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Research Article

TOXICITY AND BIOACCUMULATION OF MERCURY ON THE JUVENILE OF ASIAN SEA BASS, *LATES CALCARIFER* (BLOCH)

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ABSTRACT

Combination in aquatic ecosystems is perennial due to different toxicants entering through fabricated activities. Heavy metals may precipitate, get absorbed on sediment particles, remain soluble or suspended in water, these are absorbed by aquatic fauna upon their entry into water bodies. This study aimed to determine the levels of mercury in the gills, liver, kidney, muscles, fin and scales of Asian Seabass *Lates calcarifer*. Mercury was chosen because at higher concentrations it might become toxic to the fish and by extension to fish consuming humans too. The results demonstrate that the concentrations of mercury were higher in the liver followed by kidney, gills, muscle, fins and scales. Metal accumulation varied significantly in tissues of sea bass between different concentrations and periods. The liver is reported to be the primary organ contaminated by mercury in this fish.

Keywords: Acute toxicity, Bioaccumulation, Mercury, Juvenile sea bass.

INTRODUCTION

Heavy metal mercury in the fish is significantly toxicant and increasingly poses a crisis around the world. Mercury is a naturally occurring element present throughout the environment and in plants and animals. Human industrial activities increase the amounts of airborne mercury that eventually finds its way into lakes, rivers and the ocean, where it is guzzled up by the fish and other marine life. Once mercury gets into the marine food chain, it "bioaccumulates" in the larger predators. The larger fishes are generally riskier to eat than smaller ones. Historically, *Lates calcarifer* is an economically crucial species supporting major fisheries and has been used extensively in toxicity testing because of its cosmopolitan distribution and sensitivity to environmental contaminants.

Aquatic ecosystems are highly contaminated with dissimilar toxicants through anthropogenic activities, and some naturally present metals may be essential in lower concentrations however toxic in higher concentrations (Pereira *et al*., 2013). Some bioavailable form of pollutants can be accumulated in living organisms to a greater extent than others and there is a need to study the levels of pollutants in the organisms to predict their environmental risk (O'connell, *et al.*, 1999). Thus, chemical analyses of the tissues of aquatic organisms are used as a regime approach in studies of aquatic pollution. This provides a temporal integration of levels of pollutants with biological relevance at higher concentrations than in water or sediment, and facilitates their quantification (Rainbow & Phillips, 1993).

Monitoring sentinel fish species is popular to assess the degree of accumulation of pollutants and the effects on health status (De La Torre *et al.*, 2005). Besides, toxicant accretion in water suggests this fish as useful indicators of contamination in aquatic systems because they respond very well to aquatic changes than invertebrates and also tend to accumulate mercury often in concentrations several times higher than in the ambient media (Dhanakumar *et al*., 2015; Eagles Smith & Ackerman, 2014; Zhao, *et al.*, 2015).

Fishes of different sizes and ages are good indicators of water contamination in aquatic systems because they occupy different trophic levels; Unlike invertebrates, fishes are highly sensitive to many toxicants (Barak & Mason, 1990; Burger *et al.*, 2002). Mercury readily accumulates in tissue and is biomagnified through the aquatic food chain. Methylation of mercury is accepted to be a major process

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controlling its biological accessibility in aquatic ecosystems. Fish can accumulate mercury either through food or directly from the water. In natural ecosystems, it is most likely that fish accumulate mercury via these two vectors concurrently. However, the relative significance of these two uptake processes has not been clearly established under laboratory conditions and is probably related to species and site-specific conditions. The efficiency of mercury assimilation from food appears to vary among species.

MATERIALS AND METHODS

Experimental Fish

Healthy hatchery reared juvenile Asian sea bass; *L. calcarifer* with mean total length of 6.03 ± 0.59 cm and mean total weight of 8.76 ± 1.24 gm were obtained from the Rajiv Gandhi Centre for Aquaculture, Thirumullaivasal near Sirkali, Nagapattinam Dist, Tamil Nadu, India. Fishes were acclimatized for 2 weeks in stock tank to the experimental glass aquaria (120x50x50 cm) filled with 250 l of water with a salinity of 27 $\pm$ 2 ppt, under a natural photoperiod 12 h:12 h (light: dark) cycle. The water in the tanks was passed through a 1µm filter, UV-sterilized, and refilled daily. Fish were fed twice daily with chopped fresh fish. They were starved for 24 h before and during the experiment.

Experimental Procedure

Acute Toxicity test

A static bioassay acute toxicity test was performed to determine the 96-h LC_{50} of mercury to *L. calcarifer*, following the Standard Methods (APHA, 1995). After acclimatization period, 2 month-old fish (7.06 ± 0.15cm in length and 10.18 ± 0.24gm in weight) were transferred from the stock tank to the experimental aquaria. Ten fishes were randomly placed in each glass aquarium filled with 250 l (120 x 50 x 50 cm) of water, with loading densities of 0.69 g/l. Fishes were exposed to nominal mercury concentrations (0.8, 1.0,1.2,1.4,1.6,1.8 and 2.0 ppm). Each concentration was done in three replicates. Control fishes were held in a similar facility without exposure to mercury. The water quality characteristics were measured daily: dissolved oxygen (DO) 6.4$\pm$0.7 mg/l, temperature 27.5 $\pm$ 0.7ºC, salinity of 26 $\pm$ 3 ppt and pH 7.45 $\pm$ 0.5. The criteria for death were no gill movement and no reaction to gentle prodding. Fish mortality in each aquarium was recorded at the intervals of 24, 48, 72 and 96 hrs using the method of Sprague (1973). Dead fish were immediately removed. Based on acute toxicity, four lethal concentrations were derived for 24, 48, 72 and 96h of exposure, which were used as the experimental concentration of the mercury toxicants in the subsequent experiments.

Sublethal toxicity test

Fishes were exposed to 0.127 and 0.254 ppm sublethal concentration of mercury. Doses were theoretically sublethal, at 10% and 20% respectively, of the Maximum Acceptable Toxicant Concentration (MATC), which was 1.27 ppm. After 2 weeks of acclimatization in a holding tank, ten healthy fishes (7.76 ± 0.19 cm in length and 10.69 ± 0.84 gm in weight) were transferred to each aquarium at a loading density of 0.71 g/L. Three replicates were performed for test concentration and control. Fishes were fed twice daily with chopped fresh fish at 10:00 and 14:00 h. Uneaten food was quickly removed from the system. Fishes were starved for 24 h before sampling. The experimental water (50%) was changed every 2 weeks to keep the water quality within acceptable limits according to APHA (1995). Water quality (dissolved oxygen, temperature, pH and salinity) was measured everyday and water chemistry (ammonia nitrogen, nitrite nitrogen, nitrate nitrogen) was measured twice weekly. All chemical parameters were determined following the techniques of (APHA, 1995) using analytical grade reagents. The actual concentration of mercury was measured weekly before and after its addition to maintain concentrations at the designed level. Mortality and behavior were observed everyday in each concentration. Two fishes from each aquarium were sampled at 0, 7, 14, 21 and 28 days post exposure.

Metal analysis

Prior to metal analysis muscle, gills, liver, kidney fins and scales were dissected and were homogenized thoroughly in a food blender with stainless steel cutters (Voegborlo *et al.*, 1999). Approximately 125 mg of each undigested sample homogenate was loaded directly into a Quartz sample boat, and then the sample boat was delivered into a decomposition furnace. In brief, the working principles with respect the DMA-80 system are as follows: (1) Samples were dried and thermally decomposed in an oxygen-rich stream at 750°C with the consequent sublimation of Hg; (2) Catalytic reduction of Hg; (3) Hg is selectively trapped by gold amalgamation; (4) The amalgamation furnace is heated and Hg is quickly introduced into multiple measuring cells situated along the optical path of the spectrophotometer, then Hg is quantitatively determined by atomic absorption spectrometry(Milestone Srl, Sorisole (BG) - Italy) using a low-pressure Hg lamp at a wavelength of 253.65 nm.

RESULTS

Acute Toxicity Test

Acute toxicity study was done to determine the impact of mercury on *L. calcarifer* within a short period. In the present study the 24, 48, 72 and 96h LC_{50} value were found to be 2.22, 1.86, 1.42 and 1.27 ppm respectively. Among the test concentrations prepared from the preliminary toxicity test the mortality of 50% of the population after 96 h of exposure was observed on 1.27 ppm concentration of mercury.

Bioaccumulation

Mercury accumulation in muscle, gills, liver, kidney, fins and scales of *L. calcarifer* showed significant variations

(Figs. 1 to 6). In the muscle of untreated fish, mercury concentration was between *0.010µg*/g to 0.014µg/g . A sharp increase in mercury content was recorded after 28 days of exposure to 0.025 µg/g at 0.127 ppm and 0.034 µg/g at 0.254 ppm concentrations of mercury. In the gills of untreated fish, the mercury concentration was between 0.012 µg/g and 0.015 µg/g. A sharp increase in mercury content was recorded after 28 days of exposure. It was 0.029 µg/g at 0.127 ppm and 0.038 µg/g at 0.254 ppm concentrations of mercury. In the liver of untreated fish, the Mercury concentration was between 0.023µg/g and 0.026µg/g. At the end of the experiment (28 days), the mercury concentration of the liver increased to 0.046 µg/g at 0.127 ppm and 0.064µg/g at 0.254 ppm. In the kidney of untreated fish, mercury concentration varied between 0.018 µg/g and 0.022 µg/g. At the end of the experiment (28 days), mercury concentration of the kidney increased to 0.034 µg/g at 0.127 ppm and 0.042 µg/g at 0.254 ppm. In the fins of untreated fish, mercury concentration varied between 0.009µg/g and 0.013 µg/g. At the end of the experiment (28 days), mercury concentration of fins increased to 0.018 µg/g at 0.127 ppm and 0.021 µg/g at 0.254 ppm. In the scales of untreated fish, mercury concentration varied between 0.008 µg/g and 0.012 µg/g. At the end of the experiment (28 days), mercury concentration of scales increased to 0.015 µg/g at 0.127ppm and 0.019 µg/g at 0.254 ppm.

The results demonstrate that the concentrations of mercury were higher in the liver followed by kidney, gills, muscle, fins and scales. Metal accumulation varied significantly in tissues of sea bass at different concentrations and periods ($p < 0.05$).

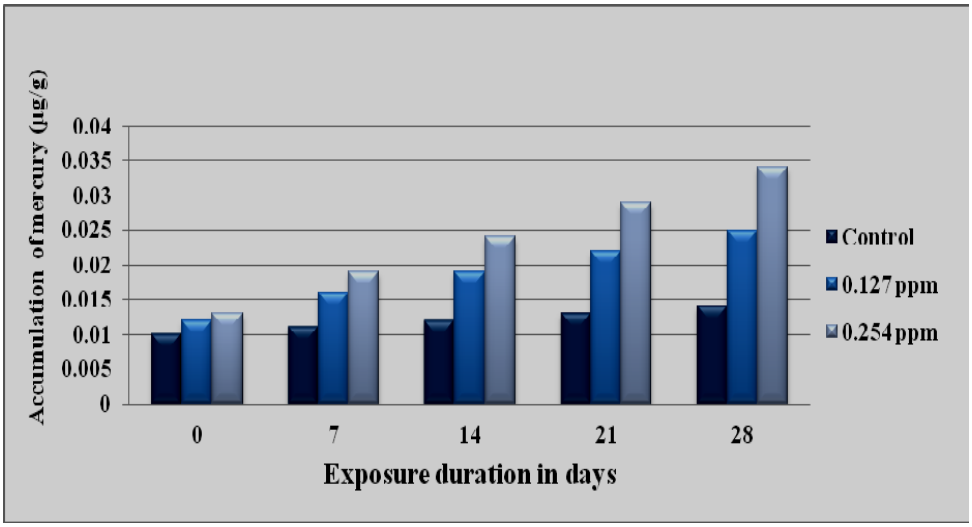

Figure 1. Accumulation of mercury in the muscle of *L.calcarifer* exposed to sublethal concentrations of mercury.

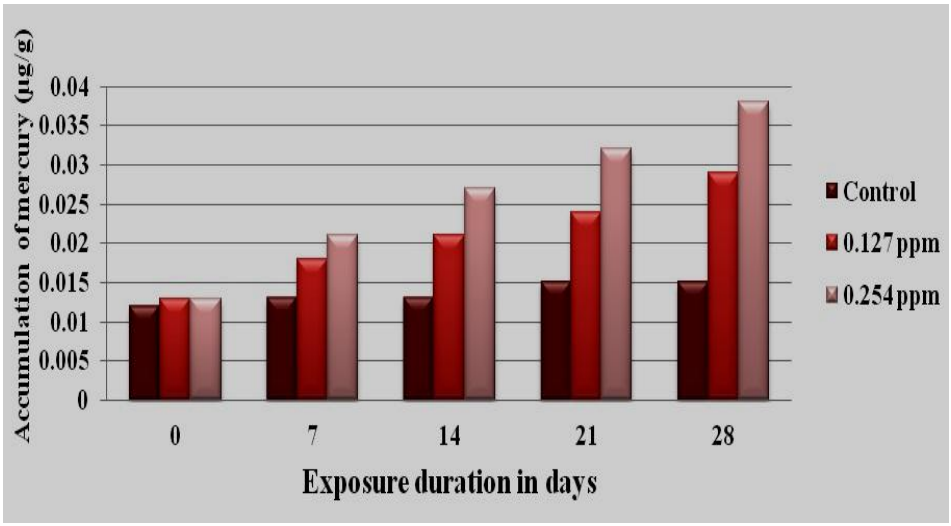

Figure 2. Accumulation of mercury in the gill of *L.calcarifer* exposed to sublethal concentrations of mercury.

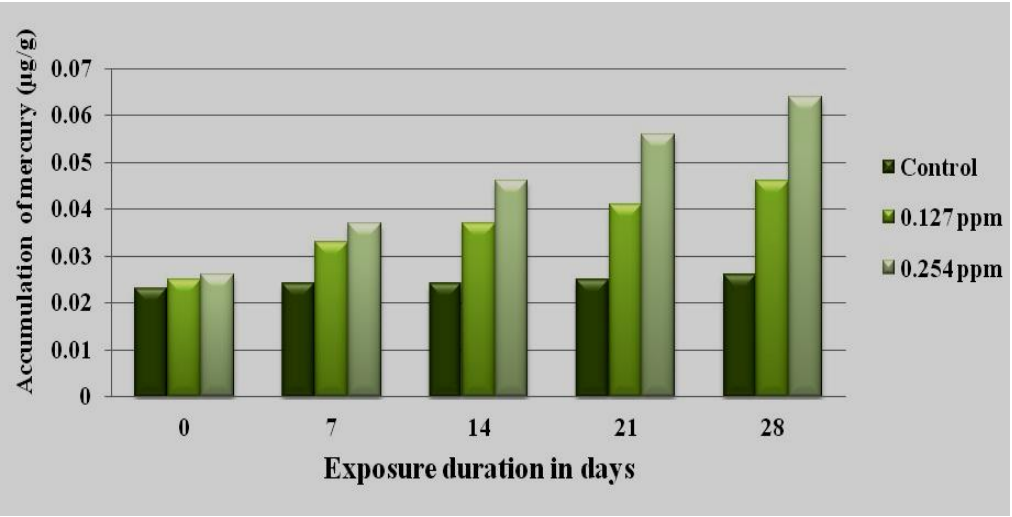

Figure 3. Accumulation of mercury in the liver of *L. calcarifer* exposed to sublethal concentrations of mercury.

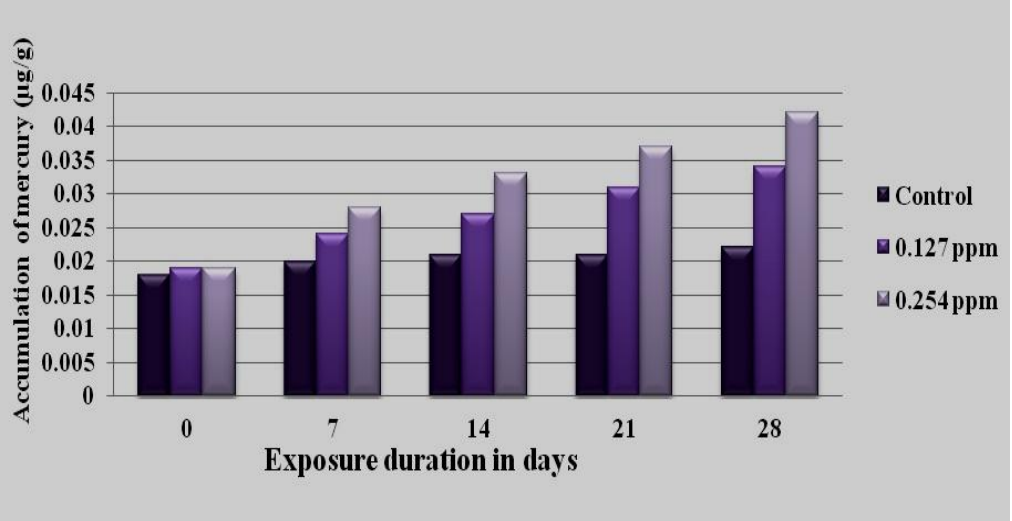

Figure 4. Accumulation of mercury in the kidney of *L.calcarifer* exposed to sublethal concentrations of mercury.

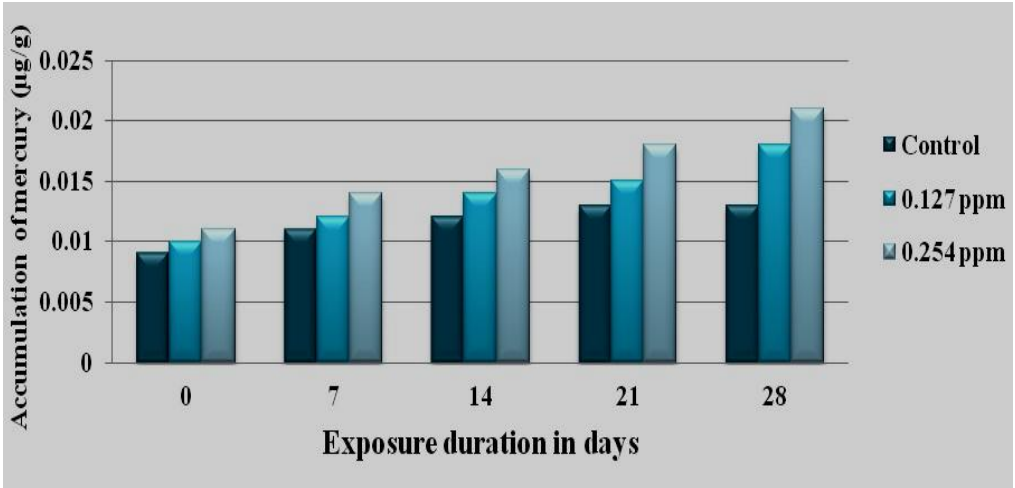

Figure 5. Accumulation of mercury in the fins of *L.calcarifer* exposed to sublethal concentrations of mercury.

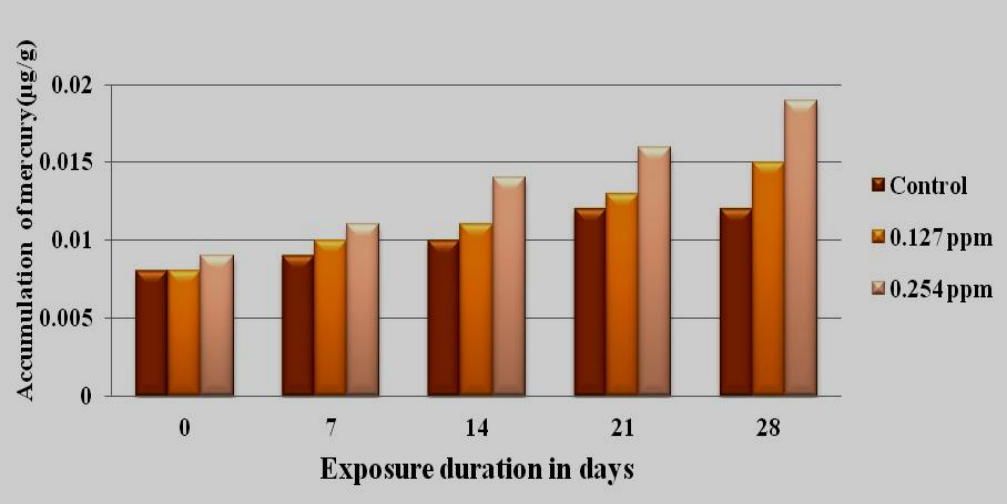

Figure 6. Accumulation of mercury in the scales of *L.calcarifer* exposed to sublethal concentrations of mercury.

DISCUSSION

The presence of xenobiotics in the nautical environment exerts well-known biological effects on marine organisms. In select sentinel species, these effects may be considered as biomarkers, a sort of early warning signal to review the quality of marine habitats (Moore *et al.*, 2004). Fish are generally exposed to toxicants via two exposure routes, waterborne: gills and derma and dietary (Sloman, 2007). In the present investigation the fingerlings of *L.calcarifer* were exposed to two sublethal concentrations of water borne mercury for four weeks only. During this exposure fish accumulated a significant level of mercury in their various tissues (liver > kidney> gills> muscle> fins >scales), in agreement with researchers (Maharajan *et al*., 2010; Parurukkumani *et al*., 2015a,b, c & d).

Toxicants are absorbed by various organs of the fish. In this process, they are concentrated at different levels in different organs of the fish (Rao & Padmaja, 2000). Therefore, in teleost fish, the gills, liver, kidney and muscles are the tissues most recurrently utilized in ecological, toxicological studies (Heier *et al.*, 2009) because they are metabolically active tissues and accumulate toxicants at higher levels.

The fish muscle is a very important, precious and recommended food in the human nutrition due to low fat and high proteins and desides mineral substances as well as optimal ratio of unsaturated fatty acids with cardio-protective effect. Conversely, fish muscle may be the repository for different contaminants, in the water ecosystem (Andres *et al.*, 2000). Such environmental pollutants like heavy metals are a global threat to food safety, thus muscles could lose these properties due to environmental contamination (Bajc *et al.*, 2005). The metal concentrations in water are positively correlated with the concentrations in fish tissues (Svobodova, *et al.*, 1996). Consumption of fish spoiled by heavy metals have deleterious effects on human health which was widely accredited after a series of events in the period from 1953 to 1960 when several thousands of people died in Minamata Bay in Japan as a result of poisoning caused by the consumption of mercury contaminated fish (Harada, 1995). Among the metals, mercury (Hg) is the most widespread concern in connection with fish consumption and advisories linked to fish consumption are issued by health authorities in many countries (Ibrahim *et al*., 2011). Therefore, the presence of these metals and other contaminants in seafood has become a matter of conceren in the last decades (Usydus *et al*., 2009).

Toxicants enter the body mainly through the gills and consequently, through blood they reach the parenchymal organs where they retain for a longer time (Terra, *et al.*, 2008). In addition toxicant concentrations, in the gills reflect the same in water where the fish live; whereas, concentrations in other organs such as the liver and kidney represent storage of toxicants (Kroglund *et al.*, 2012). Studies of metal accumulation in fish are mainly focused on the muscle tissue, while the metal accumulation patterns in other tissues have been largely neglected (Jovicic, 2014). Elemental accumulation in many fish tissues and organs and their potential use in monitoring programs have not received enough attention.

Fish gills are multifunctional organs involved in ion transport, gas exchange, acid–base regulation and waste excretion (Dang, *et al.*, 2001). One of the major target organs for waterborne toxicants is the gill (Playle, 1998; Sprague, 1973). Gills are regarded as important sites for direct uptake from the water, whereas the body surface is generally assumed to play a minor role in xenobiotics uptake of fish (Pereira, 2013). Besides, fish gills are very sensitive to physical and chemical alterations of the aquatic medium such as: temperature, acidificaion of water supply due to acid rain, salts and heavy metals, and to any change

in the composition of the environment which is an important indicator of waterborne toxicants (Saber, 2011). Fish gills are the main route of penetration of toxicants into the fish organism, thus they are the first organs coming into contact with environmental pollutants, and are also sensitive subjects for identifying the effects of water toxicants on fish organisms (Tkacheva *et al.*, 2004; Rosseland, *et al.*, 2007). Fish gills can accumulate bioavailable pollutants, and their measurement on gills can reflect the speciation of pollutants, and in particular metals in water, therefore, they are a useful tool for assessing bioavailability of elements in water (Heier *et al*., 2009). Gill surface serves as metal-binding ligands and metal bioaccumulation in particular can occur due to positively charged metal species in the water to negatively charged sites on the gills (Playle, 1998). Gills are important site for direct toxic effects to metals at high concentrations, for sub-lethal effects at lower metal concentrations, and, along with uptake from food (Cu, Zn, Se, Fe) and non-essential elements (Al, As, Cd, Cr, Ni, Pb) (Rosseland *et al*., 2007).

Once the toxicants cross the biological barriers and enter the bloodstream, they accumulate in the internal organs of fish. Numerous studies have quantified contaminants in fish organs to evaluate environmental quality, seeking causal relationships with fish health. The liver is the best choice to access contaminants, followed by the kidney and gills (Pokorska *et al.*, 2012). The liver is reported to be the primary organ for bioaccumulation and so has been extensively studied with regard to the toxic effects of xenobiotics (Simonato, *et al*., 2008). The liver is also a target organ due to its large blood supply, which causes noticeable toxicant exposure. The vertebrate kidney is the main organ involved in the maintenance of body fluid homeostasis. The morphology and function of the kidney have been modified through evolution to fulfill different physiological requirements and the widest range of kidney types is found in fishes (Hentschel & Elger, 1989). In teleosts, the kidney, together with the gills and intestine is responsible for excretion and the maintenance of the homeostasis of the body fluids (Ojeda, *et al*., 2003). Besides producing urine, it acts as an excretory route for the metabolites of a diversity of xenobiotics to which the fish may be exposed (Hinton & Lauren, 1990). The kidney also excretes other nitrogen-containing waste products from the metabolism such as ammonia and creatinine. In addition, in fish as in higher vertebrates, the kidney performs an imperative function related to electrolyte and water balance and the maintenance of a stable internal environment (Maduereira *et al*., 2012). Thus, many studies showed that different toxicants accumulate mainly in the liver and kidney (Maharajan *et al.*, 2012 & 2016). Based on the literature we can conclude that fishes are very good indicators for impaired water quality as they have different sizes and occupy different tropic levels and are long-living.

CONCLUSION

Depending on the purposes of research, bioaccumulation and biomarker levels; can be applied on different fish organs. The most frequently used are the respiratory organs the gills and parenchyma organs – liver and kidney, but in terms of human health the most appropriate tissue are the muscles

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