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# Genotoxic Effect on Hematological and Micronucleus alteration of Common Carp (*Cyprinus carpio* L.) Exposed to glyphosate-based herbicide

# Efek Genotoksik Terhadap Perubahan Hematologi dan Mikronukleus Ikan Mas (*Cyprinus carpio* L.) yang Dipapar Herbisida Berbasis Glifosat.

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## ABSTRACT

*This study aims to analyze the genotoxic potential of a glyphosate-based herbicide on common carp (Cyprinus carpio L) using Micronucleus and hematological assay. The concentration of Glyphosate-based herbicide in this study was 0 ppm, 1.35 ppm, 1.8 ppm, 2.4 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, and 8.7 ppm. Administration of herbicide based on a modification of published methods with 96 hours of exposure. This research result has shown that the average number of micronuclei was increased simultaneously with increasing the concentration of herbicide exposure. There are also other types of cell nucleus abnormalities, namely: blebbed, lobed, notched, and binuclear. In the treatment of 0 ppm of herbicide shown blebbed nuclei are 8 ‰, lobed nuclei are 6.6 ‰, notched nuclei are 10 ‰, and binuclei are 4 ‰. From the research results, it can be concluded that the $LC_{50\text{-}96}$ hours exposure of the glyphosate-based herbicide in carp (Cyprinus carpio L.) was obtained at a concentration of 8.57 ppm. Based on the evaluation of hematology, it was found that there was a decrease in the number of erythrocytes, hematocrit, and hemoglobin in fish blood, whereas the number of leukocytes, micronuclei, and other abnormal micronuclei showed an increase along with the increase in the dose of exposure to the glyphosate-based herbicide, which indicates a genotoxic effect.*

***Keywords:*** *Common carp, Genotoxic, Herbicide, Poisonous, Water quality*

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## ABSTRAK

Penelitian ini bertujuan menganalisis potensi genotoksik herbisida berbahan dasar glifosat pada ikan mas (*Cyprinus carpio* L) dengan menggunakan Micronucleus dan uji hematologi. Konsentrasi herbisida berbahan dasar glifosat pada penelitian ini adalah 0 ppm, 1,35 ppm, 1,8 ppm, 2,4 ppm, 3,2 ppm, 4,2 ppm, 6,5 ppm, dan 8,7 ppm. Pemberian herbisida berdasarkan modifikasi metode yang dipublikasikan dengan paparan 96 jam. Hasil penelitian ini menunjukkan bahwa rata-rata jumlah mikronuklei meningkat bersamaan dengan peningkatan konsentrasi pemaparan herbisida. Ada juga jenis kelainan inti sel lainnya, yaitu: blebbed, lobed, notched, dan binuclear. Pada perlakuan herbisida 0 ppm ditunjukkan nuklei yang melepuh adalah 8 ‰, nuklei bercuping adalah 6,6 ‰, nuklei berlekuk adalah 10 ‰, nukleus binuklei adalah 4 ‰. Dari hasil penelitian dapat disimpulkan bahwa paparan herbisida berbahan dasar glifosat $LC_{50\text{-}96}$ jam pada ikan mas (*Cyprinus carpio* L.) diperoleh pada konsentrasi 8,57 ppm. Berdasarkan evaluasi hematologi ditemukan adanya penurunan jumlah eritrosit, hematokrit, dan hemoglobin darah ikan, sedangkan jumlah leukosit, mikronuklei, dan mikronuklei abnormal lainnya menunjukkan peningkatan seiring dengan peningkatan dosis paparan herbisida isopropilamin glifosat, yang menunjukkan efek genotoksik.

**Kata Kunci:**, Genotoksik, Herbisida, Ikan mas, Kualitas air, Racun.

## INTRODUCTION

Farmers widely use herbicides to control weeds that are specific to plants (Solomon et al., 2013). However, the herbicide has a poisonous substance that can affect plant weeds and non-target organisms in specific concentrations (Islamy et al., 2017). On another side, the particular herbicide concentration can cause acute poisoning, even death in fish (Li et al., 2008). A study reported that methomyl pesticide causes a genotoxic effect on fish (Islamy et al., 2017; Naqvi et al., 2016; Kilawati et al., 2019).

The toxicity test is most often used to determine the toxicity of a pollutant to living things. The use of test animals for acute toxicity testing is one of the many forms of aquatic toxicology research that is useful for determining the content of toxic effluent compounds or receiving water bodies in concentrations that can cause acute toxicity (Esmiralda 2010). Generally, the measured parameter is the death of a test animal. The test results will later be expressed as a concentration that can give a 50% impact on mortality in test animals ($LC_{50\text{-}96}$) in a relatively short time of one to four days.

Generally, toxicity tests use animals with high sensitivity to environmental changes, such as common carp (*C. carpio* L.) (Syahrial et al., 2013). Fish is a common aquatic animal that provides an excellent model for monitoring pesticide toxicity in aquatic systems. They are susceptible to pollutants, can metabolize xenobiotics, and exhibit a very high bioaccumulation rate of dissolved chemicals relative to their concentration. Teleost fish are good test animals to evaluate the toxicity and effects of contaminants in animals (Modesto and Martinez 2010).

Moreover, hematology can also use as an indicator for the evaluation and assessment of cellular deviation and other genetic damage in fish caused by pesticide exposure (Islamy et al., 2017). This study aims to evaluate a lethal concentration of isopropylamine glyphosate herbicide and its impact on some hematological parameters of common carp. Genotoxicity is a chemical agent that damages the chemical and genetic info among cells inflicting mutations, resulting in cancer. Genotoxic agents will produce a deleterious effect on DNA and other cellular targets that control genetic material integrity (Lopez et al., 2012).

Moreover, fish can also be used for assessing genotoxic contaminants present in aquatic (Al-Sabti and Metcalfe 1995; Da Rocha et al., 2011; Fazio et al., 2013a; Fazio et al., 2013b). Examples of fish that can be sacrificed to assay a substance's toxicity are Cyprinidae (Valen et al 2022); and Chiclidae (Serdiati, 2022; Widodo et al., 2022). Also, phytoplankton can be used as a natural bioindicator of pesticide pollution in the environment (Soraya & Islamy, 2022). However, some fish with high adaptabilities, such as predatory fish Arapaima (Fadjar et al., 2019), Giant Snakehead (Pratama et al., 2019), and Aligator gar (Hasan et al., 2020) are not recommended to be candidates for environmental pollution bio-indicator agents because they are less sensitive to environmental changes. This study aims to analyze the genotoxic potential of a glyphosate-based herbicide on common carp (*Cyprinus carpio* L) using a micronucleus assay.

## MATERIAL AND METHODS

### Animal Preparation

Isopropylamine glyphosate herbicide was purchased from the agriculture market in Batu, East Java, Indonesia. Common carp (9 - 12 cm) were purchased from the Freshwater Laboratory of Sumberpasir, Malang, East Java. Genotoxicity tests and micronucleus assay were conducted at the Fish Cultivation Laboratory, Division of Parasites and Fish Health, Faculty of Fisheries and Marine Sciences, Brawijaya University.

The fish's acclimatization was carried out by holding them in a tank and feeding them with commercial feed once per day. After 14 day holding period, fishes were classified into 6 groups of 10 fishes, then transferred and acclimatized into the aquarium with an aeration system (size 60x30x25cm) for 2 days. If less than 3% of the fish population was dead during 48 hours, it means the Carp population treatment will be considered worthy of testing. But if over 3% of the fish population were dead, the fish should be replaced with the new fish from the holding tank and then reacclimatized for 2 days.

### Toxicity Test

Treatment Research phase 1: Prepare the aquarium (60x30x30cm) labeled for control (without treatment) and test (three replications each), then refill with fresh water. Acclimated fish are then transferred into the prepared aquarium, ten

fishes each. Dissolve Isopropylamine glyphosate herbicide that has been measured into each aquarium. The Isopropylamine glyphosate herbicide concentration in this study was 0 ppm, 1.35 ppm, 1.8 ppm, 2.4 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, and 8.7 ppm. The administration of herbicide was carried out using a modified method based on published articles (Islamy et a., 2017) with 96 hours of exposure.

*Critical Range Test*

This test was conducted to determine the upper range (N) and the bottom range (n) of the fish test pesticide. According to Guthrie and Perry's method, this section was conducted for 96 hours by observing the fish test mortality level—the used concentration of pesticide (1980).

*Definitive Test*

A definitive test was carried out to determine the pesticide concentration that causes 50% mortality of the fish population (LC50). Based on the critical range test, the pesticide concentration was 0 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, 8.7 ppm dan 10 ppm. The concentration was modified from the progressive concentration table of Bowman dan Rand (1980) with 96 hours of exposure.

**Micronucleus assay**

After 96 hours of exposure to isopropylamine glyphosate herbicide, erythrocyte blood from each fish group was sampled and smeared on clean microscope slides. After fixation in absolute methanol for about 20 min, the slides were air-dried and stained with 10% of Giemsa for about 25 minutes and then observed. Coding using a microscope (Olympus CX21) with 400x magnification to determine the frequency of micronucleus cells and another different pattern of morphologically altered erythrocytes and then counted as cells per 1000 (‰) (Güner and Muranlı 2011). The micronucleus frequency is then counted based on the published formulation below (Betancur et al., 2011).

$$\textbf{Nucleus Abnormal Cell Frequency (‰)} = \frac{\textbf{(Cell Abnormal x (1000))}}{\textbf{(Total Cell Counted)}}$$

**Hematological analysis**

*Erythrocyte*

Blood that has been given an anticoagulant is sucked with a hemocytometer pipette (there is a red grain for erythrocytes) up to 0.5 marks. The hayem solution (for erythrocytes) is sucked up to the 101 marks. For the blood to be evenly mixed, the pipette is shaken to form a figure of eight for 3-5 minutes. After that, two drops of blood are discarded to remove the air cavity. Then the blood is dripped on a hemocytometer and covered with a glass cover. The number

of erythrocyte cells is observed using a microscope on 5 small boxes on the hemocytometer. then determined the amount using the formula from an article that has been published (Kumala, 2016).

$$\textbf{Number of erythrocytes} = \Sigma\ \mathbf{N \times 10^4\ cells / mm3}$$

Where: N = number of erythrocytes counted in 5 fields of view $10^4$ = dilution factor

*Hematocrit*

The blood sample is inserted into the hematocrit capillary tube to approximately 4/5 of the tube, the test portion of the revealer is closed with a special cover, or by using a crystal, the capillaries are placed in a centrifuge (microhematocrit centrifuge). Then the microhematocrit tube was centrifuged for 5 minutes at a speed of 3000 rpm with the same volume tube facing each other so that the centrifuge rotation was balanced. After that, the percentage of the hematocrit value was measured. The hematocrit value is expressed as% the volume of blood cells (Dosim et al., 2013). Then the obtained hematocrit value is read on a special reading tool (microhematocrit reader)

*Hemoglobin*

The procedure for calculating the hemoglobin level is carried out by referring to the Sahli method. First, the sample blood is sucked using a Sahlih pipette up to a scale of 20 $mm^3$ or on a scale of 0.2 ml. Then the tip of the pipette is cleaned with tissue paper. Then, the blood in the pipette was transferred to a Hb-meter tube filled with 0.1 N HCl up to a scale of 10 (red). After that, the blood is then stirred with a stirring rod for 3 to 5 minutes. After that, distilled water was added to the tube until the blood's color became the same as the color of the standard solution in the Hb-meter. The hemoglobin level is expressed in g%.

*Leucocyte*

The procedure for calculating the number of leukocytes was measured according to Blaxhall and Daisley (1973). First, the sample blood was sucked with a pipette containing a stirring white grain up to a scale of 0.5. Then, add Turk's solution to a scale of 11. The pipette is swung in a number 8 (the same as stirring for red blood cell count) for 3-5 minutes so that the blood is evenly mixed. After that, the first two drops of the blood solution from the pipette are discarded, then the solution is dropped on a hemocytometer, after which it is closed with a closing glass. The liquid will fill the counting space capillary. The total number of white blood cells or leukocytes is calculated with a microscope's aid with a magnification of 400x. The total number of leukocytes is calculated by counting

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the cells contained in 4 small squares, and the number is calculated according to the formula:

$$\textbf{Number of Leukocyte} = \Sigma\ \mathbf{N \times 10^4\ cells / mm^3}$$

Where: N = number of erythrocytes counted in 5 fields of view $10^4$ = dilution factor

## RESULTS AND DISCUSSION

### Results

Micronuclei are one form of cell nucleus abnormalities that is used as one of the parameters that indicate the result of the entry of pollutants into the organism's body. The average micronuclei in carp (*Cyprinus carpio* L.) exposed to isopropylamine glyphosate herbicide were presented in Figure 1 and other cell nucleus abnormalities was existed in Figure 2.

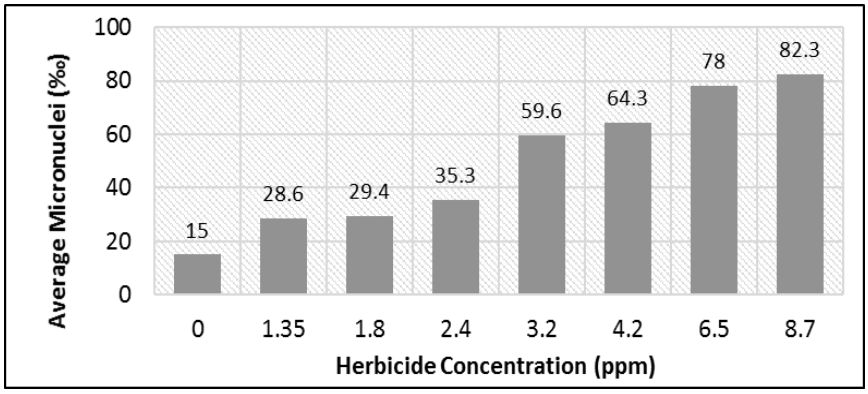


**Figure 1. The average micronuclei in common carp (*Cyprinus carpio* L.) during the research**

This research shows different results in each treatment. At the concentration of 0 ppm, frequencies of micronuclei (Figure 1) show the average number of micronuclei was 15 ‰ then increased simultaneously with increasing the concentration of herbicide exposure until 82.3 ‰ in treatment of 8.7 ppm.

In addition to micronuclei, other types of cell nucleus abnormalities, namely: blebbed, lobed, notched, and binuclear. In the treatment of 0 ppm of herbicide shown blebbed nuclei are 8 ‰, lobed nuclei are 6.6 ‰, notched nuclei are 10 ‰, and binuclei are 4 ‰. The increased exposure concentrations of herbicides simultaneously increase the number of micronuclei and other abnormal nuclei. The highest number occurs at the highest concentration of exposure, which

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is 8.7 ppm; the results show an average of blebbed nuclei of 84.2 ‰, lobed nuclei of 81.9 ‰, notched nuclei of 78.5 ‰, and binuclei of 78.4 ‰.

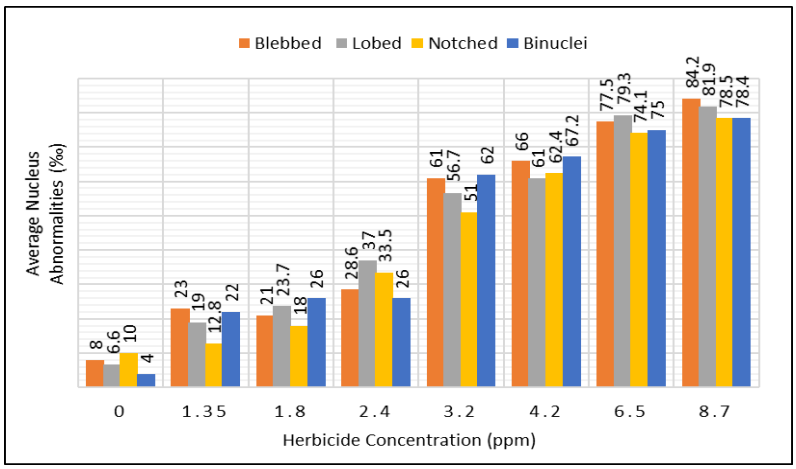


**Figure 2. The average other cell nucleus abnormalities in common carp (*Cyprinus carpio* L.) during the research**

The results of observations on the hematology include erythrocytes, leucocytes, hematocrit, and hemoglobin of goldfish, which have been carried out in this study, showing different results in each treatment. The following is a graph of the number of erythrocytes (cells / $mm^3$) (Figure 3), leukocytes (cells / mm3) (Figure 4), hematocrit (%) (Figure 5), and hemoglobin (g%) of carp (*Cyprinus carpio* L.) (Figure 6).

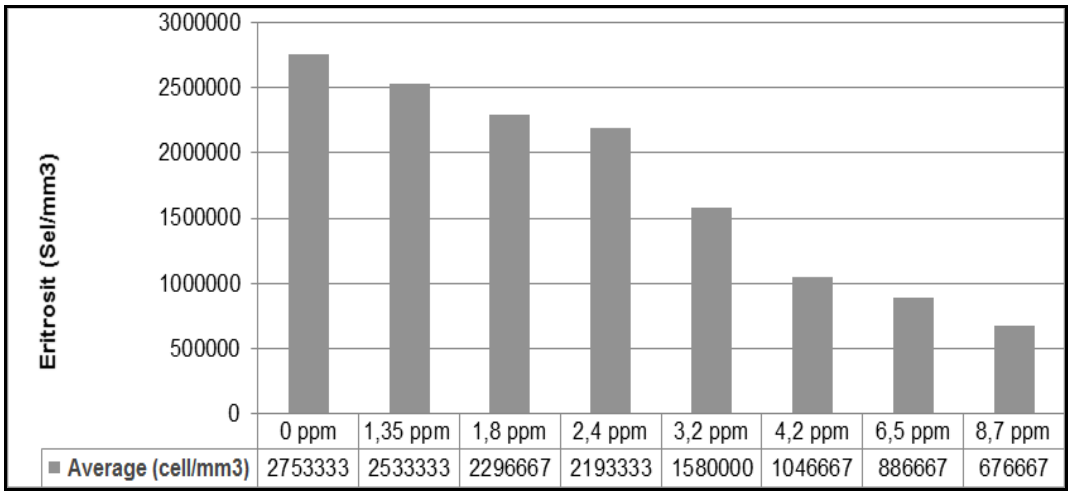


**Figure 3. The average number of carp (*Cyprinus carpio* L.) erythrocytes**

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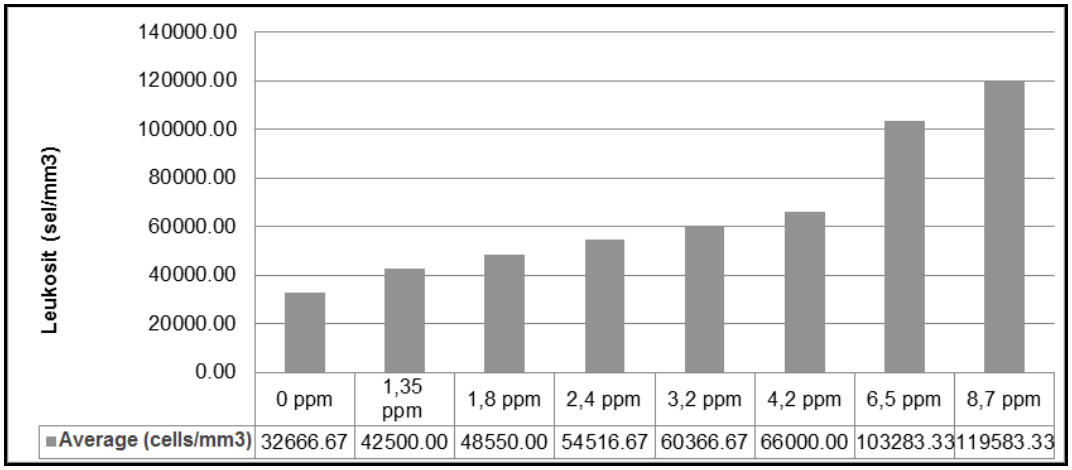


**Figure 4. The average number of carp (*Cyprinus carpio* L.) leukocytes**

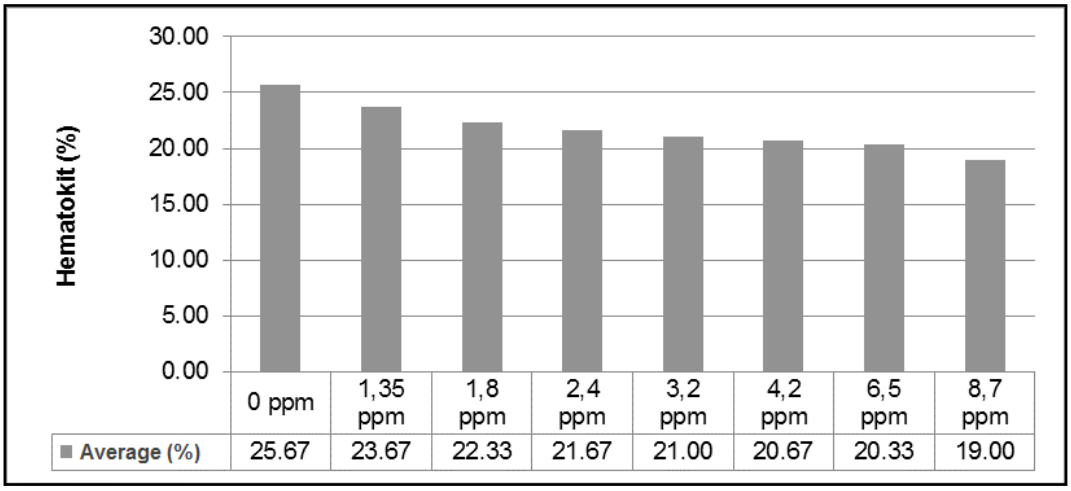


**Figure 5. The average number of carp (*Cyprinus carpio* L.) Hematocrit**

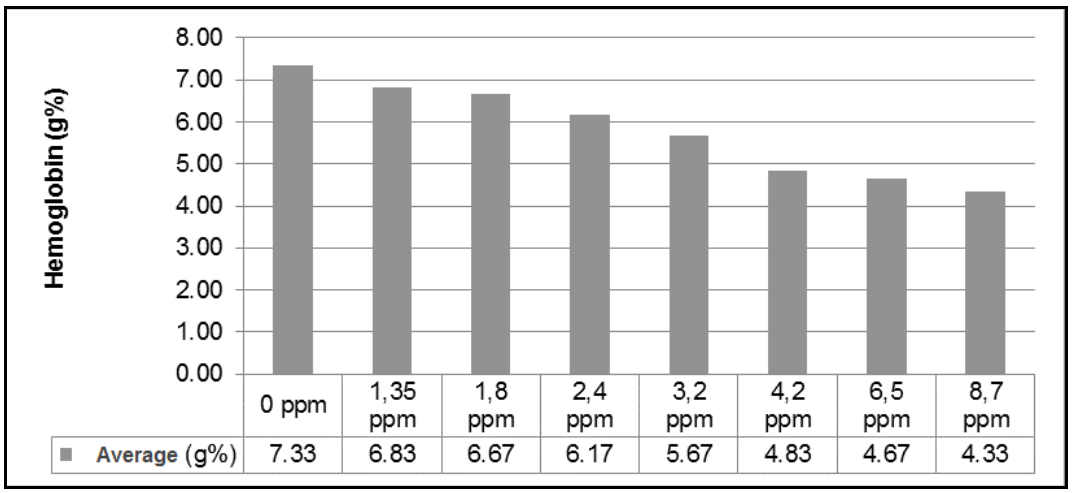


**Figure 6. The average number of carp (*Cyprinus carpio* L.) hemoglobin**

## Discussion

This study shows that Glyphosate-based herbicide exposure can cause nucleus alteration in erythrocytes cell of common carp (*C. carpio* L). It means that Glyphosate-based herbicides have genotoxic potential on common carp (*C. carpio* L) in low to a high concentrations. The appearance of nucleus alteration in fish treated with 0 ppm herbicide may cause by another factor such as water quality. However, we assume that this factor does not significantly affect the appearance of micronuclei and other nucleus alterations in fish.

According to the research data, concentrations and the exposure period of herbicide on treated fish may be the reason for relatively high micronuclei frequencies and other alterations. The present study also reports that dose and time-dependent of some pesticide exposure (Chlorpyrifos, malathion, cypermethrin, lambda-cyhalothrin, and Buctril) can increase micronuclei induction in the peripheral blood erythrocytes of fish (Naqvi et al., 2016)

Micronucleus can be interpreted as cytoplasmic chromatin, which is seen as a small nucleus. The published study explains micronuclei formation (Rangkuti et al., 2012), Micronucleus is formed from chromosome fragments isolated from the nucleus in the phase of cell division anaphase. When it reaches telophase, the centric element is transformed into a daughter cell nucleus; on the other hand, the remaining chromosome fragments will remain in the cytoplasm that forms a small nucleus. The genotoxic effect is a change in the function of gene expression that occurs due to a bond between DNA and carcinogenic substances. A genotoxic agent such as a Glyphosate-based herbicide not only produces a deleterious effect on DNA and other cellular targets (Lopes et al., 2012), but it also leads to the appearance of physiological changes in the body, such as chronic tissue damage, decreasing the immune system, hormonal changes, or binding to proteins that are repressive to specific genes (Mohammed et al., 2015).

The figure above shows a tendency for erythrocyte, hematocrit, and hemoglobin levels to show a trend of decreasing with increasing exposure dose. On the other hand, leukocyte levels increase with increasing doses of herbicide exposure. The highest average erythrocyte level was in the 0 ppm (control) treatment of 2753333 cells / $mm^3$, and the lowest was in the treatment with the highest exposure concentration of 8.7 ppm of 676667 cells / $mm^3$. Furthermore, the lowest average total number of leukocytes was at a concentration of 0 ppm of 32666.67 cells / $mm^3$. The highest concentration of exposure was 8.7 ppm, and the number of leukocytes was 119583.3 cells / $mm^3$. The highest hematocrit value was found in the treatment with a concentration of 0 ppm, namely 25.67%, while the lowest average hematocrit value was 19% at a concentration of 8.7 ppm. Furthermore, the highest average hemoglobin level was 7.33g% in the control treatment (0 ppm concentration), and the lowest was 4.33g% at a concentration of 8.7 ppm.

Changes in the hematological parameters indicate that the fish experience stress due to exposure to pesticides. This is following the opinion of Torres et al. (1986) that under stress conditions, the number of erythrocytes, hemoglobin, and hematocrit decreases, and leukocytes tend to increase. Heath (1995) adds that the increase and decrease in hematological parameters is a response to environmental stress, chemicals, or vice versa; it may be due to blood cell loss or excess water. According to Rachmawati et al. (2010), the stress response's emergence indicates an adaptation to unexpected changes and to return to homeostatic conditions. In the future, research can continue research on the mechanism of pesticide toxicity to Mitogen-Activated Protein Kinase (MAPK) (Soeprijanto & Islamy, 2023).

In Ajani and Awogbade's (2012) study, a significant decrease in the number of *Clarias gariepinus* erythrocytes occurred at 15 ppm sub-lethal diuron exposure, from $1.57 \pm 0.48 \times 10^6$ cells / $mm^3$ in control to $1.23 \pm 0.01 \times 10^6$ cells / mm3. Whereas in the research of Ramesh and Saravanan (2008), the number of carp erythrocytes decreased in exposure to acute toxicity of chlorpyrifos LC50-24 hours with a concentration of 5.28 ppm, namely from $3.24 \pm 0.046 \times 10^6$ cells / mm3 in control to $0.893 \pm 0.025 \times 10^6$ cells / $mm^3$.

According to Suhermanto et al. (2013), erythrocytes are one of the important components of fish blood cells. In erythrocytes, there is a hemoglobin substance that plays a role in binding oxygen from the environment and is carried throughout the body where it is needed. Low erythrocytes will cause fish to be unable to take in large amounts of oxygen even though the water availability is sufficient. As a result, the fish will experience a lack of oxygen (anoxia).

Total leukocytes in the blood indicate the health condition of the fish. Fish that experience stress caused by changes in environmental conditions and because of foreign bodies show a response to an increase in the number of leukocyte cells (Hastuti, 2004). Exposure to chemical pollutants and a hypoxic environment can affect the increase and decrease in hematological parameters (Heath, 1995).

Research on the number of leukocytes conducted by Ahmad (2011) stated that the total leukocyte of goldfish has decreased with each additional exposure to diazinon concentration. In contrast, in Sahetapy's (2011) research, a higher lead concentration can increase the number of tiger grouper leucocytes. El-Sayed et al. (2007) reported a decrease in the total number of tilapia leukocytes in the acute toxicity test of LC50-96 hours of deltamethrin when compared to controls. In contrast, Bojarski et al. (2015) reported that the total number of *Cirrhinus mrigala* leukocytes experienced a significant increase when compared with controls after exposure to ethofumesate 0.11 ppm for 3 days, from $13 \pm 0.7 \times 10^3$ cells / $mm^3$ in control to $36.17 \pm 5.0 \times 10^3$ cells / $mm^3$. Montanha et al. (2014), also reported that the total number of *Rhamdia quelen* leukocytes on cypermethrin 2.5 ppm exposure with an average of $134.7 \pm 21.50$ cells / µL had a significant increase when compared to 0 ppm exposure with a mean of $104.9 \pm 28.8$ cells / µL. It can be concluded that each addition of toxins to the body of fish will have a different

effect on the total number of leukocytes, and changes in the total number of leukocytes can be caused by other factors, as expressed by Yanto et al. (2015), that the number of leucocytes in fish is strongly influenced by various factors including the type and species of fish, other physiological factors such as age, muscle activity, activity, and extras period.

Research conducted by Montanha et al. (2014) reported that at 2.5 ppm lethal cypermethrin exposure, the hematocrit value of *Rhamdia quelen* experienced a significant increase when compared to 1.5 ppm and 0 ppm exposure, with an average of 35.80 ± 3.36% respectively; 31.33 ± 2.10% and 31.17 ± 4.95%, meanwhile Haider and Rauf (2014), reported that *Cirrhinus mrigala*'s hematocrit value decreased significantly with sub-lethal diazinon exposure of 1.63 ppm with a mean of 23.

## CONCLUSIONS

We concluded that the LC50-96 hour's exposure of the glyphosate-based herbicide in carp (*Cyprinus carpio* L.) was obtained at a concentration of 8.57 ppm. Based on the evaluation of hematology, it was found that there was a decrease in the number of erythrocytes, hematocrit, and hemoglobin in fish blood, whereas the number of leukocytes, micronuclei, and other abnormal micronuclei showed an increase along with the increase in the dose of exposure to the glyphosate-based herbicide, which indicates a genotoxic effect.

## SUGGESTION

Further studies are needed on the impact of other types of pesticides such as methomyl on the immune system of fish with more specific parameters.

## DAFTAR PUSTAKA

Ahmad Z. 2011. Acute toxicity and hematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure. African Journal of Biotechnology 10 (63): 13852-13859.

Ajani F, Awogbade. 2012. Hematological changes of the African catfish *Clarias gariepinus* (Burchell, 1822) juveniles induced by diuron. British Biotechnology Journal 2 (4): 247-256.

Al-Sabti K, Metcalfe CD. 1995. Fish micronuclei for assessing genotoxicity in water. Mutation Research 343: 121-135.

Betancur IP, Palacio BJA, Guerrero MC. 2009. Micronuclei test application to wild tropical ichthyic species common in two lentic environments of the low zones in Colombia. Actualidades Biológicas 31: 67-–77.

Bojarski B, Ludwikowska A, Kurek A, Pawlak K, Tombarkiewicz B, Lutnicka H. 2015. Hematological alterations in common carp (*Cyprinus carpio* L.) exposed to herbicides: pendimethalin and ethofumesate tested separately and in a mixture. Folia Biologica (Krakow) 63 (3): 167-174.

Bowman WC, Rand MJ. 1980. Textbook of pharmacology, 2nd Ed. Blackwell Scientific Publications. Oxford. 16.17-16.31.

Da Rocha CAM, da Cunha LA, Pinheiro RHS, Bahia MO, Burbano RMR. 2011. Studies of micronuclei and other nuclear abnormalities in red blood cells of Colossoma macropomum exposed to methyl mercury. Genetics and Molecular Biology 34: 694-697.

El-Sayed YS, Saad TT, El-Bahr SM. 2007. Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. Environmental Toxicology and Pharmacology 24: 212–217.

Esmiralda 2010. Uji toksisitas akut limbah cair industri biodiesel hasil biodegradasi secara aerob skala laboratorium. Teknika 1: 73-76.

Fadjar M, Islamy RA, Herawati EY. 2019. Short communication: First record of Arapaima gigas (Schinz, 1822) (Teleostei: Osteoglossomorpha), in the Brantas River, Sidoarjo, East Java, Indonesia. Biodiversitas Journal of Biological Diversity 20(12). doi:10.13057/biodiv/d201209

Fazio F, Faggio C, Marafioti S, Torre A, Sanfilippo M, Piccione G. 2013. Effect of water quality on hematological and biochemical parameters of Gobius niger caught in Faro lake (Sicily). Iranian Journal of Fisheries Sciences 12: 219-231.

Fazio F, Marafioti S, Torre A, anfilippo M, Panzera M, Faggio C. 2013. Haematological and serum protein profiles of Mugil cephalus: effect of two different habitats. Ichthyological Research 60: 36-42.

Güner U, Muranlı FD. 2011. Micronucleus test, nuclear abnormalities and accumu-lation of Cu and Cd on Gambusia affinis (Baird and Girard, 1853). Turkish Journal of Fisheries and Aquatic Sciences 11: 615-622.

Guthrie FE, Perry JJ. 1980. Introduction to environmental toxicology. Elsevier. New York.

Haider MJ, Rauf A. 2014. Sub-lethal Effects of Diazinon on Hematological Indices and Blood Biochemical Parameters in Indian Carp, Cirrhinus mrigala (Hamilton). Brazilian Archives of Biology and Technology 57(6): 947–953. https://doi.org/10.1590/s1516-8913201402086

Hasan V, Widodo MS, Islamy RA, Pebriani DAA. 2020. New records of alligator gar, Atractosteus spatula (Actinopterygii: Lepisosteiformes: Lepisosteidae) from Bali and Java, Indonesia. Acta Ichthyologica et Piscatoria 50 (2): 233-236.

Hastuti S. 2004. Respons fisiologis ikan gurami (*Osphronemus gouramy*,Lac.) yang diberi pakan mengandung kromium-ragi terhadap perubahan suhu lngkungan. *Disertasi*. Institut pertaian Bogor. Bogor.

Heath AG. 1995. Water Pollution and Fish Physiology. CRC Press. USA

Islamy RA, Yanuhar U, Hertika AMS. 2017. Assessing the Genotoxic Potentials of Methomyl-based Pesticide in Tilapia (*Oreochromis niloticus*) Using Micronucleus Assay. The Journal of Experimental Life Sciences 7(2): 88–93. doi: 10.21776/ub.jels.2017.007.02.05

Kilawati Y, Islamy RA. 2019. The Antigenotoxic Activity of Brown Seaweed (Sargassum sp.) Extract Against Total Erythrocyte and Micronuclei of Tilapia Oreochromis niloticus Exposed by Methomyl-Base Pesticide. The Journal of Experimental Life Sciences 9(3): 205–210. doi: 10.21776/ub.jels.2019.009.03.11

Li H, Jiang H, Gao X. Wang, Qu WR, Lin J, Chen. 2008. Acute toxicity of the pesticide methomyl on the Topmouth Gudgeon (Pseudorasbora parva): mortality and effects on four biomarkers. Fish Physiol. Biochem 34(3): 209-216.

Lopes G, Sousa C, Silva LR, Pinto E, Andrade PB, Bernardo J, Mouga T, Valentão P. 2012. Can Phlorotannins Purified Extracts Constitute a Novel Pharmacological Alternative for Microbial Infections with Associated Inflammatory Conditions? PLoS ONE 7(2). doi: 10.1371/journal.pone.0031145

López SL, Aiassa D, Benítez-Leite S, Lajmanovich R, Mañas F, Poletta G, Sánchez N, Simoniello MF, Carrasco AE. 2012. Chapter Two - Pesticides Used in South American GMO-Based Agriculture: A Review of Their Effects on Humans and Animal Models. Advances in Molecular Toxicology. 6: 41-75.

Modesto KA, Martinez CB. 2010. Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 81(6): 781-787. doi:10.1016/j.chemosphere.2010.07.005

Mohammed KP, Aarey A, Tamkeen S, Jahan P. 2015. Mutation Research/genetic toxicology and environmental mutagenesis. Mutat. Res. 777. 29-32.

Montanha FP., Fredianelli AC, Wagner R, Sacco SR, Rocha DCC, Pimpao CT. 2014. Clinical, biochemical and haemathological effects in *Rhamdia quelen* exposed to cypermethrin. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 66 (3): 697-704.

Naqvi GEZ, Shoaib N, Aisha MA. 2016. Genotoxic Potential of Pesticides in the Peripheral Blood Erythrocytes of Fish (*Oreochromis mossambicus*). Pakistan Journal of Zoology 48(6): 1643-1648.

Pratama WW, Nursyam H, Hariati AM, Islamy RA, Hasan V. 2020. Short Communication: Proximate analysis, amino acid profile and albumin concentration of various weights of Giant Snakehead (Channa micropeltes)

from Kapuas Hulu, West Kalimantan, Indonesia. Biodiversitas Journal of Biological Diversity 21(3). doi:10.13057/biodiv/d210346

Rachmawati FN, Susilo U, Sistina Y. 2010. Respon fisiologi ikan nila, Oreochromis niloticus, yang distimulasi dengan daur pemuasaan dan pemberian pakan kembali. National Seminat of Biology. Yogyakarta.

Ramesh M, Saravanan M. 2008. Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. International Journal of Integrative Biology 3 (1): 80-83

Rangkuti RH, Suwarso E, Hsb AZ. 2012. Pengaruh Pemberian Monosodium Glutamat (MSG) Pada Pembentukan Mikronukleus Sel Darah Merah Mencit the Effect of Monosodium Glutamate (MSG) In Mice Red Blood Cell Micronucleus Formation. Journal of Pharmaceutics and Pharmacology 1: 29–36

Sahetapy JM. 2011. Toksisitas logam berat timbal (Pb) dan pengaruhnya pada konsumsi oksigen dan respon hematologi juvenil ikan kerapu macan. Jurnal Manajemen Sumberdaya Perairan 7(2): 42– 48.

Serdiati, N., Safir, M., & Islamy, R. A. New Record of the Non-native species of Mayan Cichlid (Cichlasoma urophthalmus Günther, 1867) in Klawing River, Central Java, Indonesia.

Soeprijanto, A., & Islamy, R. A. (2023). The Mitogen-Activated Protein Kinase (MAPKs) Expression and Primary Stress Hormone (Cortisol) of Clarias sp. Juvenile in Different Light Intensity. *Journal of Aquaculture and Fish Health*, *12*(1), 49–56. https://doi.org/10.20473/jafh.v12i1.35054

Solomon KR, Dalhoff K, Volz D, Kraak GV. 2013. Effects of Herbicides on Fish. Fish Physiology Organic Chemical Toxicology of Fishes, 369-409. doi:10.1016/b978-0-12-398254-4.00007-8

Soraya, I., & Islamy, R. A. (2022). Analisis Keanekaragaman Perifiton Di Anak Sungai Brantas, Malang, Jawa Timur, Indonesia. *JFMR (Journal of Fisheries and Marine Research)*, *6*(1), 152-162.

Suhermanto A, Andayani S, Maftuch. 2011. Pemberian total fenol teripang pasir (Holothuria scabra) untuk meningkatkan leukosit dan diferensial leukosit ikan mas (Cyprius carpio) yang diinfeksi bakteri Aeromonas hydrophila. Jurnal Kelautan 4 (2): 49-56.

Syahrial A, Setyawati TR, Khotimah S. 2013. Tingkat kerusakan jaringan darah ikan mas (*Cyprinus carpio* ) yang dipaparkan pada media Zn-Sulfat (ZnSO4). Protobiont 2: 181-185.

Torres P, Planas LTJ, Flos R. 1986. Effects of confinement stress and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula.* Comparative Biochemistry and Physiology 83 (1): 89-92.

Valen, F. S., Widodo, M. S., Islamy, R. A., Wicaksono, K. P., Insani, L., & Hasan, V. (2022, July). Molecular phylogenetic of silver barb barbonymus gonionotus (bleeker, 1849)(cypriniformes: cyprinidae) in Java, Indonesia.

In *IOP Conference Series: Earth and Environmental Science* (Vol. 1036, No. 1, p. 012011). IOP Publishing.

Widodo, M. S., Hasanz, V., Faqih, A. R., Islamy, R. A., & Ottoni, F. P. (2022). THE RANGE EXPANSION OF Parachromis managuensis GUNTHER, 1867 (PERCIFORMES, CICHLIDAE) IN JAVA, INDONESIA. *Biotropia*, *29*(1), 7-11.

Yanto H, Hasan H, Sunarto. 2015. Studi hematologi untuk diagnose penyakit ikan secara dini di sentra produksi budidaya ikan air tawar sungai Kapuas kota Pontianak. Jurnal Akuatika 6 (1): 11-20