Histological Changes in the Gill Tissues of *Oreochromis* sp. from Naung Tong Lake, Kyaing Tong

Ni Ni Win[1], Moe Kyi Han[2] and Thant Zin[3]

Abstract

Naung Tong Lake was chosen as the study area to conduct the present research from June, 2017 to March, 2018. Naung Tong Lake is located in Kyaing Tong Township. Water is stored in this lake within an area of 2414.02 m in length and about 600 m in width. Presently, cyanobacterial bloom and water pollution are found in Naung Tong Lake by anthropogenic activities. In the present study, some cyanobacteria, *Microcystis* spp. were observed in four sample collection sites in Naung Tong Lake. *Microcystis* spp. are toxic cyanobacteria and can produce bioactive compounds that may be harmful called cyanotoxins. Fish specimen, *Oreochromis* sp. was used for histological investigations. The occurrences of histological changes in gill tissues of fish, *Oreochromis* sp. were recorded. Histological changes in gills were epithelial lifting, epithelial separation, dilation of gill filaments with blood congestion, incomplete and complete fusion of lamellae, shortening of lamellae, hyperplasia, curling the lamellae, necrosis, elastic cartilage and deformed structure of gill. Histological changes in gill tissues of studied fish revealed metabolic disturbance and cellular disorder due to environmental contaminations, toxic cyanobacteria, *Microcystis* spp. and water pollution in Naung Tong Lake.

Keywords: Cyanobacteria, *Microcystis* spp., cyanotoxins, *Oreochromis* sp., histological changes

Introduction

Aquatic ecosystems are exposed to excessive input of pollutants and contaminants from various sources like domestic and industrial sewage, agricultural processes, heavy metals and others. The uncontrolled discharge of these compounds into the water directly affects aquatic organisms, including fish, which are considered a bioindicator of environmental pollution or contamination (Lin *et al.*, 2010).

Phytoplanktons are very useful tool for the biomonitoring of a water body with regard to its pollution status. However, excessive growth of algae and cyanobacteria may even harmful. Because of their importance to aquatic ecosystem and susceptibility to changes in the environment, algal measurements are often key components of water quality monitoring programs. Increased occurrence of harmful algal blooms (HABs) worldwide, caused mainly by human activity, has important effects on aquatic ecosystems. In fresh-water environments, planktonic cyanobacteria are associated with eutrophication, as they are the case for *Microcystis* blooms (Moat War Dine Naw, 2001). Water pollution is one of the principal environmental and public health problems. Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment is leading to various deleterious effects on the aquatic organisms. Aquatic organisms, including fish, accumulate

[1] Associate Professor, Dr., Department of Zoology, University of Mandalay
[2] Professor, Dr., Department of Zoology, University of Mandalay
[3] Professor, Dr., Head of Department of Zoology, University of Mandalay

pollutants directly from contaminated water and indirectly via the food chain (Sasaki *et al*., 1997).

Fish are economically important as a source of income in many countries. Fish have also been the subject of extensive eco - toxicological research during the last few decades of twentieth century. In the past 25 years, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring and risk assessment programs (Sousa *et al*., 2013; Munoz *et al*., 2015).

Histology is a useful tool for assessment the effects of toxicants, in cells, tissues, and organs (Adams 2002). Today, histological biomarkers have been widely used in fish for detection and assessment the effects of exposure to pollutants (Oliveira *et al*., 2005) as well as monitoring the fish health status during exposure to pollutants (Thophon *et al*., 2003). Histological biomarkers allow evaluating specific target organs, including gills, gonad and liver, that are responsible for vital functions, and the advantage of this fact is making them as biomarkers in monitoring programs of water environments (Gernhofer *et al*., 2001).

Histological changes are more sensitive and occur earlier. They provide a better assessment of fish health, as well as the effects of pollution on each biochemical parameter. Histopathological changes have been integrated with the impact of various stressors (microbial pathogens, toxic compounds, nutritional and adverse environmental conditions) (Marchand *et al*., 2009). Gills of fish are extremely sensitive to chemical and physical modifications in the environment, mainly because of the large surface of the respiratory epithelium and the high perfusion rate that facilitates the entry of pollutants into this tissue (Cerqueira and Fernandes, 2002).

Naung Tong Lake was chosen as the study area to conduct the present work. Naung Tong Lake is located in Kyaing Tong Township. Water is stored in this lake within an area of 2414.02 m in length and about 600 m in width. Presently, environmental contaminations, cyanobacterial bloom and water pollution are found in Naung Tong Lake by anthropogenic activities. Anthropogenic activities such as industrial and vehicular emissions, agricultural operations sewage discharge, and waste disposal have been responsible for a rapid increase of the environmental pollutions. There has been an increasing awareness that the aquatic pollution and other anthropogenic impacts on water resources may have the potential to damage natural fish in lake. The research aimed to identify and record the toxic cyanobacteria found in Naung Tong Lake and to examine the histological changes in gill tissues of *Oreochromis* sp.

Materials and Methods

Study Area

Naung Tong Lake is located in Kyaing Tong Township between 21º 17′ 56.4″ North Latitude and 99º 35′ 91.2″ East Longitude. Water is stored in this lake within an area of 2414.02 m in length and about 600m in width. At the time of this study, a water depth of the lake is 1.778 m. Water samples containing microorganisms were collected from four study sites of Naung Tong Lake.

Site I (21º 29' 55.70" N and 99º 59' 91.14" E)
Site II (21º 28' 55.60" N and 99º 55' 91.06" E)
Site III (21º 28' 56.54" N and 99º 50' 91.05" E)
Site IV (21º 28' 56.56" N and 99º 50' 91.12" E)

Study Period

The study was conducted from June, 2017 to March, 2018.

Sample Collection and Preparation

Water sampling was carried out once a month from four sampling sites in Naung Tong Lake during the study period. Water samples containing cyanobacteria were collected during 7:00 – 8:00 am by using the plastic basket and then put into one-liter plastic bottle. The collected water samples were carried to the laboratory of Zoology Department, Kyaing Tong University. One drop of water sample from beaker was sucked up with micropipette and placed on a glass slide and then covered by cover slip for the observation of toxic cyanobacteria. Examination of water samples was done at least 20 slides under light microscope for each site. Water samples containing cyanobacteria were preserved in 4% formalin solution.

Identification and Examination of Specimens

Cyanobacteria were examined using a compound microscope (CE DA1 – 180M) under various magnifications of x40, x100 and x400. Taxonomic determination of cyanobacteria was performed with a light microscope on living materials and photomicrographs were taken with SONY digital camera and microscope (CE DA 1 -180M). Classification of cyanobacteria based on Bellinger and Sigee (2010), Desikachary (1959), Otsuka *et al* (2000).

Specimen Preparation for Histology

Fishes (25 specimens) were dissected and parts of gill were carefully removed and fixed in 10% formalin for histological examination.

Histological Procedures

The procedures generally include the following steps: (1) tissue processing, (2) embedding into paraffin wax, and (3) sectioning by microtome. Tissue processing was made in the present study. Wax sections were cut at 7µm by using a microtome (MRM – ST Semi Automic Rotary Microtome, Medimeas Instruments and India)

Staining (H& E Staining) and Mounting

Wax sections affixed to the slides were subjected to heat at 60 ˚C for one hour and then stained with Hematoxylin (Ehrlich) and Eosin yellowish (Merck), India. Regressive staining was practiced in the present study. The routine procedures for H&E staining include deparaffinization in xylene series, rehydration in descending alcohol series, staining in Hematoxylin, bluing in Scott's tap water, staining in Eosin, dehydration in ascending alcohol series, cleaning in xylene series. The slides were subsequently mounted into Balsam Canada (Synthetic), Avi Chem Industries, India.

Examination of the Slides

Histological changes in the gill tissue of fish were examined under a binocular microscope (BX4, Olympus, Japan), which is manually attached with a USB digital microscope camera with built –in 10X eyepiece (MA88-900, C&A Scientific, USA) and recorded on photographs.

Fig.1 Location map of study area, Naung Tong Lake (Source: Google Earth)

A. Study area (Naung Tong Lake)

B. Fish sample, *Oreochromis* sp.

C. Study site I

D. Study site II

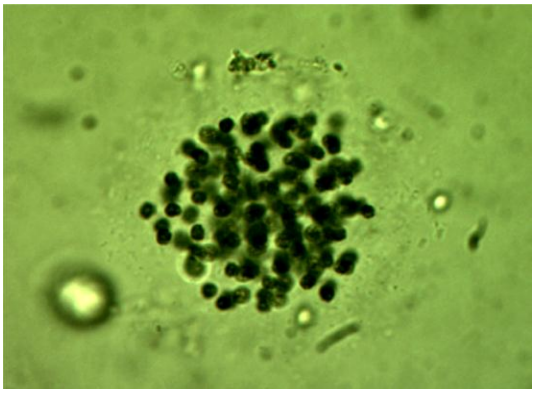
E. Study site III

F. Study site IV

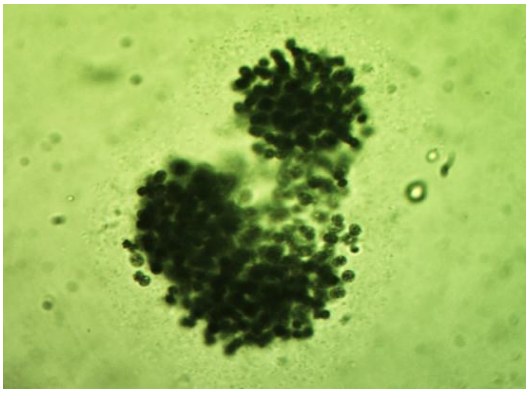
Plate 1. Study area, Naung Tong Lake, study sites and fish sample

Results

In the present study, some cyanobacteria, *Microcystis* spp. were observed in four sample collection sites in Naung Tong Lake during the study period. *Microcystis* spp. are toxic cyanobacteria and can produce bioactive compounds that may be harmful called cyanotoxins. The occurrences of histological alterations in gill tissues of sampling fish, *Oreochromis* sp. were recorded. Histological changes in the gills were epithelial lifting, epithelial separation, dilation of gill filaments with blood congestion, incomplete and complete fusion of lamellae, shortening of lamellae, hyperplasia, blood congestion, necrosis, elastic cartilage, deformed structure of gill filaments and separation of gill epithelium. Based on the present results, histological changes in gill tissues of studied fish, *Oreochromis* sp. revealed metabolic disturbance and cellular disorder due to environmental contaminations, toxic cyanobacteria, *Microcystis* spp. and water pollution in Naung Tong Lake.



A. *Microcystis* sp.



B. *Microcystis* sp.

Plate 2. Cyanobacteria, *Microsystis* spp. in Naung Tong Lake

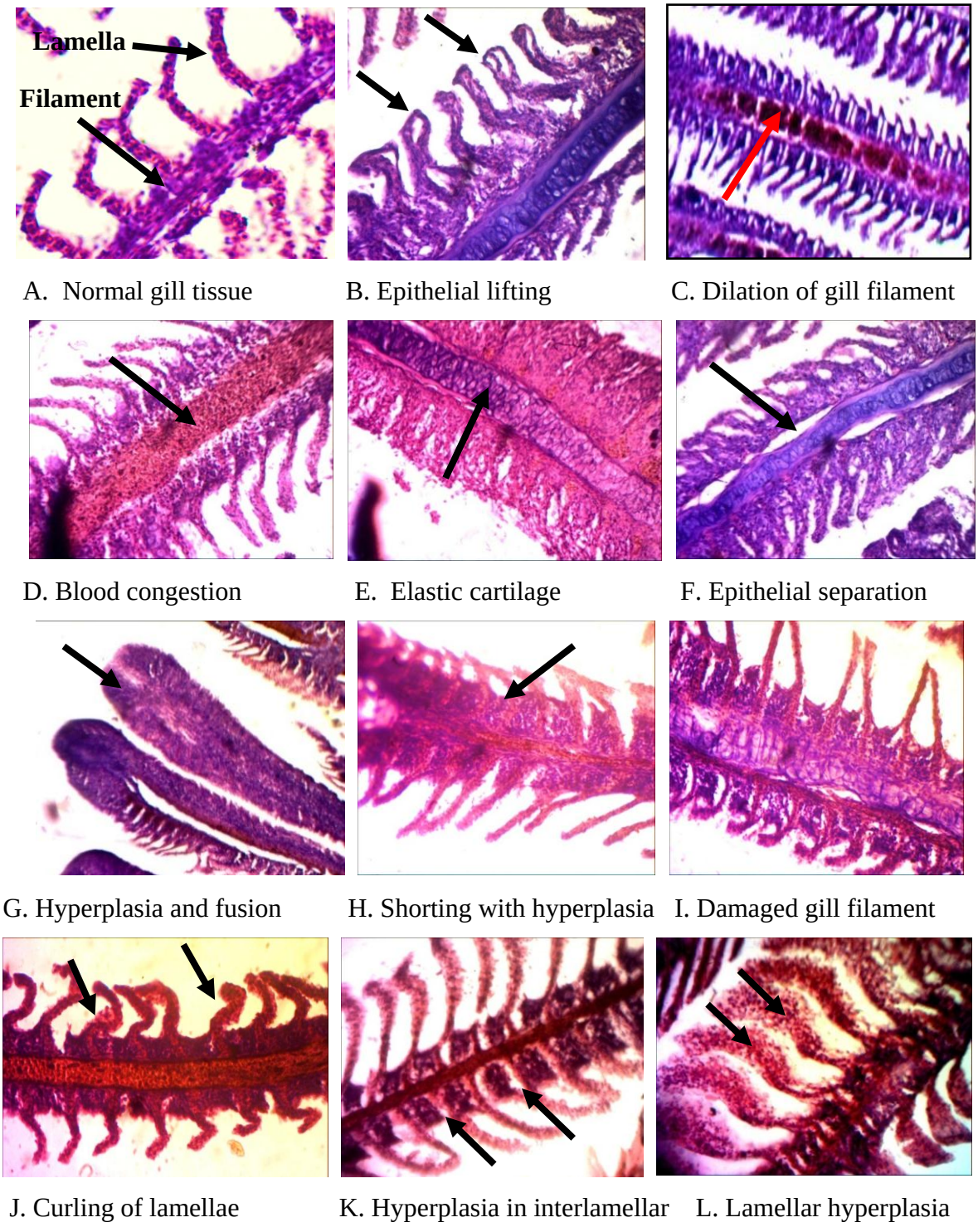


Plate 3 Histological changes in gill tissues of fish, *Oreochromis* sp. (x400)

Discussion

Fish are relatively sensitive to changes 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 their physiological changes serve as biomarkers of environmental pollution (Kock *et al*., 1996). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and kidney (Dutta, 1996). In this study, the gill histology of the fresh water fish was analyzed.

In the present study, some cyanobacteria, *Microcystis* spp. were observed in four sample collection sites within Naung Tong Lake. *Microcystis* spp. are toxic cyanobacteria and can produce bioactive compounds that may be harmful called cyanotoxins. Moat War Dine Naw (2001) first reported that the dominant genera in freshwater from Myanmar are *Microcystis*, *Anabaena*, *Oscillatoria* and *Cylindrospermopsis*. Toxic producing cyanobacteria, *Microcystis* spp. and *Oscillatoria* were found in fish culture ponds during blooming condition (Ni Ni Win, 2007). Hepatotoxins are produced by fresh water cyanobacteria, *Microcystis* spp. This toxin produced symptoms including nausea, vomiting and acute liver failure (WHO, 2004).

Cyanobacterial toxins have been implicated in human and animal illness and death in more than 50 countries. In addition, a study on cyanobacteria in Myanmar shows that almost all of toxic genera contain in freshwater body. In the present study, *Microcystis* sp. were found in all sample collection sites I, II, III and IV of Naung Tong Lake during blooming condition. This finding shows that since *Microcystis* spp. are nuisance cyanobacteria should be monitored and investigated regularly for aquatic fauna. Ni Ni Win (2007) reported *Microcystis* spp. can produce toxins which cause histological changes in gill and liver tissues of fish during *Microcystis* bloom condition in aquatic environment. In this study, the occurrence of histological alterations in gill tissues of sampling fish *Oreochromis* sp. were recorded. Histological changes in gills were recorded epithelial lifting, epithelial separation, dilation of gill filaments with blood congestion, incomplete and complete fusion of lamellae, shortening of lamellae, hyperplasia with blood congestion, necrosis, elastic cartilage, deformed structure of gill filaments and separation of gill epithelium. In the present study, epithelial lifting in gill tissues was recorded. Part *et al*., (1982) reported epithelial lifting of gill lamellae will impair oxygen transfer as a result of the increased distance between water and lamellar capillaries. The lamellar structure is optimized for exchange with the environmental medium with short diffusion distance. The lifting of the respiratory epithelium is one the earliest injuries found in fish; it is characterized by displacement of the lining epithelium of the secondary lamellae, in which the formation of a space called edema occurs.

In this study, the hyperplasia and lamellar fusion in gill tissues of sampling fish *Oreochromis* sp. were recorded. According to Brito *et al*. (2012), the occurrence of lamellar fusion is frequently found in fishes exposed to urban sewage. Similarly, Ewald (1995) reported tissue alterations are preceded by biochemical and physiological responses and once the damage is detected, the adverse effects on the organisms are incontestable. When the alterations occur in organisms exposed to environmental samples, they confirm the degradation of the area. In the present study, histological changes in gill tissues of studied fish revealed metabolic disturbance and cellular disorder due to environmental contaminations and water pollution in Naung Tong Lake.

According to Winkalar *et al*. (2001) and Tkatcheva *et al*. (2004), the exposure of gills to different contaminants may be marked by the occurrence of lamellar fusion, tissue

hyperplasia and aneurysms. In the present study, hypaplasia, fusion, dilation of gill filaments and damage of lamellae were observed. The present findings are agreement with Olurin *et al*., (2006). who reported that, vasodilation was found in fishes which seem to indicate severe exposure to polluted water. When fish are under more severe stress, vascular changes can occur in the lamellae and the response function can become dysfunctional and impair their physiology. Kock *et al.,* (1996) reported fish gills are critical organs for respiration, osmoregulation and excretion. Gills serve as a good indicator of water quality. They are sensitive to any change of water components since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water. The gills are the primary respiratory organs of fish.

In this study, histological changes in gill tissues of studied fish revealed metabolic disturbance and cellular disorder due to environmental contaminations, toxic cyanobacteria *Microcystis* spp. and water pollution in Naung Tong Lake.

Conclusion

The present study was conducted to investigate toxic cyanobacteria and histological changes in gill tissues of studied fish, *Oreochromis* sp. in aquatic habitat of Naung Tong Lake. The present study has shown that Cyanophyta, toxic cyanobacteria *Microcystis* spp. can produce toxins in water. Histological changes in gill tissues of studied fish, revealed metabolic disturbance and cellular disorder due to environmental contaminations and toxic cyanobacteria *Microcystis* spp. in Naung Tong Lake. It is believed that the present research will provide some information about pollution for further research.

Acknowledgements

The Authors wish to express their gratitude to Dr San San Mar, Rector of Kyaing Tong University, for her permission to conduct this research. We are also obliged to Dr Thein Win, Director General, Department of Higher Education, Ministry of Education and Dr Kaythi Thin, Dr Myin Zu Minn and Dr Mi Mi Gyi, Pro-Rectors of University of Mandalay for their permission to present this paper. Special thanks are also go to Dr Thant Zin, Professor and Head, Department of Zoology, Mandalay University for his encouragement.

References

Adams, 2002. Biological indicators of aquatic ecosystem stress. *Am. Fish Soc.,* 3:104-112.

Bellinger, E.G. and Sigee, D.C., 2010. *Freshwater algae*: Identification and use as bioindicators. John Wiley & Sons, Ltd, United States of America.

Brito, 2012. Monitoring water quality in reservoirs for human supply through multi-biomarker evaluation in tropical fish. *J. Environ. Monit*., 14: 615-625.

Cerqueira, C.C.C.; Fernandes, M.N., 2002. Gill tissue recovery copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicol. Environ. Safety*, 52: 83–91.

Ewald, G., 1995. Chronic measures of toxicant induced effects on fish. *Ann. Zool. Fenici.,* 32: 311-316.

Desikachary, T.V., 1959. *Cyanophyta.* Indian Council of Agricultural Research, New Delhi.

Lins, J.A.P.N., Kirschnik, P.G, Queiroz, V.S.; Cirio, S. M., 2010. Uso de peixes como biomarcadores para monitoramento ambiental aquatic. *Rev. Acad. Cien. Agarar. Ambient*. (8): 469-484.

Kock, G., Triendl, M. and Hofer, R., 1996. Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. *Canadian j. Fish. Aquat. Sci*., 53: 780-786.

Marchand, M.J., van Dyk,. Pieterse, G.M., Barnhoorn, I.E. and Bornman, M.S., 2009. Histopatological alterations in the liver of the sharptooth catfish *Calarias gariepinus* from polluted aquatic ecosystems in South Africa. *Environmental toxicology*, 24(2): 133-47.

Moat War Dine Naw, 2001. Biological studies on fresh water cyanobacteria, producing toxin. *PhD, dissertation*. Yamagata University, Japan.

Ni Ni Win, 2007. Studies on the cyanobacterial and algal blooms in aquaculture ponds of Thayetkone Fisheries Department, Mandalay. *PhD dissertation*. Department of Zoology, University of Mandalay.

Oliveira, Ribeiro, C.A., Filipack N.F., Mela M., Silva P.H, Randi M.A.F., Costa J.R.A and Pelletier, E., 2005. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead and tributyltin chloride. *Environ. Resour.*, 101: 74–80.

Olurin, K., Olojo, E. and Akindele, A., 2006. Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. *Afr. J. Biotechnol.*, 5: 2480–2487.

Otsuka, S., Matsumoto, S. and Watanabe M.M., 2000. Morphological variability of colonies of *Microcystis* morphospecies in culture. *Applied Microbiology*, (46): 39-50.

Sasaki, Y.F., Izumiyama, Nishidate, S. Ishibashi, S. Tsusa, N. Matsusaka, N. Asano, K. Saotome, T. Sofuni, M. Hayashi, 1997. Detection of genotoxicity of polluted sea water using shellfish and the alkaline single-cell gel electrophoresis (SCE) assay: A preliminary study. *Mutation Res*., 393:133-139.

Sousa, D.B.P., Almeida, Z.S. and Carvalho-Neta, R.N.F., 2013a. Histological biomarkers in two estuarine catfish species from the Maranhense Coast. Arquivo Brasileiro de Medicina Veterinariae. *Zootecnia*, 65(2): 369-376.

Thophon, S.K., Kruatrachue, M. Upathan, E.S., Pokthitiyook, P., Sahaphong, S. and Jaritkhun, S., 2003. Histopathological alterations of whitesea bass, Lates calcarifer, in acute and subchronic cadmium exposed. *Environmental Pollution*, 121: 307-320.

Tkatcheva, V., Hyvarinen, H., Kukkonen, J., Ryzhkov, L.P. and Holopainen, I.J., 2004. Toxic effects of mining effluents on fish gills in a subarctic lake system in NW Russia. *Ecotoxicol. Environ. Saf*., 57: 278-289.

Winkaler, E.U., Silva, A.G., Galindo, H.C and Martinez, C.B.R., 2001. Biomarcadores histológicose fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. *Acta Scient. Biol. Sci.*, 23, 507–514.

World Health Organization (WHO), 2004. Guidelines for safe recreational water environments. Volume 1, *Coastal and Fresh Water*. WHO, Geneva, Switzerland.