UNIVERSITI PUTRA MALAYSIA

HISTOPATHOLOGICAL EVALUATION OF GILL, LIVER AND KIDNEY OF TILAPIA (OREOCHROMIS SPP.) FROM A POLLUTED RIVER IN HULU LANGAT, SELANGOR

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HISTOPATHOLOGICAL EVALUATION OF GILL, LIVER AND KIDNEY OF TILAPIA (*OREOCHROMIS SPP.*) FROM A POLLUTED RIVER IN HULU LANGAT, SELANGOR

NOR AZZALINA BINTI MOSLIM

A project paper submitted to the

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In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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CERTIFICATION

It is hereby certified that we have read this project entitled "Histopathological Evaluation in Gills, Kidney and Liver of Tilapia (Oreochromis spp.) from a Polluted River in Hulu Langat, Selangor", by Nor Azzalina Binti Moslim and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Akhir Tahun

PENILAIAN HISTOPATOLOGI INSANG, HATI, DAN GINJAL TILAPIA (*OREOCHROMIS* SPP.) DARI SEBUAH SUNGAI TERCEMAR DI HULU LANGAT, SELANGOR

Oleh

Nor Azzalina binti Moslim

2023

Penyelia: Prof. Madya Dr. Intan Shameha binti Abdul Razak

Penyelia bersama: Dr. Mohd Fuad Matori

Ikan di dalam air yang tercemar sering mengumpul logam berat di dalam tisu mereka terutamanya di insang, hati, dan ginjal, yang boleh menyebabkan perubahan patologi. Sungai Langat dipilih kerana Kelas III Indeks Kualiti Air, menunjukkan bahawa sungai tersebut agak tercemar sementara Tilapia (*Oreochromis* spp.) digunakan sebagai bioindikator pencemaran alam sekitar. Kajian ini bertujuan untuk mengenal pasti perubahan histopatologi di dalam insang, hati dan ginjal tilapia dari Sungai Langat dan untuk membandingkan sama ada terdapat perbezaan perubahan

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histopatologi antara tilapia dewasa dan tilapia muda. Lapan ekor tilapia ditangkap, ditimbang, dan dibunuh sebelum post-mortem. Sampel insang, hati, dan ginjal diproses secara sewajarnya sebelum pewarnaan H&E. Dua puluh bidang minat dipilih secara rawak setiap slaid, dan perubahan histopatologi dinilai secara sewajarnya. Data dikumpul dan analisis statistik ujian U Mann-Whitney pada $p<0.05$ dilakukan. Identifikasi mikroelemen kualitatif bagi sampel daging dan air dilakukan dengan menggunakan analisis *energy-dispersive X-ray* (EDX). Perubahan histopatologi pada insang dicirikan oleh pengangkatan epitel pernafasan, hiperplasia epitel lamelar, penggabungan beberapa lamela, dan disorganisasi lamela. Hati menunjukkan perubahan histopatologi dalam kehadiran Pusat Melanomakrofag (MMC), kehadiran pigmen, nekrosis, dan tumpuan darah, manakala di ginjal, terdapat kehadiran MMC, tumpuan darah, degenerasi selular, dan bahan dalam lumen. Berdasarkan analisis statistik, pengangkatan epitel pernafasan dan hiperplasia epitel lamelar di insang, kehadiran MMC, kehadiran pigmen, dan nekrosis di hati, serta kehadiran MMC dan tumpuan darah di ginjal menunjukkan perbezaan yang signifikan antara tilapia dewasa dan tilapia muda.

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 - Final Year Project

HISTOPATHOLOGICAL EVALUATION OF GILL, LIVER AND KIDNEY OF TILAPIA (*OREOCHROMIS* SPP.) FROM A POLLUTED RIVER IN HULU LANGAT, SELANGOR

By

Nor Azzalina binti Moslim

2023

Supervisor: Prof. Madya Dr. Intan Shameha binti Abdul Razak

Co-supervisor: Dr. Mohd Fuad Matori

Fish living in polluted water often accumulate heavy metals in their tissues mainly in the gills, liver and kidney, which can lead to pathological changes. Langat River was chosen due to Class III of the Water Quality Index (WQI), indicating that the river is slightly polluted while Tilapia (*Oreochromis* spp.) were used as bioindicators of environmental pollution. This study aimed to identify the histopathological changes in the gills, kidneys and liver of tilapia from Langat River and to compare whether there are histopathological changes differences between adult and

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juvenile tilapia. Eight tilapias were caught, weighed and euthanized before post-mortem. Gills, liver and kidney samples were processed accordingly before being stained with H&E staining. 20 fields of interest were randomly selected per slide, and histopathological changes were scored accordingly. The data was collected and the statistical analysis of the Mann-Whitney U test at $p<0.05$ was done. Qualitative microelement identification of the muscle and water samples was performed by using energy-dispersive X-ray (EDX) analysis. Histopathological changes in gills are characterised by the lifting of respiratory epithelium, hyperplasia of lamellar epithelium, fusion of several lamellar and lamellar disorganisations. The liver showed histopathological changes in the deposition of Melanomacrophage Centres (MMC), pigment deposition, necrosis and congestion while in the kidney, there was deposition of MMC, congestion, cellular degeneration and intraluminal discharge. Based on the statistical analysis, lifting of respiratory epithelium and hyperplasia of lamellar epithelium in gills, deposition of MMC, pigment deposition and necrosis in the liver, and deposition of MMC and congestion in kidney had a significant difference between adult and juvenile tilapias. Fusion of several lamellar and lamellar disorganisations in gills, congestion in the liver, and cellular degeneration and intraluminal materials in the kidney did not reveal any significant difference between the two groups. EDX analysis of muscles revealed the presence of Na, Mg, P, Cl, K, Nb, Pt, Cu and S in both groups. There was also a presence of Hf in the muscle sample of the adult fish, and Ca and Rb in the juvenile fish. Water samples indicated the presence of Mg, K, Ca, Cu, Al, Si, Fe, Ti and W. In conclusion, the tilapia from the Langat River were exposed to pollutants and demonstrated histopathological changes. In the adult group, changes in the liver were proven to be more significant, while no significant difference in the gills and kidneys between both groups.

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1.0 INTRODUCTION

Pollution has a negative impact on the quality and quantity of fish stocks by altering the physicochemical properties, sediments, and biological components (Zeitoun, 2014). According to Nikalje et al. (2012), the detrimental effects of pollutants may become apparent at the cellular or tissue level before observable changes occur in fish behaviour or external characteristics. Thus, examining the histology of fish can serve as one of the bio-monitoring methods for assessing aquatic pollution (Muñoz et al., 2015). Histopathological investigations have been employed to assess the impact of pollutants on fish health in the environment and to establish a cause-and-effect relationship between exposure to harmful substances and diverse biological reactions (Schwaiger et al., 1997).

On the other hand, fish are extensively utilized to evaluate the quality of the aquatic environment and can function as indicators of environmental pollution (Farombi et al., 2007). In this study, tilapia (*Oreochromis spp.*) are used as bioindicators of environmental pollution. Tilapia fish are an ideal species for assessment research because they can survive in adverse environmental conditions due to their physical resistance to disease and low respiratory demands, allowing them to tolerate low oxygen and high ammonia levels (Zhou et al.,1998).

Besides, the Langat River was chosen due to the Class III the of Water Quality Index. This classification necessitates extensive treatment for water supply purposes. Additionally, the river is burdened with pollution from heavy metals like iron (Fe), zinc (Zn), manganese (Mn), and cadmium (Cd), as highlighted by Basheer et al. (2017). Furthermore, there is no report on

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the histopathological changes due to heavy metal accumulation in Tilapia (*Oreochromis* spp.) in Langat River.

Hence, the objectives of this study are:

1. To identify the histopathological changes in the gills, liver and kidney of tilapia inhabiting the Langat River.
2. To compare the histopathological changes in the gills, liver and kidney between adult and juvenile tilapia inhabiting the Langat River.

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2.0 LITERATURE REVIEW

2.1 Langat River

Figure 1: Langat River

The source of the Langat River lies in the hilly region of Hulu Langat, and the Langat River basin spans the states of Selangor and Negeri Sembilan, along with the federal territories of Kuala Lumpur and Putrajaya, before ultimately flowing into the Strait of Malacca. In Malaysia, the Langat River holds significant importance, particularly in the Selangor region, as it provides drinking water to approximately one-third of the state's population (Ahmed et al., 2019). However, due to the rapid development near the Langat River, the quality of this stream has been severely harmed. According to the Interim National Water Quality Standards for Malaysia (INWQS), the Water Quality Index (WQI) for the Langat River is Class III, indicating that the river is slightly polluted and requires extensive treatment (Basheer, et al., 2017). The sources of the Langat River pollution are identified as industrial discharge (58%), domestic sewage from treatment plants (28%), construction projects (12%) and pig farming

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(2%). Besides, industrial and construction activities are the primary sources of heavy metal pollution in the river, with contaminants including Manganese (Mn), Zinc (Zn), Iron (Fe), and Cadmium (Cd). These pollutants pose a significant threat to the well-being of living organisms within the river ecosystem.

2.2 Tilapia (Oreochromis spp.)

Figure 2: Tilapia (*Oreochromis* spp.)

Tilapia, a popular freshwater fish species, has gained significant attention in the field of pollution monitoring due to its unique physiological and ecological characteristics. Researchers and environmental scientists have increasingly utilized tilapia in studies aimed at assessing the impact of various pollutants on aquatic ecosystems. One key advantage of tilapia is its versatility and adaptability to diverse environmental conditions, making it an ideal candidate for monitoring pollution in different water bodies, ranging from rivers and lakes to ponds and aquaculture systems (Fernandes et al., 2019). Moreover, tilapia possesses a remarkable ability to accumulate contaminants in its tissues, providing a reliable indicator of the extent of pollution in its habitat (Suresh et al., 2018). The bioaccumulation of pollutants, such as heavy metals and organic compounds, in tilapia tissues serves as a valuable biomarker for assessing environmental contamination levels over time. Additionally, tilapia's relatively high reproductive rate and short life cycle contribute to its suitability for long-term monitoring

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studies, allowing researchers to observe the cumulative effects of pollutants on aquatic ecosystems (Jeyasanta et al., 2020). According to Zhang et al. (2017), the use of tilapia in pollution monitoring not only assists in understanding the ecological effects of pollution but also offers important information about possible health hazards to humans from consuming infected fish from polluted water bodies.

2.3 Histology

2.3.1 Gills of fish

Gills are specialised respiratory organs in fish that facilitate the exchange of oxygen and carbon dioxide with the surrounding water. The efficiency of respiratory processes is influenced by factors such as water flow rate and oxygen availability (Perry et al., 2010). While their primary function is respiratory, gills also play a crucial role in various other physiological processes, such as osmoregulation, acid-base balance, and nitrogen excretion. Fish gills contribute significantly to osmoregulation, maintaining the internal ion balance necessary for cellular functions. Active ion transport mechanisms in the gill epithelium play a vital role in adapting to different salinity levels (Evans et al., 2005). Besides, the elimination of nitrogenous waste, primarily in the form of ammonia, occurs through the gills. The regulation of ammonia excretion is crucial for preventing toxicity and maintaining internal homeostasis (Wright and Wood, 2009).

The respiratory apparatus of fish comprises arciform, bony structures situated in the pharyngeal region. These structures, supporting two rows of paired filaments known as primary lamellae, extend laterally from the gill arches. Additionally, secondary lamellae, characterized by folds, project perpendicularly from the primary lamellae. The primary gill

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lamellae are enveloped by stratified epithelium, consisting of mucous cells, chloride cells, and pavement cells, and feature a central venous sinus. Squamous epithelium lines the secondary lamellae, where numerous capillaries are interspersed with pillar cells running parallel to the surface. Moreover, the secondary lamellae serve as the primary interface for functions such as gas exchange, ion regulation, maintenance of plasma pH, nitrogen excretion, and the transport of water to and from the surrounding environment (Evans et al., 2005).

2.3.2 Liver of fish

The liver in fish performs a multitude of essential functions crucial for maintaining physiological balance. Involved in metabolic processes, it regulates carbohydrate metabolism, including glycogen storage and blood glucose levels, as well as lipid metabolism, encompassing synthesis, storage, and mobilisation. Furthermore, the liver is essential for protein metabolism because it affects the regulation of amino acids and synthesis of new proteins. Serving as a detoxification hub, the liver employs enzymatic pathways to eliminate harmful substances. Bile production, a key function, aids in digestion and waste excretion. The liver is integral to the immune response, housing immune cells and participating in immunomodulatory functions. Furthermore, it collaborates with other physiological systems for environmental adaptations. These functions are supported by various studies, such as those by Wood and Grosell (2008), and Farrell and Richards (2009).

The parenchyma that made up the liver was enveloped in a thin, delicate capsule. Connective tissue branches emerge into the parenchyma, dividing it into irregular lobules of the liver. Parenchymal hepatocytes are radially arranged around a central vein in interconnecting laminae or cords of two cells thickness, narrow straight blood sinusoids arising

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from the central vein separating each lamina. Each hepatocyte contained a large, round, and peripherally located nucleus with euchromatin and a prominent dark nucleolus. A thin layer of connective tissue divides the pancreatic exocrine tissue, which is dispersed throughout the liver and arranged in an acinar arrangement. The spherical nucleus, which is basally positioned in the tall columnar exocrine cells has a noticeable dark nucleolus. Zymogen granules are in the apical ends of these cells. The eosinophilic apical cytoplasm and basophilic basal pole of pancreatic cells distinguish them from hepatic tissue, according to microscopical findings (Azab, 2012).

2.3.3 Kidney of fish

Fish, like other vertebrates, possess kidneys that play a crucial role in maintaining internal homeostasis by regulating water and electrolyte balance, excreting waste products, and controlling blood pressure. The primary functions of the fish kidney include filtration of blood, reabsorption of essential substances, and secretion of waste products.

The kidney is generally made up of several renal corpuscles with tubules and well-developed glomeruli. Tall columnar epithelial cells covering the proximal convoluted tubule have brush borders along the cell apices and basal nuclei. There was little to no brush border and the distal convoluted tubules were lined with large, rather clear columnar epithelial cells with central nuclei. The glomerulus is larger in diameter than the distal convoluted tubule, containing columnar epithelial cells with basal nuclei and no brush border.

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2.4 Heavy metals as pollutants

Heavy metals have been recognized as potent biological poisons due to their toxicity, persistence, bioaccumulation, and biomagnification. Since heavy metals are readily dissolved and carried by the water, they are rapidly taken up by aquatic biota. Furthermore, heavy metals constitute a core group of aquatic contaminants that cause mutagenicity, carcinogenicity, and cellular toxicity in animals due to their high toxicity. Additionally, the form in which metals exist in water has a major impact on how poisonous they are to fish. The ionic forms of metals or simple inorganic compounds are more toxic than complex inorganic or organic compounds.

Fish can absorb heavy metals through their skin, gills, or digestive tract when they consume contaminated food. In fish, metals are essentially absorbed and then carried to the organs and tissues through the bloodstream, where they accumulate. The concentration of heavy metals in fish tissues indicates how the animals were previously exposed to water and/or food, and it can also show how they are now coping before toxicity disrupts the ecological balance of aquatic population populations. Besides, heavy metals have the potential to disrupt the integrity of the physiological and biochemical pathways in fish, which are not only an important ecosystem component but also a food source.

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3.0 MATERIALS AND METHODS

3.1 Experimental fish

This study was approved by the Institutional Animal Care and Use Committee (IACUC, UPM) with AUP number UPM/IACUC/AUP-U003/2023. A total of eight tilapias were obtained from Langat River through a casting net.

3.2 Experimental design

The fish was euthanized by deep anaesthesia using 200 mg/L clove oil using the immersion technique followed by the pithing technique. Then, the fish was weighed and the body length and width were measured. Next, the fish were divided into 2 groups which were juvenile (<120g) and adult (>120g). Gill, kidney and liver were removed from the carcass through dissection. The samples were fixed in fixatives for 24 hours. For the gill, additional decalcification solutions were followed for 10 hours. The samples were then transferred into 70% alcohol for tissue preservation. Then, all the samples were taken from 70% alcohol and processed for histological observation by using the routine histological technique (H&E staining).

3.3 Fixation and decalcification

Before euthanising the fish, 10% buffered formalin solution, 20% buffered formalin solution and buffered formic acid solution were prepared. The buffered formic acid was used to decalcify the gill after being fixed in formalin solutions. Buffered formic acid was prepared according to Table 1.

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Table 1: Composition of buffered formic acid preparation

Sodium citrate	50g
Distilled water	250ml
88% Formic acid	125ml
Distilled water	125ml

Immediately following euthanisation, from a total of 8 fish, samples of gills, kidney, and liver were obtained. The samples were put inside a 20% buffered Formalin solution at 4° C for 4 hours before being transferred into a 10% buffered Formalin solution at room temperature for 20 hours. The gills samples were then transferred into buffered formic acid for 10 hours. Next, all the samples were transferred into 70% alcohol for 2 days for preservation.

3.4 Preparation of histological slides

Tissue processing involves the usage of an automated tissue processor where the samples undergo dehydration through 100% alcohol within a few cycles. The samples were then embedded at the base of the cassette mould with paraffin wax using a Leica EG1160 paraffin-embedded station. It was carefully embedded to make sure that the sample lies straight and level at the bottom of the cassette mould. Once the wax had cooled, it was trimmed to expose tissue and the sample embedded in paraffin wax was sectioned to get 4 um thickness using a Leica RM2235 microtome. The sectioned tissue was taken carefully using a glass slide and it was left dry at 40°C on the drying cabinet overnight. The glass slide was then stained

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with H&E and dried overnight. The glass slides were then mounted with Distyrene Plasticizer Xylene (DPX). After the glass slides were cleaned from excessive DPX, it was ready to be viewed under a microscope.

3.5 Histopathological observation and scoring

All the slides were viewed under the light microscope equipped with a digital camera (Image Analyzer (Olympus BX51) with each slide viewed by 20 fields. The parameters observed in the sampled gill, kidney and liver are listed in Table 2.

Table 2: Histopathological parameters for gill, kidney and liver.

Gill	Kidney	Liver
Lifting of respiratory epithelium	Melanomacrophage Centers (MMC)	Melanomacrophage Centers (MMC)
Hyperplasia of lamellar epithelium	Cellular degeneration	Pigment deposition
Fusion of several lamellar	Congestion	Congestion
Lamellar disorganisation	Intraluminal materials	Necrosis

Parameters in each organ were semi-quantitatively graded according to the severity which can be classified as none (0), mild (2), moderate (3) or severe (4). Modified method of Shackelford et al., (2002). The scoring method is summarised in Table 3.

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Table 3: Modified method of Shackelford et al., (2002).

Grade	Description
0	No histopathological changes
1	Histopathological changes occupy less than or equal to 30% of area
2	Histopathological changes occupy more than 30% but less than or equal to 60% of area
3	Histopathological changes occupy more than 60% of area

The average from all 20-field view scoring from each parameter was taken, recorded and analysed.

3.6 Energy-Dispersive X-ray (EDX) analysis

3.6.1 Water samples

Samples were collected from two points along the river using Scott's bottles labelled A1 and A2. After collecting the water samples, they were allowed to sit for two days to let the sediments settle at the bottom. The next day, the samples underwent centrifugation to separate and obtain the sediments. These water sediments were subsequently dried in an oven. Once dried, the sediments were submitted for EDX analysis.

3.6.2 Muscle samples

Muscle samples from two adult (A1, A2) and two juvenile (J1, J2) fish were collected. The muscle tissues were cut into smaller pieces and oven-dried at 60°C for 4 days. Once the muscle samples were dried, the tissues were ground. The ground muscle samples were sent for EDX analysis.

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4.0 RESULT

4.1 Energy-dispersive X-ray (EDX) analysis

4.1.1 Water samples

Three spectrums from each water sample were taken. In the case of sample A1, Spectrum 1 identified heavy metals including Mg, Al, Si, K, Ca, Fe, and Cu. Spectrum 2 displayed the same heavy metals, excluding Cu. Spectrum 3 indicated the presence of heavy metals consistent with Spectrum 1, along with Ti. For sample A2, Spectrum 1 showed the presence of Al, Si, K, Ca, Fe, and W. Spectrum 2 revealed the presence of Mg, Al, Si, K, Ca, Fe, and W. Spectrum 3 displayed the same heavy metal presence as Spectrum 1, including Ti.

4.1.2 Muscle samples

Three spectrums from each muscle sample were taken. In the case of sample A1, both Spectrum 1 and Spectrum 2 disclosed the presence of heavy metals such as Na, Mg, P, Cl, K, Nb, and Pt. However, Spectrum 3 showed the absence of Na while maintaining the presence of other heavy metals. For sample A2, Spectrum 1 exhibited the presence of Cl, K, Cu, Nb, Hf, and Pt, uniquely found in this spectrum. Spectrum 2 displayed the same heavy metals as Spectrum 1, except for Hf. Spectrum 3 revealed the presence of S, Cl, K, Nb, and Pt. In sample J1, Spectrum 1 showed Na, Cl, K, Ca, Rb, Nb, and Pt. Spectrum 2 revealed Na, Mg, P, Cl, K, Ca, Rb, Nb, and Pt. Spectrum 3 indicated the presence of Na, Mg, P, S, Cl, K, Ca, and Cu. Lastly, for sample J2, both Spectrum 1 and Spectrum 2 unveiled Na, P, S, Cl, K, and Cu, while Spectrum 3 indicated the same presence of heavy metals as the other spectra, along with Mg.

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4.2 Histopathology

4.2.1 Gills

Histopathological findings observed from gills of juvenile and adult tilapia were lifting of respiratory epithelium, hyperplasia of lamellar epithelium, fusion of several lamellar and lamellar disorganisation. For lifting of respiratory epithelium and hyperplasia of lamellar epithelium, the lesions were more severe in adult tilapia compared to the juvenile group. On the other hand, there was no obvious difference in terms of severity for the fusion of several lamellar and lamellar disorganisation between the two groups.

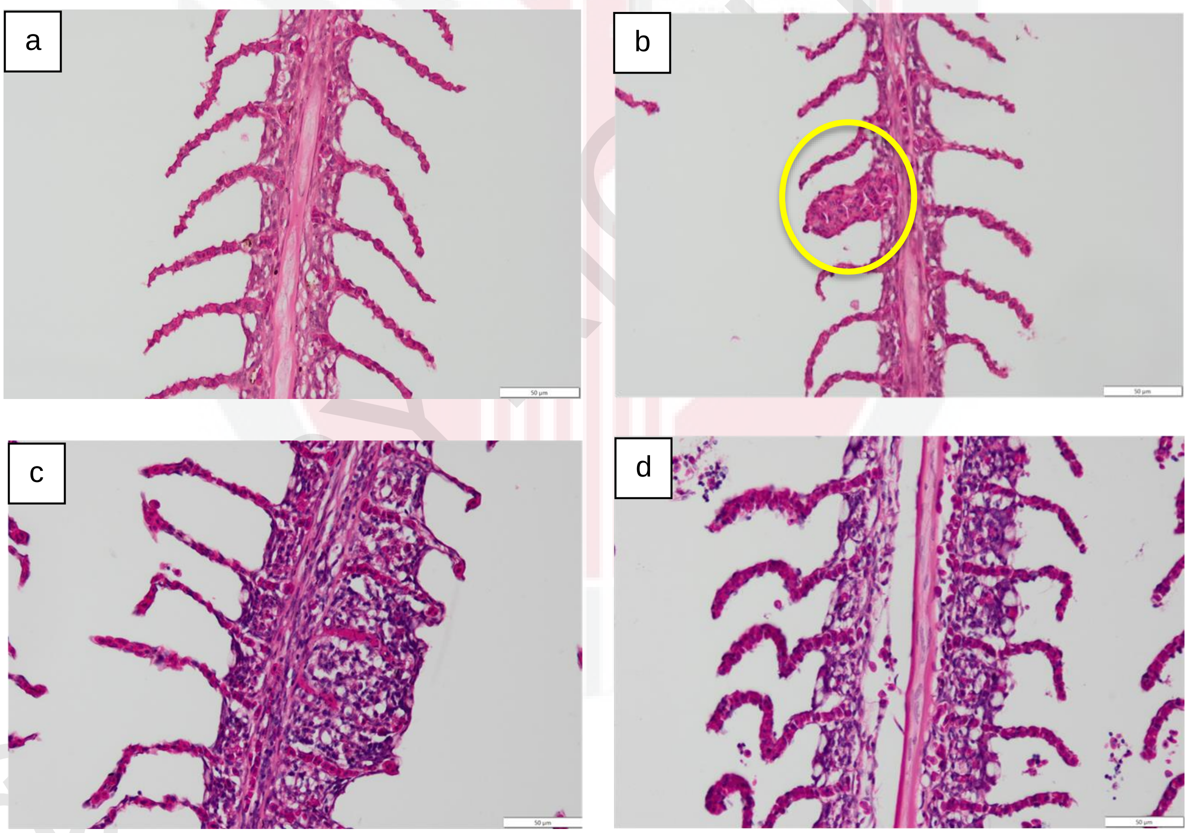

Figure 3: Photomicrographs of juvenile gills. (a) Normal histology of gills. (b) Mild hyperplasia of lamellar epithelium. (c) Fusion of several secondary lamellar. (d) The secondary lamellar are disorganised. (H&E stain, 200x magnification)

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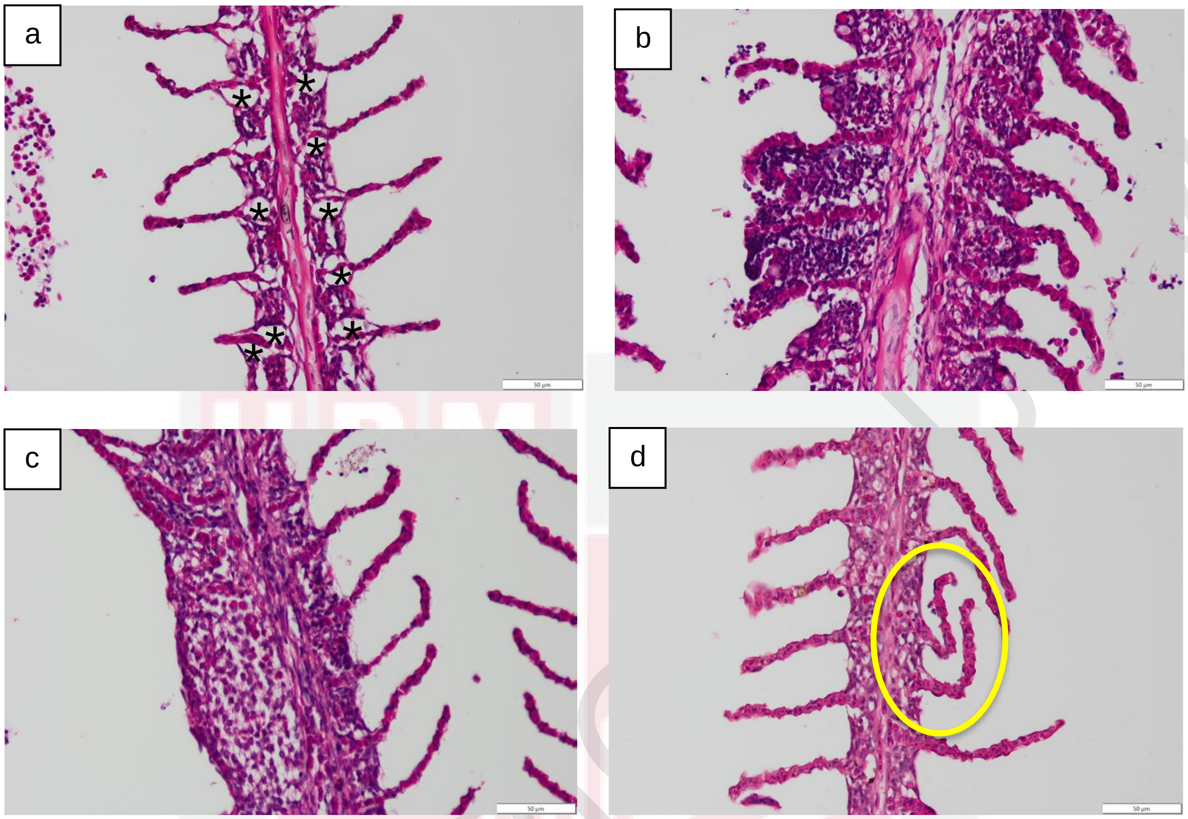

Figure 4: Photomicrographs of adult gills. (a) Lifting of respiratory epithelium between the secondary lamellar are shown by the asterisk (*). (b) Marked hyperplasia of lamellar epithelium. (c) Fusion of several secondary lamellar. (d) The secondary lamellar are disorganised. (H&E stain, 200x magnification)

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4.2.2 Liver

Histopathological findings observed from the liver of juvenile and adult tilapia were the presence of Melanomacrophage Centers (MMC), pigment deposition, congestion and necrosis. For the presence of MMC, pigment deposition and necrosis, the lesions were more severe in adult tilapia compared to the juvenile group. On the other hand, there was no obvious difference in terms of the severity of congestion between the two groups.

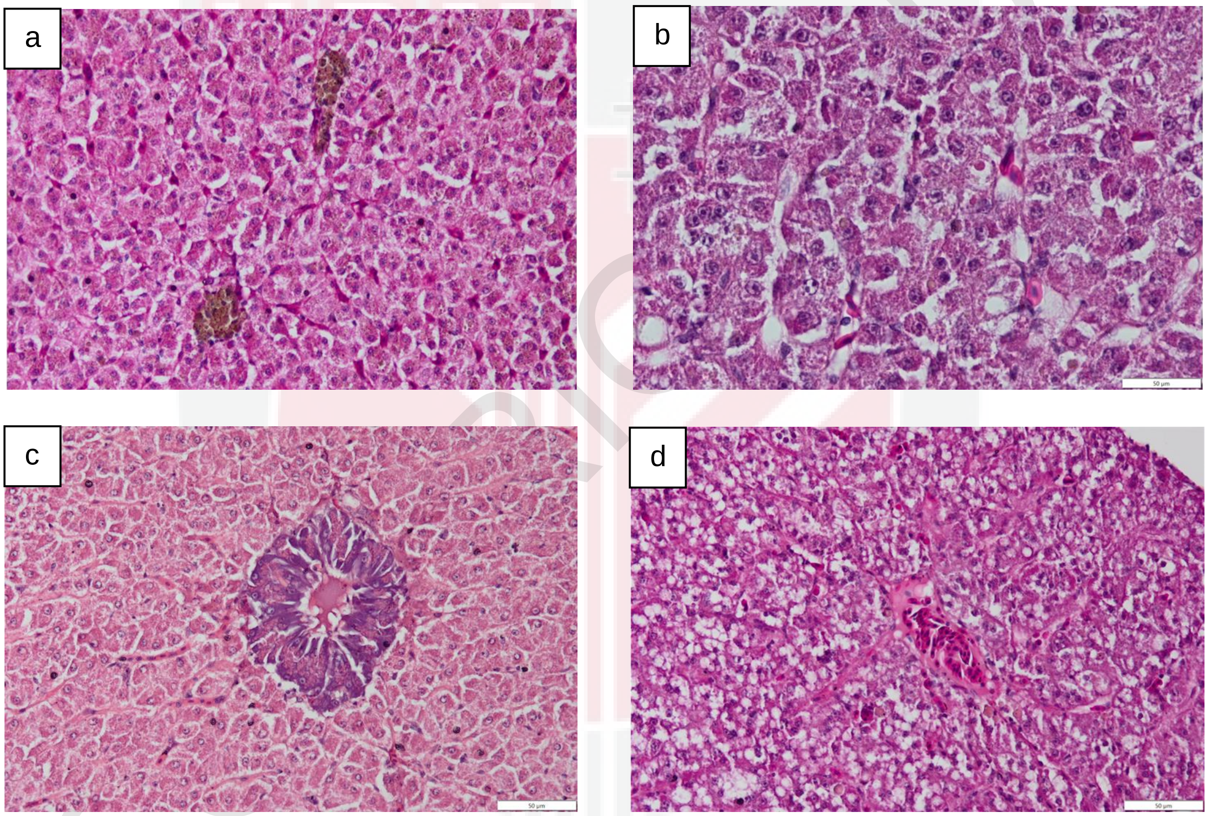

Figure 5: Photomicrographs of juvenile liver. (a) Presence of MMCs between hepatocytes. (b) (c) Normal hepatocytes of hepatocytes and pancreas. (d) Mild congestion in blood vessel. (H&E stain, 200x magnification)

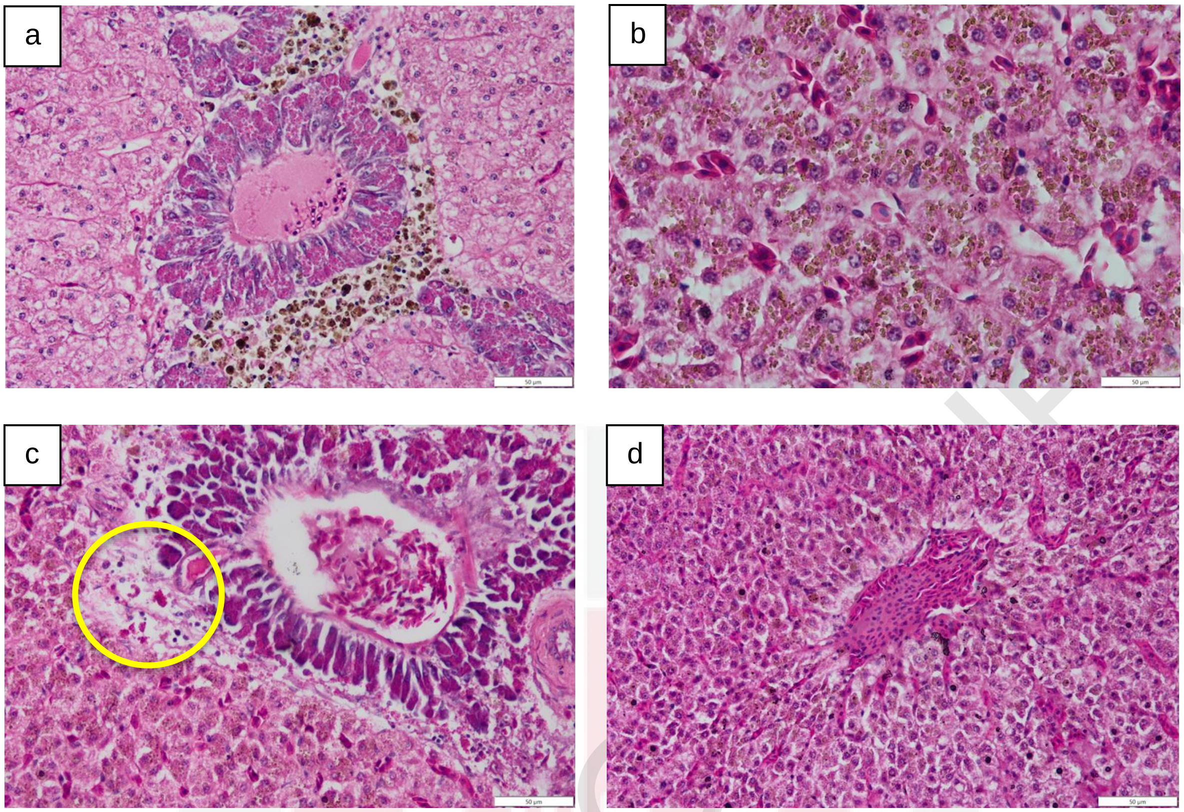

Figure 6: Photomicrographs of adult liver. (a) Presence of MMCs along the basal membrane of pancreas. (b) Diffuse pigments between hepatocytes (c) Focal necrosis area at basal membrane of pancreas. (d) Mild congestion in blood vessel. (H&E stain, 200x magnification)

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4.2.3 Kidney

Histopathological findings observed from the kidney of juvenile and adult tilapia were the presence of MMC, cellular degeneration, congestion and intraluminal materials. For presence of MMC, intraluminal materials and congestion, the lesions were more severe in adult tilapia compared to the juvenile group. On the other hand, there was no obvious difference in terms of severity the cellular degeneration between the two groups.

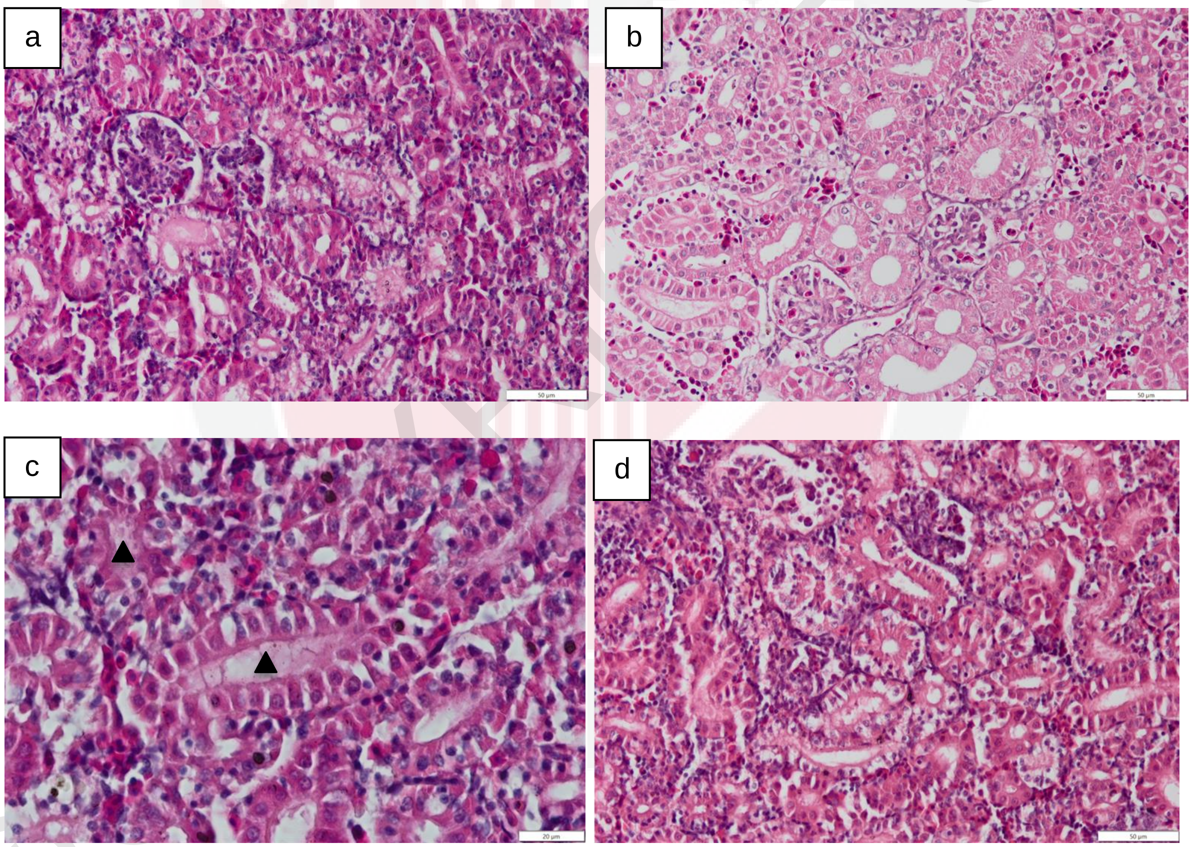

Figure 7: Photomicrographs of juvenile kidney. (a) (b) Normal histology of liver. (c) Presence of intraluminal materials in proximal and distal convoluted tubules shown by arrow heads. (d) Marked cellular degeneration. (H&E stain, 200x magnification)

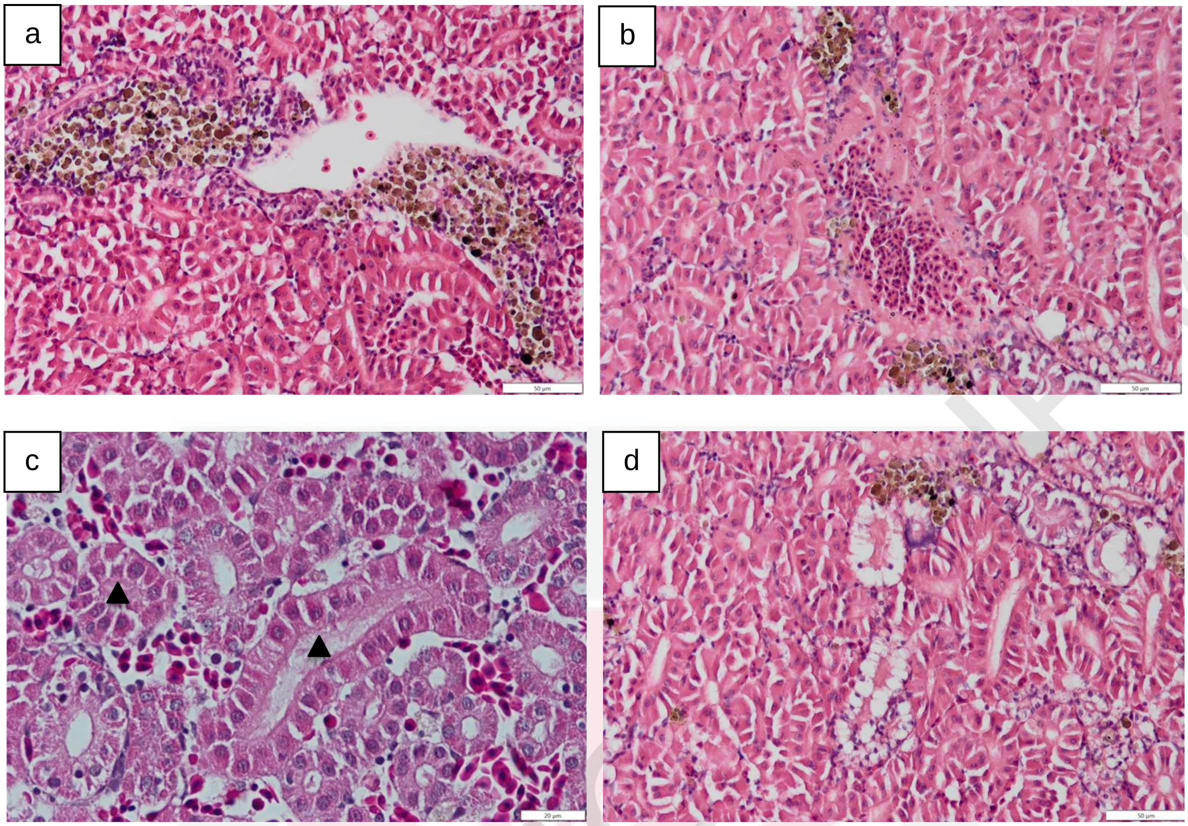

Figure 8: Photomicrographs of adult kidney. (a) Presence of MMC. (b) Marked congestion in blood vessel. (c) Presence of intraluminal materials in proximal and distal convoluted tubules shown by arrow heads. (d) Marked cellular degeneration. (H&E stain, 200x magnification)

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4.3 Statistical analysis

Table 4 shows the semiquantitative scoring done on the gills, liver and kidney. The scoring on the lesions was ranked based on Table 2. Statistical analysis between groups was done using Mann-Whitney U test in SPSS.

Organ	Histopathological Parameters	Group							
		Juvenile				Adult			
		J1	J2	J3	J4	A1	A2	A3	A4
Gill	Lifting of respiratory epithelium	0.1	0.3	0.3	0.2	1.1	1.2	0.9	0.5
	Hyperplasia of lamellar epithelium	0.8	0.3	0.6	0.6	1.4	1.5	0.8	1.1
	Fusion of several lamellar	2.0	0.2	0.2	0.1	1.5	1.1	0.4	0.8
	Lamellar disorganisation	0.3	0.5	0.2	0.5	0.6	0.6	0.3	0.5
Kidney	Presence of MMC	0	0.2	0.1	0	1.0	0.7	0.4	0.3
	Cellular degeneration	0.9	0.6	1.0	1.0	0.9	1.0	1.3	0.7
	Congestion	0.1	0.1	0.2	0.2	0.8	0.4	0.6	0.3
	Presence of intraluminal materials	0.8	1.1	1.0	1.0	2.2	2.0	1.6	1.1
Liver	Presence of MMC	0	0.3	0	0	0.6	0.6	0.3	0.3
	Pigment deposition	0	0	0	0	2.0	1.9	0.6	0.6
	Necrosis	0.1	0.2	0.2	0.1	0.3	0.3	0.4	0.3
	Congestion	0.1	0.1	0.2	0.1	0.8	0.8	0.3	0

Table 4: Semiquantitative scoring of gills, liver and kidney.

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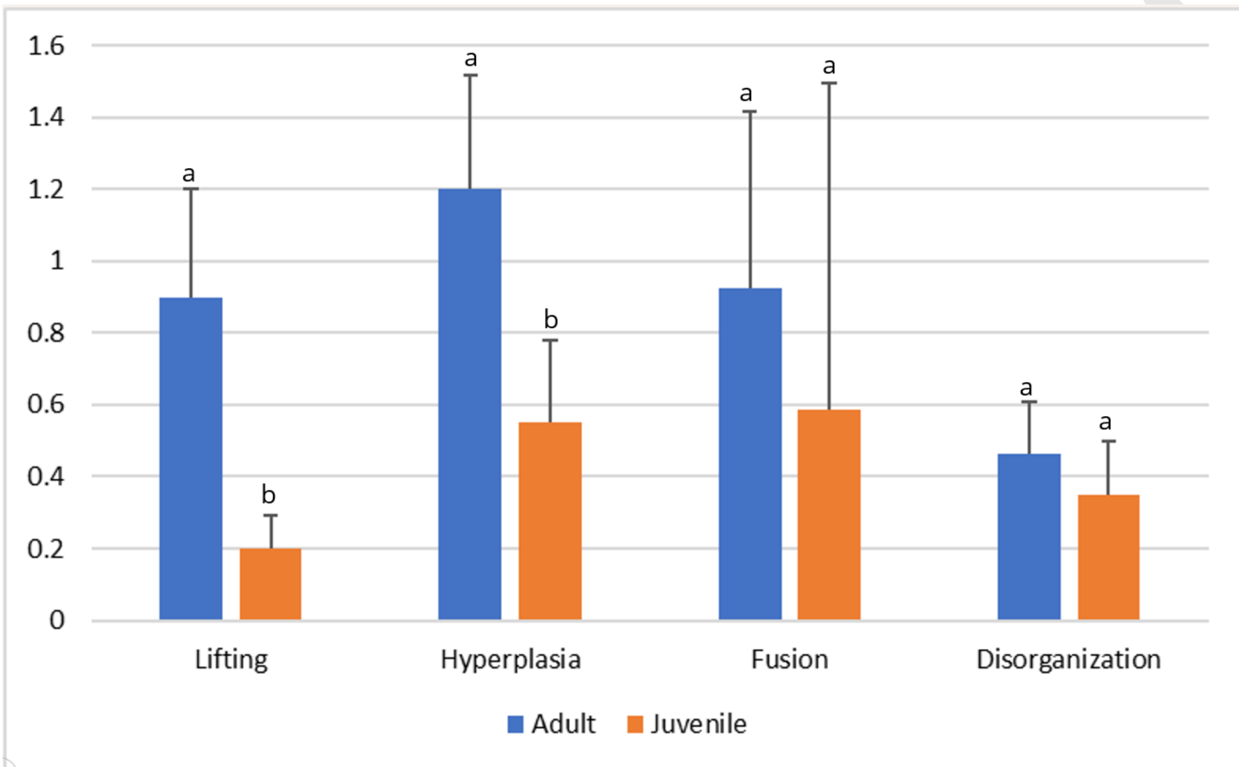

Figure 9: Histopathological scoring of gills for adult and juvenile tilapia. There is significant difference between juvenile and adult tilapia ($p<0.05$) for lifting of respiratory epithelium, hyperplasia of lamellar epithelium and fusion of several lamellar. There is no significant difference ($p>0.05$) for lamellar disorganisation.

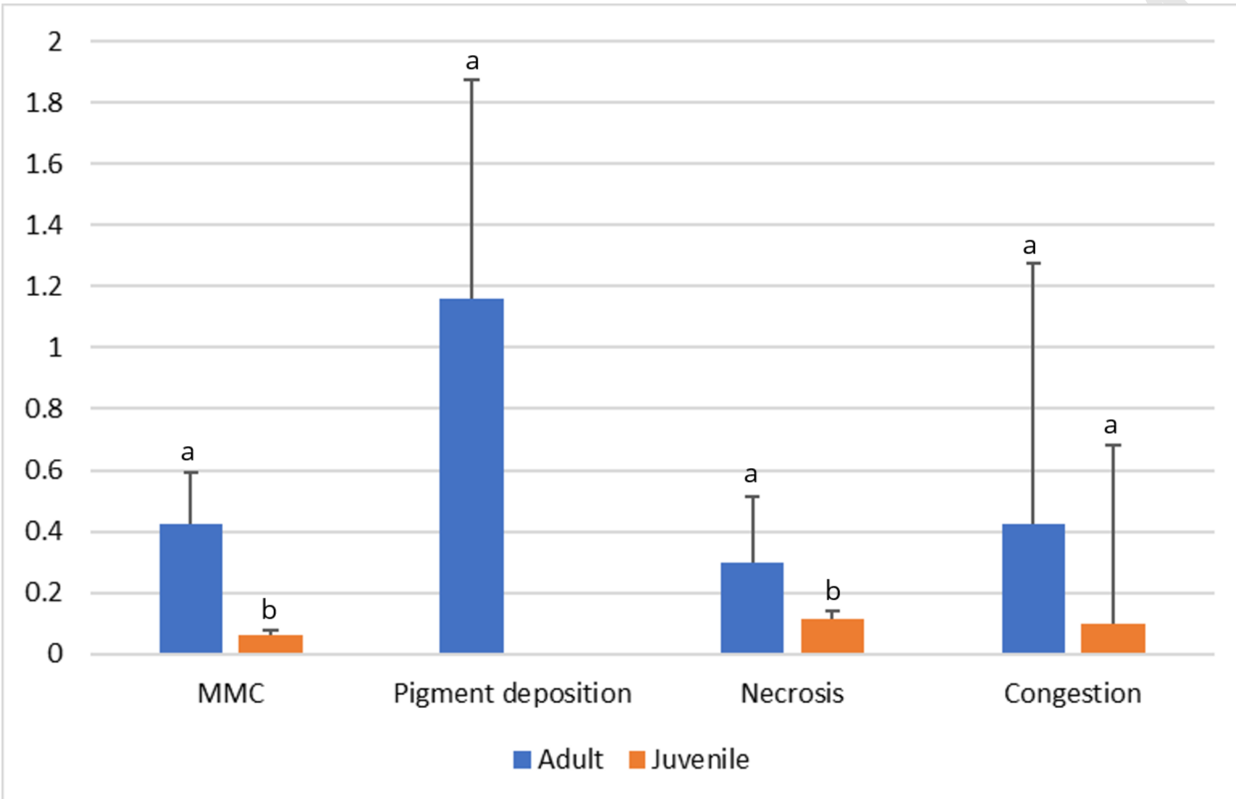

Figure 10: Histopathological scoring of liver for adult and juvenile tilapia. There is significant difference between juvenile and adult tilapia ($p<0.05$) for presence of MMC, pigment deposition and necrosis. There is no significant difference ($p>0.05$) for congestion.

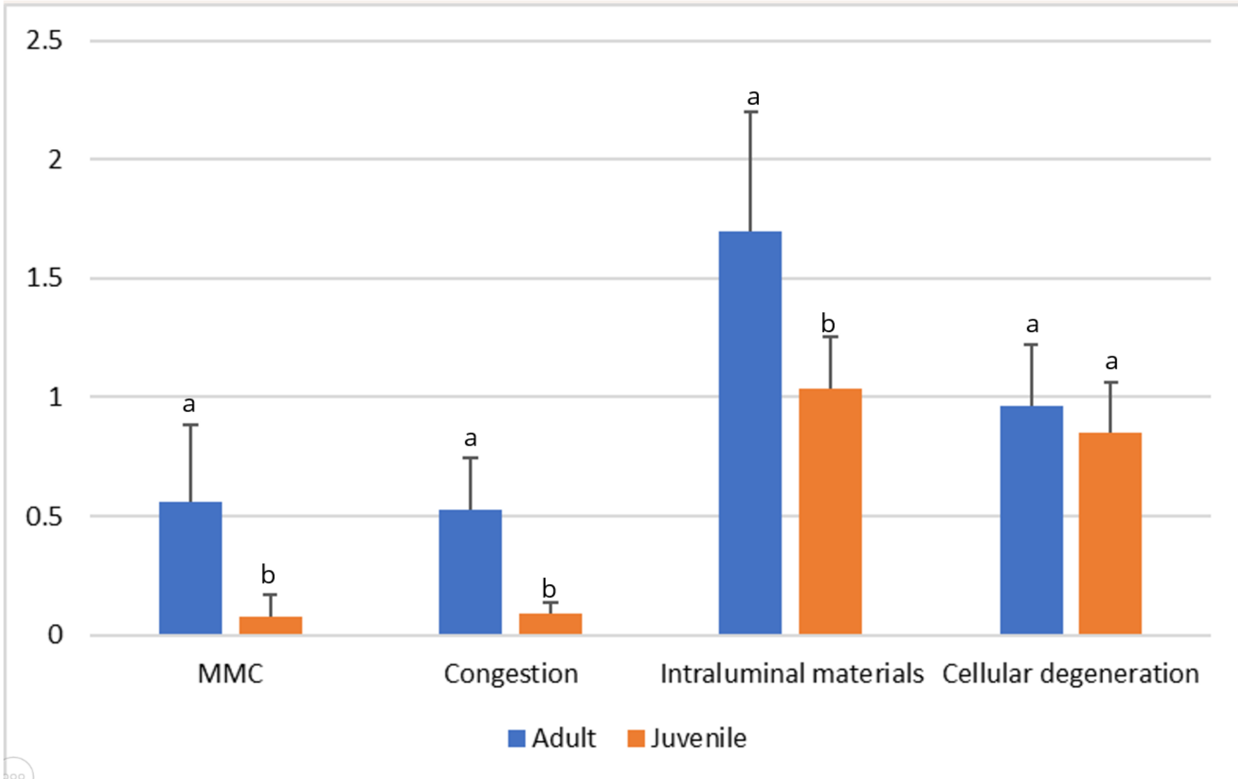

Figure 11: Histopathological scoring of kidney for adult and juvenile tilapia. There is significant difference between juvenile and adult tilapia ($p<0.05$) for presence of MMC, congestion and presence of intraluminal materials. There is no significant difference ($p>0.05$) for cellular degeneration.

5.0 DISCUSSION

Environmental pollution is a worldwide issue, with heavy metals being the most significant pollutants because of industrial expansion. Metals from the local environment are accumulated by aquatic species through their gills and skin, or through their digestive tract after consuming polluted food sources (Squadrone et al. 2013; Has-Schön et al. 2015). Metal bioaccumulation by fish and vital organs varies depending on the concentration of heavy metals in the water reservoir, the sensitivity of the fish, the age of the fish, the size of the fish, the physiological status of the fish, their habitat preference, feeding behavior, and rate of growth (Chapman et al., 1996). Furthermore, metal concentrations can be influenced by the extent of environmental contamination as well as the period of exposure.. Therefore, as organisms grow, it can be expected that larger or older individuals have accumulated higher metal concentrations than smaller or younger ones.

Fish living in polluted waters tend to accumulate heavy metals in their primary target organs mainly in the gills, liver and kidney. This accumulation, however, is dependent on their intake, storage, and excretion from the body. This is due to the fact that different metals accumulate in fish bodies in varying amounts due to differences in their affinities for fish tissue, rates of absorption, deposition, and elimination. This implies that larger levels of accumulation are anticipated for metals with high absorption and poor clearance rates in tissues. Metal pollution, on the other hand, has the potential to harm aquatic organisms at the cellular level and disrupt ecological balance (Kouba et al. 2010; Has-Schön et al. 2015). Therefore, the effect of heavy metals can be recognized via histopathological evaluation.

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Histopathological changes have been extensively employed as biomarkers for assessing the health state of fish exposed to pollutants in both field and lab research. The ability to examine specific target organs, such as the liver, kidney, and gills, which are in responsible for respiration, excretion, and the biotransformation of xenobiotics in fish, is one of the biggest benefits of using histopathological biomarkers in environmental monitoring. Moreover, changes in these organs are typically simpler to recognize than functional ones and act as warning indicators of harm to the health of the animal. In order to sustain a sustainable fishery and ensure the safety of the product for human consumption and health, biomarkers of contaminant exposure in fish species are crucial indicators. In this study, histopathological evaluation was done on gills, liver and kidney.

Gills

Fish gills are a highly recognized target organ because they are the first to respond to adverse environmental circumstances (Lemke and Mount, 1963). Fish gills are particularly sensitive because of their constant interaction with the water and the secondary epithelium's great surface area, which is less protected than the skin and mouth. Consequently, many pollutants come in contact with gill epithelium and cause injury. One of the first injuries discovered in fish is the lifting of the respiratory epithelium. It is characterized by the secondary lamella’s lining epithelium being displaced, which leads to the formation oedema. This is associated with the presence of chemical pollutants. As a result of the epithelium lifting, the distance between the water and the blood increases, reducing oxygen uptake. In these conditions, however, the fish increase their rate of respiration to compensate for the low oxygen uptake (Fernandes and Mazon, 2003). These histopathologic lesions, according to

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Winkaler et al. (2001), show that the fish respond to the effects of harmful pollutants present in the water and sediment.

Gill epithelial hyperplasia may be produced by an increase in cellular metabolism, which results in an imbalance of osmotic control by compromising ionic active transport (Mazon et al., 2002). Hyperplasia causes the proliferation of adjacent lamellae cells, limiting the inter-lamellar space and potentially causing lamellar fusion. According to Thiyagarajah et al. (1996), changes such as hyperplasia of the mucosal cells, hyperplasia of the chloride cells, and proliferation of the epithelial cells occur because of pathogenic condition or due to the presence of pollutants.

Liver

The liver is the essential organ for the metabolism and detoxification and is also one of the organs most affected by contaminants in water (Crestani et al., 2007) and due to these functions combined with its location and access to the blood supply, it is one of the organs most affected by water contaminants (Camargo and Martinez, 2007).

Melano-macrophage centres (MMC), also known as macrophage aggregates, are unique collection of pigment-containing cells within the tissues. MMCs are darkly pigmented due to high lipofuscin, melanin, and hemosiderin content, making them histologically distinguishable via light microscopy. They have a role in the biotransformation of xenobiotics and are found in the pancreas, liver, and kidney. According to Wolke et al. (1985), there is a possibility that environmental changes are the reason behind the increase in MMCs' quantity, area, and pigment content, which suggests that MMCs could be useful as possible biomarkers for monitoring healthy wild fish populations. Hence, MMC has been proposed as a valid

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biomarker for water quality in terms of both deoxygenation and iatrogenic chemical contamination, and it increases in size or frequency in conditions of environmental stress.

Furthermore, an analysis conducted by Soufy et al. (2007) suggested that the direct toxic effects of pollutants on hepatocytes could be responsible for necrotic foci, thrombosis formation in central veins, dilatation and congestion in blood sinusoids, and enhanced vacuolar degeneration in hepatocytes.

Kidney

Heavy metal accumulations in the kidney, according to one study, could disrupt the organ's detoxifying system and produce histopathological abnormalities. According to Takashima and Hibiya (1995), occlusion of the proximal and distal tubules can result from swelling of the epithelial cells as well as the accumulation of certain materials in the lumen.

Heavy metals

One of the heavy metals detected in the muscle of adult and juvenile tilapia is Copper. Copper is an important trace metal and micronutrient for cellular metabolism in organisms since it is a component of metabolic enzymes. Copper is a non-biodegradable substance that cannot be broken down once it enters the cell. Copper is classified as a potentially hazardous heavy metal in relatively high quantities since Ajani et al (2010) showed that it is a heavy metal with a density more than 5 g/cm3. Copper is often consumed by fish for metabolism activities; however, if the fish are exposed to a higher concentration for an extended length of time, it becomes harmful. The gills are the first organ that accumulate heavy metals at concentrations greater than the harmful level due to absorption along the gill surface and gut tract wall (Annabi

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et al., 2013). The copper is subsequently disseminated and bioaccumulated in the fish's vital organs and biological systems, including the liver, spleen, and kidney, via the blood. According to Khabbazi et al. (2015), copper absorption may result in epithelial hypertrophy, hyperplasia, lamella fusion, lamella aneurysm, and oedema on the gills of rainbow trout. Sublethal Cu exposure in Nile tilapia (*Oreochromis niloticus*) has also been linked to histological changes in the gills (oedema; vasodilation of the lamellar vascular axis) and livers (vacuolation and necrosis) (Pieterse, 2004).

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6.0 CONCLUSION

In conclusion, EDX analysis of the muscle and water samples showed the presence of heavy metals and there are histopathological changes in the gills, kidney and liver of tilapia (*Oreochromis spp*.) from a polluted river in Hulu Langat, Selangor. Besides, there is a significant difference in the histopathological changes of the liver between adult and juvenile, but not in the gills and kidney.

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7.0 RECOMMENDATIONS

It is recommended to evaluate water quality parameters. This comprehensive approach will strengthen the correlation between observed tissue abnormalities and specific water quality parameters, contributing to a more holistic understanding of the environmental stressors impacting the Tilapia population. Furthermore, expanding the sample size is crucial for obtaining statistically significant results and ensuring the generalizability of findings. Increasing the number of fish specimens collected from the polluted river in Hulu Langat will enhance the study's reliability and statistical power, allowing for more robust conclusions regarding the impact of pollution on Tilapia health. Lastly, to capture the variability in pollution levels across the Langat River, it is advisable to diversify the sampling sites by selecting different locations along the river.

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