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The Impact of Heavy Metal Fe, Zn, and Cu in Water and Sediment on the Histopathology of Gills and Liver of Mullet Fish (*Crenimugil seheli*) at Kamal Beach, Madura Strait

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Abstract: Kamal Beach in the Madura Strait is suspected to have experienced heavy metal contamination due to ship recycling industry activities. Heavy metals have a negative impact on the aquatic environment, aquatic biota and even humans. This study aims to analyze the impact of heavy metal content in Kamal Beach, especially heavy metals Fe, Zn, and Cu as heavy metals found in the region on the histopathological aspects of the gill and liver organs of aquatic biota, namely mullet fish (*Crenimugil seheli*). The levels of heavy metals Fe, Zn, and Cu were analyzed using the AAS (Atomic Absorption Spectometry) method in accordance with SNI 6989.4:2009. Hiatopathological analysis of the gill and liver organs was carried out using the scoring method where from the results of the analysis it was known that the damage that occurred in the gill organs was in the form of edema, hyperplasia, lamellar fusion, and necrosis while damage to the liver organs included degeneration, necrosis, and congestion. Damage to the gill and liver organs of mullet fish is caused by the high content of Zn and Cu in water and sediment.

Keywords: Copper; Histopathology; Iron; Ship recycling; Zinc

Introduction

Kamal Beach is a coastal area located in Kamal District, Bangkalan Regency, Madura. This area is famous for its many ship recycling and shipyard industry activities. The ship recycling business is a business engaged in the dismantling and destruction of old ships to recycle metal or non-metal materials (Muvariz et al., 2023). In addition to ship recycling activities, Kamal waters also have several shipyard companies such as PT Gapura Shipyard and PT Ben Santosa which are engaged in shipbuilding, repair, and inspection. Ship recycling activities and the shipyard industry contribute major pollutants in the form of heavy metals including Fe, Zn, Cu, Cd, Mn, As, Cr, Pb, Co, Ni, and Ag into seawater and soil around the industrial area (Hasan et al., 2023). As a result of these activities, the surrounding seawater will change color to brown and smell like rusty iron (Alimby & Triajie, 2021). In addition, this area also has a harbor that is still used for crossings.

Preliminary studies that have been carried out in December 2023 show that the types of heavy metal pollutants that dominate in the Kamal Beach area are heavy metals Fe, Zn, and Cu with an average content of 0.0001-0.1 ppm while the metals Cd, As, Cr, and Pb were not detected. Research conducted by Putri, *et al* (2023), stated that the heavy metal content of Cu in seawater in Kamal waters ranged from 0.2-0.4 ppm and heavy metal Zn in seawater ranged from 0.1-2 ppm. In biological terms, heavy metals Fe, Zn, and Cu are essential heavy metals that play an important role in the metabolism of organisms, but if the content exceeds the threshold, it can be toxic and adversely affect the environment, biota

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and even humans (Mehana et al., 2020). The increase in heavy metal concentration in waters causes heavy metals to settle in the water, be absorbed by tissues, and eventually enter large organisms as end-level consumers through the food chain (Sugiantari et al., 2022).

One of the aquatic biotas that can be used as an indicator of Fe, Zn, and Cu heavy metal pollution is fish. Fish are very vulnerable to pollutants because they cannot avoid the harmful effects of pollutants (Mehana et al., 2020). One type of fish that can be used as an aquatic bioindicator in Kamal Beach biomonitoring efforts is mullet. Mullet fish (*Crenimugil seheli*) is one of the fish species found in Kamal Beach (Hur et al., 2020). Histopathological assessment can describe the impact of pollutants on fish health in an ecosystem by looking at changes that occur in tissues or organs (Sari & Perwira, 2019). The purpose of this study was to analyse the impact of Fe, Zn, and Cu heavy metal pollution on the histopathology of gills and liver of mullet (*Crenimugil seheli*) in Kamal Beach, Madura Strait.

Method

Time and Location

This research was conducted in February-March 2024.

Research Location

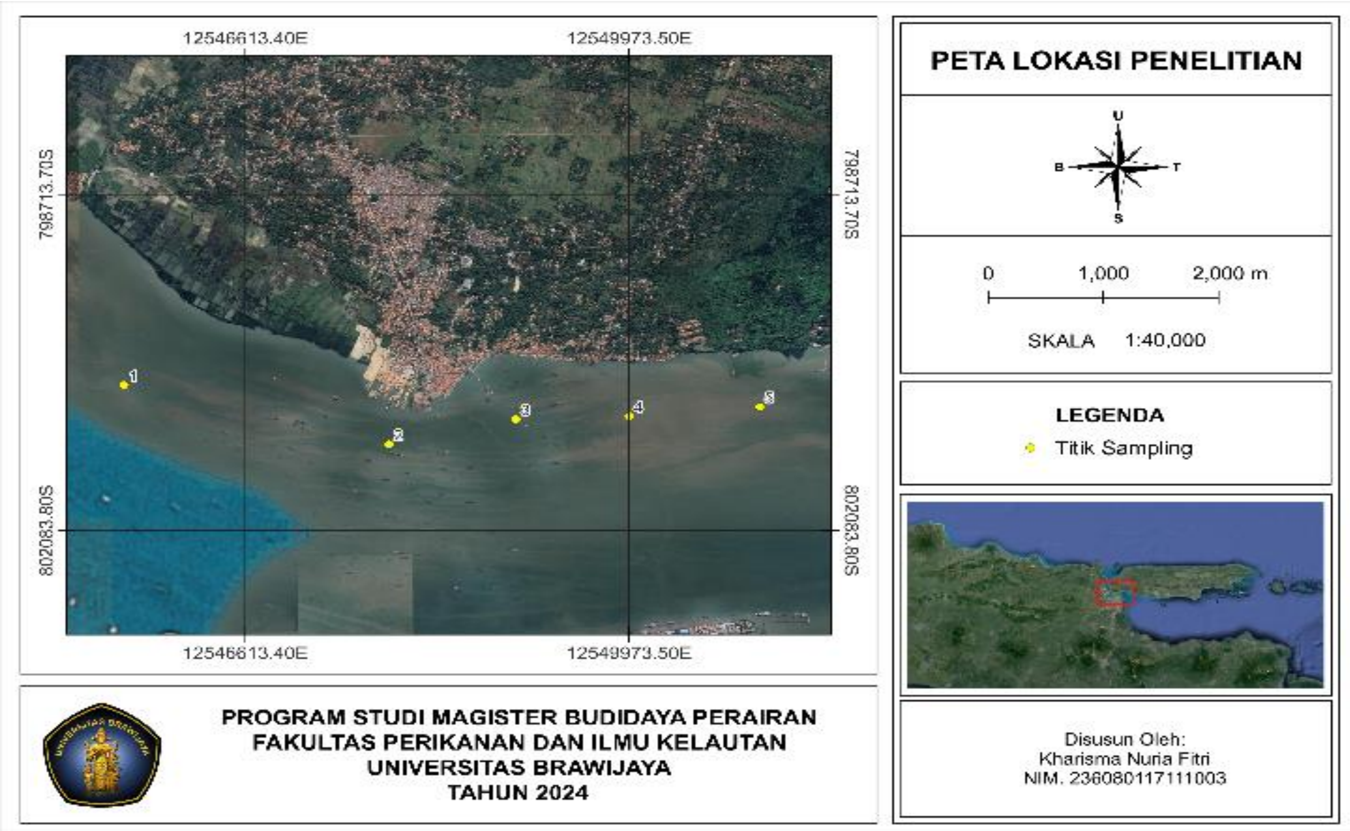

Figure 1. Location map

The research was conducted in the waters of Kamal Beach, Madura Strait (Kamal District, Bangkalan Regency, Madura Island, East Java) which was divided into 5 research locations, namely location 1, location 2, location 3, location 4, and location 5. Analysis of water samples was carried out at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, State University of Malang and for sediment samples was carried out at the Analysis and Measurement Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Histopathological analysis was conducted at the Hydrobiology Laboratory of the Division of Fish Resources, Faculty of Fisheries and Marine Science, Universitas Brawijaya. This research will be conducted from February 2024 to May 2024.Research design and method should be clearly defined**.** Determining the research location point is based on the map figure 1.

Water and Sediment Sampling

Five sampling points for seawater, sediment and mullet were determined based on the characteristics of the surrounding area with 3 replicates. Seawater and sediment samples were collected 50-100 metres from the shoreline. Water samples were collected using the composite method (Putri et al., 2023). Sediment sampling using ekman grab tool then stored in plastic bags (Pambudi & Armi, 2022).

Sampling of Mullet Fish (Crenimugil seheli)

Sampling sediment and mullet fish (*Crenimugil seheli*) using purposive random sampling technique so that the samples used are more representative (Ani et al., 2021).

The Measurement of Fe, Zn, and Cu Concentration in Seawater and Sediment

Measurement of heavy metal content of Fe, Zn, and Cu using AAS (Atomic Absorption Spectrophotometry) method in accordance with SNI 6989.4:2009.

Histopathological Analysis of Mullet Fish

Mullet fish that have been taken from 5 research locations scattered in Kamal Beach are carried out the process of preparing with the first stage, namely dissection, then fixation, dehydration, clearing impregnation, embedding, blasting, sectioning, affixing, colouring, mounting, and the last stage is observation using a microscope with a magnification of 400-1000 x (Faridah et al., 2019). The level of tissue damage was analysed using the scoring method according to Halim et al. (2022), calculation of organ damage using the following formula:

$$\% \, Damage = \frac{Number\ of\ damaged\ cells}{Number\ of\ fields\ of\ view} x\ 100\% \quad (1)$$

Scoring for organ damage is divided into categories as per the table 1.

Table 1. Scoring Organs

Score	Meaning	Category
Score 0	No damage at all	No damage at all
Score 1	Damage that occurs less than 25% of the viewing area	Light damage
Score 2	Damage occurring at 25%-50% of the field of view	Moderate damage
Score 3	Damage that occurs more than 50% of the field of view	Heavy damage

Result and Discussion

Water Quality

The results of water quality measurements including physical and chemical parameters at Kamal Beach, Madura Strait are described in the table 2. Table 2 display the water quality of Kamal Beach in Madura Strait. The temperature value at research sites 1 to 5 has no significant difference in value. A good temperature range for fish growth in tropical waters ranges from 28-32^{0}C (Azhari et al., 2018). Constant and relatively stable water temperature causes fish not to experience stress because the temperature does not change drastically. Temperature is a very important physical factor in water because it is related to the substances / elements in it that will determine the density of water, water density, water saturation, accelerate water chemical reactions and affect the amount of dissolved oxygen in the water. Fish are cold-blooded animals (poikilothermal) so that metabolism in the body depends on the temperature of its environment, including its immunity (Wangni et al, 2019). High temperatures that can still be tolerated by fish are not always lethal to fish but can cause long-term health status disorders, such as stress that causes weak, thin bodies and abnormal behavior (Cintia et al., 2023).

Table 2. Water Quality

Location	Parameter					
	Temperature (°C)	pH	DO (ppm)	Salinities (ppt)	BOD (ppm)	TDS (ppm)
Location 1	31	7.04	2.2	31.9	20.7	1981.0
Location 2	32	7.03	2.8	30.6	20.4	2305.7
Location 3	31	7.04	3.5	30.9	19.7	2033.0
Location 4	33	7.00	2.4	29.8	21.3	2668.3
Location 5	32	7.03	3.6	31.1	19.5	1691.7

pH is an important factor that affects the life of organisms. This is because it is related to the acidic, alkaline, or neutral factors of the medium in which the organism lives. The proportion of H+ and OH- ions is extensively important for regulating a physiological process of the organism (Reynalte et al., 2015). According to a statement from Robi et al. (2021), stated that the pH value also affects the level of toxicity of heavy metals to biota. Because heavy metals will easily dissolve in waters in acidic conditions. Changes in pH values in waters affect the chemical properties of water including the solubility of heavy metals.

The salinity value at Kamal Beach is still classified as below the optimum limit in accordance with the statement from Patty et al. (2018), the normal limit value in coastal water and mixed water ranges from 32-34 ppt.

The low value of water salinity in Kamal Beach is due to weather factors, namely rain. Rain brings freshwater supplies from rivers to the sea more so that there is dilution of sea water (Lisna et al., 2023). Rain causes salinity to decrease which has an impact on increasing heavy metal toxicity accompanied by increased accumulation of heavy metals in the waters (Putri et al., 2023).

DO values at all research locations are below the threshold value of dissolved oxygen levels that are good for the survival of aquatic biota according to PP No. 22 of 2021, which is > 5 ppm. Research location 5 is the location with the lowest level of pollution activity because it is adjacent to the Navy Base area which is closed to public activities. According to Azhari et al. (2018) waters that are spared from many anthropogenic activities tend to have good water quality levels, especially in dissolved oxygen parameters. Locations 4 and 1 have the lowest oxygen levels because location 4 is very close to the old ship dismantling industry which produces waste containing high heavy metals while location 1 is close to shrimp ponds which produce waste with high levels of organic matter (Ji et al., 2021). Waste disposal and industrial activities can significantly reduce DO levels due to an increase in organic matter and nutrients that cause eutrophication, which can increase oxygen demand by microorganisms to decompose these materials (Auddy et al., 2023).

The BOD value of waters can reflect the value of the pollution load that occurs around the location (Daroini & Arisandi, 2020). The amounts of pollutants from industrial activities, household waste, and other anthropogenic activities greatly affects the level of pollution of a water body. The higher the BOD value, the higher the level of pollution (Hariyanto & Larasati, 2016).

According to Kepmen Lingkungan Hidup No 51 Tahun 2004, the TDS quality standard for the survival of marine biota is 1000 ppm. Therefore, the TDS value of all research locations in Kamal Beach has exceeded the normal threshold so that it can be said that the waters of Kamal Beach have been polluted. TDS is dissolved solid matter, including all minerals, salts, metals, and cations dissolved in water. In general, the concentration of dissolved solid objects is the sum of cations and anions in water (Wibowo & Rachman, 2020). The high level of TDS is due to the large number of water-soluble organic and inorganic compounds, minerals, and salts. In seawater, the TDS value is high because it contains many chemical compounds (Umasugi et al., 2021).

Heavy Metal Content in Water

The measurement results of Fe, Zn, and Cu heavy metal content in water using the AAS method are described in the table 3.

Table 3. Heavy Metal Content in Water

Heavy Metal Parameters					Location	Quality	Standard Unit
	Location 1	Location 2	Location 3	Location 4	Location 5		
Fe	0.04	0.20	0.10	0.25	0.06	ppm	0.5*
Zn	0.017	0.081	0.053	0.169	0.037	ppm	0.05*
Cu	0.017	0.025	0.023	0.044	0.021	ppm	0.008*

Ket *= Quality standards for heavy metals in water in accordance with Peraturan Pemerintah Republik Indonesia No 22 Tahun 2021

The content of Fe heavy metal in water at all research locations is still below the quality standard value. The content of heavy metal Zn at research sites 2, 3, and 4 has exceeded the standard limit while research sites 1 and 5 are still below the quality standard value. The value of Cu heavy metal content in water at all research locations has exceeded the quality standard.

Concentration of Heavy Metal Fe in Water

The highest Fe content value is found in location 4 with an average of 0.25 ppm followed by location 2 with an average value of 0.20 ppm, location 3 with an average value of 0.10, location 5 with an average value of 0.06 and the lowest Fe heavy metal content value in water is found in location 1 with a value of 0.04 ppm. Research location 4 is a ship dismantling industrial area where waste generated from the dismantling of old ships will be directly discharged into the sea. This is in accordance with the statement of Akhir et al. (2023), heavy metal iron (Fe) in waters can be sourced from corroded iron parts of ships, domestic waste, and industrial waste. In addition, ships that have not been dismantled are left unattended. According to Supriyantini et al. (2015), heavy metal content in sediments is generally higher than in the water column because heavy metals that enter the water column will be absorbed by suspended particles.

Concentration of Heavy Metal Zn in Water

Study sites 4 and 2 had high concentrations of heavy metal Zn compared to other study sites with average values of 0.169 ppm and 0.81 ppm, respectively. This is because study site 4 is close to the ship dismantling industry while site 2 is close to the shipyard industry and West Harbour. Industrial activities and ship shipping greatly affect the concentration of heavy metal Zn in Kamal Beach. According to Yin et al. (2015), the release of heavy metal Zn from anti-fouling coatings on ship paints increases the input of heavy metal Zn into the water. Zn heavy metal is one of the substances mixed in the raw material for making colour paint for ships (Putri et al., 2016). In addition, heavy metal Zn can also be sourced from household waste, agriculture, and other domestic waste (Mustafa et al., 2021).

Concentration of Heavy Metal Cu in Water

The concentration of heavy metal Cu in the sediment measured using the AAS method showed that site 4 was the study site with the highest Cu content in the water. This occurred due to the high activity of ship dismantling. According to Putri et al., (2023), several parts of the ship's body are made of copper, including electrical cables, metal plating, wires, pipes and plumbing systems, generators and motors, cooling systems and electronic equipment installed on the ship. According to Sugiantari et al. (2022), copper (Cu) is one of the ingredients of a mixture of preservatives in the manufacture of shipyards. Many ships that are anchored or shipwrecks that are left unattended are the main source of Cu metal pollution that occurs due to corrosion of ship bodies in the waters.

The levels of heavy metals Fe, Zn, and Cu at location 1 show average values below the quality standard. this happens because there is no source of input of heavy metal Fe. The location 1 area is adjacent to ponds and mangrove forests. According to Nursagita et al. (2021), mangroves are plants that can grow in extreme places with high salinity mangroves can remediate environments that experience heavy metal pollution through phytoremediation mechanisms so that heavy metals in the waters will be absorbed by mangrove plants.

Heavy Metal Content in Sediment

The results of measuring the heavy metal content of Fe, Zn, and Cu in sediments using the AAS method are described in the table 4.

Table 4. Heavy Metal Content in Sediment

Heavy Metal Parameters	Location 1	Location 2	Location 3	Location 4	Location Location 5	Quality	Standard Unit
Fe	1266.7	1533.3	1700.0	1800.0	1366.7	ppm	20.000**
Zn	3.0	11.3	9.6	37.9	5.9	ppm	120**
Cu	60.84	175.10	72.67	180.79	67.52	ppm	32**

Ket **= Quality standards for heavy metals in sediment in accordance with the Wisconsin Department of Natural Resources, 2019

Concentration of Fe Metal in Sediment

According to Indrawan et al. (2021), heavy metal Fe in water will experience dilution resulting in precipitation and accumulation of Fe (iron) in the bottom sediments of waters. According to Supriyantini et al. (2015), water quality factors such as pH, temperature, and dissolved oxygen are thought to also affect the high concentration of heavy metal Fe in sediments compared to the concentration of heavy metal Fe in water where low pH will cause iron in the form of Fe2+ in water to oxidise into a form of Fe3+ which cannot dissolve in water so that it will settle to the bottom of the waters.

Concentration of Zn Metal in Sediment

Many Zn metals scattered in the water column have precipitated. According to Yin et al. (2015), the Kamal Beach area is still used for various activities such as ship berthing, ship repair, and shipping activities by ship. In addition, water quality parameter factors such as pH, temperature, and DO also affect the concentration of Zn metal. Measurements of water quality parameters in the form of temperature, pH, and salinity at all research locations show that the higher the levels of temperature, pH, and salinity, the speed of accumulation of heavy metal Zn in sediments increases.

According to Viet et al. (2018) and Viet et al. (2016), heavy metal Zn in the water will be absorbed by suspended particles towards the bottom of the water so that the concentration of heavy metal Zn in the water is lower than in the sediment. Heavy metal accumulation does not occur in water due to the influence of dilution and flushing by tides (Mustafa et al., 2021).

Concentration of Cu Metal in Sediment

Massive activities in the ship dismantling industry are the main source of Cu pollution in Kamal Beach, Madura Strait and port and shipyard activities increase the concentration of Cu metal in the sediment. According to Viet et al. (2016), heavy metal Cu in the water will be absorbed by suspended particles to the bottom of the water so that the concentration of heavy metal Cu in the water is lower than in the sediment.

Histopathology of Mullet Gills

Fish gills are vital organs that serve as the primary means for gas exchange, maintaining osmotic homeostasis, as well as excretion of waste substances.

The gill structure consists of rows of gill filaments lined with secondary lamellae, which expand the surface for oxygen diffusion from the water into the fish's blood. This process is very efficient because the gills have a countercurrent exchange system, where water and blood flow in opposite directions, maximising oxygen uptake and carbon dioxide removal (Thai-Hoang et al., 2022). In addition, gills also play an important role in ion regulation and ammonia excretion. This ability becomes the gills can maintain the ionic balance of the fish body even in different environments in both freshwater and seawater (Ji et al., 2021). Damage to the gills, whether by environmental pollution or parasitic infection, can disrupt these vital functions and negatively affect fish health and survival (Ngatia et al., 2023). Damage on the gill can be seen in figure 2 and table 5.

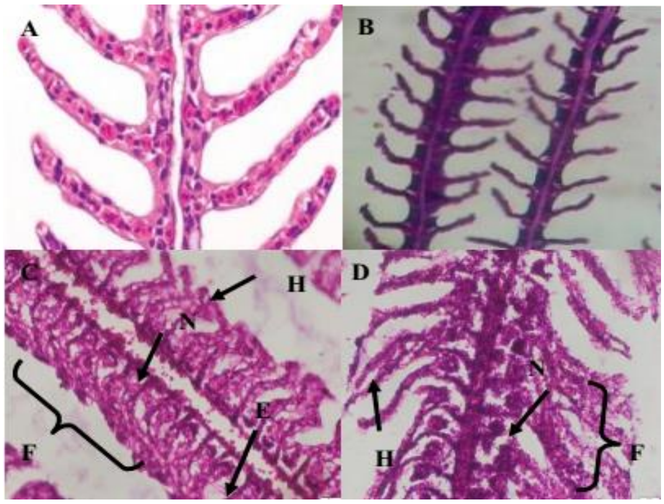

Figure 2. (A) Normal fish gill histopathology (Smith et al., 2018), (B) Normal fish gill histopathology (Noviyanto et al., 2022), (C) Mullet gill histopathology. Notes: H=Hyperplasia, FL=Lamella fusion, E=Edema, N=Necrosis

Table 5. Mullet Fish Gill Damaged

Location	Type of Damage				Mean (%)	Category
	Edema	Hyperplasia	Lamellar Fusion	Nekrosis		
1	30	26	26	31	28	Moderate damage
2	56	49	43	47	49	Moderate damage
3	35	49	45	49	45	Moderate damage
4	72	80	72	74	74	Heavy damage
5	40	31	36	32	35	Moderate damage

Edema damage to the gills is characterised by the accumulation of fluid in the gill tissue causing swelling and structural damage (Marinović et al., 2021). Fe metal can cause increased capillary permeability and cellular damage, leading to gill oedema and swelling (Barbieri et al., 2016).

Hyperplasia that occurs in primary lamellae is caused by excessive division of chlorid cells due to disruption of ion transport regulation in the gills, while hyperplasia in secondary lamellae occurs due to excessive epithelial cell division in secondary lamellae (Idzni et al., 2020). Hyperplasia that occurs continuously will stimulate an increase in mucus secretion by mucous cells. Increased mucus cell secretion in the gills can interfere with the breathing process of fish because the mucus covers the epithelium so that the oxygen osmosis process does not occur in the area (Sudaryatma et al., 2013).

Secondary lamellar fusion that occurs in gill tissue can be characterised by the fusion of two or more secondary lamellar epithelial tissues. Fusion that occurs in secondary lamellae causes the lamellae to be unable to function because the lacunae containing red blood cells are covered by damaged epithelial cells (Utami et al., 2017). Fusion in the lamellae causes the surface area of the gills to perform the respiration process to be reduced, so that the supply of oxygen and nutrients is also reduced which causes Adenosisn Tri Phosphate (ATP) produced by the metabolic process to be reduced. The condition of gill hyperplasia triggers an increase in mucous cell activity by hypersecretion and the beginning of mucous cell proliferation if there is a disturbance in the form of parasites or toxic substances and chemical disturbances in the waters. Water in the water that contains material such as toxic substances will enter through the gill lamellae so that these materials will stick to the gill mucus which has a function to capture foreign particles from the water (Juanda et al., 2024). The more material the mucus captures, the more mucous cells will produce mucus (Lestari et al., 2018). The proliferation of mucous cells that occurs in the gill lamellae is done to protect body parts that are eaten by parasites by producing a lot of mucus on the surface of the gills which will then cause fusion and cell death.

Cell necrosis is described by cells that are torn, brittle, pale in colour, and the nucleus disappears, enlarges, or shrinks (Juanda et al., 2024). Edema damage that occurs due to foreign body infiltration will cause swelling to get worse and epithelial cells will experience lifting (Epithelium lifting) which can cause impaired epithelial function and eventually necrosis (death) of cells and tissues. The epithelial layer of the gills, which is in direct contact with the external environment, can cause the gills to have a great opportunity to be exposed to contaminants in the water (Juanda et al., 2023).

Histopathology of Mullet Liver

The fish liver is an important organ that plays a role in various metabolic and biochemical functions, including lipid and carbohydrate metabolism, detoxification, as well as the synthesis of important proteins such as albumin and blood coagulation factors. Fish liver is highly responsive to environmental changes such as exposure to pollutants that can cause histopathological changes (Araujo et al., 2019). Damage on the liver can be seen in figure 2 and Table 6.

Cell degeneration occurs because of the electrolyte charge outside and inside the cell being in an unbalanced condition. The instability of the cell in pumping sodium ions out of the cell can cause an increase in the influx of extracellular fluid into the cell so that it is unable to pump enough sodium ions. This causes the cell to swell and causes the cell to secrete cell material resulting in cell death or necrosis (Wagiman et al, 2014). Swelling in the form of cell degeneration causes cells to lose membrane stability. Heavy metals such as Fe, Zn, and Cu can cause degeneration in liver tissue due to leukocyte infiltration into liver cells to fight against foreign substances that can be heavy metal pollutants (Javed & Usmani, 2013).

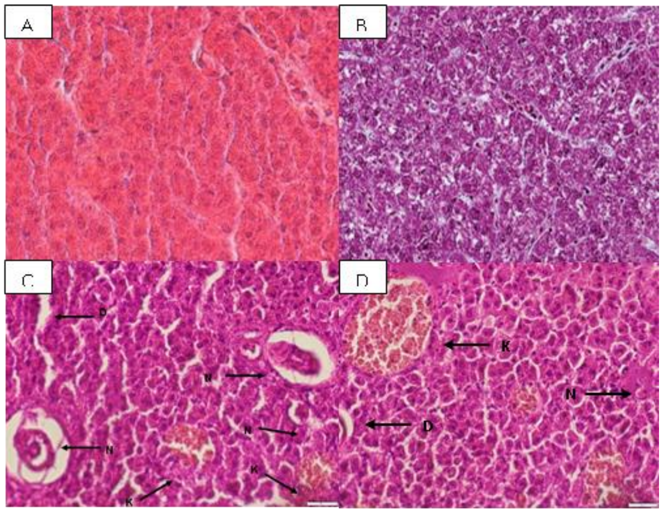

Figure 3. (A) Normal fish liver histopathology (Santos et al., 2022), (B) Normal liver histopathology (Savassi et al., 2020). (C) & (D) Liver histopathology of mullet in Kamal Beach, Madura Strait. Notes: D=Degeneration, N=necrosis, K=congestions

Table 6. Mullet Fish Liver Damaged

Location	Type of Damage			Mean (%)	Category
	Degeneration	Nekrosis	Congestion		
1	30	28	28	29	Moderate damage
2	56	45	44	48	Moderate damage
3	45	41	41	42	Moderate damage
4	75	71	71	72	Heavy damage
5	36	40	40	38	Moderate damage

Necrosis is a form of cell death that occurs along with the rupture of the plasma membrane. The sign of cells undergoing necrosis is the shape of the nucleus which is getting smaller or commonly referred to as pyknotic, enlarged, blurred, and even lost. The characteristics of necrotised tissue are that it has a paler colour than normal, loses its range or has a poor or deformed consistency (A'yunin et al., 2019). Necrosis is a type of reversible cell death that occurs when there is severe injury until at some point the cell is unable to adapt or repair itself (Sulastri et al., 2018). Necrosis occurs due to toxic materials that enter aquatic biota. In general, cells that undergo necrosis show changes in the cell nucleus and cytoplasm. Necrosis is characterised by cytoplasm filled with sediment or eusonifilik in the form of protein (Wagiman et al., 2014). Necrosis is a description of a decrease in tissue activity characterised by loss of cell parts. The loss of parts of the cell occurs one by one, so that if this happens over a long period of time, the cell will die as a result. The death of this tissue will be accompanied by degeneration of the cells (Mandia et al., 2013). Necrosis is considered a direct effect that occurs due to the presence of pollutants, especially heavy metals Fe, Zn, and Cu on certain organs (Javed & Usmani, 2013).

Congestion is the dilation of blood vessels where the number of blood cells contained in the blood vessels exceeds the normal limit. Histological changes in the form of congestion indicate an increase in the amount of blood in the blood vessels. Blood capillaries also appear dilated and filled with erythrocytes (Wagiman et al., 2014). This congestion is characterised by blood spots where blood accumulates in one area. This is in accordance with the opinion of Mutiara et al. (2013), which states that the accumulation of blood in certain areas is due to blockage of blood circulation. The characteristics of congestion are the accumulation of red blood cells that are very dense in the blood vessels. This build-up indicates an abnormal condition of the cells. Generally, congestion is caused by physical trauma, parasites, or other disorders of the circulatory system. If this condition occurs continuously and the condition of the cells is severe, the result is rupture of the blood vessels, and the effect will cause necrosis.

Conclusion

The content of heavy metal Fe in water and sediment in Kamal Beach, Madura Strait has not exceeded the threshold of 0.5ppm and 20,000ppm so that the waters of Kamal Beach are still not polluted by heavy metal Fe. The content of heavy metal Zn in water at research locations 2, 3, and 4 has passed the threshold value of 0.05ppm while the content of metal Zn in sediment has passed the threshold at all research locations with a value above 120ppm. Cu heavy metal content in water and sediment at all study sites exceeded the threshold levels of 0.008ppm and 32ppm, respectively. Histopathological analysis showed that the high content of heavy metals Zn and Cu in Kamal Beach caused structural changes in the gill and liver organs of mullet fish (*Crenimugil seheli*) in the form of liver degeneration, liver necrosis, liver congestion, gill edema, gill hyperplasia, gill lamella fusion, and gill necrosis. Damage to the gills and liver of mullet at sites 1, 2, 3, and 5 was classified as moderate damage while site 4 was classified as severe damage.

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Conflicts of Interest
No conflicts of interest

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