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Leukocyte Profiles and Phagocytic Response of the Nile Tilapia in Relation to Water Quality and Macrobenthic Indicators

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ABSTRACT

This study investigated the relationship between water quality, macrobenthic composition, and innate immune responses in Nile tilapia (*Oreochromis niloticus*) cultivated in a tropical freshwater aquaculture system. Water samples and biological data were collected from six stations at the Sumberpasir Aquaculture Laboratory, Universitas Brawijaya. Physicochemical parameters measured included temperature, pH, ammonia, and total organic matter (TOM), while macrobenthic community structure was analyzed through diversity and dominance indices. Fish hematological assessments were conducted at two culture stations (Stations 1 and 2), where leukocyte differentials (lymphocytes, monocytes, neutrophils) and phagocytic activity were quantified. Canonical Correspondence Analysis (CCA) revealed that Baetidae were associated with high pH and stable temperatures, indicating clean water conditions, whereas Potamidae, Thiaridae, and Pachychilidae were linked to elevated ammonia and TOM, suggesting pollution tolerance. In tilapia, lymphocyte counts remained within physiological norms (69–71%) under favorable water conditions, while monocytes and neutrophils declined in response to increased pollution levels. Spearman correlation analysis demonstrated strong negative associations between ammonia and immune parameters, particularly neutrophils (r = –0.992) and phagocytosis (r = –0.927). These findings highlight that macrobenthic bioindicators and fish leukocyte profiles are effective tools for detecting ecological stress and guiding sustainable management in aquaculture environment.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) aquaculture is one of the high-value fishery commodities in Indonesia, significantly contributing to farmers' income and local food security **(Saputry & Latuconsina, 2022)**. The success of tilapia farming is highly dependent on water quality, as optimal physicochemical parameters promote better growth performance and enhance fish health **(El-Sayed & Fitzsimmons, 2023)**.

Conversely, drastic fluctuations in water parameters such as temperature, pH, and ammonia levels can induce physiological stress, suppress immune responses, and increase disease outbreaks **(Liu *et al.*, 2022)**. Therefore, continuous monitoring of water quality is essential to minimize both economic and ecological risks in commercial aquaculture operations **(Yusoff *et al.*, 2024)**.

Although several studies—such as **Witeska *et al.* (2023)**—have documented the impact of water pollutants on fish hematological parameters, little attention has been given to integrated ecological and immunological assessments, particularly in tropical freshwater systems. Leukocytes, including lymphocytes, monocytes, and neutrophils, play crucial roles in innate immune defense and early inflammatory responses, while phagocytosis reflects the functional capacity of immune cells to engulf and neutralize pathogens **(He *et al.*, 2023)**. Numerous studies have reported that chemical stressors, such as elevated ammonia and dissolved organic matter, can alter leukocyte profiles and reduce phagocytic activity, making these parameters effective early-warning biomarkers for environmental stress in aquaculture systems **(Parvathy *et al.*, 2022)**.

The Sumberpasir Laboratory in Malang is a strategic research facility for investigating water quality impacts on the Nile tilapia aquaculture **(Rahayu, 2024)**. This site is equipped with adequate infrastructure for conducting water quality assessments and immuno-hematological analyses **(Sutrisno, 2025)**. However, comprehensive studies that simultaneously integrate physicochemical water parameters, macroinvertebrate community structure (as ecological and organic load indicators), and immunological responses (e.g., phagocytic activity and leukocyte profiles) in tropical freshwater systems remain limited. There is a critical gap in holistic approaches that combine these multi-level assessments to develop predictive models for fish health under dynamic tropical conditions.

This study aimed to evaluate the correlation between water quality parameters (temperature, pH, ammonia, and total organic matter), macrobenthic ecological indices, and non-specific immune responses of the Nile tilapia (*Oreochromis niloticus*), as measured by differential leukocyte counts and phagocytic activity, across six sampling stations at the Sumberpasir Aquaculture Laboratory, Brawijaya University. By integrating physicochemical, biotic, and hematological data, this research seeks to identify key environmental factors influencing fish immune resilience in tropical freshwater culture systems. The findings are expected to provide practical insights into the development of more comprehensive water quality monitoring protocols and fish health indicators, ultimately supporting sustainable tilapia aquaculture management strategies. It is hypothesized that deteriorating water quality—particularly elevated levels of ammonia and total organic matter—negatively correlates with phagocytic activity and alters leukocyte profiles in tilapia, thereby compromising their non-specific immune function.

MATERIALS AND METHODS

1. Research location

This study was conducted between May and June 2025. Water sampling and quality assessments were performed at six predetermined stations within the Freshwater Fisheries Laboratory, Sumberpasir, Brawijaya University, over a two-week period. Hematological and phagocytic analyses were carried out at the Fish Disease Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University.

2. Sampling procedure

Water and macrobenthos samples were collected from six predetermined stations, with two sampling repetitions per week over a two-week period. Stations 1 and 2 are located at the inlet points supplying freshwater to the ponds. Stations 3 and 4, active aquaculture ponds. While Stations 5 and 6 are situated at the outlet channels where wastewater is discharged. As no fish were present at these inlet and outlet points. Fish sampling for hematological analysis was conducted only at Stations 3 and 4.

Water was sampled using a standard water sampler at a consistent depth of 30 cm below surface. Macrobenthos were collected using a kicking net, followed by sieving through a 20-mesh sieve. All samples were placed in labeled containers and transported under cold conditions for laboratory analysis. Tilapia (*Oreochromis niloticus*) were used as bioindicator organisms for hematological and phagocytic assessments. At each sampling point, three fish were collected and anesthetized prior to blood collection. Although the sample size per station (n=3) was limited, it was sufficient for preliminary observational analysis. Future studies with larger replicates are recommended for more robust statistical inference. Blood was drawn via caudal vein puncture using 1 mL sterile syringes pre-coated with EDTA as an anticoagulant. The blood samples were immediately processed for differential leukocyte counts and phagocytic activity assays. This study used the Nile tilapia (*Oreochromis niloticus*) as test organisms, and all procedures involving live animals were conducted in accordance with ethical principles as outlined in Article 26, Paragraph 1 of the Government Regulation of the Republic of Indonesia No. 19 concerning the Implementation of Education. The handling, sampling, and maintenance of fish were carried out following proper welfare protocols to minimize stress, pain, and discomfort, thereby ensuring compliance with ethical standards for scientific research.

3. Water quality analysis

Temperature and pH were measured *in situ* using portable digital probes during sampling at each station **(Fitriani *et al*., 2022)**. Ammonia concentrations were determined using the phenate method (indophenol blue reaction) with UV-Vis spectrophotometry at a wavelength of 640 nm, following the Indonesian National Standard (SNI 06-6989.30-2005). Total Organic Matter (TOM) was analyzed based on the gravimetric method in accordance with SNI 06-6989.22-2004 **(Dina *et al*., 2025)**.

4. Macrobenthos analysis

Macrobenthic community assessments were conducted following the method described by **Yusal and Hasyim (2022)**, macrobenthos samples were collected using a standard kicking net. The samples were preserved in 70% ethanol and stained with Rose Bengal solution to facilitate sorting under a binocular microscope. Each sample was labeled to indicate its respective station and replicate. Taxonomic identification of macrobenthos was performed using the identification key by **Higgins and Thiel (1988)** method. Macrobenthic community analysis included diversity **(Shannon & Wiener, 1963)**, evenness and dominance **(Odum, 1971)** and calculations of abundance **(Krebs, 1989)** method. The Biological Monitoring Working Party (BMWP) index was also used to assess the biological quality of freshwater based on macrobenthic composition.

5. Differential leukocyte count

Differential leukocyte analysis was performed based on the method described by **Petit *et al*. (2024)** with minor modifications. A peripheral blood smear was prepared by placing a drop of fresh blood onto a clean glass slide and spreading it using a second slide held at a 45° angle to create a thin monolayer. The smear was air-dried, fixed in absolute methanol for 5 minutes, and subsequently stained with Giemsa solution for 30 minutes. After rinsing with distilled water and drying, the slides were examined under a light microscope at 1000× magnification using oil immersion. Leukocytes were differentiated and counted until a total of 100 cells was reached per sample. The percentages of lymphocytes, monocytes, and neutrophils were then calculated.

6. Phagocytic activity assay

Phagocytic activity was assessed following the protocol described by **El-Bab *et al*. (2022)** with slight modifications. A suspension of *Saccharomyces cerevisiae* yeast was prepared by dissolving 0.5 g of yeast in 10 mL of 0.9% NaCl solution and incubated for 20 minutes. Fresh blood was collected from fish and mixed with the yeast suspension at a 1:1 ratio. The mixture was incubated at room temperature for 30 minutes to allow phagocytosis.

A drop of the mixture was smeared onto a clean glass slide, air-dried, and fixed with methanol for 3 minutes. The slide was then stained with Giemsa solution and rinsed with distilled water. After drying, slides were examined under a light microscope at 400× magnification. For each slide, 100 leukocytes were observed, and the number of phagocytic cells was recorded. The phagocytic index was calculated as the percentage of leukocytes actively engulfing yeast cells.

7. Canonical component analysis (CCA)

Canonical correspondence analysis (CCA) was employed as a multivariate statistical technique to explore the relationships between water quality parameters and macrobenthic community composition. This method is advantageous in identifying linear associations among variable groups, accommodating heterogeneous datasets, and simultaneously analyzing multiple environmental variables. In this study, CCA was conducted using XLSTAT software to evaluate the influence of physicochemical parameters such as temperature, pH, ammonia, and total organic matter on macrobenthic diversity and distribution patterns. The CCA approach provides an integrated perspective on how environmental gradients structure benthic communities in freshwater ecosystems, as supported by the method described by **(Alhafidzoh *et al*., 2021)**.

8. Spearman rank correlation

The Spearman Rank correlation test was employed to assess the relationships between environmental parameters and immunological indicators, as the data were ordinal in nature and did not meet the assumptions of normality. This method, categorized as non-parametric statistics, is suitable for evaluating monotonic associations between variables **(Sugiyono, 2019)**. The strength and direction of the correlation were interpreted based on the Spearman correlation coefficient (r_s), while statistical significance was determined by the two-tailed *P*-value. A *P*-value less than 0.05 indicates a statistically significant relationship between variables, whereas values greater than 0.05 suggest no significant correlation. All statistical analyses were performed using IBM SPSS Statistics version 26.

Table 1. Interpretation of Spearman's correlation coefficient (r) **(Sugiyono, 2019)**

Correlation Coefficient (r)	Strength of Association
0.00-0.025	Very weak correlation
0.26-0.50	Fair correlation
0.51-0.75	Strong correlation
0.76-0.99	Very strong correlation
1.00	Perfect correlation

RESULTS

1. General description of the research site

The study was conducted at the Sumberpasir Freshwater Fisheries Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, located in Sumberpasir Village, Malang Regency, East Java, Indonesia. The facility spans approximately 1.1 hectares and includes earthen and concrete ponds, laboratory facilities, and supporting infrastructure for freshwater aquaculture research. Water quality assessments and biological sampling were carried out at six stations representing different hydrological zones. These included inlet points (before water enters the aquaculture pond), internal pond locations (within the active culture system), and outlet points (post-pond flow into the adjacent river). This spatial sampling design was intended to capture potential gradients in water quality and associated ecological conditions. A schematic layout of the sampling stations is provided in Fig. (1).

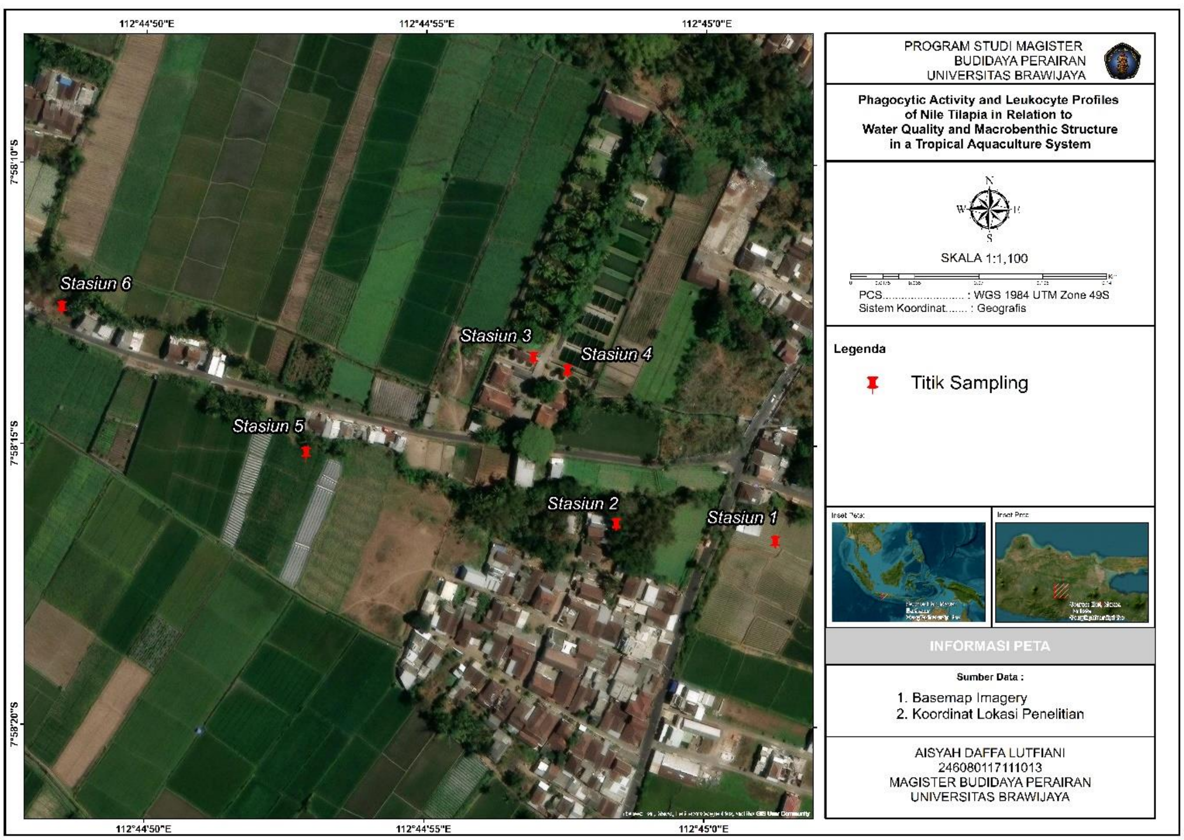

Fig. 1. Research location

2. Water quality

Temperature measurements across the six stations ranged from 25.5 to 26.8°C, with the highest temperature observed at station 5 (26.8°C) and the lowest at station "3"

(25.5°C). The pH values varied slightly, with the highest pH recorded at station 6 (8.48) and the lowest at station "1" (7.92), indicating slightly alkaline conditions across all stations. Ammonia concentrations showed greater variability, ranging from 0.049 mg/L at station "3" to 0.645 mg/L at station "1", suggesting potential localized pollution. Total Organic Matter (TOM) levels were highest at station "6", increasing from 65.72 mg/L to 70.1 mg/L between sampling events, while the lowest values were observed at station "3", increasing modestly from 13.94 mg/L to 21.95 mg/L. These variations suggest differences in organic load and nutrient input among the sampled locations.

3. Ecological index

The macroinvertebrate diversity index across the six stations was categorized as moderate, with values ranging from 0.99 to 1.15. Station "1" exhibited a slight decline in diversity from 1.09 to 1.08 between the first and second sampling periods, but remained within the moderate range. The lowest diversity was observed at station "2" (0.99–1.00), likely due to the dominance of the Pachychilidae family, which may have suppressed the presence of other taxa. In contrast, station "3" recorded the highest diversity (1.13–1.14), followed by station "4", which showed an increase from 1.09 to 1.15, attributed to the decline in Baetidae abundance, resulting in a more even species distribution.

At station "1", the macroinvertebrate community was composed mainly of Baetidae, Potamidae, Thiaridae, and Pachychilidae. The most abundant taxa were Pachychilidae and Thiaridae, accounting for 53.57%–51.43% and 28.57%–34.29%, respectively, while Potamidae exhibited the lowest abundance (4.44% to 2%). Although Baetidae (insecta) were also present, their contribution remained comparatively low.

The results of macroinvertebrate observations show that the dominance index values vary across stations. Station "1" has the same dominance value between the first and second samples, which is 0.39, classified as low dominance. Stations "3 and 4" also showed low dominance indices across all sampling periods, ranging from 0.35–0.37 and 0.33–0.38. This indicates that the distribution of macroinvertebrate species is relatively even, with no dominant species. Meanwhile, the dominance value at station "2" ranged from 0.43 to 0.45, which falls into the moderate category and reflects the presence of a dominant species, albeit in small numbers. The high dominance value is due to the presence of the Pachychilidae family, which has a higher abundance than other species.

The Canonical correspondence analysis (CCA) plot illustrates the relationship between the abundance of macroinvertebrate families and environmental variables, including ammonia, pH, temperature, and total organic matter (TOM). The green vector lines indicate the direction and magnitude of influence exerted by each water quality parameter on macroinvertebrate distribution. The family Baetidae was closely associated with higher pH and warmer temperatures, suggesting a preference for cleaner and more stable aquatic conditions. In contrast, families Potamidae, Thiaridae, and Pachychilidae were positioned near the ammonia and TOM vectors, implying a greater tolerance to suboptimal or polluted environments. Additionally, the proximity of Gomphidae to the

TOM vector suggests that this family can thrive in habitats with elevated organic matter concentrations. These spatial associations underscore the utility of macroinvertebrate assemblages as bioindicators for assessing the ecological status of freshwater systems.

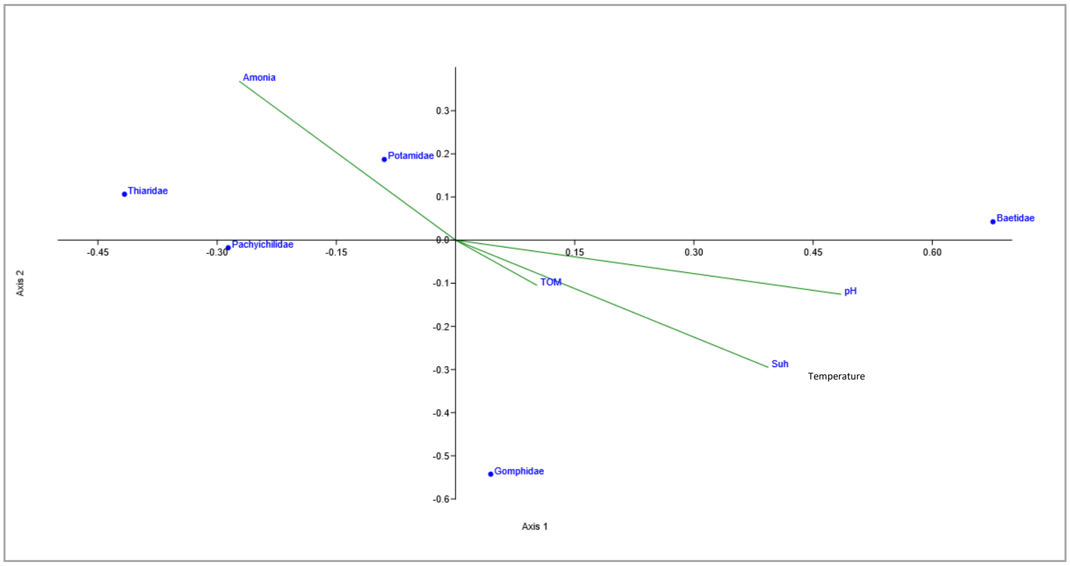

Fig. 2. CCA analysis results of water quality and macrobenthos

4. Leukocyte differential analysis

Based on the two differential leukocyte graphs presented for station “3” and station “4”, it can be seen that there are differences in the percentages for each type of white blood cell. The graph for station “3” shows that lymphocytes dominate with the highest percentage at 71%, followed by monocytes at 6.1%, and neutrophils at 5.5%. Meanwhile, the graph from Station “4” shows a slightly lower percentage of lymphocytes at 69%, with monocytes at 5.3% and neutrophils at 4.5%. These differences reflect variations in the immunological composition of fish between stations. This range of values is important in indicating the balance of fish immune responses to their environment.

4.1 Lymphocyte cell

Lymphocyte percentages in the blood of the Nile tilapia (*Oreochromis niloticus*) across the sampling stations ranged narrowly between 69 and 71%. Station “3” exhibited the highest average lymphocyte proportion at 71%, while station “4” showed a slightly lower average of 69%. These values fall within the normal physiological range for this species (68%–86%), indicating that the fish maintained relatively stable immune conditions under the observed environmental settings. The consistency in lymphocyte levels suggests that the environmental stressors across stations may not have significantly disrupted the adaptive immune component. Spearman correlation test on lymphocytes showed moderate-to-strong positive correlations with temperature (r = 0.971) and pH (r =

0.918), although not statistically significant ($P > 0.05$), indicating that stable physicochemical conditions may support adaptive immune components. Conversely, negative correlations were observed between lymphocytes and ammonia (r = –0.195 to –0.605) and total organic matter (TOM) (r = –0.061 to –0.755), suggesting mild suppressive effects of nitrogenous and organic pollutants on lymphocyte profiles. Given that lymphocytes play a critical role in antigen-specific responses, their maintained dominance across samples reflects baseline immune preparedness in the studied populations. Further interpretation of these values in relation to water quality parameters is discussed in the subsequent sections. Graphs and images related to lymphocyte can be seen in Fig. (3).

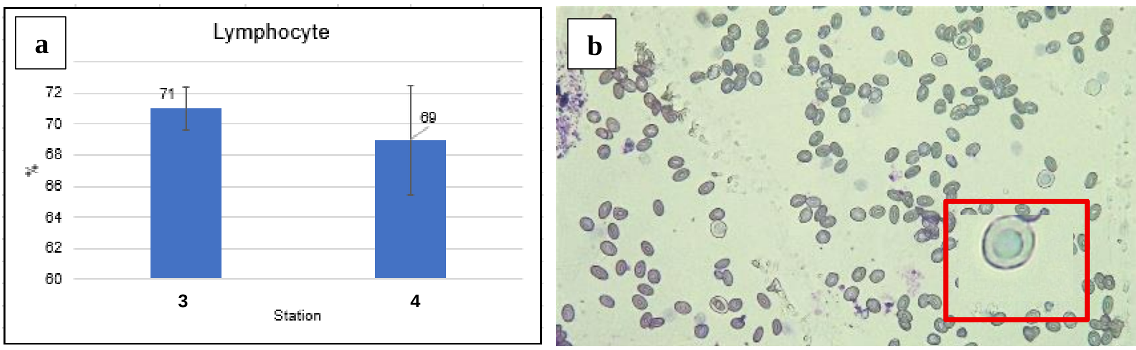

Fig. 3. Lymphocyte: a) Value Graphic; b) Research Image (10x zoom on red square)

4.2 Monocyte cell

The percentage of monocyte cells in the Nile tilapia (*Oreochromis niloticus*) blood samples ranged from 5.3 to 6.1% across the two sampling stations. Station "3" recorded the highest average monocyte proportion (6.1%), while station "4" had a slightly lower average of 5.3%. These values fall within the normal physiological range for the Nile tilapia (*Oreochromis niloticus*) and suggest relatively stable immune function under the observed conditions. Spearman correlation test on monocyte levels, on the other hand, exhibited a significant positive correlation with neutrophils (r = 0.865, $P <$ 0.05), highlighting a possible synchronized activation of innate immune cells under stress conditions. Monocytes also displayed strong positive correlations with ammonia (r = 0.995 at Station 3) and TOM (r = 1.000 at Station 4), indicating that these cells may increase as a defensive response to environmental degradation. However, the modest variation between stations may reflect differences in environmental stress levels, such as fluctuations in ammonia or organic matter. Monocytes play a crucial role in innate immunity through phagocytosis and antigen presentation; thus, their proportion can serve as an indicator of immunological activation. A slightly elevated monocyte count at station 1 may indicate heightened immune surveillance in response to local environmental conditions, which is further explored in the discussion section. Graphs and images related to monocyte can be seen in Fig. (4).

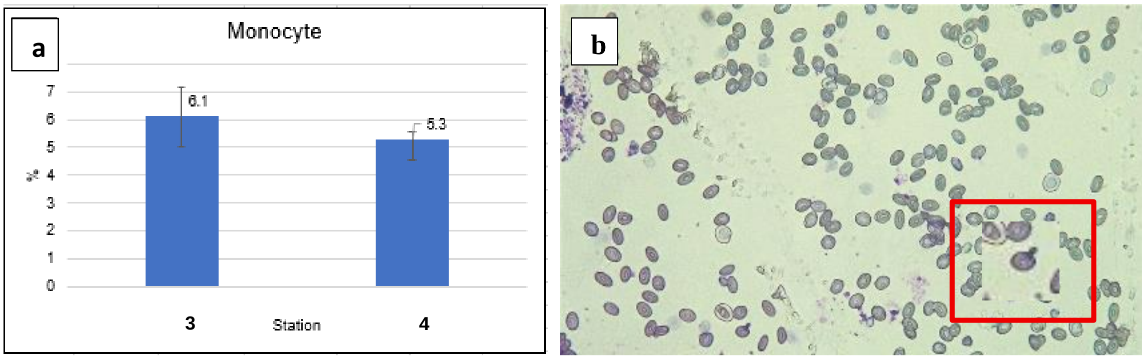

Fig. 4. Monocyte: a) Value Graph; b) Research Image (10x zoom on red square)

4.3 Neutrophil cells

The proportion of neutrophils in the Nile tilapia blood ranged from 4.5% at station "4" to 5.5% at station "3", with a narrow overall variation between sampling sites. These values fall slightly below the typical physiological range for tilapia, which is generally reported to be 6–8%. A lower neutrophil count may suggest reduced innate immune stimulation, potentially reflecting favorable environmental conditions or a lack of recent pathogenic exposure. Neutrophils demonstrated strong negative correlations with TOM (r = –0.946) and ammonia (r = –0.992), suggesting a potential suppression of neutrophilic response under elevated organic and nitrogenous loads. While these correlations were not statistically significant, their strength may reflect underlying immunotoxic effects of water pollutants. However, considering station "4" exhibited the lowest neutrophil proportion, this may indicate a transient suppression of immune activity potentially influenced by elevated levels of total organic matter (TOM) or ammonia, as observed in water quality data. Neutrophils are key players in early immune responses, particularly in bacterial clearance and inflammation; thus, deviations from the normal range either upward or downward can provide insights into the immunophysiological state of the fish under varying environmental stressors. Graphs and images related to leukocytes can be seen in Fig. (5).

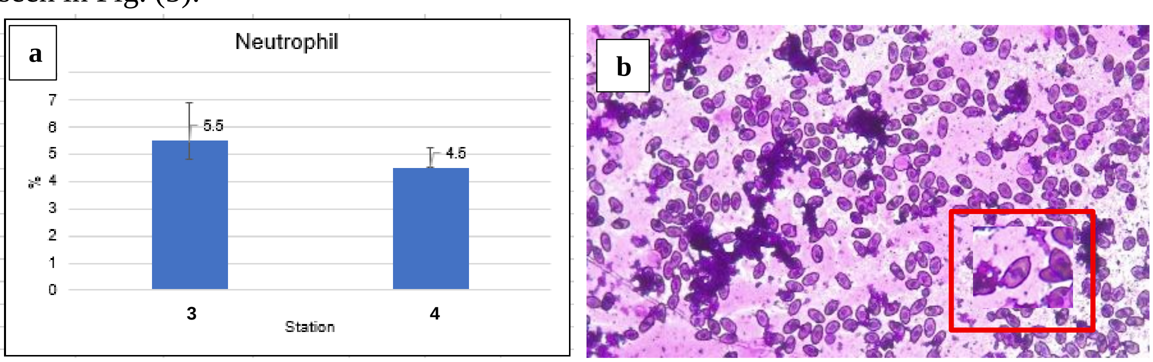

Fig. 5. Neutrophil: a) Value Graph; b) Research Image (10x zoom on red square)

5. Phagocytosis activity

Phagocytosis activity was evaluated across six different stations, with two replicates per station. The percentage of phagocytic cells was calculated from leukocyte smears stained and observed microscopically. The highest average phagocytic activity was recorded at station "4" with a mean value of 43.1% and a standard deviation of 7.1. This suggests that immune cells in fish sampled from this station demonstrated a stronger functional response, potentially as an adaptive mechanism to counterbalance environmental stressors such as elevated organic matter or ammonia concentrations. Interestingly, although stations "3" and "4" both showed the same mean phagocytic activity (37.7%), their standard deviations were notably different 7.3 at station "3" and 7.9 at station "4". This indicates that even with comparable averages, the consistency of cellular immune responses between samples at each site varied greatly. Such variation may reflect microenvironmental differences that influence immune regulation, such as temporal fluctuations in water quality or pathogen exposure.

A closer look at replicate differences reveals that station "3" showed an increase in phagocytosis between the first (38.7%) and second replicate (43.5%), indicating a temporal shift in immune responsiveness. This could be linked to transient changes in water parameters or biological stress such as microbial presence. Meanwhile, station "3" recorded the most consistent response with the lowest standard deviation (±1.5), suggesting that environmental conditions in this location may be more stable and less immunologically challenging for fish. Additionally, when analyzing total counts of active phagocytes, station "3" displayed the highest number of active cells during the second replicate (n=51), although its average percentage was slightly lower than that of station "4". On the other hand, station "4" showed relatively stable values across both replicates, indicating a moderately consistent immune activation. The varying patterns of phagocytic activity across stations highlight the influence of environmental quality on fish innate immunity. Most notably, phagocytic activity was consistently and negatively correlated with ammonia and TOM at both stations (e.g., $r = –0.927$ and $r = –0.830$ at Station 4), although not significant at $P < 0.05$. The data suggest that increased environmental stress from nutrient and organic enrichment may reduce the functional competence of innate immune cells. Additionally, phagocytosis had a weak association with pH and temperature, highlighting that extreme organic and chemical fluctuations rather than physical parameters are more influential in altering this immune function. Elevated ammonia or total organic matter (TOM), as observed in some stations, may act as immunostimulants or stressors, triggering different levels of leukocyte activation. Hence, phagocytic activity serves not only as a functional marker of immune competence but also as a biological indicator of habitat condition. Such findings underscore the importance of integrating immune parameters into water quality monitoring frameworks to assess ecological risks and fish health in aquaculture environments.

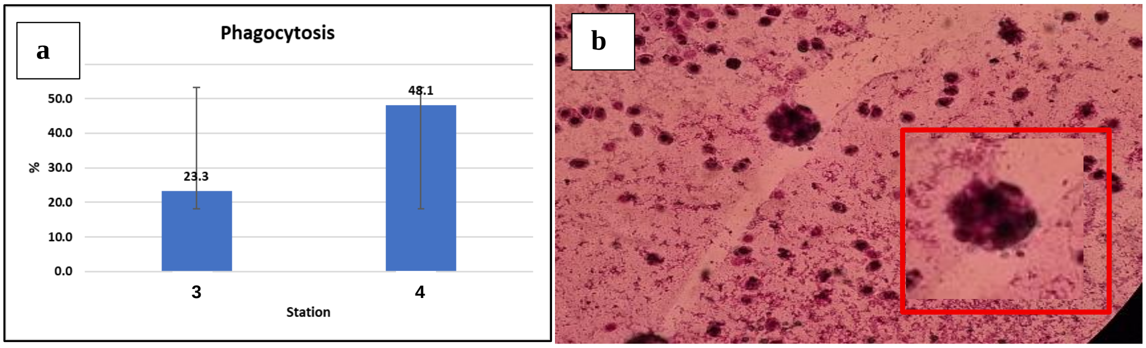

Fig. 6. a) Phagocytosis b) Microscope Image (10x zoom on red square)

DISCUSSION

The distribution of Baetidae, closely associated with vectors of high pH and warm temperatures in the CCA analysis, indicates this family's preference for stable aquatic environments with optimal physicochemical quality. This pattern is consistent with findings from the Luvuvhu River in South Africa, where Baetidae dominated reference sites characterized by neutral to alkaline pH (6.5–8.0) and temperatures ranging from 18–24°C **(Munyai *et al*., 2025)**. Due to its sensitivity to environmental fluctuations, Baetidae is considered a reliable bioindicator for detecting habitat degradation, as reflected in its marked decline in organically polluted areas. Supporting this, a study conducted in Benin demonstrated a negative correlation between Baetidae abundance and ammonium (NH_4^+) as well as phosphate (PO_4^{3-}) concentrations, reinforcing its role as a sensitive taxon to eutrophication **(Gouissi *et al*., 2019)**.

The association of Potamidae, Thiaridae, and Pachychilidae with the ammonia and total organic matter (TOM) vectors in the CCA ordination reflects their physiological adaptations to degraded environmental conditions. Thiaridae, for instance, exhibits high tolerance to elevated ammonia concentrations (>2 mg/L) through ammonotelic excretion and gill modifications that mitigate hypoxic stress, as observed in urban streams in Nigeria heavily impacted by agricultural and domestic waste **(Munyai *et al*., 2025)**. Similarly, the dominance of Pachychilidae in TOM-rich substrates is linked to their ability to exploit biofilms on organic sediments, while Potamidae function as specialized detritivores in habitats with soil organic carbon levels exceeding 5%. These adaptive strategies align with findings from the Affon River in Benin, where these families served as key indicators of organic pollution **(Gouissi *et al*., 2019)**.

The findings from the differential leukocyte analysis align with various national studies highlighting the critical role of leukocyte composition in evaluating fish health. As reported by **Seibel *et al*. (2021)**, a predominance of lymphocytes in fish blood often indicates heightened immune preparedness and a strong capacity to respond to pathogenic challenges. Similarly, **Safitri and Rahma (2020)** suggested that elevated lymphocyte levels are indicative of the fish’s resilience against environmental stressors. **Mustafa**

(2023) emphasizes that fluctuations in monocyte and neutrophil percentages are reflective of the immune system's modulation, particularly in response to water quality and nutritional status. This observation is supported by **Ristea and Zărnescu (2020)**, who affirm that leukocyte ratios serve as reliable indicators of the interaction between environmental factors and the immunophysiological state of fish.

The lymphocyte profiles observed in the Nile tilapia (71% at Station 3 vs. 69% at Station 4), while varying slightly between stations, demonstrate compelling correlations with water quality parameters that support their role as environmental biomarkers. Despite the variation, the narrow range of values suggests stable immunological conditions, as all values remain within the normal physiological range for the species **(Luthfiannisa *et al*., 2024)**. Though statistically non-significant ($P > 0.05$), the strong positive correlations with temperature ($r = 0.971$) and pH ($r = 0.918$) align remarkably well with **(Iyiola *et al*., 2024)** findings that optimal physicochemical conditions enhance lymphocyte proliferation in tilapia. Furthermore, **Schröter *et al*. (2021)** note that stable lymphocyte levels are a sign of good environmental adaptation in cultured systems. Conversely, the consistent negative correlations with ammonia (r = -0.195 to -0.605) and total organic matter (r = -0.061 to -0.755) provide mechanistic evidence for observation that lymphocyte counts serve as sensitive indicators of aquaculture management efficacy **(Munni *et al*., 2023)**.

The observation results indicate a variation in the average percentage of monocytes in tilapia blood across different sampling stations, with station "3" exhibiting the highest value (6.1%) and station "4" the lowest (5.3%). Monocytes are crucial components of the fish innate immune system, and elevated counts are generally associated with a heightened immune response and better physiological condition. The lower monocyte percentage observed at station "4" may reflect exposure to suboptimal environmental conditions or increased stress levels. As highlighted by **He *et al*. (2023)**, fluctuations in immune cell populations are commonly influenced by external stressors such as declining water quality and pollutant load. This is further supported by **Segev-Hadar *et al.* (2020)**, who note that environmental stress can compromise immune function in fish, thereby affecting their health and survival. In line with this, **Joseph *et al*. (2022)** emphasize that elevated concentrations of aquatic pollutants are often associated with alterations in hematological parameters, including monocyte levels. Consequently, monitoring monocyte dynamics in relation to environmental parameters is essential for maintaining fish health and ensuring sustainable aquaculture practices.

Although neutrophil counts remained within normal physiological ranges, the observed variations between stations - particularly the strong negative correlations with total organic matter (TOM: $r = -0.946$) and ammonia ($r = -0.992$) suggest potential immunosuppressive effects of these pollutants. In addition, although the average neutrophil count remains close to the normal physiological range, the observed variations across stations warrant further attention. As noted by **Cerqueira *et al*. (2021)**, documented similar neutrophil suppression in fish exposed to organic pollutants, attributing this to either direct immunotoxicity or migration to peripheral tissues during stress. **Samaddar *et al*. (2022)** contention that neutrophilic fluctuations serve as sensitive indicators of environmental stress, even when values remain within normative ranges. Moreover, **Iyiola *et al*. (2023)** highlight that effective water quality management plays a critical role in maintaining the stability of leukocyte populations, including neutrophils. Therefore, assessing neutrophil profiles as part of routine monitoring can offer valuable insight into the immunological status of fish and serve as an early indicator for implementing sustainable aquaculture management strategies.

The observed negative correlations between phagocytic activity and both ammonia (r = -0.927) and total organic matter (r = -0.830), though not statistically significant (P > 0.05), align with established patterns of immune modulation under environmental stress. These findings corroborate **Wu *et al.* (2020)** and underscore the significance of microenvironmental variability in interpreting immunological responses. Elevated phagocytic activity is frequently linked to increased pollutant concentrations or the presence of pathogenic organisms. As noted by **Ma *et al.* (2022)**, phagocytic responses are highly sensitive to microenvironmental variability, particularly to organic pollutants. Our results show a stronger association with chemical parameters (ammonia and total organic matter) than with physical factors (pH and temperature). This is further supported by **Ha *et al.* (2021)**, who reported enhanced phagocytosis during acute environmental changes; however, our data suggest that chronic exposure to nutrient enrichment may instead suppress this immune function—a distinction that highlights the importance of exposure duration in ecological immunology. In line with **Ogungbile *et al.* (2022)**, a comprehensive interpretation of phagocytosis data requires an ecological framework to ensure accurate conclusions. Integrating insights from these studies provides a broader understanding of the interplay between microbial activity and environmental stressors within aquatic systems.

CONCLUSION

This study reveals that key water quality parameters ammonia, total organic matter, pH, and temperature jointly shape both macroinvertebrate assemblages and innate immune function in the Nile tilapia. Macroinvertebrate families such as Baetidae reliably signal optimal habitat conditions, whereas Potamidae, Thiaridae, and related taxa indicate tolerance to pollution through their association with elevated ammonia and organic loads. In tandem, tilapia immune profiles demonstrate clear patterns: stable lymphocyte proportions reflect environmental stability, while reductions in monocytes, neutrophils, and phagocytic activity correspond to increasing organic and nitrogenous stress. Together, these results underscore the value of combining benthic bioindicators with fish leukocyte dynamics into an integrated monitoring framework, offering a powerful toolset for early detection of ecological degradation and the development of sustainable aquaculture management strategies. Further studies are recommended to validate the use of macroinvertebrate indices alongside hematological biomarkers under different aquaculture intensification levels and stressor gradients, particularly in tropical freshwater systems.

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