, although the sediment composition is still dominated by clay, the sand content is higher (22.5% for Station 1 and 21.91% for Station 2), indicating differences in sedimentation processes and a higher potential for water infiltration.

In the industrial area, both stations (*A. alba* and *E. agallocha*) showed a dominance of clay fractions with a very high percentage. The dominant clay fractions indicate that the sediment in this area consists of fine particles, which may be caused by the accumulation of fine particles from industrial activities around this location. Industries such as fertilizer processing, oil and gas, crude palm oil production, agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households contribute to the presence of fine particles in sediments [3, 5, 6, 7]. Port activities involve frequent vessel movement, dredging, and cargo handling, all of which can resuspend fine particles and increase sedimentation rates [69, 70]. Crude oil processing and petroleum industries may contribute to fine particle deposition through air emissions, which settle via atmospheric deposition [72]. Additionally, agricultural activities, particularly palm oil plantations, can contribute to

increased fine particle accumulation through soil erosion and runoff carrying clay-rich sediments into adjacent water systems, particularly during heavy rainfall [72, 73].

Fine particles such as clay are usually carried by water and can accumulate in areas with slow water movement, such as near mangrove roots [74, 75]. In the conservation zone, the clay fraction also dominates, although with a slightly lower percentage than the industrial area. The conservation zone may also have less influence from human activities, so the sediment pattern is more natural than the industrial area. Clay is a sediment particle with a very fine grain size and a large surface area [76, 77]. Due to its small size and its tendency to be negatively charged, clay has a high adsorption capacity, which allows clay particles to attract and bind heavy metal ions such as Hg, Pb, Cd, Cu, and others [78, 79, 80]. Consequently, sediments dominated by clay fractions tend to accumulate more heavy metals than larger sediment fractions [81, 82].

Determination of heavy metals

The results of the heavy metal concentration analysis for Pb and Cu in sediments and mangrove leaves from both areas are summarized in Table 3. The concentrations of heavy metals, specifically Pb and Cu, in sediments from both the industrial area and the conservation zone exhibit variability; however, they generally remain below hazardous thresholds (ERL, ERM, TEL, and PEL). In the industrial area, the highest Pb concentration was found at Station 2 (18.70 \pm 0.48 mg/kg), while in the conservation zone, the highest concentrations of Pb and Cu were each at Station 2 (Pb 14.22 \pm 0.16 mg/kg; Cu 5.17 \pm 0.17 mg/kg). For metal accumulation in mangrove leaves, Cu was recorded higher than Pb at all stations. In the industrial area, *A. alba* (Station 1) had Pb 0.67 \pm 0.17 mg/kg and Cu 3.39 \pm 0.20 mg/kg, while *E. agallocha* (Station 2) showed Pb 1.27 \pm 0.31 mg/kg and Cu 3.73 \pm 0.16 mg/kg. In the conservation zone, the highest accumulation of Cu in mangrove leaves was 3.69 \pm 0.23 mg/kg at Station 2.

Tabel 3

Average concentrations of heavy metals (mg/kg) in mangrove sediments and leaves

	Pb	Cu
Sediments		
Station.1 Industry area	12.63±0.01	5.58±0.05
Station.2 Industry area	18.70 ± 0.48	6.07±0.37
Station.1 Conservation zone	12.61±0.32	4.21±0.03
Station.2 Conservation zone	14.22±0.16	5.17±0.17
ERL	46.7	34
ERM	218	270
TEL	30.2	18.7
PEL	112	108.2
Mangrove leaves		
Station.1 Industry area (A. alba)	0.67±0.17	3.39±0.20
Station.2 Industry area (E. agallocha)	1.27±0.31	3.73±0.16
Station.1 Conservation zone (A. alba)	$0.84{\pm}0.12$	3.50±0.35
Station.2 Conservation zone (E. agallocha)	0.99±0.37	3.69±0.23

The industrially impacted area in the Musi River Estuary is affected by high anthropogenic activities, making it susceptible to accumulating pollutants, especially heavy metals such as Pb and Cu. Sediments in this area tend to contain higher pollutants than water and biota, influenced by domestic, industrial, and river transportation activities that pollute the environment [83, 84]. Ship and coastal building maintenance activities, including the use of anti-rust materials, electronic waste, and pipe corrosion, are the main sources of Pb, while sources of Cu in the aquatic environment come from antifouling paint, agricultural pesticides, and industrial waste [85, 86]. In addition, previous studies report that cleaning ship hulls can release Cu into the marine environment [87, 88]. Fisheries sector that uses Cu-coated nets to prevent biofouling can also contribute to increasing Cu levels in waters [89, 90].

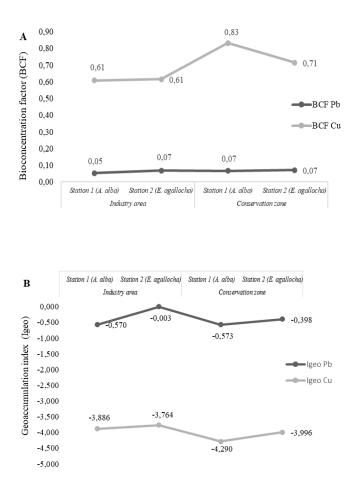
The conservation area in the Barong River is also exposed to heavy metal pollution, although at a lower level, considering that some human activities such as fishing are still ongoing [92]. Unmanaged anthropogenic activities, including unregulated industrial waste disposal, improper wastewater treatment, and uncontrolled agricultural runoff, have contributed to the increasing Cu concentrations observed in both locations, as indicated by the findings of this study and previous research [7, 50]. These activities introduce Cu into the aquatic system, where it binds to suspended particles and accumulates in sediments, further exacerbating environmental pollution.

In mangrove leaves, Pb was detected at low concentrations. Plants regulate Pb primarily by limiting its uptake and translocation. Because Pb is a non-essential and highly toxic metal, most of it accumulates in the roots rather than being transported to the leaves [93]. In contrast, Cu an important micronutrient for plants, is regulated through controlled absorption and detoxification mechanisms [93, 94]. Plants manage excess Cu by binding it to metallothionein and phytochelatin, storing it in vacuoles, and activating the antioxidant defense system to fight oxidative stress [96]. Although heavy metal concentrations vary between locations, they are still below the threshold, indicating a relatively low risk of contamination. However, long-term monitoring is essential to track bioaccumulation trends in mangrove ecosystems.

Sediment quality indices

The results of the sediment quality index assessment are summarized in Figure 4. The results of the leaf bioconcentration factor (BCF) in bioaccumulating Pb and Cu metals from sediment with a BCF value <1 indicating low bioaccumulation. The geoaccumulation index

shows uncontaminated properties for Pb and Cu with an Igeo value <0 indicating uncontaminated. The contamination factor (Cf) shows that contamination is low and moderate in Pb and Cu with a value of 1 < Cf < 3 indicating low contamination. The PLI ranges from 0 to 2 indicating that both areas are not polluted.



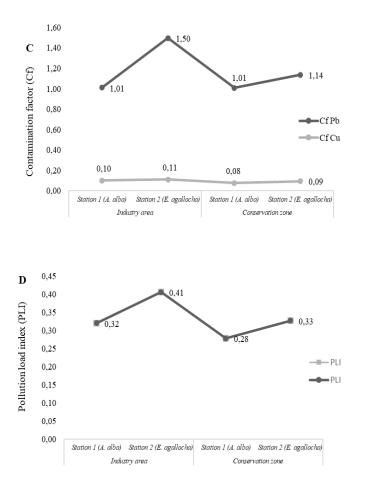


Fig.4. Sediment quality indices. A). Bioconcentration factor (BCF), B). Geoaccumulation index (Igeo), C). Contamination factor (Cf), and D). Pollution load index (PLI).

The difference in bioconcentration factor (BCF) between Cu and Pb can be explained by the chemical properties of each metal. Cu accumulates more easily in biota tissues than Pb. Cu is an essential element for organisms, although at higher concentrations it can be toxic [97]. In contrast, Pb is a non-essential heavy metal that tends not to accumulate much in biota tissues [98]. The previous studies stated that essential heavy metals are more easily absorbed by organisms because they have physiological mechanisms to regulate the concentration of these elements [98, 99]. Analuddin et al. (2023) have also examined BCF in mangrove ecosystems, with results showing that the BCF values for Hg, Cu, Mn, Pb, and Zn > 1. This finding is thought to be related to the impact of anthropogenic activities in Kendari City, which has a high population density.

The Igeo index shows higher Pb contamination than Cu in industrial areas. Pb is thought to originate from human activities such as ports, agriculture, ship transportation, and household waste that tends to settle in sediments [8, 32]. A high Igeo value indicate an anthropogenic contamination and show sediments contaminated heavy metal [102]. In addition, long-term exposure to these heavy metals can change community structure and disrupt ecosystem function through bioaccumulation and biomagnification in the food chain [102, 103].

The high value of the Pb contamination factor (Cf) in industrial areas indicates that this environment is more susceptible to Pb pollution than Cu. Relevan study by Hasan et al. (2023) that the CF value of Pb (0.76) > Cu (0.68) in core sediment from a mangrove at the Pasur River. Cu is more likely to be bound to organic particles and accumulate in the tissues of benthic organisms, which may explain the lower Cf Cu value. The areas suspected of being polluted tend to have higher anthropogenic activity than conservation zones, which causes significant differences in the levels of contamination and accumulation of heavy metals [106]. Industries around mangrove areas may contribute to elevated levels of heavy metals. Meanwhile, the conservation zone which is relatively protected from industrial activities, shows lower contamination values, although there are still traces of pollution due to remote pollution sources [106, 107].

The PLI value in the industrial area is higher than the conservation zone. This indicates that industrial activities play a role in elevating heavy metal pollution in the area. Industrial

areas are usually exposed to pollution sources such as factory waste, air pollution, and surface runoff that carry heavy metals into the sediment [108, 109]. Although both stations are in the same area, there is a difference in the PLI value between Station 1 and Station 2 at both locations. Local factors, including water movement, sediment composition, and proximity to pollution sources, significantly influence the distribution of heavy metals. [111]. The PLI in the conservation zone still shows heavy metal pollution. This could be due to atmospheric deposition from industrial activities in the surrounding area or pollutants carried by water currents from more contaminated areas [111, 112]. This suggests that although the conservation zone has better protection, it is not completely protected from the impacts of nearby industrial pollution.

The study indicate that both areas are classified as not polluted. In line with these findings by Karmakar et al. (2025), the PLI value in mangrove planting areas due to heavy metals from ship demolition activities is still below 1. Even though the PLI reflects low levels of pollution over time, it can increase the potential for absorption by aquatic organisms and pose ecological risks. therefore, continuous monitoring is required to identify dynamic changes in heavy metal concentrations

Antioxidant non-enzyme activities

The results of percentage of depreciation data for the *A. alba* species taken from the industrial area were 66%, and the conservation zone was 65.8%. While for the *E. agallocha* species from the industrial area it was 68.5% and the conservation zone was 67.9% in the conservation zone. Conversely, the findings of the percentage of dry weight of *A. alba* in the industrial area were 34%, and the conservation zone was 34.3%. In the *E. agallocha*, the percentage of dry weight in the industrial area was recorded at 31.5% and the zone was 32.1% (Table 4).

Table 4

Depreciation percentage of weight

Location	Sample	Sample weight (g)		Depreciation	Weight
Location	leaves	Wet	Dry	percentage (%)	percentage (%)
Industry area	A. alba	800	272	66	34
	E. agallocha	800	252	68.5	31.5
Conservation	A. alba	800	274	65.8	34.3
zone	E. agallocha	800	257	67.9	32.1

The removal of water content from the sample can be achieved by drying it until all moisture is eliminated, as the presence of water can influence the stability of bioactive compounds during extraction. Certain compounds may remain more stable or be less prone to chemical degradation or oxidation in dry conditions. The extraction of leaf samples from *A*. *alba* and *E. agallocha* was performed using ethanol as the solvent. The results indicated that the extract yield from the *A. alba* leaves was the highest at 8.80%, which was obtained from the conservation area (Table 5).

Table 5

Percentage of etanol extract

	Sample	Extract v	veight (g)	Depreciation	Extract
Location	leaves	Dry	Crude	percentage (%)	percentage (%)
		powder	extract		
Industry area	A. alba	250	22.01	91.20	8.80
	E. agallocha	250	17.33	93.07	6.93
	A. alba	250	13.17	94.73	5.27

Conservation	E. agallocha				
zone	0	250	21.42	91.43	8.57

Based on Table 5, these results indicate that environmental conditions, both in industrial areas and conservation zones, have the potential to affect the weight of crude extracts and the percentage of depreciation of *A. alba* and *E. agallocha* leaves, with the possibility of differences in the composition of bioactive compounds in each location. The maceration and extraction processes are important steps in testing the content of bioactive compounds in samples, especially in separating compound components from mangrove extracts [115]. The use of solvents such as ethanol, which are amphipathic, allows the dissolution of both polar and nonpolar compounds, so that it is optimal for obtaining various bioactive compounds from mangroves, which contain various types of compounds with these properties [115, 116, 117]. A high percentage of extraction weight indicates the effectiveness of the extraction method, indicating the method's ability to obtain active compounds from the sample optimally [119]. High extraction results also indicate a high content of active compounds in the sample, which possess the capability to have biological value and other practical applications [120].

The potential antioxidant content is illustrated by the percentage value of free radical scavenging inhibition along with the IC₅₀ value. The results of the antioxidant test on mangrove leaves using the DPPH radical scavenging method using ethanol solvent (Table 6). The IC₅₀ value content in the industrial area for *A. alba* of 137.8 µg/ml is classified as a moderate and *E. agallocha* of 21.63 µg/ml is classified as a very strong. While in the conservation area, *A. alba* of 64.32 µg/ml is classified as a strong and *E. agallocha* of 41.43 µg/ml is also classified as a very strong.

Table 6

Classification of IC50

Location	Sample	Linear regression			IC ₅₀	Category
Location	leaves	a	b	R ²	(µg/ml)	
In duction and	A. alba	36,277	128,7	0,9429	137,8	Moderate
Industry area	E. agallocha	30,953	45,165	0,9419	21,63	Very strong
Conservation	A. alba	28,726	69,611	0,8905	64,32	Strong
zone	E. agallocha	18,425	18,661	0,904	41,43	Very strong

The IC₅₀ classification results indicate that *A. alba* leaves from both areas fall into the strong-moderate category, while *E. agallocha* is classified as very strong. According to Kodikara et al. (2020), the difference in the strength of antioxidant activity in each species is thought to be because mangroves have different tolerances to certain environmental conditions, and this can affect the extent to which they can overcome heavy metal toxicity. Previous research explained that the genus Avicennia is a mangrove found in the front zone and directly facing the waters [122]. *Avicennia spp.* has strong and dense aerial roots so that it is able to efficiently capture and bind mud and various pollutants carried by water [122, 123]. As a type of plant that is periodically submerged in water, the roots of mangroves are able to take, absorb, or reduce contaminants through the dilution process [124, 125]. Therefore, it is hypothesized that contaminants absorbed by roots do not induce excessive oxidative stress and do not increase the production of secondary metabolites.

Another study in the Island of Weno area, Chuuk State of Micronesia, for the antioxidant activity of *Rhizophora stylosa* roots was 41.3% and *Sonneratia alba* 40.7% [61]. While the IC_{50} value of the *E. agallocha* in both areas is included in the high category. *E. agallocha* in this study was found in the ladward zone. This zone is rarely submerged by seawater and is

more often affected by lower tides. This is thought to be the cause of the low water content in the leaves of *E. agallocha* as presented in Table 4, so that the pollutants absorbed are greater and last longer in the leaves. Therefore, the roots act to mitigate stress effectively by producing antioxidant activity [127]. The concentration of antioxidant activity (IC_{50}) in the leaves showed different values in the two areas. The differences that occur in the ability to produce antioxidant activity in each mangrove as a form of self-defense against oxidative stress are due to differences in morphology, habitat, tides, sediment substrates, and environmental conditions [127, 128]. Kumar et al. (2019) also found that mangrove sediments in intertidal zones are rich in organic matter, including phenolic compounds and triterpenoids, which contribute to antioxidant potential. The presence of triterpenoids such as <u>taraxerol acetate</u>, germanicol, and β -amyrin suggests a strong chemotaxonomic link between mangrove-derived organic matter and plant defense mechanisms against oxidative stress. Differences in IC₅₀ classification results can reflect differences in the level of heavy metal exposure in the two locations.

In addition to testing antioxidant activity using the DPPH method, this activity can also be analyzed by calculating total phenol. Measuring the total phenol content is done by adding Folin-ciocalteu reagent to the solution sample being tested (Table 7). Phenols possess antioxidant properties that play a role in protecting plant tissues from damage induced by free radicals. Therefore, the total phenol test can provide information about the potential antioxidant activity of mangrove leaf extracts. In this study, the highest quantitative phenol value was found in *E. agallocha* at 398.80 mg GAE/gr from the industrial area and the smallest in *A. alba* at 21.85 mg GAE/gr from the conservation forest area.

Table 7

Total phenol of mangrove leaves extract

Location

Sample leaves

Phenol (mg GAE/g)

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	A. alba	36.68
Industry	E. agallocha	398.80
Conservation	A. alba	21.85
	E. agallocha	320.44

The total phenol obtained in this study has a positive relationship with antioxidant activity, as indicated by the IC₅₀ value in Table 7. The antioxidant activity of this mangrove is influenced by its total phenol content. The total phenol content is positively correlated with antioxidant activity, where the higher the total phenol content, the higher the antioxidant activity in the sample [131]. Based on this study, A. alba has a lower total phenol content than E. agallocha, which is strongly suspected due to differences in environmental factors. Mangroves in the pioneer zone more pressure from pollutants and the physicochemical conditions of the habitat. This is in line with previous findings, where the total phenol content in the roots of A. marina in the pioneer zone was 26.11 mg GAE/g, lower than B. gymnorrhiza in the landward zone with 344.02 mg GAE/g [132]. Mangrove ecosystems located in the pioneer zone tend to have special adaptations to survive in coastal environments that are often inundated by sea tides [131, 132]. Mangrove sediments in intertidal zones are rich in organic matter originating from terrestrial vascular plants, including phenolic compounds and triterpenoids, which contribute to their antioxidant potential [130]. Mangroves mitigate pollutants by reducing their concentration and toxicity through internal water content regulation, preventing excessive accumulation of absorbed contaminants [135]. According to Laoué et al. (2022)], non-enzymatic antioxidant activity is not produced exclusively because there is a certain limit for excess free radicals. However, the non-enzymatic antioxidant system is usually activated when free radical levels or oxidative stress exceed normal defense capacity [26].

GC-MS analysis using *E. agallocha* mangrove leaf samples from industrial areas because they are included in the IC₅₀ classification is very strong among others. The graph revealed 15 peak points identifying compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids (Figure 5). The identified compounds, based on chromatogram peak heights and mass spectra from the analysis, match those in the WILEY 7 database library (Table 8).

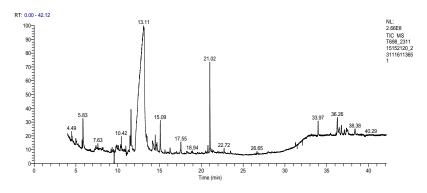


Figure 5. GC-MS chromatogram of bioactive compounds in mangrove leaves E. agallocha (Industry area)

Table 8

Retetion time, peak area, compound name, formula, and compound group (Daun E. agallocha)

Ret.	Peak	Compound name	Formula	Compound group
time	Area %			
5.84	2.45	4H-Pyran-4-one, 2	2,3- C ₆ H ₈ O ₄	Flavonoid
		dihydro-3,5-dihydrox	xy-	
		6-methyl		
9.49	1.68	2-Myristynoyl	$C_{23}H_{45}N_2O_4$	S Lipid
		pantetheine		

9.77	1.65	Paromomycin	$C_{23}H_{45}N_5O_{14}$	Glikosida
9.87	1.17	2-Myristynoyl	$C_{23}H_{45}N_2O_4S$	Lipid
		pantetheine		
11.46	1.16	Desulphosinigrin	$C_{11}H_{21}NO_9S_2 \\$	Glukosinolat
11.59	3.31	2-O-Methyl-D-	$\underline{C_7H_{14}O_6}$	Glikosida
		mannopyranosa		
13.10	73.97	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Glukosa
14.16	1.84	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Glukosa
14.48	0.99	7-Methyl-Z-tetradecen-	C ₁₇ H ₃₄ O ₂	Ester
		1-ol acetate		
14.69	1.05	9-Octadecenoic acid, (2-	$\underline{C_{28}H_{44}O_4}$	Ester
		phenyl-1,3-dioxolan-4-		
		yl)methyl ester, trans-		
15.09	2.29	2,6,8-	$C_{11}H_{18}O_2$	Terpenoid
		Trimethylbicyclo[4.2.0]		
		oct-2-ene -1,8-diol		
17.55	0.98	Hexadecanoic acid,	C ₁₇ H ₃₄ O ₂	Asam lemak
		methyl ester		
21.01	4.87	Phytol	$C_{20}H_{40}O$	Terpenoid
33.97	0.94	9-Desoxo-9-x-acetoxy-	$C_{21}H_{30}O_9$	Glikosida
		3,8,12-tri-O-ac		
		etylingol		
36.27	1.65	1-	$C_{21}H_{44}O_4Si$	Ester
		Monolinoleoylglycerol		
		trimethylsilyl ether		

Based on Table 8, 8 groups of compounds were found. The groups of compounds that are thought to be formed in response to the environment that increases antioxidant activity, such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl found in these leaves is classified as a flavonoid. Flavonoids are specialized metabolites commonly found in plants, serving multiple functions such as defense and signaling, particularly under stress conditions [137]. Flavonoids are categorized into several groups, including chalcones, aurones, flavanonols, flavones, isoflavones, flavanols, flavonols, anthocyanins, proanthocyanidins, and leucoanthocyanidins. They can exist as aglycones, glycosides, and methylated derivatives. The compounds 2-Myristynoyl pantetheine and 2-O-Methyl-D-mannopyranose are classified as lipids. Lipid compounds can exhibit antioxidant activity, especially through mechanisms involving phenols and other structures that modulate oxidative stress and lipid peroxidation processes [138].

The compounds Paromomycin, 2-O-Methyl-D-mannopyranose, and 9-Desoxo-9-xacetoxy-3,8,12-tri-O-ac etylingol are classified as glycoside compounds. Based on the results of the study of Yang et al. (2018), flavonoid glycosides are widely distributed in plants, where they function as phytoalexins to combat biotic stress. Desulphosinigrin is a glucosinolate known to exhibit anticancer and antimicrobial properties [140]. 4-methylsulfinylbutyl glucosinolate is a glucosinolate derived from the amino acid methionine, which has antioxidant, antifungal, and antimicrobial activities [141]. The compounds 7-Methyl-Ztetradecen-1-ol acetate, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, and 1-Monolinoleoylglycerol trimethylsilyl ether are classified as esters. Clearly show that ester groups with different aromatic and alkyl chains will increase antioxidant capacity. e compound 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1 carboxaldehyde is categorized as an aldehyde. This type of compound is commonly found in various essential oils and contributes a distinctive aroma to certain plants. Several phenolic aldehydes and derivatives have antioxidant activity [142].

Compounds 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene -1,8-diol and phytol belong to the terpenoid compound group. Terpenoids are promising lead compounds for further structural modification and optimization because of their potent anti-inflammatory effects [141, 142]. Terpenoids (such as monoterpenes and carotenoids) and polyphenols (such as quercetin and other flavonoids) are important phytochemicals with various antioxidant effects [145]. Hexadecanoic acid, methyl ester compounds are classified as fatty acid compounds. Fatty acids have been found to be associated with various biological activities such as anti-inflammatory, antioxidant, antifeedant, antimicrobial, and neuroprotective [146]. While compounds that have no relationship with antioxidant activity are the glucose compound group found in leaf extracts. Glucose produced through photosynthesis and other carbohydrate processes can be used as an energy source to maintain cell vitality [147].

Correlation of heavy metal concentrations and biomarkers

The relationship between heavy metal concentrations and antioxidant activities in mangrove leaves in both areas using Pearson correlation analysis, which begins with assumption testing (Table 9). The test results were obtained for all variables with significance > 0.05, and if the skewness and quasi-sequence ratios are in the range of -1.96 and +1.96, it can be concluded that the data distribution is normal.

St.Dev

Table 9

Assumption test results

Sample Variable Mean

Sig.2 tailed Skewness

Values

					Kurtosis	
	Pb	0.94	0.12	0.927	0.55 dan 0.55	Normal
Leaves	Cu	3.57	0.080	0.498	0.33 dan 1.35	Normal
Leaves	IC50	66.35	25.19	0.457	1.31 dan 0.69	Normal
	Total Phenol	194.44	193.48	0.182	0.13 dan 1.93	Normal

Based on the results of the assumption test, the normal distribution of the data can explain that the statistical parameters used in the correlation analysis provide an accurate picture of the center and distribution of the data. Furthermore, the results of the Pearson correlation test (r) and the coefficient of determination (Kd) are summarized in Table 10.

Table 10

Results of the Pearson correlation test (r) and coefficient of determination (Kd)

Sample	Variable (X-Y)	r	Kd (%)	Interpretation
	$Pb - IC_{50}$	-0.906	82.08	Strong correlation
Leaves	$Cu-IC_{50} \\$	-0.937	87.79	Strong correlation
Louves	Pb – Total Phenol	0.904	81.72	Strong correlation
	Cu – Total Phenol	0.949	90.06	Strong correlation

The results of the correlation test is a significant correlation or relationship between heavy metals and physiological responses ($r \neq 0$). The relationship between Pb and Cu to antioxidant activity in mangrove leaves produced from both areas has a very high negative correlation direction of -0.906 and -0.937. The relationship between Pb and Cu to total phenol in leaf samples is also very strong, with a very high positive correlation value of 0.904 and 0.949. In addition, the percentage of the determination coefficient (Kd) indicates that variables X and Y have a strong relationship. The Kd value of mangrove leaf samples ranges from 81.72% to 90.06%. This indicates that most of the variations in IC₅₀ and total phenol can be explained by the Pb and Cu variables in both types of samples.

A high correlation indicates a strong relationship between the variables concerned and significantly supports the hypothesis. A negative relationship with IC₅₀ indicates that the higher the concentration of Pb or Cu, the lower the IC₅₀ value (higher antioxidant potential). A positive relationship with total phenol indicates that the higher the concentration of Pb or Cu, the total phenol content also increases. Furthermore, the results of GCMS screening also showed the presence of compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Previous studies have shown that some of these compounds, especially the flavonoid and terpenoid groups, have significant antioxidant activity [148]. Therefore, increasing concentrations of heavy metals can indirectly affect the profile of secondary metabolite compounds in mangrove plants, which in turn can affect antioxidant activity and response to oxidative stress. Excessive concentrations of heavy metals cause the formation of ROS and affect the activity of antioxidants involved in plant metabolism [149]. According to Georgiadou et al. (2018), detoxification of ROS due to heavy metal contamination by producing antioxidant enzymes plays a central and vital role in protection in mangrove species.

In line with the research by [151], that under abiotic stress conditions, such as heavy metal contamination, the production of reactive oxygen species (ROS) increases in plants, resulting in the induction of oxidative stress, and plants initiate antioxidant production that significantly delays or prevents oxidative stress. According to Angon et al. (2024), secondary metabolite compounds are involved in plant responses to biotic and abiotic stresses and contribute significantly to the antioxidant activity of plant tissues. Antioxidant activity is a common approach used to increase heavy metal tolerance, strengthening the defense system

against oxidative stress [151, 152]. Several previous studies have found a relationship between heavy metal pollution and the physiological response of plants, especially mangroves. The decline in sediment quality due to heavy metal pollution in a gradual pattern that has the potential to have a negative impact on the biogeochemical cycle, with potentially fatal consequences for the survival of biodiversity (*A. marina*) [155]. Furthermore, the results of the study by Ghosh et al. (2021) also stated that there was a statistically significant relationship between the activity of antioxidant enzymes, photosynthetic pigments, and heavy metal contamination, resulting in the biotic response of riparian mangroves characterized by reduced photosynthetic pigments (chlorophyll a and b) and increased activity of antioxidant stress enzymes (POD, CAT, and SOD). The response of two tropical medicinal plant species to heavy metal accumulation can increase hydrogen peroxide (H₂O₂) activity, malondialdehyde content, enzymatic activity, and nonenzymatic antioxidants [156].

Mangroves cause trigger antioxidant defenses to overcome heavy metal absorption and normalize excessive production of oxidative stress mediated by reactive oxygen species (ROS) [157]. However, antioxidant responses in mangroves vary depending on the concentration and type of heavy metals, plant species, and duration of exposure [158]. Previous findings related to plant reactions to higher concentrations of heavy metals in the soil. For example, Kulbat-Warycha et al. (2020) observed that an increase in the concentration of heavy metals (Ni, Cu, Zn) caused a decrease in the concentration of phenols in oregano, which was associated with the induction of severe oxidative stress. According to Mansoor et al. (2023), excessive ROS production due to severe oxidative stress can cause damage to the mitochondrial respiratory chain, uncoupling of oxidative phosphorylation, and mitochondrial death in plants. However, this can also experience a decrease in the antioxidant activity defense system of the mangrove itself if the contamination of absorbed pollutants exceeds the threshold and severe oxidative stress occurs, which can cause damage and death to the mangrove ecosystem [159, 160].

The correlation between heavy metals and antioxidant activity in mangroves illustrates the complex relationship between heavy metal pollution and plant responses to oxidative stress. In this context, high concentrations of heavy metals can trigger ROS production, which in turn affects plant antioxidant activity. Excessive ROS can induce oxidative stress that activates the plant defense system to increase the production of antioxidant compounds. Thus, the relationship between heavy metals and antioxidant activity, total phenols, and secondary metabolite compound profiles in mangroves provides a deeper understanding of the mechanism of the plant's response to heavy metal pollution and oxidative stress. Therefore, if there is an indication that pollutant contamination exceeds the threshold and causes severe oxidative stress, some coastal environmental management policies can be expected in response to these findings.

To ensure the sustainability of mangrove ecosystems and mitigate the impact of heavy metal pollution, routine monitoring is recommended every 3–6 months to capture seasonal variations in heavy metal concentrations and antioxidant responses. Additionally, long-term monitoring (≥5 years) is necessary to identify trends in heavy metal accumulation and its effects on coastal ecosystems. Supplemental monitoring is also advised following specific events, such as industrial waste spills or land-use changes, to assess their immediate environmental impact. The data from this study can serve as a basis for environmental policy development, including updating regulations on heavy metal thresholds in sediments and coastal biota, strengthening conservation and mangrove rehabilitation policies, and improving industrial zone management in coastal areas. Furthermore, these findings can be utilized to raise public awareness about the importance of protecting coastal ecosystems and promoting sustainable resource management practices.

Conclusion

Heavy metal pollution of Pb and Cu resulting from areas affecting industrial and conservation activities has a significant effect on antioxidant activity in mangroves, especially *A. alba* and *E. agallocha*. Sediment pollution assessment showed that the Igeo value was at a low level, while the concentration factor (Cf) and Pollution Load Index (PLI) showed a relatively moderate level of pollution (Cf between 1 and 3, and PLI between 0 and 2). The bioaccumulation value of heavy metals in mangrove leaves was low (BCF < 1), indicating moderate accumulation of heavy metals in leaf tissue. The antioxidant activity of *E. agallocha* leaves from the industrial area was very strong and had the highest total phenol content. The compounds identified as having high antioxidant activity included flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Correlation analysis showed that increasing heavy metal concentrations were directly proportional to increasing antioxidant activity and total phenol content in mangrove leaves. This study contributes to our understanding of the potential of mangroves to respond to heavy metal exposure through increased antioxidant activity, which can support conservation efforts and sustainable management of coastal natural resources.

Author statement

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

CRediT authorship contribution statement

Rozirwan: Writing – review & editing, Supervision, Project administration, Conceptualization. Redho Yoga Nugroho: Resources, Formal analysis, Data curation. Nadila Nur Khotimah: Validation, Resources, Data curation. Fauziyah: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. Wike Ayu Eka Putri: Writing – original draft, Software, Investigation. **Riris Aryawati**: Methodology, Data curation. **Gusti Diansyah**: Software, Investigation.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The data supporting the findings of this study can be obtained from the corresponding author upon a reasonable request.

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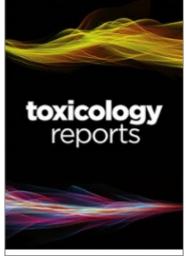
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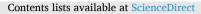
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ABSTRACT

Heavy metal contamination from industrial activities in coastal regions can lead to pollution in mangrove ecosystems. Mangroves produce antioxidant compounds to mitigate the impact of free radicals. This study aimed to analyze the correlation between the concentration of heavy metals Pb and Cu and antioxidant activity in Avicennia alba and Excoecaria agallocha mangroves from areas affected by industrial activities and conservation areas, Banyuasin, South Sumatra, Indonesia. This study was conducted in September 2023 with sampling locations in the Payung Island area and the Barong River conservation area, Berbak Sembilang National Park. The samples taken included sediment and mangrove leaves. The concentration of heavy metals Pb and Cu was measured by atomic absorption spectrometry. Antioxidant activity test using the DPPH test, total phenol using the Folin-Ciocalteu method, and phytochemical profile screening using GCMS. Statistical analysis of the correlation between antioxidant activity and heavy metal concentration using the Pearson correlation. The results showed that the highest concentration of heavy metals in sediment and mangrove leaves was found in the area affected by industrial activity, with a range of Pb values of 0.67 ± 0.16 –18.70 ± 0.48 mg/kg and Cu values of 3.39 \pm 0.20–6.07 \pm 0.37 mg / kg. The results of sediment pollution assessment for heavy metals Pb and Cu at Igeo < 0 indicates uncontaminated, 1 < Cf < 3 indicates low contamination, and PLI 0–2 indicates not polluted. While the results of heavy metal bioaccumulation in leaves were BCF < 1, indicates low bioaccumulation. E. agallocha leaves from the Pulau Payung area showed very strong antioxidant activity of 21.63 µg/ml, and the highest total phenol content reached 398.80 mg GAE/g. Analysis of compounds with the highest antioxidant activity identified the presence of esters, aldehydes, alcohols, fatty acids, glycosides, flavonoids, terpenoids, and steroids. Correlation analysis shows that higher heavy metal concentrations correspond to increased antioxidant activity and total phenol content (r \neq 0). These findings are expected to contribute to scientific knowledge that enhances environmental sustainability, supporting effective management of coastal natural resources.

1. Introduction

Coastal areas are transitional areas between land and sea that have abundant biodiversity and unique ecosystems [1,2]. Coastal areas face great pressure from various anthropogenic activities that can cause pollution [3,4]. Previous studies report that industrial activities like fertilizer processing, oil and gas, and crude palm oil production contribute to coastal pollution [3,5,6]. In addition, there are also agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households [7]. Continuous anthropogenic activities in coastal areas can produce pollutants, such as microplastics, heavy metals, as well as various organic and inorganic contaminants [8–10]. Among various pollutant types, heavy metals are categorized as persistent pollutants due to their resistance to decomposition [11]. Heavy metals initially present in the water column gradually settle to the sediment and eventually accumulate in aquatic organisms [12]. This condition may have adverse impacts, particularly if it exceeds environmental quality standards. These adverse impacts can affect aquatic ecosystems, including mangroves [13,14]. According to Xu et al., [15], as the largest plant community in coastal areas, mangroves are also directly affected by pollution.

Mangrove ecosystems play a vital role in coastal protection,

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supporting biological diversity, and contributing to the socio-economic development of local communities [16,17]. Additionally, their capacity to accumulate pollutants makes them valuable indicators for assessing pollution levels in coastal waters, as they can absorb and store these pollutants in their tissues, enhancing their role in monitoring environmental health [18-20]. Roots and leaves are important parts of mangroves in the absorption, accumulation, and response to pollutants [21]. Roots are the first part exposed to pollutants from their growth media. Furthermore, roots also have the ability to translocate pollutants to the leaves. Leaves are the primary site for photosynthesis in plants, supplying the energy essential for cell development, and overall plant function [22]. High concentrations of pollutants in roots and leaves can potentially increase excessive reactive oxygen species (ROS), resulting in oxidative stress in mangroves [23,24]. Oxidative stress arises from an imbalance between ROS production and detoxification, potentially leading to harmful cellular damage [25,26]. Although oxidative stress can be detrimental, plants also have a resistance response mechanism against free radicals [27]. This process involves producing antioxidant enzymes and molecules to counteract the harmful effects of free radicals. In response to environmental changes, plants enhance the activity of antioxidant defenses, including both enzymatic and non-enzymatic components such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPx), and phenolic compounds. These antioxidants serve as protective mechanisms against various environmental stress [28,29].

Research on the specific physiological adaptations of various mangrove species to pollutants is still limited. Most previous studies only focused on the accumulation of heavy metals in mangroves without exploring in depth the biochemical defense mechanisms they employ [30–32]. However, studies on how different mangrove species respond to industrial pollution in environments with varying levels of pollution have not yet been conducted. In addition, most studies only examine one mangrove species without comparing the adaptability of different species in the face of heavy metal contamination [33,34].

This study aimed to evaluate the accumulation of heavy metals (Pb

and Cu) in two mangrove species (*Avicennia alba* and *Excoecaria agallocha*) and assess their antioxidant activity in industrial and conservation zones. The selection of these two species was based on their prevalence in the research location as well as differences in habitat zones and morphological characteristics [35,36]. This study was carried out in the mangrove ecosystem, which includes areas influenced by industrial activities such as Payung Island as well as conservation areas in the Berbak Sembilang National Park [37,38].

By assessing biomarkers, new insights are provided into how mangrove species adapt to environmental stress caused by heavy metal pollution. Additionally, the research explores the impact of heavy metal contamination on the physiological responses of mangroves, focusing on their biochemical defense mechanisms. The findings aim to enhance understanding of mangrove adaptation strategies in response to pollution and offer valuable implications for coastal ecosystem conservation and environmental pollution management.

2. Materials and method

2.1. Leaf sampling

This study was conducted in September 2023. The samples included *Avicennia alba, Excoecaria agallocha*, and sediments collected from industrial and conservation zones in Banyuasin, South Sumatra, Indonesia (Fig. 1). The first area is the mangrove ecosystem on Payung Island. This area was chosen due to the high accumulation of heavy metals from industrial activities along the Musi River. Additionally, the area includes agricultural activities, ports, fish ponds, and settlements [39,40]. The second area is the Barong River conservation area, Berbak Sembilang National Park, which represents a natural area and protects flora and fauna from the threat of damage, scarcity, or deforestation [41–43].

The sampling stages include collecting sediment samples and mangrove leaves. Sediment samples were taken as supporting data to determine the concentration of heavy metals in the mangrove growth media. The availability of heavy metals in sediments has a direct effect

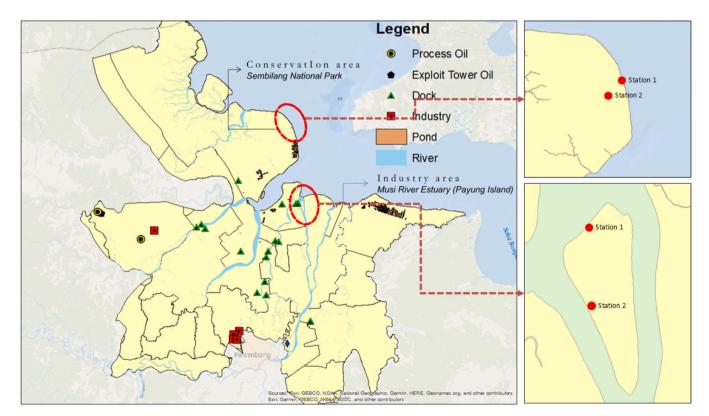


Fig. 1. Map of sample collection.

on the bioaccumulation and biomagnification processes in aquatic organisms. Sediment data helps understand the level of risk and potential impacts to organisms in mangrove ecosystems. Sediment samples were taken using a grab pipe at a depth of \pm 10 cm from the surface [44]. Sediment depth shows a very significant impact on heavy metal content, with a greater decrease in heavy metal content as sediment depth increases [45]. Samples were taken at three location points for each station, which were considered as replications. Samples were taken compositely together (taken as needed, 500 g) and placed into a polyethylene plastic container and stored in a cool box for analysis in the laboratory.

The method for collecting mangrove leaves taken from the field uses a random sampling method [46]. The random sampling method can be used if the sample studied is homogeneous. The mangrove species taken were *A. alba* and *E. agallocha*. The samples taken consisted of ± 1 kg of leaves and were put in polyethylene plastic.

2.2. Sediment grain size analysis

Grain size analysis was conducted using the sieving and pipetting methods as outlined by [47]. Sediment types (gravel, sand, silt, and clay) were classified using Shepard's triangle analysis and processed with Microsoft Excel V.2021, following the protocols established by [48,49]. The sediment fraction type was determined by identifying the most dominant composition from the analysis results.

2.3. Sample preparation

Sediment sample preparation involved removing foreign objects such as plastic fragments and leaves. The sediment was then air dried at room temperature for 72 hours until fully dry, ground to a homogeneous consistency, and stored in a tightly sealed polyethylene bottle. They were then air-dried in a shaded, well-ventilated area for five days, ensuring indirect exposure to sunlight to prevent the degradation of bioactive compounds. The drying process was conducted at ambient temperature with sufficient airflow to facilitate moisture evaporation. Once dried, the samples were ground into a fine powder and stored in sealed containers for further analysis. The extraction of heavy metals (Pb and Cu) from the sediment samples and mangrove leaves was performed using the wet destruction method, following the procedures outlined by [8,50].

2.4. Atomic absorption spectroscopic measurement

Measuring the concentration of heavy metals Pb and Cu using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Operational parameters: Pb (283.3 nm, 5 mA lamp current) and Cu (324.7 nm, 4 mA), slit width 0.5 nm, air-acetylene flame (2.0 L/min air; 1.5 L/min acetylene), burner height 5–7 mm. After 15–20 min warm-up, calibration was performed using blank and standard solutions (0.1–2.0 ppm Pb; 0.05–1.0 ppm Cu), achieving $R^2 \ge 0.995$. Samples were aspirated in triplicate with 15-sec distilled water rinsing between measurements and acid blank checks every 5 samples. Quality control included spike recovery (85–115 %), duplicate analyses (RSD <5 %). LODs: 0.02 ppm Pb; 0.01 ppm Cu (3 ×SD blank) [51].

2.5. Determination of heavy metals in leaves and sediments

2.5.1. Determination of sediment pollution

2.5.1.1. Geoacumulation index (I_{geo}). The Igeo (geo-accumulation index) quantitatively evaluates the degree of heavy metal contamination and classifies the level of pollution based on detailed categorization [52].

$$Igeo = \log_2 (Cn/1.5 Bn) \tag{1}$$

The classification of Igeo values includes the following categories: uncontaminated (Igeo \leq 0), uncontaminated to moderately contaminated (Igeo 0–1), moderately contaminated (Igeo 1–2), moderately to highly contaminated (Igeo 2–3), highly contaminated (Igeo 3–4), highly contaminated to very highly contaminated (Igeo 4–5), and very highly contaminated (Igeo \geq 5) [53].

2.6. Contamination factor (Cf)

The contamination factor is determined experimentally as the ratio of the element concentration in the sample to its background concentration [54].

$$Cf = (Cn/Bn)$$
(2)

The contamination factor (Cf) classifications are as follows: [55]: Cf < 1 = low contamination; 1 < Cf < 3 = moderate contamination; 3 < Cf < 6 = sufficient contamination; Cf > 6 = very high contamination.

2.7. Pollution load index (PLI)

The pollution load index (PLI) is utilized to assess pollution quality in a given area. The pollution load index value uses the formula [56].

$$PLI = [Cf1 \ x \ Cf2 \ x \ Cf3... \ x \ Cfn]^{1/n}$$
(3)

Pollution load index (PLI) criteria: PLI 8-10 = severely polluted; PLI 4-8 = heavily polluted; PLI 2-4 = moderately polluted; PLI 0-2 = not polluted to lightly polluted; PLI < 0 = not polluted.

2.8. Bioaccumulation of metal in leaves

2.8.1. Bioconcentration factor (BCF)

The absorption of metals by leaf from sediment occurs through a process known as bioaccumulation. The bioconcentration factor (BCF) values are utilized to assess the extent of metal bioaccumulation in mangrove leaf originating from sediment [57].

$$BCF = (Cn.leaf/Cn. sediment)$$
 (4)

BCF > 1 hyperaccumulator; BCF = 1 indicator; BCF < 1 is an excluder [58].

2.9. Analysis of antioxidant non-enzymes in leaves

2.9.1. Antioxidant activity evaluated by DPPH assay

Antioxidant activity analysis was carried out using ethanol solvent based on a method adapted from [59]. A 50 ml 0.1 μ M DPPH solution was prepared, followed by the preparation of a sample stock solution and a 10 ml pure ascorbic acid stock solution of 2000 ppm, which was homogenized. Furthermore, a series of solutions were made with concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm. At each concentration, 1 ml of 0.1 μ M DPPH solution was added to the mixture, which was then homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) at a wavelength of 517 nm. The antioxidant activity of the extract is expressed as IC₅₀, which quantifies the strength of its antioxidant capacity (Table 1). The

Table 1Characteristic value of IC50.

Concentration (µg/ml)	Characteristic
< 50	Very strong
50-100	Strong
100–150	Moderate
150-200	Low

IC₅₀ value is calculated using the following formula:

$$\% inhibition = \frac{blank \ abs - sample \ abs.}{blank \ abs} x100\%$$
(5)

The IC₅₀ value was derived by inputting the data into a linear regression equation, where the sample concentration was plotted on the X-axis and the percentage of inhibition of antioxidant activity on the Y-axis. The regression equation used is represented as y = ax + b [60].

2.10. Determination of phenol content

The analysis of total phenol content in the samples was conducted using the Folin-Ciocalteu method, as outlined in the literature [60-62]. A standard solution of 1000 ppm gallic acid as much as 50 ml was prepared, then variations in concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were made, each as much as 5 ml. For each concentration variation, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were pipetted into a 10 ml measuring flask containing a standard solution of 100 ppm gallic acid. A total of 50 mg of sample was weighed, then 2 ml of methanol and 5 ml of distilled water were added, then homogenized in a 10 ml measuring flask. In both the standard series and sample variations, 0.5 ml of 50 % Folin-Ciocalteu reagent was added, followed by the addition of distilled water up to the mark. The mixture was then allowed to stand for 5 minutes. Next, one ml of a 5 % Na₂CO₃ solution was added and incubated in a dark place for one hour. After incubation, the absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 750 nm.

2.11. Pearson correlation analysis (correlation bivariate)

The use of pearson correlation analysis (bivariate correlation) is a

method used to evaluate the relationship between two variables [63, 64], in this case to see the relationship between antioxidant activity and heavy metal concentrations. This analysis was carried out using SPSS software version 28.

3. Result and discussion

3.1. Description of mangrove leaves

The mangrove species *A. alba* and *E. agallocha* found at the sampling location exhibit distinct characteristics. Fig. 2 shows the characteristic differences between *A. alba* and *E. agallocha* leaves.

Leaves are the part that characterizes a mangrove species. When identifying each type of mangrove, observation of the morphology of the leaf shape is very important to understand the characteristics and differences in each type of leaf [65,66]. A. alba leaves have a green surface with a smooth and slippery texture, while the underside is yellowish green with a rough texture. The characteristics of the leaves is elliptical, almost oval, with a tapered tip. Based on observations, the length of the leaves ranges from 10 to 13 cm, and the width of the leaves ranges from 4 to 5 cm. E. agallocha leaves are elliptical and dark green in color, with finely serrated edges and tapered tips. The observed leaf sizes ranged from 8 to 10 cm in length and 3-4.5 cm in width. Old leaves were selected as samples for the study of heavy metal content and bioactive compounds due to several considerations related to their maturity and potential accumulation of pollutants and compounds of interest. According to [67], plants tend to produce bioactive compounds in higher amounts in older parts. This could be a plant strategy to protect itself from pests, diseases, or the external environment [68,69]. Older leaves may have more stable chemical conditions, thus facilitating analysis and minimizing variability in results.

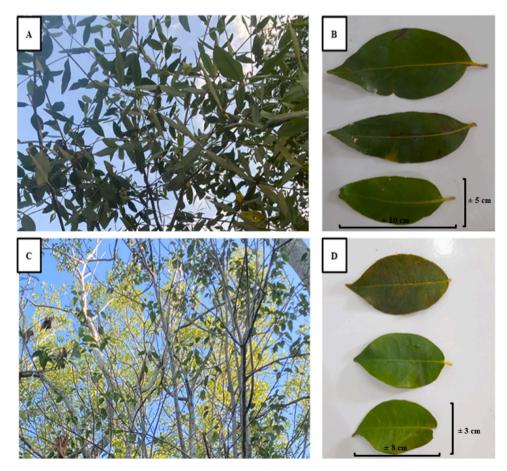


Fig. 2. Leaves description. A-B). A. alba, C-D). E. agallocha.

3.2. Sediment grain size

The determination of substrate types in the sampling was conducted using the Shepard triangle method (Fig. 3). In the mangrove ecosystem of both industrial and conservation areas, sediment substrates were categorized into four types: gravel, sand, mud, and clay. The results indicated that the predominant substrate type in both areas was clay.

The sediment substrate surrounding the mangrove ecosystem in both areas is predominantly clay, with clay percentages ranging from 80.5 % to 84.03 %. The highest clay content was observed at station 1 in the industrial area (Table 2).

Based on the results of Table 2, distribution of sediment fractions and grain sizes at two different locations, which represents two stations with different mangrove species (*A. alba* and *E. agallocha*). In the industrial area, most of the sediments consist of clay with a very low sand content (3.6 % for Station 1 and 3.36 % for Station 2), which indicates the predominance of fine materials that can influence the mobility of heavy metals and nutrients in the sediments. In contrast, in the conservation zone, although the sediment composition is still dominated by clay, the sand content is higher (22.5 % for Station 1 and 21.91 % for Station 2), indicating differences in sedimentation processes and a higher potential for water infiltration.

In the industrial area, both stations (*A. alba* and *E. agallocha*) showed a dominance of clay fractions with a very high percentage. The dominant clay fractions indicate that the sediment in this area consists of fine particles, which may be caused by the accumulation of fine particles from industrial activities around this location. Industries such as fertilizer processing, oil and gas, crude palm oil production, agricultural activities, ports, shipping, loading and unloading of coal raw materials and their products, and households contribute to the presence of fine particles in sediments [3,5–7]. Port activities involve frequent vessel movement, dredging, and cargo handling, all of which can resuspend fine particles and increase sedimentation rates [70,71]. Crude oil processing and petroleum industries may contribute to fine particle deposition through air emissions, which settle via atmospheric deposition [72]. Additionally, agricultural activities, particularly palm oil plantations,

Table 2
Sediment grain size in each station.

Location	Station	Sedimen	Sediment fraction (%)			Grain
		Gravel	Sand	Mud	Clay	size
Industry area	1 (A. alba)	0.00	3.6	12.37	84.03	Clay
	2 (E. agallocha)	0.00	3.36	16.14	80.5	Clay
Conservation	1 (A. alba)	0.00	22.5	1.95	75.55	Clay
zone	2 (E. agallocha)	0.00	21.91	2.02	76.07	Clay

can contribute to increased fine particle accumulation through soil erosion and runoff carrying clay-rich sediments into adjacent water systems, particularly during heavy rainfall [73,74].

Fine particles such as clay are usually carried by water and can accumulate in areas with slow water movement, such as near mangrove roots [75,76]. In the conservation zone, the clay fraction also dominates, although with a slightly lower percentage than the industrial area. The conservation zone may also have less influence from human activities, so the sediment pattern is more natural than the industrial area. Clay is a sediment particle with a very fine grain size and a large surface area [77, 78]. Due to its small size and its tendency to be negatively charged, clay has a high adsorption capacity, which allows clay particles to attract and bind heavy metal ions such as Hg, Pb, Cd, Cu, and others [79–81]. Consequently, sediments dominated by clay fractions tend to accumulate more heavy metals than larger sediment fractions [82,83].

3.3. Determination of heavy metals

The results of the heavy metal concentration analysis for Pb and Cu in sediments and mangrove leaves from both areas are summarized in Table 3. The concentrations of heavy metals in sediments from both the industrial area and the conservation zone exhibit variability; however, they generally remain below hazardous thresholds (ERL, ERM, TEL, and PEL). In the industrial area, the highest Pb concentration was found at Station 2 (18.70 \pm 0.48 mg/kg), while in the conservation zone, the

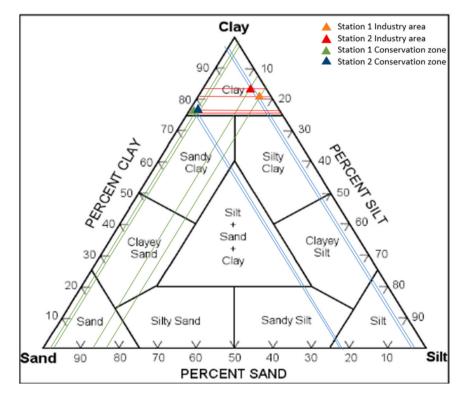


Fig. 3. Classifications of sediment type with shepard triangle method.

Table 3

Average concentrations of heavy metals (mg/kg) in mangrove sediments and leaves.

	Pb	Cu
Sediments		
Station.1 Industry area	12.63 ± 0.01	$\textbf{5.58} \pm \textbf{0.05}$
Station.2 Industry area	$\textbf{18.70} \pm \textbf{0.48}$	6.07 ± 0.37
Station.1 Conservation zone	12.61 ± 0.32	$\textbf{4.21} \pm \textbf{0.03}$
Station.2 Conservation zone	14.22 ± 0.16	$\textbf{5.17} \pm \textbf{0.17}$
ERL	46.7	34
ERM	218	270
TEL	30.2	18.7
PEL	112	108.2
Mangrove leaves		
Station.1 Industry area (A. alba)	0.67 ± 0.17	$\textbf{3.39} \pm \textbf{0.20}$
Station.2 Industry area (E. agallocha)	1.27 ± 0.31	3.73 ± 0.16
Station.1 Conservation zone (A. alba)	0.84 ± 0.12	3.50 ± 0.35
Station.2 Conservation zone (E. agallocha)	$\textbf{0.99} \pm \textbf{0.37}$	$\textbf{3.69} \pm \textbf{0.23}$

highest concentrations of Pb and Cu were each at Station 2 (Pb 14.22 \pm 0.16 mg/kg; Cu 5.17 \pm 0.17 mg/kg). For metal accumulation in mangrove leaves, Cu was recorded higher than Pb at all stations. In the industrial area, *A. alba* (Station 1) had Pb 0.67 \pm 0.17 mg/kg and Cu 3.39 \pm 0.20 mg/kg, while *E. agallocha* (Station 2) showed Pb 1.27 \pm 0.31 mg/kg and Cu 3.73 \pm 0.16 mg/kg. In the conservation zone, the highest accumulation of Cu in mangrove leaves was 3.69 \pm 0.23 mg/kg at Station 2.

The industrially impacted area in the Musi River Estuary is affected by high anthropogenic activities, making it susceptible to accumulating pollutants, especially heavy metals such as Pb and Cu. Sediments in this area tend to contain higher pollutants than water and biota, influenced by domestic, industrial, and river transportation activities that pollute the environment [84,85]. Ship and coastal building maintenance activities, including the use of anti-rust materials, electronic waste, and pipe corrosion, are the main sources of Pb, while sources of Cu in the aquatic environment come from antifouling paint, agricultural pesticides, and industrial waste [86,87]. In addition, previous studies report that cleaning ship hulls can release Cu into the marine environment [88, 89]. Fisheries sector that uses Cu-coated nets to prevent biofouling can also contribute to increasing Cu levels in waters [90,91].

The conservation area in the Barong River is also exposed to heavy metal pollution, although at a lower level, considering that some human activities such as fishing are still ongoing [92]. Unmanaged anthropogenic activities, including unregulated industrial waste disposal, improper wastewater treatment, and uncontrolled agricultural runoff, have contributed to the increasing Cu concentrations observed in both locations, as indicated by the findings of this study and previous research [7,50]. These activities introduce Cu into the aquatic system, where it binds to suspended particles and accumulates in sediments, further exacerbating environmental pollution.

In mangrove leaves, Pb was detected at low concentrations. Plants regulate Pb primarily by limiting its uptake and translocation. Because Pb is a non-essential and highly toxic metal, most of it accumulates in the roots rather than being transported to the leaves [93]. In contrast, Cu an important micronutrient for plants, is regulated through controlled absorption and detoxification mechanisms [93,94]. Plants manage excess Cu by binding it to metallothionein and phytochelatin, storing it in vacuoles, and activating the antioxidant defense system to fight oxidative stress [95,96]. Although heavy metal concentrations vary between locations, they are still below the threshold, indicating a relatively low risk of contamination. However, long-term monitoring is essential to

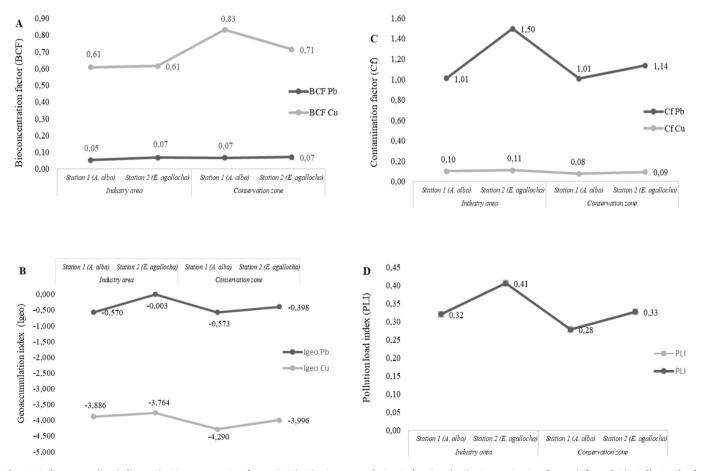


Fig. 4. Sediment quality indices. A). Bioconcentration factor (BCF), B). Geoaccumulation index (Igeo), C). Contamination factor (Cf), and D). Pollution load index (PLI).

track bioaccumulation trends in mangrove ecosystems.

3.4. Sediment quality indices

The results of the sediment quality index assessment are summarized in Fig. 4. The results of the leaf bioconcentration factor (BCF) in bioaccumulating Pb and Cu metals from sediment with a BCF value < 1 indicating low bioaccumulation. The geoaccumulation index shows uncontaminated properties for Pb and Cu with an Igeo value < 0 indicating uncontaminated. The contamination factor (Cf) shows that contamination is low and moderate in Pb and Cu with a value of 1 < Cf < 3 indicating low contamination. The PLI ranges from 0 to 2 indicating that both areas are not polluted.

The difference in bioconcentration factor (BCF) between Cu and Pb can be explained by the chemical properties of each metal. Cu accumulates more easily in biota tissues than Pb. Cu is an essential element for organisms, although at higher concentrations it can be toxic [97]. In contrast, Pb is a non-essential heavy metal that tends not to accumulate much in biota tissues [98]. The previous studies stated that essential heavy metals are more easily absorbed by organisms because they have physiological mechanisms to regulate the concentration of these elements [98,99]. Analuddin et al. [100] have also examined BCF in mangrove ecosystems, with results showing that the BCF values for Hg, Cu, Mn, Pb, and Zn > 1. This finding is thought to be related to the impact of anthropogenic activities in Kendari City, which has a high population density.

The Igeo index shows higher Pb contamination than Cu in industrial areas. Pb is thought to originate from human activities such as ports, agriculture, ship transportation, and household waste that tends to settle in sediments [32,79]. A high Igeo value indicate an anthropogenic contamination and show sediments contaminated heavy metal [101]. In addition, long-term exposure to these heavy metals can change community structure and disrupt ecosystem function through bio-accumulation and biomagnification in the food chain [102,103].

The high value of the Pb contamination factor (Cf) in industrial areas indicates that this environment is more susceptible to Pb pollution than Cu. Relevan study by Hasan et al. [104] that the CF value of Pb (0.76) > Cu (0.68) in core sediment from a mangrove at the Pasur River. Cu is more likely to be bound to organic particles and accumulate in the tissues of benthic organisms, which may explain the lower Cf Cu value. The areas suspected of being polluted tend to have higher anthropogenic activity than conservation zones, which causes significant differences in the levels of contamination and accumulation of heavy metals [105]. Industries around mangrove areas may contribute to elevated levels of heavy metals. Meanwhile, the conservation zone which is relatively protected from industrial activities, shows lower contamination values, although there are still traces of pollution due to remote pollution sources [105,106].

The PLI value in the industrial area is higher than the conservation zone. This indicates that industrial activities play a role in elevating heavy metal pollution in the area. Industrial areas are usually exposed to pollution sources such as factory waste, air pollution, and surface runoff that carry heavy metals into the sediment [107,108]. Although both stations are in the same area, there is a difference in the PLI value between Station 1 and Station 2 at both locations. Local factors, including water movement, sediment composition, and proximity to pollution sources, significantly influence the distribution of heavy metals [109]. The PLI in the conservation zone still shows heavy metal pollution. This could be due to atmospheric deposition from industrial activities in the surrounding area or pollutants carried by water currents from more contaminated areas [110,111]. This suggests that although the conservation zone has better protection, it is not completely protected from the impacts of nearby industrial pollution.

The study indicate that both areas are classified as not polluted. In line with these findings by Karmakar et al. [112], the PLI value in mangrove planting areas due to heavy metals from ship demolition activities is still below 1. Even though the PLI reflects low levels of pollution over time, it can increase the potential for absorption by aquatic organisms and pose ecological risks. therefore, continuous monitoring is required to identify dynamic changes in heavy metal concentrations

3.5. Antioxidant non-enzyme activities

The results of percentage of depreciation data for the *A. alba* species taken from the industrial area were 66 %, and the conservation zone was 65.8 %. While for the *E. agallocha* species from the industrial area it was 68.5 % and the conservation zone was 67.9 % in the conservation zone. Conversely, the findings of the percentage of dry weight of *A. alba* in the industrial area were 34 %, and the conservation zone was 34.3 %. In the *E. agallocha*, the percentage of dry weight in the industrial area was recorded at 31.5 % and the zone was 32.1 % (Table 4).

The removal of water content from the sample can be achieved by drying it until all moisture is eliminated, as the presence of water can influence the stability of bioactive compounds during extraction. Certain compounds may remain more stable or be less prone to chemical degradation or oxidation in dry conditions. The extraction of leaf samples from *A. alba* and *E. agallocha* was performed using ethanol as the solvent. The results indicated that the extract yield from the *A. alba* leaves was the highest at 8.80 %, which was obtained from the conservation area (Table 5).

Based on Table 5, these results indicate that environmental conditions, both in industrial areas and conservation zones, have the potential to affect the weight of crude extracts and the percentage of depreciation of A. alba and E. agallocha leaves, with the possibility of differences in the composition of bioactive compounds in each location. The maceration and extraction processes are important steps in testing the content of bioactive compounds in samples, especially in separating compound components from mangrove extracts [113]. The use of solvents such as ethanol, which are amphipathic, allows the dissolution of both polar and nonpolar compounds, so that it is optimal for obtaining various bioactive compounds from mangroves, which contain various types of compounds with these properties [113-115]. A high percentage of extraction weight indicates the effectiveness of the extraction method, indicating the method's ability to obtain active compounds from the sample optimally [116]. High extraction results also indicate a high content of active compounds in the sample, which possess the capability to have biological value and other practical applications [117].

The potential antioxidant content is illustrated by the percentage value of free radical scavenging inhibition along with the IC₅₀ value. The results of the antioxidant test on mangrove leaves using the DPPH radical scavenging method using ethanol solvent (Table 6). The IC₅₀ value content in the industrial area for *A. alba* of 137.8 µg/ml is classified as a moderate and *E. agallocha* of 21.63 µg/ml is classified as a very strong. While in the conservation area, *A. alba* of 64.32 µg/ml is classified as a very strong and *E. agallocha* of 41.43 µg/ml is also classified as a very strong.

The IC_{50} classification results indicate that *A. alba* leaves from both areas fall into the strong-moderate category, while *E. agallocha* is classified as very strong. According to Kodikara et al. [118], the difference in the strength of antioxidant activity in each species is thought to be

Depreciation percentage of wei	ght.	
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Location	Sample leaves	Sample weight (g)		Depreciation percentage (%)	Weight percentage	
		Wet	Dry		(%)	
Industry area	A. alba	800	272	66	34	
	E. agallocha	800	252	68.5	31.5	
Conservation	A. alba	800	274	65.8	34.3	
zone	E. agallocha	800	257	67.9	32.1	

Table 5

Percentage of etanol extract.

Location	Sample leaves	Extract weight (g)		Depreciation percentage (%)	Extract percentage (%)
		Dry powder	Crude extract		
Industry area	A. alba	250	22.01	91.20	8.80
	E. agallocha	250	17.33	93.07	6.93
Conservation zone	A. alba	250	13.17	94.73	5.27
	E. agallocha	250	21.42	91.43	8.57

Table 6

Classification of IC50.

Location	Sample leaves	Linear regression	Linear regression			Category
		a	b	R ²		
Industry area	A. alba	36.277	128.7	0.9429	137.8	Moderate
	E. agallocha	30.953	45.165	0.9419	21.63	Very strong
Conservation zone	A. alba	28.726	69.611	0.8905	64.32	Strong
	E. agallocha	18.425	18.661	0.904	41.43	Very strong

because mangroves have different tolerances to certain environmental conditions, and this can affect the extent to which they can overcome heavy metal toxicity. Previous research explained that the genus *Avicennia* is a mangrove found in the front zone and directly facing the waters [119]. *Avicennia spp.* has strong and dense aerial roots so that it is able to efficiently capture and bind mud and various pollutants carried by water [119,120]. As a type of plant that is periodically submerged in water, the roots of mangroves are able to take, absorb, or reduce contaminants through the dilution process [121,122]. Therefore, it is hypothesized that contaminants absorbed by roots do not induce excessive oxidative stress and do not increase the production of secondary metabolites.

Another study in the Island of Weno area, Chuuk State of Micronesia, for the antioxidant activity of Rhizophora stylosa roots was 41.3 % and Sonneratia alba 40.7 % [61]. While the IC_{50} value of the *E*. agallocha in both areas is included in the high category. E. agallocha in this study was found in the ladward zone. This zone is rarely submerged by seawater and is more often affected by lower tides. This is thought to be the cause of the low water content in the leaves of E. agallocha as presented in Table 4, so that the pollutants absorbed are greater and last longer in the leaves. Therefore, the roots act to mitigate stress effectively by producing antioxidant activity [123]. The concentration of antioxidant activity (IC₅₀) in the leaves showed different values in the two areas. The differences that occur in the ability to produce antioxidant activity in each mangrove as a form of self-defense against oxidative stress are due to differences in morphology, habitat, tides, sediment substrates, and environmental conditions [124,125]. Kumar et al. [126] also found that mangrove sediments in intertidal zones are rich in organic matter, including phenolic compounds and triterpenoids, which contribute to antioxidant potential. The presence of triterpenoids such as taraxerol acetate, germanicol, and β -amyrin suggests a strong chemotaxonomic link between mangrove-derived organic matter and plant defense mechanisms against oxidative stress. Differences in IC50 classification results can reflect differences in the level of heavy metal exposure in the two locations.

In addition to testing antioxidant activity using the DPPH method, this activity can also be analyzed by calculating total phenol. Measuring

Table 7

Location	Sample leaves	Phenol (mg GAE/g)
Industry area	A. alba	36.68
	E. agallocha	398.80
Conservation zone	A. alba	21.85
	E. agallocha	320.44

the total phenol content is done by adding Folin-ciocalteu reagent to the solution sample being tested (Table 7). Phenols possess antioxidant properties that play a role in protecting plant tissues from damage induced by free radicals. Therefore, the total phenol test can provide information about the potential antioxidant activity of mangrove leaf extracts. In this study, the highest quantitative phenol value was found in *E. agallocha* at 398.80 mg GAE/gr from the industrial area and the smallest in *A. alba* at 21.85 mg GAE/gr from the conservation area.

The total phenol obtained in this study has a positive relationship with antioxidant activity, as indicated by the IC₅₀ value in Table 7. The antioxidant activity of this mangrove is influenced by its total phenol content. The total phenol content is positively correlated with antioxidant activity, where the higher the total phenol content, the higher the antioxidant activity in the sample [66]. Based on this study, A. alba has a lower total phenol content than E. agallocha, which is strongly suspected due to differences in environmental factors. Mangroves in the pioneer zone more pressure from pollutants and the physicochemical conditions of the habitat. This is in line with previous findings, where the total phenol content in the roots of A. marina in the pioneer zone was 26.11 mg GAE/g, lower than B. gymnorrhiza in the landward zone with 344.02 mg GAE/g [127]. Mangrove ecosystems located in the pioneer zone tend to have special adaptations to survive in coastal environments that are often inundated by sea tides [128,129]. Mangrove sediments in intertidal zones are rich in organic matter originating from terrestrial vascular plants, including phenolic compounds and triterpenoids, which contribute to their antioxidant potential [126]. Mangroves mitigate pollutants by reducing their concentration and toxicity through internal water content regulation, preventing excessive accumulation of absorbed contaminants [130]. According to Laoué et al. [131], non-enzymatic antioxidant activity is not produced exclusively because there is a certain limit for excess free radicals. However, the non-enzymatic antioxidant system is usually activated when free radical levels or oxidative stress exceed normal defense capacity [132].

GC-MS analysis using *E. agallocha* mangrove leaf samples from industrial areas because they are included in the IC_{50} classification is very strong among others. The graph revealed 15 peak points identifying compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids (Fig. 5). The identified compounds, based on chromatogram peak heights and mass spectra from the analysis, match those in the WILEY 7 database library (Table 8).

Based on Table 8, 8 groups of compounds were found. The groups of compounds that are thought to be formed in response to the environment that increases antioxidant activity, such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids.

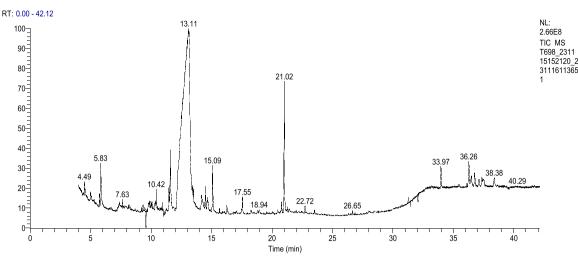


Fig. 5. GC-MS chromatogram of bioactive compounds in mangrove leaves E. agallocha (Industry area).

Table 8

Retetion time, peak area, compound name, formula, and compound group (*E. agallocha*).

Ret. time	Peak Area %	Compound name	Formula	Compound group
5.84	2.45	4H-Pyran—4-one, 2,3- dihydro—3,5-dihydroxy—6- methyl	$C_6H_8O_4$	Flavonoid
9.49	1.68	2-Myristynoyl pantetheine	$C_{23}H_{45}N_2O_4S$	Lipid
9.77	1.65	Paromomycin	C23H45N5O14	Glikosida
9.87	1.17	2-Myristynoyl pantetheine	$C_{23}H_{45}N_2O_4S$	Lipid
11.46	1.16	Desulphosinigrin	$C_{11}H_{21}NO_9S_2$	Glukosinolat
11.59	3.31	2-O-Methyl-D- mannopyranosa	$C_7H_{14}O_6$	Glikosida
13.10	73.97	3-O-Methyl-d-glucose	C7H14O6	Glukosa
14.16	1.84	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Glukosa
14.48	0.99	7-Methyl-Z-tetradecen–1-ol acetate	$C_{17}H_{34}O_2$	Ester
14.69	1.05	9-Octadecenoic acid, (2- phenyl–1,3-dioxolan–4-yl) methyl ester, trans-	$C_{28}H_{44}O_4$	Ester
15.09	2.29	2,6,8-Trimethylbicyclo [4.2.0]oct–2-ene –1,8-diol	$C_{11}H_{18}O_2$	Terpenoid
17.55	0.98	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Asam lemak
21.01	4.87	Phytol	$C_{20}H_{40}O$	Terpenoid
33.97	0.94	9-Desoxo–9-x- acetoxy–3,8,12-tri-O-ac etylingol	$C_{21}H_{30}O_9$	Glikosida
36.27	1.65	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{21} H_{44} O_4 Si$	Ester

The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl found in these leaves is classified as a flavonoid. Flavonoids are specialized metabolites commonly found in plants, serving multiple functions such as defense and signaling, particularly under stress conditions [131]. Flavonoids are categorized into several groups, including chalcones, aurones, flavanonols, flavones, isoflavones, flavanols, flavonols, anthocyanins, proanthocyanidins, and leucoanthocyanidins. They can exist as aglycones, glycosides, and methylated derivatives. The compounds 2-Myristynoyl pantetheine and 2-O-Methyl-D-mannopyranose are classified as lipids. Lipid compounds can exhibit antioxidant activity, especially through mechanisms involving phenols and other structures that modulate oxidative stress and lipid peroxidation processes [133].

The compounds Paromomycin, 2-O-Methyl-D-mannopyranose, and 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-ac etylingol are classified as

glycoside compounds. Based on the results of the study of Yang et al. [134], flavonoid glycosides are widely distributed in plants, where they function as phytoalexins to combat biotic stress. Desulphosinigrin is a glucosinolate known to exhibit anticancer and antimicrobial properties [135]. 4-methylsulfinylbutyl glucosinolate is a glucosinolate derived from the amino acid methionine, which has antioxidant, antifungal, and antimicrobial activities [136]. The compounds 7-Methyl-Z-tetradecen-1-ol acetate, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, and 1-Monolinoleovlglycerol trimethylsilyl ether are classified as esters. Clearly show that ester groups with different aromatic and alkyl chains will increase antioxidant capacity. e compound 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1 carboxaldehyde is categorized as an aldehyde. This type of compound is commonly found in various essential oils and contributes a distinctive aroma to certain plants. Several phenolic aldehydes and derivatives have antioxidant activity [137].

Compounds 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene -1,8-diol and phytol belong to the terpenoid compound group. Terpenoids are promising lead compounds for further structural modification and optimization because of their potent anti-inflammatory effects [138, 139]. Terpenoids (such as monoterpenes and carotenoids) and polyphenols (such as quercetin and other flavonoids) are important phytochemicals with various antioxidant effects [140]. Hexadecanoic acid, methyl ester compounds are classified as fatty acid compounds. Fatty acids have been found to be associated with various biological activities such as anti-inflammatory, antioxidant, antifeedant, antimicrobial, and neuroprotective [141]. While compounds that have no relationship with antioxidant activity are the glucose compound group found in leaf extracts. Glucose produced through photosynthesis and other carbohydrate processes can be used as an energy source to maintain cell vitality [142].

3.6. Correlation of heavy metal concentrations and biomarkers

The relationship between heavy metal concentrations and antioxidant activities in mangrove leaves in both areas using Pearson correlation analysis, which begins with assumption testing (Table 9). The test results were obtained for all variables with significance > 0.05, and if the skewness and quasi-sequence ratios are in the range of -1.96 and + 1.96, it can be concluded that the data distribution is normal.

Based on the results of the assumption test, the normal distribution of the data can explain that the statistical parameters used in the correlation analysis provide an accurate picture of the center and distribution of the data. Furthermore, the results of the pearson correlation test (r) and the coefficient of determination (Kd) are summarized in Table 10.

Table 9

Assumption test results.

Sample	Variable	Mean	St.Dev	Sig.2 tailed	Skewness Kurtosis	Values
Leaves	Pb	0.94	0.12	0.927	0.55 dan 0.55	Normal
	Cu	3.57	0.080	0.498	0.33 dan 1.35	Normal
	IC50	66.35	25.19	0.457	1.31 dan 0.69	Normal
	Total Phenol	194.44	193.48	0.182	0.13 dan 1.93	Normal

Table 10

Results of the Pearson correlation test (r) and coefficient of determination (Kd).

Sample	Variable (X-Y)	r	Kd (%)	Interpretation
Leaves	$Pb - IC_{50}$	-0.906	82.08	Strong correlation
	$Cu - IC_{50}$	-0.937	87.79	Strong correlation
	Pb – Total Phenol	0.904	81.72	Strong correlation
	Cu – Total Phenol	0.949	90.06	Strong correlation

The results of the correlation test is a significant correlation or relationship between heavy metals and physiological responses (r \neq 0). The relationship between Pb and Cu to antioxidant activity in mangrove leaves produced from both areas has a very high negative correlation direction of -0.906 and -0.937. The relationship between Pb and Cu to total phenol in leaf samples is also very strong, with a very high positive correlation value of 0.904 and 0.949. In addition, the percentage of the determination coefficient (Kd) indicates that variables X and Y have a strong relationship. The Kd value of mangrove leaf samples ranges from 81.72 % to 90.06 %. This indicates that most of the variations in IC₅₀ and total phenol can be explained by the Pb and Cu variables in both types of samples.

A high correlation indicates a strong relationship between the variables concerned and significantly supports the hypothesis. A negative relationship with IC₅₀ indicates that the higher the concentration of Pb or Cu, the lower the IC₅₀ value (higher antioxidant potential). A positive relationship with total phenol indicates that the higher the concentration of Pb or Cu, the total phenol content also increases. Furthermore, the results of GCMS screening also showed the presence of compounds such as flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Previous studies have shown that some of these compounds, especially the flavonoid and terpenoid groups, have significant antioxidant activity [143]. Therefore, increasing concentrations of heavy metals can indirectly affect the profile of secondary metabolite compounds in mangrove plants, which in turn can affect antioxidant activity and response to oxidative stress. Excessive concentrations of heavy metals cause the formation of ROS and affect the activity of antioxidants involved in plant metabolism [144]. According to Georgiadou et al. [145], detoxification of ROS due to heavy metal contamination by producing antioxidant enzymes plays a central and vital role in protection in mangrove species.

In line with the research by [146], that under abiotic stress conditions, such as heavy metal contamination, the production of reactive oxygen species (ROS) increases in plants, resulting in the induction of oxidative stress, and plants initiate antioxidant production that significantly delays or prevents oxidative stress. Secondary metabolite compounds are involved in plant responses to biotic and abiotic stresses and contribute significantly to the antioxidant activity of plant tissues [103]. Antioxidant activity is a common approach used to increase heavy metal tolerance, strengthening the defense system against oxidative stress [146,147]. Several previous studies have found a relationship between heavy metal pollution and the physiological response of plants, especially mangroves. The decline in sediment quality due to heavy metal pollution in a gradual pattern that has the potential to have a negative impact on the biogeochemical cycle, with potentially fatal consequences for the survival of biodiversity (*A. marina*) [148]. Furthermore, the results of the study by Ghosh et al. [149] also stated that there was a statistically significant relationship between the activity of antioxidant enzymes, photosynthetic pigments, and heavy metal contamination, resulting in the biotic response of riparian mangroves characterized by reduced photosynthetic pigments (chlorophyll a and b) and increased activity of antioxidant stress enzymes (POD, CAT, and SOD). The response of two tropical medicinal plant species to heavy metal accumulation can increase hydrogen peroxide (H_2O_2) activity, malondialdehyde content, enzymatic activity, and nonenzymatic antioxidants [149].

Mangroves cause trigger antioxidant defenses to overcome heavy metal absorption and normalize excessive production of oxidative stress mediated by reactive oxygen species (ROS) [150]. However, antioxidant responses in mangroves vary depending on the concentration and type of heavy metals, plant species, and duration of exposure [151]. Previous findings related to plant reactions to higher concentrations of heavy metals in the soil. For example, Kulbat-Warycha et al. [152] observed that an increase in the concentration of heavy metals (Ni, Cu, Zn) caused a decrease in the concentration of phenols in oregano, which was associated with the induction of severe oxidative stress. According to Mansoor et al. [153], excessive ROS production due to severe oxidative stress can cause damage to the mitochondrial respiratory chain, uncoupling of oxidative phosphorylation, and mitochondrial death in plants. However, this can also experience a decrease in the antioxidant activity defense system of the mangrove itself if the contamination of absorbed pollutants exceeds the threshold and severe oxidative stress occurs, which can cause damage and death to the mangrove ecosystem [154,155].

The correlation between heavy metals and antioxidant activity in mangroves illustrates the complex relationship between heavy metal pollution and plant responses to oxidative stress. In this context, high concentrations of heavy metals can trigger ROS production, which in turn affects plant antioxidant activity. Excessive ROS can induce oxidative stress that activates the plant defense system to increase the production of antioxidant compounds. Thus, the relationship between heavy metals and antioxidant activity, total phenols, and secondary metabolite compound profiles in mangroves provides a deeper understanding of the mechanism of the plant's response to heavy metal pollution and oxidative stress. Therefore, if there is an indication that pollutant contamination exceeds the threshold and causes severe oxidative stress, some coastal environmental management policies can be expected in response to these findings.

To ensure the sustainability of mangrove ecosystems and mitigate the impact of heavy metal pollution, routine monitoring is recommended every 3-6 months to capture seasonal variations in heavy metal concentrations and antioxidant responses. Additionally, long-term monitoring (\geq 5 years) is necessary to identify trends in heavy metal accumulation and its effects on coastal ecosystems. Supplemental monitoring is also advised following specific events, such as industrial waste spills or land-use changes, to assess their immediate environmental impact. The data from this study can serve as a basis for environmental policy development, including updating regulations on heavy metal thresholds in sediments and coastal biota, strengthening conservation and mangrove rehabilitation policies, and improving industrial zone management in coastal areas. Furthermore, these findings can be utilized to raise public awareness about the importance of protecting coastal ecosystems and promoting sustainable resource management practices.

4. Conclusion

Heavy metal pollution of Pb and Cu resulting from areas affecting industrial and conservation activities has a significant effect on antioxidant activity in mangroves (*A. alba* and *E. agallocha*). Sediment

pollution assessment showed that the Igeo value was at a low level, while the contamination factor (Cf) and pollution load Index (PLI) showed a relatively moderate level of pollution (Cf between 1 and 3, and PLI between 0 and 2). The bioaccumulation value of heavy metals in mangrove leaves was low (BCF < 1), indicating moderate accumulation of heavy metals in leaf tissue. The antioxidant activity of E. agallocha leaves from the industrial area was very strong and had the highest total phenol content. The compounds identified as having high antioxidant activity included flavonoids, lipids, glycosides, glucosinolates, glucose, esters, terpenoids, and fatty acids. Correlation analysis showed that increasing heavy metal concentrations were directly proportional to increasing antioxidant activity and total phenol content in mangrove leaves. This study contributes to our understanding of the potential of mangroves to respond to heavy metal exposure through increased antioxidant activity, which can support conservation efforts and sustainable management of coastal natural resources.

Author statement

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

CRediT authorship contribution statement

Rozirwan: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Nadila Nur Khotimah: Writing – original draft, Validation, Resources, Formal analysis, Data curation. Wike Ayu Eka Putri: Software, Investigation. Fauziyah: Supervision, Data curation. Riris Aryawati: Methodology, Data curation. Gusti Diansyah: Software, Investigation. Redho Yoga Nugroho: Writing – review & editing, Resources, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

Declaration of competing interest

The authors declare no conflict of interest.

Data Availability

No data was used for the research described in the article. The data supporting the findings of this study can be obtained from the corresponding author upon a reasonable request.

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