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Bioconcentration of cadmium (CD) in rohu (*Labeo rohita*) collected from Sealdah, Garia and Sonarpur fish market in West Bengal

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Abstract
The fish, meat, eggs, milk, etc. are being sold and purchased in markets every day without any knowledge of pollution threats of heavy metals in human health. To judge the pollution status, the bioconcentration of Cd in meat, offal and whole body (dry weight) of *Labeo rohita* (Rohu) collected from Sealdah (SDH), Garia (GRA) and Sonarpur (SNP) fish markets around Kolkata of West Bengal were analyzed from November 2016 to April 2017 by Atomic Absorption Spectrophotometer. The highest Cd content in meat and offal of Rohu was 0.882µg/g and 5.488µg/g respectively. The Cd accumulated Rohu sold in those markets were in the order of SNP>SDH>GRA. As the accumulated metal stored mainly in offal, which is often discarded before preparation of the dish, the chances of risks of the fish eaters are comparatively less. The level of this metal in fish meat was below the prescribed permissible limits, but the cumulative effect of all the heavy metals together can be harmful. So, it is the time to aware the people about the threats of pollution and to equip them to overcome future threats for their sustenance.

Keywords: bioconcentration, cadmium, fish, *Labeo rohita*

1. Introduction
Fish is consumed by the majority of the Indian population on a daily basis. It is a very good source of animal protein, omega-3 fatty acids and varieties of vitamins and minerals including vitamin A, vitamin D, calcium, phosphorus, iron, zinc, magnesium, selenium and iodine [1, 2]. But, at the present time, consumption of fish can cause serious health problems in human being due to their high level of pollutants including toxic metals. Worldwide aquatic environments are being profoundly polluted by different contaminants including toxic metals; with rapid economic development, population explosion and modernization of the society [3]. Pollution from heavy metals (e.g., Cd, Hg, Cr, Pb, etc.) has become a critical issue due to their stable, persistent, and non-biodegradable nature [4]. Among them, Cadmium (Cd) is released to the biosphere from both natural and anthropogenic sources like smelting operations, the use of phosphate fertilizer, cigarette smoking, automobiles, etc. [5]. Afterward, it enters the fish and other aquatic organisms through the food chain and causes serious problem to all members of an ecosystem including human being. In fish, its concentration can become tens to thousands-fold greater than that in water [6], which is conventionally called “bio magnifications”. The increasing concentration of metal burden in aquatic environment and bioaccumulation and thereby bio magnifications of metals in fish triggered to undertake a comprehensive study on Cadmium (Cd) concentration present in the muscle tissues of *Labeo rohita* collected from three commercially important fish markets, namely Garia, Sealdah and Sonarpur fish market in and around Kolkata Metropolitan city of West Bengal, India. Several other studies have already revealed about the bioaccumulation of Cd and other heavy metals in fish tissue at different parts of India. West Bengal contains a large fish-eating population and the quality of the fish and the probable health risk from them should also be assessed. The species was selected because peoples of West Bengal preferred this species and consumed large quantity at least 3-5 days per week. The three fish markets (Sealdah, Garia and Sonarpur) were chosen because huge quantity of different types of fish was imported there from the different parts of the state as well as from the different countries and by nature, they are urban (Sealdah), Semi-urban (Garia) and rural (Sonarpur) fish markets. The preliminary work was designed only for

six months starting from November 2016 to April 2017. With this background, the present study was designed to quantify the Cadmium (Cd) level in *Labeo rohita* collected from the fish markets in Kolkata of West Bengal and to assess the health risk of those fish consumers of those localities due to consumption of those fish.

2. Materials and Methods

2.1 Selection of fish species

One most important, highly demandable and consumable and extensively cultured fin-fish species, namely *Labeo rohita* popularly called Rohu was selected for the study. Both production and consumption of Rohu as regular fish meal and different occasions or ceremonies is more than other fin-fish species. Therefore, this species is consumed by the maximum population of the West Bengal.

2.2 Markets Selection for Sampling

The fish was collected from three popular and important fish markets namely Sealdah (22°34′03″N 88°22′15″E), Garia (22.4662° N 88.4049°E) and Sonarpur (22.43°N 88.42°E) fish markets abbreviated as SDH, GRA and SNP respectively, situated in and around Kolkata of West Bengal. As per the quantity of fish selling, Sealdah market is the largest among the three followed by Garia and Sonarpur markets. By nature, Sealdah fish market is supplied fishes basically for the urban population in Kolkata. Whereas, Garia and Sonarpur fish markets are supplied fishes for urban, semi-urban and rural peoples inhabited in and around the markets.

2.3 Collection and preservation of fish samples

The Rohu (*Labeo rohita*) was sampled thrice-a-week in every month from each market randomly from different retailers. The fresh sampled fish were carried out to the laboratory for analysis of metals. The muscle (meat), livers, gills, fins, scales and gut of the sampled fish were dissected out. The livers, gills, fins, scales and gut of the sampled fish were merged to form a sample of offal. The muscle and offal of the fish were weighted in electronic balance and dried in hot air oven at 103 ºC for 24 hours. Then these samples were kept at room temperature for further analysis.

2.4 Digestion of the Samples

A modified dry-weight method of Churn off (1975) was followed to prepare the fish tissue samples for the determination of Cd [7]. The dried meat and offal of the sampled fish was crushed with mortars and pestles to form a composite sample. The dry weight of each composite sample (5.0 g for meat and offal) in triplicate was kept in a 100 ml beaker. The 10 ml concentrated Nitric Acid (HNO_3) 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 70ºC for complete digestion and extraction of metals from the sample. The digestion was done until the solution turned into pale yellow to transparent colour. The 1.0-2.0 ml of Perchloric Acid ($HClO_4$) 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 Assessment of Estimated Weekly Intake (EWI µg/kg/w)

In the present investigation, the estimated weekly intake (EWI) was calculated based on the guidelines of USEPA (1989) by the following equation [8]:

$$EWI= [(IR \times C) / BW] \times 5$$

Where,
IR = the daily fish intake rate or meal size of fish (i.e., 31.54 g/person/day)
C = the metal concentration (µg/kg of fish in wet weight) of each metal
BW = Average body weight of an adult male (57 kg) or female (50 kg) of West Bengal
5 = Constant (fish intake for 5 days/week/person in West Bengal)
In this calculation, 8.2 kg/person/year of fish and shell-fish consumption rate in West Bengal was considered based on the national report [9]. An average 57 kg weight of an adult male (>18 years) and 50 kg of an adult female (>18 years) were considered for the calculation for estimation of human health hazard [10]. It was assumed that the intake rate of fish by a person of West Bengal (either male or female) was 5 days/week or 260 days/year. Hence, all calculations in this study were made based on the above reference data for fish consumption in West Bengal. Therefore, the daily fish intake rate in West Bengal was 31.54 g/day considering the intake frequency of 5 days/week.

2.7 Statistical analysis

Descriptive statistics and χ^2 test for normalization of the generated data, the two-way analysis of variance (ANOVA) among the data, comparison between the mean differences both as month-wise and market-wise were done using the statistical software like Microsoft Office Excel 2007 and MedCalc Statistical Software (MedCalc Software bvba and version 14.8.1, 2014, Ostend, Belgium).

3. Results

The maximum Cd content in the meat of *L. rohita* collected from Sealdah, Garia and Sonarpur fish markets from November 2016 to April 2017 was 0.882µg/g (Fig. 1). However, the overall average mean value of Cd contents was 0.153µg/g ±0.032 (Fig. 1). In the case of offal, the maximum Cd content was 5.488µg/g (Fig. 2) and the overall average mean value of it was 0.935 µg/g ±0.190 (Fig. 2). Similarly, the highest Cd content in the whole body of Rohu was 6.355µg/g (Fig. 3) and the overall average mean value was 1.088 µg/g ±0.219 (Fig. 3). We found significant differences between Cd contents in meat, offal and whole body (*P*<

0.05). We also observed significant differences in Cd contents in all tissue types between markets and between different months ($P< 0.05$).

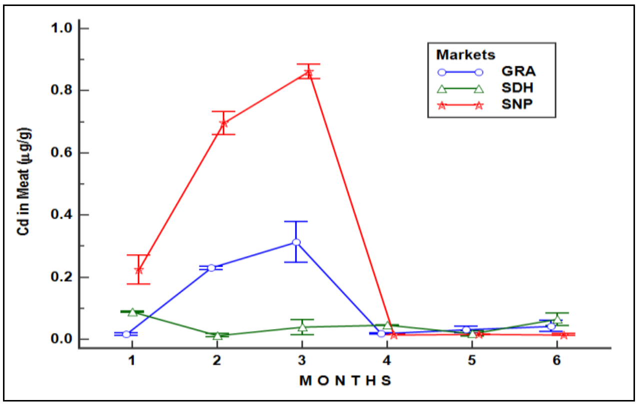

Fig 1: The average monthly variations of Cd in the meat of *L. rohita* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata during Nov-2016 to April-2017.

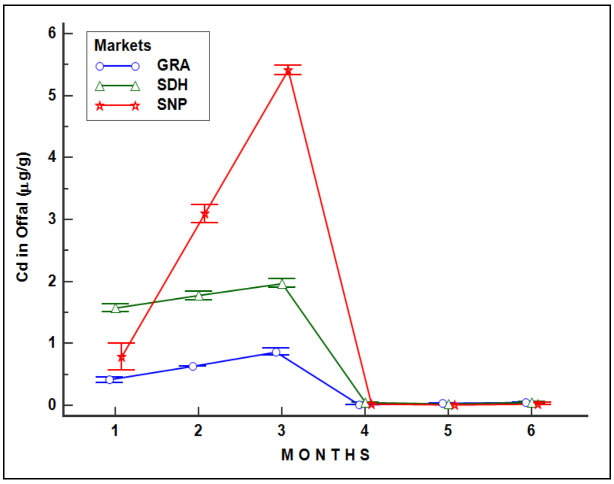

Fig 2: The average monthly variations of Cd contents in offal of *L. rohita* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata during Nov-2016 to April-2017.

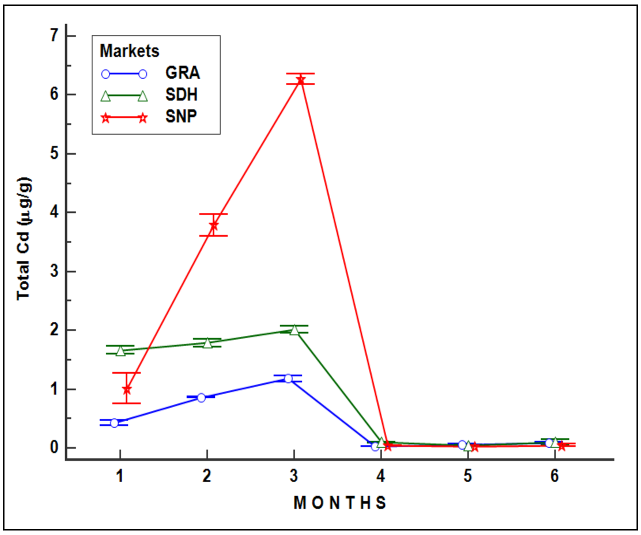

Fig 3: The average monthly variations of Cd contents in the whole body (total) of *L. rohita* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata from November 2016 to April 2017.

Regarding Cd concentration in different markets, the maximum Cd content was observed in the meat of *L. rohita* collected from SNP market followed by GRA and SDH markets (i.e. in the order of SNP>GRA>SDH). Considering the markets, the Cd contents in fish meat were significantly varied ($P<0.05$) among all the markets except in between GRA and SDH markets. The present results showed that the highest Cd content was in offal collected from SNP market followed by SDH and GRA fish markets (i.e., in the order of SNP>SDH>GRA). Like meat, the Cd contents in fish offal were significantly varied ($P<0.05$) among all the markets. The bio-concentrations of Cd in the whole body (total) of fish showed a similar trend like offal. The metals content in the whole body of fish was also significantly varied ($P<0.05$) among all the markets. The highest Cd contents were observed in the whole body (total) of *L. rohita* collected from SNP market followed by SDH and GRA markets (i.e., in the order of SNP>SDH>GRA).

The pair-wise comparisons of mean difference of Cd contents in the meat of *L. rohita* varied significantly ($P<0.05$) among all months of study except Feb vs Mar, Apr; Mar vs Apr. In later cases, they were not varied significantly ($P> 0.05$). Like meat, the comparisons of mean difference of Cd contents in offal also varied significantly ($P< 0.05$) among the months of study except for Feb vs Mar, Apr; Mar vs Apr, where their variations were not significant ($P> 0.05$). Similarly, the pair-wise comparisons of mean difference of Cd contents in the whole body (Total) were varied significantly ($P< 0.05$) among all months of study except Feb vs Mar, Apr and Mar vs Apr, where they were varied insignificantly ($P> 0.05$).

We also calculated the Estimated Weekly Intake (EWI) to assess the possible human health risk. We found that the maximum EWI of Cd was 1.106 µg/kg/wk in female (Fig.4) and 0.970 µg/kg/wk in male (Fig.5) from rohu. However, the overall average means EWI of Cd were 0.151±0.037 µg/kg/wk in female and 0.133±0.032 µg/kg/wk in the male.

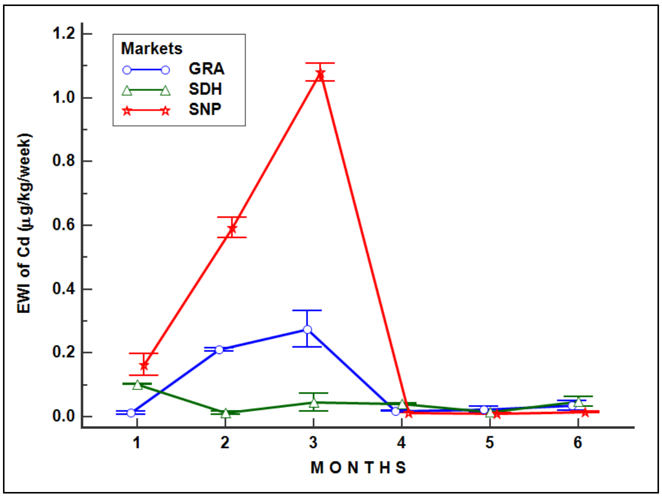

Fig 4: The estimated weekly intake (EWI) of Cd by a female of West Bengal through the consumption of *L. rohita* meat marketed from Sealdah, Garia and Sonarpur fish markets in Kolkata from Nov-2016 to April-2017.

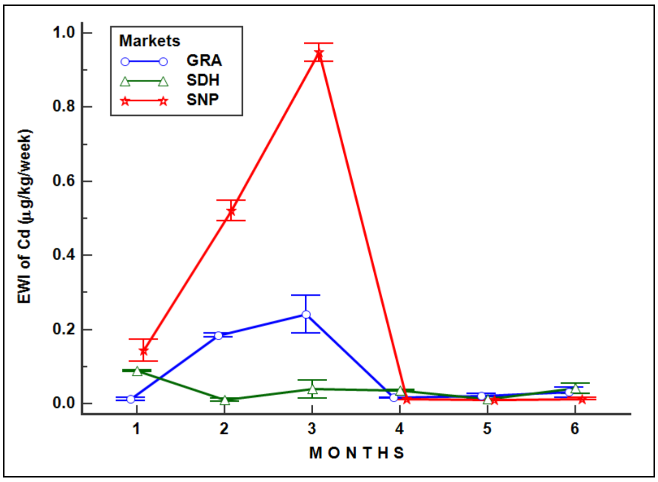

Fig 5: The estimated weekly intake (EWI) of Cd by a female of West Bengal through the consumption of *P. monodon* meat marketed from Sealdah, Garia and Sonarpur fish markets in Kolkata from Nov-2016 to April-201

4. Discussion

It is clear from the above results that Cd accumulates in different tissues of fish and can become a serious threat to the human being. Fish acquires the metal from the environment through different sources. The possible areas of absorption of dissolved metal are the gill, intestine and skin. Perera and co-workers (2015) also opined that Cadmium (Cd) may be considered as one of the most toxic metal and intake of Cd by fish may create serious implications for fish consumers [11]. It can come to the environment from a large number of sources and has a trend to accumulate in aquatic organisms including fish. After entering the organism it interacts with different cytoplasmic components and produces toxic effects [12]. Though, sometimes it may not cause any harm to the fish at low concentration; but becomes a threat to the human being due to gradual bio magnifications through different tropic levels. High concentration of Cd (70-100 ng/ml) has been detected in many cities of India including Mumbai [13]. Though in our experiment, it was not that much high. We also observed that the least amount of Cd was recorded in meat and maximum content was recorded in offal of fish. Hence, the consumption of fish meat after discarding offal (i.e. gut, gill, liver, kidney, scale, fins, etc.) are more hygienic and low risk for human health, because maximum accumulated metals are disposed of through fish offal. Elias and co-workers reported the status of Cd accumulation in the muscle of some salted fish namely kembong (*Psettodes Erumei* sp.), bulu ayam (*Thryssa mystax* sp.), gelama (*Decapterus russelli*) and parang (*Makrochirichthys makrochirus*) were quite high (0.367 µg/g of Cd) [13]. But Cd content was within the permitted level (i.e., 1 µg/g). In our study, we observed the highest Cd concentration in whole fish as 6.355 µg/g and the maximum EWI was 1.106 µg/kg/wk in female (Fig.4) and 0.970 µg/kg/wk in male (Fig.5). According to the Joint FAO/WHO Expert Committee on Food Additives, the Provisional Tolerable Weekly Intake (PTWI) Cd for an average adult (70 kg) is 7 µg/kg [14, 15]. So, the EWI in our cases was quite lower than the prescribed limit and the fishes can be considered safe for consumption. But, the results also alert us about the increasing rate of heavy metal accumulation in fishes which can cause a severe problem in the near future.

5. Conclusion

The maximum Cd contents in the meat, offal and the whole body of *L. rohita* was 0.88µg/g, 5.488µg/g and 6.355µg/g respectively and among markets, these were in the order of SNP>GRA>SDH, SNP>SDH>GRA and SNP>SDH>GRA. The present study clearly indicates that the fish meats are accumulating least quantity of metals and their offal's' are accumulating the highest quantity of metals from their surrounding environment. However, the level of the metal does not cross the permissible limits prescribed by WHO and FAO [14–16]. Hence, it can be suggested that the conventional method of the preparation of a fish meal in West Bengal by discarding the offal (i.e., liver, kidney, gills, alimentary canal, fins and scales) should be followed by everybody and everywhere to minimize the threat of secondary metal accumulation in the human body. People should also be aware of the threat of pollution in the coming days. Accordingly, they may equip themselves to overcome the threats for their sustenance. There are many options to keep our environment clean and pollution free. The green technology for aquaculture, organic farming in agriculture and aquaculture, use of organic manures in place of chemical fertilizers, use of bio-pesticides in place of pesticides, herbicides and insecticides, banning of chemical and nuclear weapons, reducing the use of fossil fuels, etc. are some of the options in this journey.

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