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Bioaccumulation and Histopathological Changes induced by Toxicity of Mercury ($HgCl_2$) to Tilapia Fish *Oreochromis niloticus*

(Perubahan Bioakumulasi dan Histopatologi Teraruh oleh Ketoksikan Merkuri ($HgCl_2$) pada Ikan Tilapia *Oreochromis niloticus*)

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ABSTRACT

*In this paper we have studied the acute toxicity effect of Hg on hybrid tilapia (*Oreochromis niloticus*). For this, the tissues of tilapia have been digested by means of acids in microwave oven and was analyzed by flameless atomic absorption spectrophotometer (FAAS). We have identified that the levels of Hg varied significantly in different tissues and the metal concentration was in the following order: liver > gills > muscles; of which the maximum level recorded for Hg was 0.799 mg/kg. We have also observed the alterations towards histopathological aspects in the gills and liver of treated fishes were studied using light and electron microscopy, subjected to the exposure of Hg for 24 h and furthermore we have also noticed the extent of the increased alterations during the 96 h of exposure to median lethal concentration LC50 (0.3 mg/L) a severe disorganization of epithelial cells and modifications of the structure of the secondary lamellae. Moreover the severity has also found to increase to sub-lethal concentration (0.03 mgHg/L) in 21 days of exposure; Liver was slightly affected by the contamination of Hg. Ultimately, histopathology is considered as a sensitive technique of bioaccumulation and for the observing the potential damage from Hg exposure.*

Keywords: Acute exposure; bioaccumulation; histopathology; Mercury; Oreochromis niloticus

ABSTRAK

*Dalam penyelidikan ini, kami mengkaji kesan ketoksikan akut Hg pada tilapia hibrid (*Oreochromis niloticus*). Tisu tilapia telah dicerna menggunakan asid di dalam ketuhar gelombang mikro dan telah dianalisis menggunakan spektrofotometer atom serapan tidak bernyala (FAAS). Kami telah mengenal pasti bahawa, paras Hg berubah dengan ketara dalam tisu yang berbeza dan susunan kepekatan logam adalah seperti berikut: hati> insang> otot; dengan paras maksimum Hg yang direkodkan adalah 0.799 mg/kg. Kami juga dapati perubahan terhadap aspek histopatologi dalam insang dan hati ikan terawat dan kajian dijalankan menggunakan mikroskop cahaya dan elektron, tertakluk kepada pendedahan Hg selama 24 jam. Kami turut mengenal pasti peningkatan perubahan semasa 96 jam pendedahan terhadap kepekatan maut LC50 (0.3 mg/L) ketidakaturan sel-sel epitelium yang rosak dan pengubahsuaian struktur lamela sekunder, kerosakan teruk juga berlaku pada kepekatan sub-maut (0.03 mgHg/L) dalam masa 21 hari pendedahan; kesan pencemaran Hg terhadap hati hanya sedikit sahaja. Histopatologi dianggap sebagai teknik yang sensitif bagi bioakumulasi dan pemerhatian terhadap potensi kerosakan akibat pendedahan Hg.*

Kata kunci: Bioakumulasi; histopatologi; Merkuri; Oreochromis niloticus*; pendedahan akut*

INTRODUCTION

Developments have brought lots of good things to the human kind, on the other hand it has also created a huge adverse effects towards the environment, of which the aquatic environment has been continually subjected to numerous contaminants, consequently a number of chemical contaminants, such as heavy metals have significantly polluted the water sources, which has turned out to be a substantial obstacle and severe danger. Toxicity of metal happens when the amount of metal intake into the body surpasses the combined rate of excretion and detoxification of metabolically available metal (Rainbow 2002). Aquatic animals have different capabilities to maintain their internal chemical composition, depending on type of species and the physiological function of trace elements (Qiu et al. 2011; Sloman 2007). Generally fish populations are indirectly affected either negatively or positively, based on the direct metal toxicity at any trophic levels (Couture & Pyle 2011). Among the various metals, due to possible dangers posed to aquatic organisms, few heavy metals, such as, mercury (Hg) has gained exclusive consideration. This element is classified as one of the most toxic metals, which is introduced to the natural environment by anthropogenic sources (Buhl 1997). Mercury is a hazardous substance and its acute chemical releases cause the most emergency events (ATSDR 2004). Basically, mercury is released into the atmosphere through a number of sources such as, surface water and soil from pulp and paper, chlorine factories, electrical industries and combustion of fossil fuels; apart from

these, human activities are also considered responsible for the mercury contamination (Friberg & Vostal 1974). Hg^{+2} have no known role in biological systems, it is considered as inessential, imperishable and lasting heavy metal and moreover the amalgamations of Hg are extremely poisonous. Additionally, constant low-level exposure towards Hg, might result in serious health complications, which are categorized as carcinogenic and mutagen (DiFrancesco & Shinn, Jr. 2002; Zahir et al. 2005). Generally, industries are one of the main sources of releasing inorganic mercury into the atmosphere, which creates an intensive impact on fish tissues as opposed to the organic form of mercury (Oliveira-Ribeiro et al. 2002; Sunderland & Chmura 2000).

A very important biological property of metals is their tendency of bioaccumulation; which is very important aspect in hazard evaluation strategies; furthermore, fishes have the ability to collect the element from water to the highest level for the environment and therefore bioaccumulation of metals is considered as an evidence of metal pollution index (Osman 2012).

As humans are on the top of food chain, it is highly possible for them to get contaminated with high levels of Hg by the consumption of polluted foods (WHO 2012), moreover, increasing amount of Hg in the human tissues can cause Minamata disease (Harada 1995).

The level of metal in water and the period of subjection are the main aspects for the accumulation of heavy metals in the tissues of aquatic organisms; apart from these, salinity, pH, hardness and temperature of water are few other factors that caused the collection of metals (Alhashemi et al. 2012; Carvalho & Fernandes 2006; Costa et al. 2009; Mohan et al. 2012). Generally fish contaminated by Hg suffer histopathological alterations, with consequent inhibition of metabolic processes (Silva et al. 2012), including gills damage, which is considered as the most affected member, such as, hyper-secretion of mucus, moreover, ensuing mortalities is related to secondary physiological respiratory disturbance (Sharma et al. 2001).

Lots of researchers have considered histo-pathological lesions of natural water fish as typical signs of toxic-damage, subjected to the exposure of mercury contaminants or other metals; however, under the same conditions, the histological changes of exposed aquatic organisms differ in severity, depending on the type of organism and concentration of the chemicals (Gehringer et al. 2013; Greenfield et al. 2008; Oliveiro-Ribeiro et al. 2002; Triebskorn et al. 2008).

Although laboratory studies of Hg toxicology used tilapia as the animal model in lethal or sub-lethal tests and structural damage of gills and liver in *Oreochromis niloticus* (Kaewamatawong et al. 2013), still there are lack of data about the effects of mercury over tilapia.

Tilapia (Cichlidae family) is regarded as one of the leading ten species with high expansion rate amongst the aquaculture fisheries food supply, with regards to volume of production (FAO 2007), because of its superior attributes such as quick growth, huge size, palatability and effortless reproduction, which does not need any exclusive hatching technologies (Nandlal & Pickering 2004). On top of that, tilapia can tolerate a wide range of environmental conditions, especially low dissolved oxygen, high ammonia level and a wide range of pH(5-11) (Watanabe et al. 1997).

In this present study, we have investigated hybrid tilapia, which has become popular through collaborative program with world fish centre on genetic improvement of farmed Tilapias as GIFT. The hybrid tilapia has been selected due to the following reasons: High productivity, significant improvements in growth rate in successive generations, as well as remarkable survival rates in the Malaysian aquacultures, which became an important food source for human beings (Ponzoni et al. 2010, 2005); in addition, the ability to respond against environmental pollution is also another reason for the selection (Low et al. 2011; Mokhtar et al. 2009).

The objectives of the present work were: To determine the acute toxicity of mercury in hybrid red tilapia *Oreochromis* sp.; to evaluate bioaccumulation of mercury in different tissues; and to examine the structural damage of gills and liver of the studied fish.

Materials and Methods

We have purchased the fingerling tilapias 7±1 g in mean weight and 7.5±2 cm in length from a commercial aquaculture facility in Serendah, Selangor, Malaysia. The fishes were acclimated in group of 25 in 50 L glass aquarium filled with tap water for one week, the fish were fed with a dry commercial food (pellets with 25% of crude protein). Air pump with aquarium was used as the aeration system.

Temperature, pH, salinity and dissolved oxygen (DO) were recorded daily during the experiment and the average was 26.8±2°C, 7.65±0.5, 0.085±0.022 g/L and 7.0 mg/L, respectively. Tap water in aquaria was replaced every 24 h. Afterwards, fingerlings were transferred to assay aquaria (20 × 20 × 40 cm) 5 L glass aquaria, which provided aeration via air pumps and air stone diffusers. Each group was at a stocking density of 10 fish /aquarium.

The chemical product used in this study was inorganic mercury chloride ($HgCl_2$) Analar BDH chemicals with 99.5% purity dissolved in double deionized water, to prepare the stock solution (1000 $mg.L^{-1}$) of Hg. A series of six concentrations were prepared by adding a calculated volume from the stock solution with local tap water into test containers. The Tilapias were semi statically exposed to different concentrations (control (0), 0.1, 0.3, 0.5, 0.7, 0.9 and 1.2 ppm) of mercury metal during 96 h (range determined by preliminary tests) with three simultaneous replicates. No food was supplied during the experiment. The test solutions were replaced with fresh ones of the same respective concentrations every 24 h, according to the renewal method recommended in APHA et al. (1998). Mortality were recorded at 6, 12, 24, 48, 72 and 96 h of exposure and dead organisms were removed regularly from the test solutions, for estimating the median lethal

concentration (LC_{50}) and median lethal time (LT_{50}) from the probit transformed concentration - response curves (U.S. EPA 2002).

MERCURY BIOACCUMULATION TEST

Tilapia fish fingerlings were exposed to various concentrations of mercury chloride 0 (control), 0.3, 0.7 and 1.2 ppm during median lethal time (LT_{50}) of a higher concentration (21 h). The active fish was collected and dissected using stainless steel knife (scalpels) to cut the tissues (muscles, gills and liver) after drying for 24 h at 105°C. The dried samples were weighed by three duplicate thawed of 0.5 g for muscles and gills and 0.1 g for liver, because liver is small compared to the gills and muscles. Microwave digestion method has been applied using closed vessel (Nguyen et al. 2005) in a microwave oven (Milestone model Start D, Italy) by adding 6 mL HNO_3 (65%) and 1 mL H_2O_2 (35%) mixture. The samples were then diluted by deionized water and mercury analysis was performed by flameless atomic absorption spectrophotometer (AOAC 1995). Standard stock solutions of mercury were prepared from Titrasol 1000 mg/L. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solution. The certified reference materials DORM-2 was used as quality control samples.

HISTOPATHOLOGICAL STUDY

Histopathological analysis was conducted on liver and gills of post exposure fish to 96hLC50 (0.3 ppm) over 96 h and fish were exposed to sub-lethal concentration 96hLC50/10 (0.03 ppm) for 21 days. The tissues were dried out in a graded ethanol series and inlayed in paraffin, after being exposed to neutrally buffered formalin for 48 h. Each block of tissue has been cut into serial sections (7 µm thick) and stained with hematoxylin and eosin (H&E). The tissues were later tested for wide range of histopathological characteristics and lesions, via general measures of morphology and health alterations were qualitatively described. Next, it was semi quantitatively evaluated by ranking the severity of lesions belonging to grades 1 to 5, based on the procedure described by Triebskorn et al. (2008).

After examining the tissues, the digital images were obtained by using a light microscope Nikon type Eclipse E200, equipped with a Dino eye camera Ø30 mm, employing 10, 20 and 40× objectives.

Fish used for scanning electron microscope were exposed to inorganic Hg. Gills and liver were prepared as described by Pandey et al. (2008) and washed with NaCl 0.9%, fixed with 8% glutaradehyde in (0.1 M phosphate buffer, pH7.4). Then, it was post fixed in buffered 1-2% Osmium tetraoxide to increase the electron density. After dehydration in ascending series of ethanol, specimens were mounted onto aluminum stubs and coated with gold paint and placed in vacuum evaporator. After processing, gills were examined using JSM-7001F field emission scanning electron microscope (JEOL Electron Microscopy, Japan).

RESULTS AND DISCUSSION

Clinical signs of tilapia, affected by mercury exposure were observed in the first experimental session, mainly at the higher concentrations (0.7, 1.2 and 1.4 mg Hg L^{-1}). The following aspects were identified: Hyperactivity, aggressiveness, followed by respiratory distress and death. Similar behaviors have also been reported by Ishikawa et al. (2007) in *Oreochromis niloticus* exposed to $HgCl_2$ (0.370, 0.740 and 0.925 mg Hg L^{-1}).

The LC_{50} values of Hg within 24, 48, 72 and 96 h recorded for *Oreochromis* sp. in the present study, with 95% confidence limits were 1.09 (0.92 - 1.40 mg/L), 0.75 (0.47 - 1.32 mg/L), 0.54 (0.12- 0.96 mg/L) and 0.30 (0.17- 0.44 mg/L), respectively (Figure 1). Furthermore, we have identified that, the tolerance to mercury decreases with the increased in time of exposure. The $LC_{50\text{-}96h}$ 0.30 mgL^{-1} was very similar to those estimated with Nile tilapia *Oreochromis niloticus* (0.24 mg Hg/L) (Kaoud & Mekawy 2011); while it had higher value (1.15 mg/L) in air-breathing fish *Channa punctatus* (Pandey et al. 2005) due to some differences in type of species; moreover, older and larger aquatic organisms were more resistant to toxicants.

A safe concentration estimated in the present study (LC50-96h × 0.01) was 0.003 mg L^{-1}. This value is very similar to those recommended by Malaysian National Water Quality Standards (DOE-UM 1986), which has considered Hg level (0.0001 mg L^{-1}) as safe water quality requirement for fish; however this recommendation is lower than the safe mercury concentration in this study. The concentrations of mercury in different tissues (muscle, gills and liver) of fish exposed to 0.3, 0.7 and 1.2 mgHg/L are given in Table 1.

In *Oreochromis* sp., the mercury concentrations in muscle, gills and liver of control fish were 0.0008, 0.004 and 0.004 mg/kg, respectively. Relatively, similar levels have been found in the same tissues of the *Oreochromis niloticus* (0.0005, 0.001 and 0.005 mg/kg) (Osman 2012); and in goliath grouper *Epinephelus itajara* maximum mercury concentration have been recorded, ranging at 22.68 µg/g in liver with a mean of 0.63 µg/g in muscle (Adams & Sonne 2013).

After the exposure towards mercury (0.7 mg/L), our results have showed that, the concentration of mercury has increased in different tissues. The highest mercury accumulation was observed in liver (0.799 mg/kg), followed by the gills (0.679 mg/kg) and the muscle (0.164 mg/kg). A similar hierarchy of accumulation was observed in *Oreochromis niloticus* muscle (3.21 mg/kg), gills (20 mg/kg) and visceral organs in abdomen (45.75 mg/kg), after exposing to 1 mg Hg/L for 3 days (Kaewamatawong et al. 2013). Hg concentration in control fish muscles were within the approved limits for human consumption and lower maximum level limit, which has been reported by Malaysian Food Act 1983 and Food Regulations 1985 (Ministry of Health Malaysia 2012); whereas, the metal content in muscles tissues of treated fish has significantly varied ($p<0.05$) higher than in the control groups.

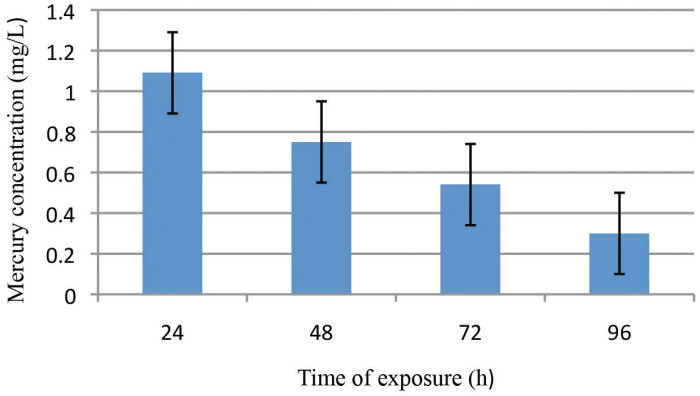

FIGURE 1. The 24, 48, 72 and 96 h LC_{50} of mercury in *Oreochromis* sp.

TABLE 1. Concentration of mercury in different tissues of *Oreochromis* sp. (mg/kg dry weight) after exposure to different concentration of mercury

	[c]control(0)	[b]0.3	[a]0.7	[b]1.2
[b]muscle	0.0008±0.0002	0.055±0.057	0.164±0.018	0.024±0.018
[a]gills	0.004±0.0002	0.357±0.023	0.679±0.141	0.526±0.082
[a]liver	0.004±0.003	0.371±0.207	0.799±0.126	0.335±0.172

The data expressed as Mean ± Standard deviation, *n*=3

Metal concentration in the liver might originate from a progressive transfer of mercury from the gills to the liver via the blood (Firat & Kargin 2010) However, the higher mercury concentration has been observed in the liver of post exposure fish, with levels reaching up to 10 times higher than the values measured for control group, because liver plays multifunctional role in detoxification mechanism and storage process and may be due to their strong binding with cystine residues of metallothionein (MT), where the lower molecular weight protein has high affinities for heavy metals and its storage as a constituent of hepatic cytoplasm, trigger increased accumulation of metal in the liver (Ashraf et al. 2011; Montaser et al. 2010; Yacoub 2007).

Similar finding was also demonstrated in Hg contaminated fish *Gymnotus carapo,* after acute exposure to Hg^{+2}; the highest mercury level was found in the liver, followed by the gills and lowest concentration was observed in the muscle (Vergilio et al. 2012). Muscle was found to accumulate small amounts of all the heavy metals and might have received them through circulation. It was suggested that, the low accumulation of metals in muscle may be due to lack of binding affinity of these metals with the proteins of muscle. This is particularly important, because muscles contribute the greatest mass of the flesh that is consumed as food (Osman 2012).

The toxic effects and accumulation of all forms of inorganic mercury are ascribed to the action of ionic mercury, because elemental mercury (Hg^0) cannot form chemical bonds, nevertheless, ionic mercury exists in mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}) forms, the mercurous ion is unstable and dissociates further into the mercuric ion, gradually ionic mercury forms complexes with SH group and other ligands in the tissues of the body and only a very small fraction exists in the free from (Ashraf et al. 2012; Friberg & Vostal 1974).

Histological observations were performed on the *Oreochromis* sp. organs, to demonstrate the toxic effect of the Hg concentrations, however, morphological analysis of the gills and liver showed alterations after 24 h of Hg exposure which showed increased in severity over the 96 h of exposure.

The untreated gills showed a characteristic arrangement (Figures 2(a) & 4(a)), the gill comprised of four sets of gill lamellae and both sides had been reinforced by bony structure and primary lamellae. When viewed in vertical section, the secondary lamellae comprised of several blood capillaries, which were segmented by single layered pillar cells. The laminar epithelium was thicker, accompanied by basement membrane, underneath the pillar cells had enclosed the blood spaces, we have also observed lots of mucous cells on the epithelial gill rackers; in contrast the primary lamellae had relatively smaller and lesser number of mucous cells.

Alterations were observed in this organ at 24-96 h Hg exposure points under concentration (0.3 ppm), showed some areas with focal proliferation (Figure 2(b)), occasionally resulting in fusion of adjacent secondary lamellas (Figure 2(c)). Epithelial cells also showed vacuolization after 96 h of exposure. The fusion of the secondary lamellae may work to protect the affected gills and thus helps to reduce the entry of the toxic substance, which increases suffocation and death of fish (Hamid et al. 2015) and this is consistent with studies carried out by Silva et al. (2012) with predator fish *Hoplias malabaricus*.

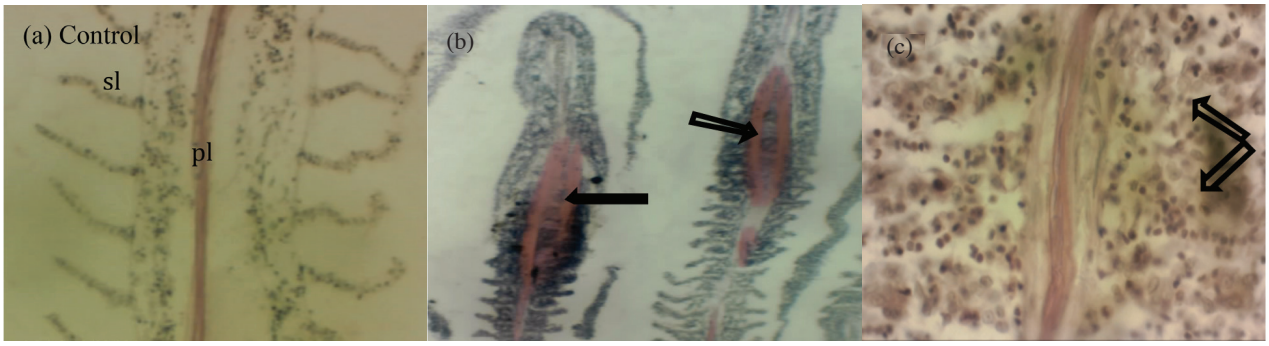

FIGURE 2. (a) Regular shape d secondary lamellae and primary lamella in gills of controlled *Oreochromiss* sp., (b) some areas with focal proliferation in primary lamella (arrows) and (c) fusion of adjacent secondary lamellas (arrows)

The histopathology of experimented fish gill, treated with sub-lethal concentration (0.03 ppm) has showed slight damage in 10th day of mercury chloride exposure. Nevertheless, we have observed that the gill had sore in the epithelial layer, hypertrophy, due to mucous cells and vacuolation in gill membrane. Figure 3(a) illustrates the histopathology of fish gill exposed to mercury for 21 days; which showed noticeable edema and effective secretion of mucous, elevated in size, but reduced in number and majority of them were either vacuolated or almost empty. The secondary lamellae have also confirmed damage of, either epithelial cells, or few lamellae have curled, which causes congestion and hemorrhage of gills (Figure 3(b)). The gills of the experimented fish turned out to be reddish. This is consistent with the observations of Cerqueira and Fernandes (2002), where the gill of the tropical fish *Prochilodus scrofa* exposed to different concentrations of heavy elements, has become coated with a mucosa layer, due to a defensive reaction of the fish, against the presence of contaminants, which reduces the absorption of these pollutants through gills; and consequently causes an increased amount of mucus, which affects the breathing process. Some of the observed histological and healthy changes in this study have also been similar with alterations that have been reported with contamination with other metals (Jalaludeen et al. 2012).

Morphological alterations on the secondary lamellae of the experimental fish were easily detected even at the beginning of the experiment. Hence, after 24 h of exposure, hypertrophied cells and alterations on secondary lamellae surface (Figure 4(b)). After 96 h, the presence of squamous epithelium and extensive epithelial hyperplasia resulted in modifications of the structure of the secondary lamellae represented in the formation of an interlamellar bridge (Figure 4(c)) this is similar to the study of Oliveira Ribeiro et al. (2000) who attributed this bridge due to the fusion in the adjacent lamellae that caused reduction of the water space. According to Oliveira Ribeiro et al. (2002), the dissolved inorganic mercury at 0.015 mg$HgCl_2.L^{-1}$ caused major morphological alterations on respiratory lamellae, decreasing their gas exchange capability with the environment.

The gills of fishes play lots of vital activities including respiratory, osmoregulation and excretion functions; furthermore the gills have close contact with the surrounding environment and predominantly delicate to changes in the quality of the water, therefore, they are regarded as the primary target of the contaminants (Ahmed et al. 2014; Pereira et al. 2013).

Damage in epithelial membranes is primary reaction of gills with variant pollutants, whereas mercury element pick-up charge Hg^{+2}, which is similar to many of the ions charges Ca^{+2} and Mg^{+2} and competing on the union and transit through the chloride cells, which are important in the process of ion balance, causing damage to those cells, affecting the process of osmotic regulation of the

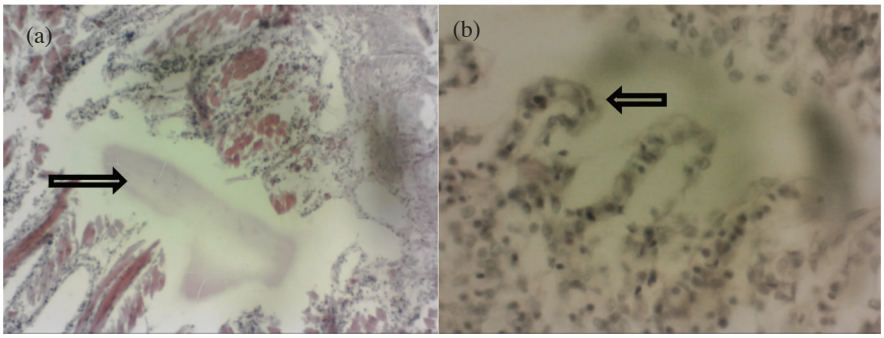

FIGURE 3. (a) The gill shows lesion in the epithelial layer with marked edema and active secretion of mucous in treatment with sub lethal concentration (0.03 ppm), (b) showing curled in secondary gill lamellae during 21 days in sublethal concentration (0.03 mgHg/L) (arrow ×400)

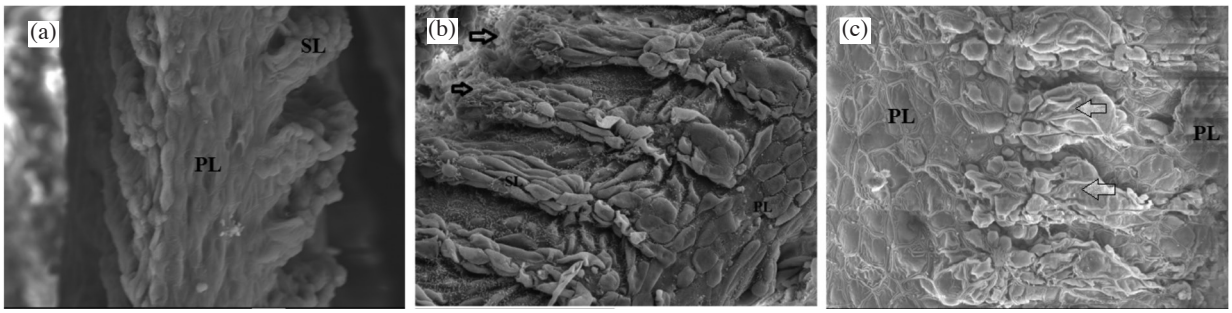

FIGURE 4. Control material from gill of *Oreochromis* sp. (4a) (300×), primary (PL) and secondary lamellae (SL). Partial view of the secondary lamellae showing the lamellae fusion (arrow) and hyperplasia on lamellae surface (4b)(600×) after 24 h of exposure. The damage are more severe after 96 h (4c) (600×) arrow head show the interlamellar fusion

fish and results in multiple damages, such as electrolytic imbalances, disruption and necrosis in gill tissues and thus resulting in a lack of oxygen uptake and ultimately suffocation and death (Chang et al. 2003; Olivera-Ribeiro et al. 1996; Wu et al. 2008).

Morphological alterations in liver were observed after 24 h, when exposed to acute concentration 0.3 mg/L Hg. The untreated liver showed typical compact structure, where the hepatocytes presented a characteristic cytoplasmic distribution and nuclear morphology (Figure 5(a)). The 24 h Hg treatment induced disorganization of hepatic tissue; severe loss of lipid has been characterized by low fat vacuolation in cytoplasm, coupled with changes in cytoplasmic and nuclear morphology (Figure 5(b)). The 72 and 96 h treatments have displayed dilate and congestion of blood vessels. Areas with severe degradation of the liver parenchyma were also observed, usually in close proximity to the blood circulation, as well as, lymphocytic and macrophage infiltration in the liver (Figure 5(c)). Furthermore, necrosis has occurred in the liver over 96 h (Figure 5(d)). Advanced micronecrosis was observed after 10 days of exposure of sub-lethal concentration 0.03 mgHg/L (Figure 5(e)), moreover, small regions of necrosis under 21 days exposure have also been observed (Figure 5(f)).

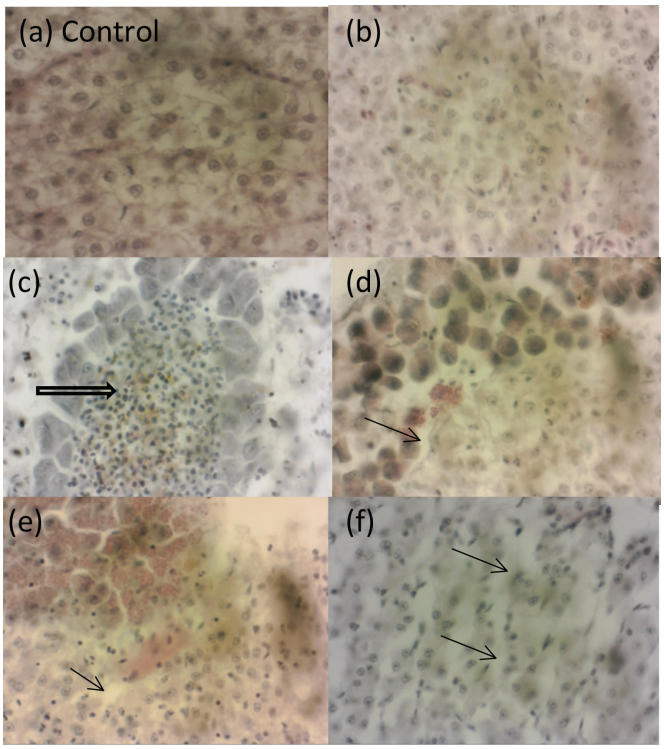

FIGURE 5. (a) Normal liver showing the normal location and morphology of the nucleus and the cytoplasm of the hepatocytes (×400), (b) 24 h Hg treatment induced disorganization of hepatic cells, (c) 72 and 96 h treatments, showing areas with severe degradation of the liver parenchyma, lecucytic infiltration (arrows) (×400), (d) necrosis occurred in the liver over 96 h, (e) micronecrosis after 10 days of exposure of sublethal concentration 0.03 mgHg/L and (f) small regions of necrosis under 21 days exposure (arrows ×400) (H&E)

When the fish were subjected to severe exposure of inorganic Hg, the metabolism of those fish had increased, due to loss of stored lipid substances in hepatocytes; furthermore we have witnessed alarming quick and primary response of the cells, as well as the liver alterations, including multiple necrotic sites; and these conditions are considered as potential biomarkers. However, histological changes seen in the liver is not considered as a specific biomarker of mercury exposition, but are generally associated with the response of hepatocytes to toxicants. This trend likely indicates that the liver is a sensitive organ for the evaluation of damage, after exposure to pollutants (Senthamilselvan et al. 2011; Velcheva et al. 2010). In addition, these induced alterations agree with other studies of Hg contamination in fish liver (Oliveira Ribeiro et al. 2002; Raldu´a et al. 2007). Researches focused on toxicology have showed that, the accumulations of contaminants might affect the plasma blood biochemistry, including activities of plasma enzyme and directly cause cell damage in particular tissues (Ahmed et al. 2015; Fernandes et al. 2008; Yang & Chen 2003).

Conclusion

The present study showed that the exposure of inorganic Hg for a short period of time could cause serious toxic influence on survival of fish. Based on the different concentrations of exposure, the studied fish is capable of concentrating the Hg in their bodies. In addition, this study has focused on enhancing the knowledge of tissue damage of the organs of tilapia *Oreochromis* sp. such as gills and liver, due to lethal and sub-lethal concentration exposure of waterborne mercury chloride. These results were very important factor in assessing the seriousness of toxicity and also important for the references of future studies.

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References

Adams, D.H. & Sonne, C. 2013. Mercury and histopathology of the vulnerable goliath grouper, *Epinephelus itajara*, in U.S. waters: A multi-tissue approach. *Environmental Research* 126: 254-263.

Ahmed, Q., Yousuf, F., Sarfraz, M., Mohammad, Q.A., Balkhour, M., Safi, S.Z. & Ashraf, M.A. 2015. *Euthynnus affinis* (little tuna): Fishery, bionomics, seasonal elemental variations, health risk assessment and conservational management. *Frontiers in Life Science* 8(1): 71-96.

Ahmed, Q., Yousuf, F., Sarfraz, M., Bakar, N.K.A., Balkhour, M.A. & Ashraf, M.A. 2014. Seasonal elemental variations of Fe, Mn, Cu and Zn and conservational management of *Rastrelliger kanagurta* fish from Karachi fish harbour, Pakistan. *Journal of Food, Agriculture and Environment* 12(3&4): 405-414.

Alhashemi, A.H., Karbassi, A., Kiabi, B.H., Monavari, S.M. & Sekhavatjou, M.S. 2012. Bioaccumulation of trace elements in different tissues of three commonly available fish species regarding their gender, gonadosomatic index, and condition factor in a wetland ecosystem. *Environmental Monitoring and Assessment* 184: 1865-1878.

AOAC 1995. *Official Methods of Analysis of AOAC*. 16th ed. Rockville: AOAC International.

APHA; AWWA; WPCF. 1998. *Standard Methods for the Examination of Water and Waste Water.* 20th ed. Washington, D.C.: APHA – American Public Health Association; AWWA- American Water Works Association; WPCF- Water Pollution Control Federation. p. 140.

Ashraf, M.A., Maah, M.J. & Yusoff, I. 2012. Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. *International Journal of Environmental Research* 6(1): 209-218.

Ashraf, M.A., Maah, M.J. & Yusoff, I. 2011. Assessment of heavy metals in the fish samples of mined out ponds Bestari Jaya, Peninsular Malaysia. *Proceedings of the Indian National Science Academy* 77(1): 57-67.

Agency for Toxic Substances & Disease Registry (ATSDR). 2004. Hazardous substances emergency events surveillance. Division of health studies surveillance and registries branch Atlanta, Georgia. http://www.atsdr.cdc.gov/HS/HSEES.

Buhl, K.J. 1997. Relative sensitivity of three endangered fishes, Colorado Squawd fish, Bony tail, and Razorback Sucker, to selected metal pollutants. *Ecotoxicology and Environmental Safety* 37: 186-192.

Carvalho, C.S. & Fernandes, M.N. 2006. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture* 251: 109-117.

Cerqueira, C.C. & Fernandes, M. 2002. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicology and Environmental Safety* 52: 83-91.

Chang, I.C., Wei, Y., Chou, F. & Hwang, P. 2003. Stimulation of Cl- uptake and morphological changes in gill mitochondria rich cells in fresh water tilapia (*Oreochromis mossambicus*) *Physiological and Biochemical Zoology* 76: 544-552.

Costa, M.F., Barbosa, S.C., Barletta, M., Dantas, D., Kehrig, H., Seixas, T. & Malm, O. 2009. Seasonal differences in mercury accumulation in *Trichiurus lepturus* (catlassfish) in relation to length and weight in a northest Brazilian estuary. *Environmental Science and Pollution Research* 16: 423-430.

Couture, P. & Pyle, G. 2011. Homeostasis and toxicology of essential metals, 9 - Field studies on metal accumulation and effects in fish. *Fish Physiology*. Vol 31, Part A. pp. 417-473.

DiFrancesco, D.T. & Shinn, Jr. R.C. 2002. New Jersey mercury task force report. *Exposure and Impacts*. Volume II , January. p. 147.

DOE-UM. 1986. Water quality criteria and standards for Malaysia. Executive Summary. Final Report. Department of Environment, Ministry of Science, Technology and Environment, Malaysia Consultant group on water quality, Institute of Advanced Studies, Univ. of Malaya, Kuala Lumpur, Malaysia, 1, V-Xl1.

Fisheries and Aquaculture Department (FAO). 2007. The state of world fisheries and aquaculture 2006. Food and Agriculture Organization of the United Nations. Electronic publishing policy and support Branch, Rome. ISSN 1020- 5489.

Fernandes, C., Fontainhas-Fernandes, A., Rocha, E. & Salgado, M. 2008. Monitoring pollution in Esmoriz- Paramos lagon, Portugal: Liver histological and biochemical effects in *Liza saliens*. *Environmental Monitoring and Assessment* 145: 315-322.

Firat, O. & Kargin, F. 2010. Individual and combined effects of heavy metals on serum biochemistry of nile tilapia *Oreochromis niloticus*. *Archives of Environmental Contamination and Toxicology* 58: 151-157.

Friberg, L. & Vostal, D. 1974. *Mercury in the Environment, an Epidemiological and Toxicological Appraisal*. Boca Raton: CRC press. p. 215.

Gehringer, D.B., Finkelstein, M.E., Coale, K.H., Stephenson, M. & Geller, J.B. 2013. Assessing mercury exposure and biomarkers in largemouth bass (*Micropterus salmoides*) from a contaminated river system in California. *Archives of Environmental Contamination and Toxicology* 64: 484-493.

Greenfield, B.K., Swee, J., Teh, S.J., Ross, J.R., Hunt, J., Zhang, G.H., Davis, J.A., Ichikawa, G., Crane, D., Hung, S., Deng, D., Teh, F. & Green, P. 2008. Contaminant concentrations and histopathological effects in sacramento splittail (*Pogonichthys macrolepidotus*). *Archives of Environmental Contamination and Toxicology* 55: 270-281.

Hamid, M.A., Mansor, M. & Nor, S.A.M. 2015. Length-weight relationship and condition factor of fish populations in Temengor reservoir: Indication of environmental health. *Sains Malaysiana* 44(1): 61-66.

Harada, M. 1995. Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology* 25(1): 1-24.

Ishikawa, N.M., Ranzani-Paiva, M. & Lombardi, J. 2007. Acute toxicity of mecury (HgCl2) to Nile tilapia *Oreochromis niloticus*. *B. Inst. Pesca, Sao Paulo* 33(1): 99-104.

Jalaludeen, M.D., Arunachalam, M., Raja, M., Nandagopal, S., Bhat, A. & Sundar, S. 2012. Histopathology of the gill, liver and kidney tissue of the fresh water fish tilapia mossambica exposed to cadmium sulphate. *International Journal of Advance Biological Research* 2(4): 572-578.

Kaewamatawong, T., Rattanapinyopituk, K., Ponpornpisit, A., Pirarati, N., Ruangwises, S. & Rungsipipat, A. 2013. Short-term exposure of Nile tilapia *Oreochromis niloticus* to mercury histopathological changes, mercury bioaccumulation, and protective role of metallothioneins in different exposure routes. *Toxicologic Pathology* 41: 470-479.

Kaoud, H.A. & Mekawy, M.M. 2011. Bioremediation the toxic effect of mercury-exposure in Nile tilapia (*Oreochromis niloticus*) by using Lemna gibba L. *Journal of American Science* 7(3): 336-343.

Low, K.H., Zain, S.Md. & Abas, M.R. 2011. Evaluation of metal concentrations in red tilapia (*Oreochromis* spp) from three sampling sites in Jelebu, Malaysia using principal component analysis. *Food Analytical Methods* 4: 276-285.

Ministry of Health Malaysia. 2012. Food Act 1983 and Food regulations 1985. Updated until December 2011 (P.U.(A) 435/2010). Kuala Lumpur, Malaysia. fsq.moh.gov.my

Mohan, M., Deepa, M., Ramasamy, E.V. & Thomas, A.P. 2012. Accumulation of mercury and other heavy metals in edible fishes of Cochin backwaters, Southwest India . *Environmental Monitoring and Assessment* 184: 4233-4245.

Mokhtar, M.B., Aris, A.Z., Munusamy, V. & Praveena, S.M. 2009. Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis* spp. in selected aquaculture ponds of high densities development area. *European Journal of Scientific Research* 30(3): 348-360.

Montaser, M., Mahfouz, M., El-Shazly, S., Abdel-Rahman, G. & Bakry, S. 2010. Toxicity of heavy metals on fish at Jeddah Coast KSA: Metallothionein expression as a biomarker and histopathological study on liver and gills. *World Journal of Fish and Marine Sciences* 2(3): 174-185.

Nandlal, S. & Pickering, T. 2004. *Tilapia Fish Farming in Pacific Island Countries. Vol. 1. Tilapia Hatchery Operation*. Noumea, New Caledonia: Secretariat of the Pacific Community.

Nguyen, H.L., Leermakersa, M., Kurunczic, S., Bozod, L. & Baeyens, W. 2005. Mercury distribution and speciation in Lake Balaton, Hungary. *Science of the Total Environment* 340: 231-246.

Oliveira-Ribeiro, C.A., Belger, L., Pelletier, E. & Rouleau, C. 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). *Environmental Research* 90: 217-225.

Oliveira Ribeiro, C.A., Pelletier, E., Pfeiffer, W.C. & Rouleau, C. 2000. Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. *Environmental Research Section A* 83: 286 -292.

Oliveira Ribeiro, C.A., Fanta, E., Turcatti, N.M., Cardoso, R.J. & Carvalho, C.S. 1996. Lethal effects of inorganic mercury on cells and tissues of *Trichomycterus brasiliensis* (Pisces, Siluroidei). *Biocell* 20: 171-178.

Osman, A.G. 2012. Biomarkers in Nile tilapia *Oreochromis niloticus niloticus* (Linnaeus, 1758) to assess the impacts of river Nile pollution: Bioaccumulation, biochemical and tissues biomarkers. *Journal of Environmental Protection* 3: 966-977.

Pandey, S., Parvez, S., Ansari, R., Ali, M., Kaur, M., Hayat, F., Ahmad, F. & Raisuddin, S. 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of afreshwater fish, *Channa punctata* Bloch. *Chemico-Biological Interactions* 174: 183-192.

Pandey, S., Kumar, R., Sharma, S., Nagpure, N., Srivastava, S. & Verma, M. 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). *Ecotoxicology and Environmental Safety* 61: 114-120.

Pereira, S., Pinto, A.L., Cortes, R., Fontaı´nhas-Fernandes, A., Coimbra, A.M. & Monteiro, S.M. 2013. Gill histopathological and oxidative stress evaluation in native fish captured in Portuguese northwestern rivers. *Ecotoxicology and Environmental Safety* 90: 157-166.

Ponzoni, R.W., Khaw, H.L. & Yee, H.Y. 2010. *GIFT: The Story since Leaving ICLARM (now known as the World Fish Center – Socioeconomic, Access and Benefit Sharing and Dissemination Aspects*. Fridtj of Nansen Institute FNI Report 14/2010. p. 47.

Ponzoni, R.W., Hamzah, A., Tan, S. & Kamaruzzaman, N. 2005. Genetic parameters and response to selection for live weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 247: 203-210.

Qiu, Y-W., Lin, D., Liu, J. & Zeng, E. 2011. Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment. *Ecotoxicology and Environmental Safety* 74: 284-293.

Rainbow, P.S. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environmental Pollution* 120: 497-507.

Raldu´a, D.; Dı´ez, S.; Bayona, J. M.; and Barcelo, D., 2007. Mercury levels and liver pathology in feral fish living in the vicinity of a mercury cell chlor-alkali factory. *Chemosphere* 66: 1217-1225.

Senthamilselvan, D., Chezhian, A., Kabilan, N. & Sureshkumar, T. 2011. Synergistic impact of heavy metals (Ni and Hg) and histopathological alterations in the organ (gill) of the fish, *Lates calcarifer* (Bloch, 1790). *European Journal of Experimental Biology* 1(2): 198-205.

Sharma, R.R., Pandey, A.K. & Shukla, G.R. 2001. Histopathological alterations in fish tissues induced by toxicity. *Aquaculture* 2(1): 31-43.

Silva, G.S., Neto, F.F., Assis, H.C., Bastos, W.R. & Oliveira Ribeiro, C.A. 2012. Potential risks of natural mercury levels to wild predator fish in an Amazon reservoir. *Environmental Monitoring and Assessment* 184: 4815-4827.

Sloman, K.A. 2007. Effects of trace metals on almonid fish: The role of social hierarchies. *Applied Animal Behaviour Science* 104: 326-345.

Sunderland, E.M. & Chmura, G.L. 2000 An inventory of historical mercury emissions in Maritime Canada: Implications for present and future contamination. *The Science of the Total Environment* 256: 39-57.

Triebskorn, R., Telcean, I., Casper, H., Farkas, A., Sandu, C., Stan, G., Colarescu, O., Dori, T. & Kohler, H.R. 2008. Monitoring pollution in river Mures, Romania, part II: Metal accumulation and histopathology in fish. *Environmental Monitoring and Assessment* 141: 177-188.

U.S. Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving water to fresh water and marine organisms, 5th ed. EPA-821-R-02-012. Final report, Office of water, Washington, D.C.

Velcheva, I., Tomova, E., Arnaudova, D. & Arnaudov, A. 2010. Morphological investigation on gills and liver of fresh water fish from Dam lake 'Studen Kladenets'. *Bulgarian Journal of Agricultural Science* 16(3): 364-368.

Vergilio, C.S., Carvalho, C.E. & Melo, E.J. 2012. Accumulation and histopathological effects of mercury chloride after acute exposure in tropical fish *Gymnotus carapo*. *Journal of Chemical Health Risks* 2(4): 01-08.

Watanabe, W.O., Wicklund, R.I., Olla, B.I. & Head, W.D. 1997. Saltwater culture of the Florida red and other saline tolerant tilapias: A review. In *Tilapia Aquaculture in the Americans*. Vol. 1, edited by Costa-Pierce, A.B. & Rakocy, J.E. Baton Rouge, Louisiana: World Aquaculture Society. pp. 54-141.

World Health Organization (WHO). 2012. Mercury and Health. Fact sheet N°361. http://www.who.int/mediacentre/factsheets/fs361/en. Accessed on April 2012.

Wu, S.M., Ding, H., Lin, L. & Lin, Y. 2008. Juvenile tilapia (*Oreochromis mossambicus*) strive to maintain physiological functions after waterborne copper exposure. *Archives of Environmental Contamination and Toxicology* 54: 482-492.

Yacoub, A.M. 2007. Study on some heavy metals accumulated in some organs of three river Nile fishes from Cairo and Kalubia Governorates. *African Journal of Biological Science* 3: 9-21.

Yang, J.L. & Chen, H.C. 2003. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. *Zoological Studies* 42: 455-461.

Zahir, F., Rizwi, S.J., Haq, S.K. & Khan, R.H. 2005. Low dose mercury toxicity and human health. *Environmental Toxicology and Pharmacology* 20(2): 351-360.

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