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Hematological Profile of Bader Fish (*Barbonymus altus*) in the Brantas River, Blitar Region in Measurement of River Water Quality

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Abstract: Brantas River is habitat to a fish species known as the bader fish (*Barbonymus altus*). Research aims to evaluate the hematological profile of the bader fish as a means to assess the quality of the Brantas River in the Blitar region. Determining the waste flow from community activities that influences blood quality of bader fish is another goal of this research. To achieve this, various water quality indices were measured in the river and hematological parameters measured in the fish. Data collection was conducted once every week for three weeks during 17 January to 17 March 2022. The measurements aimed to observe any changes in the fish's hematological profile over this period. Two data analysis techniques were employed for the research: Pollution Index (IP) and Canonical Correlation Analysis (CCA). IP measurements reveal that all three stations share a common assessment, classifying them as having light pollution. When analyzing correlation measurements through CCA, it was found that erythrocytes exhibited a robust positive correlation with a pH value of 0.549, while conversely, DO (Dissolved Oxygen) showed a strong negative correlation with leukocytes with value -0.717. In summary, the water conditions in the Brantas region remain conducive for the habitat of bader fish.

Keywords: Bader fish; Brantas river; CCA; Hematology; IP; Water quality

Introduction

Rivers have always been important suppliers of water for a variety of social uses. As industries developed, so did the impact of their activities on how rivers functioned. Regrettably, one common use of rivers has been as landfills for trash, particularly from major industrial enterprises. Industrial waste's organic and inorganic components have a negative impact on river ecosystems, producing environmental harm and imbalances.

For the locals of East Java, the Brantas River serves as a vital water source. It starts in Sumber Brantas Village, Batu City, in the Bumiaji District. The river, which travels in a clockwise path around the volcano and has a length of 320 km, rises from a spring near the foot of Mount Arjuno (Sujono, 2019). Blitar City is bordered on the north by Kec. Nglegok and Kec. Garum, Kab. Blitar, east by Kec. Garum and Kec. Kanigoro, Kab. Blitar, south by Kec. Kanigoro and Kec. Sanankulon, Kab. Blitar (Nur'aini, 2012).

As the environment changes because of numerous human activities, river water quality will also change. Human activity as river users and the life on the river itself both contribute to river pollution (Yuliastuti, 2011). An endemic spesies called bader fish (*Barbonymus altus*) can be found in the Brantas River (Hertika et al., 2021). The caudal fin lobes of baders lack a black submarginal line and have a broad red distal border. The anal and pelvic fins of the bader fish are crimson. The larger fish's

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dorsal fin is dark in the distance. On the lateral line, there are 31–33 scales from bader fish. Bader fish typically reach lengths of 15 cm, with a 25 cm maximum (Phen et al., 2005).

Because blood transports waste products to the gills, liver, and kidneys, where waste can be excreted from the body, hematological status can be a sign of health in fish. Blood also provides nutrients and oxygen to the tissues (Habenicht, 2020). Blood consists of erythrocytes, leukocytes, hemoglobin and hematocrit (Tkachuk et al., 2007). In the Baderbank Blitar protection area, research has been done on the hematology and water quality of bader fish in the Brantas River (Hertika et al., 2021), but not in the vicinity of local residents.

The goal of this study was to evaluate the water quality of the Brantas river in the Blitar area near residential areas by observing the concentration of factors in waters with the hematological status of bader fish.

Method

The main study materials for this investigation were river water and Bader Fish (*Barbonymus altus*). The Brantas River sample site in Blitar is separated into three sites. The coordinates of the first station are -8.1587, the second station is -8.1544, and the third station is -8.1532. Local fisherman sampled fish at three different places, repeating the process three times over the course of three weeks, which were conducted from January to March 2022.

Observations were carried out in two places, namely directly in the field and in the laboratory. Temperature, pH, and DO are measured directly in the field to determine the quality of the water. Observations of fish blood carried out directly in the field were hemoglobin. An immersion thermometer is used to measure temperature, a pH meter is used to detect pH, and a digital titration instrument is used to perform the Wrinkler method for DO. Blood observations observed directly in the field are hemoglobin parameters. Using a syringe, fish blood was initially drawn in the lateral linear segment to measure the hemoglobin characteristics. Hemoglobin observation using an HB meter consisting of 0.01 N HCL solution, thoma pipette, drop pipette, and hemoglobin scale.

Laboratory observations for water quality are BOD, TSS, TDS, Ammonia. BOD was observed using the wrinkler method with digital titration. The difference in DO and BOD observations is that the sample solution is first incubated in an incubator for five days at a temperature of 20 C. The water sample for the TSS parameter is first dried in an oven at a temperature of 103-105°C for an hour. Meanwhile, TDS is dried in an oven at a temperature of 103-105°C for 24 hours (Nasrabadi et al., 2016). The results of these two parameters are weighed with an analytical balance. Observation of ammonia using a spectrometer (Guspita & Ulianas, 2020). Observations of fish blood parameters carried out in the laboratory are erythrocytes, leukocytes, hematocrit, phagocytosis and micronuclei. Fish blood samples that have been taken in the linear lateral section using a syringe, are dropped into an Eppendorf tube that has been coated with EDTA solution with the aim of preventing the blood sample from clotting (Sheikh & Ahmed, 2020). Eppendorf tubes containing fish blood samples are stored in a coolbox.

Erythrocyte observation with an Olympus BH2 microscope, hemocytometer kit, and Hayem solution. The hemocytometer set consists of a hemocytometer and a thoma pipette. Turk's solution is utilized instead of erythrocyte solution for examining leukocytes. The technique is nearly same (Lulijwa et al., 2019). Observation of hematocrit using a microhematocrit tube, centrifuge, and microhematocrit scale (Zainun, 2017). Phagocytosis observation using an Olympus BH2 microscope, glass objects, and Giemsa solution. Observation of micronuclei using a glass object, ethanol solution, 10% Giemsa solution, and an Olympus BH2 microscope.

Figure 1. Bader fish (*Barbonymus altus*)

Table 1. Pollution Index Evaluation (Kementerian Lingkungan Hidup, 2003)

IP Polution	Water quality
0 ≤ IP ≤ 1.0	Good condition
1.0 < IP ≤ 5.0	Lightly polluted
5.0 < IP ≤ 10.0	Moderately polluted
IP > 10.0	Heavily polluted

Among the data analysis techniques employed are the Pollution Index (IP) and Canonical Correlation Analysis (CCA). The Pollution Index is used to assess the degree of pollution in relation to the allowable water quality indicators. Pollution Index (IP) is determined for a designation, after which it can be produced for a variety of functions for all areas of a water body or section of a river. This technique can directly relate the amount of pollution to whether or not the river can be used for specific purposes and with specific parameter values (Djoharam et al., 2018).

The data analysis method used is called CCA (Canonical Corelation Analysis). To identify and break down the connection between the two variables, CCA analysis is a powerful multivariate technique (Hussein et al., 2018). Fish hematology is the dependent variable, and water quality is the independent variable.

Result and Discussion

Water Quality

The average river water temperature readings at the first, second, and third stations are 21.9-28 °C, 21.9-29.5 °C, and 22-29.5 °C, respectively. With no clouds and scorching weather, the third station recorded the maximum temperature, which was around 29.5 °C. At the second station, where it was raining, the lowest temperature, around 21.9 °C, was recorded. Land use, hydrological systems, geography, and climate can all have an impact on temperature variations. Temperature has a significant impact on the functions of aquatic species, including respiration, food consumption, growth, and reproduction. Rivers should be between 27 and 30 °C, with 29°C being the ideal range for fish growth (Tang et al., 2017).

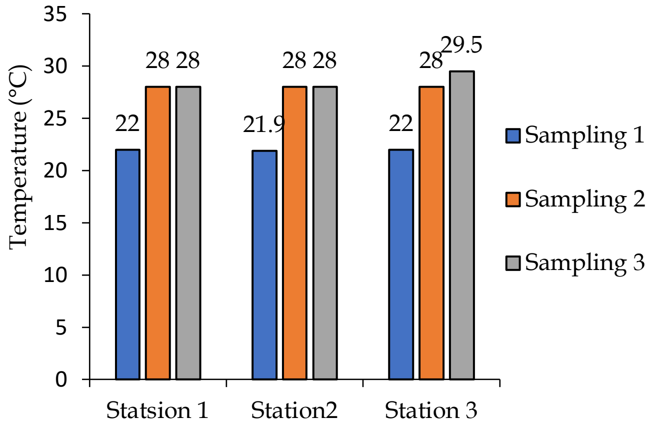

Figure 2. Graphic of temperature

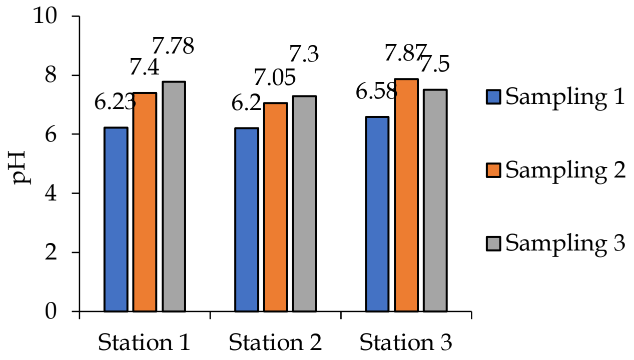

Figure 3. Graphic of pH

Tropical fish require a temperature range of 28 to 31 °C to survive (Erika et al., 2018), such that the Brantas River's temperature continues to be considered ideal. Fish will lose their appetite at temperatures between 18 and 25 degrees Celsius, which will cause them to starve to death (Warman, 2017). However, too hot temperatures will stress fish and negatively affect their hematological health (Saparuddin, 2019).

At the first, second, and third stations, the average results of measuring the pH of the waters were 6.23–7.78, 6.2–7.3, and 6.58–7.87. The third station recorded the greatest pH, which was roughly 7.87, and station two recorded the lowest pH, which was roughly 6.2. Second-class water has a pH value between 6.2 and 7.40 (Machairiyah et al., 2020). The optimal pH value is 6-9. These values indicate the optimal balance between oxygen and carbon dioxide. As a result, harmful microorganisms will have difficulty thriving when the pH is less than 4.8 or greater than 9.2. Water with a river pH value less than 4.8 or greater than 9.2 can be concluded to be polluted (Putri et al., 2019).

The pH of the waters of the Brantas river can be inferred to be still regarded as ideal. A pH that is too acidic will make fish more prone to illness, reduce production, and interfere with fish growth (Kurniawan et al., 2020). The survival rate of fish will drop due to too-acidic water since it can interfere with fish respiration, which is another effect (Arizuna et al., 2014).

The average DO measurement results in the first, second and third stations were 6.51-7.15 mg/L, 5.46-7.62 mg/L, and 6.2-7.47 mg/L. The highest DO sample value at the second station was 7.62 mg/L and the lowest station value at the second station was also around 5.46 mg/L. The quality standard of DO is around 5 mg/L (Saraswati et al., 2017), consequently, it can be said that the DO in the water of the Brantas River is still ideal. The lack of phytoplankton that performs photosynthesis is what causes the DO value to fall (Sari & Wijaya, 2019). When the DO value falls to 2 mg/L, the fish will experience hypoxia and die from a lack of oxygen (Yulistia et al., 2018).

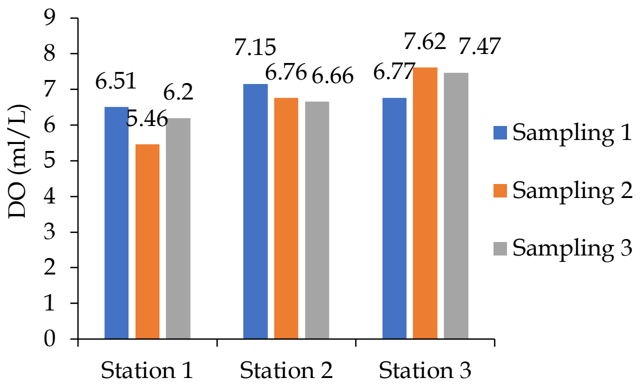

Figure 4. Graphic of DO

At the first, second, and third stations, the average BOD measurement results were 1.39 to 3.02 mg/L, 0.02 to 1.45 mg/L, and 0.12 to 1.74 mg/L. At the third station, the BOD level was approximately 1.74 mg/L, while at the second station, it was 0.02 mg/L. Less than 20 mg/L of BOD is required for aquatic biota to survive (Putri et al., 2019). The Brantas River's waters still have an ideal BOD value, it can be said. Dissolved oxygen (DO) is used by aerobic bacteria in the decomposition process, which results in a fall in BOD value (Simbolon, 2016). The amount of plankton, bacteria, and organic matter in the waters are factors that influence the BOD value (Zahroh et al., 2019).

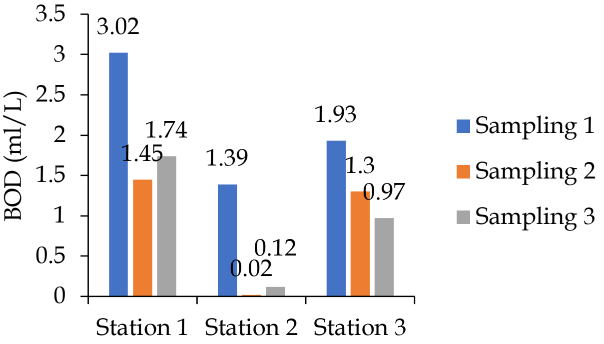

Figure 5. Graphic of BOD

The first, second, and third stations' average ammonia measurement findings, respectively, varied from 0.67 to 0.154 mg/L, 0.047 to 0.206 mg/L, and 0.045 to 0.229 mg/L. At station three, the ammonia reading ranged from 0.045 mg/L, which was the lowest, to 0.229 mg/L, which was the highest. Ammonia levels in aquatic environments should not exceed 0.3 mg/L (Putri et al., 2019). It might be said that the Brantas River's ammonia waters are still at their best. Ammonia is produced by fish metabolism and the bacterial breakdown of organic materials (Hamuna et al., 2018). Waste from industries, households, and agriculture contributes to high ammonia concentrations (Nasir & Baiduri, 2018).

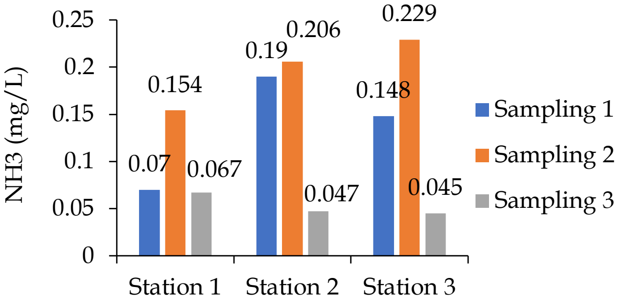

Figure 6. Graphic of NH_3

Fish respiratory problems are a result of elevated ammonia levels (Zhang et al., 2013). The nitrogen cycle in nature contains ammonia. Ammonia is regarded as a water pollution since it can be hazardous at specific amounts. If the pH is below 7.0, most ammonia takes the form of NH_3, and if the pH is over 7.0, it takes the form of NH^{+4} or ammonium salt. Ammonia has the characteristic of being soluble in water (Roney & Llados, 2004).

The results of the average TSS measurements at the first, second, and third stations were 5-23 mg/L, 9-24 mg/L, and 6-30 mg/L, respectively. A TSS concentration between 20 and 110 mg/L is required for class II water (Ofiyen & Puryanti, 2022). According to the sampling's results, the Brantas River's water is still classified as class II water. Silt, fine sand, and soil-eroding microorganisms make up the TSS content. The movement of river water bodies carries the information (Djoharam et al., 2018). A high TSS number will have the effect of preventing light from entering the river body, which will hinder photosynthesis (Pasisingi et al., 2014).

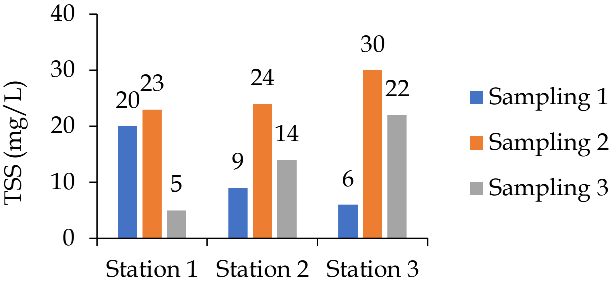

Figure 7. Graphic of TSS

The average TDS measurement findings at the first, second, and third stations were 32-183 mg/L, 79-171 mg/L, and 77-165 mg/L, respectively. Because the recovery operation was conducted during a time of rain, the first station recorded the highest TDS value. The class II standard TDS value is 1000 mg/L. Given that the TDS concentration at each of the first three stations is less than 1000 mg/L, it can be said that they are still considered to be in class II. The season affects the TDS value. The TDS value will rise during the wet season and fall during the dry season (Purwono et al., 2019). This occurs by organic and colloidal substances that enter streams during the rainy season (Effendi, 2003).

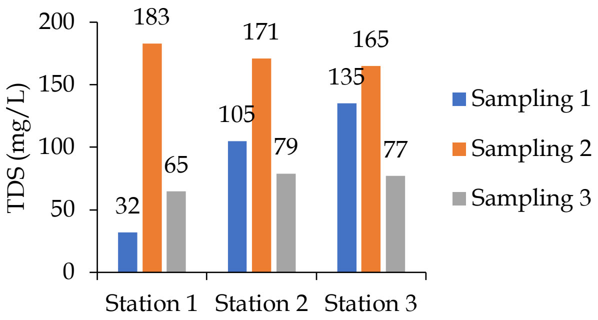

Figure 8. Graphic of TDS

Table 2. Brantas River Pollution Index

Research Station	Sampling to	IP Pollution	Quality Standard	Category
Station 1	1	3.25	1.0 ≤ IP ≤ 5.0	Lightly Polluted
Station 1	2	2.13		Lightly Polluted
Station 1	3	2.58		Lightly Polluted
Station 2	1	2.16		Lightly Polluted
Station 2	2	4.52		Lightly Polluted
Station 2	3	1.99		Lightly Polluted
Station 3	1	2.41		Lightly Polluted
Station 3	2	1.76		Lightly Polluted
Station 3	3	1.56		Lightly Polluted

According to Table 2 analysis of the Brantas River's Pollution Index data, the first through third stations fall into the category of lightly polluted areas. As a result of the IP value being larger than one and less than five, the indicator falls under the lightly polluted category. The third station recorded the lowest IP value, 1.58, whereas the second station on the second take had the highest IP value, 4.52. Stations 1-3 are considered to be lightly polluted by the Decree of the Minister of the Environment No. 115 of 2003 because the IP values range from 2-4 (Sari & Wijaya, 2019). Due to Station Two's large population, there is a chance that the river water may be mildly polluted.

Fish Hematology Observations

The average erythrocyte count in fish at the first, second, and third stations was 1.790.000-3.67.000 cells/mm^3, 1,100,000-2,540,000 cells/mm^3, and 1,240,000-3,420,000 cells/mm^3, respectively. The first station produced the most erythrocytes, 3,670,000 cells/mm^3, and the second station produced the fewest, 1,100,000 cells/mm^3. Fish erythrocyte density should range between 1,050,000 and 3,000,000 cells/mm^3. It can be said that the quantity of erythrocytes in fish is still largely normal (Rimalia & Iskandar, 2016). Heavy metals that build up in the blood will bond to erythrocytes, making them more easily damaged, which is one of the mechanisms that reduces erythrocytes. Fish may develop anemic as a result of the weak erythrocytes that result (Sari & Rahmawati, 2020).

The average fish leukocyte observation values at the first, second, and third stations were 23,550-475,300 cells/mm^3, 165,100-243,000 cells/mm^3, and 54,750-218,900 cells/mm^3, respectively. The first station's 475,300 cell/mm^3 and 23,550 cell/mm^3 leukocyte counts were the highest and lowest, respectively. Leukocyte density in healthy fish ranges from 20,000 to 150,000 cells/mm^3 (Lestari et al., 2019). The abundance of leukocytes suggests that the bader fish's physiology is defending itself by fending off poisons in these waters (Vaiyanan et al., 2015).

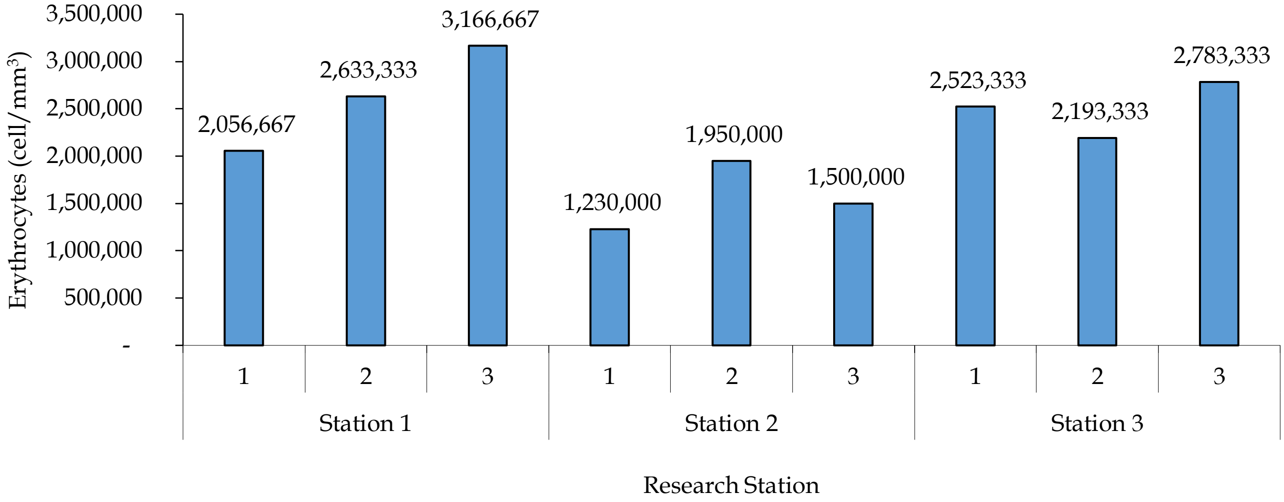

Figure 9. Graphic of Erythrocytes

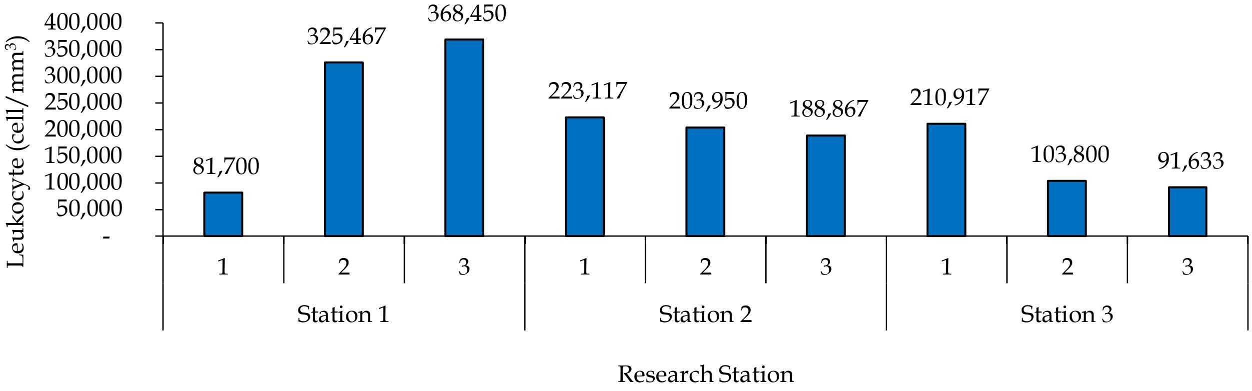

Figure 10. Graphic of leukocyte

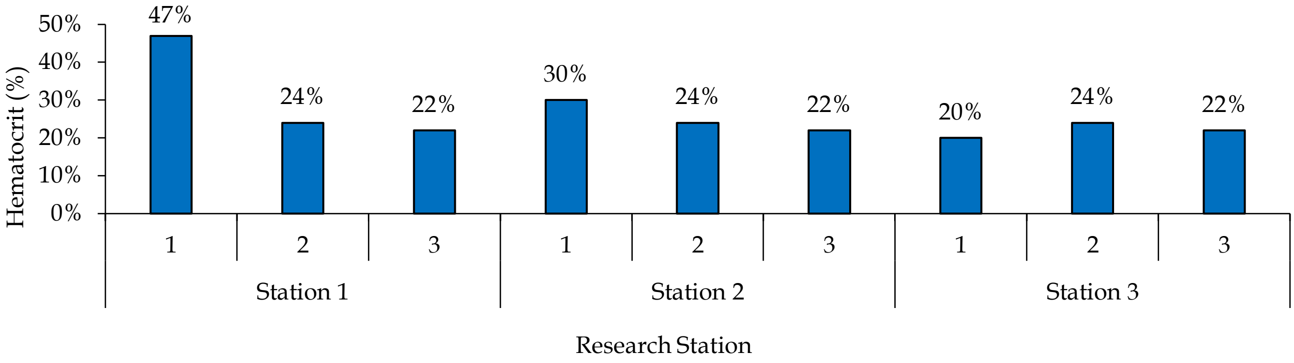

Figure 11. Graphic of hematocrit

The average hematocrit measured at the first, second, and third stations ranged from 22-47%, 22-30%, and 20-24%, respectively. The third station revealed lowest hematocrit percentage. Because the ideal hematocrit percentage in fish is above 30%, this is caused by ertorocyte malfunction (Hertika et al., 2021). Anemia will occur in fish with a low hematocrit percentage of less than 30%, while stress will occur in fish with a high hematocrit percentage of more than 30% (Mahardika et al., 2020).

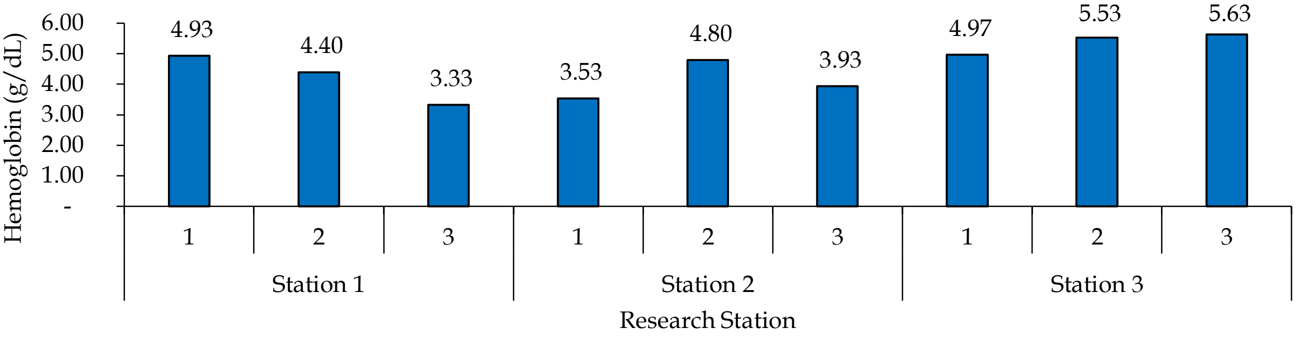

Figure 12. Graphic of hemoglobin

The average hemoglobin concentration measured at the first, second, and third stations ranged from 3-5.6 g/dL, 2-5 g/dL, and 4.3-6.2 g/dL, respectively. The third station's percentage value is the highest at 6.2 g/dL, while station two's is the lowest at 2 g%. The obtained hemoglobin percentage value is still in excellent shape. Because fish's ideal hemoglobin percentage is 2–16 g/dL (Alipin & Sari, 2020). The level of hemoglobin in an organism's blood is a reflection of its ability to control its oxygen supply and maintain stability. The oxygen supply to the tissues of fish may diminish due to decreased hemoglobin concentration brought on by industrial pollution, which would also reduce fish activity (Hertika et al., 2021).

The average number of micronuclei cells found at the first, second, and third stations varied from 11-38 ‰, 10-20‰, and 7-26‰, respectively. The first station has the highest concentration of micronuclear cells due to the presence of numerous human activities, such as plantations and rice fields, which result in the administration of pesticides that end up in water bodies. The quantity of leukocytes, or white blood cells, affects the number of micronuclei; the more white blood cells there are, the more significant the micronuclei (Islamy, 2017). Inconsistencies in the number and structure of chromosomes produced by different agents in the cell are represented by the growing number of micronuclei; this condition might result in the possible cytotoxic effects of metals and genotoxic chemicals on the body of organisms (Hertika et al., 2021).

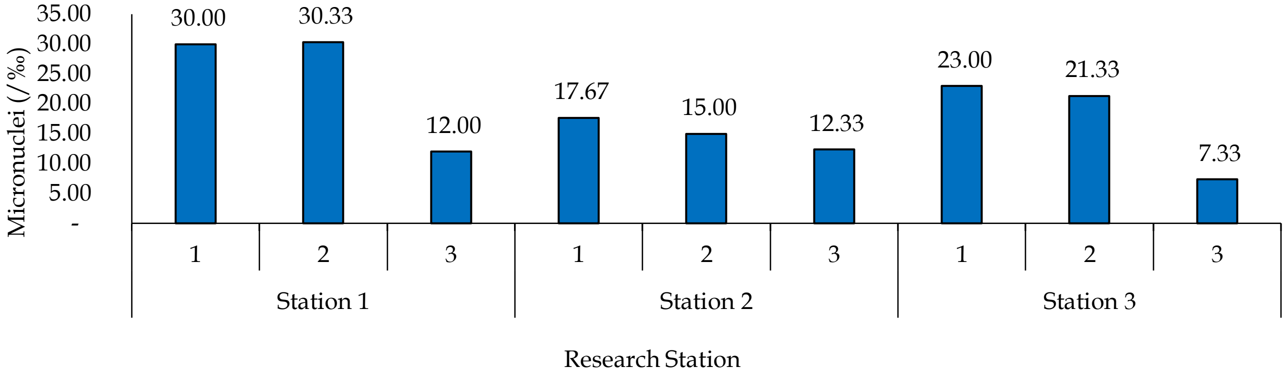

Figure 13. Graphic of micronuclei

Phagocytosis is the defense mechanism used by the body using leukocyte to devour and digest infections or foreign particles that enter the body. Phagocytic activity is a measurement of how many phagocytic cells are active or engaged in phagocytosis relative to the total number of cells present (Jamal et al., 2013). The average results of observations of phagocytosis in bader fish obtained values of 12-25%, 9-16%, and 11-21%, respectively, at the first, second, and third stations. The lowest phagocytosis value in fish was 4%, and the highest was 10% (Utami et al., 2013). The second station has the lowest score of 9-16%, and the highest is the first station in the range of 12-25%. Although the phagocytosis value was low, the value at the second station had already passed the threshold. An increase in the value of the phagocytosis index indicates that there is an increase in the fish's immune system. Phagocytic activity is the first line of defense of the cellular response carried out by monocytes (macrophages) and granulocytes (neutrophils). The increase in fish immunity can be seen from the increased activity of phagocytic cells in the blood (Wu et al., 2022).

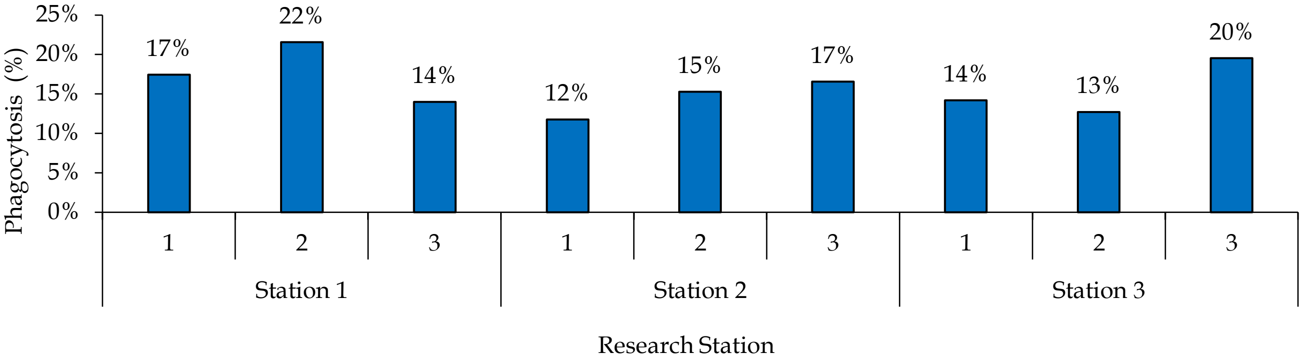

Figure 14. Graphic of Phagocytosis

Analysis CCA

The goal of CCA analysis is to ascertain the relationship between fish blood parameters and water quality indicators. XLSTAT software is used for CCA analysis. The measurement results revealed a number of fish blood characteristics that were strongly correlated with water quality indicators, including erythrocytes with pH, leukocytes with temperature, hemoglobin with DO, hemoglobin with TSS, and hematocrit with BOD. In particular, micronuclei and DO, hematocrit and TDS, and phagocytosis and ammonia and DO parameters, were found to be severely at odds with each other. The aquatic environment has a physical and chemical impact on the components of fish blood. If exposed to specific chemical agents, fish hematology can change in either direction (Lestari et al., 2019). As an illustration, fish can become anemic due to disruptions in the osmoregulatory network (Heath, 2018).

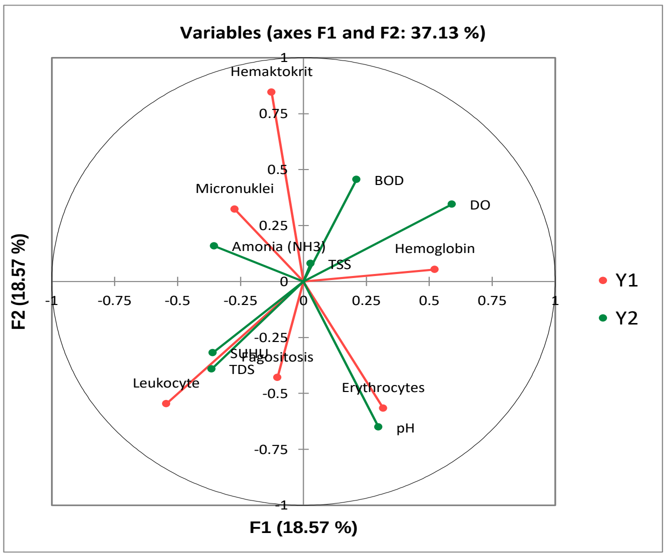

Figure 15. Graphic CCA (Canonical correlation analysis) between fish hemalogy and water quality parameter

Conclusion

The parameters of temperature, pH, DO, BOD, Ammonia, TSS, and TDS are still optimal for second class water quality. However, for the heavy metal Hg, the parameter value has exceeded the threshold. In assessing water quality using the Pollution Index (IP), it was concluded that the waters of the Brantas River at three stations were still classified as lightly polluted. The hematological parameters observed in bader fish were erythrocytes, leukocytes, hemoglobin, hematocrit, micronuclei and phagocytic activity. Of all these parameter values, only leukocytes have parameter values that exceed the threshold for normal fish. This happens because in the physiology of bader fish there is leukocyte activity which is fighting pathogens. The strongest parameter in CCA analysis is hemoglobin with TSS, while the strongest opposing parameter is leukocytes with DO.

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Author Contributions

Alby Ghifari Azhar played a role in conceptualization, methodology, formal analysis, investigation, and original draft preparation. Asus Maizar Suryanto Hertika and Andi Kurniawan were involved in validation, writing review, and supervision.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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