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ORIGINAL ARTICLE

Bioaccumulation and histopathological alteration of total lead in selected fishes from Manila Bay, Philippines

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Abstract This study aims to assess the bioaccumulation of total lead and the effect of heavy metal on the muscles of fish obtained in the coastal lagoon of the Manila Bay. Fish species muscles were assessed for lead concentrations and were examined for histological alterations. Results showed that lead bioaccumulation in the muscles, and a degree of disintegration in the muscle fibers of all the fish examined were found.

1. Introduction

Manila Bay is one of the famous water bodies in the country known for its picturesque view. The Manila Bay has provided a number of benefits. However, the concern of heavy metal pollution continues to haunt the existence of the organisms and the state of the environment. The occurrence and persistence of heavy metals in the environment is one of the crucial problems affecting the general populace. Several studies have indicated that heavy metals may bring about several consequences on both the ecosystem and human health. The entry of heavy metals into the food chain is one of those issues that bring about concern, especially that these heavy metals may end up in each and everyone's table, resulting to morbidities and mortalities.

A previous study (Sia Su et al., 2009) has shown that heavy metals do exist in the waters of and in the fish and macroinvertebrates obtained in the Manila Bay. Other studies (Olojo et al., 2005; Fadel and Gaber, 2007) have indicated that the persistence of heavy metals in the environment contributes to the accumulation of the metals in the organism's tissues and bring about induced morphological, histological, and biochemical alterations in the organism's tissues. Commonly, tissue lesions, apoptosis, and necrotic alterations in the fish are the cellular changes that continue to affect most fish, as they continue to be exposed to heavy metals.

The histological biomarkers are constantly in use in most studies, as this indicates the overall health of the environment and are important indicators of pollutants in the environment.

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This present study aims to assess the bioaccumulation of total lead in the fish muscle and to investigate the histological changes in the muscles, as this is the part of the fish that is commonly consumed by most people. No study has explored on looking at the histological alterations of the organisms exposed to metals locally. Results of this study may contribute significantly to our current knowledge, particularly on the present state of the Manila Bay. Likewise, this study provides baseline information on the induced alterations occurring in the fish obtained in the Manila Bay due to their exposures to heavy metals persisting in the environment. The investigation presents a reliable indicator on the state of the aquatic ecosystem and on the impact of xenobiotic substances on the organisms and the general environment.

2. Materials and methods

A local fisherman caught the fish samples using a trawl net at the coastal lagoon of the Manila Bay. The collection of the fish samples using the bottom trawl fishing method started from the northern end of the lagoon going to the south and then back to the starting point again, which is the northern end. Fish collected were immediately processed and identified. All fish obtained were identified through the website www.fishbase.org. A total of five fish per species were randomly selected from the pool of fish obtained. About one gram sample of the fish muscles dissected was ash and the wet digestion method was used in the analysis of the heavy metals total lead following the standard procedures (APHA, AWWA, WEF, 1998) before using the Shimadzu AA-6300 atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). Appropriate controls and standards and calibration curves were prepared for the variables tested in the fish. Duplicate samples were tested and read in triplicates. Consequently, the remaining fish muscles obtained from the fish species were dissected and fixed in 10% formalin for 24 h, dehydrated in graded ethanol concentrations, and embedded in paraffin wax. Sagittal sections (5 μm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol, and stained with hematoxylin–eosin (HE) method, and approximately two to four sections of each individual fish were analyzed by the light microscope.

3. Results and discussion

A total of five fish species were obtained at the coastal lagoon of the Manila Bay. The fish species caught were Barracuda (*Sphyraena qenie*), Deep body sardinella (*Sardinella brachysoma*), Yellowstripe scad (*Selaroides leptolepis*), Greenback mullet (*Liza subviridis*), and Otomebora mullet (*Mugil melinopterus*). Table 1 shows the listing of fish caught, their local and common names

The heavy metal total lead was detected in the fish muscles obtained from the coastal lagoon of the Manila Bay. Among all the fish muscle examined, the *L. subviridis* had the lowest mean ± SD total lead detected ($0.172 \pm 0.006\ mg\ kg^{-1}$), whereas the *S. brachysoma* had the highest mean ± SD total lead detected at $0.395 \pm 0.030\ mg\ kg^{-1}$. The other fish muscle examined showed a mean ± SD total lead of $0.246 \pm 0.003\ mg\ kg^{-1}$ for *S. leptolepis,* $0.252 \pm 0.016\ mg\ kg^{-1}$ for *S. brachysoma*, $0.395 \pm 0.030\ mg\ kg^{-1}$ for *S. qenie*, and $0.183 \pm 0.011\ mg\ kg^{-1}$ for *M. melinopterus*. The heavy metals, particularly the mean total lead in the muscle of fish species examined was above the permissible limit of lead for food at $0.05\ mg\ kg^{-1}$ (WHO, 1971) and the permissible limit of lead in fish at $0.05\ mg\ kg^{-1}$ (FAO, 1983).

All the fish examined had no gross lesions. Light micrographic sections of the normal fish (*Oreochromis niloticus*) muscle as previously obtained by one of the authors showed well-organized, closely packed muscle bundles. As compared with those of our study, the entire muscle sections of the fish caught in the coastal lagoon in Manila Bay showed slightly similar histological appearance but with a degree of evident disintegration of the muscle fibers. Spaces in between muscle bundles are remarkable (Fig. 1). While it is true that the normal fish muscle H&E staining have deteriorated relative to the newly obtained fish muscle samples, the integrity of the muscle bundles are clearly exhibited as shown in Fig. 1. Despite that the bioaccumulated lead concentrations at the fish muscles were different, similar histological alterations were observed. At lower magnifications, the Yellowstripe scad and the Greenback mullet muscles showed closely packed muscle bundles, but individual muscle fibers within the bundles showed a degree of disintegration. At higher magnifications, the disintegration among the individual muscle fibers was obvious. Likewise, the Yellowstripe scad muscle sections showed muscle fibers that have distinct peripherally located nuclei. For fish muscles that were obliquely cut, similar histological appearance with the cross-sectioned sections were observed. All the muscle tissues showed disintegration, and individual muscle fibers within bundles appeared to be loosely packed and exhibited shrinkage away from the surrounding perimysium.

This was a cross-sectional study, and its scope is limited to assessing the heavy metal, particularly total lead, and the histological change of the muscles of the fish obtained at the coastal lagoon of the Manila Bay. In the Philippines, fish is an important commodity and an important source of protein as 38 kg of fish and fishery products is per capita consumed per year (BFAR-DA, 2011). The range of fresh fish consumed by most Filipinos is from less than 0.5 g to about 5 kg per year (BFAR-DA, 2011). According to the BFAR-DA (2011), about

Table 1 Common, local, and scientific names of fish obtained at the coastal lagoon of the Manila Bay, Philippines.

Scientific names	Common names	Local names
Sphyraena qenie	Barracuda	Torsillos
Sardinella brachysoma	Deep body sardinella	Daing, Lapad, Tamban-yapad
Selaroides leptolepis	Yellowstripe scad	Salay-salay
Liza subviridis	Greenback mullet	Buwan-buwan, Banak,
Mugil melinopterus	Otomebora mullet	Kapak

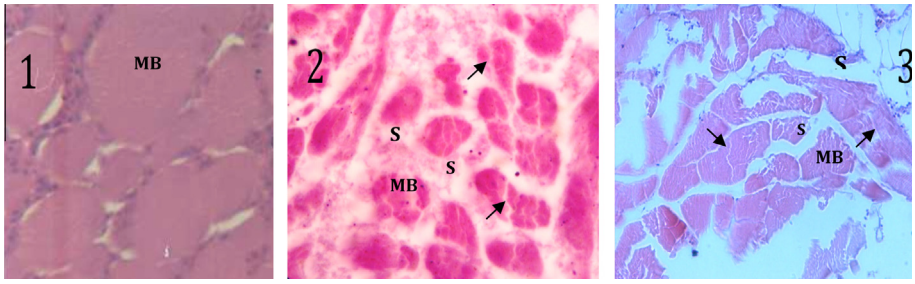

Figure 1 Photomicrograph of muscle showing the closely packed bundles in a normal fish, 400×(1), shrinkage away from the surrounding perimysium, 400×(2), and the disintegration of the muscle fibers, 400×(3). MB (muscle bundle); S (spaces between MB); arrow (disintegrated fibers).

a mean of 104 g of fish and its products is normally consumed by most Filipinos per day. This study has shown that the muscle of the fish examined bioaccumulated the total lead. The total lead present in the muscles of the fish may be due to its uptake of the heavy metal coming from its surrounding environment (Jeffree et al., 2006) and the incorporation of the chemical in its tissue (Dural et al., 2006). The result of this study is vital, as the muscle of the fish is the most commonly consumed part. The differences in the lead concentrations in the fish muscle examined may be attributable to the metal uptake and on the animal species (Olojo et al., 2005; Sia Su et al., 2009). The histological assessment of this study corroborates to the findings on the heavy metals accumulating in the muscles as a degree of disintegration of the muscle fibers was observed. Similar results by other studies (Thophon et al., 2003; Kaoud and El-Dahshan, 2010) present that the fish exposed to the heavy metal may reflect histological alterations where the muscles would reflect degeneration in its muscle bundles with certain focal areas of necrosis. Similar results as described by Kaoud and El-Dahshan (2010) present that the fish exposed to heavy metals show degeneration of the muscle bundles with atrophy and splitting of the muscle fibers. Our results showed no gross lesions in the fish species examined and this result is corroborated in a study (Ebrahimi and Taherianfard, 2011) where they found that as fish samples were exposed to heavy metals in the environment, accumulation of the metals in the fish tissues took place and consequent muscle degeneration was found but no pathological changes in the muscle tissues were also observed.

4. Conclusion

All the fish examined had lead bioaccumulation in their muscles, and a degree of disintegration in the muscle fibers was observed. Despite the differences in the bioaccumulated lead concentrations in the muscles of the fish, similar alterations on the histology of all the fish examined were evident.

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