Original Article

Bioaccumulation and Eco toxicity of Mercury and Lead in Cyprinus Carpio

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Abstract

One of the most important scientific disciplines is determining the toxicity of heavy metals in freshwater, and there is a rising concern about the development of methodologies for identifying toxic effects in aquatic creatures. To test the efficacy of this approach, an experimental study was conducted on the fish Cyprinus carpio from a pond in Bengaluru, Karnataka. Surface bioaccumulation of mercury and lead on Cyprinus carpio was studies using Scanning Electron microscopy. Molecular bioaccumulation of Mercury and lead were analysed by ICPMS. Brain showed highest level of mercury (1780 ppb) when compared to kidney (1365 ppb) and liver (1150 ppb) tissues of C. carpio. Similarly, highest accumulation of lead noticed in brain (1490 ppb) followed by kidney (1245 ppb) and liver (1100 ppb). Moreover, mercury and lead surface accumulation and molecular bioaccumulation were highest in the brain compared to the liver and kidney Cyprinus carpio. The surface morphology of kidney, liver and brain is studied by scanning electron microscopy (SEM). This study has elicited a strong response from freshwater aquaculture to implement toxicity control measures and avoid future infiltration into the food chain. The present study has been undertaken to explore the toxic effects of lead and mercury on kidney, liver and brain of fish Cyprinus carpio and to detect the spectral biochemical and surface morphological changes.

Keywords: Cyprinus carpio, Mercury, Lead, Molecular bioaccumulation, Surface accumulation.

1. INTRODUCTION

Heavy metals that could remain in the environment after the source has been removed can build up in aquatic creatures' tissues. Heavy metals have an important role in the biological functions of aquatic species and are only found in tiny quantities in the body, i.e., less than 1g/g [Esakku et al,2005]. Consequently, even a small rise in concentration causes significant damage to various organs. Despite their capacity to react with nuclear proteins and nucleic acids, heavy metals can cause oxidative degradation of biomolecules, producing toxicity to living organisms [Cooke et al, 1996]. However, industrial wastes carrying toxic and hazardous compounds, such as heavy metals, pollute aquatic ecosystems as a consequence. Heavy metal contamination in marine ecosystems is increasing at an alarming rate and has become a major global issue. They are produced by a variety of anthropogenic activities, including sewerage drainage systems, discharging of hospital and other wastes, idol immersion, recreational activities, and so forth [Bernet et al, 1999]–[Linnik et al,2000].

Metal bioavailability is influenced not just by species, but also by metal's content in water, their speciation, and other water functional properties including pH and dissolved organic carbon. Heavy metals such as Mercury (Hg) are widely regarded as major pollutants worldwide due to the tenacity, bioaccumulation, and toxicity of their constituent elements [Shastri et al 2008], [Pack et al, 2014]. As a result, mercury contamination causes severe damage to human beings and the environment. The identification of Hg as either a priority hazardous substance in the aquatic environment has prompted several study results in the last few decades to evaluate the consequences of Hg pollution for human and aquatic organisms [Black et al 2007]–[Garai et al, 2021].

Fish survival and growth are dependent on stable internal factors of the environment, which would include a well-balanced immune system. In freshwater aquaculture, cultured aquatic animals are exposed to environmental stresses like high temperatures, congestion, poor water quality, and pathogen infection [Benson et al,2007]–[Zheng et al, 2019].

Due to its toxicity, persistence, surface accumulation and molecular bioaccumulation, heavy metals are a significant source of water contamination. Owing to biomagnification, metals accumulated in the aquatic environment may accumulate in the food chain, causing ecological damage as well as carcinogenic and other bad impacts on human health [Tao et al,2012]. These health problems are significant and given the increasingly globalized demand for mercury and the resulting potential for higher natural and anthropogenic sources, it is critical to have a better knowledge of its impacts on fish. However, compared to other more dangerous heavy metals less research has been done on mercury toxicity in fish [Fernandez-Leborans et al,2000], [Clasen et al, 2018], [Palermo et al, 2005]–[Bawuro et al, 2018], and additional research on how it impacts fish is needed.

The main purpose of this study was to better understand the toxic effects of mercury exposure on the, and to determine the acute toxicity of mercury, surface accumulation and molecular bioaccumulation for the brain, liver and kidney in common carp (Cyprinus carpio) from a pond in Bengaluru, Karnataka. This species being a while consumed by the local population, an attempt was made to understand the level of heavy metal toxicity and severe health hazards associated by consuming the toxic fish. The effects of mercury on oxidative stress induction and immune response were also investigated. This study aimed to provide references for mercury toxicity research, aquatic environmental protection, and the stable development of aquaculture.

Histopathological as biomarkers in the estimation of the health of fish exposed to contaminants, both in the laboratory and field studies [Schwaiger J et al, 2007]. According to [Gernhofer M et al,2001] the Great advantage of using histopathological biomarkers in ecosystem monitoring is that this category of biomarkers allows examining specific target organs including gills that are responsible for vital function such as respiration. In addition, the changes found in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Lauren 1990). With a small sample size and quick preparation, FTIR spectroscopy is a useful and well-liked method for obtaining the quantitative profile of a biological sample's biochemical composition. Surface morphological features are provided by FE-SEM.

2. Materials and Methods

2.1 Experimental sample collection

Cyprinus carpio weighing approximately (18±2.0g) was obtained from a freshwater pond in Bengaluru, Karnataka. The criteria for selecting healthy fish were based on morphological characteristics. Fishes were acclimatized at 32°C with natural photoperiod and fed commercial feed once a day before the actual experiment began. The fish's body weight and length were initially measured. Standard protocols were used to check for the presence of total dissolved solids and other physicochemical characteristics in the water (APHA, 1995 and 2005).

2.2 Sample Preparation and water analysis

The tissue samples of 500gm each from the liver, brain and kidney of C. Carpio were extracted and partially defatted protein powders were obtained by acetone extraction/microwave digestion. The physicochemical properties of the freshwater used to grow the C. Carpio in the laboratory conditions were tested to confirm the absence of mercury (Hg) and lead (Pb).

2.3 Bioaccumulation

2.3.1 Surface Accumulation

EDAX analysis was performed to detect the surface accumulation of Mercury and Lead using Oxford Link ISIS-300 detector. The result obtained was tabulated and calculated and the surface bioaccumulation in ppb levels was determined.

2.3.2 Scanning Electron Microscopic Analysis

The liver, kidney and brain of the target organisms were dissected and fixed in 4% Glutaraldehyde in Phosphate Buffer (0.2 M and 6.9 pH for 2 h followed by dehydration in graded series of ethanol). Samples were air-dried and mounted on aluminium stubs using double adhesive tape coated with gold in Hitachi HUS-5 GB Vacuum Evaporator and observed in Hitachi S-520 Scanning Electron Microscope (SEM) by Bruslé, 1987 and SEM photographs were taken.

2.3.3 Molecular Bioaccumulation

ICPMS analysis for molecular bioaccumulation was carried out to identify the amount of Mercury bioaccumulation, 500 mg of the cryo-preserved tissues were subjected to microwave digestion. The samples were treated with concentrated HNO3↓or H2O2↓ before microwave digestion. The digested tissue samples were made up to 25ml and 1ml of the sample was introduced into an inductively coupled plasma mass spectroscopy device (Ultra Mass 700, Varian). The obtained data were tabulated and values were calculated to get molecular bioaccumulation at the ppb level.

2.3.4 sample preparation using microwave digestion procedure

Prepared partially defatted protein powders by acetone extraction. Spray dry or freeze dry residue was made. Weighed 500mg into a weighing boat and transferred both to a microwave digestion vessel. Slowly added 6.0 ml concentrated HNO3↓, remnant powder from the surface of the weighing boat and the sides of the digestion vessel washed with the acid. Discard the weighing boat. Located the release valve on the top of the vessel, cap and screw finger tight using the capping station, applied a torque of 16 Nm to the cap. Placed a total of four vessels (including samples) equally spaced, in the sample turntable. Set the exhaust fan to maximum, heated at 100% power until a pressure of 550 kPa (80 Psi) was attained and maintained at this level for 25 min.

Transferred the vessels and turntable to a cold water bath and allowed to return to room temperature. Vent by hand and any remaining vapor into a fume cupboard, and loosen the caps with the capping station. Carefully removed caps and transferred the vessel contents to a PTFE beaker, washed residual liquid from cap vessel interiors with de-ionized water. Evaporated on a hot plate at 900C to incipient dryness, added 2.0ml 16 M HNO3 and warmed gently. Carefully added 1.0ml of H2O2 drop wise to the solution, allowing effervescence to lied down between additions. Evaporated to near dryness and repeated the HNO3/ H2O2 addition and evaporation twice more. Added 0.5ml 16m HNO3 to the residue and warm gently until digested. Allowed the solution to cool, dilute to 25ml with de-ionized water in a volumetric flask producing 0.3M HNO3 solution and transferred immediately to new polypropylene bottle for storage. The sample was analyzed by ICP-MS without further dilution.

3. Results

3.1 Physicochemical analysis of freshwater

The physical and chemical properties of the freshwater in which C. carpio was found and analyzed for the presence of dissolved salts and toxic metals. The dissolved oxygen content was found to be 6.2 0.4 mg/l, with a neutral pH of 7.3 0.01. The total hardness of the water was determined to be 345 99 mg/l, whereas the free CO2 concentration was calculated to be 2.1-0.12 mg/l. In the tested water, there were no residues of mercury or cadmium, though there were traces of calcium (81 88 mg/l) and magnesium (34 mg/l). In addition, the water sample contained high levels of sulphates and chlorides (Table 1). This test showed that C. carpio had not been exposed to mercury before the trial began.

Table 1. The physicochemical characteristics of water were analyzed by using standard methods (APHA, 1995 and 2005).

Parameters	Values
Dissolved Oxygen	6.2 ± 0.4 mg/l
pH	7.3 ± 0.01 m
Temperature	28 ± 2°C
Total hardness	345 ± 99 mg/l
Free CO_2	2.1 ± 0.12 mg/l

Ca	81 ± 88 mg/l
Mg	34 ± 0.0 mg/l
Hg	Nil
Sulphates	112 ± 0.9 mg/l
Chlorides	234 ± 22 mg/l
Pb	Nil
Specific conductance	2340 (Micro siemens/cm) at 2°C

3.2 Bioaccumulation

3.2.1 Surface accumulation

EDAX analysis was performed to detect the surface accumulation of the control and Mercury-induced C. carpio on the 28th day of exposure (Figure 1). The results revealed that the surface accumulation in the control was 115, 105 and 205 in the kidney, liver, and brain respectively. Whereas, the mercury-induced fish revealed that the surface accumulations were 1365, 1150 and 1780 ppb in the kidney, liver, and brain respectively. In case of lead accumulation, brain showed highest accumulation of 1490 ppb when compared to kidney (1245 ppb) and liver (1100 ppb). Significantly, the surface accumulation in three organs had increased abruptly.

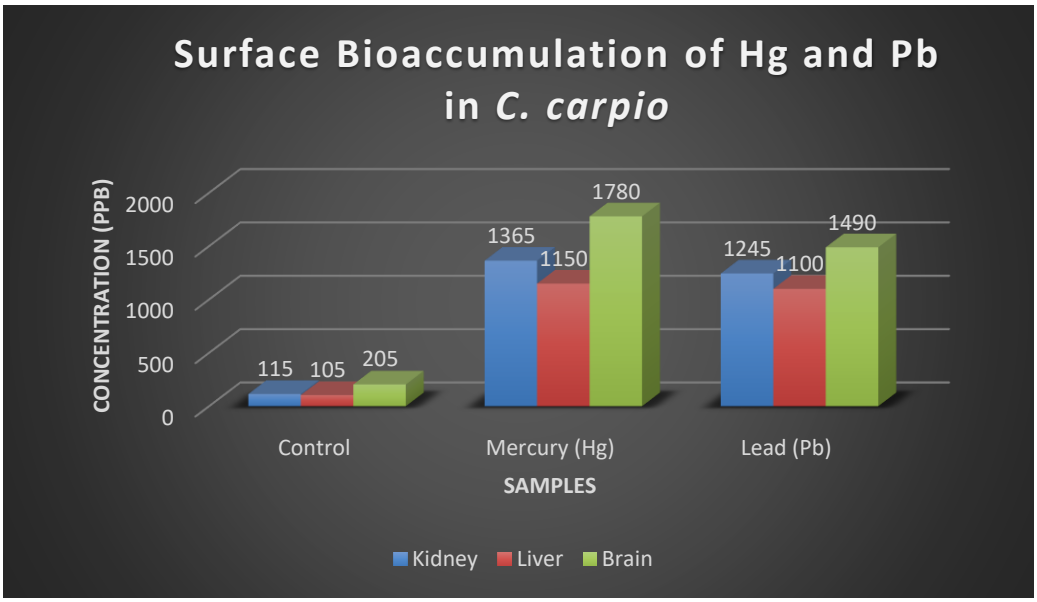

Figure 1. Surface Accumulation of Mercury and Lead by EDAX in the control and treated tissues of C. carpio on the 28th day of exposure.

3.2.2 Molecular Bioaccumulation of Mercury and Lead

Molecular Bioaccumulation of Mercury and lead (in ppb levels detected by ICP-MS) in the control and treated tissues Cyprinus carpio on the 28th day of exposure showed high levels. The results for mercury and lead accumulation in the liver, brain and kidney of the fish C. carpio are represented in Figure 2. Mercury and lead accumulation in the fish was in the following order: liver < kidney < brain. The mercury and lead levels in three organs grew significantly on the 28th day of the exposure.

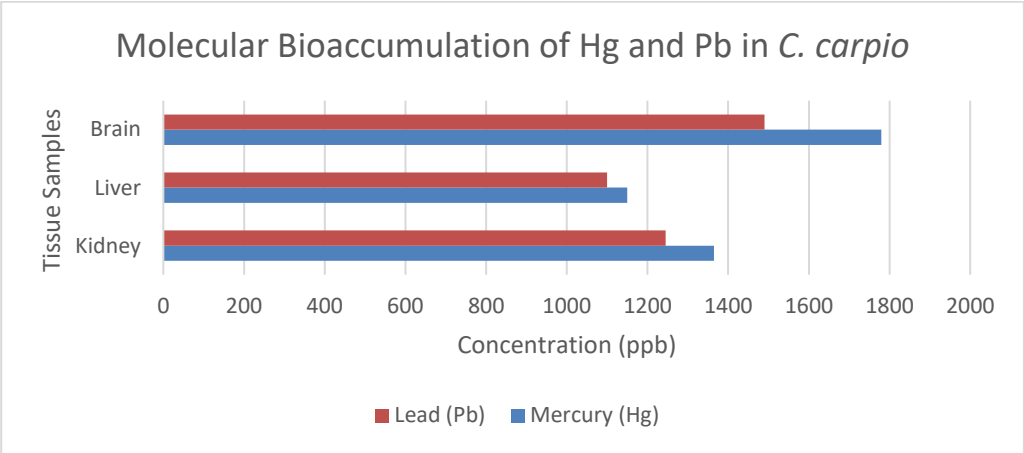

Figure 2. Molecular Bioaccumulation of Mercury and Lead (in ppb levels detected by ICP-MS) in the control and treated tissues C. carpio on 28th day of exposure.

3.2.3 Scanning electron microscopic Analysis

On the 28th day of exposure, scanning electron microscopic examinations of the kidney, liver, and brain were performed on Cyprinus carpio. The kidney, liver, and brain of fish are just a few of the organs where lead and mercury can accumulate. The untreated fish's brain, liver, and kidney samples all came back normal. Many histologic abnormalities were seen in the kidney, liver, and brain of Cyprinus carpio after exposure to lead and mercury. The buildup of fish gonads impeded their growth and development, which had an effect on the reproductive system, neurodevelopment, and mortality. Cyprinus carpio's muscles and brain were shown to have very low levels of methyl mercury. Surface morphological investigations were conducted with lead and mercury control, and treated fish were released after 28 days of exposure. Scanner electron microscopy (SEM) images corroborate a variety of histological alterations picked up by low-resolution light microscopy. Lead and mercury tissue levels were measured using ICP-MS spectrophotometric techniques. The 28th day of the experiment saw no mortality at any of the Mercury and Lead concentrations examined. In comparison to the control values, mercury and lead levels were significantly higher in all of the tissues examined. The target organ for collecting Mercury and Lead at the chosen concentrations and exposure times was discovered to be kidney tissue in C. carpio. The tissues that were mercury and lead accumulating showed the following relationships: Kidney>Liver>Brain.

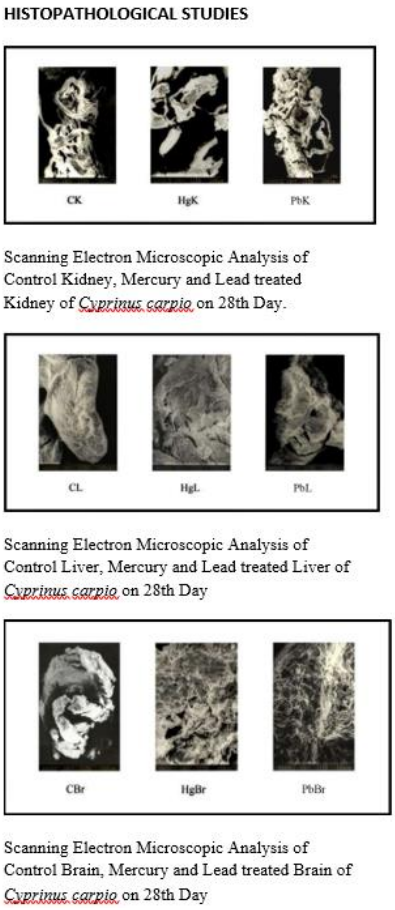

Figure 3. Scanning Electron Microscopic Analysis of Mercury and Lead in the control and treated tissues C. carpio on 28th day of exposure

4. Discussion

Heavy metals cause significant harm to aquatic environments. Some human activities, in addition to agricultural, industrial, and urban pollution, can lead to heavy metal deposition in aquatic organisms [Ratn et al,2018]. Since mercury accumulates in numerous organs and affects antioxidant defenses, the research findings made it apparent that mercury causes major changes in C. carpio. This study will be significant in establishing the harmful effects of mercury on the fish, which is a rare occurrence.

The nature of management and human eating of fish necessitates an understanding of levels of heavy metals in various tissues of fish. Metals deposition in fish tissues varies depending on intake, retention, and excretion rates. This indicates that metals with high absorption but low clearance rates in fish tissues are likely to acquire at greater levels.

On the 28th day of exposure, the highest concentration of mercury accumulated on the surface was found in the brain (1780 ppb), followed by the kidney (1365 ppb) and the last was liver (1150 ppb) when compared to the controls. The mercury surface accumulation pattern in the liver, kidney and brain of the fish C. carpio revealed that the highest mercury concentrations were mostly found in the brain, while the lowest amounts were found liver. Meanwhile, the accumulation of lead is comparatively lower than that of mercury. However, similar observations were found in the bio accumulation studies of lead. This study revealed that the increased mercury accumulation in the brain, the attention, memory, behavior, cognition, and even physical coordination are all affected, raising the likelihood of neurodegenerative illnesses including Parkinson's and dementia. So, mercury is another prevalent toxin that can damage cognitive abilities and induce neuroinflammation.

Molecular accumulation of mercury in ppb levels was identified in the brain, kidney, and liver. In the current study, mercury accumulation was found to be 309.78 ppb in the kidney, 241.38 ppb in the liver, and 911.67 ppb in the brain (Figure 2). However, the bioaccumulation in the kidney and liver were lesser compared to accumulation of lead in other tissues. The molecular bioaccumulation of mercury and lead exposure can cause oxidative stress in fish, and persistent mercury contamination is harmful to the central nervous system, resulting in behavioral and cognitive problems. Due to the mercury-induced brain and cognitive function damages caused by morphological alterations in the brain, neurodegenerative disorders, cell signaling dysregulation, and central nervous system dysfunction result. In aquatic toxicology, tissue deposition can be a sensitive sign of acute toxicity. mercury accumulates significantly in numerous tissues of the fish after exposure to waterborne mercury, with the largest accumulation in the brain, which is neurotoxic. Similar studies, i.e., bioaccumulation of mercury in other fishes L. rohita than in C. idella also showed that accumulation was less in the kidney and liver [Malik et al, 2010; Brandão et al, 2015]. Hence, the results obtained in this study are consistent with the previous studies.

Metal levels in the gills of C. carpio were compared to the WHO permitted limits for human consumption to assess public health concerns to fish eaters. According to FAO/WHO (FAO/World Health Organization) (2002), the levels of Hg and Pb were found to be greater than the permitted levels of 0.5 mg/kg (of fresh weight) and 0.2 mg/kg, respectively [WHO 2002]. The gill is an essential location for heavy metals entrance into the fish body, according to Rajeshkumar and Li et al., 2018. Despite the fact that gills are rarely eaten, they are excellent bio-monitors of metals in the environment [Rajeshkumar and Li et al., 2018]. Also, Pb enters the circulation of the fish and accumulates in bodily tissues, bones, gills, kidneys, liver, and scales.

The levels of trace components of mercury and lead in the brain, kidney, and liver of C. carpio were measured using inductively coupled plasma-mass spectrometry (ICP-MS). As a result, mercury can enter the food chain and concentrate in fish, which are at the top of the pecking order and have a proclivity for accumulating mercury. As such, molecular bioaccumulation in fish can be used as an indicator of metal pollution in aquatic bodies, which could be a valuable method for investigating the biological function of metals present at greater concentrations in fish. Mercury contamination in marine products has become a major global problem. To determine the synergistic effects of metals at the molecular level, a comprehensive investigation with vast data sets, including genomes and proteomics data, is necessary.

Histopathological changes in more than one tissue are always instructional in assessment of the biological effects of a toxicant and allow for diagnoses of the observed changes(Adeyemo,2007). The accumulation of metals in fish may cause several pathological effects such as change in enzyme activities and damage to organ structure (Alkshab,2017). The main organ that regulates these processes in fish is the brain. The brain's anatomical and histological structure differs in different fishes, but the number of parts of the brain is similar (Abdelnaeim and Cao,2018). The lead exposure increased the production of reactive oxygen species observed in the brain of the fish Clariasbatrachus this may lead to the same effects that appeared in this study (Maiti et al., 2010). Some studies indicate that the accumulation of heavy metals, especially lead, in the brain of some types of fish leads to decreased reproductive capacity in fish (Tulasi et al., 1989; Doaa and Hanan,2013). The primary organ for detoxification and metabolic waste excretion is the kidney. Histological alterations in various components of teleost fish kidney after heavy metal exposure are described by (Ortiz,2003). SEM studies of fish brain after mercury toxicity revealed change in surface morphology and spectroscopic studies showed fluctuations in absorbance areas and intensities to detect biochemical changes (Chavan et al,2015) Toxic responses to mixture of trace metals by SEM reported structural and functional alterations in fish gills (Pandey et al 2008). Necrosis, haemorrhage, degeneration of hepatocytes and pyknosis in the liver tissue were witnessed in Labeo rohita exposed to zinc [Loganathan et al,2006] and Heteropneustes fossil is exposed to thiodan [Narayan et al,1999]. Chronic exposure to CdNP resulted in histological and cytological changes in the kidney of Fish, Dicentrarchus labrax and Centropomus undecimales, [Giari, 2006].

5. Conclusion

To conclude, mercury had a considerable effect on the liver, brain and kidney of the C. carpio. This study will examine a variety of endpoints in a single fish species., surface accumulation, and molecular bioaccumulation in distinct organs following mercury exposure. Mercury concentrations in the internal organs of the examined C. carpio fish species were measured. The brain tissue had the most mercury accumulations, followed by the kidney and liver. However, mercury levels in C. carpio fish species make them unfit for human consumption and pose a health risk. Also, this study could be used to quantify pollution stress in the aquatic environment and its inhabitants, allowing for the development of policies and initiatives to limit the flow of chemical compounds and heavy metals into freshwater. The evidence suggests that mercury can have a deleterious impact on behavior in environmentally relevant quantities.

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