Aquatic Environmental Analysis of the Hematological Profile of *Barbonymus altus* in the Brantas River, Jombang, East Java

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Abstract

The Brantas River is the longest in East Java. One of the areas included in the flow of the Brantas River is the Jombang Regency. The Jombang Brantas River is used by the community to meet their daily needs in agriculture, plantations, industry, and fishing grounds. The input of waste generated from several community activities will result in the survival of fish in the waters. Fish as a biomarker agent is quite significant in estimating the level of heavy metal pollution. Red Bader Fish (*Barbonymus altus*) is often found and lives around the Jombang Brantas River. This study aims to determine the condition of the aquatic environment by observing the health of the fish through hematological observations of the Red Bader Fish (*B. altus*). Sampling was carried out at three stations with three replications every two weeks, which was carried out in February – March 2022 in the Jombang Brantas River. Data analysis used the Canonical Correspondence Analysis (CCA) method as an analytical method to determine the correlation between the dependent variable (hematological profile) and the independent variable (water quality conditions). In this study, the results showed that the water quality at Station 1 and Station 2 was classified as normal, while at Station 3, it was classified as polluted. Data analysis showed that erythrocytes, hemoglobin, and hematocrit had a relationship with temperature, pH, DO, BOD, ammonia, TSS, and TDS in low concentrations. Meanwhile, leukocytes and micronuclei showed a relationship with high concentrations of ammonia, BOD, TDS, and TSS.

Keywords: Brantas River, Bader fish (*Barbonymus altus*), CCA, hematology, water quality.

INTRODUCTION

The Brantas River is one of the longest rivers in East Java, with a length of ±320 km and an area of ±12,000 km^2 [1]. The Brantas River is often used by the surrounding community to meet water needs in agriculture, households, and industry, as well as a place to find fish for consumption and trade [2]. Community activities in meeting their needs will produce waste that causes a decrease in river water quality.

Jombang is one of the districts in the central part of East Java. Jombang has a very strategic position because it is at the crossroads of the north and south of the Island of Java [3]. This condition is accompanied by an increasingly rapid industrial development so that it cannot be separated from the problem of environmental pollution due to the waste generated. Waste is a by-product of the production process that is not used in the form of liquid, solid, gas, and so on. This condition will result in natural and environmental degradation that affects the quality of life of the community [4]. Water pollution produced in the form of heavy metals can accumulate in the body quickly and is easily absorbed by aquatic organisms, one of which is fish that live in the waters.

Fish is a biomarker that is quite significant in estimating the level of heavy metal pollution and can describe changes in water characteristics [5]. The fish that is often found in the Jombang Brantas River is the Red Bader fish (*Barbonymus altus*). Red Bader Fish is included in the Cyprinidae species, which is generally used in toxicity tests so that it can be used as a biomarker agent in determining river pollution that gets input from the pollutant load from community activities [6]. Heavy metals enter the body's tissues through the respiratory tract, digestion, and penetration through the skin. The entry of heavy metals into the body will be absorbed by the blood and distributed to all body tissue [7].

Blood is one of the parameters used to see fish abnormalities that occur due to disease or environmental conditions [8]. Blood has an important function in an organism's life. Blood plays a role in circulating food substances from digestion, carrying oxygen, and carrying enzymes and hormones to all parts of the body [9]. Therefore, information is needed to determine the health status of fish based on the hematological profile used as an indicator of the aquatic environment in the Jombang Brantas River.

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MATERIAL AND METHOD

Sampling Area

This research was conducted in the Jombang Brantas River, East Java. Sampling was carried out using the purposive sampling method [10]. The location of the research was carried out at three stations suspected of receiving different waste inputs, including Station 1, adjacent to agricultural land and boat crossings; Station 2, adjacent to land use in agriculture; and Station 3, is adjacent to the waste disposal from the animal feed industry. The research locations of the three stations can be seen in (Fig. 1). Water and fish blood samples were taken three times every two weeks from February – March 2022. Fish blood sampling was taken as many as three fish for each time at each station.

Water Quality Measurement

Water quality measurements were carried out in situ and *Ex Situ*. The water samples were measured *in situ*, such as temperature using a water thermometer (SNI) [11], pH using a pH meter (SNI) [12], and Dissolved Oxygen (DO) using the Winkler method [13]. Meanwhile, water samples were measured ex-situ by inserting the sample that had been taken in a bottle container and then put into a coolbox for testing at the Freshwater Fisheries Laboratory, Brawijaya University. The parameters are Biological Oxygen Demand (BOD) using the Winkler method, which had been incubated for five days [14], ammonia (NH_3) using a spectrophotometer, Total Suspended Solid (TSS), and Total Dissolved Oxygen (TDS) [15].

Fish blood sampling and observation of hematological profile

A sampling of fish was carried out using fishing nets by taking three fish from each location. The Red Bader Fish (*Barbonymus altus*) used in this study can be seen in (Fig. 2). Blood was drawn by wetting the syringe and Eppendorf tube with a 3.8 % Na-citrate anticoagulant, then puncturing the linea lateralis at a slope of 45°C and slowly taking the blood with a 1 mL syringe, then putting it into the Eppendorf tube [16]. After that, it was put into a cool box that has been given ice gel for observations at the Freshwater Fisheries Laboratory, Universitas Brawijaya.

Observation of the hematological profile was carried out by counting the number of erythrocytes and leukocytes under a microscope using a hemocytometer [17]. Hemoglobin using a Hb meter and hematocrit using a hematocrit scale [18]. Then micronuclei by counting cell damage using a microscope [19].

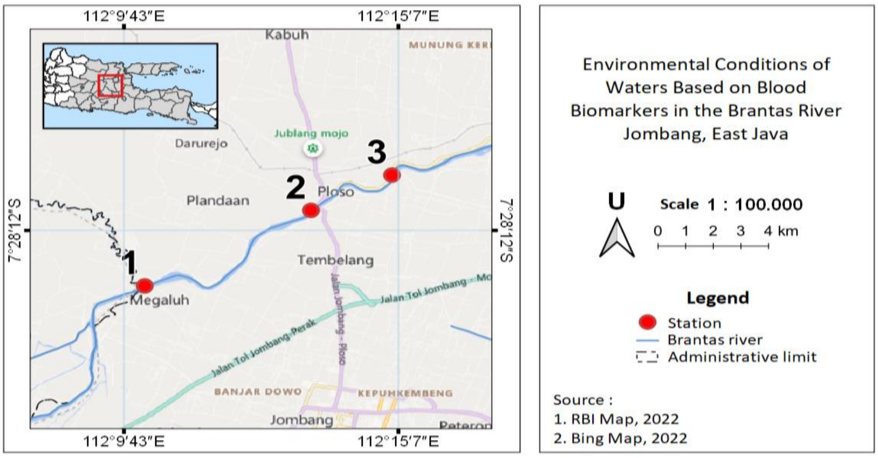

Figure 1. Map of sampling location

Figure 2. Red Bader fish (*Barbonymus altus*) sample (body length ±20 cm)

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Data Analysis

This study connects water quality and hematological profiles as variables in the analytical test using the Canonical Correspondence Analysis (CCA) method [20]. This analysis is a multivariate statistical model that identifies and quantifies the relationship between two variables. This analysis focuses on the correlation between a linear combination of one set of variables with a linear combination of another set of variables. The independent variables in this study were all water quality parameters, while the dependent variable was the observation of the hematological profile of the red Bader fish (*B. altus*).

RESULT AND DISCUSSION

Water Quality Measurement

Water quality measurements are carried out with parameters of temperature, pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), ammonia (NH_3), Total Suspended Solid (TSS), and Total Dissolved Oxygen (TDS) (Fig. 3). In this study, the average temperature parameter at the three research locations was 28-30.5°C. At each station, the values are 28-29.5°C (Station 1), 28-30°C (Station 2), and 28-30.5°C (Station 3). The highest temperature increase was in the third sampling, with an average of 30°C due to the hot weather. This value is still considered optimal in waters where the temperature range is 28-32°C, while at a temperature of 18-25°C, it will cause a decrease in fish appetite and death if the temperature is too cold [21]. An increase in temperature that is too high will result in fish stress, which can affect fish hematology [22].

The pH parameters of the waters at the three stations were obtained in the range of 7.12-7.95. The permissible pH value in the waters according to the Class II quality standard is 6-9 [23]. The pH value of the three stations is still considered optimal in the waters. Water conditions that have a very acidic or alkaline pH will endanger the survival of organisms because it causes metabolic and respiratory disorders [24]. Other effects of pH on fish physiology include stunted fish growth, sensitivity to bacteria and parasites, and water toxicity to fish [25].

Parameters of DO were obtained in the range of 7.11-10.77 $mg.L^{-1}$ at the three stations. With values at each station of 7.11-9.01 $mg.L^{-1}$ (Station 1), 7.9-10.77 $mg.L^{-1}$ (Station 2), 8.11-8.76 $mg.L^{-1}$ (Station 3). The value of DO allowed in the waters according to the Class II quality standard is 4 $mg.L^{-1}$ [23]. The main source of oxygen in waters can come from the process of diffusion of free air and the results of photosynthesis of organisms that live in the waters. Increasing the depth will result in a decrease in DO levels due to the reduced photosynthesis process and oxygen levels that are too much used for respiration and oxidation of organic and inorganic materials [26]. When dissolved oxygen has a low concentration, the fish will respond in various forms, including blood flow can be increased by opening the lamellae and then a secondary response to increase the area of effective breathing. Furthermore, the concentration of red blood cells can be increased by carrying oxygen from the blood per unit volume [27].

The BOD parameter is the amount of dissolved oxygen needed by microorganisms to decompose organic matter under aerobic conditions. BOD levels in waters are influenced by temperature, an abundance of plankton, the presence of microbes, and the content of organic matter in the waters [28]. In this study, the measurement of BOD parameters in the waters ranged from 9.15-28.25 $mg.L^{-1}$. Measurement of BOD obtained the highest value at the time of the second sampling at Station 3 of 28.25 $mg.L^{-1}$ and the lowest value at the time of the third sampling at Station 1 of 9.15 $mg.L^{-1}$. BOD values in high waters can reduce the availability of dissolved oxygen in the water because it is used in the oxidation process of organic matter that can be decomposed by microorganisms [29]. The value of BOD of river water quality standard with Class II category is 3 $mg.L^{-1}$ [23].

Ammonia (NH_3) is one of the inorganic nitrogen that can be dissolved in water. Ammonia in water generally comes from urine and feces, oxidation of organic matter, industrial waste and community activities [30]. Ammonia values at the three stations ranged from 0.056-0.45 $mg.L^{-1}$. The increase in ammonia value occurred at Station 3 with a range of 0.266-0.45 $mg.L^{-1}$. The value of Ammonia (NH_3) in river waters according to the Class II quality standard is 0.2 $mg.L^{-1}$ So, Station 3 obtained a value that exceeds the quality standard, which is suspected to be sampling close to the industrial waste disposal site.The high concentration of ammonia in the waters can come from the animal metabolism and the decomposition of organic matter by bacteria. Relatively high levels of ammonia indicate contamination of organic matter from industrial, agricultural, and domestic waste [31].

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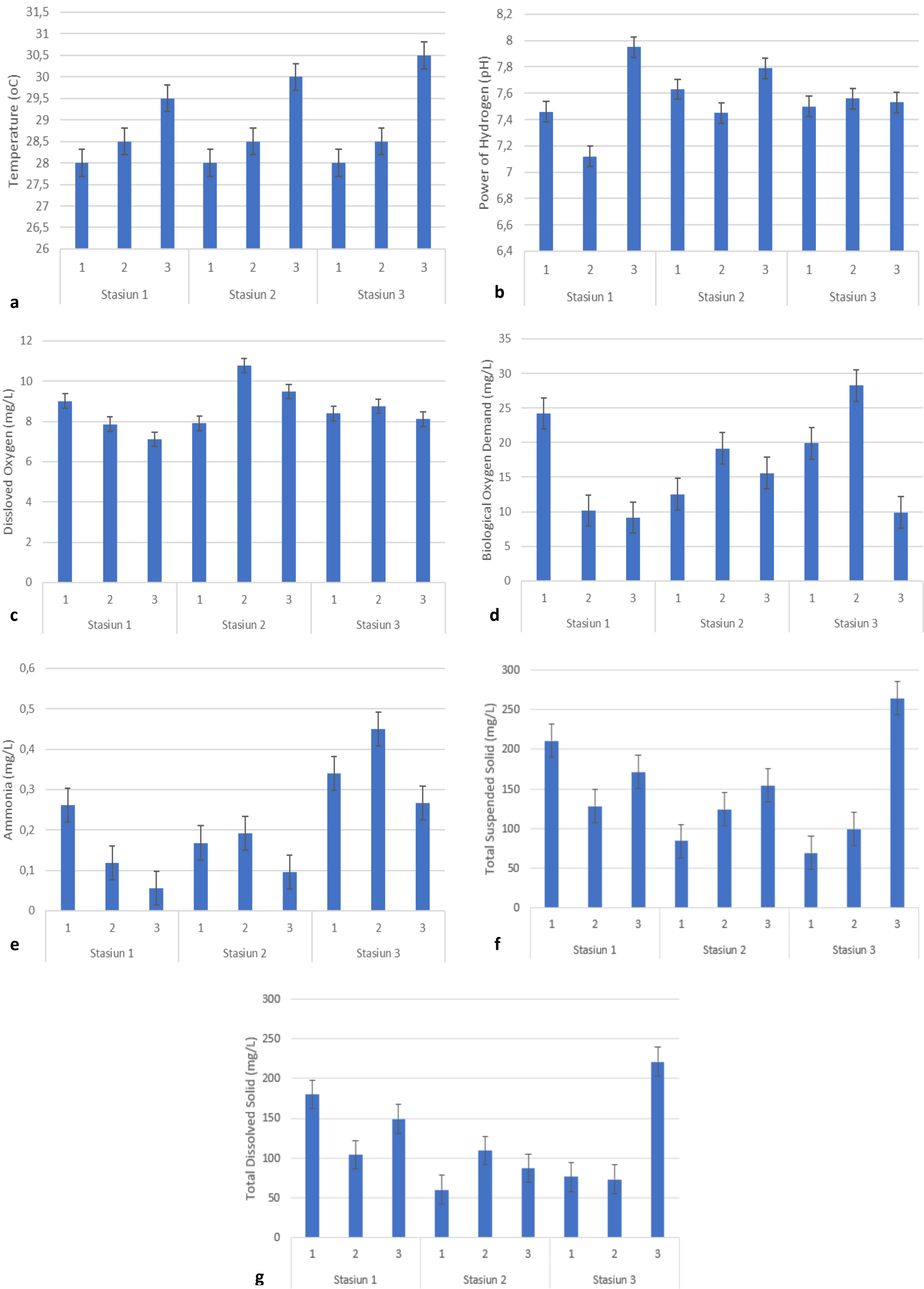

Figure 3. Water quality measurement result. a) Temperature, b) pH, c) *Dissolved Oxygen* (DO), d) *Biological Oxygen Demand* (BOD), e) *ammonia* (NH_3), f) *Total Suspended Solid* (TSS), and g) *Total Dissolved Solid* (TDS).

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The excessive concentration of Total Suspended Solid (TSS) is due to the low quality of the waters due to the presence of heavy metals that can bind to suspended sediments in the waters. It will result in a high value of TSS in the waters, thereby increasing the value of turbidity, which has an impact on inhibiting the life of aquatic biota. Penetration of light into water bodies will be reduced, thereby inhibiting the photosynthesis process thereby reducing water productivity [32]. TSS consists of mud and fine sand, and micro-organisms caused by soil erosion and soil erosion carried into water bodies [33]. In this study, the TSS values of the three stations ranged from 69-264 mg.L^{-1}. The highest TSS value indicates Station 3, which is near the disposal of industrial waste, which is 154-264 mg.L^{-1}. Allowable TSS value in waters, according to class II, is 50 mg.L^{-1} [23]. This condition illustrates that the TSS value still meets the water quality standards.

The value of the concentration of Total Dissolved Solids (TDS) in waters is a natural component of surface water whose main constituents can be organic salts, organic materials, and various other dissolved materials found in waters. The TDS value in the waters is based on the Class II quality standard of 1000 mg.L^{-1} [23]. The TDS values at the three research stations showed that the average was 60-221 mg.L^{-1} and the highest value was obtained at station 3 of 87-211 mg.L^{-1}. So it can be concluded that the TDS value is still in the optimal range in the waters. However, for station 3, the highest score was obtained due to the location adjacent to industrial waste disposal. Changes in TDS concentration in waters can be caused by industrial waste, increased rainfall, or seawater intrusion [34]. The high value of TDS can be caused by weathering of rocks and water runoff that carries soil erosion. The process of rock weathering is related to strong currents so that it can erode rocks and sediments at the bottom of the water [35].

Observation of Hematological Profile of Red Bader Fish (*Barbonymus altus*)

Hematological observations were carried out by looking at several blood profiles of the Red Bader Fish (*B. altus*), such as erythrocytes, leukocytes, hemoglobin, hematocrit, and micronuclei. Hematology is often used to detect physiological changes caused by environmental stress conditions and fish health status [22]. Erythrocytes play an important role in the transportation of oxygen throughout the body and are the largest component of blood cells and contain Hemoglobin [36]. Erythrocyte counts at the three stations obtained an average of 870.000-2.560.000 cells.mm^{-3} (Fig. 4). At each station, the erythrocyte values were 1.180.000-2.270.000 cells.mm^{-3} (Station 1), 1.670.000-2.560.000 cells.mm^{-3} (Station 2), 870.000-1.190.000 cells.mm^{-3} (Station 3). The number of erythrocyte levels in fish ranges from 1.050.000-3.000.000 cells.mm^{-3}. So the research shows that the lowest value occurs at Station 3, which can be presumed because of the input of industrial waste, while at Stations 1 and 2, the erythrocyte values are still in the normal range.

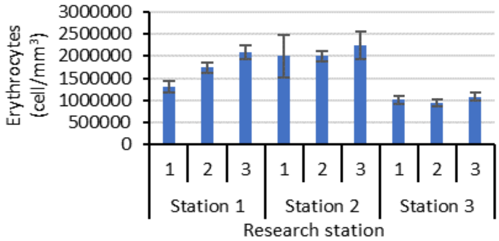

Figure 2. Erythrocyte in Bader fish hematological profile

Leukocytes are components of blood cells that act as the defense system of the fish body. The number of leukocytes in fish ranges from 20.000-150.000 cells.mm^{-3}. An increase in the number of leukocytes is called leukocytosis. The number of leukocytes is influenced by several factors, including fish species, age, nutrition, and stress [8]. The leukocyte count at the three stations yielded an average of 71.400-190.800 cells.mm^{-3} (Fig. 5). At each station, the leukocyte values were 77.400-158.400 cells.mm^{-3} (Station 1), 71.600 – 117.400 cells.mm^{-3} (Station 2), 130.000-190.800 cells.mm^{-3} (Station 3). The highest increase in the number of leukocytes occurred at Station 3, while the lowest value was at stations 1 and 2. The increase in the number of leukocytes at Station 3 may be due to industrial waste disposal. An increase in the number of leukocytes is an increase in antibody production to help fish survive and fight toxins as a form of immunological reaction [37].

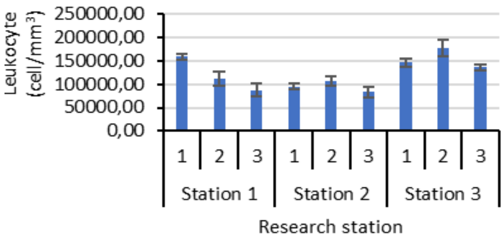

Figure 3. Leukocyte in Bader fish hematological profile

Hemoglobin (Hb) in the fish body functions to bind oxygen, so the presence of heavy metals will interfere with Hb synthesis which causes the ability to bind oxygen to be small. The lack of oxygen bound in the fish's body will affect the metabolic process [38]. The amount of hemoglobin in fish is influenced by the type of species, gender, age, physical condition, season, air pressure, and living habits [39]. Measurement of hemoglobin at the three stations obtained an average result of 3.4-8 g% (Fig. 6). At each station, the hemoglobin value was 4.8-8.6 g% (Station 1), 6.2-7.6 g% (Station 2), and 3.6-4.8 g% (Station 3). The optimal amount of hemoglobin in fish in fish is 6 - 11 g% [40]. So at station 3, the value is below the normal threshold for the amount of hemoglobin, while Stations 1 and 2 are relatively normal. Anemia can occur due to increased destruction of erythrocytes or reduced release of erythrocytes in the blood circulation. The impact of anemia is the inhibition of fish growth due to the low number of erythrocytes which results in reduced food supply to cells, tissues, and organs so that the metabolic process of fish will be hampered [41].

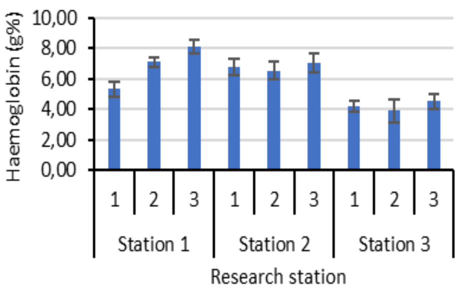

Figure 4. Hemoglobin in Bader fish hematological profile

Hematocrit is influenced by season, diet, and hormonal factors. Fish hematocrit levels are generally relatively constant between 10 - 30% [42]. Hematocrit calculations at each station obtained hematocrit values of 18 - 28% (Station 1), 21 - 30% (Station 2), and 10 - 21% (Station 3). The results of the hematocrit calculation are shown in Figure 7. The lowest hematocrit value comes from Station 3, which is thought to be due to the location close to the waste disposal site. The content of a relatively low hematocrit value describes a low erythrocyte volume which causes fish to experience anemia. On the other hand, high erythrocytes indicate homeostatic efforts in the fish body (pathogenic infection) in the body to produce more blood cells to replace infected erythrocytes, which will cause stress in fish [43].

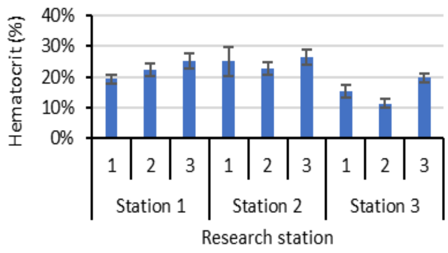

Figure 5. Hematocrit in Bader fish hematological profile

Micronuclei are extra-nuclear bodies containing chromosomal fragments and whole chromosomes that do not fuse with the nucleus after cell division [44]. Micronuclei calculations at the three stations obtained an average result of 18-64 cells.1000^{-1} (Fig. 8). At each station, the micronuclei values obtained were 17-34 cells.1000^{-1} (Station 1), 19-30 cells.1000^{-1} (Station 2), 31-64 cells.1000^{-1} (Station 3). Micronuclei observations obtained the highest results at station 3 with a range of 31-64 cells.1000^{-1}. The high levels of micronuclei at this station are caused by the location of the extraction, which is close to the industrial waste disposal site.

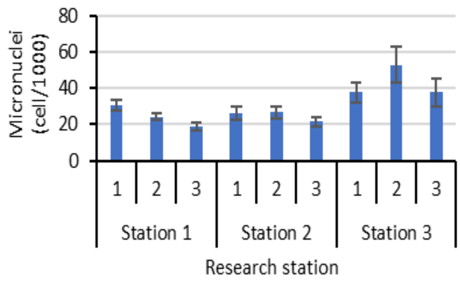

Figure 6. Micronuclei in Bader fish hematological profile

Relationship of Water Quality with Hematological Profile of Red Bader Fish (*B.altus*)

This research was conducted by analyzing data using the CCA (Canonical Correlation Analysis) method. Data analysis using the Canonical Correspondence Analysis (CCA) method is a multivariate analysis that can explain the relationship between biological communities and environmental parameters in the form of ordinances [45]. This method related to the dependent variables including erythrocytes, leukocytes, hemoglobin, hematocrit, and micronuclei with independent variables including temperature, pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), ammonia (NH3), Total Suspended Solid (TSS), and Total Dissolved Solids (TDS) (Fig. 9). The results of the data analysis showed three conclusions. First, erythrocytes, hematocrit, and hemoglobin correlated with temperature, pH, DO, BOD, ammonia, TSS, and TDS with moderate concentrations. Second, leukocytes and micronuclei have a strong association with ammonia, BOD, TSS, and TDS. Third, leukocytes and micronuclei showed a correlation with temperature and pH parameters with low concentrations. The number of erythrocytes affected by overall air quality is due to not being able to display all air quality parameters closely. Hematological parameters as a toxicity index are used in monitoring the aquatic environment [37]. Blood characteristics used in unifying fish physiological and pathological changes are effective and sensitive. Blood is an essential tool in diagnosing health with different levels of fish blood response and different stress factors [46].

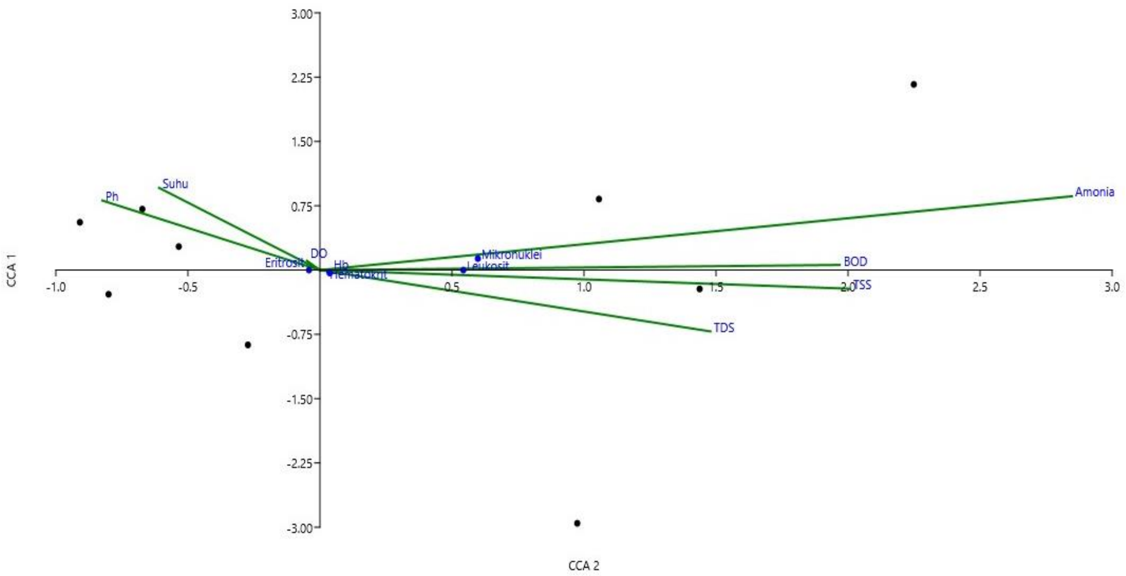

Figure 9. Result Canonical Corelation Analysis (CCA)

CONCLUSION

Based on the results of the analysis of the water quality parameters of the Jombang Brantas River, it was found that Station 1 and Station 2 were classified in normal conditions, while Station 3 was classified in relatively abnormal/polluted conditions. This condition is related to the hematology of Red Bader fish (*Barbonymus altus*), such as the values of erythrocytes, hemoglobin, and hematocrit, which are too low at Station 3, while Stations 1 and 2 have optimal values. The increase in the value of leukocytes and micronuclei was quite significant at Station 3, which was close to the location of industrial waste disposal. The results of data analysis show that the value of water quality affects the number of erythrocytes, hemoglobin, and hematocrit, which are moderate/low. Meanwhile, the values of ammonia and BOD at high concentrations were strongly correlated with leukocytes and micronuclei.

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