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Histological studies of *Selaroides leptolepis* and *Sardinella* sp. kidneys as a biomarker of water pollution in Pasuruan Waters, Indonesia

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ABSTRACT

The current study aimed to determine the histological of the selar (*Selaroides leptolepis*) and the tembang (*Sardinella* sp.) kidneys of Pasuruan waters (Kraton, Lekok and Nguling), Indonesia, during the dry and rainy seasons. Water quality parameters (temperature, pH, DO, current velocity and salinity) and heavy metal content (Hg) were measured. Kidney histology was processed using Haematoxylin-Eosin staining. In both seasons, cell necrosis, hypertrophy/degeneration and cell congestion were recorded. In April, mild tissue damages were detected in the kidneys of the species under study (Selar & Tembang) in Nguling and Kraton (25.7%, 36.3% and 37.5%, 45.4%, respectively). While in Lekok waters, the damage was moderate (53.1% and 65.9%, respectively). Notably in August, selar fish collected from Nguling and Kraton waters recorded severe damage (79.9% and 76.6%), whereas tembang fish were moderately damaged (61.7% and 67.5%). Water quality parameters were within the standard values, while heavy metal (Hg) content surpassed the limits. The damage in the kidneys of selar and tembang fish was attributed to the impact of heavy metals. Therefore, better management is recommended to decrease the pollution in Pasuruan waters.

INTRODUCTION

The Selar (*Selaroides leptolepis*) and the tembang (*Sardinella* sp.) are seawater fishes with a high economic value. They spread almost throughout the whole waters of Indonesia and even to the western Pacific such as the Philippines, Arafuru, Australia, Japan and Persia **(Paxton *et al*., 2012)**. The Pasuruan sea waters form the center of capture fisheries in Nguling, Kraton and Lekok. The selar and tembang fishes are found in abundance in this area. In this research, the fixed chart was the main tool used to catch these species. Pasuruan Regency is located between Mojokerto, Sidoarjo, Probolinggo and Malang Regencies. Pasuruan Regency lies at 7.30'- 8.30 'south latitude and 112^{o}30' - 113^{o}30 'east longitude. Pasuruan Regency is characterized with flat and mountainous areas, with an altitude of 0 to more than 1,000 m above the sea level. A number of 14 districts have an average height up to 100 m above sea level (above sea level) ; namely, Kejayan, Wonorejo, Gempol, Beji, Bangil, Rembang, Kraton, Pohjentrek, Gondangwetan, Rejoso, Winongan, Grati, Lekok and Nguling **(Bappeda Pasuruan, 2013)**.

The water environment in Pasuruan Regency, including the coastal area in the Kraton, Lekok and Nguling Districts has been contaminated with heavy metals during the dry and rainy seasons. The pollution of the Lekok Beach is related to the residues originated from the inputs of the Rejoso River and the discharge of several tributaries with their residential areas, in addition to the industrial and agricultural activities that dispose remnants into the river **(Haryono *et al*., 2017)**. Thus, organic and inorganic wastes released from tributaries flow reaching the Lekok Beach where they are buried. Pollution caused by heavy metals would negatively impact marine ecosystem and its organisms as well **(Wicaksono *et al*., 2013)**. On the other hand, **Pramleonita *et al*. (2018)** reported that, most fish are very sensitive to water quality changes and referred to thesignificant relation between water quality fish growth and survival. The afore-mentioned authors added that fish are influenced by internal factors, such as genetic, physiological and external conditions, which are related to the availability of feed and environmental conditions. In this context, **Mandia *et al*. (2013)** related the decrease in water quality to structural and physiological damages in fish organs. Given that the kidney is an organ that is closely related to the circulatory system, its function in excreting materials and metabolic wastes for the wellness of fish is essential.

Ortiz *et al.* (2013) postulated that, abnormal fish kidneys is an indicator of changes in the aquatic environment since they receive large amounts of blood from arteries (post-branchial). Hence, the current study was conducted to analyze the histological structure of the kidneys of selar (*Selaroides leptolepis*) and tembang (*Sardinella* sp.) fishes in different seasons (dry & rainy) in the Pasuruan sea waters. Furthermore, this research would provide informative data on the histology of fish kidneys in the species under study for evaluating the environmental quality of the study sites.

MATERIALS AND METHODS

1. Research location

A marine survey was conducted in the Pasuruan District during April 2020 (dry season) and August 2020 (rainy season). This research was conducted in the Kraton, Lekok and Nguling waters, Pasuruan Regency, East Java Province. Selar and tembang fish samples were taken from the existing chart in the waters of the study sites.

2. Data collection

The physical and chemical qualities of water were measured including temperature, pH, dissolved oxygen, salinity and current velocity. The analysis of heavy metal mercury

(Hg) was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia. Physical and chemical water parameters were determined at the research location. Selar and tembang fishes were taken randomly from the "chart" in each sampling area of Kraton, Lekok, and Nguling districts. Fish specimens were stored in a coolbox before transferring to the laboratory. The kidney tissues of both species were stained using haematoxylin and eosin. The histology of kidneys was examined using a microscope, and then the level of damage in the kidney tissue was identified.

Data analysis

The tissue damage was measured by scoring the level of organ damage. The percentage of damage can be calculated using the following formula of **Indrayani *et al*. (2014):**

$$Damage\ (\%) = \frac{number\ of\ damaged\ cells}{number\ of\ analyzed\ cells} \times 100\% \qquad (1)$$

RESULTS

1. Histological analysis of selar and tembang kidneys

A significant alteration was detected in the kidney structure of selar and tembang fishes from Pasuruan waters at the three different locations in April (dry season) (Table 1) and August (rainy season) (Table 2).

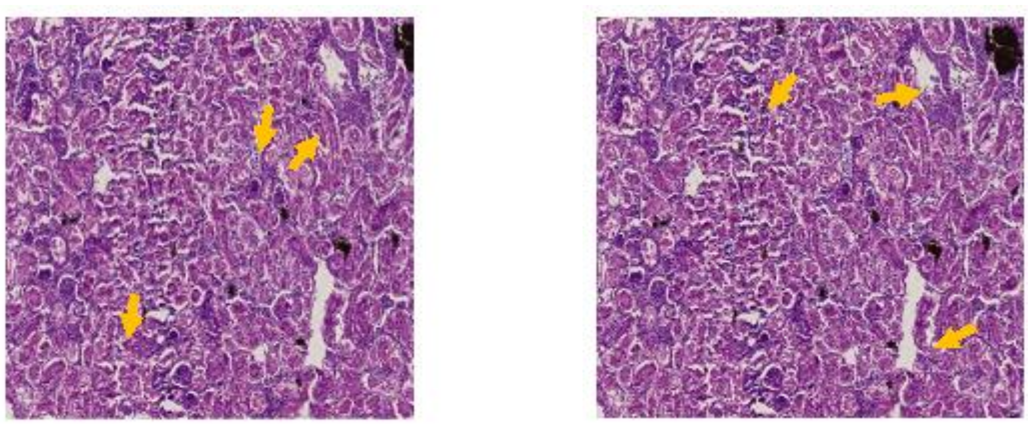
Table 1. Percentage of kidney tissue damage in selar and tembang fishes (April 2020)

Location	Selar Fish			Average (%)	Kidney Damage Status
	1	2			
Kraton	36.7	38.7	Light	36.3	Light
Lekok	53.3	54.2	Moderate	53.1	Moderate
Nguling	25.3	25.1	Light	25.7	Light
Location	**Tembang Fish**			**Average (%)**	**Kidney Damage Status**
	1	**2**			
Kraton	45.0	45.6	light	45.4	Light
Lekok	65.1	65.0	moderate	65.9	Moderate
Nguling	37.9	37.1	light	37.5	Light

Table 2. Percentage of kidney tissue damage in selar and tembang fishes (August 2020)

Location	Selar Fish			Average (%)	Kidney Damage Status
	1	2	3		
Kraton	76.6	76.7	76.6	76.6	Heavy
Lekok	82.7	91.2	90.0	88.0	Heavy
Nguling	81.9	78.0	79.8	79.9	Heavy
Location	**Tembang Fish**			**Average (%)**	**Kidney Damage Status**
	1	**2**	**3**		
Kraton	67.7	67.8	67.1	67.5	Moderate
Lekok	77.0	75.1	76.7	76.3	Heavy
Nguling	61.1	61.3	62.6	61.7	Moderate

In April 2020, the kidney tissue damages of selar and tembang fishes at Nguling and Kraton waters were marked as light damages with percentages of 25.7, 36.3, 37.5 and 45.4, respectively. Meanwhile, in winding waters (Lekok water), moderate damage of kidney was recorded, with averages of 53.1% and 65.9%.3. Meanwhile, in August 2020, both species at Kraton and Nguling waters were severely damaged, with damage percentages of 79.9 and 76.6, indicating that the kidney structure was in a bad condition. Tembang fish at Nguling and Kraton locations were moderately damaged with percentages of 61.7 and 67.5, respectively, and the Lekok location showed severe damage of 76.3%. If 25≤ P <50% and 50% ≤P <75, then the kidney structure was in a mild to moderate damaged condition. If P <25%, then the condition of the kidney structure was normal with no damage.



(**b**)

(**a**)

Fig. 1. Kidney Histology at Kraton Location showing **(a)** Selar Fish (*Selaroides leptolepis*); **(b)** Tembang Fish (*Sardinella* sp.)

HE staining (magnification 40x10; Bar = 5 µm) Ds; Cell degeneration / Hypertrophy; K; Congestion N; Necrosis.

(**a**) (**b**)

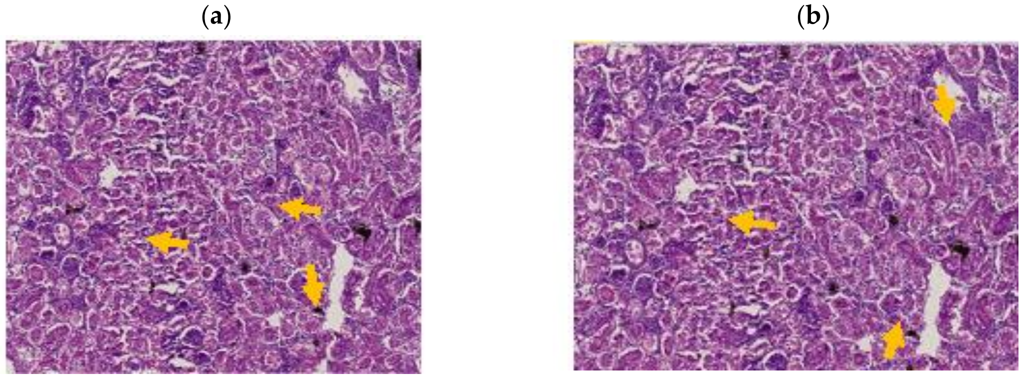
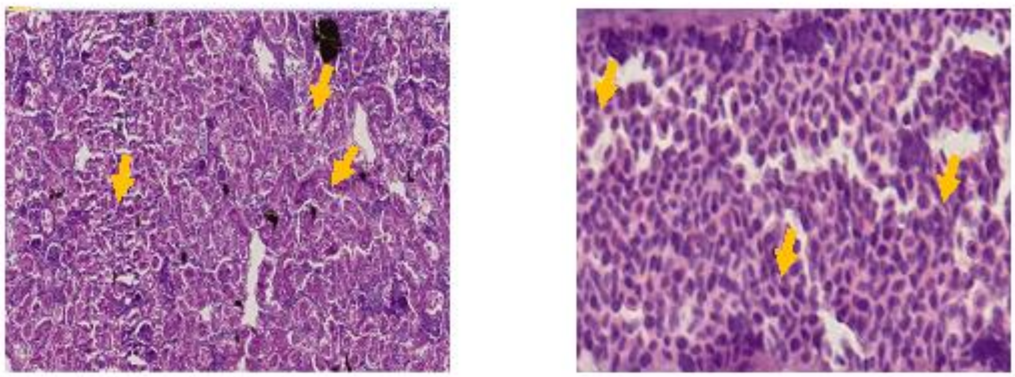


Figure 2. Kidney Histology at Lekok location showing **(a)** Selar Fish (*Selaroides leptolepis*); **(b)** Tembang Fish (*Sardinella* sp.)

HE staining (magnification 40x10; Bar = 5 µm) Ds; Cell degeneration/Hypertrophy; K; Congestion N; Necrosis.

Figs. (1-3) show that, the kidney of *Selaroides leptolepis* and *Sardinella* sp. witnessed a tubules and glomeruli damage indicated by cell degeneration or hypertrophy or swelling in the tubular cells. Hypertrophy/glomerular occurs due to blockage of toxic compounds. Though their concentration might be low, time factor has its considered impact on their effect on the body of the fish. The longer the fish is contaminated, the harder the effect of toxic compounds. Hypertrophy is an early symptom of necrosis. In addition, congestion is a symptom of the appearance of red spots on the tissue, indicating an increase in the amount of blood in the tissue. This affects the kidney function and metabolism. Damage to the cell wall or inhibition of cell wall synthesis results in cell lysis. The longer the kidneys are exposed to toxic compounds, the greater the number of kidney tissue cells experience necrosis.



(**b**)

(**a**)

Fig. 3. Kidney Histology at Nguling Location showing **(a)** Selar Fish (*Selaroides leptolepis*); **(b)** Tembang Fish (*Sardinella* sp.)

HE staining (magnification 40x10; Bar = 5 µm) Ds; Cell degeneration / Hypertrophy; K; Congestion N; Necrosis.

2. Water quality

2.1 Temperature

The highest temperature value was found in the Kraton location in April (32.4^{o}C), while the lowest temperature was found in the Nguling location in August (29.2^{o}C) (Fig. 4). The temperatures obtained at 3 different locations and different months did not show any significant difference since they were relatively identical in the study sites. Water temperature in August at the three sites is low because during the study season (rainy), the weather is cloudy.

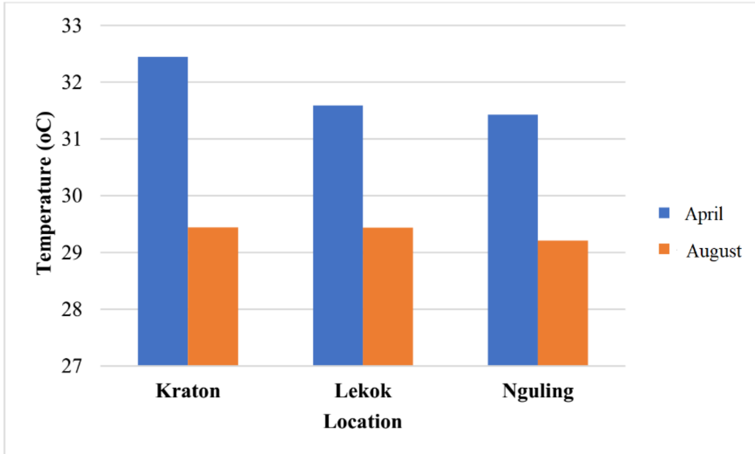


Fig. 4. Temperature at three different locations in Pasuruan waters

2.2 Current velocity

The presence of fish in the Pasuruan water is marked by current velocity. The current velocity ranged from 0.02- 0.07 m/s. The highest current velocity was found at Nguling location, specifically in April recording a value of 0.02 m/s. While, the lowest current velocity was found at Kraton location in August (0.07 m/s) (Fig. 5).

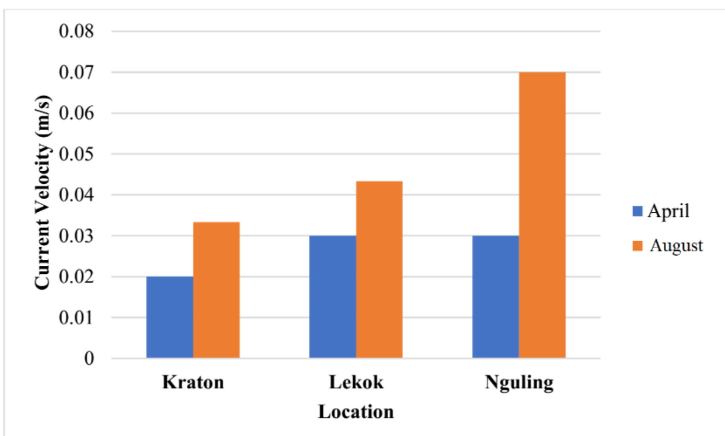


Fig. 5. The current velocity at three different locations in Pasuruan waters

2.3 The pH value

The pH value ranged from 8.1- 8.6. The pH value at all stations and points is relatively the same. Marine waters generally have a pH ranging from 6.5 to 9.0 (Fig. 6).

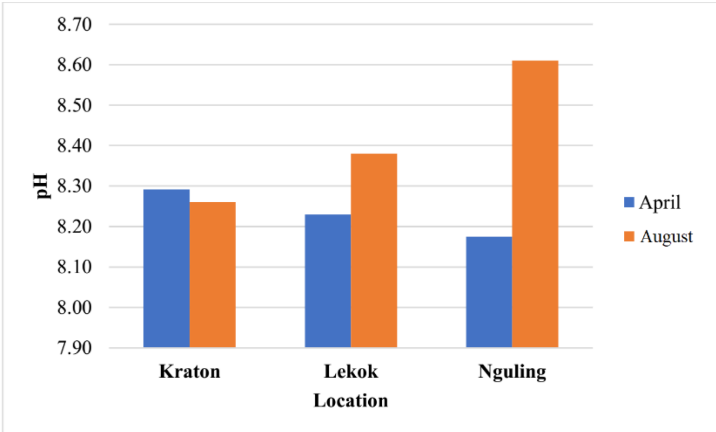


Fig. 6. The pH value at three different locations in Pasuruan waters

2.4 Salinity

The salinity values during the study ranged from 30.4- 33.56 ppt (Fig. 7). Salinity in Lekok waters is lower than Kraton and Nguling since these waters are close to the river's mouth.

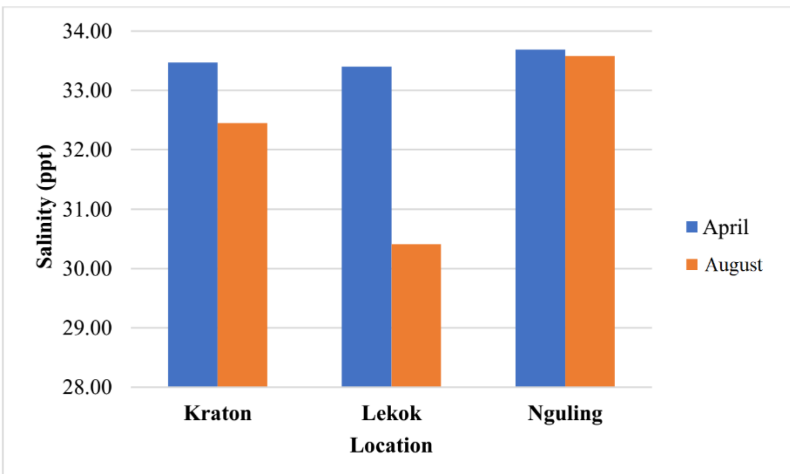


Fig. 7. The salinity value at three different locations in Pasuruan waters

2.5 Dissolved oxygen (DO)

Dissolved oxygen values ranged from 6.3 to 7.1 mg/l. Dissolved oxygen (DO) parameters play an important role, which is strongly influenced by temperature and the diversity of organisms. Fig. (8) shows that the DO value in Pasuruan waters was within the normal range. The DO value in April was higher than that in August because of the a dry season, where the temperature is relatively high in April.

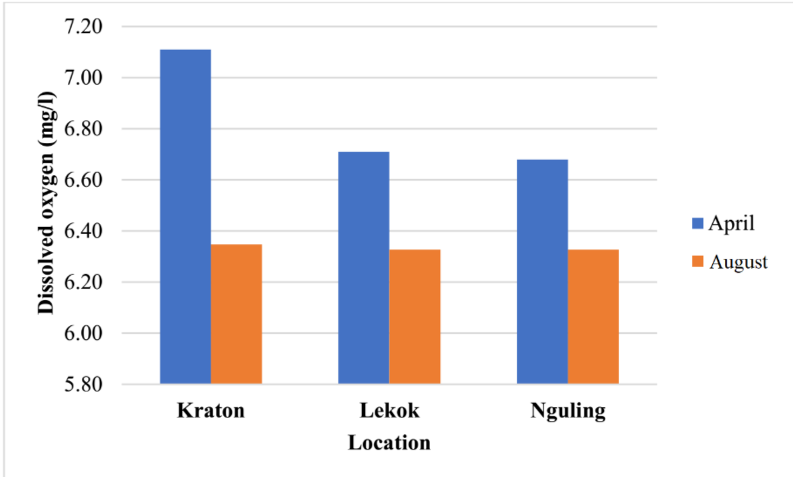


Fig. 8. The dissolved oxygen (DO) value at three different locations in Pasuruan waters

2.6 Heavy metal (Hg)

Hg quality standards of water was determined by the Ministry of Environment Decree No. 51 of 2004 is 0.002 mg/l. The dissolved Hg during the study ranged from 0.1 to 0.21 mg/l (Fig. 9). The level of Hg tends to be higher in August or the beginning of the rainy season, which can reduce the concentration of heavy metals in the waters.

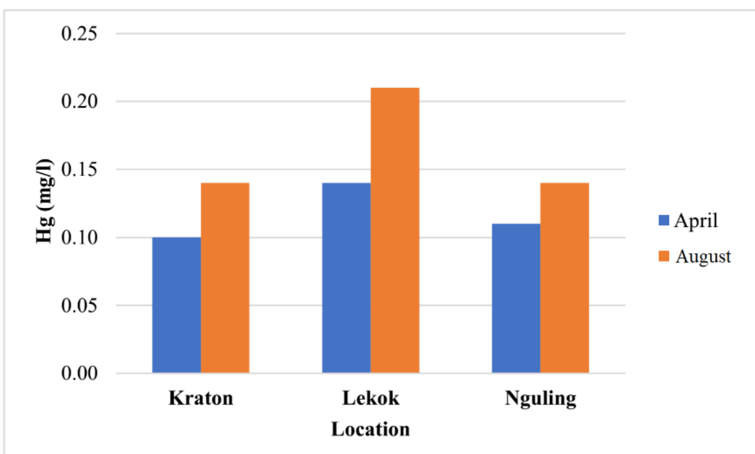


Fig. 9. The Hg content at three different locations in Pasuruan waters

DISCUSSION

The histological changes in the selar and tembang fishes collected from Pasuruan waters were observed. Cell hypertrophy/degeneration, cell congestion and cell necrosis were detected. The histological damage of the kidneys of selar fish in the Pasuruan waters (Kraton, Lekok and Nguling waters) is categorized as moderate to severe in accordance with the classifications of **Indrayani *et al.* (2014)**. In addition, **Ibrahim (2013)** attributed the kidney damage of the tilapia fish to the changes in water quality. Necrosis and degeneration are identified as forms of damages. Necrosis is a condition in which a decrease in tissue activity is marked by the loss of some parts of the cell indicating that death is taking place shortly. Necrosis can occur due to the activity of microorganisms, feed and metabolic disorders, in which cells generally change the cell nucleus. Degeneration is a disorder status in which empty space are present and marked by the swelling of the tubular epithelium, which leads to necrosis **(Ibrahim, 2013).**

Wagiman *et al.* (2014) reported that, necrosis is characterized by pycnosis, karyorrhexis and karyolysis. Pycnosis is characterized by shrinkage of the cell nucleus, while karyorrhexis is the rupture or destruction of the cell nucleus whose fragments are scattered in the cell. Furthermore, karyolysis is mostly known as cell shrinkage. Necrosis is characterized by unclear or missing cell boundaries and nuclei. In accordance to **Lubis *et al*. (2017)**, necrosis results from the lack of oxygen or extreme temperatures.

Kidney damage causes disturbance in the balance of the fish's body fluid volume. In this essence, **Coulibaly *et al*. (2012)** identified hypertrophy as the tissue damage and an increase in the organ size due to increased cell size causing detachment of cells. The characteristics of this hypertrophy can be seen by the disruption of the function of filtrate production and control of the filtrate composition itself that occurs in the kidney tubules.

In this study, the fish under study were exposed to water pollution in Pasuruan waters (Kraton, Lekok and Nguling) caused by toxic compounds such as organic waste, industrial waste, household waste and pesticides from rivers that enter marine waters. Water pollution in these three locations is supported by quantitative data from the histology of the kidneys, such as the percentage of kidney tissue damage. The results of this study can confirm that histopathological changes are good indicators of water quality. This demonstrates that histopathology can be used to evaluate the initial contamination in the water against chemical compounds that cause stress to aquatic organisms.

Tissue damage can also be used as a biomarker control for a polluted environment which can be specifically tested in organs such as gills, liver and kidneys. These three organs mainly play a role in the respiration, excretion and the accumulation of biotransformation of pollutants. In this study, kidney defects occurred in the glomerulus. According to **Mu'jijah *et al*. (2019)**, kidney function begins in the glomerulus, which is the ultrafilter formation of plasma. The ultrafilter enters the Bowman's capsule and goes to the lumen of the tubule. Filtering through various segments of the tubule results in changes in the volume and composition of the filtration fluid due to reabsorption and secretion along the tubule. Glomerulus composed of blood capillaries functions as a selective filter of blood, especially in normal blood filtering. After going through the filtering of the glomerulus, it is reabsorbed in the tubule which produces urine in normal conditions as a result of secretion. Therefore, due to pollution in the waters under study, the blood content proved to be abnormal, causing the development of the kidney organs to become abnormal with various damages that occur.

According to **Hamuna *et al.* (2018)**, seawater quality standards for marine biota shows that a good temperature for marine life ranges from 28^{o}C- 32^{o}C. In addition, **Bayhaqi *et al*. (2017)** postulated that, the movement of currents in coastal areas tends to be dominated by tidal conditions. The movement of water tends to move towards land at high tide. On the other hand, **Siburian *et al*. (2017)** assessed that, the degree of acidity is very important in determining the use-value of water for organisms and other purposes, generally influenced by several factors ,such as photosynthetic activity, temperature and the presence of cation anions. Additionally, **Souhoka and Patty (2013)** deduced that the difference in salinity values is influenced by weather, wind and land.

Based on the research of **Usman *et al*. (2015)**, Hg concentration fluctuates with respect to DO water. The highest heavy metal concentration was found at DO 8.3 mg/l, while the lowest heavy metal concentration was recorded at 9.3 mg/l. Mercury is a heavy metal that is highly toxic and harmful to aquatic organisms as well as humans. Bacteria cannot degrade mercury, so it can accumulate in the water. Mercury can enter the water due to mining activities, coal-burning residues, factory waste, fungicides, pesticides, household waste, etc. In 1956, in the Minamata Bay of Japan, there was a case of mercury poisoning from a chemical factory (Chisso Co. Ltd.). Factory waste containing mercury enters Minamata Bay and then accumulates in fish and shellfish. Fish and shellfish are then consumed by humans so that mercury also accumulates in humans. The high level of Hg^{2+} ions generated from the overhaul of mercury compounds in sediment deposits of water bodies, with the help of bacteria, would change to dimethylmercury $(CH_3)_2Hg$ and methylmercury ion (CH_3Hg) **(Zulfahmi *et al*., 2014)**.

The high condition of Hg in the waters of Lekok needs to be pointed out because it will spread and accumulate in sediment, then accumulate in the body of aquatic organisms. In addition, Hg is a persistent heavy metal, so that if it contaminates living things, it will endanger the body. This finding is in line with that of **Riani (2017)** who stated that, heavy metal concentrations that are not lethal (chronic, not lethal dose) causes damage to body organs, especially organs related to immunity. Heavy metal also disrupts physiological processes in the body of the biota leading to reproduction problems and congenital defects in the embryo **(Riani, 2017)**.

Rahman *et al.* (2012) postulated that high rainfall is synonymous with increased water discharge and can accelerate purification. Discharge is a diluting factor, the higher the discharge passes through the river, the lower the concentration of dissolved heavy metals. Decreasing river water discharge causes an accumulation of pollutant concentrations in the water. However, in fact, there is an increase in heavy metal concentrations in the rainy season (August). The results of interviews by researchers with fishermen in Pasuruan waters indicated that there are indications that there are still many industries pouring waste containing heavy metals into the water in rainy days, so that the concentration of pollutants, especially B3 waste such as heavy metals, increases in the rainy season. The high and low concentrations of heavy metals in Pasuruan waters are caused by domestic community activities, industrial factories and transportation activities.

CONCLUSION

The alteration of kidney histology was found in the kidney of selar and tembang fishes in Pasuruan waters during the dry and rainy seasons, markedly by cell necrosis, hypertrophy/degeneration and congestion. There is a significant difference in the kidney damage scores of selar and tembang fishes in Pasuruan waters with a minor to severe damage. The damage percentage increased in the species under study in the rainy season (August). Water quality parameters such as temperature, Ph, DO, velocity, and salinity are within water quality standards. However, the content of heavy metal (Hg) is high. Pasuruan water is classified as polluted water, and thus better management is required to decrease pollution in those water bodies. There is also need for further research on other heavy metal compounds as well as other sensitive organs such as gills and liver. Therefore, the pollution status in Pasuruan waters could be regularly monitored.

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