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IMPACTS OF MICROPLASTIC POLLUTION ON CELLULAR BLOOD INDICATORS IN NILE TILAPIA (*Oreochromis niloticus*) FROM THE JAGIR RIVER, SURABAYA

Dampak Cemaran Mikroplastik Terhadap Indikator Seluler Darah Ikan Nila (*Oreochromis niloticus*) Di Kali Jagir, Surabaya

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ABSTRACT

Microplastics (<5 mm) are increasingly recognized as pervasive pollutants in urban rivers and may induce sublethal physiological stress in freshwater fish. This study assessed the impacts of microplastic contamination on cellular/hematological blood indicators in Nile tilapia (Oreochromis niloticus) from the Jagir River, Surabaya, Indonesia. Field sampling was conducted from July to August 2025 at three river segments (upstream near the sluice gate, midstream near fishing-vessel mooring and small-scale activities, and downstream approaching the estuarine outlet). Water was collected at each site for microplastic quantification, and tilapia were sampled three times at two-week intervals for blood analysis. In situ and laboratory assessments of water quality included temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), and total suspended solids (TSS). Microplastics were detected at all sites, ranging from 2,365,333.3 to 2,541,333.3 particles/m³, with site-specific dominance of film/fiber upstream, fragment/film midstream, and fiber/fragment downstream. Water quality was relatively stable in temperature (27.7–28.8°C) and DO (5.5–5.9 mg/L), while BOD indicated organic loading (3.5–4.06 mg/L) and TSS showed clear spatial differences (8–17 mg/L). Blood profiles varied across sites: erythrocytes, hemoglobin, and hematocrit were lowest at the midstream site, whereas leukocyte counts were highest downstream, suggesting spatially heterogeneous stress and immune activation. One-way ANOVA indicated significant differences in microplastic abundance among sites ($p < 0.05$),

and Pearson correlation results showed very strong associations between microplastic abundance and most blood parameters, supporting the use of integrated microplastic metrics, water quality indicators, and blood biomarkers for urban river monitoring prior to discharge into the Madura Strait.

INTRODUCTION

Microplastics (particles less than 5 mm in size) have been recognized as emerging pollutants that are widely distributed in aquatic systems, including rivers, drainage channels, estuaries, and raw water bodies. Rivers play a key role as primary corridors, transporting plastic fragments from anthropogenic activities on land to coastal and marine environments, leading to high microplastic loads in river ecosystems, particularly in urban areas with dense populations and intensive industrial activities (Lebreton *et al.,* 2017). In urban areas, sources of microplastics typically include the degradation of secondary plastics (such as packaging, plastic bags, and textile fibers), infrastructure abrasion, and inputs from domestic waste, which are then dispersed through surface runoff, stormwater, and drainage systems (Lebreton *et al.,* 2017). This situation makes river waters a relevant exposure medium for freshwater biota and an important issue for cities that still rely on surface water as part of their raw water supply system (Lestari *et al.,* 2020).

In the context of Surabaya, scientific evidence shows the presence of microplastics across various environmental compartments (water, sediments, and/or estuary locations) related to river networks and urban channels. A study on the Surabaya River (a tributary of the Brantas River and a source of raw water) reported the abundance of microplastics at various depths and sampling points, with the dominance of specific forms and the identification of common polymers such as polyethylene and polypropylene (Lestari *et al.,* 2020). Similar findings have been reported on other segments of the Surabaya River (Driyorejo–Karang Pilang) regarding the abundance of meso- and microplastics (Wijaya and Trihadiningrum, 2019), as well as on other systems in Surabaya and its surroundings, such as the sediments in East Surabaya (Ni'am *et al.,* 2022), Kali Mas (Fitriyah *et al.*, 2022), Kanal Mangetan (Faqih *et al.,* 2022), and water from the Surabaya River in local journal studies (Putri *et al.,* 2022). Specifically, in the Jagir River, reports indicate the presence of microplastics in river water and drinking water, as well as studies on the performance of treatment process units (pre-sedimentation-aeration) in reducing microplastics in Jagir River raw water (Radityaningrum *et al.,* 2024). This body of evidence underscores the urgency of evaluating the impact of microplastics on local aquatic biota, especially consumption organisms and/or bioindicators in the Jagir River, Surabaya.

Nile tilapia (Oreochromis niloticus) is an important species in aquaculture and natural waters, commonly used to assess the effects of environmental stressors on fish health and food safety. Microplastic exposure in fish is not only related to ingestion and accumulation in the digestive tract but also to the potential translocation of particles/associated components (including adsorbed additives or contaminants), which can trigger systemic physiological disturbances (Kardgar *et al.,* 2024). Toxicological literature shows that microplastics can affect the fish immune system (Li *et al.,* 2024), induce oxidative stress through the imbalance of ROS formation and antioxidant capacity (Subaramaniyam *et al.,* 2023), and provoke inflammatory responses in juvenile O. niloticus under certain exposure scenarios (Kardgar *et al.,* 2024). Since blood is a sensitive tissue to internal homeostasis changes, approaches based on hematological indicators and cellular blood markers are relevant for detecting early or sublethal responses to pollutants, including microplastics.

Specifically, cellular blood indicators offer more "mechanistic" insights into cellular stress compared to general hematological parameters. In juvenile O. niloticus, exposure to microplastics at certain concentrations has been reported to be associated with changes in hematological indices and biochemical blood disturbances, some of which may persist despite a recovery phase (Hamed *et al.,* 2019). Other studies on juvenile *O. niloticus* also suggest eryptosis and erythron profiles (poikilocytosis and nuclear abnormalities) as new biomarkers responsive to microplastic exposure (Hamed *et al.,* 2021). However, most of the evidence comes from laboratory experimental designs, and field studies linking the presence of microplastics in urban rivers with cellular blood indicators in local fish are still relatively limited. Therefore, this article aims to analyze the impact of microplastic contamination on cellular blood indicators in Nile tilapia (*O. niloticus*) in the Jagir River, Surabaya, as a basis for ecological risk assessment and input for urban water quality management.

METHODS

This study was conducted from July to August 2025. Sampling activities and in situ measurements of water quality parameters were carried out at the study site, namely the Jagir River, Surabaya. Subsequent laboratory analyses were performed at the Sumber Pasir Freshwater Fisheries Laboratory and the Fish Culture Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya.

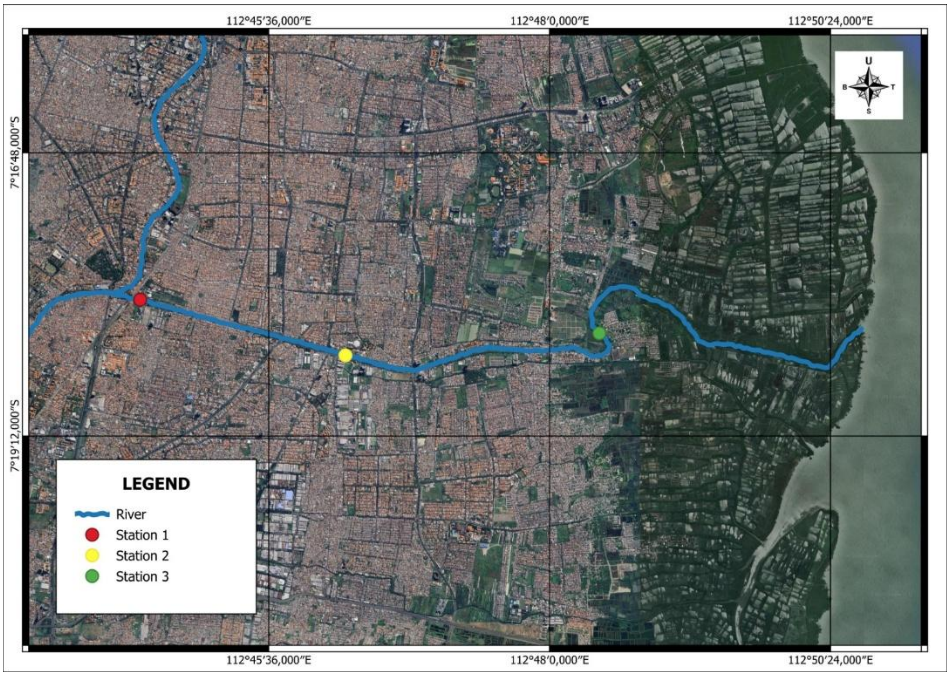

Figure 1. Location of the study area.

Sample collection

The water samples were collected at three stations along the Jagir River, Surabaya, using clean bottles as sampling containers. To maintain sample integrity during transport to the laboratory, the bottles were placed in an ice-filled cooler. Fish were sampled three times at the same three stations, with a two-week interval between sampling events, using appropriate fishing gear. To keep the fish alive during transport, they were placed in plastic bags containing river water and supplemented with oxygen.

Measuring microplastic abundance

The procedure described by Yanuhar et al. (2025) was applied to quantify microplastic abundance. Water samples from each observation station were first sieved through a stainless-steel mesh (0.5 mm) to remove larger debris. The retained material was transferred into a test tube using a funnel and rinsed with distilled water to ensure complete recovery of microplastic particles. The collected residue was then oven-dried at 80°C for 24 h. After drying, 2 mL of 30% hydrogen peroxide (H_2O_2) was added to each sample, which was subsequently covered with aluminum foil to minimize contamination. The samples were incubated in a water bath at 80°C for 24 h to facilitate the full degradation of organic matter. The treated suspension was then filtered through Whatman No. 41 filter paper and allowed to dry. Microplastics were examined and identified using an Optilab device to ensure reliable characterization. Microplastic abundance in the water samples was calculated using Eq. 1 following Buwono et al. (2021):

$$Microplastics\ Abundance = \frac{Number\ of\ microplastic\ particles(i)(particles)}{filtered\ water\ volume\ (m3)} \text{...............}(1)$$

Blood cell analysis

Fish were anesthetized by immersion in clove oil solution at a dose of 0.3 mL/L (approximately 5–6 drops). Blood was then withdrawn from the caudal vein using a 5 mL syringe. Hematological assessments comprised red and white blood cell counts measured with a hemocytometer, hemoglobin (Hb) concentration determined using a Sahli haemometer, and hematocrit values obtained with microhematocrit tubes followed by centrifugation at 6000 rpm. Differential leukocyte counts (monocytes, lymphocytes, eosinophils, and neutrophils) were evaluated from blood smears stained with Giemsa (Khan *et al.,* 2022; Rasheed *et al.,* 2023). Each blood parameter was counted in triplicate to improve measurement accuracy.

Water quality analysis

Water quality measurements were conducted both in situ and ex situ at each monitoring station along the Jagir River. Water temperature was directly measured in the field using a digital thermometer or temperature probe, while dissolved oxygen (DO) was measured in situ using a portable DO meter that was calibrated prior to use. For laboratory analysis, water samples were collected in clean bottles, stored in a cooler with ice, and immediately transported to the laboratory to prevent any changes in sample characteristics. Total Suspended Solids (TSS) were analyzed gravimetrically using the filtration method with filter paper (e.g., Whatman GF/C or equivalent), then the residue was dried to a constant weight and calculated in mg/L. Biochemical Oxygen Demand (BOD_5) was determined by the 5-day incubation method at 20°C, measuring initial and final DO using the Winkler titration method or a DO meter. The DO difference was used to calculate BOD_5 (mg/L) according to standard procedures (APHA, 2023).

RESULTS

Results of microplastic abundance

Based on Table 1, the abundance of microplastics in the Jagir River was detected at all observation stations with a range of 2,365,333.3–2,541,333.3 particles/m³. Station 1 showed the highest abundance (2,451,333.3 particles/m³) with dominant film and fiber types. Station 2 had the lowest abundance (2,365,333.3 particles/m³) with fragment and film types. Station 3 again showed a high abundance (2,541,333.3 particles/m³) with fiber and fragment types, indicating a combination of microplastic sources from upstream to midstream and potential accumulation before entering the estuary.

Table 1. Results of microplastic abundance

Location	Type of microplastics	Microplastics abundance (partikel/m^3)
Site 1	Film, fiber	2,451,333.3
Site 2	Fragmen, film	2,365,333.3
Site 3	Fiber, fragmen	2,541,333.3

Results of blood cells analysis

Based on Table 2, the blood cell parameters of tilapia showed variations between locations. The highest erythrocyte count was found at Site 3 (3,460,000 cells/mm^3), followed by Site 1 (2,567,500 cells/mm^3), and the lowest at Site 2 (1,475,000 cells/mm^3). A similar pattern was seen for hemoglobin and hematocrit, where Site 3 was also the highest (Hb 8.5; Ht 18.5%), Site 1 was at an intermediate value (Hb 7; Ht 15%), and Site 2 was the lowest (Hb 5.25; Ht 10.5%). For leukocytes, the highest value was recorded at Site 3 (171,500 cells/mm^3), followed by Site 1 (158,750 cells/mm^3), and the lowest at Site 2 (146,000 cells/mm^3). The differential leukocyte composition was relatively stable: lymphocytes predominated at all sites (60–64%), whereas monocytes (8–9%), neutrophils (6–9%), and eosinophils (2.5–3%) showed little change between stations.

Table 2. Results of blood cells analysis

Blood cells parameters	Site 1	Site 2	Site 3
Eritrosit (sel/mm^3)	2,567,500	1.475.000	3.460.000
Leukosit (sel/mm^3)	158,750	146,000	171,500
Hematokrit (%)	15	10.5	18.5
Hemaglobin	7	5.25	8.5
Limfosit (%)	62	64	60
Monosit (%)	9	8	9
Neutrofil (%)	8	6	9
Eusinofil (%)	3	2.5	3

Results of water quality analysis

Based on Table 3, the water temperature in the Jagir River is in the range of 27.7–28.8°C, with Site 1 being the lowest (27.7°C) and Site 2–Site 3 both at 28.8°C. The DO value is relatively stable at all locations (5.5–5.9 mg/L), with the highest DO at Site 2 (5.9 mg/L) and the lowest at Site 3 (5.5 mg/L). The organic load parameter represented by BOD shows a value of 3.5–4.06 mg/L, namely the lowest at Site 1 (3.5 mg/L) and the highest at Site 2 (4.06 mg/L), while Site 3 is 4.0 mg/L. TDS is relatively uniform (332–337.6 mg/L) with the highest value at Site 1 (337.6 mg/L). Meanwhile, TSS showed the clearest differences between locations, namely the highest at Site 1 (17 mg/L) and lower at Site 2 (9 mg/L) and Site 3 (8 mg/L).

Table 3. Results of water quality analysis

Water quality parameters	Site 1	Site 1	Site 3
Temperature (°C)	27.7	28.8	28.8
DO (mg/L)	5.8	5.9	5.5

BOD (mg/L)	3.5	4.06	4.0
TDS (mg/L)	337.6	332	336
TSS (mg/L)	17	9	8

Data analysis

The results of the statistical analysis using One-Way ANOVA indicated a significant difference in microplastic abundance among the study groups/locations. This was evidenced by a calculated F value of 2,330.008, which was substantially higher than the critical F value of 7.708647, and a p-value of 0.000001, which was lower than the significance level of α = 0.05. The variation among groups (Between Groups) showed a Sum of Squares (SS) value of 9.023, reflecting the contribution of spatial differences to the variability in microplastic abundance. Meanwhile, the variation within groups (Within Groups) had an SS value of 15.490, representing natural variability or other uncontrolled factors in the study, such as water current dynamics, tidal influences, and natural plastic degradation processes.

Table 4. Results of One-Way ANOVA Test on Microplastic Abundance.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	9.023	1	9.023	2.330,008	0,000001	7,708647
Within Groups	15.490	4	3.872			
Total	9.038	5				

The results of the Pearson correlation analysis indicated that microplastic abundance exhibited a very strong relationship with almost all blood parameters of Nile tilapia, as reflected by correlation coefficient (r) values approaching ±1. Microplastic abundance showed very strong positive correlations with erythrocytes (r = 0.997), leukocytes (r = 1.000), hematocrit (r = 0.996), hemoglobin (r = 0.998), monocytes (r = 0.859), neutrophils (r = 0.979), and eosinophils (r = 0.859), indicating that increasing microplastic contamination in the water was accompanied by increases in hematological parameters and non-specific immune cells as part of the fish's physiological and immunological responses to environmental stress. In contrast, microplastic abundance exhibited a very strong negative correlation with lymphocyte percentage (r = −1.000), suggesting a decline in adaptive immune responses with increasing microplastic exposure. This pattern reflects the role of microplastics as a chronic stressor that stimulates inflammatory and non-specific defense-related blood cells while potentially suppressing the adaptive immune system of Nile tilapia. Overall, these correlation results strengthen the assumption that microplastic contamination significantly affects the physiological balance and blood immune system of Nile tilapia in the Jagir River, Surabaya.

DISCUSSION

Ecologically, the dominance of fibers at Station 1 (near the Jagir sluice gate) is consistent with the characteristics of an urban river strongly influenced by dense residential areas and domestic discharges, including contributions of textile fibers from wastewater systems and laundry activities as major sources of microplastic fibers in urban environments (McCormick *et al.,* 2016; Wang *et al.,* 2024). The presence of films at Stations 1–2 is likely associated with the degradation of plastic packaging/sheet materials (plastic bags, food wrappers) entering the river through surface runoff and urban drainage, with a fiber–film–fragment composition pattern that is also commonly reported in urban rivers in Indonesia (Sari *et al.,* 2022), including large rivers used as raw water sources and affected by domestic–industrial wastewater inputs (Lestari *et al.,* 2020). The renewed high abundance at Station 3 (downstream) can be explained by the accumulation of loads from the upstream–midstream sections and transport processes that enhance contamination toward the downstream/estuarine segment a pattern frequently observed in rivers discharging into the sea due to cumulative inputs from the entire catchment and hydrodynamic conditions that promote particle accumulation before entering the estuary (Sulistyowati *et al.,* 2022). Meanwhile, the occurrence of fragments at Stations 2–3 may be linked to shipping activities and fishing docks (degradation of ropes, nets, and operational plastic materials) as well as secondary plastic fragments transported by the current; studies on urban rivers also indicate that particles can remain mobile within wastewater infrastructure and be detected as far as downstream segments (Wang *et al.,* 2024).

Lower erythrocyte–hemoglobin–hematocrit values at Site 2 indicate a tendency toward anemia or reduced oxygen-carrying capacity, whereas increased leukocyte counts at Site 3 may reflect stress responses or immune activation. These findings are consistent with experimental evidence in Oreochromis niloticus showing that microplastic exposure can reduce key hematological indices (RBC, Hb, and Ht) and alter leukocyte profiles as responses to microplastic stressors (Hamed *et al.,* 2019). Moreover, open-access studies on Nile tilapia demonstrate that microplastics can induce more mechanistic alterations in erythrocytes, such as eryptosis, poikilocytosis, and erythrocyte nuclear abnormalities (including micronuclei), supporting the notion that changes in blood profiles may occur through cellular stress pathways and red blood cell damage (Hamed *et al.,* 2021). Other studies have also reported reductions in hemoglobin, erythrocytes, and total leukocytes in O. niloticus exposed to specific microplastics, accompanied by indications of physiological disturbances (including oxidative stress), suggesting that spatial hematological variations observed in field studies may reflect combined effects of microplastic exposure and co-occurring aquatic stressors across different river segments (Raza *et al.,* 2023).

The combination of BOD ~3.5–4.06 mg/L and DO ~5.5–5.9 mg/L indicates the persistence of organic matter stress, as BOD represents the oxygen demand required for organic matter decomposition and its increase is commonly associated with reduced oxygen availability for aquatic biota, particularly in freshwater systems receiving domestic wastewater inputs (Vigiak *et al.,* 2019). The pattern of highest BOD at Site 2 is consistent with the characteristics of the midstream station influenced by vessel mooring activities, domestic waste, and small-scale industries conditions that often elevate organic loading and drive DO–BOD fluctuations in urban rivers (Mawaddati *et al.,* 2021; Kurnianto *et al.,* 2024). In Surabaya, studies on major river systems used as raw water sources further emphasize that parameters such as DO, BOD, and TSS are key indicators of pollution dynamics and require continuous monitoring due to their close association with domestic industrial wastewater inputs and temporal changes in water quality (Razif *et al.,* 2022). The relatively higher TSS at Site 1 may reflect local hydrodynamic effects (e.g., turbulence or flow retention near the sluice gate), sediment resuspension, or suspended particle runoff from densely populated areas, which can generally deteriorate the physical condition of the water body and are characteristic of urban river segments (Novita *et al.,* 2020). Overall, the water quality results for the Jagir River indicate relatively warm waters with moderate DO, accompanied by signs of organic loading (BOD) and spatially variable suspended solids (TSS) corresponding to activity pressures in each river segment.

CONCLUSION

Based on the results of this study, the Jagir River in Surabaya is indicated to be under pollution pressure, as reflected by the high abundance of microplastics at all stations (2,365,333.3–2,541,333.3 particles/m³), with variations in fiber, film, and fragment types corresponding to segment-specific anthropogenic sources (dense domestic activities in the upstream area, vessel mooring activities and domestic small industrial waste in the midstream, and cumulative loading toward the downstream/estuarine segment). Water quality conditions showed relatively stable temperatures (27.7–28.8°C) and moderate dissolved oxygen levels (5.5–5.9 mg/L), accompanied by indications of organic loading (BOD 3.5–4.06 mg/L) and marked differences in suspended solids (TSS 8–17 mg/L), reinforcing the influence of wastewater inputs and local hydrodynamic dynamics. The biological responses of Nile tilapia exhibited spatial variations in blood parameters, with the lowest erythrocyte, hemoglobin, and hematocrit values observed at Site 2 and increased leukocyte counts at Site 3, collectively indicating physiological disturbances and stress/immune responses potentially associated with microplastic exposure and environmental conditions. Therefore, these findings confirm that microplastic contamination in the Jagir River is associated with alterations in cellular/hematological indicators of Nile tilapia, suggesting that microplastic metrics, water quality parameters, and blood biomarkers may be integrated as a comprehensive monitoring basis for urban water quality management prior to discharge into the Madura Strait.

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