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# Accumulation of cadmium (Cd) and lead (Pb) in tissues of rohu fish (*Labeo rohita*) collected from the sewage-fed pond of Kolkata

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**Abstract**
Sewage-fed aquaculture is a well-known method of fish cultivation. As the wastewater is generally contaminated with heavy metals and other pollutants; fishes and other aquatic organisms tend to accumulate them. It creates a serious health problem when these are consumed by a human being. The objective of our study was to assess the concentration of two potentially toxic metals namely cadmium (Cd) and lead (Pb) in tissues of rohu fish (*Labeo rohita*) collected from the sewage-fed pond of Kolkata. Samples were collected fortnightly from January to June 2014 and the level of metals was determined by Atomic Absorption Spectrophotometer. We observed that accumulation of Cd and Pd in fish tissue ranged from 0.002-0.013µg/g and 0.23-1.84µg/g respectively. Higher concentration was found in internal organs like liver, kidney and gill compared to muscle. It indicates that the conventional method of fish preparation in West Bengal by discarding the offal (i.e., liver, kidney, gills etc) should be followed everywhere. Though the concentration of metals was below the prescribed limit, people should be aware of the possible threats in the near future.

**Keywords:** Bioaccumulation, cadmium, fish, lead, rohu, sewage-fed pond

## 1. Introduction

Fish comprise a major part of the human diet due to high protein content, low saturated fat and sufficient omega-3 fatty acid which are known to support good health. It is cultivated and consumed in a large quantity by the Indian population. There are several culture systems used for fish production in India. Among them, sewage-fed aquaculture has been practiced for the last 70 years in our country. It is also one of the most widely recommended biological wastewater treatment methods. In India, 35 metropolitan cities produce 15,644 Million Liters per Day (MLD) of sewage. But, the sewage treatment capacity of our country is only 51% i.e., 8,040 MLD of sewage. Kolkata itself produces 705.86 MLD of sewage and it has the treatment capacity of only 24% or 172 MLD of sewage [1]. From the above information, it is clear that about 76% of sewage generated from Kolkata Metropolitan City is discharged every day without any treatment. Due to widespread developmental activities and industrial blooming of the country, wastewater generated from domestic sewage is often contaminated by different toxicants including metals. Those metals are highly toxic owing to their persistent and non-degradability in nature besides their toxicity is revealed even at lower concentrations. Moreover, freshwater organisms are especially vulnerable to these toxicants which tend to accumulate in fish tissues and other biota and subsequently transported to the human food chain. Metals like Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb) and Zinc (Zn) exhibit toxic effects on aquatic organisms [2]. Therefore, metal contamination in reared fish is a major concern throughout the world. The increasing concentration of metal burden in sewage water and bio-accumulation of metals in fish provoked to undertake a comprehensive study on metal concentration in the various tissues of a commercially important fish namely rohu (*Labeo rohita*) reared in sewage-fed aquaculture pond of Bonhooghly Fisherman's Co-operative Society Limited, Kolkata, West Bengal, India. Quantitative estimation of metals in this species is crucial as it forms a major part of the food for the people of this part of West Bengal. The preliminary work was designed only for six months starting from January 2014 to June 2014. With this background, the present study was designed to assess the level of cadmium (Cd) and lead (Pb) in fish tissues of a sewage-fed aquaculture system in Kolkata and also to formulate some managemental strategies of such culture systems for the safety of human health.

## 2. Materials and methods

### 2.1 Location of the study area

The study was conducted in a sewage-fed aquaculture pond belonging to 'The Bonhooghly Fishermen's Cooperative Society Ltd., located at $22.64^0$N and $88.38^0$E in a densely populated area of Kolkata. The lakes of the 'Bonhooghly' and 'Noapara' receive a huge quantity of sewage coming from the Baranagar Municipality area. This sewage is being used for scientific pisciculture. As there is no sewage treatment plant, only biological treatment with water hyacinth and regular liming enables good fisheries activity. The general practice of pisciculture is the regular stocking of fingerlings and harvesting.

### 2.2 Selection of species

One most commercially important, highly demandable and consumable and extensively cultured fin-fish species, namely *Labeo rohita* popularly called Rohu was selected for the study. It is found throughout the Bangladesh, India, Pakistan, Nepal, Myanmar and Srilanka. It is found in freshwater bodies, rarely in brackish water. Some common habitats are ponds, ditch, canals, beels, flood plains, haors, boars, rivers, lakes etc. It is a very popular fish and largely consumed on a daily basis and on occasions by the people of West Bengal.

### 2.3 Collection of samples

The study was carried out only for a period of six months from January 2014 to June 2014. The samples were taken fortnightly at 1st day and 15th day of a month containing 12 sampling days. The sampling days were denoted as $D_1$ to $D_{12}$ starting from 1st January 2014 to 30th June 2014 respectively. The sampled fresh fish were carried out to the laboratory for analysis of metals. The liver (L), kidney (K), gills (G) and muscle (M) of the sampled fish were dissected out. They were weighted in electronic balance and dried in a hot air oven at 103 °C for 24 hours. Then these samples were kept at room temperature for further analysis.

### 2.4 Digestion of the Samples

A modified wet-digestion method of Churnoff (1975) was followed to prepare the fish tissue samples for the determination of Cd and Pb [3]. The dried samples were crushed with mortars and pestles to form a composite sample. The dry weight of each composite sample in triplicate was kept in a 100 ml beaker. The 10 ml concentrated Nitric Acid (HNO3) was added to each sample and kept overnight for digestion. On the very next day, beakers with samples were placed on a hot plate at 70 ºC for complete digestion and extraction of metals from the sample. The digestion was done until the solution turned into pale yellow to transparent color. The 1.0-2.0 ml of Perchloric Acid (HClO4) was added drop-wise to the sample to make a transparent solution. After complete digestion, the solutions were cooled at room temperature, diluted with ion-free double distilled water and filtered in Whatmann filter paper No.1 (110 mm) and kept in sample bottles (Tarson®) with a final volume of 30 ml of each.

### 2.5 Detection of metals by atomic absorption spectrophotometer

The metal content of the samples was detected in Atomic Absorption Spectrophotometer (Varian AA 240) using hollow cathode lamps of Pb and Cd. Three standard solutions (0.5 mg/l, 1.0 mg/l and 1.5 mg/l) Cd were prepared from stock solutions (1,000 mg/l) procured from analytical grade Merck India Pvt. Ltd. The metal concentration of each sample was calculated from the standard curve prepared by plotting the absorption values of the standard solutions at Y-axis and concentration of the standard solution at X-axis. The final concentration of each sample was expressed in µg of metal/g (d wt).

### 2.6 Statistical Analysis

The data generated from the study were tested for significance of variance by single-factor ANOVA (Analysis of Variance) with replication as per the structure of the data. The graphical presentation of the data was also made. All the statistical analysis and graphical presentation were done with the help of Microsoft Excel 2007.

## 3. Results

### 3.1 Bioaccumulation of Cadmium in fish tissue

Among the four tissues, the maximum bio-accumulation of Cd was observed in the kidney (0.006µg/g) and the minimum bio-accumulation was observed in the muscle (0.002µg/g) of rohu (Fig 1). The concentrations of Cd in gill (0.004µg/g) and liver (0.005µg/g) was moderate in rohu. In the kidney, the maximum concentration was observed during February ($D_3$) and May ($D_9$ and $D_{10}$) followed by March ($D_6$), April ($D_8$) January ($D_1$ and $D_2$) and minimum bio-accumulation were observed during June ($D_{12}$). In liver, the maximum concentration was observed during January (0.006µg/g) followed by March (0.005µg/g), April (0.005µg/g), May (0.005µg/g), June (0.005µg/g) and minimum bio-accumulation was observed February (0.004µg/g). In muscle the average bio-accumulation was observed during February (0.002µg/g), March (0.002µg/g), April (0.002µg/g), May (0.002µg/g), June (0.002µg/g) and January (0.001µg/g). In Gill, the average concentration was observed during January (0.004µg/g), February (0.004µg/g), March (0.004µg/g), April (0.004µg/g), June (0.004µg/g) and May (0.003µg/g).

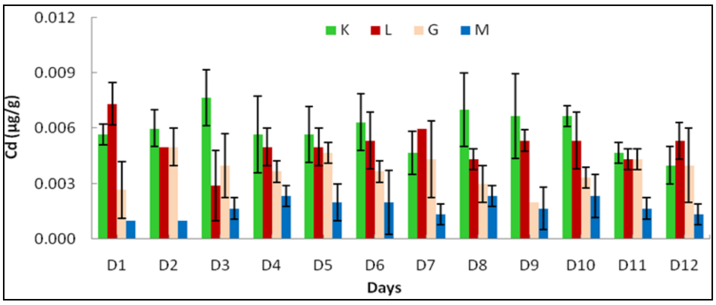


**Fig 1:** Cadmium accumulation (mean ± SD, n=3) throughout the experimental period in different tissues of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (K- Kidney, L- Liver, G- Gill, M- Muscle)

### 3.2. Bioaccumulation of Lead in fish tissue

There was significant variation (P<0.05) of Lead accumulation throughout the study period. The average concentration of lead in different fish organs kidney, liver, gill and muscle was 1.48µg/g, 0.76µg/g, 0.56µg/g and 0.23µg/g respectively in Rohu (Fig 2). Pb showed the highest concentration in the kidney (1.71 µg/g) and the lowest concentration in muscles (0.16 µg/g). In the kidney, the maximum accumulation was observed during June (1.63µg/g) followed by February (1.57µg/g) and March (1.48µg/g) and the minimum bio-accumulation was observed in April (1.38µg/g). In liver, maximum bio-accumulation was observed during January (0.92µg/g) followed by February (0.79µg/g), May (0.79µg/g), June (0.74µg/g), March (0.68µg/g) and April (0.67µg/g). In muscle, the average accumulation of Pb was recorded about 0.21µg/g-0.26µg/g and in gill, the average accumulation was recorded 0.051µg/g-0.61µg/g.

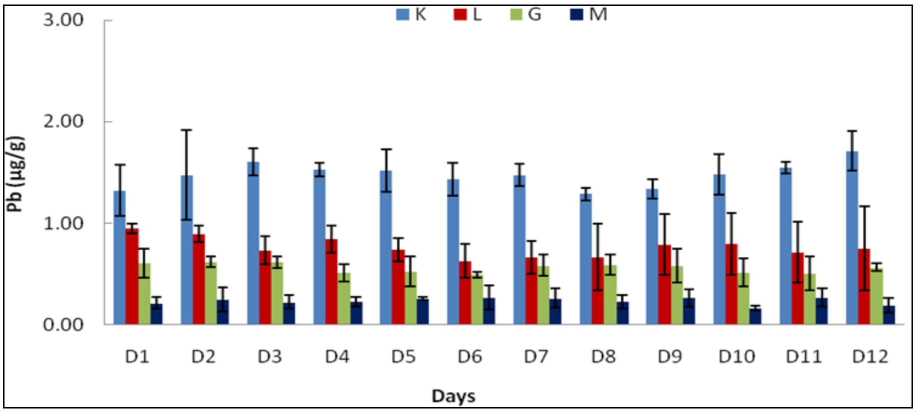


**Fig 2:** Lead accumulation (mean ± SD, n=3) throughout the experimental period in different tissues of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (K- Kidney, L- Liver, G- Gill, M- Muscle)

### 3.3. Tissue-wise accumulation of Cd and Pb

Average Cadmium and Lead concentrations were found to be 0.006µg/g and 0.708µg/g in fish kidney samples of rohu respectively (Fig 3). Heavy metals concentration of fish samples of February had been reported higher than other Months. The concentration levels of Cd and Pb were found to be 0.005µg/g and 0.664µg/g in the liver and 0.004µg/g and 0.715µg/g in gill (Fig 4, 5). The average concentration of Cd and Pb were found 0.002µg/g and 0.217µg/g in the muscle (Fig 6).

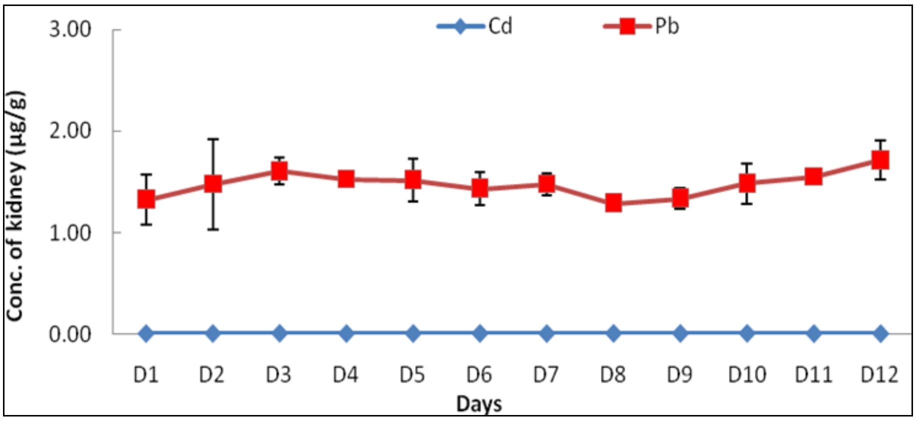


**Fig 3:** Cadmium (Cd) and Lead (Pb) accumulation throughout the experimental period in the kidney of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (mean ± SD, n=3).

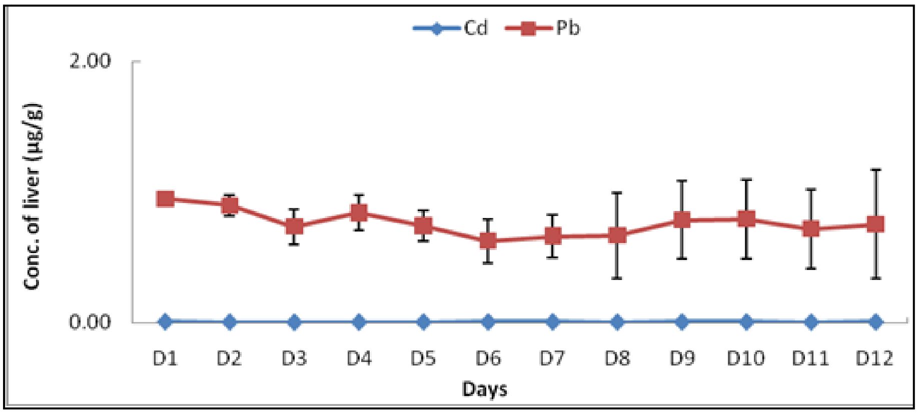


**Fig 4:** Cadmium (Cd) and Lead (Pb) accumulation throughout the experimental period in the liver of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (mean ± SD, n=3).

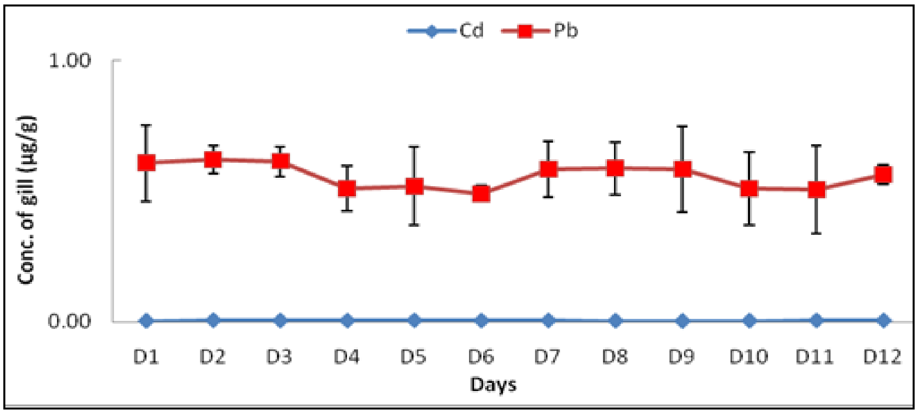


**Fig 5:** Cadmium (Cd) and Lead (Pb) accumulation throughout the experimental period in the gill of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (mean ± SD, n=3).

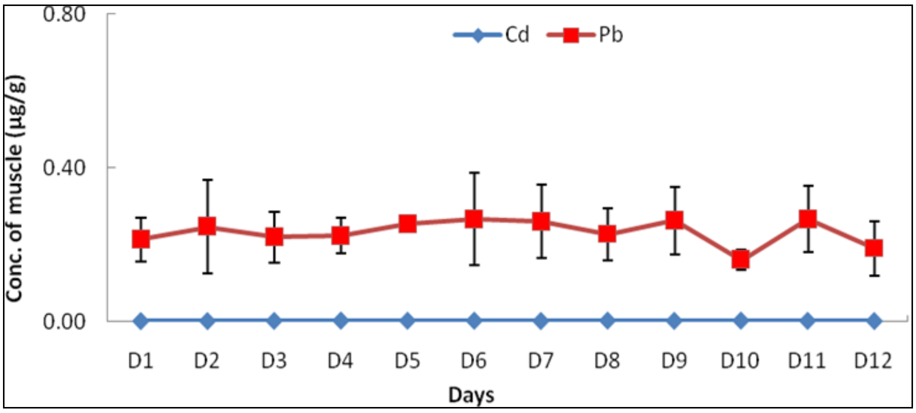


**Fig 6:** Cadmium (Cd) and Lead (Pb) throughout the experimental period accumulation in the muscle of Rohu (*Labeo rohita*) cultured in a sewage-fed aquaculture pond (mean ± SD, n=3).

## 4. Discussion

### 4.1 Accumulation of cadmium

Cadmium is a non-essential toxic metal and may accumulate in human being from food chain with biomagnification [4]. The minimum Cd accumulated in the muscle (0.002µg/g) are within the permissible limits issued by WHO [5]. Overall the bioaccumulation of Cd in the tissues was less. In addition, the muscle contains least Cd content among all the tissues which is the main edible part of the fish. According to Abdel-Baki and team, the liver accumulated the highest concentration while muscles accumulated the lowest which was comparable with present results [6]. In accordance with some authors [7, 8], liver and kidney are the main sites of storage for these metals. Low level of Cd induces metallothionein formation but with chronic exposure, Cd binding capacity of metallothionein decreases. Thus the low accumulation of the metal in the tissues is possible due to the binding of the metal with metallothionein molecules with its subsequent depuration from the organs. Cadmium uptake is non-linear and the accumulation factor decreases with its increasing concentration in water [9]. Cadmium is toxic to virtually every system. It is almost absent in the human body at birth, however, accumulates with age [10, 11]. In general, kidney, liver and gill of fish are discarded during the preparation of fish for food for human consumption. Therefore, there is no acute threat about the Cd accumulation into the fish of sewage-fed aquaculture pond. But caution may be imposed to check the entry of Cd-contaminated sewage in the sewage-fed aquaculture pond.

### 4.2 Accumulation of Lead

Like cadmium, lead is also a non-essential and toxic even in low concentration in human lead delays physical and mental health [12]. In the present study, the maximum bioaccumulation of Pb observed kidney in rohu was 1.48±0.12µg/g. Comparing the observed values with the guideline values of WHO [5], the results showed that the values of Pb are lower than guideline values. As the water and sediment were the primary sources of Pb, the reared fish in these ponds also accumulated a high level of Pb in different tissues. The maximum Pb accumulation observed in the kidney followed by liver, gill and muscle in all the fishes. Abdel-Baki and co-workers also opined that kidney accumulated the highest concentration of Pb while muscles accumulated the lowest [6]. Lead (Pb) does not induce metallothionein (MT) formation in tissues. It is also reported that Pb accumulation does not vary significantly with age and liver, kidney and scales are the main sites for Pb accumulation [13, 14].

## 5. Conclusion

It is clear from the above discussion that both Cd and Pb accumulate in internal organs of fish and eventually can cause harm to human being. The bioaccumulation of Cd and Pd in fish tissue of the sewage-fed pond range from 0.002-0.013µg/g and 0.23-1.84µg/g respectively. It can be concluded that the levels of Cd and Pb in fish tissue don't cross the permissible limit. But a thorough study is required to know the interaction of such low levels of metals with the biochemical function of fish and their levels of transformation into the human body. In general, the domestic sewage is free from any toxic metals, pesticides, herbicides and industrial effluents. But sewage water is often contaminated by pesticides, herbicides, industrial effluents and metals through different means. So contaminated sewage water cannot be used directly for aquaculture practices. Prior to its application in aquaculture, it must be treated properly. Moreover, care should be taken for reducing the input load of metals for a safe culture. Otherwise, the aquaculture products will be contaminated by metals, which may create long term harmful impacts on human society. Sewage-fed aquaculture is a novel approach for the conversion of waste to valuable wealth. It is nothing but a biological treatment of waste-water. The present study clearly indicates that the maximum amounts of metals are accumulating in liver, kidney and gill of fish. Fortunately, the peoples are generally discarding the liver, gill and kidney during the preparation of fish for consumption. Hence, the chances of toxic metal accumulation into the human body are very less [15]. Apparently, the consumption of such fish cultured in this water body is not harmful to the human body. Still, it is alarming to consume the fish cultured in the sewage-fed aquaculture system. Therefore, clean water technology must be adopted in aquaculture to get quality-fish for consumption. This will ensure the safety of human health as well as for trading the contaminant-free aqua-product in different countries.

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