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Bioaccumulation of lead (PB) in tiger prawn (*Penaeus monodon*) collected from fish markets of Kolkata

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Abstract
Every day a good amount of fish and fish products are being purchased and consumed without knowing the pollution threat of heavy metals from them. The aim of our study was to assess the pollution status in terms of Lead (Pb) accumulation in a common shellfish namely *Penaeus monodon* (tiger prawn). For that purpose samples were collected from Sealdah (SDH), Garia (GRA) and Sonarpur (SNP) fish markets around Kolkata of West Bengal, India from November 2016 to April 2017 and the bioaccumulation of Pb in different tissues was analyzed by Atomic Absorption Spectrophotometer. The maximum concentration of Pb in meat, shell and the whole body of prawn was 10.00μg/g, 10.620μg/g and 17.290μg/g respectively. Though the level of this metal in this shellfish was below the prescribed permissible limits, the cumulative effect of all the heavy metals together can be harmful. So, it is the time to make people conscious about the possible damage from heavy metal contamination in fish and to prepare them to overcome future threats for their sustenance.

Keywords: Bioaccumulation, Lead, shellfish, *Penaeus monodon*, tiger prawn

1. Introduction

Both finfish and shellfish are sources of excellent nutritional value and largely consumed by the Indian population. It provides good quality nutrients including protein, fatty acids and varieties of vitamins and minerals [1, 2]. But, nowadays these nourishing food items has emerged as a major source of heavy metal contamination. With rapid industrialization, modernization and population explosion; environmental pollution has also increased by leaps and bounds. Several anthropogenic activities have resulted in the release of a lot of pollutants into the environment. Heavy metals are also not an exception. They are released to the biosphere from different sources like fertilizers, automobiles, smelting operations, etc. After that, they enter the human and animal food chain mainly through the aquatic ecosystem. Due to the persistent and non-biodegradable nature, these metals accumulate in different tissue of aquatic organisms. The level increases further in next tropic level and finally can cause harm when consumed by the human being. It has been observed that the concentration of certain heavy metals can be thousand-fold greater in fish tissue compared to its concentration in water. This increasing concentration of heavy metal load in aquatic environment and bioaccumulation and thereby bio magnifications of metals in aquatic organisms provoked to undertake a comprehensive study on Lead (Pb) concentration present in the tissues of *Penaeus monodon* collected from three commercially important fish markets, namely Garia, Sealdah and Sonarpur fish market in and around Kolkata Metropolitan city of West Bengal, India. There are reports of Pb bioaccumulation in several aquatic organisms at different parts of India. In West Bengal, prawn is a common food item consumed very often. So, the possible health risk from heavy metal in prawn should 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 work was planed only for six months starting from November 2016 to April 2017. With this background, the present study was designed to quantify the Lead (Pb) level in *P. monodon* 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 shellfish species, namely *P. monodon* popularly called tiger prawn was selected for the study. Both production and consumption of Prawn as regular meal and different occasions or ceremonies is very common. 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 prawn (*P. monodon*) was sampled thrice-a-week in every month from each market randomly from different retailers. The fresh sampled shellfish were carried out to the laboratory for analysis of metals. The shell was dissected out and made a separate sample; the remaining portion was considered as meat. The muscle and shell of the fish were weighted in electronic balance and dried in hot air oven at 103ºC 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 Churnoff (1975) was followed to prepare the prawn tissue samples for the determination of Pb [3]. The dried meat and shell of the sampled prawn was crushed with mortars and pestles to form a composite sample. The dry weight of each composite sample (5.0 g for meat and shell) 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. Three standard solutions (0.5 mg/l, 1.0 mg/l and 1.5 mg/l) Pb 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 [4]:

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

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 [5]. 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 [6]. 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 Pb content in the meat of *Penaeus monodon* collected from Sealdah, Garia and Sonarpur fish markets from November 2016 to April 2017 was 10.00µg/g (Fig 1). However, the overall average mean value of Pb contents was 3.988µg/g±0.241 (Fig. 1). In the case of the shell, the maximum Pb content was 10.620µg/g (Fig. 2) and the overall average mean value of it was 4.299µg/g±0.239 (Fig. 2). Similarly, the highest Pb content in the whole body of prawn was 17.290µg/g (Fig. 3) and the overall average mean value was 8.287µg/g±0.430 (Fig. 3). We found significant differences between Pb contents in meat, shell and whole body ($P < 0.05$). We also observed significant differences in Pb contents in all tissue types between markets and between different months ($P < 0.05$).

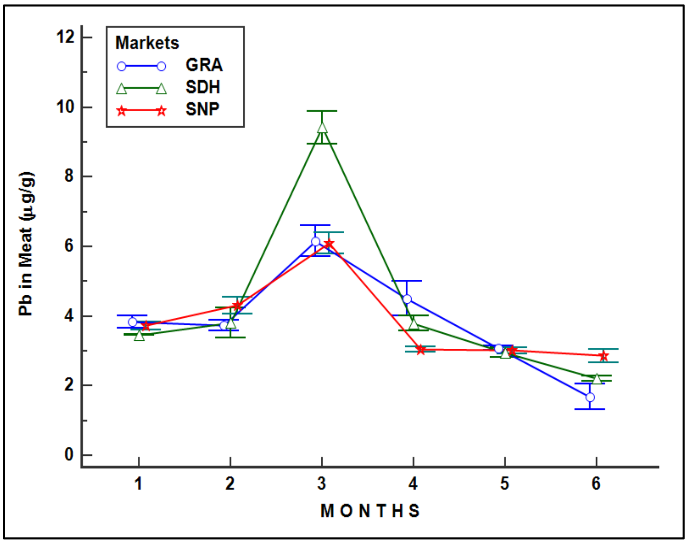

Fig 1: The average monthly variations of Pb in the meat of *Penaeus monodon* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata during Nov-2016 to April-2017.

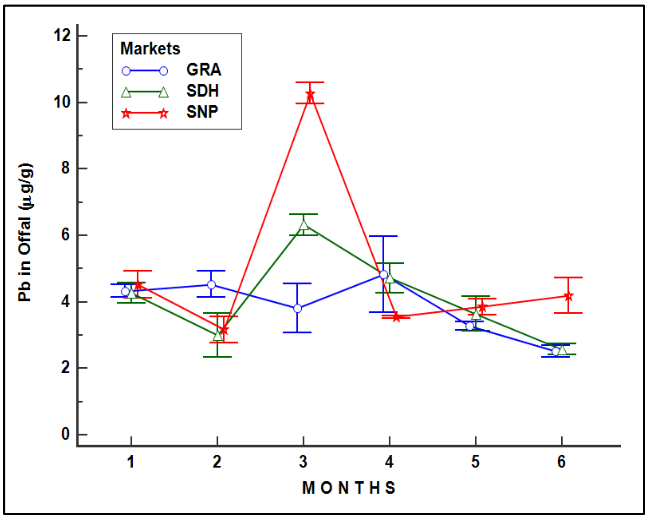

Fig 2: The average monthly variations of Pb contents in the shell of *Penaeus monodon* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata during Nov-2016 to April-2017.

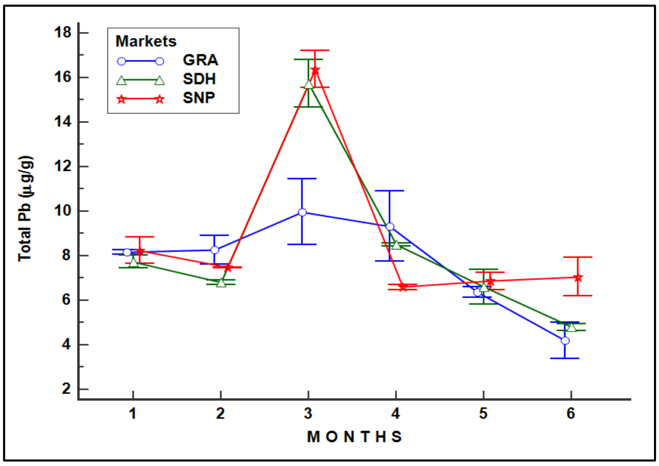

Fig 3: The average monthly variations of Pb contents in the whole body (total) of *Penaeus monodon* collected from Sealdah, Garia and Sonarpur fish markets in Kolkata from November 2016 to April 2017.

Regarding Pb concentration in different markets, the maximum Pb content was observed in the meat of *Penaeus monodon* collected from SDH market followed by SNP and GRA markets (i.e. in the order of SDH>SNP>GRA). Considering the markets, the Pb contents in meat were significantly varied (P<0.05) among all the markets except in between GRA and SNP markets. The present results showed that the highest Pb content was in shell collected from the SNP market followed by SDH and GRA fish markets (i.e., in the order of SNP>SDH>GRA). Like meat, the Pb contents in the shell were significantly varied (P<0.05) among all the markets except between SDH and GRA. The bio-concentrations of Pb in the whole body (total) of fish showed a similar trend like shell. The metals content in the whole body was also significantly varied (P<0.05) among all the markets except in between SDH vs SNP, where it was comparable to each other (P> 0.05). The highest Pb contents were observed in the whole body (total) of *P. monodon* 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 Pb contents in the meat of *P. monodon* varied significantly (P<0.05) among all months of study except Nov vs Feb, Mar, Dec; Dec vs Feb. In later cases, they were not varied significantly (P> 0.05). Like meat, the comparisons of mean difference of Pb contents in shell also varied significantly (P< 0.05) among the months of study except for Nov vs Feb; Dec vs Mar, Apr, where their variations were not significant (P> 0.05). Similarly, the pair-wise comparisons of mean difference of Pb contents in the whole body (Total) were varied significantly (P< 0.05) among all months of study except Nov vs Dec, Feb; Dec vs Feb, Mar, 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 Pb was 1.896 µg/kg/wk in female (Fig.4) and 1.663 µg/kg/wk in male (Fig.5) from prawn. However, the overall average means EWI of Pb were 0.682±0.051 µg/kg/wk in female and 0.598±0.045 µg/kg/wk in the male.

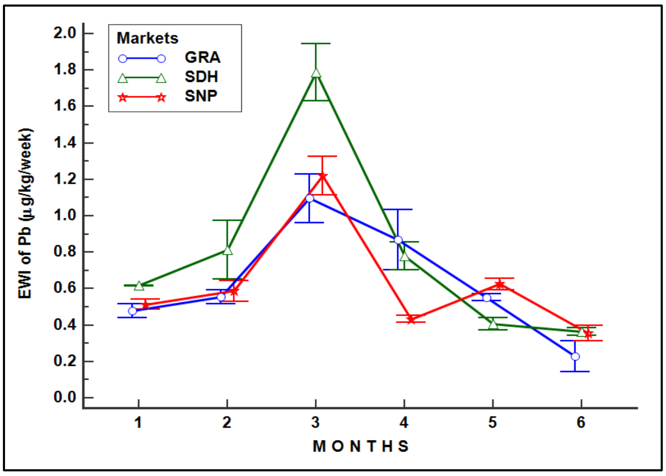

Fig 4: The estimated weekly intake (EWI) of Pb by a female of West Bengal through the consumption of *Penaeus monodon* meat marketed from Sealdah, Garia and Sonarpur fish markets in Kolkata from Nov-2016 to April-2017.

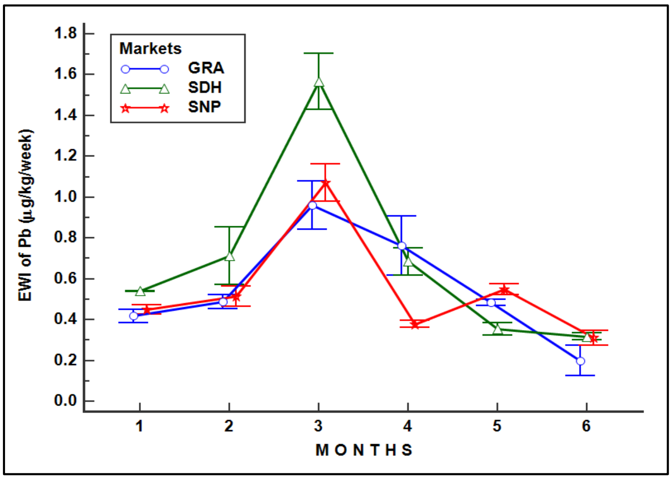

Fig 5: The estimated weekly intake (EWI) of Pb by a female of West Bengal through the consumption of *P. monodon* meat marketed from Sealdah, Garia and Sonarpur fish markets in Kolkata from Nov-2016 to April-201

4. Discussion

It is clear from the above results that Pb accumulates in tissues of shellfish and can become a serious threat to the human being. The prawn acquires the metal from the environment through different sources. The major route of Pb accumulation in prawn is ingestion. After intake, it has a tendency to accumulate in different internal organs. When consumed by a human it causes several problems. The Pb is known to reduce cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in the adult [7, 8]. It is a cumulative poison that causes both chronic and acute intoxication. Chronic exposure to lead (Pb) results in its deposition and immobilization in bone from where lead (Pb) can be mobilized to other tissues [9]. The accumulation of lead in fish depends upon the concentration of metal in water and exposure time [7]. But, this accumulate metal can be excreted out if the organism is placed in fresh water. Several types of researches have reported accumulation of Lead in prawns and fishes in different parts of the World [11, 12]. Regarding finfish, Sary and Mohammadi reported that the Pb content in muscle of *Barbus xanthopterus*, *Liza abu*, *Barbus grypus*, *Acanthopagrus latus*, *Platycephalus indicus*, *Otolithes ruber* collected from the river and marine environment in Khouzestan was more in riverine fish than marine fish [13]. In contrast, in the present investigation, Pb accumulation in the meat of the saline tiger shrimp/prawn (*P. monodon*) was more than that of freshwater fish *L. rohita* [14]. However, the mean content of Pb in shrimp was significantly higher than that of their observation. In our study, we observed the highest Pb concentration in whole fish as 17.290µg/g and the maximum EWI was 1.896 µg/kg/wk in female (Fig.4) and 1.663 µg/kg/wk in male (Fig.5). According to the Joint FAO/WHO Expert Committee on Food Additives, the Provisional Tolerable Weekly Intake (PTWI) Pb for an average adult (70 kg) is 25 µg/kg [15, 16]. So, the EWI in our cases was quite lower than the prescribed limit and the prawns available in the market can be considered safe for consumption. But, the results also alert us about the increasing rate of heavy metal accumulation in aquatic organisms which can cause a serious problem in the coming days.

5. Conclusion

The maximum Pb contents in the meat, shell and the whole body of *L. rohita* was 10.00µg/g, 10.620µg/g and 17.290µg/g respectively and among markets, these were in the order of SDH>SNP>GRA, SNP>SDH>GRA and SNP>SDH>GRA. However, the level of the metal does not cross the permissible limits prescribed by WHO and FAO. But, simultaneously it gives an indication of the threat in the future. With increasing modernization, we have increased pollution also. Our future generation will not be safe unless we take the necessary steps in reducing contaminants in the environment. There are several options to make the earth clean again. Use of green technology, adopting organic agriculture and aquaculture, reduction in carbon footprint are some examples in the way for a better future.

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