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Macrozoobenthos Community Structure in Rivers Receiving Tofu Industry Wastewater in Madiun Regency

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Abstract: This study aimed to analyze the macrozoobenthos community structure in two rivers receiving tofu industry wastewater and to examine its relationship with aquatic environmental conditions. Sampling was conducted using purposive sampling at six stations representing upstream, impacted, and recovery zones. Macrozoobenthos community structure was assessed using ecological indices, the Biological Monitoring Working Party–Average Score Per Taxon (BMWP-ASPT), Bray–Curtis similarity analysis with Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering, and Canonical Correspondence Analysis (CCA). The results showed that stations receiving direct inputs of tofu industry wastewater exhibited greater biological stress than the upstream and recovery zones. This pattern was characterized by the dominance of the family Thiaridae, a taxonomic group tolerant of organic pollution, and lower BMWP-ASPT scores at stations directly receiving wastewater inputs. BMWP-ASPT values indicated biological conditions ranging from lightly to moderately polluted. Bray–Curtis UPGMA analysis revealed clustering of communities according to similarities in community structure among stations. CCA indicated a tendency toward an association between macrozoobenthos community structure and environmental parameters; however, the overall relationship was not statistically significant ($p = 0.139$). These findings suggest that macrozoobenthos community structure has potential as a biological indicator for rivers receiving tofu industry wastewater, although its association with the measured environmental parameters was not statistically significant.
Keywords: Macrozoobenthos; BMWP-ASPT; tofu industry wastewater; water quality

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INTRODUCTION

Rivers play a fundamental role in maintaining ecosystem balance, supporting diverse ecological processes, and providing essential ecosystem services for human well-being. However, increasing anthropogenic pressures have intensified the degradation of river ecosystems, making their protection an increasingly urgent environmental concern (Adam et al., 2025). One of the major drivers of river degradation is the input of organic matter from domestic and industrial activities. Elevated organic loads are commonly reflected by increased biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which enhance oxygen consumption during microbial decomposition and consequently reduce dissolved oxygen (DO) concentrations (Maddah, 2023). Prolonged or excessive organic loading may therefore promote deoxygenation and alter the physicochemical characteristics of aquatic environments. These changes can subsequently modify aquatic community structure by reducing pollution-sensitive organisms and favoring taxa that are more tolerant of environmental stress. Consequently, biological assessment is needed to complement physicochemical measurements and provide a more integrated evaluation of ecological condition.

Macrozoobenthos are widely used as biological indicators of river ecological quality because their community structure responds to both water quality and habitat conditions. Unlike physicochemical measurements, which generally represent environmental conditions only at the time of sampling, aquatic organisms can integrate the effects of environmental disturbances over longer periods (Cruz et al., 2022). Macrozoobenthos are particularly useful because of their relatively low mobility, close association with benthic habitats, and comparatively long life cycles, which limit their ability to avoid localized pollution and allow their communities to reflect accumulated environmental stress (Pong-Masak & Pirzan, 2006). Changes in dissolved oxygen, organic matter, suspended materials, and sediment characteristics can therefore influence their taxonomic composition, abundance, diversity, and community structure (Aulia et al., 2020). Recent studies also continue to demonstrate the usefulness of macrozoobenthic community metrics for assessing pollution and ecological condition in freshwater environments (Izzati & Retnaningdyah, 2022; Ibrahim et al., 2023; Nurhaliza et al., 2025). Accordingly, changes in macrozoobenthic assemblages can provide biologically meaningful evidence of environmental disturbance.

In Madiun Regency, tofu production represents an important small-scale community-based economic activity. Field observations identified six tofu-processing industries operating near the study sites and discharging wastewater into nearby water bodies. Tofu production generates wastewater during soybean washing, soaking, grinding, filtering, and pressing processes, and this wastewater typically contains substantial concentrations of biodegradable organic matter and suspended solids. Studies of tofu-industry effluents in Indonesia have reported high BOD, COD, and TSS concentrations, indicating a considerable organic pollution potential when wastewater is discharged without adequate treatment (Isnaeni, 2022; Zalfain et al., 2024; Yuniar et al., 2024). Such discharges may adversely affect receiving waters by increasing oxygen demand and degrading aquatic habitat quality (Pagoray et al., 2021).

The ecological conditions of rivers receiving tofu-industry wastewater may be further complicated by other anthropogenic inputs. At the study sites, upstream sections are also influenced by surrounding residential activities. Domestic wastewater can introduce organic matter, detergents, nutrients, and suspended materials into river systems, whereas tofu-industry wastewater provides an additional source of concentrated organic loading (Hastika & Surtikanti, 2024; Isnaeni, 2022). Therefore, macrozoobenthic communities in these rivers are potentially exposed to combined pressures from domestic activities, industrial wastewater, and local habitat characteristics. This complexity makes biological assessment particularly relevant because observed community patterns may reflect the cumulative effects of multiple environmental stressors rather than a single pollution source.

Numerous studies have used macrozoobenthos to evaluate the ecological effects of pollution in rivers and other freshwater ecosystems. Changes in environmental quality are commonly associated with increasing dominance of pollution-tolerant taxa, declining diversity, shifts in biotic index values, and variation in organism abundance across habitats exposed to different levels of environmental stress. Macrozoobenthic distribution can also vary in response to dissolved oxygen, organic matter, suspended solids, and benthic substrate characteristics (Aulia et al., 2020; Ibrahim et al., 2023; Izzati & Retnaningdyah, 2022; Nurhaliza et al., 2025). Recent Indonesian studies further indicate that macrozoobenthic diversity and community composition can effectively reflect differences in water quality and pollution conditions in aquatic ecosystems (Qurrotu & Kuntjoro, 2024; Djunaidi, 2024).

Nevertheless, most previous studies have focused primarily on pollution arising from domestic wastewater, agriculture, livestock activities, or mixed anthropogenic sources. Relatively little attention has been given to macrozoobenthic responses specifically associated with tofu-industry wastewater, particularly along an upstream–impact–recovery gradient. In addition, river habitat characteristics can influence benthic community composition independently of pollution. Differences in current velocity, sediment texture, substrate stability, and organic matter accumulation may create contrasting microhabitats and modify organism distribution. Recent evidence has also demonstrated relationships among sediment characteristics, organic matter, and macrozoobenthic abundance, reinforcing the need to interpret pollution responses within their habitat context (Hikmah et al., 2024).

This knowledge gap is particularly relevant when comparing rivers with contrasting physical characteristics. In the present study, the two investigated rivers differ in substrate composition and flow conditions, providing an ecological context for examining whether macrozoobenthic responses to organic pollution are consistent across different river habitats. The study therefore extends previous biomonitoring research by evaluating macrozoobenthic community structure along upstream, wastewater-impact, and downstream recovery zones in two rivers receiving tofu-industry wastewater.

We hypothesized that macrozoobenthic community structure would vary along the organic pollution gradient and would be associated with ambient water conditions and river habitat characteristics. Accordingly, this study aimed to evaluate macrozoobenthic community structure as a biological indicator in two rivers receiving tofu-industry wastewater in Madiun Regency. Specifically, the study assessed spatial patterns in macrozoobenthic community structure and biological quality along upstream–impact–recovery gradients and explored their associations with ambient environmental conditions during the study period.

METHOD

This study employed a descriptive-comparative design with a quantitative approach to compare the biological responses of macrozoobenthic communities along a tofu wastewater pollution gradient in two river ecosystems in Madiun Regency, East Java (Nurhaliza et al., 2025). The study was conducted in August 2025, during the dry season. Accordingly, the results represent the ecological conditions of the study rivers during the dry-season sampling period.

The study was conducted in two rivers receiving tofu industry wastewater: the Bangunsari River (Station E) in Mejayan District, located at coordinates −7.5387270, 111.6606771, and the Sawahan River (Station S) in Sawahan District, located at coordinates −7.5913313, 111.5198809. A total of six sampling stations were selected purposively based on the gradient of exposure to tofu wastewater and the surrounding land-use characteristics. Three sampling zones were established in each river: an upstream station located before the tofu wastewater input, an impacted station located near the wastewater outfall, and a downstream station representing the recovery zone. In general, the Bangunsari River was characterized by substrates dominated by rocks, gravel, and sand, whereas the Sawahan River was dominated by mud and fine sand. This spatial design was intended to represent the pollution gradient from the upstream zone through the wastewater-impacted zone to the downstream recovery zone, thereby enabling evaluation of changes in macrozoobenthic community structure along the gradient.

Field procedures included in situ and ex situ measurements of physicochemical parameters and the collection of macrozoobenthos samples. The equipment and materials used included a Global Positioning System (GPS), pH meter, thermometer, water sampling containers, cool box, kick net, Ekman grab, 1-mm mesh sieve, Petri dishes, forceps, fine brushes, droppers, a camera equipped with a magnifying lens for taxonomic documentation, and 70% alcohol for specimen preservation. The geographic locations of all sampling stations are presented in Figure 1.

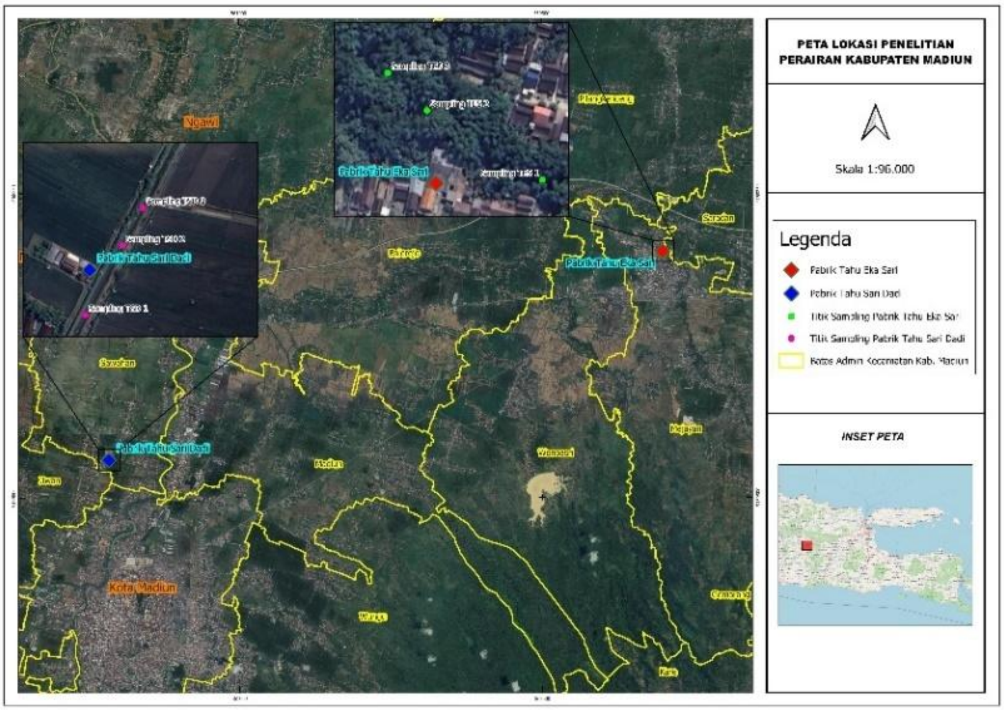

Figure 1. Study location

Water sampling and measurements of aquatic environmental parameters were conducted before macrozoobenthos collection during the first biological replicate at each station. These measurements were intended to characterize the ambient water conditions prevailing at the time of biological sampling. Water temperature and pH were measured in situ, whereas dissolved oxygen (DO), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity, ammonia, organic matter, and nitrate were analyzed ex situ.

Water samples were collected using sterile 2-L high-density polyethylene (HDPE) containers at a depth of approximately 20 cm below the water surface, with the container opening facing the direction of incoming flow. The samples were stored in a cool box and transported to the Environmental Health Laboratory of the Ministry of Health for analysis. Environmental parameters were analyzed using standard methods. pH was determined according to SNI 06-6968.11-2019, whereas TSS was analyzed according to SNI 6989.3:2019. Turbidity was determined using the turbidimetric method; organic matter, DO, BOD, and COD were determined using titrimetric methods; and ammonia and nitrate were analyzed spectrophotometrically.

Environmental parameters were measured once at each station concurrently with the collection of the first macrozoobenthos replicate. Therefore, the environmental data were treated as site-level measurements representing a snapshot of ambient water conditions at the time of biological sampling rather than as estimates of temporal variation in water quality. These measurements were used as site-level environmental descriptors and as supporting variables for interpreting patterns in macrozoobenthic community structure.

Three biological replicate samples of macrozoobenthos were collected at each station during the dry-season study period. Each replicate was collected from a different sampling point separated by approximately 1–5 m while remaining within the same habitat zone and under relatively similar substrate conditions. The replicates were intended to capture local biological variation within each station and were treated as biological replicates in subsequent analyses.

Macrozoobenthos sampling methods were adapted to the dominant substrate characteristics of each river to obtain representative samples of the benthic community under the contrasting habitat conditions. The Bangunsari River was dominated by rocks, gravel, and sand; therefore, macrozoobenthos were collected using a kick net over a 1-m sampling reach. In contrast, the Sawahan River was dominated by muddy substrate and fine sand; therefore, an Ekman grab with a 15 × 15 cm mouth opening was used (Ibrahim et al., 2021). The use of different sampling devices reflected differences in substrate characteristics between the two rivers.

Samples were separated according to station and replicate and subsequently sieved through a 1-mm mesh. The retained macrozoobenthos were cleaned of coarse debris and transferred to sample containers labeled with the station, sampling date, and replicate information. Samples were preserved in 70% alcohol, stored in tightly sealed containers, and transported to the laboratory for examination.

Macrozoobenthos were identified to the genus level based on observable morphological characteristics at the Biology Laboratory of Universitas PGRI Madiun. For BMWP-ASPT assessment, specimens were classified at the family level according to the scoring requirements of the index. Taxonomic identification followed the morphological characteristics described in *Benthic Macroinvertebrates: Field Identification Guide* (Smith et al., 2020).

Data analysis was conducted to integrate ambient environmental conditions with macrozoobenthic community structure. Water-quality data obtained from in situ and ex situ measurements were compared with the Class IV water-quality standards stipulated in Government Regulation of the Republic of Indonesia No. 22 of 2021. Because each environmental parameter was measured only once at each station, these comparisons were used descriptively to characterize the environmental conditions at the time of sampling.

Macrozoobenthic community structure was evaluated using individual abundance, the Shannon–Wiener Diversity Index (H′), Pielou's Evenness Index (J), and Simpson's Dominance Index (C). Biological water quality was assessed using the Biological Monitoring Working Party–Average Score Per Taxon (BMWP-ASPT) index. This index assigns scores according to the relative tolerance of macroinvertebrate families to organic pollution and therefore enables biological water-quality assessment without requiring identification to the species level. BMWP-ASPT has been applied and adapted in various tropical river ecosystems as an indicator of biological water quality and can reflect the responses of local macroinvertebrate assemblages to environmental stressors (Ochieng et al., 2020; Santos et al., 2023; Tampo et al., 2021).

Differences in ecological indices among stations were evaluated using the non-parametric Kruskal–Wallis test. Ecological index values are presented as mean ± standard deviation (SD) based on three biological replicates. Community similarity was assessed using the Bray–Curtis similarity index and visualized using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis. The agreement between the resulting dendrogram and the original similarity matrix was evaluated using the Cophenetic Correlation Coefficient (CCC), while cluster robustness was assessed by bootstrap analysis with 1,000 resamplings.

Associations between macrozoobenthic community composition and environmental conditions were explored using Canonical Correspondence Analysis

(CCA). The CCA was conducted at the station level because environmental parameters were measured only once at each station. The environmental variables included DO, BOD, TSS, and ammonia. The significance of the ordination was evaluated using a Monte Carlo permutation test with 999 permutations.

All statistical analyses were performed using PAST (Paleontological Statistics) version 5.3 at a 95% confidence level. Given the limited number of sampling stations and environmental observations, the CCA results were interpreted exploratorily to identify potential patterns of association between macrozoobenthic community structure and environmental conditions. They were not interpreted as evidence of causal relationships.

RESULTS AND DISCUSSION

Aquatic Environmental Characteristics

This study was conducted in two river ecosystems affected by tofu-industry activities in Madiun Regency. Both study sites are located within residential areas and are therefore potentially exposed to anthropogenic inputs from both industrial and domestic sources. The two rivers differed in several physical characteristics, particularly substrate type, riparian vegetation, and flow conditions. A comparison of the physical characteristics of the Bangunsari and Sawahan Rivers is presented in Table 1.

Table 1. Comparison of the physical characteristics of the study sites in the Bangunsari and Sawahan Rivers

Characteristic	Bangunsari River (Station E)	Sawahan River (Station S)
Flow	Fast/turbulent	Slow/stagnant
Substrate	Rocks, gravel, and coarse sediment	Fine sand and mud
Vegetation	Riparian vegetation (yellow bamboo)	Aquatic macrophytes (water hyacinth and water spinach)
Morphology	Open, with natural shading	Partially covered by aquatic vegetation
Pollution sources	Tofu-industry and domestic wastewater	Tofu-industry and domestic wastewater

As shown in Table 1, the Bangunsari River was characterized by relatively fast flow, rocky and gravelly substrates with coarse sediment, and riparian vegetation dominated by yellow bamboo. In contrast, the Sawahan River had slower flow, substrates dominated by fine sand and mud, and abundant aquatic macrophytes such as water hyacinth and water spinach. Both rivers received pollutant inputs from tofu-industry activities and domestic wastewater generated in the surrounding residential areas.

Table 2. Ambient water conditions in the Bangunsari River

Parameter	Unit	E1	E2	E3	Water Quality Standard	Method
Temperature	°C	26	28	26	Dev. 3	SNI 06-6968.23-2005
Turbidity	NTU	15.42	59.90	14.96	–	Turbidimetry
TSS	mg/L	85	73	42	400	SNI 6989.3:2019
pH	–	7.1	6.3	6.9	6–9	SNI 06-6968.11-2019
Organic matter	mg/L	8.1	11	8.6	–	Titrimetry
Ammonia	mg/L	0.08	0.8	0.1	–	Spectrophotometry

Parameter	Unit	E1	E2	E3	Water Quality Standard	Method
Nitrate	mg/L	0.1	0.2	0.3	20	Spectrophotometry
DO	mg/L	3.0	0.2	2.9	1	Titrimetry
COD	mg/L	99	98	79	80	Titrimetry
BOD	mg/L	22	28	26	12	Titrimetry
Saprobic zone	–	α-mesosaprobic	Polysaprobic	α-mesosaprobic	–	–

Table 3. Ambient water conditions in the Sawahan River

Parameter	Unit	S1	S2	S3	Water Quality Standard	Method
Temperature	°C	27	29	26	Dev. 3	SNI 06-6968.23-2005
Turbidity	NTU	27.75	111.7	31.30	–	Turbidimetry
TSS	mg/L	63	74	42	400	SNI 6989.3:2019
pH	–	6.8	6.0	7.1	6–9	SNI 06-6968.11-2019
Organic matter	mg/L	9.4	16	8.0	–	Titrimetry
Ammonia	mg/L	0.2	0.8	0.2	–	Spectrophotometry
Nitrate	mg/L	0.07	0.2	0.2	20	Spectrophotometry
DO	mg/L	0.6	0	2.4	1	Titrimetry
COD	mg/L	95	109	74	80	Titrimetry
BOD	mg/L	27	42	10	12	Titrimetry
Saprobic zone	–	Polysaprobic	Polysaprobic	α-mesosaprobic	–	–

Tables 2 and 3 show spatial variation in ambient water conditions among the sampling stations in both rivers. Overall, the stations located near the tofu-wastewater discharge points (E2 and S2) exhibited less favorable conditions than the upstream and recovery stations. Water temperature increased at the impacted stations, reaching 28°C at E2 and 29°C at S2. This increase was accompanied by a decline in pH to 6.3 at E2 and 6.0 at S2.

Dissolved oxygen (DO) concentrations declined markedly at the impacted stations. In the Bangunsari River, DO decreased from 3.0 mg/L at E1 to 0.2 mg/L at E2, whereas S2 in the Sawahan River exhibited an extremely low DO concentration of 0 mg/L. In contrast, BOD, COD, turbidity, TSS, and ammonia generally showed higher values at stations receiving direct wastewater inputs. At the recovery stations (E3 and S3), several parameters showed signs of improvement relative to the impacted stations, particularly increased DO and decreased concentrations of several organic-pollution indicators. Collectively, these measurements indicate a spatial environmental gradient associated with the tofu-wastewater discharge zones.

The ambient water data further indicate that stations located near wastewater discharge points generally contained higher concentrations of organic matter than the upstream and recovery stations. Tofu-industry wastewater is known to contain substantial organic loads, including proteins and amino acids, which can contribute to elevated COD, BOD, and TSS concentrations and consequently degrade receiving-water quality (Amri et al., 2020).

In addition to organic wastewater inputs, differences in habitat characteristics between the two rivers may have contributed to the observed environmental conditions. The Bangunsari River, which is characterized by rocky substrates and relatively fast flow, likely experiences greater natural aeration than the slow-flowing Sawahan River, where muddy substrates predominate. These habitat differences can influence dissolved oxygen distribution, organic-matter accumulation, and microhabitat conditions, which may subsequently affect macrozoobenthos community structure. In dynamically flowing waters, suspended solids can make a substantial contribution to turbidity because turbulence maintains particles in suspension (Siregar et al., 2021). Conversely, slow-flow conditions facilitate the deposition of fine particles, resulting in substrates dominated by mud and fine sand (Wahyuningsih et al., 2024).

Macrozoobenthos Taxonomic Composition

A total of eight macrozoobenthos genera belonging to six families were recorded in the Bangunsari and Sawahan Rivers. Their distribution among sampling stations is presented in Table 4.

Table 4. Distribution of macrozoobenthos in the Bangunsari and Sawahan Rivers

No.	Genus	Family	E1	E2	E3	S1	S2	S3	Total	%
1	*Pilsbryoconcha*	Unionidae	14	3	7	6	1	4	35	1.24
2	*Corbicula*	Cyrenidae	28	8	11	3	0	4	54	1.91
3	*Lymnaea*	Lymnaeidae	3	2	0	0	0	2	7	0.25
4	*Pila*	Ampullariidae	4	0	1	5	1	6	17	0.60
5	*Bellamya*	Viviparidae	6	2	1	2	0	1	12	0.42
6	*Thiara*	Thiaridae	57	30	37	27	12	22	185	6.55
7	*Tarebia*	Thiaridae	215	194	169	156	159	118	1,011	35.79
8	*Melanoides* sp.	Thiaridae	260	269	250	257	233	235	1,504	53.24
Total individuals			**587**	**508**	**476**	**456**	**406**	**392**	**2,825**	

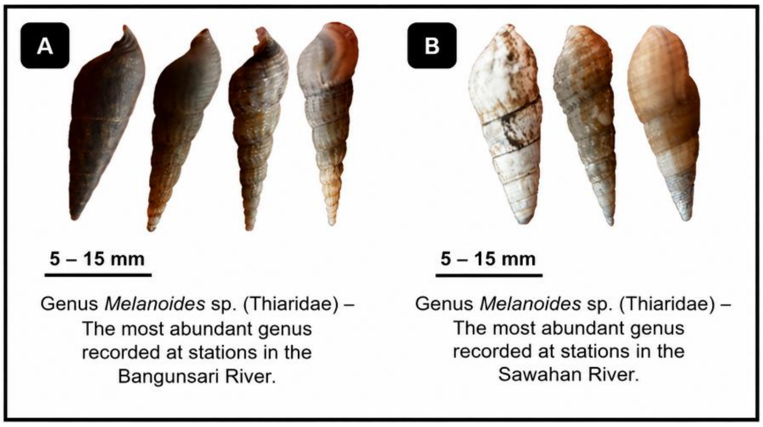

Figure 2. Genus *Melanoides* sp.

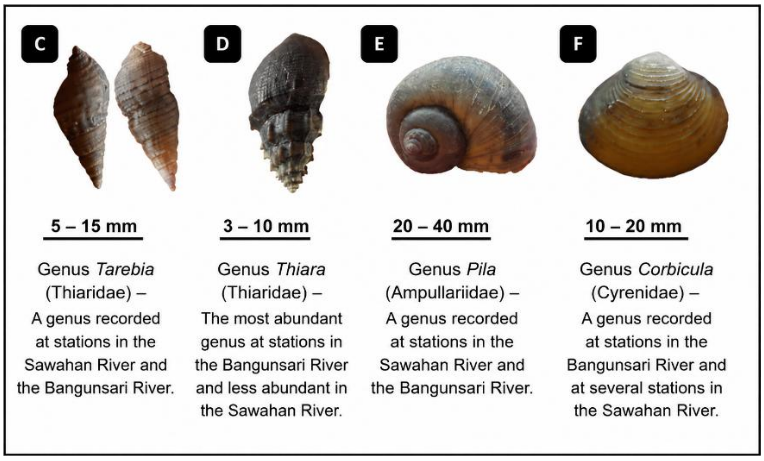

Figure 3. Macrozoobenthos recorded at the Bangunsari and Sawahan River stations

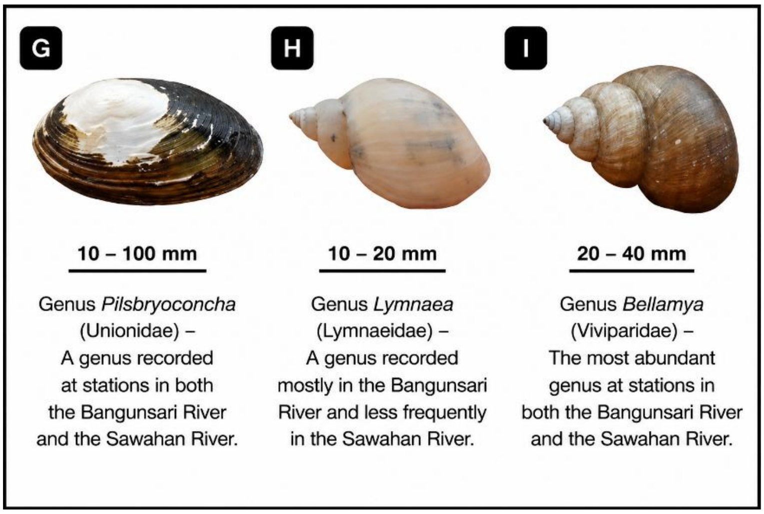

Figure 4. Macrozoobenthos recorded at the Bangunsari and Sawahan River stations

A total of 2,825 macrozoobenthos individuals were collected from the six sampling stations. Gastropods were the dominant taxonomic group, particularly members of the family Thiaridae, represented by *Melanoides*, *Tarebia*, and *Thiara*. Together, these taxa accounted for 95.58% of all individuals recorded. *Melanoides* sp. was the most abundant genus, with 1,504 individuals, followed by *Tarebia* with 1,011 individuals and *Thiara* with 185 individuals. In contrast, *Lymnaea*, *Bellamya*, *Pila*, *Corbicula*, and *Pilsbryoconcha* occurred at considerably lower abundances.

Melanoides sp. occurred at all sampling stations and consistently showed high abundance, whereas *Bellamya* and *Pila* occurred only at several stations and in relatively small numbers. *Pilsbryoconcha* and *Corbicula* also exhibited more restricted distributions than members of the family Thiaridae.

The dominance of Thiaridae may reflect a combination of physiological tolerance to organic enrichment, high reproductive capacity, substrate characteristics, and local habitat conditions. Members of Thiaridae are relatively tolerant of hypoxic conditions and can therefore persist in waters exposed to high organic loads (Athifah et al., 2019). Their reproductive strategies may also facilitate successful colonization. These gastropods are ovoviviparous, with embryos developing within a brood pouch, and some species are capable of parthenogenetic reproduction. In addition, their operculum may enhance their ability to survive unfavorable water conditions, including severe oxygen depletion or anoxia (Isnaningsih et al., 2021).

In contrast, several other genera occurred at relatively low proportions. *Corbicula* and *Pilsbryoconcha* accounted for only 1.91% and 1.24% of the total individuals, respectively. *Corbicula* is a filter-feeding organism that can function as a biofilter by removing organic and inorganic particles from the water column, potentially contributing to stabilization of bottom substrates (Bahtiar et al., 2023). *Bellamya* and *Pila* were also recorded at low proportions, representing 0.42% and 0.60%, respectively, which may reflect their association with habitats characterized by more favorable dissolved oxygen conditions. *Lymnaea* was the least abundant genus, accounting for only 0.25% of all individuals, and is commonly associated with aquatic vegetation used for shelter and feeding.

Thiaridae were also present at upstream stations located before the tofu-wastewater discharge points. This pattern suggests that the occurrence of tolerant taxa cannot be attributed solely to tofu-industry wastewater. Both rivers flow through residential areas, and their upstream sections may already receive domestic wastewater inputs capable of affecting habitat quality. Wahyuningsih et al. (2024)

similarly reported that waters receiving domestic wastewater were dominated by gastropods that are relatively tolerant of variation in physicochemical conditions. Thus, the presence of Thiaridae at upstream stations may reflect the combined influence of natural habitat characteristics and anthropogenic pressures occurring before the tofu-wastewater discharge points.

The dominance of Thiaridae at the study sites may therefore be associated not only with high organic loads but also with local habitat characteristics (Isnaningsih et al., 2021). Differences in substrate type between rivers can influence the distribution and composition of benthic communities because different substrates provide distinct microhabitat conditions for benthic organisms (Apriadi et al., 2020). The use of sampling methods adapted to substrate characteristics was intended to provide the most representative sampling of the benthic community at each river. Overall, the observed dominance of Thiaridae likely reflects an interaction between organic-pollution pressure and local habitat conditions.

Macrozoobenthos Ecological Indices

The ecological characteristics of the macrozoobenthos communities were evaluated using abundance, the Shannon–Wiener diversity index (H′), Pielou's evenness index (J′), and Simpson's dominance index (C), as presented in Table 5.

Table 5. Macrozoobenthos ecological indices in the Bangunsari and Sawahan Rivers

Index	E1	E2	E3	S1	S2	S3
Abundance (ind/m²)	8,696.30	7,525.93	7,051.85	6,755.56	6,014.81	5,807.41
Diversity (H′)	1.291 ± 0.057	1.017 ± 0.077	1.058 ± 0.160	1.003 ± 0.136	0.730 ± 0.166	0.975 ± 0.295
Evenness (J′)	0.644 ± 0.032	0.560 ± 0.042	0.630 ± 0.006	0.553 ± 0.039	0.559 ± 0.176	0.549 ± 0.048
Dominance (C)	0.349 ± 0.008	0.428 ± 0.026	0.420 ± 0.068	0.450 ± 0.058	0.570 ± 0.179	0.485 ± 0.098

Macrozoobenthos abundance ranged from 5,807.41 to 8,696.30 ind/m². The highest abundance was recorded at E1, whereas the lowest occurred at S3. Overall, abundance was higher in the Bangunsari River than in the Sawahan River. Shannon–Wiener diversity values ranged from 0.730 ± 0.166 to 1.291 ± 0.057, with the highest value at E1 and the lowest at S2. Pielou's evenness index ranged from 0.549 ± 0.048 to 0.644 ± 0.032, whereas Simpson's dominance index ranged from 0.349 ± 0.008 to 0.570 ± 0.179. Dominance was highest at S2 and lowest at E1.

Table 6. Differences in ecological indices among sampling stations

Ecological index	Kruskal–Wallis statistic	p-value	Interpretation
Diversity (H′)	10.740	0.0569	$p > 0.05$
Evenness (J′)	8.205	0.1453	$p > 0.05$
Dominance (C)	9.982	0.0757	$p > 0.05$

The Kruskal–Wallis test indicated no statistically significant differences among sampling stations in Shannon–Wiener diversity (H′), Pielou's evenness (J′), or Simpson's dominance (C) ($p > 0.05$). The test statistics were 10.740 for H′ ($p = 0.0569$), 8.205 for J′ ($p = 0.1453$), and 9.982 for C ($p = 0.0757$).

Although macrozoobenthos abundance varied among stations, high abundance does not necessarily indicate favorable ecological conditions because community status also depends on the taxonomic composition of the assemblage. In this study, most individuals belonged to Thiaridae, a group known to tolerate environmental stress (Athifah et al., 2019). Consequently, ecological interpretation should consider community composition together with diversity, evenness, and dominance indices rather than abundance alone.

The relatively low Shannon–Wiener diversity values indicate that the macrozoobenthos communities were dominated by a limited number of taxa and that individuals were unevenly distributed among genera. Based on the classification of Wilhm and Dorris (1968), H′ values ranging from 0.730 to 1.291 correspond to conditions ranging from moderate to severe pollution. Low diversity suggests that environmental conditions at the study sites favored taxa with relatively high tolerance to environmental stress over more sensitive taxa.

Evenness values of 0.549–0.644 indicated a moderately uneven distribution of individuals, with abundance concentrated in several dominant taxa. This pattern was also reflected in Simpson's dominance values of 0.349–0.570, particularly because of the predominance of Thiaridae. The combination of low diversity, moderate evenness, and dominance by tolerant taxa is commonly associated with ecosystems experiencing pollution pressure, including organic enrichment. Similar patterns have been reported in studies showing that anthropogenic organic inputs can reduce community diversity while increasing the dominance of pollution-tolerant taxa (Ramadhan et al., 2024; Wijayanti et al., 2025; Yanti et al., 2022).

Nevertheless, the Kruskal–Wallis analysis showed that H′, J′, and C did not differ significantly among stations ($p > 0.05$). This suggests that the overall macrozoobenthos community structure, as represented by these indices, remained relatively similar among sampling locations. The widespread dominance of Thiaridae likely contributed to the relative homogeneity of community-index values and reduced the magnitude of differences in diversity, evenness, and dominance among stations (Athifah et al., 2019; Isnaningsih et al., 2021). In addition, the limited number of sampling stations and biological variation among replicates may have reduced the statistical power to detect significant differences.

Biological Water Quality Assessment Using BMWP-ASPT

The Biological Monitoring Working Party (BMWP) metric was originally developed in the United Kingdom in the 1970s as a biological index for assessing river quality based on the pollution tolerance of benthic macroinvertebrate families. The Average Score Per Taxon (ASPT) represents the mean tolerance score across the families recorded and therefore provides an assessment that is less sensitive to differences in taxon richness (Ibrahim et al., 2021). The BMWP-ASPT results for the study sites are presented in Table 7.

Table 7. BMWP-ASPT scores for macrozoobenthos at the study sites

Station	BMWP score	No. of families	ASPT score	Category
E1	30	6	5.0	Lightly polluted
E2	24	5	4.8	Moderately polluted
E3	27	5	5.4	Lightly polluted
S1	27	5	5.4	Lightly polluted
S2	15	3	5.0	Lightly polluted
S3	30	6	5.0	Lightly polluted

Notes:
ASPT > 6: Clean water (very good)
ASPT 5–6: Lightly polluted (good)
ASPT 4–<5: Moderately polluted (fair/doubtful)
ASPT < 4: Heavily polluted (poor/very poor)

BMWP-ASPT assessment classified the study sites as ranging from lightly to moderately polluted, with ASPT values of 4.8–5.4. E2 was classified as moderately polluted, whereas E1, E3, S1, S2, and S3 fell within the lightly polluted category. Although S2 remained within the lightly polluted category based on an ASPT score of 5.0, its lower BMWP score and reduced family richness indicate biological impairment relative to several other stations.

The relatively low biological scores at the directly impacted stations suggest greater ecological pressure where tofu-industry wastewater entered the rivers. Such changes are consistent with a reduction in sensitive taxa and the increasing predominance of organisms that are more tolerant of organic pollution.

The BMWP-ASPT assessment yielded a more moderate evaluation of biological water quality than that inferred from the Shannon–Wiener diversity index. This difference may reflect the presence of several macrozoobenthos families with relatively favorable tolerance scores, which can increase the ASPT value even when the overall assemblage is numerically dominated by tolerant taxa. BMWP-ASPT was originally developed for temperate rivers using family-level sensitivity to pollution; however, it has subsequently been adapted and applied to tropical river ecosystems because of its ability to characterize gradients of biological disturbance (Ochieng et al., 2020; Tampo et al., 2021). Applications in tropical Indonesian rivers have similarly indicated that BMWP-ASPT can provide a useful assessment of biological water quality because it incorporates information from the macroinvertebrate families present and their relative pollution sensitivity (Nurman et al., 2025).

Macrozoobenthos Community Similarity Analysis (Bray–Curtis and UPGMA)

To evaluate patterns of similarity in macrozoobenthos community composition among sampling units, cluster analysis was performed using the Bray–Curtis similarity index and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The resulting dendrogram illustrates the degree of similarity among sampling units based on their macrozoobenthos composition and abundance, as presented in Figure 5.

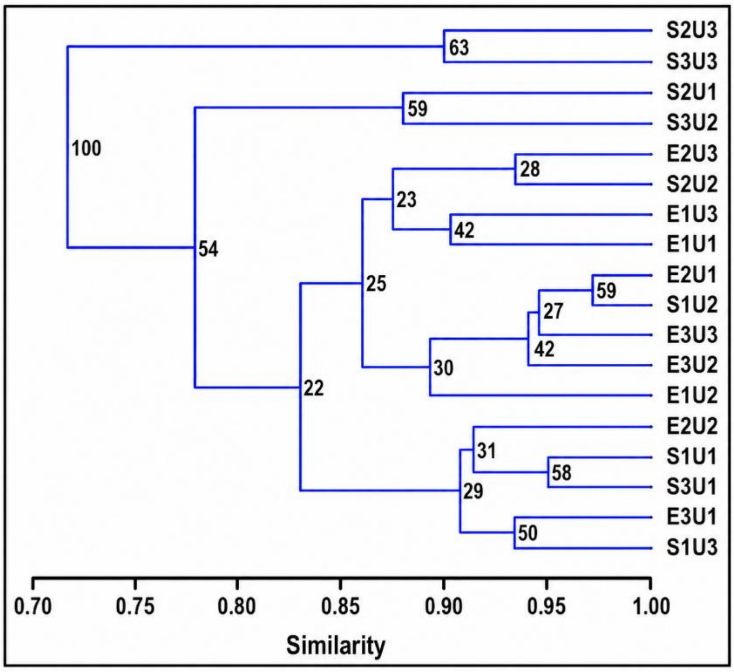

Figure 5. Dendrogram of macrozoobenthos community similarity based on the Bray–Curtis index using the UPGMA method

Cluster analysis based on Bray–Curtis similarity and the unweighted pair-group method with arithmetic mean (UPGMA) revealed patterns of grouping according to macrozoobenthos community composition. The Cophenetic Correlation Coefficient (CCC) was 0.7855, indicating a reasonably good correspondence between the dendrogram and the original Bray–Curtis similarity matrix. CCC values closer to 1 indicate that the dendrogram more effectively preserves the original dissimilarity relationships among objects (Gere, 2023).

Bootstrap analysis with 1,000 resamplings produced cluster-support values ranging from 22% to 100%. Overall, the sampling units exhibited relatively high community similarity (>70%). Samples from the Sawahan River tended to form a major group with relatively homogeneous community composition, whereas samples from the Bangunsari River formed several subclusters, indicating somewhat greater variation in community composition.

The high similarity among sampling units indicates that macrozoobenthos composition was broadly comparable across much of the study area. This pattern was largely associated with the dominance of genera belonging to Thiaridae, which are known for their relatively high environmental tolerance. Their widespread occurrence reduced compositional variation among sites.

Community similarity may also have been influenced by the taxonomic resolution used in this study. Organisms were identified to the genus level; consequently, species-level variation was not captured by the analysis. Differences among sampling units may therefore appear smaller than they would if identification were conducted to species level. The combination of broadly comparable habitat characteristics and the dominance of several tolerant genera likely contributed to the high community similarity observed across most sampling locations.

Canonical Correspondence Analysis (CCA)

To explore the association between macrozoobenthos community composition and measured water-quality variables, Canonical Correspondence Analysis (CCA) was performed using DO, BOD, TSS, and ammonia as environmental predictors. The resulting ordination illustrates the relative positions of sampling stations, macrozoobenthos taxa, and environmental gradients, as shown in Figure 6.

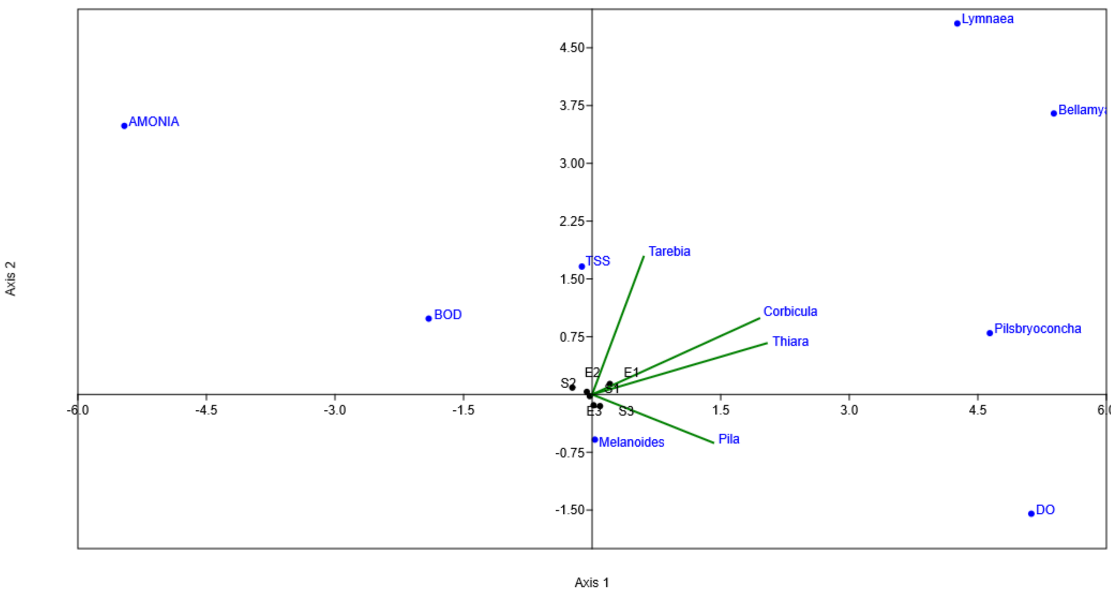

Figure 6. CCA ordination of water-quality variables and macrozoobenthos

Table 8. Scores of environmental variables on the first two CCA axes

Variable	Axis 1	Axis 2
DO	5.124	-1.548
BOD	-1.907	0.984
TSS	-0.120	1.660
Ammonia	-5.458	3.486

Canonical Correspondence Analysis (CCA) showed that the first two axes accounted for 71.85% of the variation represented in the ordination between macrozoobenthos community composition and the environmental variables. Axis 1 explained 49.85% of the variation, whereas Axis 2 accounted for 22.00%.

Based on the environmental-variable scores, Axis 1 was primarily characterized by the opposing gradients of DO (5.124) and ammonia (-5.458), which had the largest absolute scores among the variables analyzed. Axis 2 was associated mainly with ammonia (3.486), TSS (1.660), and BOD (0.984). BOD and ammonia were positioned on the negative side of Axis 1 and were associated with the impacted stations, particularly E2 and S2. In contrast, DO was positioned on the positive side of Axis 1 and was associated with *Bellamya*, *Lymnaea*, and *Pilsbryoconcha*. *Melanoides*, *Thiara*, and *Tarebia* were located close to the center of the ordination, consistent with their relatively broad distributions across the sampling stations.

However, the Monte Carlo permutation test showed that the overall relationship between macrozoobenthos community composition and the environmental variables was not statistically significant (Trace = 0.02684; $p = 0.139$).

The CCA nevertheless suggested an ecological gradient between waters characterized by higher DO and those characterized by higher concentrations of ammonia, BOD, and TSS. This descriptive pattern suggests that variation in water quality may be associated with macrozoobenthos distribution at the study sites. A comparable pattern was reported by Imroatushshoolikhah et al. (2021), who identified DO, temperature, pH, and turbidity as environmental factors associated with macrozoobenthos distribution in a CCA ordination.

Because the Monte Carlo permutation test was not significant ($p = 0.139$), however, the measured environmental variables cannot be considered strong statistical predictors of macrozoobenthos community variation in this dataset. Several factors may have contributed to the lack of statistical significance. First, macrozoobenthos communities across the sampling stations were strongly dominated by Thiaridae, resulting in relatively homogeneous taxonomic composition. Second, the number of stations included in the analysis was limited. Third, water-quality variables were measured only once at each station, restricting the ability of the analysis to account for temporal environmental variability.

In addition, habitat variables that were not quantitatively measured, such as current velocity, river discharge, and riparian vegetation cover, may also have influenced macrozoobenthos community structure. Accordingly, the CCA results should be interpreted as exploratory evidence of potential associations between macrozoobenthos communities and environmental conditions rather than as evidence of causal relationships.

CONCLUSION

Based on the results and discussion, the macrozoobenthos community structures in the Bangunsari and Sawahan Rivers showed descriptive changes along the gradient of tofu industry wastewater influence. Stations directly receiving wastewater inputs tended to exhibit poorer ambient water conditions and a greater dominance of pollution-tolerant taxa. Biological water quality assessment using the BMWP-ASPT index indicated conditions ranging from lightly to moderately polluted, with the lowest ASPT score recorded at the directly impacted station in the Bangunsari River. Bray-Curtis similarity analysis and UPGMA clustering revealed grouping patterns based on similarities in macrozoobenthos community composition. Canonical Correspondence Analysis (CCA) further indicated a tendency for macrozoobenthos community structure to be associated with ambient water variables, particularly DO, BOD, TSS, and ammonia; however, this relationship was not statistically significant according to the Monte Carlo permutation test ($p > 0.05$).

This study provides baseline information on macrozoobenthos community structure, biological water quality, and community–environment relationships in the Bangunsari and Sawahan Rivers in Madiun Regency. These findings may serve as an initial reference for monitoring the ecological condition of rivers receiving tofu industry wastewater and provide a basis for further research incorporating broader spatial coverage, seasonal observations, and additional habitat variables.

RECOMMENDATION

Based on the findings of this study, the implementation of wastewater treatment systems capable of reducing organic loads before effluent is discharged into receiving waters is recommended. Waste stabilization ponds may be considered as a treatment option suitable for household-scale and small-scale tofu industries. In addition to controlling pollution at its source, periodic biological monitoring should be conducted during both the dry and rainy seasons. Biomonitoring can complement physicochemical measurements in evaluating wastewater treatment effectiveness and the ecological recovery of receiving waters.

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