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Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia

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Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia --Manuscript Draft--

Manuscript Number:	CHNAES-D-22-00089R1
Article Type:	Full Length Article
Keywords:	Accumulation; Bioindicators; Gastropods; Heavy metals; Shrimp ponds
Corresponding Author:	Rozirwan Rozirwan, Ph.D Indralay, INDONESIA
First Author:	Yossy Fitria
Order of Authors:	Yossy Fitria Rozirwan Rozirwan, Ph.D Mirna Fitrani, Ph.D Redho Yoga Nugroho
Manuscript Region of Origin:	Asia Pacific
Abstract:	<p>The supply of shrimp ponds water sources in this research utilizes water input from the waters of the Banyuasin River Estuary, which brings in heavy metal pollutant particles due to anthropogenic pressure. The potential for accumulation of heavy metals can occur in gastropods which are bioindicators of the pond environment. In this study, we present data on gastropods abundance and community structure concerning environmental physicochemical factors and heavy metal accumulation. Sampling was carried out in November 2021 at five observation stations spread over three shrimp ponds. The collection of gastropod samples used two methods based on achievement targets. The gastropod ecology index calculated the abundance, diversity (H') values of heavy metals analyzed, namely Pb, Hg, Cu, and Cd. Wet digestion with an acid solution of HNO₃ and HClO₄ was performed before measuring the concentration on the Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Furthermore, all data on environmental variables were analyzed by principal components analysis (PCA) to determine the leading variable group for each station. A cluster was formed based on the Bray-Curtis similarity analysis. The results of the gastropod ecology index as a bioindicator showed moderate and low diversity (H') and evenness (J) values, while dominance (D) was high and moderate. From two species analyzed for heavy metals, <i>Nerita violacea</i> and <i>Telescopium telescopium</i> contained Pb, Hg, and Cu concentrations, but no Cd levels were detected, including in water and sediment. Accumulation of heavy metals in shrimp ponds is not only due to the mass movement of tidal water, some of which contribute, namely the location of the inlet and outlet waterways and the position of the land to the main canal source. In general, these results indicate that the environmental conditions of the pond have accumulated heavy metal pollutants, but its whereabouts cannot be ascertained all the time. These results are sufficient to describe the current situation but need further study its impact on aquaculture production.</p>
Suggested Reviewers:	Hefni Effendi Professor, IPB University: Institut Pertanian Bogor hefni_effendi@yahoo.com Bintal Amin, Ph.D Professor, Riau University: Universitas Riau bintalamin@gmail.com
Opposed Reviewers:	
Response to Reviewers:	

COVER LETTER

Indralaya, April 4, 2022

To: **Professor Bojie Fu, PhD**
Editor in Chief
Acta Ecologica Sinica

Dear Prof.,

I wish to submit an original research article entitled “**Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia**” for consideration by *Acta Ecologica Sinica*.

I confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we report on / show that the distribution of gastropods in the shrimp ponds of this study only consisted of five species. Two of them were found in all observation stations so that both could provide information on the ecological assessment of the shrimp ponds water environment. The high abundance level at the observation station showed a significant positive correlation with the gastropod Pb accumulation of heavy metals. These physicochemical-biological components produced various relationships, but no single element significantly influenced the accumulation of heavy metals in water, sediments, and gastropods.

We believe that this manuscript is appropriate for publication by *Acta Ecologica Sinica* because it is an aquatic ecology related to the Gastropods as a bioindicators of marine culture area in estuarine and marine environments.

We have no conflicts of interest to disclose in regard to this research or its funding. All authors have read and approved the manuscript and take full responsibility for its content.

Please address all correspondence concerning this manuscript to me at email:
rozirwan@unsri.ac.id or rozirwan@gmail.com.

Thank you for your consideration of this manuscript.

Sincerely,



Dr. Rozirwan

Department of Marine Science
Faculty of Mathematics and Natural Sciences
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Manuscript Title: Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia

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Manuscript Number: CHNAES-D-22-00089

Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia

Dear Dr Rozirwan,

Thank you for submitting your manuscript to Acta Ecologica Sinica.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by May 01, 2023.

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Editor and Reviewer comments:

Reviewer #1: Bioindicators of heavy metal pollution is an important scientific issue. This study focused on heavy metal accumulation in gastropods in pond environment and tried to test if the gastropod ecology index could be a bioindicator of heavy metal in the pond environment. Two species, *Nerita violacea* and *Telescopium telescopium* were found good response to heavy metal (Pb, Hg and Cu) pollution in the pond. However, there are some issues should be concerned.

Major comment:

1. The enrichment of heavy metals by benthic organisms is the key to judging whether they can be used as biological indicators. So, the bioconcentration factor (BCF) should be calculated and discussed to support the statement.
2. Considering Cu and Cd is trace element in environment, ICP-OES analysis will be better than AAS.

Minor comment

Line 102-105. The brand, model and origin of the instrument used to test the physicochemical parameters need to be provided.

Section 2.4. What grade of acid is used for digestion? Do you have any reference to support AAS measuring heavy metal concentrations? How to conduct QC? Please add the detection limit, standard material measurement, etc.

Section 2.5. All the letter in the equation should be defined here.

As shown in Table 3, the values of Cd and Cu are negative. Strongly suggest the authors use ICP-OES to monitor Cu and Cd in the samples.

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Response to Reviewers

Reviewer #1	Response to Reviewer
Major Comment	
1. The enrichment of heavy metals by benthic organisms is the key to judging whether they can be used as biological indicators. So, the bioconcentration factor (BCF) should be calculated and discussed to support the statement.	The bioconcentration factor (BCF) had been calculated and presented as Table 5.
2. Considering Cu and Cd is trace element in environment, ICP-OES analysis will be better than AAS.	Considering that ICP-OES was very good, but we didn't have this tool at our institution, so we tried to following some of references that used AAS in the method of measuring Pb, Cu, Cd and Hg heavy metals. A. Soegianto, H. I. Wahyuni, B. Yulianto, and L. A. Manaf, "Health risk assessment of metals in mud crab (<i>Scylla serrata</i>) from the East Java Estuaries of Indonesia," <i>Environ. Toxicol. Pharmacol.</i> , vol. 90, p. 103810, Feb. 2022. K. Analuddin et al., "The carrying capacity of estuarine mangroves in maintaining the coastal urban environmental health of Southeast Sulawesi, Indonesia," <i>Egypt. J. Aquat. Res.</i> , 2023.
Minor Comment	
1. Line 102-105. The brand, model and origin of the instrument used to test the physicochemical parameters need to be provided.	We have added the brand and model of the instrument: - pH meter Hanna HI 9811-5 - DO meter Hanna HI 98193
2. Section 2.4. What grade of acid is used for digestion? Do you have any reference to support AAS measuring heavy metal concentrations? How to conduct QC? Please add the detection limit, standard material measurement, etc.	– Pro analyst grade acid used for digestion – Here some reference to support AAS measuring heavy metal concentration: A. Soegianto, H. I. Wahyuni, B. Yulianto, and L. A. Manaf, "Health risk assessment of metals in mud crab (<i>Scylla serrata</i>) from the East Java Estuaries of Indonesia,"

	<p>Environ. Toxicol. Pharmacol., vol. 90, p. 103810, Feb. 2022.</p> <p>K. Analuddin et al., “The carrying capacity of estuarine mangroves in maintaining the coastal urban environmental health of Southeast Sulawesi, Indonesia,” Egypt. J. Aquat. Res., 2023.</p> <p>– QC conducted : Analytical grade chemicals were used in the study without further purification. All glassware was washed with 10% HNO₂ solution or acid washing and then rinsed 3 times with distilled water. Water, sediment and biota samples were stored directly into the sample storage to avoid heavy metal contamination. Water sample was taken using a 500 mL glass bottle, the bottle is tilted and drowned then the bottle cap was released, the bottle cap was replaced when it was full. Sediment sample was taken with a 10 x 30 cm PVC sediment core, then the sediment was put in clean and dry plastic bag to avoid heavy metal contamination. Biota was collected directly, then put into clean and dry plastic bag. All samples were extracted and measured in 3 replicates. Measurement of heavy metal concentrations using Atomic Absorption Spectrophotometer (Shimadzu AA-7000)</p> <p>– LoD and LoQ of heavy metal analysis were presented in Table 1.</p>
<p>3. Section 2.5. All the letter in the equation should be defined here.</p>	<p>Here, N was abundance of species, H' was diversity, D was dominance, J was evenness, A was sampling surface area, n_i was the number of individuals of species i, and N was the total number of individuals per station.</p>
<p>4. As shown in Table 3, the values of Cd and Cu are negative. Strongly suggest the authors use ICP-OES to monitor Cu and Cd in the samples.</p>	<p>Based on the results of the analysis, the concentrations of Cd and Cu were very low, meaning that the accumulation of</p>

	<p>Cd and Cu in biota was still very low and did not pose a major threat to the food chain in shrimp ponds. Meanwhile, we could not do measurements using ICP-OES due to limited availability of tools at our institution or our closest institutions that did not have this tool. Based on that consideration, we analyzed it using AAS and followed by a negative result / undetectable / very low concentration</p>
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18 Mei 2023 pukul 09.09

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Gastropods as bioindicators of heavy metal pollution in the Banyuasin Estuary shrimp pond area, South Sumatra, Indonesia

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Gastropods as bioindicators of heavy metal pollution in the Banyuasin estuary shrimp pond area, South Sumatra, Indonesia

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ARTICLE INFO

Keywords:

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Gastropods
Heavy metals
Shrimp ponds

ABSTRACT

The supply of shrimp ponds water sources in this research utilizes water input from the waters of the Banyuasin River Estuary, which brings in heavy metal pollutant particles due to anthropogenic pressure. The potential for accumulation of heavy metals can occur in gastropods which are bioindicators of the pond environment. In this study, we present data on gastropods abundance and community structure concerning environmental physico-chemical factors and heavy metal accumulation. Sampling was carried out in November 2021 at five observation stations spread over three shrimp ponds. The collection of gastropod samples used two methods based on achievement targets. The gastropod ecology index calculated the abundance, diversity (H') values of heavy metals analyzed, namely Pb, Hg, Cu, and Cd. Wet digestion with an acid solution of HNO_3 and $HClO_4$ was performed before measuring the concentration on the Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Furthermore, all data on environmental variables were analyzed by principal components analysis (PCA) to determine the leading variable group for each station. A cluster was formed based on the Bray-Curtis similarity analysis. The results of the gastropod ecology index as a bioindicator showed moderate and low diversity (H') and evenness (J) values, while dominance (D) was high and moderate. From two species analyzed for heavy metals, *Nerita violacea* and *Telescopium telescopium* contained Pb, Hg, and Cu concentrations, but no Cd levels were detected, including in water and sediment. Accumulation of heavy metals in shrimp ponds is not only due to the mass movement of tidal water, some of which contribute, namely the location of the inlet and outlet waterways and the position of the land to the main canal source. In general, these results indicate that the environmental conditions of the pond have accumulated heavy metal pollutants, but its whereabouts cannot be ascertained all the time. These results are sufficient to describe the current situation but need further study its impact on aquaculture production.

1. Introduction

The coast of South Sumatra has two large estuaries, namely the Musi River Estuary and the Banyuasin River Estuary. Both are brackish water areas with fresh water from the river and seawater from the Bangka Strait. The surroundings are vast tidal wetlands with mangrove vegetation [1–3]. Local people use wetlands in this area as traditional shrimp farming areas. The traditional pond irrigation system utilizes the ups and downs of the water in order to carry out regular pond water changes [4,5]. The source of water for shrimp ponds in this area comes from the

two estuary water, while the water comes from the upstream of the river, which has received pollutant pressure from human activities [6–8]. Pollutants such as heavy metals, pesticides, organic pollutants, hydrocarbons, and microplastics can enter the waters due to poor waste management performance, lack of standard infrastructure, and increasing community and industrial waste volume. Meanwhile, the Musi River and Banyuasin River are also one of the busiest rivers with small and large ship traffic activities and industrial and mining cargo ships [9,10]. Of course, this indicates the presence of heavy metal pollutants in rivers in higher quantities than other pollutants.

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As the only water source for irrigating the community's traditional shrimp ponds, indications of pollution have been found around the Musi River and Banyuasin River watersheds. These conditions can accumulate at any time into the waters and the biota. Based on the research results related to the level of accumulation of pollutants into biota, it was found that pollutants accumulated in higher concentrations in benthic biota than fish in the water column [11–14]. Benthic communities live around shrimp ponds because of the suitable environmental conditions as their habitat and adjacent to mangrove vegetation, especially gastropods [15,16]. An assessment of pollution in the Musi River waters has been carried out on fish and the water column [6,17,18]. However, until now, the pollution assessment of benthic communities, which is suspected of having a higher accumulation of contamination than other environmental matrices, has not been reported. Regarding heavy metal pollution and its implications for the shrimp ponds environment, the accumulation of heavy metals can occur in gastropods through bioaccumulation from pond water and sediments. In contrast, heavy metals in shrimp ponds water can accumulate to individual pond commodities. Furthermore, the shrimp produced will be consumed by humans, and a contamination biomagnification process occurs through the food chain cycle [19,20].

This study aims to assess aspects of environmental bioindicators in shrimp ponds aquaculture by assessing the level of heavy metal pollution in macrobenthos biota, especially gastropods. This result is also expected to assess the food safety of shrimp ponds aquaculture production on the coast of South Sumatra as an area that has a significant potential impact on human health and safety.

2. Materials and methods

2.1. Study area

Gastropod samples were taken in November 2021 at shrimp ponds aquaculture site in Banyuasin estuary, South Sumatra, Indonesia. This aquaculture area was a mangrove forest area that had changed land use over the last two decades. The physicochemical components of this land were still categorized as suitable gastropod habitats. As a result of the input of anthropogenic waste that accumulated in the river water posed a potential risk of pollution in waters used as the primary water source for aquaculture on the coast of South Sumatra.

Gastropods sampling was carried out at five station points spread over three shrimp ponds. Station 1 was at the outlet and inlet channel for the first pond, stations 2 and 3 were at the outlet and inlet channel for the second pond, stations 4 and 5 were at the inlet and outlet channel for the third pond. The sampling map is presented in Fig. 1.

2.2. Sample collection

Gastropods sampling for biodiversity data was carried out in a quadratic transect, while heavy metal analysis data was taken by random sampling around the station point [1,13]. Then, the samples were put into sample plastics and labeled at each station, then put in a cooler until they arrived at the laboratory. Gastropods were cleaned of sediment by washing with clean water. The identification process followed [21].

In addition to biota samples, pond water and sediment samples were

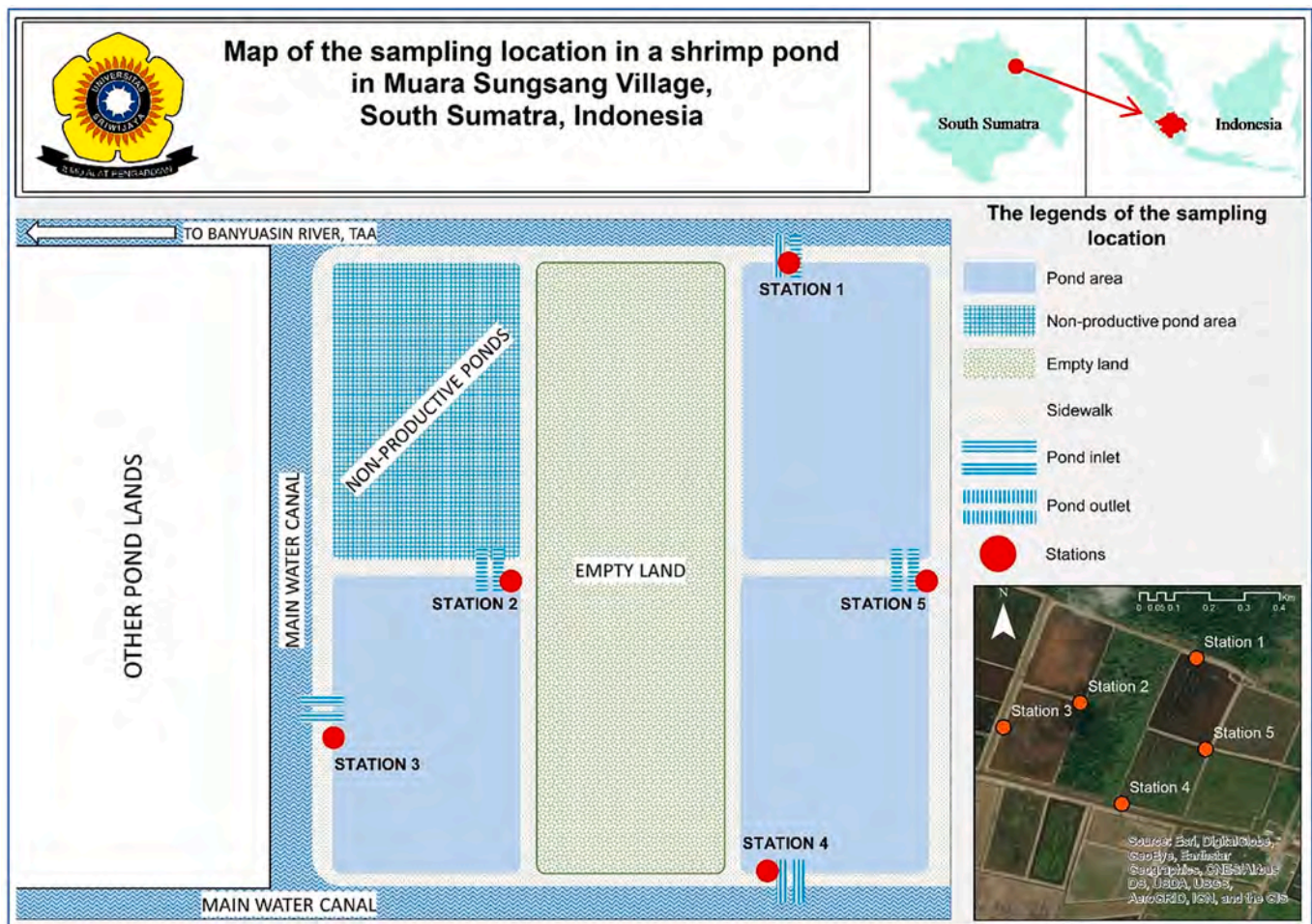


Fig. 1. Map of sampling locations in shrimp ponds.

also taken for heavy metal analysis. The 250 mL of water sample was taken using a PP bottle and then put into a cooler box. Sediment samples were taken using a grab pipe to a depth of 30 cm, and then the sample was put into a plastic sample of 1 kg [12].

In-situ measurements were also carried out for environmental physicochemical parameters (such as temperature, salinity, pH, and dissolved oxygen), repeated three times at each station. Salinity measurement using a hand refractometer, pH measurement using a pH meter (Hanna HI 9811-5), and temperature and dissolved oxygen measurement using a DO meter (Hanna HI 98193) [2,22,23].

2.3. Identification of gastropods

The collected gastropod samples were identified in the laboratory based on their morphological characteristics. Determination of taxon based on WORMS (World Register of Marine Species) data accessed in December 2021 (WoRMS, 2021). Gastropods samples were also measured for their morphometric size and biomass.

2.4. Heavy metal analysis and quality control

The gastropod soft tissues of each species at each station were separated from their shells and washed with distilled water. Soft tissue was pulverized with a mortar before reacting with an acid solution. Sample preparation for measuring the heavy metal content of Cd, Cu, Hg, Pb in all samples (gastropods, water, and sediments) using wet digestion referred to [24,25]. Acid digestion using strong acid solutions of HNO₃ and HClO with pro analyst grade [26]. Measurement of heavy metal concentrations of Cd, Cu, Hg, and Pb using Atomic Absorption Spectrophotometer as carried out by [27,28]. The measurement values of LoD (limit of detection) and LoQ (limit of quantitation) of heavy metals were presented in Table 1.

Bioconcentration factor (BCF) was calculated by dividing the value of each heavy metal concentration in the sediment by the heavy metals accumulated in biota based on the formula.

$$BCF = \frac{\text{heavy metal concentration in sediment}}{\text{heavy metal concentration in biota}}$$

The BCF value represents the concentration of heavy metals in biota in a contaminated environment. BCF <1 indicates that the accumulation of heavy metals in the environment is higher than that absorbed by biota, while BCF of >1 indicates that the absorption of heavy metals in biota is higher than in the environment.

Analytical grade chemicals were used in the study without further purification. All glassware was washed with 10% HNO₂ solution or acid washing and then rinsed 3 times with distilled water. Water, sediment and biota samples were stored directly into the sample storage to avoid heavy metal contamination. Water sample was taken using a 500 mL glass bottle, the bottle is tilted and drowned then the bottle cap was released, the bottle cap was replaced when it was full. Sediment sample was taken with a 10 x 30 cm PVC sediment core, then the sediment was put in clean and dry plastic bag to avoid heavy metal contamination. Biota was collected directly, then put into clean and dry plastic bag. All samples were extracted and measured in 3 replicates. Measurement of heavy metal concentrations using Atomic Absorption Spectrophotometer (Shimadzu AA-7000).

Table 1
LoD and LoQ values of heavy metals.

Heavy metal	LoD	LoQ
Pb	0.0327	0.0991
Hg	1.0830	3.2818
Cu	0.0807	0.2445
Cd	0.0483	0.1464

2.5. Data analysis

Biodiversity index refers to previous research [1,2]. The gastropod abundance data was calculated using this equation:

$$N = \frac{\sum ni}{A} \times 100\%$$

The gastropod diversity index was calculated using the formula of Shannon-Wiener (H') [27]:

$$H' = - \sum_{i=1}^s \left(\frac{ni}{N} \right) \left(\ln \frac{ni}{N} \right)$$

The gastropod dominance index was calculated using the dominance index formula of Simpson (D) [28]:

$$D = \sum_{i=1}^s \left(\frac{ni}{N} \right)^2$$

The gastropod evenness index was calculated using the formula [29]:

$$J = \frac{H'}{\log 2S}$$

here, N was abundance of species, H' was diversity, D was dominance, J was evenness, A was sampling surface area, ni was the number of individuals of species i, and N was the total number of individuals per station.

Principal component analysis (PCA) was an analysis used to examine the relationship between components of shrimp ponds environment (such as temperature, salinity, pH, and dissolved oxygen), gastropods biodiversity index (such as abundance, diversity, and dominance), and heavy metals water, sediments, and gastropods. In addition, data were analyzed for the similarity of Bray-Curtis to calculate the similarity of environmental factors and the structure of the gastropods community. This analysis was carried out using the XLSTAT 2022 software.

3. Results and discussion

3.1. Physicochemical quality of the shrimp ponds environment

The physicochemical conditions of shrimp ponds water were influenced by water input from the Banyuasin Estuary, such as temperature, salinity, pH, and dissolved oxygen (Table 2). Based on the geographical point, the mass of brackish water came from the west side through the main canal and then entered the ponds through the inlet channel. The salt content of brackish water in ponds was on average 14.13 ± 2.59 ppt. The average pool water surface temperature was 31.76 ± 2.94 °C, increasing at midday. The pH and dissolved oxygen conditions were quite good, with an average of 7.18 ± 0.20 and 6.96 ± 0.56 mg/l, categorized as usual and evenly distributed in all observation stations.

Physicochemical parameters of pond water were in normal condition, and there was no indication of water quality degradation. The surface water temperature ranged from 27.90 to 34.57 °C, the salt content of brackish water ranged from 10 to 17 ppt, the acidity of the water ranged from 6.92 to 7.45, which tended to be at an alkaline level,

Table 2

In-situ measurements of physicochemical parameters of the pond water environment.

Parameters	Stations					Average (± Stdev)
	1	2	3	4	5	
Temperature (°C)	27.90	29.60	32.40	34.57	34.33	31.76 ± 2.94
Salinity (ppt)	15	14	10	13	17	14.13 ± 2.59
pH	7.08	6.92	7.23	7.22	7.45	7.18 ± 0.20
Dissolved oxygen (mg/l)	6.31	6.61	6.78	7.63	7.45	6.96 ± 0.56

and dissolved oxygen levels were quite normal for pond needs, which ranged from 6.31 to 7.63 mg/l.

3.2. The composition and abundance of gastropods in the shrimp ponds

The composition of types of gastropods found in shrimp ponds was not much, consisting of five species with 214 individuals, including *Cerithidea quadrata* 41.59%, *Neritina violacea* 40.19%, *Telescopium telescopium* 13.55%, *Cerithidea cingulata* 3.27%, and *Clithon oualaniense* 1.40%. The highest percentage was *C. quadrata*, mostly found attached to substrate and wood surfaces, while *C. oualaniense* was rare species in ponds (Fig. 2).

Only two gastropod species were found at all observation stations, namely *N. violacea* and *T. telescopium*. Although *C. quadrata* had the highest quantitative number of individuals, this species was not found at station 4. Based on this quantitative, two gastropod species (*N. violacea* and *T. telescopium*) were the most likely bioindicators of the pond environment. *N. violacea* was found in more significant numbers (16–30 ind/m²) at stations 1, 4, and 5 while *T. telescopium* was found in equal numbers at each station (1–15 ind/m²). Scarce species, namely *C. cingulata* and *C. oualaniense*, were only found at station 2 and station 1 (Table 3).

3.3. Gastropod diversity as a bioindicator in shrimp ponds

In general, gastropod diversity was found in the low category ($H' < 1$) at stations 3, 4, and 5 ($0.436 < H' < 0.636$), while at stations 1 and 2 ($1.117 < H' < 1.123$) were included in the medium category ($1 < H' < 3$). The value of gastropod diversity showed an R-squared value of 0.7369 with a decreasing trendline. The evenness index had a similar pattern to diversity. Stations 1 and 2 ($0.694 < J < 0.697$) were in the high category and stations 3, 4, and 5 ($0.271 < J < 0.395$) were in the low category ($0 < J < 0.4$). The gastropod evenness value showed an R-squared value of 0.7369 with a decreasing trendline (Fig. 3).

Based on Fig. 3, the dominance value showed the opposite pattern to the diversity and evenness values. In general, high dominance ($0.6 < D < 1$) was found at stations 3, 4, and 5 ($0.661 < D < 0.734$), while at stations 1 and 2 ($0.388 < D < 0.403$) in the low category ($0 < D < 0.4$). The dominance value resulted R-squared value of 0.7794 with an increasing trendline.

3.4. Gastropod characteristics bioindicators of heavy metals in the shrimp ponds environment

There were six species of gastropods found in the ponds, but only two species could be used as bioindicators of heavy metal accumulation, namely *N. violacea* and *T. telescopium* (Fig. 4). The *N. violacea* species were mainly attached to the substrate and wood surfaces, while

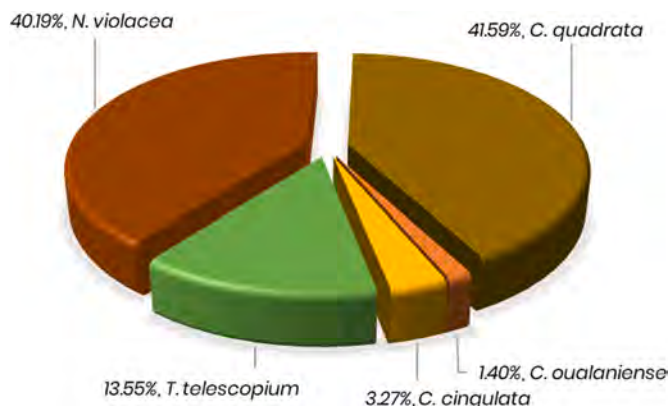


Fig. 2. Gastropod composition found in all pond stations.

Table 3

The abundance of gastropod species at each station.

Class	Species	Stations				
		S1	S2	S3	S4	S5
Gastropods	<i>Cerithidea cingulata</i>	–	+	–	–	–
Gastropods	<i>Cerithidea quadrata</i>	+	+++	+++	–	+
Gastropods	<i>Clithon oualaniense</i>	+	–	–	–	–
Gastropods	<i>Neritina violacea</i>	++	+	+	++	+++
Gastropods	<i>Telescopium telescopium</i>	+	+	+	+	+

Information: – (not found), + (1–15 ind/m²), ++ (16–30 ind/m²), +++ (> 30 ind/m²).

T. telescopium was mainly found on the substrate surface. These two species were able to live in submerged and non-submerged zones of pond water, so the accumulation of heavy metals was thought to be transferred by water and sediment.

The largest species of *N. violacea* had an average length of 21 mm, a width of 15 mm, and a biomass weight of 1.85 g. This species had a dark brown to the black, round, short shell with a blunt tip (Fig. 4A). The species of *T. telescopium* had an average length of 92 mm, a width of 37 mm, and a biomass weight of 50.92 g. This species has a long, conical, dark brown to the black shell with a pointed tip (Fig. 4B).

3.5. Heavy metal content in the shrimp ponds environment

The components assessed in studying the accumulation of heavy metals consisted of water, sediment, and primary gastropod organisms. The assessed heavy metals (Pb, Hg, Cu, and Cd) could come from various external and internal sources of ponds. Interestingly, the measurement of heavy metal Cd in this study was below the limit of the quality value for the ability to measure spectrophotometry, which was indicated by a negative value. In addition, the accumulation of Cu heavy metal in water also resulted in lower values than the instrument could measure (Table 4).

The highest average accumulation value of Pb was detected in sediments, namely 8.929 ± 2.236 ppm, and the lowest was *T. telescopium*, namely 0.026 ± 0.034 ppm. The highest accumulation of heavy metal Hg was *N. violacea* with an average accumulation value of 0.197 ± 0.032 ppm, while the lowest was water with an average value of 0.004 ± 0.003 ppm. Heavy metal Cu was only found in sediments and both species of gastropods, the highest was in sediments at 2.724 ± 0.603 ppm, and the lowest was in *N. violacea*, namely 0.006 ± 0.003 ppm.

3.6. Heavy metal bioconcentration in N. violacea and T. Telescopium

The bioconcentration factor of the gastropods *N. violacea* and *T. telescopium* was related to the benthic environment of their habitat. BCF explains bioavailability and transfer of heavy metals from the environment to the biota that live in it. $BCF > 1$ means that the biota absorbs more heavy metals than the environment, while $BCF < 1$ means that the environment becomes a better absorber of heavy metals. Gastropod bioconcentration conditions found in the pond environment are presented in Table 5.

3.7. Relationship of environmental physicochemical parameters, gastropod biodiversity index, and accumulation of heavy metals in water, sediments, and gastropods

The principal component analysis formed three axes with a cumulative eigenvalue of 96.62%. Groups one and two were formed at the axes relationship F1 and F2 with cumulative eigenvalues of 41.32% and 30.97%, respectively (Fig. 5A), while group three was formed at F3 with an eigenvalue of 24.33% (Fig. 5B). The results of the Bray-Curtis dissimilarity analysis formed two groups, namely Cs1 and Cs2. Group Cs1 consists of observation stations 4, 5, and 1, while Cs2 consists of

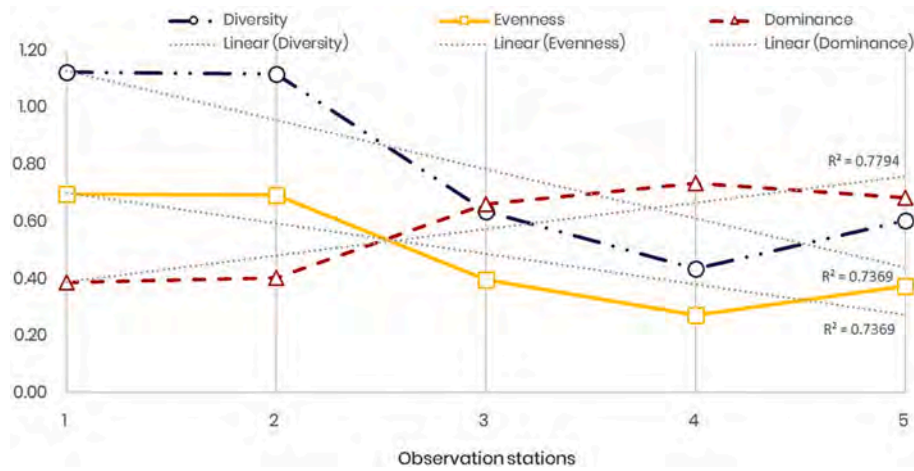


Fig. 3. Gastropod biodiversity index in ponds.

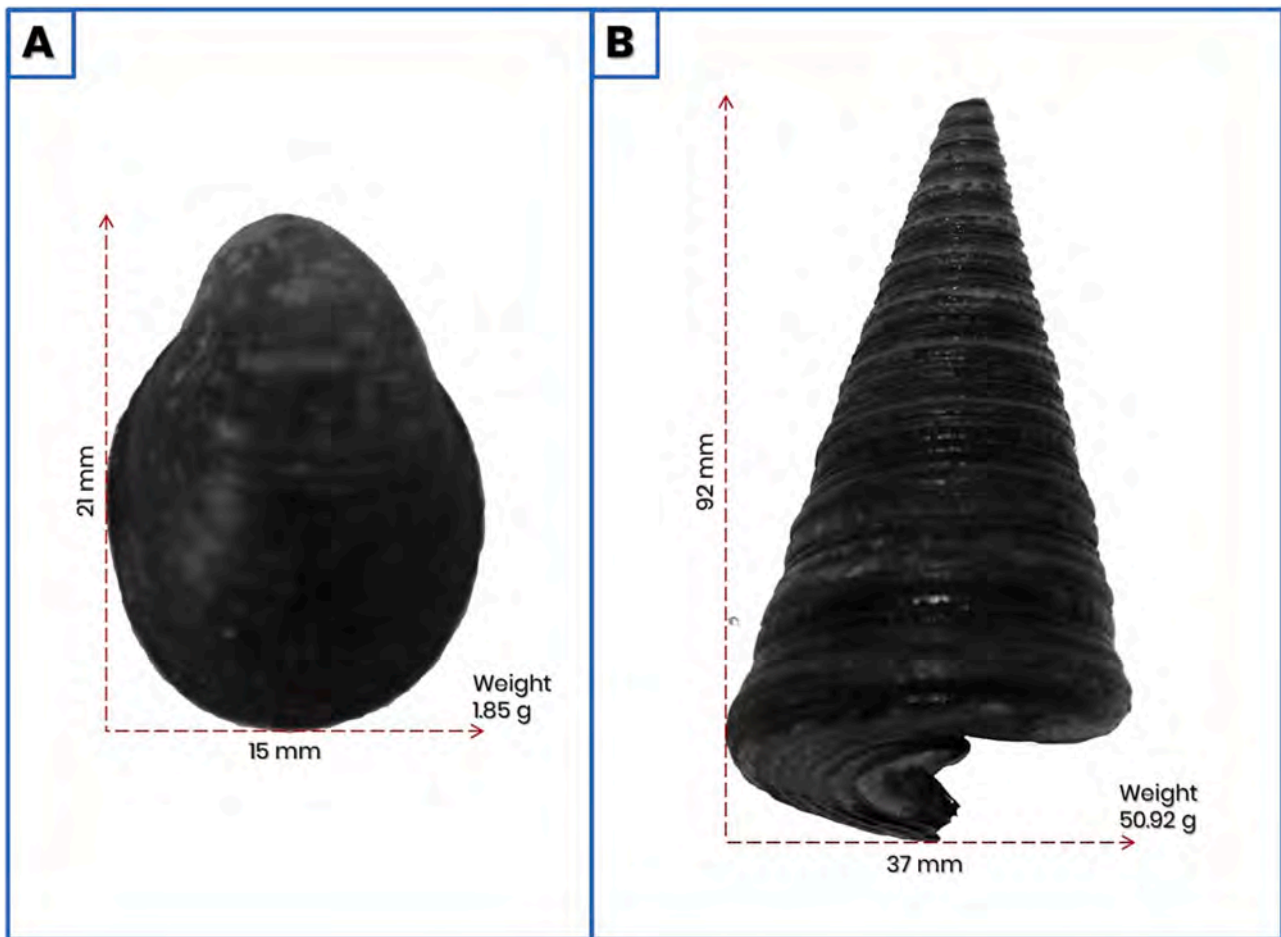


Fig. 4. Gastropod species bioindicator of heavy metals, A) *N. violacea*; B) *T. telescopium*.

stations 2 and 3 (Fig. 5C).

The positive F1 axes group showed a positive relationship between stations 4 and 5 with temperature, dissolved oxygen, and pH identifiers which increased the heavy metal content of Cd species *T. telescopium*. In contrast, the negative F1 axes showed a relationship between stations 4 and 5 on the diversity and evenness of gastropods and heavy metal content of Cu (water, sediment, *N. violacea*), Hg of *N. violacea*, and Cd of sediment. Group F2 showed station 1 with the primary identifier of salinity but had an inverse relationship with abundance, dominance,

and heavy metals Hg (water and sediment), Pb (water, *N. violacea*, and *T. telescopium*), and water Cd, which was positively related to station 3. Group F3 on positive axes identified station 2 with heavy metal identifiers Hg and Cu *T. telescopium* and Cd *N. violacea* but inversely related to Pb in water and sediment.

The similarity of each observation station was presented in Fig. 5C. The cophenetic relationship was in the powerful category with a value of 0.886. The similarity calculated using Bray-Curtis dissimilarity resulted in two clusters, namely Cs1 and Cs2. The similarity of Cs1 was 98.6%,

Table 4
Measurement of heavy metals Pb, Hg, Cu, and Cd in the pond environment.

Accumulations	Heavy metals (mg/l)	Observation stations					Average (Stdev)
		1	2	3	4	5	
Water	Pb1	0.026	0.013	0.088	0.056	0.046	0.046 ± 0.029
	Hg1	0.001	0.005	0.008	0.003	0.001	0.004 ± 0.003
	Cu1	-0.035	-0.045	-0.027	-0.050	-0.046	-0.041 ± 0.009
	Cd1	-0.229	-0.019	-0.022	-0.020	-0.019	-0.062 ± 0.093
Sediment	Pb2	11.630	6.014	10.670	8.210	8.120	8.929 ± 2.236
	Hg2	0.122	0.129	0.160	0.125	0.107	0.129 ± 0.019
	Cu2	3.510	2.450	3.220	2.280	2.160	2.724 ± 0.603
	Cd2	-0.023	-0.022	-0.022	-0.025	-0.024	-0.023 ± 0.001
<i>N. violacea</i>	Pb3	0.001	0.120	0.330	0.003	0.001	0.091 ± 0.143
	Hg3	0.225	0.205	0.227	0.176	0.153	0.197 ± 0.032
	Cu3	0.011	0.005	0.006	0.002	0.004	0.006 ± 0.003
	Cd3	-0.017	-0.006	-0.014	-0.012	-0.012	-0.012 ± 0.004
<i>T. telescopium</i>	Pb4	0.002	0.063	0.063	0.001	0.001	0.026 ± 0.034
	Hg4	0.001	0.430	0.183	0.135	0.071	0.164 ± 0.164
	Cu4	0.014	0.031	0.020	0.020	0.020	0.021 ± 0.006
	Cd4	-0.013	-0.014	-0.012	-0.007	-0.009	-0.011 ± 0.003

Table 5
Bioconcentration factor of Pb, Hg, Cu, Cd in *N. violacea* and *T. telescopium*.

Species	Heavy metal	Observation Stations				
		1	2	3	4	5
<i>N. violacea</i>	Pb	0.0001	0.0200	0.0309	0.0004	0.0001
	Hg	1.8443	1.5891	1.4188	1.4080	1.4299
	Cu	0.0031	0.0020	0.0019	0.0009	0.0019
	Cd	0.7391	0.2727	0.6364	0.4800	0.5000
<i>T. telescopium</i>	Pb	0.0002	0.0105	0.0059	0.0001	0.0001
	Hg	0.0082	3.3333	1.1438	1.0800	0.6636
	Cu	0.0040	0.0127	0.0062	0.0088	0.0093
	Cd	0.5652	0.6364	0.5455	0.2800	0.3750

covering stations 4, 5, and 1. The similarity of Cs2 was 98.2%, covering stations 2 and 3.

4. Discussion

Physicochemical factors of shrimp ponds water environment affect the life of macrobenthos communities as ecological supports [30,31]. Salinity, temperature, pH, and dissolved oxygen had varying measurements at each observation station in each pond. Temperature values differed due to differences in the intensity of solar radiation throughout the day [32–34]. The value of salinity and dissolved oxygen was affected by tidal mass conditions due to pond water sources derived from brackish estuary water masses and continued to be volatile dynamic throughout the day [4,35–37]. In addition, the turbulence that causes friction between the wind and the water surface significantly increases dissolved oxygen [38,39]. Wind gusts supported the physical function of ecology for aquatic organisms, especially in the rainy season, high-intensity winds appeared due to increased pressure on the atmosphere [40,41]. The condition of the acidity degree was lower in pond water due to internal factors of pond activity. Various organic components of pond activity accumulated in water and sediments [42,43]. Traditional shrimp ponds water that was rarely fully circulated decreased in the value of environmental parameters [44].

Previous studies often found that the distribution of gastropods in pond land adjacent to mangrove land cover changes [45,46]. As one of the classes of macrobenthos, gastropods were most adaptable to pond environments that were poor sources of internal nutrients. The advantage was that as a detritus organism, that was easiest to maintain life compared to other classes in that condition [12,13,47]. Polychaeta and Oligochaeta of the annelid phylum did not easily live on pond substrate that tended to be hard and lacked nutrients [48,49]. In addition, pond sediments had been plowed to stabilize the structure of the pond. Some

species of crabs could be found, but in ponds overgrown with mangrove trees in the outside area. All gastropods in this study had been found in native mangrove land areas. The life of the gastropod was maintained because the process of land-use change did not affect the authenticity of the habitat widely. Indications of the distribution and abundance of gastropods on pond land made it easier to identify it as the most appropriate bioindicator against the accumulation of heavy metals resulting from pond activity [50,51].

Ponds got the input influence of external materials that affected the presence of heavy metals. The transfer of heavy metals from water coming from the pond is outside the environment, allowing a high accumulation of long-term processes [12,52,53]. The existence of macrobenthos organisms in the pond environment had the opportunity to occur bioaccumulation. It would reduce the level of its population in nature through natural selection [54]. The presence of macrobenthos was at a low level of diversity which means that certain species dominated in the pond area in this study. Several previous studies reported these conditions from areas subjected to environmental degradation due to heavy metal pressure [55,56].

Environmental assessment utilizing the presence of organisms was needed to record the entire process of bioaccumulation that entered the body during its life [13,50]. Species *N. violacea* and *T. telescopium* were found at all observation stations in ponds. These species were possible as bioindicator assessors for heavy metal pollution Pb, Hg, Cu, and Cd in pond environments. In addition to these two species, several species of gastropods had status as environmental bioindicators, including *Patella* spp., *Bursa spinosa*, *Tibia curta*, *Lanistes carinatus*, and *Murex trapa* [12,57,58]. Assessment through gastropods was better than other types due to the nature of life that sticks and forages on the surface of the substrate [13,50]. This habit assumed a faster rate of accumulation of heavy metals to it while, in general, very high accumulation had occurred in the base sediments.

Some heavy metals accumulated faster to the bottom of the waters, not that physical factors always had a significant effect, but atomic mass also affected the accumulation rate. The Pb heavy metal, which had an enormous atomic mass of 207.2 g/mol, was at the highest concentration in sediments of 8.929 ppm. Some studies stated that Pb became a heavy metal that was very commonly found in various waters because of its diverse source of origin [11,59]. Cu sediments concentrations were also measured the most due to accumulation process in the long run so that it had accumulated at the bottom of the pond substrate. The increase in accumulation was also accelerated by the presence of heavy metals Cu on the organism's body naturally, which was then mixed at the bottom of the water when the organisms died [60,61]. The discovery of mercury or Hg in pond environments was quite worrying because of its impact on health in the long run. Mercury in the estuary environment was

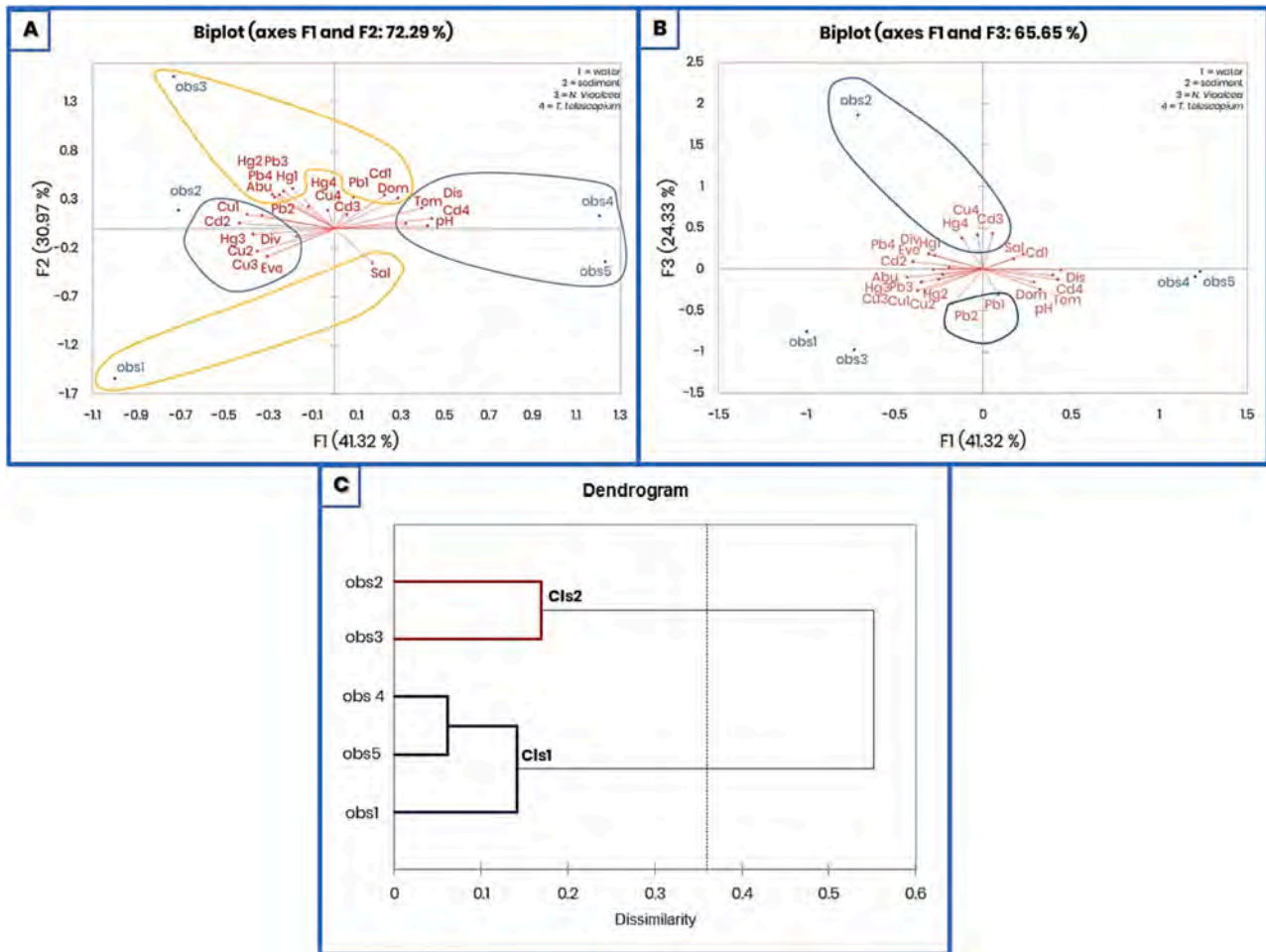


Fig. 5. The physicochemical relationship of the pond environment, gastropod biodiversity, and accumulation of heavy metals in water, sediments, and gastropods, A) F1 dan F2 axes; B) F1 dan F3 axes; C) Dendrogram of the Bray-Curtis dissimilarity.

associated with organic matter in sediments [62,63]. The presence of mercury in sediments and both species were thought to come from outdoor activities of ponds and cultivated feed used. [64,65]. However, low concentrations of Hg in the water in low-circulation conditions allowed Hg to come from activity in ponds and had accumulated at the bottom of the substrate that was the habitat of both gastropod species. Research from [66,67] Hg in benthic biota came from water, phytoplankton, and sediments with a relative bioavailability of 10:5:1. Furthermore, the presence of Cd in this pond might be found in minimal concentrations or even nonexistent. Its existence was recognized as an indication of pollution from coal and agricultural, industrial activities [68,69]. As was known, the location of this pond was flanked by two large rivers. The Banyuasin River, a pond water source, was very rare for coal and oil transportation activities, but there was still domestic ship traffic [9,11]. The assumption was that Cd concentrations were still so low that they were not readable spectrophotometric tools.

The distribution of observation stations was identified that stations 1, 4, and 5 belonged to the same cluster. These stations were related to the similarity of the source of the aqueduct input. Stations 4 and 5 were on the first water inlet land, while station 1 was on the ground after the first land outlet channel. The location of stations 4 and 5 allowed environmental parameters to be at better values for temperature, pH, and dissolved oxygen than station 1. In general, the condition could reduce some of the content of heavy metals, including Hg, Cu, and Cd. In station 1, a phenomenon referred to the abundance of gastropods also increased the Pb content in gastropods. The increase was based on the increased accumulation in the biomass of organisms [70,71]. Stations 2

and 3 were on the same ponds, and station 2 was on the exit line while station 3 was in the inlet. Pb sediments were found higher in the inlet. This accumulation could be related to the high accumulation rate of Pb atoms, making it possible to fall faster to the bottom of the substrate. At station 2, located in the outlet, there was a process of accumulation of heavy metals Hg, Cu, and Cd high in gastropods. The circulation of pool water leading towards the outlet gave rise to a buildup of organic matter and heavy metals. Piles of the organic matter directly triggered an increase in the abundance of gastropods and indirectly heavy metals accumulated in gastropods at the site of this station [72,73].

5. Conclusion

The distribution of gastropods in the shrimp ponds of this study only consisted of five species. Two of them were found in all observation stations so that both could provide information on the ecological assessment of the shrimp ponds water environment. Based on the community structure, the species *C. quadrata* was found in the highest number of individuals, but its presence was not present at station 4. Two and three order was the number of individuals, namely *N. violacea* and *T. telescopium*, which were found at all stations to be selected as environmental bioindicators. The high abundance level at the observation station showed a significant positive correlation with the gastropod Pb accumulation of heavy metals. The Shannon-Wiener index (H') was moderate at stations 1 and 2 and low at stations 3, 4, and 5. The evenness index (J) showed a positive relationship pattern with the higher H' index at stations 1 and 2. The dominance index (D) negatively correlated with

the two previous indices. The stations scattered in the inlet and outlet of shrimp ponds water showed variations in environmental physicochemical parameters and the accumulation level of heavy metals Pb, Hg, Cu, and Cd on the distribution of gastropods based on principal component analysis (PCA). Based on the similarity analysis, the Bray-Curtis showed similarities at stations 1, 4, and 5, which were located in one water source, and stations 2 and 3 in one pond. These physicochemical-biological components produced various relationships, but no single element significantly influenced the accumulation of heavy metals in water, sediments, and gastropods. Further studies from other aspects are urgently needed to find the main factors that must be considered to facilitate a better understanding of the distribution of heavy metal pollution in the shrimp ponds environment.

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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