Heavy Metal Analysis and Histopathology of Gills of Nile Tilapia (Oreochromis niloticus) in Selected Areas of Candaba Swamp Pampanga
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ABSTRACT
Aquatic ecosystem is now greatly dominated with various heavy metals as harmful chemical pollutants. Different organisms are mainly affected to this environmental problem due to ceaseless discharge of pollutants in their natural habitat. In this study the Nile tilapia (Oreochromis niloticus) that were collected from different stations at Candaba, Swamp, Pampanga were subjected to heavy metal analysis for the presence and level of Lead, Cadmium and Mercury in their gills. Histopathology was also done to determine histological damages in gills. Using Shimadzu Atomic Absorptions Spectrophotometry the study revealed that there is Cadmium toxicity in all stations in which station 1 and 3 are beyond the method detection limit (MDL) which is 0.02 mg/kg, whereas Lead toxicity which is beyond MDL (0.01 mg/kg) is also detected in Station 1 and 2. The level of mercury from the gill samples are also beyond the MDL (0.01 mg/kg) in stations 1 and 3 but mercury is not detected in station 2, this was carried out using DMA- Mercury Analyzer. Additionally, the result in histopathological manifestations in the gills of Nile Tilapia were revealed to have a shortening of secondary lamellae, lamellar fusion, hyperplasia, epithelial lifting, epithelial desquamation, cell mass damage, epithelial necrosis, epithelial degeneration, edema, proliferation of mucous cells, blood vessels dilation. The study provides an informative knowledge with regards to the status of contaminations of chemical pollutants in the aquatic ecosystem.

INTRODUCTION
Consistent dispersion of toxic chemical pollutants is one of the serious environmental issues, nowadays. Most of the developing countries are suffering from these problems due to rapid industrializations and increase in number of population [1]. Heavy metal depositions were primarily derived from the industrial sites that release toxic chemicals, domestic wastes products, mining activities and agricultural wastes products [2-4]. The petroleum from the shipping vessels and the harmful substances and the harmful substances from the dump sites may also contribute to water pollution [5]. Heavy metal contamination may also be due to natural events such as volcanic eruptions and interactions of abiotic factor in the ecosystem [6]. Heavy metals that are highly prevalent in the aquatic ecosystem are the Cadmium (Cd), Mercury (Hg), and Lead (Pb) and Nickel (Ni). Candaba swamp in Pampanga is a major source of livelihood due to fishing, is at risk of contaminations with heavy metals because it serves as a catch basin of water from Penaranda River, Sierra Madre and Pampanga River that is connected to Manila de Bay which is one of the polluted marine water in the country [7,8]. Nile Tilapia (Oreochromis niloticus) is one of the freshwater fishes in the Philippines that provide a primary source of food and employment to the people [9]. This organism can act as a good bioindicator of different toxic chemical pollutants in the aquatic ecosystem [10]. Hence, this study aimed to investigate the histological abnormalities due to heavy metal in the water that will persist in the gills of the fish.
MATERIALS AND METHODS

Description of Study Area

Candaba Swamp lies in the municipality of Candaba Pampanga, Philippines, nearby to the town of San Ildefonso and San Miguel Bulacan in Central Luzon Island. It is located 50 km away NNW from the city capital of the Philippines, Manila. This swamp covers the area for almost 32,000 ha. and is elevated 11 m. above sea level. Candaba swamp is composed of freshwater ponds, swamps and grassland that are submerged during wet season (June to October) whereas dry season starts from November to May 10. The swamp are suitable area for the agricultural farm, fishponds and also important for flood control and a source of irrigation.

Collection of Fish Sample

Nine pieces of Nile Tilapia in each of the stations were randomly collected in Candaba Swamp with the help of the hired fisherman for the heavy metals determination and histopathological examination.

Preparations and Heavy Metal Determinations of Fish Sample

Nine selected Nile Tilapia samples from the three different stations were sacrificed and cleaned first for the removal of gills. The gills were stored in a cooler and were sent to private environmental laboratory for the determinations of Lead and Cadmium using the Shimadzu Atomic Absorptions Spectrophotometry. Mercury contamination was determined through DMA Mercury Analyzer.

Tissue Preparations for Histopathological Examinations

Fish gills were collected and stored in a clean container. It was fixed in 10% formalin for 24 hours and then was sent to a private medical center for tissue embedding and staining. Eosin and haematoxylin was the solutions used for the tissue preparation. Prepared tissue slides were used to observe the damages occurred caused by the contaminations of heavy metals.

RESULTS AND DISCUSSION

Gill is a unique respiratory organ that usually found in all species of fishes. This organ regulates various functions including respirations, osmoregulation, nitrogenous wastes excretions and pH level regulation. Gills are the main site where the gases exchange in fish occur which has an intimate contact with the surrounding water. Direct exposure to water is vulnerable to different kind of infectious disease, heavy metals contamination and other several factors. Toxic pollutants were the focus of this study and the results revealed that gills were found to have minute concentrations of harmful heavy metals.

Lead

The finding of the study reveals that there is a high contamination of Lead in Station 1 with the concentration of 2.1 mg/kg. Gills of fish from Station 2 was also detected with 0.8 mg/kg level of Lead toxicity whereas Station 3 no contamination of lead is detected.

Cadmium

Two stations in Candaba were found to have contamination of Cadmium. Station 1 has been reported with the highest level of toxicity which is 1.2 mg/kg, while Station 3 was also detected of 0.8 mg/kg unit concentration of Cadmium. Heavy metal concentration in Station 3 was still beyond the given Method Detection Limit (MDL) which is 0.02 mg/kg (Figure 1).

Mercury

Mercury toxicity was reported in Station 1 and 2 having the heavy metal concentration of 0.9 mg/kg and 0.04 mg/kg respectively which are beyond to the Method Detection Limit. No contamination of Mercury is detected in Station 2 (Table 1).
Figure 1. A graph representation the heavy metal concentrations in the Gills of Nile Tilapia in three selected stations.

Heavy Metal Concentrations

Table 1. Heavy metal concentrations in three selected stations.

<table>
<thead>
<tr>
<th></th>
<th>Lead MDL (0.1 mg/kg)</th>
<th>Cadmium MDL (0.02 mg/kg)</th>
<th>Mercury MDL (0.01 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station 1</td>
<td>2.1</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Station 2</td>
<td>0.8</td>
<td>0.02</td>
<td>ND</td>
</tr>
<tr>
<td>Station 3</td>
<td>ND</td>
<td>1.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Histopathological Observation

Histological aberrations are the following evidences that the bodies of water in Candaba Swamp might be contaminated with different hazardous and toxic chemical pollutants. Various abnormal tissues that had observed from the gills tissue of Nile Tilapia indicate that there is a continuous and increasing discharge of chemicals in the aquatic ecosystem. Abnormal tissues caused by the contaminations of lead, cadmium and mercury are the shortening of secondary lamellae, lamellar fusion, hyperplasia, epithelial lifting, epithelial desquamation, epithelial necrosis, epithelial degeneration, oedema, proliferation of mucous cells and blood vessels dilation (Figure 2).

Figure 2. (A) Epithelial hyperplasia (EH); epithelial lifting (EL); blood vessel dilation (BVD); (H and E, HPO, 1000x).
(B) Shortening of secondary lamellae (SSL); Proliferation of mucous cells (PMC); Necrosis (N). (C) Blood vessel dilation (BVD); Damage cell mass (DCM); Desquamation of secondary lamellae (DSL). (D) Epithelial degeneration (ED); Fusion of secondary lamellae (FSL); Oedema (O); Shortening of secondary lamellae (SSL). (E) Epithelial lifting (EL); Fusion of secondary lamellae (FSL); Damage cell mass (DCM); (LPO H and E, 250x).

CONCLUSION

Candaba Swamp in Pampanga is affected with the discharge of different chemical toxicity such as mercury lead and cadmium. Moreover, abnormal tissues observed in gills are lamellar fusion, shortening of secondary lamellae, damage cell mass, oedema, proliferation of mucous cells, desquamation of secondary lamellae and blood vessels dilation due to harmful heavy metals and by considering other harmful factors.

REFERENCES