

Hematological Response of Tilapia (*Oreochromis niloticus*) in Laundry Wastewater

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ABSTRACT

The high concentration of detergent in the aquatic ecosystem potentially affects the fish's physiological condition by disrupting the respiration process and changing the concentration of blood components and chemistry. This study aimed to determine the condition of the hematological parameters of tilapia (*Oreochromis niloticus*) exposed to wastewater from the laundry industry. Each treatment was stocked with five fish per aquarium (50x30x30 cm). This study used a completely randomized design (CRD) technique with treatments include P0 (0%) as a control, P1 (1%), P2 (2%), P3 (3%), P4 (4%), and P5 (5%) with each treatment exposed to a specific concentration of wastewater and residues. The results showed that the hemoglobin levels of treatments decreased, with the lowest mean of hemoglobin level found in the P2 (7.05 gr%), and the lowest concentration on the 30th day was 7.71 gr%. There were no significant effects of wastewater on erythrocytes and leucocytes number among treatments ($P > 0.05$). While there were increasing hematocrit levels, the largest mean level was found in the P4 treatment with a value of 24.11 gr%, and the largest mean on the 20th day of observation showed a value of 23.51 gr%. Wastewater from the laundry industry can affect tilapia's hematological condition by decreasing the hemoglobin concentration and increasing the hematocrit levels above the normal condition.

Keywords: detergent; fish; hematology; laundry industry, wastewater

INTRODUCTION

Detergent is a type of water-soluble surfactant used to remove impurities from laundry in the household and laundry industries. The laundry industry's work process is simple and straightforward: to dissolve detergents in water because detergents have a better hardness than soap (Yuliani *et al.*, 2015). Therefore, this industry will produce wastewater that contains detergents, which are discharged directly to the nearest aquatic environment. (Ardiyanto & Yuantari, 2016; Yusmidiarti, 2016).

Detergent addition from the laundry industry wastewater cause water quality reduction (Sumisha *et al.*, 2015; Uzma *et al.*, 2018). The negative effect of detergents deteriorates water quality due to their different chemical components (Giagnorio *et al.*, 2017; Goel & Kaur, 2020), led toxicity and genotoxic effects on aquatic life (Adewoye, 2010; Sobrino-Figueroa, 2013), and tend to be the

most resilient to biodegradation (Hidaka *et al.*, 2010; Verdia *et al.*, 2016). Surfactant is one of the main ingredients of the detergent, causing foam in the water and creating a layer that inhibits the process of transferring oxygen from the air to water (Sugito *et al.*, 2014; Srinet *et al.*, 2017).

Fishes as bioindicator play a role in monitoring the effect of heavy metals contamination (Authman, 2015; Łuczyńska *et al.*, 2018). Tilapia (*Oreochromis* sp.) used as biomarker to assess water pollution (Osman, 2012), bioaccumulation (Abdel-Baki *et al.*, 2011; Eneji *et al.*, 2011), and measure the risk of pollution to humans (Adel *et al.*, 2016).

The accumulation of detergent from the laundry industry will cause a low supply of dissolved oxygen (DO) in the water. This condition will disrupt air-breathing fish (Lee *et al.*, 2012; Franklin, 2014), reduce the energy as DO declined (Tran-Duy *et al.*, 2012), and cause death for a longer period of time (Hobbs &

McDonald, 2010). Death can occur due to physiological deviations of blood components. Changes in blood components and blood chemistry, both qualitatively and quantitatively, can affect the fish's condition. Therefore, hematological conditions can be used as indicators to detect and determine a fish's health status (Sabilu, 2010).

Only a few research on the laundry industry's wastewater and residues affect *Oreochromis niloticus* hematological condition, so further studies are necessary. This research contribution is expected to provide effective strategies for controlling laundry wastewater's negative impact on the aquatic life and environment, mainly to fish.

MATERIALS AND METHODS

Tilapia were obtained from the Undulako Fish Seed Center, Kolaka Regency, Southeast Sulawesi. This research was conducted by using a completely randomized design (CRD) method consisting of six treatments and three repetitions: P0 (0%), P1 (1%), P2 (2%), P3 (3%), P4 (4%) and P5 (5%) with each treatment exposed to a specific concentration of wastewater and residues from laundry industry.

Acclimatization and culture. Four months old tilapia (5 ± 0.3 - 7 ± 0.2 g, 7 ± 0.4 - 9 ± 0.1 cm) were acclimatized in two larger aquariums (100x80x80 cm) before stocked in the experimental aquarium (50x30x30 cm). After acclimatization, tilapia were weighed and measured. Tilapia were stocked to the 18 experimental aquariums with a stocking density of five fish. Continuous aeration was performed homogenously to maintain a stable oxygen concentration in each tank (Sibirian *et al.*, 2019). A total of 5% of water volume was siphoned and exchanged daily to remove the uneaten feed and the fish feces. Tilapia culture was conducted for 30 days. The food used is a commercial tilapia feed with a protein content of 40% (Sibirian *et al.*, 2019). Tilapia was fed on a limited basis, twice a day, at 08.00 and 17.00 WITA. The feed is distributed evenly and is given up to 5% of tilapia weight per day.

Parameters. The parameters observed in this study include water quality, surfactant analysis of wastewater from the laundry

industry, and hematological parameters. The water quality parameters consist of dissolved oxygen, temperature, and pH, measured every day during the study. The anionic surfactant test consists of a test tube filled with 10 ml of methylene blue solution, 5 ml of chloroform was added, and then 1% of the detergent solution was added and stirred, resulting in a color change. The cationic surfactant test included a test tube with 10 ml of 0.002% blue bromine phenol solution in Na acetate buffer pH 3.6-3.9, adding 1% of the detergent. It was stirred until distributed uniformly, and the color was observed. The blood was sampled through the caudal vein near the tail between tilapia scales. Blood samples are slowly suctioned up to 2 ml each tail, then transferred to a 4 ml vacuum tube that has been moistened with anticoagulation. Blood samples were taken on the first day as a control, then on the 10th, 20th, and 30th days. Hemoglobin concentration was measured by using the Sahli method. Total erythrocyte and total leukocyte were counted by using an improved Neubauer hemocytometer. The hematocrit measurement was conducted using microhematocrit tubes and then centrifuged at 1500 rpm.

Data analysis. The data obtained from the hematological observations of tilapia were analyzed using ANOVA and followed by the Duncan test using IBM SPSS version 23. Differences were considered as being significant at $p < 0.05$.

RESULT AND DISCUSSION

Water quality analysis. According to Table 1, it is shown that water quality parameters of control P0 have not changed from the beginning to the end of the experiment. There were some differences in water quality data between various concentration treatments. In general, DO values of treatments decreased during the 30 days of the study. In control P0, the DO value was quite stable from the beginning to the end of the study. In the treatment of P1, the DO value decreased, starting from day 10 to day 30. The pH value in P1 treatment has increased from day 10 to day 30. The other treatments of P2, P3, P4, and P5 showed a similar trend by decreasing the DO

concentrations and increasing the pH. In contrast, the temperature of all treatments during the investigation tends to be stable.

Table 1. Results of the analysis and measurement of water analysis

Concentration	Day 0			Day 10			Day 20			Day 30		
	DO (mg/L)	Temp. (°C)	pH	DO (mg/L)	Temp. (°C)	pH	DO (mg/L)	Temp. (°C)	pH	DO (mg/L)	Temp. (°C)	pH
0%	6.6	26.7	7.1	6.6	26.3	7.1	6.6	26.8	7.7	6.5	25.3	7.2
1%	6.7	26.6	7.2	6.4	26.5	7.4	5.2	26.5	7.6	4.3	23.4	7.8
2%	6.9	26.4	7.2	5.8	26.3	7.5	4	27.1	8	3.8	23.6	7.6
3%	6.8	26.6	7.1	6.1	26.5	7.5	4.2	26.5	7.6	3.6	24.8	8.3
4%	6.7	26.5	7.1	6.2	26.4	7.8	4.4	25.6	8.3	3.9	24.5	8.5
5%	6.6	26.2	7.1	5.8	25.7	7.4	4.8	26.2	8.2	3.7	23.1	8.3
Average	6.7	26.5	7.1	6.2	26.3	7.5	4.9	26.5	7.9	4.3	24.1	8.0

This study indicates that wastewater treatment from the laundry industry reduces the water quality by lowering the DO value. The higher the concentration of treatment correlates to the lower DO value. Furthermore, the longer the duration of the study, the lower the DO value. These water quality parameters support the primary data on the hematological parameter condition of tilapia. These data were obtained from DO, pH, and temperature measurements during the study from the beginning to the end. According to table 1, it is shown that there is a decrease in DO in higher concentration. This is due to the effect of the laundry industry's wastewater that causes a reduction in DO transfer, resulting in a decrease in the DO. The laundry industry wastewater contains detergents that accumulate the surfactants in surface and ground water (Ghose *et al.*, 2009; Meffe & de Bustamante, 2014), causing problems in the sedimentation of water (Rebello *et al.*, 2014; Hassan *et al.*, 2017), and reducing the system free energy at higher concentration (Ivanković & Hrenović, 2010; Gao & Sharma; 2013). Besides, the phosphate from detergents in the upper part of the river can also stimulate aquatic macrophytes and float weeds growth (Rajan, 2015; Ramachandra *et al.*, 2017). The abundant of aquatic plants will increase phosphorus decomposition, affect aeration and water quality (Rajan, 2015), and deficiency of DO levels (Patty *et al.*, 2015). This adversely affects the physiological, biochemical, and ionregulatory responses of fish (Velisek *et al.*, 201; Rajan, 2015). Water temperature during the study fluctuated during

the study, except in control P0 at a concentration of 0%. An increase in water temperature also causes a reduction in DO levels in the water. The optimal temperature for tilapia growth is range 22-30°C (Zuhrawati, 2014; Nivelles *et al.*, 2019). pH values of water at various treatments increase with the high detergent concentration due to bases chemical of detergent.

Surfactant analysis of wastewater from laundry industry. The anionic surfactant test showed a solid blue color in the chloroform layer, and the cationic surfactant test showed a natural blue color. This is consistent with Utomo *et al.* (2018), if the high anionic surfactant content will show blue indicator in the chloroform phase. Detergent solution in the first press laundry industry used in this study contained concentrated surfactants.

Hemoglobin Concentration. The results of the study show a decrease in hemoglobin levels in all treatments, except control P0, up to the 30th day of measurements. The treatments that have been exposed to the wastewater from the laundry industry have varied responses to the level of hemoglobin in each treatment. The lowest hemoglobin levels were found in treatments P2, P3, P5, P1, and P4 with an average of hemoglobin levels, respectively 7.05 gr%, 7.06 gr%, 8.79 gr%, 8.98 gr%, and 9.32 gr%, respectively (Figure 1). The P0 had quite a constant hemoglobin level (10.45 gr%) since beginning to the end of the experiment. Statistical analysis showed that hemoglobin levels of P2 was not significantly different from P3 ($P > 0.05$) but was significantly different to

the three other concentrations (P1, P3, P5) and control treatments ($P < 0.05$). The hemoglobin level of test fish in P5 was not significantly different from P1 and P4 ($P > 0.05$) but was significantly different from P2, P3 and control treatments. The P0 was significantly different from the other five treatments. This fact shows

that waste from the laundry industry increases the accumulation of surfactants on the surface water that inhibits the transfer of oxygen to the fish, thus reducing the level of hemoglobin in tilapia blood under normal conditions (Saparuddin & Arbain, 2019).

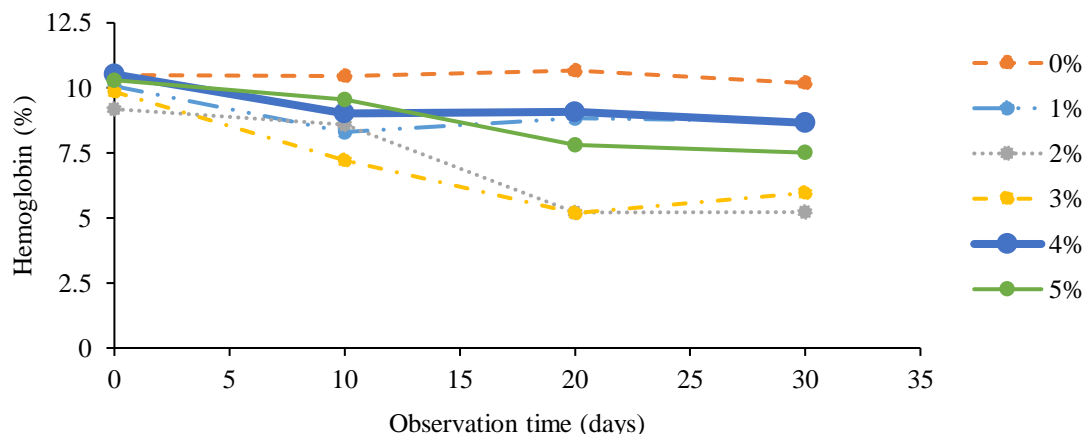


Figure 1. The average level of tilapia hemoglobin in P0 (0%), P1 (1%), P2 (2%), P3 (3%), P4 (4%) and P5 (5%)

The lowest hemoglobin level is in days 30, 20, 10, and 0, with the following percentage of 7.71 gr%, 7.80 gr%, 8.86 gr%, and 10.07 gr% (Figure 2). The hemoglobin level on day 30 did not differ with the hemoglobin level on day 20, but was different from the hemoglobin level on day 10 and 0 ($p < 0.05$). The hemoglobin level on day 20 was significantly different from the hemoglobin level on day 10 and day 0. Similarly, the hemoglobin level on day 10 was significantly different from the apparent rate of

hemoglobin on day 0 ($p < 0.05$). While the level of hemoglobin on day 0 varies in real with all observed days. This indicates that time exposure to the laundry wastewater affects the level of hemoglobin tilapia. Low hemoglobin levels associated with the low active fishes (Satheeshkumar *et al.*, 2012), affect oxygen carrying capacity of the blood (Atkins & Benfey, 2008), lower metabolic rate, and lower energy demand (Chapman *et al.*, 2002).

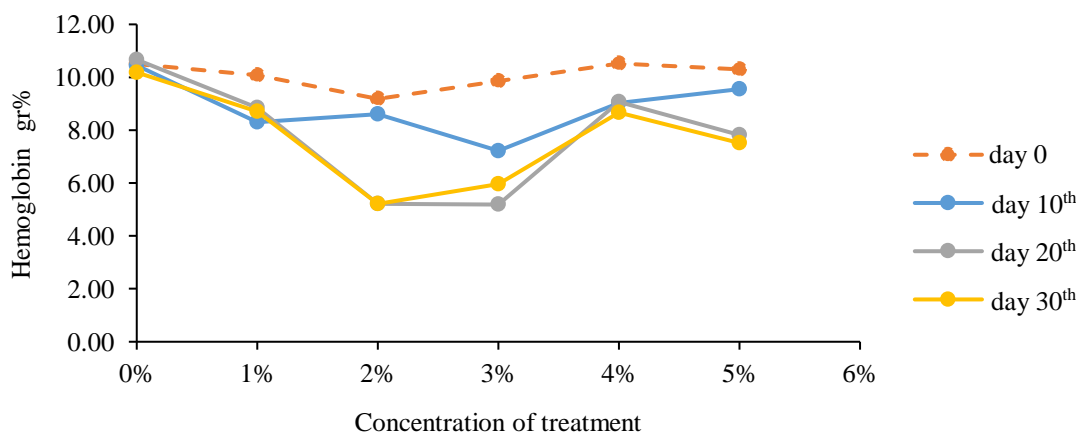


Figure 2. The average of tilapia hemoglobin levels under observation day 0, day 10th, day 20th and day 30th

Number of Erythrocytes. Figure 3 shows that the amount of tilapia's erythrocytes is not subjected to significant quantity changes with

detergent from the laundry industry. The highest erythrocytes number can be found in P1 (1.64×10^6 cells/mm³), followed by P4 ($1.64 \times$

10^6 cells/mm³), P3 (1.53×10^6 cells/mm³), P5 (1.52×10^6 cells/mm³), P2 (1.51×10^6 cells/mm³), and the smallest can be found in control P0 (1.47×10^6 cells/mm³).

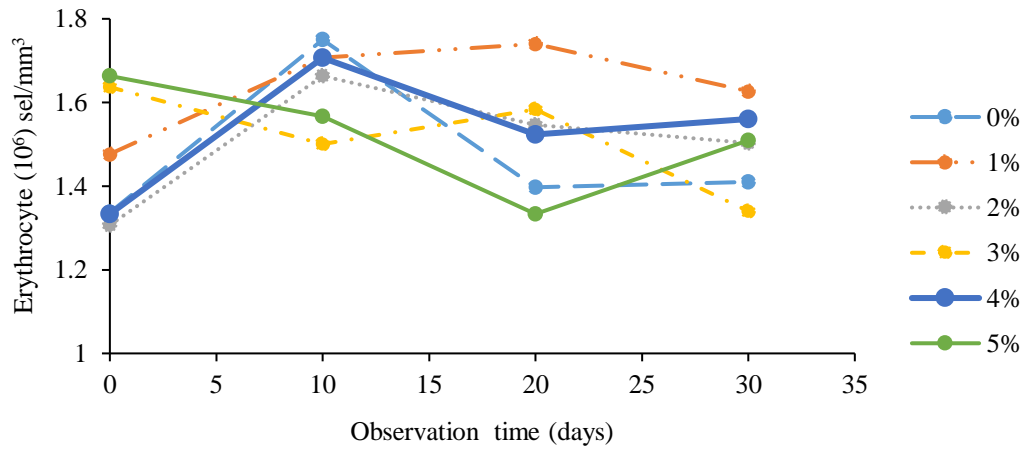


Figure 3. The average number of tilapia erythrocyte in the treatment P0 (0%), P1 (1%), P2 (2%), P3 (3%), P4 (4%) and P5 (5%)

The erythrocytes number in tilapia among the treatment were not different ($p > 0.05$). The total amount of erythrocytes was not affected by any particular concentration of wastewater from the laundry industry. All treatments had average levels of erythrocytes' abundance. The erythrocytes concentration in studied fish were within the range $0.47-1.78 \times 10^6/\text{mm}^3$ described by Maftuch (2018), but was lower than those $1.13-1.31 \times 10^6/\text{mm}^3$ reported by Ismain & Mahboub (2016). The erythrocytes number still within range of health tilapia indicates the hematopoiesis process is still happening in tilapia even though it has been exposed to the laundry's wastewater industry.

Figure 4 shows the erythrocytes number at the lowest hemoglobin level at day 0, 30th, 20th, and 10th with a consecutive number of 1.46×10^6 cells/mm³, 1.49×10^6 cells/mm³, 1.52×10^6 cells/mm³, and 1.65×10^6 cells/mm³. The total number of tilapia erythrocytes linked with the wastewater from the laundry industry on day 0 did not actually differ with the erythrocytes number on the day 30th and the 20th. However, it is different from the day 10th. The erythrocytes number on day 30th was not significantly different from day 20th and day 10th. It shows that the wastewater from the laundry industry did not affect the number of tilapia erythrocytes.

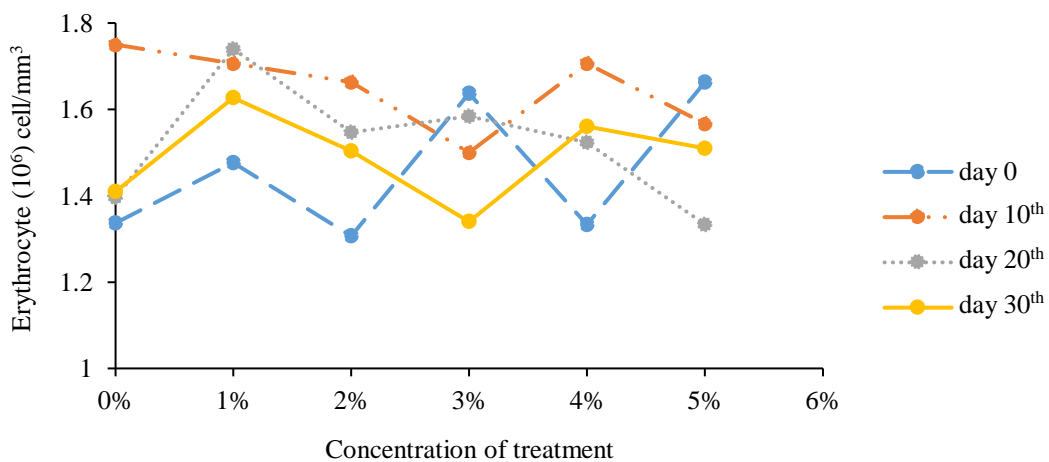


Figure 4. The average number of tilapia erythrocyte under observation on day 0, day 10th, day 20th, and day 30th

Number of Leukocytes. Figure 5 shows that the tilapia leukocytes number is aligned

with the waste detergent laundry and won't undergo significant quantity changes. The

smallest leukocytes can be found in P2 (10.25×10^4 cells/mm³) followed P3 (11.00×10^4 cells/mm³), P4 (11.28×10^4 cells/mm³), P5

(11.39×10^4 cells/mm³), while the highest leukocytes can be found in P0 (11.39×10^4 cells/mm³) and P1 (11.39×10^4 cells/mm³).

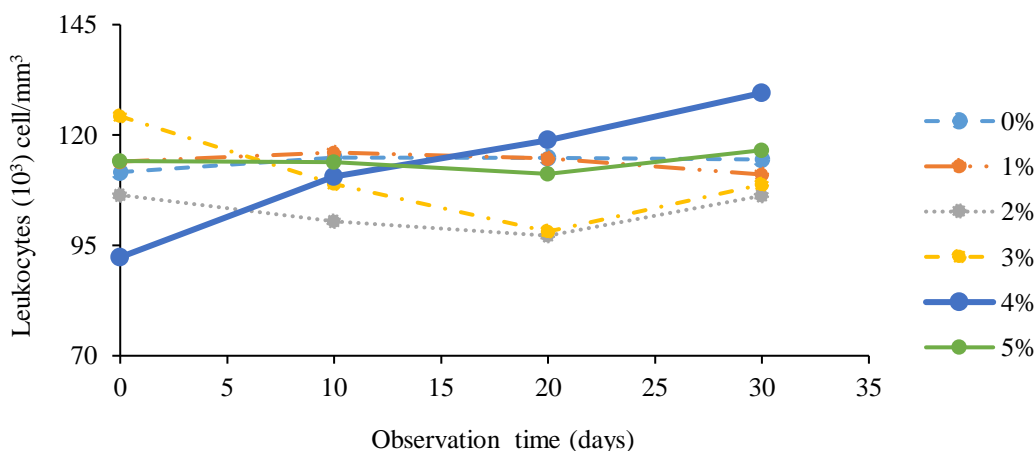


Figure 5. The average number of tilapia leukocytes in the treatment P0 (0%), P1 (1%), P2 (2%), P3 (3%), P4 (4%), and P5 (5%)

The lowest leukocytes number are found on day 20th, day 0, day 10th, and day 30th with 10.91×10^4 cells/mm³, 11.04×10^4 cells/mm³, 11.07×10^4 cells/mm³, and 11.44×10^4 cells/mm³, respectively. The number of tilapia leukocytes lined with the waste laundry detergent from day 0 to day 30th has no real difference. The leukocytes number in this study indicates that the hematopoiesis process continues to occur in tilapia even though it has been exposed to the waste detergent laundry. Several factors affect the number of leukocytes in fish, consisting of species (Sadauskas-

Henrique *et al.*, 2011; Seriani *et al.*, 2013; Ribas *et al.*, 2016), sex steroid (Milla *et al.*, 2011; Krams *et al.*, 2013; Chaves-Pozo *et al.*, 2018), and lymphoid organs activity (Tort, 2011; Scapigliati, 2013). Leukocytes will decrease if the fish is in a response to stress, such as heat stress (Davis *et al.*, 2008; Zafalon-Silva *et al.*, 2017), while elevated leukocytes number due to the immune response of stress syndrome, inflammatory processes, and oxidative stress (Lazado *et al.*, 2010; Tort, 2011; Nardocci *et al.*, 2014).

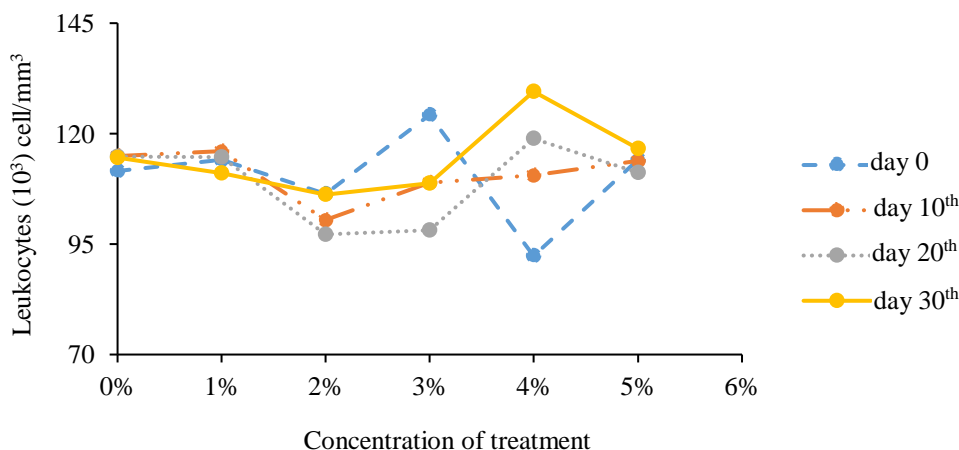


Figure 6. The average number of tilapia leukocytes under observation on day 0, day 10th, day 20th, and day 30th

Hematocrit Level. The results showed an increase in hematocrit levels across exposure to the laundry detergent treatment up to the day 30th. The lowest hematocrit level can be found

in P0 (21.77%). The highest hematocrit level can be found in P4 (24.11%), followed by P5 23.64%, P2 (22.83%), P1 (21.77%)(Figure 7).

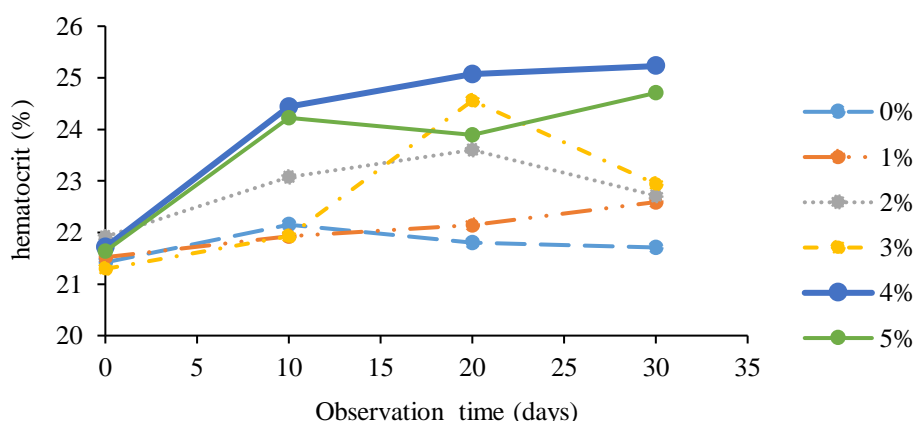


Figure 7. The average hematocrit value of tilapia in P0 (0%), P1 (1%), P2 (2%), P3 (3%), P4 (4%) and P5 (5%)

Figure 8 shows that the lowest hematocrit concentration on day 0, day 10, day 20, and day 30 are 21.58%, 22.96%, 23.31%, and 23.51%, respectively. The statistical analyses showed that tilapia hematocrit levels are aligned with

the concentration of wastewater from the laundry industry from day 0 until day 30th, and there were no significant differences among treatments ($p > 0.05$).

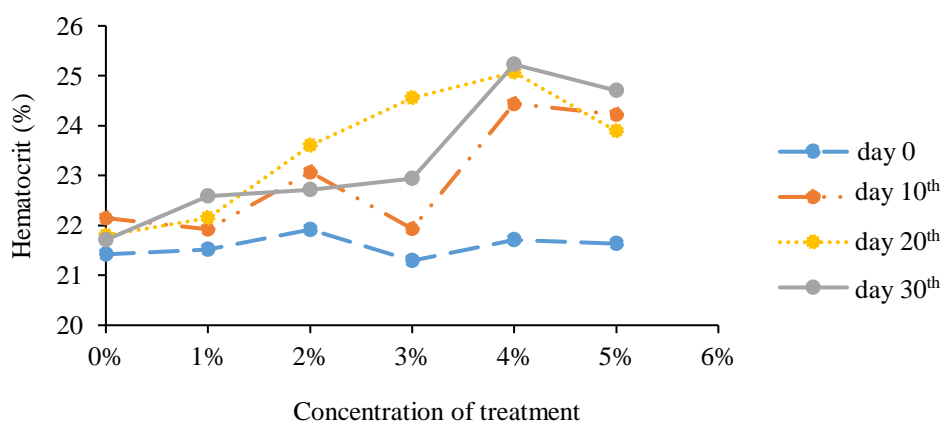


Figure 8. The average hematocrit value of tilapia under observation on day 0, day 10th, day 20th, and day 30th

The higher concentration of the wastewater of the laundry correlates to a higher increase of the hematocrit level. The normal hematocrit range of tilapia is between 21.00%-22.67%, as reported by Richard *et al.*, 2003; Aly *et al.*, 2008; Giron-Perez *et al.*, 2008; Yue & Zhou, 2008). The high value of hematocrit (above normal levels) indicates that the hematopoiesis process in tilapia began to be interrupted due to exposure from the laundry industry's wastewater. The calculation of the hematocrit value and hemoglobin level reflects the oxygen that carries the blood's carrying power. A low concentration of hematocrit can cause damage or defects in the osmoregulation process, while a high value indicates an increased demand for oxygen or hypo-osmotic conditions (Oğuz,

2015; Zainuddin *et al.*, 2017). The contamination of the water by detergent residues affects the local ecosystem with stable properties in the sediments, ease of absorption, and accumulation in the body tissue of fish, relevant to human health implication.

CONCLUSION

Wastewater from the laundry detergent industry affects tilapia's hematological condition by decreasing the hemoglobin concentration and increasing hematocrit levels higher than the normal condition, while the number of erythrocytes and leukocytes are still in the normal level.

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