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Research article

Accumulation of heavy metal (Mercury) in the different tissues of walking catfish (C*larias batrachus*)

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Abstract

In the present investigation bioaccumulation of mercury was studied in the gill, liver, muscle, kidney and brain of *Clarias batrachus*. *C.batrachus* were exposed to 10, 20 and 30 days in sub-lethal concentration of mercury (0.5 mg/l) at four different concentration levels of mercuric chloride (0.19, 0.09, 0.05 and 0.03 mg/l) for acute toxicity study. Results observed that heavy metal is predominantly accumulated in liver followed by kidney, muscle, brain and gills of *C.batrachus* used in this study.

Key words: Mercury, Bioaccumulation, catfish, *Clarias batrachus.*

Introduction

Due to growing urbanization, environmental pollution and various anthropogenic activities, such as Industrial effluents, domestic sewage, pesticides, waste chemical and sewage effluents are discharged into the water bodies. Discharged effluents affect the biological system (Arunachalam, 1980). Most organisms are exposed via the direct uptake of free ions from water through respiratory surfaces, but exposure may also occur through accumulation along the food chain or in the benthos through ingestion of contaminated sediments (Brungs and Mount 1978; Chamoli et al. 1987). Mercury enter aquatic systems through atmospheric deposition and in liquid effluents release from mining, smelting, refining, metal processing, fuel combustion and waste incinerations operations (Cheng and Kang 1994). Freshwater fish residing in contaminated systems are exposed to Hg directly through the water and through the ingestion of contaminated food and sediments (Chitra and Sree 1997). Increased concentration of Hg have also been reported in invertebrates and fish fishes comes under invertebrates modify the sentence residing in Hg contaminated environments (Chitra and Sree 1997; Dallinger et al. 1987; Eisler 1970; Goel 1997; Jung and Kim 1997; King 1992).

Bioaccumulation of metals reflects the amount ingested by the organism, the way in which the metals are distributed among the different tissues and the extent to which the metal is retained in each tissue type. There are many studies of fish response to metal contamination and fish are generally recognized as one of the most sensitive indicators of the changes in the quality of water (Krishnamurthy and Subramaniam 1995). In some cases, however, the metals may alter the biochemical composition of tissues, rendering it unfit for human consumption. Fish are good bioaccumulation and they concentrate metals thousands of times greater than in the ambient medium (Negllski 1976). The present study was aimed to investigate the bioaccumulation of mercury (Hg) in gill, liver, muscle, kidney and brain tissues of freshwater walking catfish *C. batrachus* at laboratory conditions.

Materials and methods

Fish Healthy living specimens of *C. batrachus*

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weighing 10 ± 0.5 gm and 10 ± 1 cm in length have been brought from Department of Tamil Nadu fisheries, Poondi, Thiruvallur District, Tamil Nadu, India. They were brought to the laboratory in well-aerated containers, to avoid hyperactivity, physical injuries and stress to the fish. The fishes were screened for any pathological symptoms and washed with 1% $KMnO_4$ solution. Infected fishes were isolated and discarded immediately.

The glass aquaria were washed with a 1% $KMnO_4$ solution to avoid fungal contamination and then sun dried. The healthy specimens were then transferred to glass aquaria (50x25x25cm) containing tap water. The fishes were acclimatized to the laboratory conditions for 15-20 days prior to experimentation.

Fishes were fed with artificial pelleted diet; water was replaced with clean water whenever necessary. During acclimatization, if mortality exceeded 5% in a batch, it was discarded. Stock solutions in experimental tanks were change once in alternative days. Stock solution of Hg was prepared using mercury chloride. Mercury solution in experimental tanks were changed once in alternative days. Fish were exposed to 30 days in a glass through containing 10L of water having sublethal concentration of mercury i.c 0.19, 0.09, 0.05, and 0.03 mg/l in individually. After 10, 20 and 30 days of exposure period, bioaccumulation of heavy metals on gills, liver muscle, kidney and brain of the fish were studied. The fish were sacrificed and the respective tissues were dissected out, weighed, dried and the tissues were subjected to wet digestion with nitric acid and perchloric acid until a clear solution was abstained. The digestion samples were made up to made 10 ml deionised water. Metal concentration was determined using Perkin-Elmer 2380 atomic absorption spectrophotometer.

Result

In this study the accumulation of Hg was found to increase with increase in concentration and exposure durations Table 1. Minimum accumulation of Hg was found to be observed in gills (3.45 µg/g) at 0.19 mg/l Hg on day 10 of exposure. Maximum accumulation of Hg was observed in liver (15.45 µg/g) at 0.03 mg/l on day 30 of exposure. While in muscle, kidney and brain tissues in Hg accumulation values were two folder higher than the gill. The general trend in the Hg accumulation in various tissues was found to be observed in following order gill < kidney < muscle < liver < brain.

Table 1. Bioaccumulation of heavy metal mercury (µg/g wet wt) in various tissues of *C. batrachus* exposed to different sublethal concentrations and exposure periods.

Tissue	Exposure period (days)	Heavy metal concentration (mg/l)			
		0.19 ppm	**0.09 ppm**	**0.05 ppm**	**0.03 ppm**
Gill	10	3.45±0.43	5.87±0.40	7.96±0.33	9.77±0.99
	20	4.52±0.32	6.96±.0 .46	8.72±0.43	10.55±0.87
	30	5.93±0.45	7.56±0.77	9.32±0.54	11.45±0.47
Liver	10	6.73±1.02	8.99±1.45	10.11±1.33	12.56±2.45
	20	7.20±1.01	10.71±2.60	11.12±2.87	14.35±2.25
	30	10.43±0.76	11.33±1.66	12.20±1.23	15.45±1.65
Muscle	10	12.76±1.24	13.32±2.30	14.21±2.27	16.38±2.70
	20	13.32±1.42	14.14±3.14	15.20±2.29	19.42±2.77
	30	16.55±1.82	17.16±1.11	18.21±2.23	21.36±1.32
Kidney	10	4.99±0.60	18.42±2.12	19.28±1.43	22.48±2.46
	20	7.68±0.38	19.40±2.10	20.23±3.45	24.51±1.64
	30	14.48±2.42	20.21±1.17	21.54±1.56	26.41±2.84
Brain	10	9.42±1.72	22.21±2.21	23.22±2.66	28.77±1.99
	20	10.66±0.32	23.12±3.22	24.28±2.75	30.64±2.74
	30	11.21±0.45	24.23±2.56	27.30±1.70	32.94±1.84

Discussion

Metal accumulation in fish and their tissues is dependent upon targets organ and species of fish and other factors such as exposure time, temperature salinity, and type of metal. Fish and other aquatic animals were exposed to heavy metals accumulation dominantly occurs in metabolically active organs such as liver and kidney. In the present investigation maximum Hg accumulation was found to be observed in liver tissues and lesser accumulation of Hg was observed in gill tissues. Toxicity is a relative property of a chemical which refers to its potency to induce harmful effects on an organism. It is a

function of concentration of the toxicant and the length of exposure of the animal (Wilkinson, 1976).

The characteristic of an individual organism that responds to the heavy metal at a particular concentration or dose for a specific period of time. The toxic effects may include both lethal and sublethal concentration which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behavior (Preston et al. 2000). Further, metal pollution may make mercury shift in the community structure to metal tolerant organisms, which are often capable of accumulating large amounts of metals (Dallinger et al. 1987).

Metal ions and their complex exhibit widening toxicity to the organism that ranges from sublethal to lethal depending upon the time of exposure and the prevailing conditions in the ambient water (Goel 1997). A low concentration of the heavy metal salt does not trigger a quick and marked toxicopathological manifestation as otherwise observed in the case of acute toxicity (Rajan and Banerjee 1993a,b). The evaluation of acute toxicity in the environment, the LC_{50} values, (Median Tolerance Limit) are useful indices in measuring acute toxicity of the test heavy metal, under certain environmental conditions. The application of LC_{50} values has gained wide acceptance among toxicologists and is generally the most reliable test for assessing the potential hazards to aquatic life (Brungs and Mount 1978). LC_{50} values differ from species to species for the same toxicant due to the mode of action and responses of the animals (King 1992). The toxicity tests have also been influenced by the size, age (Sanders 1993), sex (Victoriamma and Radhakrishnan 1982) and the nutrient supply (Arunachalam 1980; Eisler 1970; Trivedy 1978).

According to Negllski (1976) the acute incipient lethal level for zinc was 9 mg/l, the value for cadmium was 16 mg/l and the values for tri and hexavalent chromium are 53 and 24 mg/l respectively in juvenile mullet. Joshi and Chamoli (1987) calculated the 24 hrs, 48 hrs, 72 hrs and 96 hrs median tolerance limit values to be 2.37, 1.50, 0.95 and 0.62 ppm respectively for the Fish *Noemacheilus Montanus* exposed to Zinc sulphate (Shakoori et al. 1992).

The lethal copper concentration, expressed as LC_{50}, found in *Clarias gariepinus*, ranged from 1.29 mg/l during summer to 1.38 mg/l in winter (Van Der Merwe et al. 1993). Cheng and Kang, (1994) had tested the sensitivities of the freshwater species *Acrossocheilus paradoxus* to the 95% confidence limits of LC_{50} and it was found to be 0.032 - 0.050 mg/l for copper (24 hrs), 0.439 - 0.634 mg / l for cadmium (24 hrs) and 0.732 - 1.056 mg / l for zinc (96 hrs). This was the comparative toxicity strength for *A. paradoxus* with copper> cadmium >zinc (Sharma et al. 1999). Jung and Kim (1998) had determined LC 50 at 24 hrs, 48 hrs, 72 hrs and 96 hrs to be 209 ppm, 182 ppm, 158 ppm and 141 ppm, when Olive flounder finger lings, *Paralichthys olivaceus* were exposed to (37%) formaldehyde at concentrations ranging from 50 ppm to 500 ppm. Paolini and Leone, (2001) had studied the acute toxicity effects of potassium dichromate to rainbow trout and Zebrafish. The LC_{50} values found out were 0.13 g/l for trout and 0.11 g/l for Zebrafish (*Danio rerio*).

In the present study of mercury (Hg) and cadmium (Cd) treated liver and muscle, there is also reduction in protein bands from 20 to 16 in liver and 12 to 10, in muscle respectively till 30 days of exposure. But in 30 days of exposure, there is a marked increase in the protein bands in both Hg and Cd treated liver (20 to 18) and muscle (12 to 10) respectively. Hg and Cd treated 30 days of kidney bands (10 to 12) and brain (10 to 12) respectively. Further exposure doesn't bring about any change in the protein bands in the gill, liver and muscle of *C. batrachus***.** This may explain the fact that the adaptive immune response is triggered in gill (30 days), liver and muscle (30 days) of *C. batrachus.*

Notably, in the brain there is a decline in protein bands observed throughout the exposure period with no subsequent increase in protein bands. This indicates that the tolerance limit of mercury and cadmium stress in brain is not reached. Sahai (1990) has studied the serum proteins of *Channa punctatus* and *Channa stratus* using SDS PAGE Electrophoresis, when treated with toxicant, either the number of protein fractions decrease or sometimes new fractions appear. Protein profile variation has been studied Electrophoretically by Krishnamoorthy and Subramanian (1995) and they have recorded the protein profile variations after accumulation and duration periods.The disappearance of existing polypeptide bands, the appearance of new bands and changes in the intensity of protein fractions indicate these influences of toxicant on the physiology of the animal and on the energy demands for the detoxification and elimination of toxicants. Chitrq and Sree (1997) have studied the enzyme protein in kidney tissue of *Channa gachua* using Electrophoretically. The intensities of band are much lesser than to control. This reduction in intensity clearly indicates the decreased level of enzyme protein due to pollution.

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