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Heavy Metal Contamination into the Muscle Tissue of *Labeo rohita* Fishes (Rohu) Collected from Jaipur, Rajasthan India

Running Title: Heavy Metal Contamination in Fishes

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Abstract Heavy metal contamination affects physiology, immune system and reproductive potential of organisms from microscopic level to higher trophic levels. Biomagnifications along the food chains create concern to human exposure and associated health risks on regular consumption of these heavy metals. Direct sewage discharge and untreated waste disposal in open waters make aquatic organism more prone to heavy metal exposure. This study focuses on analysis of two heavy metals (Lead and Cadmium) into the muscle tissue of fish species, *Labeo rohita* (Rohu) which was collected in a municipality of Jaipur district, Rajasthan in the month of September and October, 2023. Rohu is a preferred choice among the fish-eating population because it is cheaper and tastier in comparison to other varieties of fishes being produced and sold in the area. Fishes were dissected and processed through Wet Digestion Method for extraction of heavy metals. Further analysis was done using Atomic Absorption Spectroscopy. Both lead and cadmium were present in higher amount than the permissive limit of the Joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO). Target Hazard Quotient (THQ) was calculated to be <1 limiting the value of Hazard index (HI) and Target Cancer Risk (TCR) to be negligible for the concerned area. This research indicates that in order to prevent future metal contamination, effective preventive measures must be implemented. Further studies with large sample size must be done in the area to access the contamination of other heavy metals including mercury, chromium, arsenic etc.

Keywords Atomic Absorption Spectroscopy, Cadmium, Heavy Metal, Lead, Wet Digestion

1. Introduction

Elements with a density more than 5 g/cm^3 are often referred to as heavy metals. These elements occur naturally in the earth crust but their extensive environmental dispersion stems from their numerous industrial, household, agricultural, medicinal, and technical applications, which have raised concerns about their possible consequences on the health of human beings and the environment [1]. Concerns over environmental pollution by these metals have grown in recent years, affecting both worldwide public health and ecology. Additionally, the usage of these materials in numerous agricultural, industrial and technical applications has grown exponentially, greatly increasing human exposure [2]. There are around 56 elements which come under the category of heavy metals out of which five are considered as most toxic heavy metals (THMs) [3]. They are non-threshold toxins and they show toxicity even at very low concentrations. Lead (Pb), mercury (Hg), and arsenic (As) are among the top 20 dangerous compounds, with the Environmental Protection Agency (EPA) and the Agency for Toxic compounds and Diseases Registry (ATSDR) reporting that these substances are in first, second, and third place, respectively. The seventh place is cadmium (Cd). Thus, since they are not biodegradable, heavy metals are found to be the most hazardous to people, animals, and aquatic life, particularly fish, as well as their surroundings [4]. The five THMs are arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb). This study focuses on two THMs namely cadmium (Cd) and lead (Pb) because of their higher potential toxicity.

Cadmium (Cd) is a non-essential trace element which occurs naturally and it has a tendency to bioaccumulate in living organisms at hazardous levels, which is a cause of environmental concern [5]. There has been an increase in the rate of production and consumption of Cd in recent times due to which environmental emissions have increased exponentially, leading to contamination of aquatic habitats [6]. Cadmium containing pesticides, fertilizers and sewage waste is the leading cause of contamination in aquatic habitats. Because of higher bioavailability than other metals, Cd tends to bioaccumulate into the tissues of aquatic organisms. Toxicity levels increase with interaction of cadmium with other toxic chemicals present in the water resources [7]. Cadmium poses a significant threat to human health and the preservation of biodiversity in impacted ecosystems, making it a priority environmental pollutant [8]. Cadmium is particularly harmful to the proximal tubular cells present in the kidney. Additionally, it may result in bone demineralization directly from bone injury or indirectly from renal insufficiency [9].

Lead is a persistent hazardous metal that has no biological function and can cause cancer in humans and aquatic systems [10]. It may enter aquatic systems by precipitation, lead-containing pesticides, smelter and industrial discharges, lead-dust dispersion, street runoff, and municipal wastewater. Pb may bioaccumulate in fish from food and water [10]. Pb can induce a variety of symptoms that differ from individual to individual, exposure duration, and the dosage when it accumulates in the body's major organs. Lead in adults can lead to high blood pressure, sluggish nerve conduction, weariness, mood changes, sleepiness, reduced sex desire, migraines, constipation, and, in extreme situations, encephalopathy or even death [11].

Chronic or acute heavy metal poisoning can result in immunodeficiency, osteoporosis, dementia, and organ failures, among other possible health problems. There have also been reports of possible connections between the concentration level of heavy metals in blood and other human body organs and estrogen-dependent disorders like endometrial cancer, breast cancer, endometriosis, spontaneous abortions, preterm births, stillbirths, and hypotrophy [12]. Heavy metals are considered to be the most prevalent pollutants in the present world of industrialization and these have everlasting impacts on human life. There are reports on the recent last few years' heavy metal poisoning of freshwater and marine fish. Fish samples taken from three reservoirs in the Cauvery River Delta region of India were found to contain higher than allowed levels of heavy metals, including lead, chromium, and zinc [13]. The intake of popular fish species from the Gomti River in Uttar Pradesh, India, including *Clariasbatrachus*, has also been linked to health risks. Studies have examined the contamination of these fish species with Ni, Pb, Zn, C, and Cu [14]. It was reported that five marine edible puffer fish species were found to have high concentrations of heavy metals in the samples taken from the Mandapam Fish Landing Center on India's southeast coast [15]. Similar studies have been done in various other parts of the country including Sundarban wetlands and Thondi fish landing, southeast coast [16, 17].

In this study, we have analyzed the most preferred fish species (*Labeo rohita*) in the area of Chaksu (Jaipur), Rajasthan. Fish consumption rate in north Indian states is lower in comparison to the southern coastal states but in spite of this, a significant proportion of non-vegetarians in this area consume fish on aregular basis. As Jaipur area is an education hub, people throughout the country are staying here for studies and jobs, and preference for sea food is also increasing accordingly. Source of sea-food in this area includes both the transportation from coastal areas and local fish production. As per the information given on the website of the Department of Fisheries, Govt. of India, inland fish production in Rajasthan has increased from 16390 tons in 2004-05 to 66000 tons in 2021-22 [18]. This sector will further see more increase in the upcoming years. But along with the increasing rate of production whether the quality of sea food is up to the mark or not is still questionable. While substantial data is available on the contamination and quality of fishes in the coastal areas, there has not been any study done in this area to the best of our knowledge. We have accessed two heavy metals, namely Cadmium (Cd) and Lead (Pb) in the fish samples of Chaksu lake area as an initial step in this direction. Our study fills the knowledge gap about heavy metal contamination in the Rajasthan area and it proves that further studies are required to assess the quality of fish produced and have some measures to check the same.

2. Material and Methods

2.1. Sampling and Description of Sampling Site

The investigation was conducted at Jaipur National University, Jaipur, Rajasthan from September to October 2023 and for this study, a total number of 30 fishes (*Labeo rohita*) were purchased from local fishermen of Chaksu lake (26 °36′ 0″ N, 75 °57′ 0″ E) (Figure 1), for assessment of metals contamination, particularly, lead (Pb) and cadmium (Cd). This municipality is about 40 kilometers from Jaipur district. It is a continuous destination of household wastewater and discharge from nearby factories. People also throw the remains of temple offerings directly into the lake water. In addition to this, there is also a trend of idol immersion into the waters after worshipping. Paints of idols are known to contain heavy metals [19]. So, this water reservoir is undercontinuous pressure of waste and pollutants. In spite of the fact, fishes are grown and captured into the lake and supplied to the nearby areas for consumption. Rohu is one of the most available and acceptable fish in the region and it was the only species considered for the present study. Fishes were collected from the local fisherman of the lake and transported to the university lab. While transferring the fishes, they were wrapped inside the aluminum foils and put into the ice box. Fishes were kept at -20 ℃ for further processing. Before processing for dissection, length and weight of the fishes were measured. The fishes ranged from 28cm to 32 cm in length and 260g to 320g in weight. Data was compared with the control fishes being produced in University fish pond. Control fishes were being fed with a standard nutrient diet and water quality was continuously under check.

2.2. Dissection and Wet-Digestion

Dissection of the fishes was done to separate the edible and inedible tissues. Edible part (muscle tissue) was further processed for heavy metal extraction because it is the actual source of passing pollutants and toxins to humans. Muscles are the main edible part in this fish and this study was not conducted on other inedible tissues. The muscle tissue from each fish was taken from the region behind the dorsal fin area [20]. For digestion of tissues, the method given by Ali and Khan [21] was followed. A 100 ml beaker containing 1.0 g of wet weight muscle sample was filled with 7.5 ml HNO_3 and 2.5 ml $HClO_4$. After this, the mixture was heated (80 ℃) on a hot plate until full digestion. The transparentyellow solution indicated that whole muscle tissue had been digested. After allowing the solution to cool to ambient temperature, it was filtered using Whatman No. 41 filter paper. Analyte-free water was used to dilute the filtrate to a final amount of 50 milliliters. The solution was further preserved for flame absorption spectrophotometer [21].

2.3. Metal Analysis

The samples were analyzed by The Atomic Absorption Spectrometer (AA500) in MRC-MNIT (Material Research Center-Malviya National Institute of Technology), Jaipur. The analytical technique AAS is used to determine the concentration of a certain element in a sample. By measuring the amount of light absorbed, the concentration of the element in the sample can be calculated [22].

Heavy metal concentration in each sample was calculated by the formula given by Akila [23] and Maurya [24].

$$\textbf{Heavymetalconcentration}\left(\frac{\textbf{mg}}{\textbf{l}}\ \textbf{or}\ \frac{\boldsymbol{\mu}\textbf{g}}{\textbf{g}}\right) = \frac{\textbf{AASreading} \times \textbf{V}}{\textbf{Volume(ml)orweight(g)ofthesample}}$$

where, V is the volume of diluted solution.

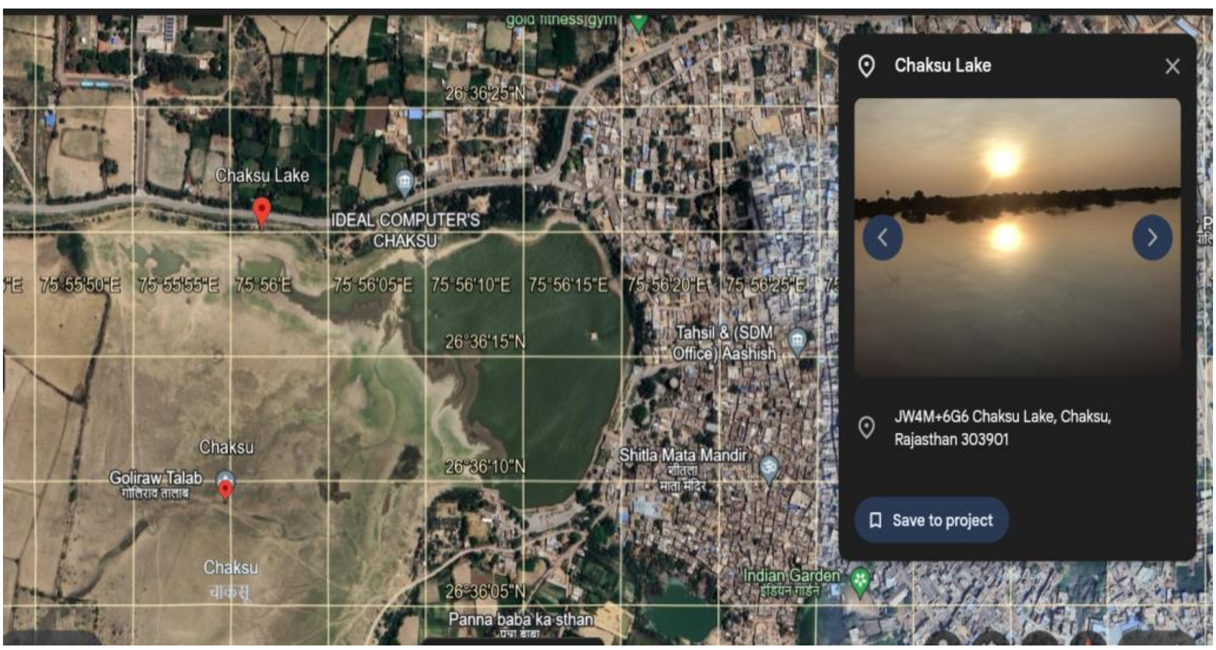

Figure 1. Chaksu lake map with coordinates (26 °36′ 0″ N, 75 °57′ 0″ E). This lake is situated in the Chaksu municipality of Jaipur district, Rajasthan

2.4. Health Risk Assessment

In addition to the heavy metal concentration, certain health risk assessment parameters were also calculated to know the potential risk of the consumption of these fishes. These parameters, namely, Target Hazard Quotient (THQ), Hazard Index (HI) and Target Cancer Risk (THQ) were measured. In order to check the potential risk to human health associated with the consumption of fish that contains both carcinogenic and non-carcinogenic components, the U.S. Environmental Protection Agency (USEPA) developed the target hazard quotient (THQ) and hazard index (HI) [25].

2.4.1. Target Hazard Quotient

THQ is a metric used for evaluating the possible health risks—that are not cancer-causing—that might arise from being exposed to a certain chemical or pollutant. The estimated exposure dosage is divided by the reference dose (RfD) or a comparable reference value to determine it. The RfD is a lifetime exposure estimate to a chemical that is probably not going to have a significant risk of negative consequences [25]. THQ was calculated for both the metals by using the formula given by Djedjibegovic [26] and Kumar [7].

$$\mathbf{THQ} = \frac{(\mathbf{EF} \times \mathbf{ED} \times \mathbf{FIR} \times \mathbf{C})}{\mathbf{RfD} \times \mathbf{BW} \times \mathbf{TA}} \times \mathbf{10^{-3}}$$

Where,
THQ = Target Hazard Quotient
EF = Exposure Frequency (365/year)
ED = Exposure Duration (70 years)
FIR = Fish Ingestion Rate (0.36g/person/day in Rajasthan, India) [18]
C = Metal Concentration in mg/kg
RfD = Reference dose of metal in mg/kg
BW = Average Body Weight (70kg)
TA = Exposure time for non-carcinogens (365 days/year)

Reference doses for Pb and Cd were 0.006 and 0.001 mg/kg/day respectively according to the US EPA IRIS (2011) [27] and WHO (2011) [28].

2.4.2. Hazard Index

The total of the individual Target Hazard Quotients for each pollutant that contributes to a certain health endpoint (such as kidney damage or liver toxicity) is known as the Hazard index. When there are several pollutants in a particular environment, the Hazard Index may be used to assess the total non-carcinogenic health risk that comes with being exposed to them all. HI = Σ THQ is the formula for HI (for all pollutants) [29].

$$\mathbf{HI} = \mathbf{THQ_{Pb}} + \mathbf{THQ_{Cd}}$$

Where,
HI = Hazard Index
THQ_{Pb} = Total Hazard Quotient of Lead
THQ_{Cd} = Total Hazard Quotient of Cadmium

2.4.3. Cancer Risk

Cancer risk, which is usually represented as a lifetime risk, is a metric used to evaluate the likelihood that a person would get carcinogenic growth as a result of being exposed to a certain carcinogenic material. This formula is commonly used to compute it:

$$\mathbf{CancerRisk} = \mathbf{CancerSlopeFactor} \times \mathbf{LifetimeAverageDailyDose} \quad [30]$$

The estimated daily dose of a carcinogen that a person is exposed to throughout the duration of their lifetime is known as the lifetime average daily dose. The ability of a drug to cause cancer is indicated by the Cancer Slope Factor. Target Cancer Risk was calculated by the formula.

$$\mathbf{TCR} = \frac{(\mathbf{EF} \times \mathbf{ED} \times \mathbf{FIR} \times \mathbf{C} \times \mathbf{CSF})}{\mathbf{BW} \times \mathbf{TA}} \times \mathbf{10^{-3}}$$

Where, CSF is the Cancer Slope Factor (0.0085mg/kg/day for Pb and 6.3mg/kg/day for Cd)

2.5. Statistical Analysis

T-Test was used to see the significant difference between heavy metal contamination in the test samples and control samples. A significant difference (P<0.05*) was found between the samples indicating the prevalence of heavy metal contamination in the test samples.

3. Results and Discussion

3.1. Heavy Metal Analysis

This study was done to check the heavy metal contamination present in the fishes collected from an open water resource prone to every kind of pollution and they were compared with the control fishes (*Labeo rohita*) being produced into the university pond which is a covered water body and is being checked regularly for the quality of water. Heavy metal contamination in the test samples was more than the permissible levels of FAO. The maximum permissible limit of Pb given by FAO is 0.2 mg/Kg and that of Cd is 0.05 mg/kg. (FAO, "Heavy Metals Regulations Legal Notice No 66/2003"). Pb concentration into the sample fishes ranged from 6.1 to 8.5 mg/kg and Cd concentration into the sample ranged from 0.15 to 2.75 mg/kg (Table 1). The mean concentration of lead and cadmium in the test samples was found to be 7.046 mg/Kg and 1.178 mg/kg respectively (Table 2). The control samples showed heavy metal concentration within the permissible limits (0.105 mg/kg Pb and 0.003 mg/kg Cd). This significant difference in the concentrations of control and test samples indicates that fish or sea food quality could be improved by applying proper measures to check the water quality in the resources.

Table 1. Concentration of Pb and Cd into the muscles of fishes collected from the Chaksu Lake and the University Pond

Sample	Pb content in mg/kg (Control)	Pb content in mg/kg (Test)	Cd content in mg/kg (Control)	Cd content in mg/kg (Test)
1	0.1	6.3	0.004	2
2	0.2	7.65	0.003	2
3	0	6.77	0.002	0.8
4	0.1	6.65	0.001	0.65
5	0.09	7.5	0.004	0.8
6	0.09	6.86	0.005	0.55
7	0.2	6.4	0.004	0.85
8	0.1	7.73	0.003	0.7
9	0.2	7.1	0.003	0.85
10	0.09	6.15	0.003	1.1
11	0.1	6.85	0.004	1.65
12	0.2	6.65	0.002	0.6
13	0.1	7.05	0.005	1.1
14	0.09	8.5	0.004	1.5
15	0.08	6.34	0.003	1.6
16	0.11	6.5	0.004	2.2
17	0.08	7.45	0.005	1.6
18	0.06	6.54	0.003	0.15
19	0.11	7.34	0.004	1.05
20	0.07	7.5	0.003	2.75
21	0.07	7.66	0.002	1.1
22	0.2	6.4	0.004	0.55
23	0.06	6.3	0.003	0.8
24	0.08	7.6	0.004	0.75
25	0.05	6.1	0.003	1.1
26	0.08	7.75	0.004	1.7
27	0.2	7.8	0.004	2.05
28	0.08	7.35	0.002	1.15
29	0.08	7.8	0.001	1.1
30	0.1	6.8	0.005	0.55

Table 2. Mean concentration of Pb and Cd into the control and test sample

	Test	Control
Pb content in mg/kg	7.046333* ±0.622617	0.105667 ±0.05237
Cd content in mg/kg	1.178333* ±0.602821	0.003367 ±0.001098

±means Standard Deviation (SD); Asterisks represent significant changes between the control and test concentrations. The mean difference is significant at the 0.05 level ($P<0.05$)*.

3.2. Health Risk Assessment

Target Hazard Quotient and Hazard Index are calculated to see the potential risk of any health hazards. THQ value >1 is considered to be potentially hazardous whereas THQ <1 is considered to be safe. THQ for both the heavy metals was <1 despite the fact that contamination was exceeding the FAO limits. Accordingly, HI was also found to be <1. Target Cancer Risk was negligible for both lead and cadmium. These values of THQ, HI and TCR indicate that there is no potential health risk. This can be justified by low ingestion rate of fish in Rajasthan i.e. only 0.36g/person/day [18]. The same concentration of heavy metals can be hazardous if the ingestion rate increases. This provides a scope for future studies with a larger sample size.

Our results are in line with the similar studies done in various other parts of the country. A similar study in East Kolkata Wetland was done in which the concentration of 16 heavy metals including Pb and Cd was determined in the different fish species. The results showed bioaccumulation of heavy metals in muscle tissues within the safe level [7]. Another seasonal study in Harike wetlands showed the seasonal variations in the level of heavy metal contamination in surface waters of the area, peaking in the winter season but HI was still < 1 in spite of high Pb and Cd contamination [31]. A similar study conducted on seventeen different species of marine fishes in Mumbai showed presence of heavy metals but within the limit of recommended levels of WHO/FAO and European Union [32]. Similar results have been published in other parts of the country which claim that fishes have been found to be contaminated with heavy metals but there is not any associated health hazard [14, 24, 33].

Our result signifies that the fishes collected from Chaksulake, Jaipur are fine for human consumption with no associated significant health risk. But continuous monitoring is needed to ensure the safety of aquatic organisms in the area because the heavy metals tend to accumulate in the tissues of fishes and with time, even a small dose of these pollutants can accumulate to dangerously high levels and could cause serious health concerns. Also, the rate of fish consumption is very low in this area. If the rate of consumption increases, the toxicity is going to increase and there could be severe health risks.

4. Conclusions

Heavy metal contamination is a prevalent throughout the globe as a side effect of the industrialization and other anthropogenic activities. But this problem could be dealt with proper legislation and management. To minimize the contamination. Rather than completely shutting down the industries, we could take steps to make industrial expansion less risky and more sustainable.

Sewage water discharge in open water resources is one of the major problems in our country and this is the most common reason for introduction of toxins into the water. Our control fish samples were found to be safer than the test samples because of proper checks on water quality and nutrient supply to the fishes. In conclusion, controlling heavy metal pollution is critical to protecting human health and natural ecosystems. It requires an all-encompassing strategy that incorporates cutting-edge science, innovative technology, and comprehensive legislation to reduce pollution sources, clean up impacted regions, and guarantee a sustainable and healthy future for our world.

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Authors' Contribution

K. Yadav: Conceptualization, methodology, data preparation and drafting manuscript.

M. Godha: Planning research, data analysis, manuscript review.

P. Jethwani: Providing the research idea and sample analysis.

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Research Content

Research content of the submitted manuscript is original and has not been published elsewhere.

Ethical Approval

There is no ethical issue for this research article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability

Not applicable.

Consent to Publish

All authors agree to publish the paper in Environment and Ecology Research journal.

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