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Assessment of Macronutrient Dynamics in a Tropical Watershed: A Study from Bengkulu River and Estuary Indonesia

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Abstract
This study assesses the sources, transport, and distribution of macronutrients, including nitrate, nitrite, silicate, and phosphate, starting from the upper stream of the Bengkulu River, which is the biggest river in Bengkulu Province, to the coastal seawater of the eastern tropical Indian Ocean. The sampling was conducted using an acid-washed polypropylene beaker and placed in acid-cleaned 5 L polyethylene containers during the dry season in July 2024. The water samples were collected from the surface waters of 15 stations. The macronutrients in the water samples were determined using a spectrophotometer UV-Vis following the Strickland and Parsons method. Macronutrient analysis was conducted in LATERIO BRIN, Jakarta, Indonesia. The concentration of analysed macronutrients ranged from 0.005 - 0.058 mgN-NO_3/L for nitrate, <0.001 - 0.014 mgN-NO_2/L for nitrite, 0.163 - 9.314 mgSi-$Si(OH)_4$/L for silicate, and 0.01 - 0.085 mgP-PO_4-/L for phosphate. Several stations, including Stations 6, 10, and 14, exhibited relatively elevated nitrate concentrations, which were further supported by similarly high phosphate levels. Stations 6, 10, and 14, situated near areas of intense anthropogenic activity in Bengkulu City, suggest that human-induced inputs have enriched the water environment with macronutrients. The highest silicate concentration was observed at Station 1, located upstream in the Bengkulu River. This aligns with previous findings and underscores the role of weathering processes in controlling silicate distribution. Compared to the macronutrient concentration guidelines for river water set by Indonesian Government Regulation 2021 No. 22, the nitrite concentrations at certain river stations exceeded the safety threshold for aquatic biota. In contrast, nitrate and phosphate levels remained within the safe range.

Keywords: Bengkulu River, environmental assessment, estuary, macronutrients, UV-Vis spectrophotometry.

1. INTRODUCTION

Estuaries are the transition zone between the river and the marine environment. The characteristics of estuaries are unique because they are influenced by the characteristics of the rivers and seas that form them. As a transition zone, the water mass contained in the estuary undergoes changes in physical and chemical properties that are influenced by the season, river water discharge, primary productivity, water circulation, and human activities around it. River flow carries nutrients, sediments, and pollutants to the estuary before finally reaching the open ocean. Changes in physical properties, especially salinity in estuarine areas, affect the biogeochemical cycles of materials in estuarine waters.

The distribution and biogeochemical cycling of dissolved materials, including macronutrients, are highly dependent on the nature and conditions of aquatic ecosystems, such as mixing between freshwater and seawater [1, 2]. In general, the behavior of dissolved materials during the mixing process can be categorized as conservative or non-conservative [3]. Mixing behavior is considered conservative if only physical processes (mixing of water mass) affect the distribution of dissolved material concentrations during the mixing process. Meanwhile, non-conservative behavior indicates the existence of other processes that affect the distribution of dissolved material concentrations during the mixing process, which results in a decrease or increase in the concentration of these materials in the water body. For materials exhibiting non-conservative behavior, salinity gradients in estuaries can induce the release of macronutrients such as nitrate, nitrite, silicate, and phosphate from sediments into water bodies [4]. Increased dissolved nitrate nutrient concentrations can lead to aquatic fertilization and hypoxic conditions [5, 6].

Given the importance of estuarine waters in carrying nutrients into aquatic ecosystems, a comprehensive study of the physical and biogeochemical aspects in the broader estuarine region, including waters (rivers, estuaries, and seas), needs to be carried out. Previous studies have been conducted in other countries, including the Mississippi River estuary [4] (the distributions of nitrate, nitrite, phosphate, and silicate in surface waters); Peconic River, USA [7] (the distribution of inorganic nutrients PO_4 and H_4SiO_4); Rhône and Seine Rivers, France [8] (the distribution of dissolved trace elements in the mixing zone); Ariake Bay, Japan [9] (input of anthropogenic nutrients and contamination through rivers); Otsuchi Bay, Japan [10, 11] (the distribution of nitrate and phosphate of river waters); and Bay of Bengal, India [12] (anthropogenic contributions through industrial and municipal waste disposal in the waters). However, comprehensive studies on the biogeochemistry and variability of macronutrients in estuarine waters in Indonesia are scarce. Likewise, limited studies assess macronutrient behavior in estuary-forming systems (rivers, estuaries, and seas). Studying comprehensive macronutrient dynamics in estuary-forming systems from the Bengkulu River to the coast of the eastern tropical Indian Ocean is important for three main reasons. Firstly, profoundly limited studies of these topics in Indonesian waters compared to those in other regions indicate that this study can add important insight into the existing knowledge, mostly found in different areas. Secondly, it is important to understand the behavior of each macronutrient during estuarine mixing to assess their biogeochemical cycle and the factors that govern their behavior. Thirdly, assessing the sources and transport of macronutrients in the Bengkulu River and its estuary is critical for identifying potential anthropogenic influences, such as agricultural runoff, wastewater discharge, and urban pollution. Elevated nitrate and phosphate concentrations in certain locations, particularly near densely populated and agriculturally active regions, suggest human-induced nutrient enrichment. Such findings provide valuable information for water resource management and environmental policies to mitigate eutrophication and maintain water quality. By systematically analyzing macronutrient dynamics from the upper Bengkulu River to the coastal seawater of the eastern tropical Indian Ocean, this study contributes to a more comprehensive understanding of biogeochemical processes in Indonesian estuaries. The results can serve as a baseline for future research and inform sustainable management strategies for riverine and coastal ecosystems in the region.

In this study, we assess the sources, transport, and distribution of macronutrients, including nitrate, nitrite, silicate, and phosphate, from the upper stream of the Bengkulu River, which is the biggest river in Bengkulu Province, to the coastal seawater of the eastern tropical Indian Ocean. Macronutrients were measured using a spectrophotometer, Shimadzu UV-1800. This study used a mixing diagram to evaluate macronutrients' conservative or non-conservative behavior during the estuarine mixing process. Understanding whether these nutrients exhibit conservative mixing, where their concentrations change solely due to the physical mixing of freshwater and seawater, or non-conservative mixing, where additional biogeochemical processes such as biological uptake, remineralization, or anthropogenic inputs play a role, is essential for assessing nutrient cycling in the system.

2. RESEARCH METHODS

Sampling location and storage

Water samples from the Bengkulu River and estuaries were collected at 15 stations using an acid-washed polypropylene (PP) beaker and placed in acid-cleaned 5 L polyethylene (PE) containers following the methods of Wiwit et al. (2023) and Wong et al. (2018) [11, 13] (Figure 1). Surface water samples were collected from four sampling sites in the river (S1, S2, S6, and S7), three sampling sites in the lakes (S3, S4, and S5), six sampling sites in the estuaries (S8, S9, S10, S11, S13, and S14), and two stations connected to the Indian Ocean (S12 and S15).

Water samples that were used for analyzing the concentration of macronutrients (nitrate, nitrite, silicate, and phosphate) were filtered through a cellulose acetate membrane filter (Whatman, 0.45 μm pore size filter) into acid-cleaned 500 mL high-density polyethylene (HDPE) bottles.1 mL of concentrated $HgCl_2$ was added to preserve the samples. Then the samples were kept cool and refrigerated at 4 °C before analysis in the laboratory.

Analytical method

The macronutrients in the water samples were determined using a spectrophotometer UV-Vis (Shimadzu UV-1800) following the Strickland and Parsons (1972) method [14]. Macronutrient analysis was conducted in the Integrated Marine Biosphere Research Laboratory (LATERIO) of BRIN Jakarta, Indonesia.

The phosphate concentration was measured using the molybdenum blue method, where phosphate reacts with ammonium molybdate in an acidic medium to form a phosphomolybdate complex. This complex is subsequently reduced by ascorbic acid to produce a blue-colored compound, which is measured at an absorption wavelength of 885 nm [14]. The silicate was quantified based on the forming a silicomolybdate complex, where silicate reacts with ammonium molybdate under acidic conditions to form a yellow silicomolybdate complex. This complex is then reduced by ascorbic acid to produce a blue-colored complex, measured at 810 nm [14]. Nitrite was analyzed using the colorimetric diazotization method, in which nitrite reacts with sulfanilamide under acidic conditions to form a diazonium salt. This salt then couples with NED to form a pink azo dye, which is detected at 543 nm [14]. Like nitrite, nitrate was also analyzed using the colorimetric diazotization method and detected at 543 nm [14]. However, nitrate in the solution was first reduced to nitrite by passing the sample through a cadmium column coated with copper. The nitrite then reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to form an azo dye, then quantified at 543 nm [14].

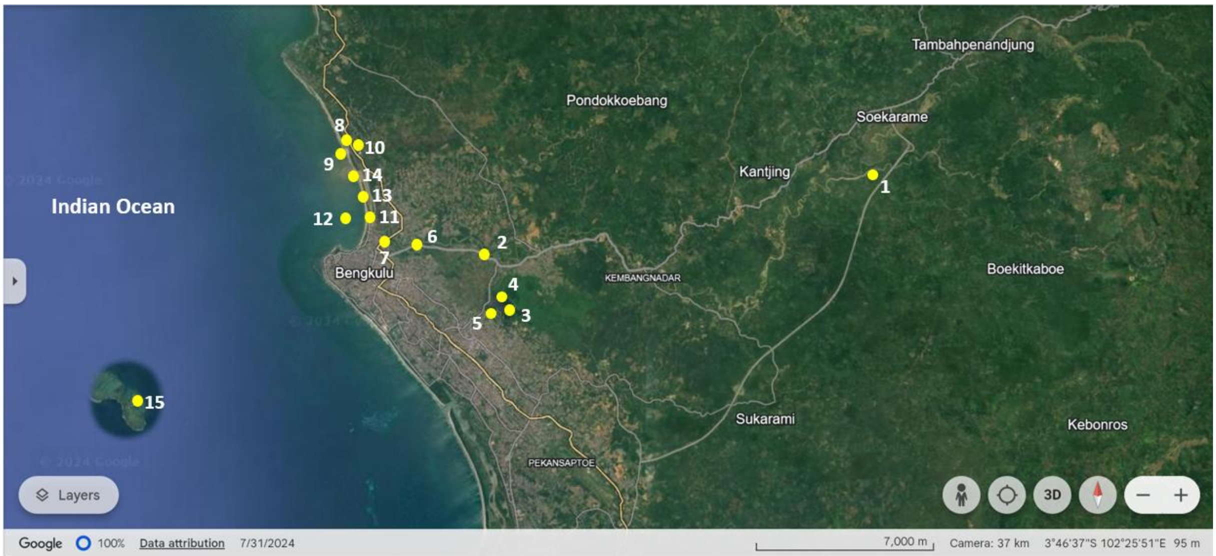


Figure 1. Map of sampling location. The map was created using the Google Earth application.

Milli-Q water (MQW), containing all reagents used in the analysis but without the sample, was used as a blank. All blank measurements were below the detection limit, indicating negligible background interference in the analysis. Due to the limited volume of water samples, replicate measurements were not performed. For quality control, deep seawater (DSW) samples from the Sulawesi Sea at a depth of 1000 m were used, based on the assumption that there is no biological utilization, no external inputs, and that the concentration remains stable during storage. Measurements of deep seawater were conducted repeatedly before, during, and after sample loading to ensure instrument stability and measurement consistency. The results showed consistent absorbance values throughout the analysis and were provided in **Supplementary Table 1.**

The limit of detection (LoD) and limit of quantification (LoQ) were determined based on the standard deviation from the standard curve rather than from blank measurements. The standard curve for each parameter was—provided in Supplementary **Figure 1**. This approach better represents real sample measurement variability, accounts for instrumental and procedural variations, and ensures more reliable detection at low concentrations. The LoD was calculated as 3.3σ (3.3 times the standard deviation) divided by the slope of the calibration curve, while the LoQ was 10σ divided by the slope. LOD for phosphate was 0.002 mg $P\text{-}PO_4$/L; nitrate was 0.004 mg $N\text{-}NO_3$/L; nitrite was 0.001 mg $N\text{-}NO_2$/L; and silicate was 0.014 mg $Si\text{-}Si(OH)_4$/L. Meanwhile, LOQ for phosphate was 0.007 mg $P\text{-}PO_4$/L; nitrate was 0.013

mg $N\text{-}NO_3$/L; nitrite was 0.002 mg $N\text{-}NO_2$/L; and silicate was 0.043 mg $Si\text{-}Si(OH)_4$/L.

3. RESULTS AND DISCUSSION

The data for salinity, temperature, pH, and the concentrations of macronutrients, including nitrate, nitrite, silicate, and phosphate, starting from the upper stream of the Bengkulu River (S1) to the coastal seawater of the eastern tropical Indian Ocean (S15) are shown in **Table 1**. The concentration of analyzed macronutrients ranged from 0.005 - 0.058 mgN-NO_3/L for nitrate, <0.001 - 0.014 mgN-NO_2/L for nitrite, 0.163 - 9.314 mgSi-$Si(OH)_4$/L for silicate, and 0.01 - 0.085 mgP-PO_4-/L for phosphate. The nitrite concentrations at certain river stations exceeded the safety threshold for aquatic biota, whereas nitrate and phosphate levels remained within the safe range [17]. The nitrite concentrations were not detected (N.D.) or below 0.001 mg/L in Stations S2, S12, and S15, indicating that the sources of nitrite were not from human activities. Several stations, including Stations S6, S10, and S14, exhibited relatively elevated nitrate concentrations, which were further supported by similarly high phosphate levels. Stations S6, S10, and S14, situated near areas of intense anthropogenic activity in Bengkulu City, suggest that human-induced inputs have enriched the water environment with macronutrients [19, 20].

Table 1. Data for salinity, temperature, pH, and the concentrations of macronutrients in the Bengkulu River and estuary

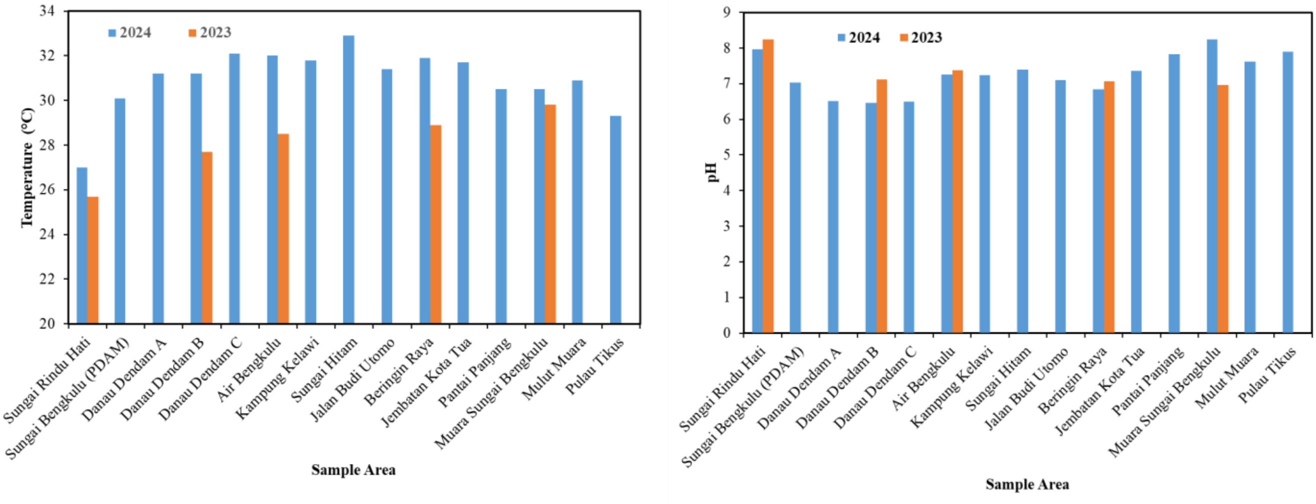
Station	Sample Area	Temperature (°C)	Salinity (‰)	pH	Phosphate (mg P-PO_4/L)	Nitrate (mg N-NO_3/L)	Nitrite (mg N-NO_2/L)	Silicate (mg Si-$Si(OH)_4$/L)
S1	Sungai Rindu Hati	27.0	0.0	7.96	0.076	0.019	0.001	9.314
S2	Sungai Bengkulu (PDAM)	30.1	0.0	7.04	0.039	0.028	N.D	8.372
S3	Danau Dendam A	31.2	0.0	6.51	0.011	0.008	0.004	1.831
S4	Danau Dendam B	31.2	0.0	6.46	0.019	0.006	0.002	1.703
S5	Danau Dendam C	32.1	0.0	6.50	0.011	0.027	0.003	1.378
S6	Air Bengkulu	32.0	0.0	7.26	0.060	0.058	0.001	8.680
S7	Kampung Kelawi	31.8	0.0	7.24	0.077	0.019	0.003	8.357
S8	Sungai Hitam	32.9	28.6	7.40	0.015	0.006	0.007	0.714
S9	Jalan Budi Utomo	31.4	23.0	7.10	0.023	0.017	0.014	1.473
S10	Beringin Raya	31.9	21.5	6.84	0.050	0.034	0.013	2.325
S11	Jembatan Kota Tua	31.7	0.5	7.36	0.085	0.005	0.003	8.332
S12	Pantai Panjang	30.5	31.8	7.82	0.010	0.007	N.D	0.163
S13	Muara Sungai Bengkulu	30.5	2.8	8.24	0.057	0.006	0.002	7.860
S14	Mulut Muara	30.9	2.0	7.62	0.073	0.052	0.002	8.776
S15	Pulau Tikus	29.3	31.9	7.90	0.079	0.005	N.D.	0.616

N.D. not detected

A comparison of the macronutrient concentration data of phosphate, silicate, nitrate, and nitrite in a previous study taken during the wet season in March 2023 at some of the same sample points in this study was carried out, as shown in **Figures 2** and 3, respectively. The difference in the sampling period resulted in water temperature and pH differences. Lower water temperatures during the wet season are probably due to the intense rainfall and lower air temperature. Water pH in the dry season was slightly more acidic than in the wet season, except for Muara Sungai Bengkulu (S13).

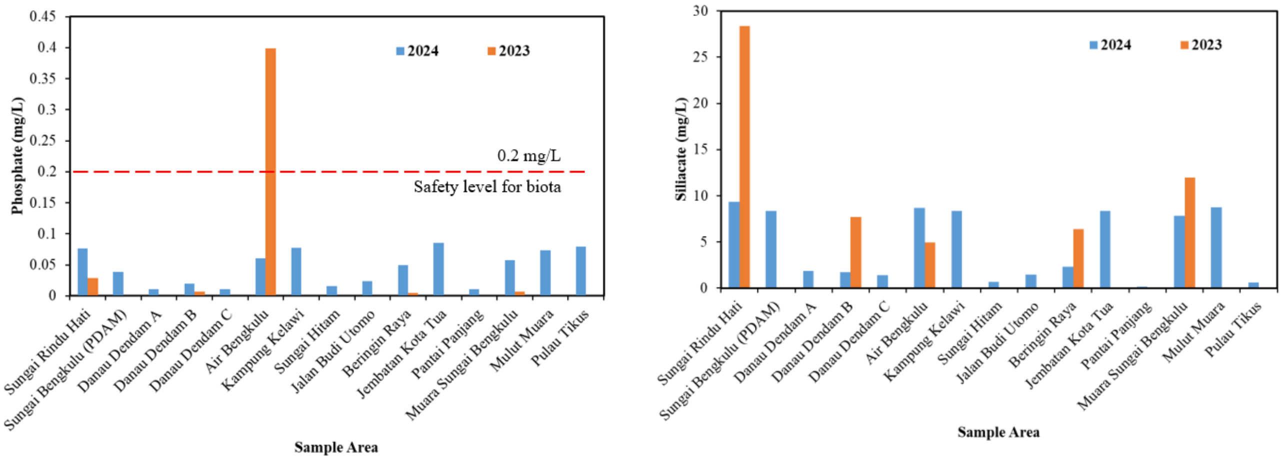
In general, phosphate and silicate showed temporal variation during wet and dry seasons (**Figure 3**). Anomalous high phosphate in Air Bengkulu (S6) during the wet season indicated a point source from agricultural activities nearby [19], while higher silicate in Rindu Hati (upper stream, S1) might be due to the more intense rock weathering in 2023 than in 2024.

Nitrate and nitrite showed temporal variation during the wet season in March 2023 and the dry season in July 2024 (**Figure 4**). Nitrate and nitrite (N+N) concentrations were higher during the wet season, suggesting natural runoff e.g. agricultural runoff near Air Bengkulu (S6), and anthropogenic runoff such as urban and agricultural wastes in other stations [18]. Seasonal changes (drought and rain) and anthropogenic activities will impact the distribution and biogeochemical cycling of macronutrients in the Bengkulu River flow towards the estuary. Nutrient loads can originate from upstream of the watershed, entering the watershed as part of the inflow. Meanwhile, rainfall can cause a runoff that washes nutrients from the soil and into the water body.



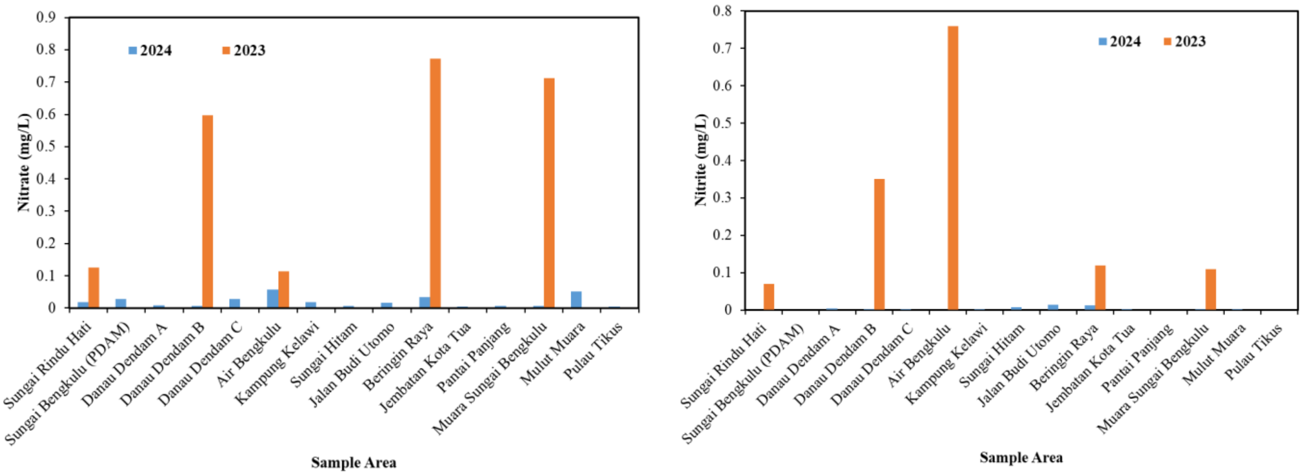
(a) **(b)**

Figure 2. Comparison of temperature **(a)** and pH **(b)** of water samples in two different seasons (dry season in 2024 and wet season in 2023).



(a) **(b)**

Figure 3. Comparison of phosphate **(a)** and silicate **(b)** concentrations in two different seasons (dry season in 2024 and wet season in 2023)



(a) **(b)**

Figure 4. Comparison of nitrate **(a)** and nitrite **(b)** concentrations in two different seasons (dry season in 2024 and wet season in 2023).

Diagram of mixing analysis

A diagram of mixing analysis showed a linear correlation (R^2 = 1) between silicate concentrations and salinity, and the relationship can be expressed as y = -0.273× + 9.3138, where y represents silicate concentrations (in mg/L) and x represents salinity (in ‰), as shown in **Figure 5**. Using surface silicate concentration data from Ikhsani et al. (2023) in the eastern tropical Indian Ocean (5°S) as a seawater end-member, silicate behaves conservatively during the mixing [3]. Physical processes such as water mass mixing are probably the main factors governing silicate distributions in the Bengkulu River.

Figure 6 shows the correlation between nitrate and nitrite concentrations versus salinity in the water samples. Additional nitrate and nitrite were observed in the simple mixing diagrams using data for the nitrate and nitrite concentrations in the eastern tropical Indian Ocean (5°S) as a seawater end-member [15]. Strong nitrate and nitrite concentrations-salinity relationship (R^2 = 1) was observed across the Bengkulu River and estuary (Figure 6), and the relationship can be expressed as y = -0.0005× + 0.0186 for nitrate and y = -2e-05× + 0.001 for nitrite, where y represents the nitrate and nitrite concentration (mg/L) and x represents the salinity (in ‰). It showed that nitrate and nitrite behave non-conservatively during the mixing. Remineralizing organic matter probably enriched N+N during the dry season [15, 16, 18]. Lower pH during the dry season suggests higher remineralization activities.

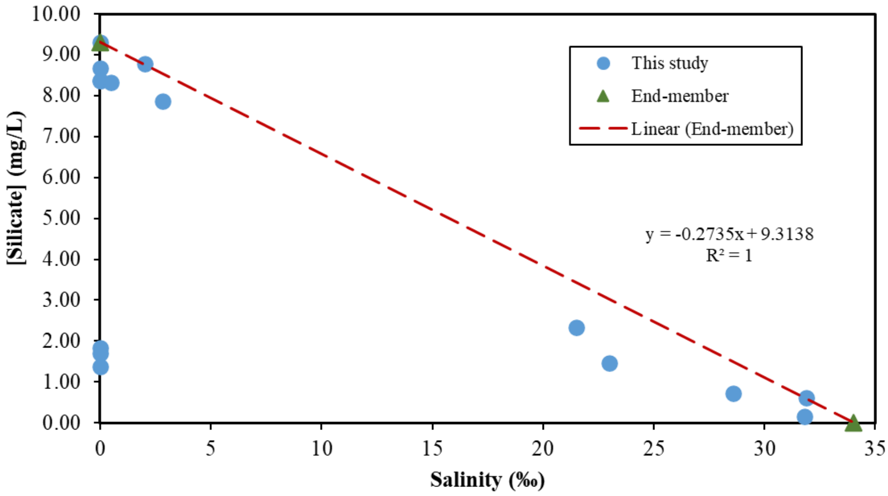
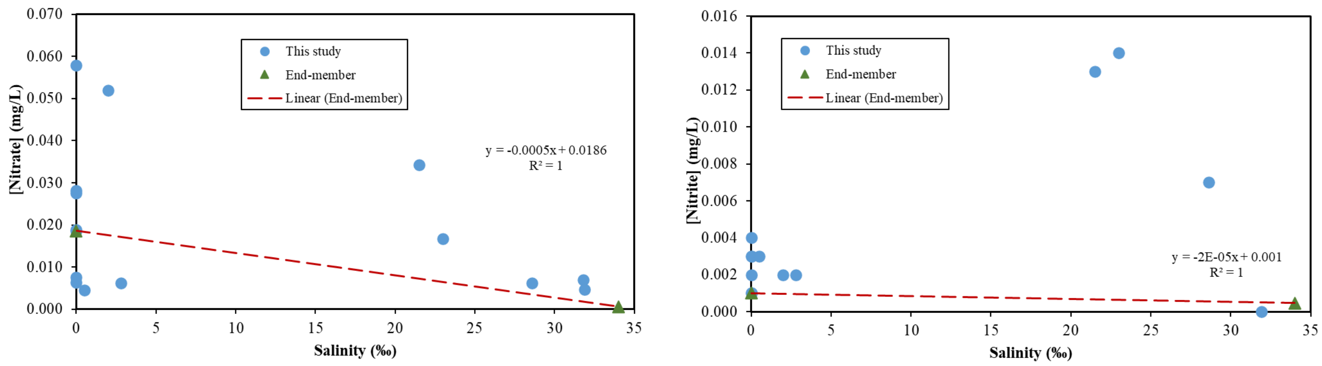


Figure 5. Correlation between silicate concentrations and salinity in the water samples.



(a) **(b)**

Figure 6. Correlation between nitrate **(a)** and nitrite **(b)** concentrations versus salinity in the water samples.

Using surface phosphate data from Ikhsani et al. (2023) [15] in the eastern tropical Indian Ocean (5°S) as a seawater end-member, phosphate in the Bengkulu River behaves non-conservatively. It can be shown in **Figure 7**. **Figure 7** presents the strong correlation between the phosphate concentration and salinity (R^2 = 1). Phosphate (P) removal was mainly observed in the low salinity region, probably due to biological uptake [19, 20, 21]. Conversely, additional P was also observed in the mid and high-salinity regions, which indicated P sources in these stations, possibly from agricultural runoffs [19, 20].

Moreover, a one-way ANOVA test was conducted to assess whether there are significant differences in the physical parameters (temperature, salinity, and pH) and macronutrient concentrations between sampling sites. It is worth noting that only temperature and pH were assessed with one-way

ANOVA for physical parameters, since salinity was not normally distributed and violated the normality assumption for one-way ANOVA ($p < 0.05$), while all macronutrients were tested. The results showed a significant effect of sampling site on water temperature and all analyzed macronutrients ($p < 0.05$). On the other hand, pH did not show significant differences at sampling locations ($p > 0.05$). The p-values of the one-way ANOVA test are provided in **Supplementary Table 2**.

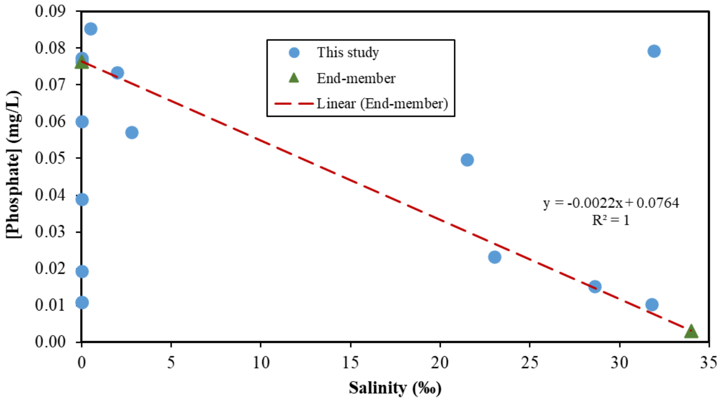


Figure 7. Correlation between phosphate concentrations and salinity in the water samples.

The significant effect of sampling site on temperature aligns with the expectation that riverine, urban, and marine stations would exhibit differing thermal profiles. Coastal stations, such as Pulau Tikus (S15) and Pantai Panjang (S12), are more thermally stable due to oceanic buffering. In contrast, riverine and urban sites, such as Sungai Rindu Hati (S1), Air Bengkulu (S6), and Beringin Raya (S10), may experience greater temperature fluctuations due to freshwater inputs and urban runoff. The significant spatial differences in macronutrient concentrations ($p < 0.05$) further emphasize the influence of land-based nutrient inputs, particularly from agricultural and urban areas. Elevated phosphate and nitrate concentrations in urban and riverine sites, such as Air Bengkulu (S6), Beringin Raya (S10), and Mulut Muara (S14), likely reflect nutrient loading from domestic wastewater, agricultural runoff, or industrial discharge [19, 20]. Meanwhile, silicate concentrations are higher in riverine stations like Sungai Rindu Hati (S1) and Sungai Bengkulu (S2), suggesting increased terrestrial inputs from weathering and erosion processes. On the other hand, the lack of significant differences in pH ($p > 0.05$) across sampling sites indicates that this parameter remains relatively consistent throughout the study area and further indicates a buffered system potentially regulated by carbonate and silicate weathering, preventing extreme pH fluctuations despite varying nutrient inputs [22].

These findings highlight the role of anthropogenic activities and hydrological processes in shaping the spatial distribution of macronutrients and physical parameters in the Bengkulu watershed system. While temperature and macronutrient concentrations exhibit marked spatial variability, the overall buffering capacity of the water body appears to mitigate significant changes in pH. Further analysis should focus on identifying specific nutrient sources, assessing nutrient ratios to determine potential nutrient limitation, and evaluating the influence of hydrological connectivity on the spatial distribution of these parameters.

4. CONCLUSIONS

Our findings revealed the temporal variations of macronutrients, including nitrate, nitrite, phosphate, and silicate, along the Bengkulu River watershed, compared with a previous study in the exact location. The analyzed macronutrient concentrations ranged from 0.005 - 0.058 mgN-NO_3/L for nitrate, <0.001 - 0.014 mgN-NO_2/L for nitrite, 0.163 - 9.314 mgSi-$Si(OH)_4$/L for silicate, and 0.01 - 0.085 mgP-PO_4-/L for phosphate. These compounds generally have lower concentrations during the dry season. All macronutrients during this study (dry season) were within the safety level threshold for biota in rivers, lakes, and coastal regions. The one-way ANOVA results showed a significant effect of sampling site on water temperature and all macronutrients ($p < 0.05$). Meanwhile, pH did not show significant differences at sampling locations ($p > 0.05$). During the estuarine mixing, silicate behaves conservatively while nitrate, nitrite, and phosphate behave non-conservatively. However, more data is needed to evaluate these processes in more detail.

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