



Experimental study on the mercury accumulation in Asian Seabass *Lates calcarifer* (Bloch) Fingerlings

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Abstract

Numerous toxicological studies have gained increasing interest in order to understand the impact of mercury on aquatic communities especially fish. In the present study bioaccumulation of heavy metal mercury was tested in the selected organs viz. muscle, gills and liver of the commercially important fish Asian seabass *Lates calcarifer*. Fishes were exposed to sublethal concentrations of 5% (0.05ppm), 10% (0.1ppm) and 15% (0.2ppm) mercury for 15, 30, 45 and 60 days of experimental period and were examined for the determination of mercury in selected organs. The result shows the order of accumulation of Hg in the the seabass tissue was Liver> gill> muscle. The accumulation of mercury in the liver tissue was significantly higher than the muscle and gill tissues. Thus the result shows that accumulation of mercury in seabass *L. calcarifer* tissues is dose and duration dependant.

Keywords: toxicity, heavy metals, bioaccumulation, biomagnification

1. Introduction

Anthropogenic activities continuously increase the amount of heavy metals in the environment, especially in aquatic ecosystem. Pollution of heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem [16]. Increase in population, urbanization, industrialization and agriculture practices have further aggravated the situation [9, 10]. As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals [15] and thus, causing heavy metal pollution in water bodies [17]. Therefore, heavy metals can be bioaccumulated and biomagnified as the tropic level increases and eventually transfer via the food chain and finally assimilated by human consumers resulting in health risks [1, 25].

Mercury is one of the most toxic elements among the heavy metals and causes hazardous environmental pollutants. Numerous toxicological studies have gained increasing interest in order to understand the impact of Hg on aquatic communities. Mercury tends to concentrate in various organisms including fish due to reduced biodegradation of its derivatives. Exposure to high level of this element could permanently damage the brain, kidneys and developing foetus [29]. It is important to always determine the bioaccumulation capacity for heavy metals by organisms especially the edible ones, in order to assess potential risk to human health [20]. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems [7, 1]. Consideration of prolonged toxicity studies is clearly important because in the aquatic environment, organisms usually expose to low pollutant concentration with long period of time due to their long biological half-life [14, 24]. Asian seabass *Lates calcarifer* (Bloch), one of the most suitable finfish for brackish water and fresh water farming in earthen ponds and in floating net cages, is a fast growing and highly economic fish cultured

extensively in Southeast Asian countries and the Indo-Pacific region [27]. In India, the interest to farm seabass is growing day by day [13]. In this respect the objective of the present study was to investigate the bioaccumulation of heavy metal mercury in the selected organs viz. muscle, gills and liver of the economically important edible fish seabass *L. calcarifer*.

2. Materials and Methods

The experiment was conducted at the Central Institute of Brackishwater Aquaculture (CIBA) Fish Hatchery Muttukkadu near Chennai, India. Healthy *L. calcarifer* fingerlings (8.06±0.05cm in length and 6.38±0.14g weight) were maintained for a period of one week in a 10 ton capacity rectangular (Reinforced Concrete Cement) RCC tank for acclimatization to the experimental condition. During this period, they were fed with minced fish meat of *Tilapia* and *Sardines* @ 3% body weight.

The heavy metal mercury (HgCl₂.H₂O) mercuric chloride monohydrate (Merck with purity of 99%) was prepared with deionized water. Test solutions were made by diluting the stock solution in exposure tanks along with filtered seawater 27ppt to produce desired Hg concentration for each treatment. Seawater without addition of chemical was used as control. The 96 h LC₅₀ of mercury calculated by probit analysis method [8] was 1.00ppm for *L. calcarifer*. Fishes were exposed to sublethal concentrations of 5% (0.05ppm), 10% (0.1ppm) and 15% (0.2ppm) of mercury from 96 h LC₅₀ value for 60 days of experimental period. The experiments were carried out in 100 lt. capacity Fibre reinforced plastic (FRP) tanks, with 10 fishes for each concentration in triplicates. Fishes were fed twice daily with minced fish meat of *Tilapia* and *Sardines* @ 3% body weight. The concentration of mercury was below detectable level (BDL) in the control tank. The water in the control and mercury containing tank was renewed every day in order to minimize decrease in the metal concentrations. Water quality parameters like (dissolved oxygen, temperature and pH) was

measured daily and (ammonia, nitrogen and nitrite) was measured weekly once. Two fishes from each tank were sampled at 15, 30, 45 and 60 days of exposure periods and were examined for the determination of mercury in selected organs. The fish were dissected and different organs like muscle, gill and liver were taken from the experimental fish as well as control fish separately. The tissues were dried in an oven at 60°C until constant weight was recorded. Dried samples of individual tissue were weighed and subjected to dry digestion with 20 ml of nitric acid and perchloric acid mixture (1:1) until a clear solution was obtained. The digested samples were then made upto 100 ml with double distilled water and were analyzed in Atomic Absorption Spectrophotometer (Varion spectra, 2000) for mercury by the method described by [21]. The data was analyzed by Duncan's multiple range test (DMRT) comparing with test concentrations and period of exposure days by SPSS (10.0) software.

3. Results and Discussion

After 15 days of experimental period mercury accumulation in muscle tissue shows significant difference. In the muscle of control fish, concentration of mercury was below detectable limit (BDL). In 0.05ppm of mercury the concentration recorded was 0.121µg/g for 15 days of exposure, while in 0.1ppm and 0.2ppm it was 0.157µg/g and 0.190µg/g respectively, indicating the gradual accumulation of the heavy metal in muscle tissue and the accumulation was proportionate to dose and duration Depandant. Likewise the bioaccumulation of mercury in the muscle was recorded high after 60 days of exposure period 0.633 µg/g in 0.05ppm, 0.661 µg/g and 0.745 µg/g in 0.1ppm and 0.2ppm (Table 1). In the gill highest concentration of mercury was recorded after 60 days of exposure period in all the three concentrations 0.883µg/g, in 0.05 ppm, 1.019 µg/g and 1.236µg/g in 0.1ppm and 0.2ppm (Table 2). Whereas in the liver tissue of control (normal) fish mercury content was below detectable level throughout the experimental period, while on exposure to sublethal concentrations of mercury after 15 days of experimental period, the accumulation level of mercury in the liver tissue raises to 0.407µg/g, 0.456 µg/g and 0.542 µg/g in 0.05, 0.1 and 0.2 ppm (Table 3). After 60 days of experimental period the mercury level increased to 1.648µg/g, 1.650µg/g and 1.700µg/g in 0.05, 0.1 and 0.2ppm respectively. The accumulation of mercury in the liver tissue was significantly higher than the muscle and gill tissues.

The quality and quantity of the heavy metals available in the environment significantly affect the organisms. Since the affinities of heavy metals vary in different organs, studies on the distribution of heavy metals in the different organs of an organism are important to understand not only the extent of environmental pollution but also the role of these metal ions in metabolic processes. It is generally advised to analyze the various organs for their affinity to heavy metals [6, 31]. During the process of intake, some of the metals actually get accumulated in the body by the organs because of affinity between certain metal and certain organ system. In the process of bioconcentration, many of these heavy metals are accumulated at different levels in different organs of the organisms. In the present investigation the order of accumulation of Hg in the the seabass tissue was Liver> gill> muscle.

Muscle is the major tissue of interest under routine

Monitoring of metal contamination because it is consumed by people. However muscle is the tissue that usually has the lowest essential and nonessential metal concentrations in fish [30]. When comparing the control fish increase of Hg accumulation in the muscular tissue of tested fish, advertises about the danger of its biomagnification into human beings (the last level of the trophic chain) consuming contaminated fish. Likewise, the other authors [5, 11] observed high concentrations of total mercury in muscle tissues of various fish species.

Gill is the main place for gas exchange in fish, they are pathways of metal ion exchange from water, because gills have very wide surface area that fastens diffusion of metals rapidly [22, 4]. Hence, it is suggested that metals bioaccumulated in gills are basically concentrated from water. The Hg taken up through gills will be directly transported to the circulation system and subsequently stored in the liver and kidney [3]. In the present study there is no accumulation of Hg in the control fish whereas in all the three different concentrations and throughout the experimental period an increasing trend of Hg accumulation was shown. Previous studies have shown that an exposure to dissolved Hg disrupts gill epithelium, potentially affecting gas exchange and permeability of cell membranes to cations [12, 19]. Such disruptions may result in compensatory changes in ventilation frequency, increased energy demands, or altered gas exchange efficiency, possibly resulting in the increase in metabolic rate of the fish [26].

In the present investigation, when the heavy metal (Hg⁺⁺) was available in the medium, the fishes tend to accumulate the same, and preferential accumulation is seen. The liver tissue was found to accumulate more than the muscle and gill tissues. The excessive accumulation of heavy metals by the liver tissue might be probably because of the affinity of the metal to the soft liver tissues and various other biochemical changes performed by the liver tissue. Many authors [7, 2, 1, 25] have reported that metal accumulation by liver and gills occur in higher magnitude than muscle. This is in agreement with the present study comparing with the muscle and gill the Hg accumulation was high in all the three concentrations throughout the experimental period, except the control fish which was below detectable limit. Metal accumulation in each tissue considerably depends upon the accumulation capacity of the tissue. Liver being a storage and detoxification organ for metals and also acts as an active site of pathological effects induced by contaminants [28]. Actually, chronic mercury intoxication leads to severe hepatic damages [17, 18] in fish. This is the reason why liver is more often recommended as an indicator of water pollution than any other organs in fish [2]. The metal accumulation affinity may vary depending on size, age, habitat, feeding habit and growth stage [23].

Table 1: Bio accumulation of mercury on the muscle tissue of seabass *Lates calcarifer*

Conc. ppm	Days of exposure			
	15	30	45	60
Control	BDL	BDL	BDL	BDL
0.05	0.1210 ¹ ±0.00	0.2063 ¹ ±0.00	0.4363 ¹ ±0.00	0.6327 ^c ±0.01
0.1	0.1573 ^b ±0.00	0.2613 ^b ±0.00	0.4607 ^c ±0.01	0.6613 ^b ±0.00
0.2	0.1903 ¹ ±0.00	0.2967 ² ±0.00	0.5070 ^d ±0.01	0.7417 ^a ±0.01

The values with different superscript alphabets (Duncan's Multiple Range Test) are significant (P≤ 0.05).

Table 2: Bio accumulation of mercury on the gill tissue of seabass *Lates calcarifer*

Conc. ppm	Days of exposure			
	15	30	45	60
Control	BDL	BDL	BDL	BDL
0.05	0.1877 ⁱ ±0.00	0.3890 ⁱ ±0.01	0.6290 ^f ±0.00	0.8830 ^d ±0.01
0.1	0.2363 ^k ±0.01	0.4853 ^h ±0.01	0.7767 ^e ±0.01	1.0191 ^b ±0.01
0.2	0.3150 ^j ±0.01	0.5817 ^g ±0.01	0.9160 ^c ±0.01	1.2363 ^a ±0.01

The values with different superscript alphabets (Duncan's Multiple Range Test) are significant (P≤ 0.05).

Table 3: Bio accumulation of mercury on the liver tissue of seabass *Lates calcarifer*

Conc. ppm	Days of exposure			
	15	30	45	60
Control	BDL	BDL	BDL	BDL
0.05	0.4070 ^k ±0.01	0.7917 ^h ±0.01	1.218 ^e ±0.00	1.648 ^b ±0.00
0.1	0.4557 ^j ±0.00	0.8933 ^g ±0.00	1.325 ^d ±0.00	1.650 ^b ±0.00
0.2	0.5423 ⁱ ±0.00	0.9777 ^f ±0.00	1.491 ^c ±0.00	1.7001 ^a ±0.01

The values with different superscript alphabets (Duncan's Multiple Range Test) are significant (P≤ 0.05).

5. Conclusion

Healthy eating has been heavily promoted during the last few decades, and considerable attention has been focused on the benefits of consuming fish. The regular consumption of fish provides proteins of high biological value, unsaturated essential fatty acids, certain minerals, and vitamins. Considering the nutritional and health benefits, fish consumption should be encouraged. However, an understanding of the links between heavy metal (mercury) concentrations in fish, and intake concentrations of this contaminant is crucial for consumers to make decisions about eating fish; they have to find out healthy fish to eat. The FDA level of concern for mercury in fish is 1ppm. The fish with level higher than this should probably be avoided by everyone.

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7. References

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