HISTOLOGICAL CHANGES IN THE GILLS OF *OREOCHROMIS* SP. GUNTHER, 1889 FROM NYAUNG KAING IN (LAKE), MONYWA TOWNSHIP

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Abstract

The histological changes found in the gills of the *Oreochromis* sp. were investigated from June 2018 to July 2019, in Nyaung Kaing In (Lake), Monywa Township. The seasonal histological investigation in the gills of *Oreochromis* sp. revealed abnormalities such as curling and club shaped of lamellae, incomplete and complete fusion of lamellae, shortening of lamellae, degeneration and desquamation of lamellae, dilation of gill filaments and arches, hyperplasia and hypertrophy of gill filaments, separation of gill filaments, elastic cartilage, haemorrhage of gill filaments**,** edema of gill arch, blood congestion of gill filaments and arches, cysts with parasites in gill filaments, aneurysms and epithelial lifting were observed. Physicochemical parameters of water quality analyzed seasonally revealed that parameters such as dissolved oxygen, total alkalinity, and ammonia nitrogen were beyond the acceptable limits in some seasons of study period .The other parameters showed within the normal range. Biochemical oxygen demand was higher than the permissible limit during three seasons. Heavy metals such as arsenic, copper, cadmium and lead values analyzed seasonally within the permissible limits.

Keywords: *Oreochromis* sp., gills, histological changes, Nyaung Kaing In (Lake)

Introduction

All living organisms attain ability to adapt themselves to change in the environment such as temperature, humidity, oxygen supply or toxicant exposure. There are wide varieties of toxicants present in the environment in the form of metals, nanoparticles, pesticides, insecticides etc. Such toxicants may reach water bodies such as freshwaters, rivers, lakes or streams in a variety of ways. Thus, the pesticides on reaching the aquatic ecosystem greatly influences the non-target organisms, especially fish (Helfrich, 2009).

Fish gills comprise a large part of fish body that contacts the external environment and play an important role in the gas and ion exchange between the organism and environment (Oliva *et al.*, 2009). The gill surface is more than half of the entire body surface area. In fish the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus the branchial function is very sensitive to environmental contamination (Cengiz, 2006).

Fishes are considered to be the most significant biomonitors in aquatic systems for the estimation of metal pollution level; they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats. In addition fish were located at the end of the aquatic food chain and may accumulate metals and pass them to human being through food causing chronic or acute diseases. Studies from the field and laboratory works accumulation of heavy metals in a tissue on water concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect significantly into fish (Authman, 2015).

Fish are relatively sensitive to change in their surrounding environment including an increase in pollution. Fish health may reflect and give a good indication of the health status of a specific aquatic ecosystem. Fish are widely used to evaluate the health of aquatic ecosystem and

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their physiological changes serve as biomarkers of environmental pollution (Kock *et al.*, 1996).

Along the connecting channel of Chindwin River into the Nyaung Kaing In (Lake), cultivated paddy fields appeared during the hot season. Pesticide usage on the paddy fields may reach the water body and have impact on aquatic organisms including fish. Therefore, it is required to examine whether the pollution of water has affected on the organs of some fish. Because, the quality of water may be considered very important to maintain the health of aquatic biota and human population health. The present work was undertaken with the following objectives:

- to determine the histological changes in the gill tissues of selected fish species
- to investigate the physicochemical parameters and heavy metal contents in the water of Nyaung Kaing In (Lake)

Materials and Methods

Study area

Fish specimens were collected from Nyaung Kaing In (lake), Monywa Township and situated between 22°5'22"- 22°6'2"N and 95°8'45"- 95°9'24"E and covered an area of 27.44 hectares, (Plate 1).

Study period

The study period was from July 2018 to June 2019.

Collection of fish specimen

The *Oreochromis* sp. was selected for this study based on the availability throughout the years. Fishes were collected seasonally and at least five specimens of selected species were preserved in 10% formalin solution for identification and histological studies.

Measurement of fishes

Total length and body weight of fish specimens were measured in the range of (17.8-26.5 cm) and (200 - 280 g) respectively during the study period.

Histological procedures

Collected fishes were dissected directly in the field. After dissection of fish samples, parts of gill were carefully taken out. Gill tissues were preserved in Bouin's solution in glass bottle for histological studies. The collected fish were brought to the Department of Zoology, University of Mandalay for further studies. Histological procedures generally include the following steps; (1) tissue processing, (2) embedding into paraffin wax, and (3) sectioning by microtome, (4) mounting onto the glass slide and (5) staining. The gill tissues were sectioned at 5-7µm thickness by a rotary microtome.

Water analysis

The water samples were collected in dark bottle (one-liter capacity) with no airspaces. The collection was made on three seasons (rainy season, cold season and hot season) during the study period.

The analysis of water was conducted seasonally at Laboratory of Water and Sanitation Department, Mandalay City Development Committee (MCDC) for determination of physicochemical parameters such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total alkalinity, ammonia nitrogen, nitrite nitrogen and nitrate were analyzed in Freshwater

Aquaculture Research Yangon, Department of Fisheries. Heavy metals such as copper, arsenic, lead, and cadmium were examined in Department of Research and Innovation, Yangon. Water temperature was examined directly at field by thermometer.

(**Source:** Google Earth, 2020)

Plate 1 Location map of the study area

Results

In the present study, *Oreochromis* sp. from Nyaung Kaing In (Lake), Monywa Township was examined to determine the histological changes of gills (Plat 2).

Seasonal histological changes in the gill tissues of *Oreochromis* sp.

Histological changes observed during the rainy season in the gill tissues of *Oreochromis* sp. included curling of lamellae, club-shaped lamellae, incomplete and complete fusion of lamellae, shortening of lamellae, degeneration and desqumation of lamellae, dilation of gill filament and gill arch, hyperplasia and hypertropy of gill filament, separation of lamellae, elastic cartilage of lamellae, haemorrhage in gill filament, edema of gill arch, blood congestion of filaments, epithelial lifting, aneurysms were observed (Table 1, Plate 3).

Histological changes in the gill tissues of *Oreochromis* sp. during the cold season showed the same as found in the rainy season and the hypertropy of gill filament and epithelial lifting were not observed in the cold season. Cyst with parasites in gill filament occurred during this season only (Table 1, Plate 3).

Histological changes in the gill tissues of *Oreochromis* sp. during the hot season are the same as observed in the rainy and cold season. The complete fusion of lamellae, cyst with parasites in gill filament were not observed in the hot season. Epithelial elastic cartilage was mostly found during the hot season and edema of gill filaments at the top of epithelial lifting was also found during this season (Table 1, Plate 3).

Water parameters of Nyaung Kaing In (Lake)

The water parametrs and heavy metal concentrations of Nyaung Kaing In (Lake) was analysed seasonally during July 2018 to June 2019.

Water parameters value of standard for Aquaceclture (Bhatnagar and Devi, 2013) and permissible limits of heavy metals concentration of WHO (2011) standards were also described (Table 2, Fig. 4.1).

Table 1 Seasonal histological change in the gill tissues of *Oreochromis* sp. from Nyaung Kaing In (Lake)

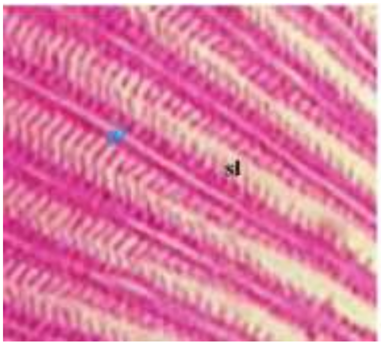
Sr no.	Histological changes	Rainy	Cold	Hot
1	Curling of lamellae	+	+	+
2	Club shaped lamellae	+	+	+
3	Incomplete fusion of lamellae	+	+	+
4	Complete fusion of lamellae	+	+	-
5	Shortening of lamellae	+	+	+
6	Degeneration of lamellae	+	+	+
7	Desquamation of lamellae	+	+	+
8	Dilation of gill filaments	+	+	+
9	Dilation of gill arch	+	+	+
10	Hyperplasia of gill filaments	+	+	+
11	Hypertropy of gill filaments	+	-	+
12	Separation of lamellae	+	+	+
13	Elastic cartilage of lamellae	+	+	+
14	Haemorrhage in gill filaments	+	+	+
15	Edema of gill arch	+	+	-
16	Blood congestion of filaments	+	+	+
17	Aneurysms (telangiectasia)	+	+	-
18	Cyst with parasites in gill filaments	-	+	-
19	Edema of gill filaments at the top	+	+	+
20	Epithelial lifting	+	-	+

+ = observed, - = Not observed

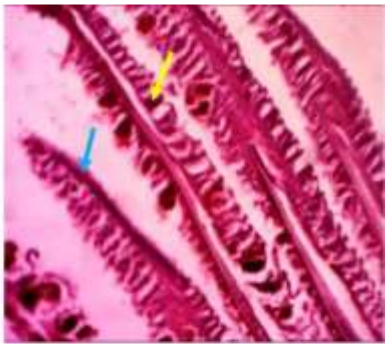
Table 2 Water parameters and heavy metal contents of water from Nyaung Kaing In (Lake) during study period

Physicochemical parameter and heavy metals	Rainy season			Cold season			Hot season			Standard for Aquaculture (Bhatnagar and Devi, 2013)			WHO (2011)
	Aug	Sep	Mean	Dec	Jan	Mean	Apr	May	Mean	Acceptable range	Desirable range	Stress range	
Water temperature (ºC)	32°C	30°C	31°C	22°C	17°C	19.5°C	34°C	35°C	34.5°C	15-35	20-30	<12, >35	
pH	7.1	7.1	7.1	7.1	7.5	7.3	8.1	8.1	8.1	7-9.5	6.5-9	<4, >11	
Dissolved oxygen (DO) (mg/L)	4.35	2.62	3.485	5.83	3.94	4.885	0.94	2.45	1.695	3-5	5	<5, >8	
Biochemical oxygen demand (BOD) (mg/L)	9.80	12	10.9	25	28	26.5	28	30	29	3-6	1-2	>10	
Total alkalinity $CaCO_3$ (mg/L)	160	160	160	296	328	312	420	400	410	50-200	25-100	<20, <300	
Ammonia nitrogen (mg/L)	0.136	0.110	0.123	0.069	0.032	0.05	0.144	0.370	0.26	0-0.05	0-<0.025	>0.3	
Nitrite nitrogen (mg/L)	0.399	0.370	0.38	0.048	0.016	0.03	0.033	0.107	0.07	0.02-2	<0.02	>0.2	
Nitrate nitrogen(mg/L)	0.136	0.122	0.13	0.036	0.044	0.04	0.101	0.25	0.18	0-100	0.1-4.5	>100, 0.01	
Arsenic (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01				0.05
Copper (mg/L)	0.018	0.014	0.016	0.012	0.010	0.011	0.040	0.022	0.031				2.0
Cadmium (mg/L)	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008				0.01
Lead (mg/L)	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015				0.05

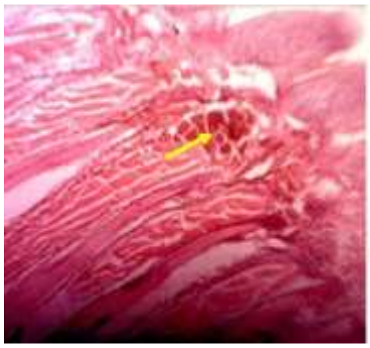
Plate 2 *Oreochromis* sp.



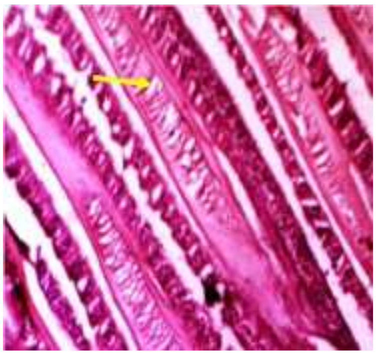
A. Normal gill structure
(pl = primary lamellae)
(sl = secondary lamellae)



B. Aneurysm (yellow arrow) and degeneration lamellae (blue arrow) (100x)

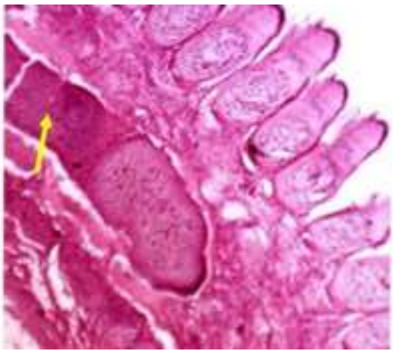


C. Blood congestion at the base of filament (400x)



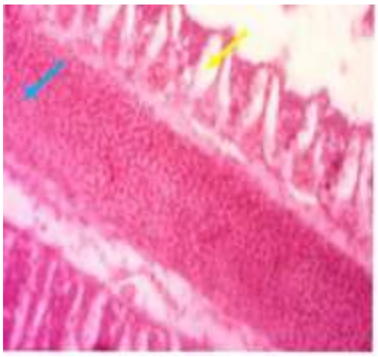
D. Elastic cartilage of gill filament (100x)

E. Complete fusion at the top lamellae (blue arrow) and elastic cartilage (yellow arrow) (100x)

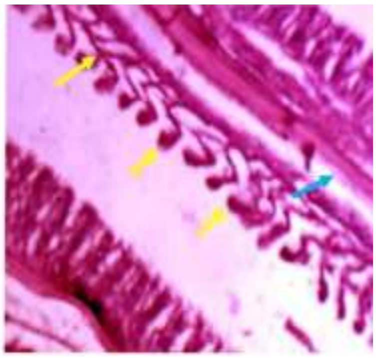


F. Haemorrahage in gill arch (400x)

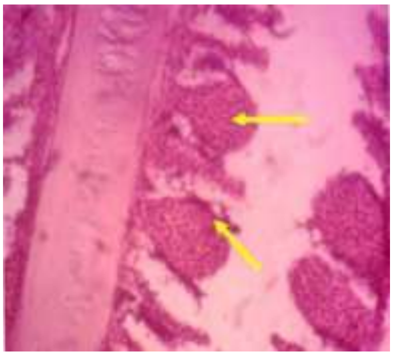
Plate 3 Histological changes in the gill tissues of *Oreochromis* sp



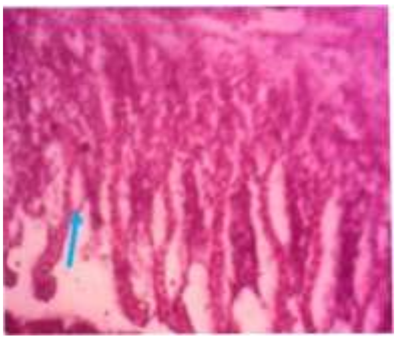
G. Blood congestion (blue arrow) and dilation of gill filament (yellow arrow) (400x)



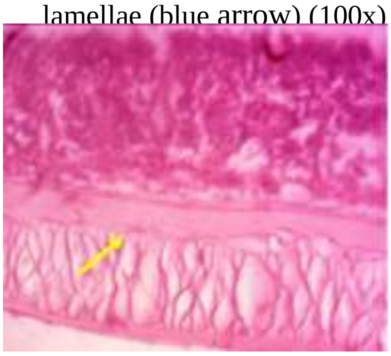
H. Club-shaped lamellae (yellow arrows) and separation of lamellae (blue arrow) (100x)



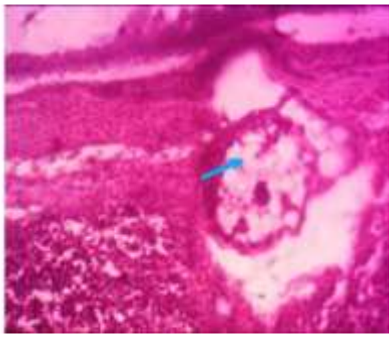
I. Aneurysms (telangiectasia) (400x)



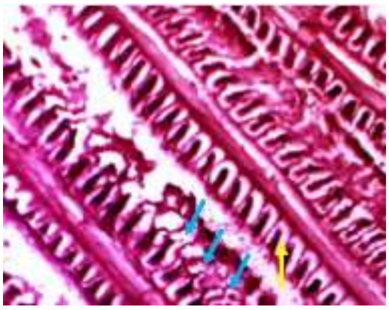
J. Epithelium lifting (400x)



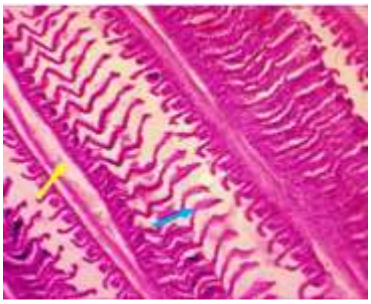
K. Elastic cartilage of primary lamella (400x)



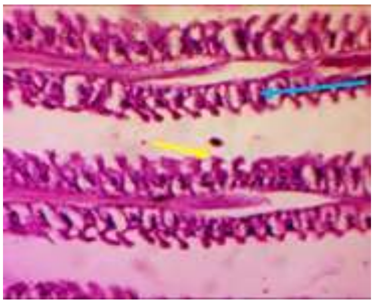
L. Cyst with parasite in gill filament (400x)



M. Hyperplasia of lamellae (blue arrows) and edema of lamellae (yellow arrow) (100x)



N. Desquamation of lamellae (yellow arrow) and curling of lamellae (blue arrow) (100x)



O. Club shaped lamella (yellow arrow) and curling (blue arrow) (100x)

P. Bulging at the tip of lamellae (black arrow) and elastic cartilage (yellow arrow) and lifting (blue arrow) (400x)

Plate 3 Continued

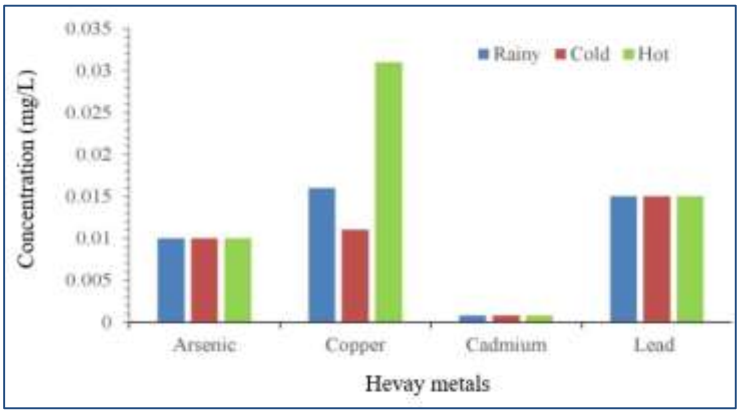


Figure 1 Heavy metals contents in the water of Nyaung Kaing In (Lake), Monywa Township during different seasons

Discussion

Histological changes in fish gills have been increasingly studied as bioindicators for assessing aquatic contamination in environmental monitoring studies (Fricke *et al.,* 2012). In the present study histological changes in the gills of *Oreochromis* sp. was investigated during July 2018 to June 2019 in Nyaung Kaing In (Lake), Monywa Township.

Histological changes in the gill tissues of *Oreochromis* sp. during three seasons were observed. The present findings are in agreement with Fernandes and Mazon (2003) who reported that, the major changes in fish are hypertorpy and hyperplasia of the epithelial cells, partial fusion of some secondary lamellae, lamellar aneurysm, beside epithelial lifting and edema. This may be early responses of the gill to the harmful substances. These alterations are examples of defense mechanisms because the lifting lamellar epithelium and edema increased the distance between external environment and the blood, thus serving as a barrier to the entrance of contaminants. Lwin Mar Oo (2017) studied the histological changes in the gills of *Oreochromis* sp. Tilapia was infected with myxosporean parasites in Taungthaman in (Lake), Amarapura Township. In the present study cyst with parasites in the gill filament occurred during the cold season only. The present histological changes characters were similar with myxosporean parasites studied by Lwin Mar Oo (2017).

Hughes and Perry (1976) reported the mild hyperplastic condition of the cell which is an indication of stress due to soap and detergent contamination. In the present study, the most occurrences of characters were hyperplasia of secondary and primary lamellae in examined fish species during the whole study period. This may be assumed that the study area is situated nearest the urban dwelling.

The water sample analysis was made on three seasons (rainy season, cold season and hot season) during the study period. Based on the water quality of Nyaung Kaing In (Lake) analyzed with respect to seasons, pH can affect fish health. According to standard of Bhatnagar and Devi (2013), the results of observation range (7.1, 7.3, 8.1) during three seasons are the suitable pH values for freshwater fishes.

Dissolved oxygen affects the growth, survival, distribution, behavior and physiologyof shrimps and other aquatic organisms (Solis, 1988). In the present study, mean values of the dissolved oxygen (3.485, 4.885, 1.695) mg/L were observed according to seasons. According to results of Bhatnagar and Devi, 2013, the hot season was also stressed but the rainy and the cold seasons are in the desirable range. In the present result of BOD based on season, (10.9, 26.5, 29) mg/L were observed. According to Bhatnagar and Devi (2013), the present results of BOD are higher than the permissible limit during the three seasons. Mallat (1985) recorded several alterations are non-specific and may be induced by different types of contaminant. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. The present study agreed with Mallat (1985) according to the results of DO and BOD. In the present study, the seasonal mean values of total alkalinity (160, 312, 410) mg/L were recorded. The rainy season of total alkalinity was attained within the acceptable range and in cold and hot seasons were higher than the permissible limit according to Bhatnagar and Devi (2013).

In the present study, the seasonal mean values of ammonia nitrogen (0.123, 0.05, and 0.280) mg/L were recorded. According to Bhatnagar and Devi (2013), the values of ammonia are higher than the permissible limit in the rainy and hot season. The ammonia concentrations of cold season was attained within the acceptable range. The nitrite nitrogen and nitrate mean values (0.38, 0.03, 0.07) mg/L and (0.13, 0.04, 0.16) mg/L respectively were recorded seasonally and these values were within the permissible limit during the seasons of study period. Pereira *et al.* (2017) reported that chronic exposure to nitrate also affects the swimming behavior as well as the health of fish. The important histopathological effects of ammonia on the gill were hyperplasia and fusion of the lamellae. Several authors have reported similar alterations to the gills of a range of fish species when exposed to ammonia. Therefore, in the present study the histological changes in the gill tissues of *Oreochromis* sp. similar to the characters exposed to ammonia. Thus, the present results of histological changes may be due to ammonia.

Arsenic, a naturally occurring element, is a worldwide contaminate that is found in rock, soil, water, air and food. Drinking water but for most people, the major exposure source is the diet, mainly fish and seafood (Castro-Gonzales, and Mendez-Armenta, 2008). In the present study, the mean values of Arsenic analyzed seasonally were observed (<0.01, <0.01, <0.01) mg/L respectively. In accordance with the results, the value of Arsenic is within the permissible limit according to WHO (2011). Toxicity induced by high concentrations of copper in the surrounding is responsible for growth reduction and negative effects on survival and reproduction (Sorensen, 1991). In the present study the mean values of copper (0.016, 0.011, 0.031) were observed seasonally. In accordance with the result, the values of copper was variable values during three seasons and these values were within the permissible limit according to WHO (2011).

The mean values of cadmium (<0.0008, <0.0008, <0.0008) mg/L were observed during the three seasons respectively. In accordance with the result, the values of cadmium is within the permissible limit according to WHO (2011) during the study period. The mean values of lead (<0.015, <0.015, <0.015) mg/L are within the permissible limit according to WHO (2011).

Lead contamination of the surface waters might be the result of entry from old plumbing, household sewages, agricultural runoff carrying lead containing pesticides and phosphate fertilizers, fall out of lead dust and human and animal excreta. The agriculture drainage water rich in phosphate fertilizers is considered the main source of cadmium (IARC, 1993). Monteiro *et al.*, (2005) recorded aneurysms might be used as a sensitive and reliable biomarker of acute copper exposure. Degenerative changes and necrosis in the fish gill epithelium were reported by Hasan *et al.*, (2014) after heavy metal and pesticide exposure. In the present study aneurysms were found in the rainy and cold seasons while degeneration of lamellaes were found during three seasons. This fact matches with the situation in which the paddy field was cultivated in the hot season and the farmers used some fertilizers and pesticides according to surveys of paddy farmers. Thus, the histological changes in the gill tissues of *Oreochromis* sp. in the present study may be due to the heavy metal mixture that causes similar histopathological changes.

Conclusion

The present study showed that histology is a useful biomarker for environmental contaminations such as heavy metals, parasites and changes of water quality. Histological alterations of gill tissues indicated that the fish responds to polluted water. Therefore, it can be concluded that the histological changes found in gills of the studied fish indicated environmental pollutions in the study area.

Nyaung Kaing In (Lake) is a leasable In and is replenished annually by the water from the Chindwin River. However, during the study period, water entering from Chindwin River was temporarily halted. As a consequence of no input and output of water, the water characteristics in Nyaung Kaing In (Lake) become deteriorated. Therefore, it is needed to replenish the Nyaung Kaing In (Lake) with water from Chindwin River in order to maintain the freshwater ecosystem of the lake.

Acknowledgements

We are deeply grateful to Rector Dr Thuya Oo, Monywa University for his permission to submit this research in MAAS. We are greatly indebted to Dr Khin Soe Win, Professor and Head, and Dr Thet Thet Lwin, Professor, Zology Department, Monywa University, for their supervision.

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