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Assessment of copper (cu) accumulation in giant freshwater prawn (*Macrobrachium rosenbergii*) marketed in greater Kolkata of West Bengal

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Abstract
Shellfish are being consumed and exported in large amount from India. But it can cause health problems in human due to the accumulation of heavy metals in them. So, a study was carried out to assess the accumulation of Cu in different tissues of giant freshwater prawn (*Macrobrachium rosenbergii*) and their probable human health hazards in adult females and males of West Bengal, India. Samples were collected from Sealdah, Garia and Sonarpur fish markets in and around Kolkata of West Bengal during January-June 2016 and Cu concentration was determined through Atomic Absorption Spectrophotometer. The mean concentration of Cu in the meat, offal and whole body were 17.74 ± 9.00 µg/g, 32.70 ± 19.26 µg/g and 50.44 ± 27.00 µg/g respectively. The maximum Cu contents were 33.12 µg/g, 63.43 µg/g and 93.01 µg/g and the maximum EWI reported in female and male were 4.61 µg/kg/w and 4.05 µg/kg/w. All these values were below the permissible limits. But the cumulative effect of all the metals can be harmful and we should equip ourselves to overcome the future threats from the heavy metal pollution.

Keywords: Bioaccumulation, Copper, giant freshwater prawn, heavy metal, *Macrobrachium rosenbergii*, shellfish

1. Introduction
Shell-fishes (shrimps and prawns) are generally considered as a very important and delicious protein source of food as it contains an enormous amount of digestible protein and essential amino acids with high nutritional value. Its consumption is largely recommended in all countries including India [1]. Giant freshwater prawn (*Macrobrachium rosenbergii)* is a commercially important seafood species. There is an increasing demand for it in world markets exported from India due to its large size, unique taste, high nutritive value and persistent consumers' demand. But, very often, the culture of prawns in the polluted aquatic environment of India become one of the major hurdles for its exporting. Heavy metals (As, Cd, Pb, Cu etc) are one of the major pollutants that accumulate in their tissues and organs including muscle [2]. Rapid industrialization and urbanization are the major causes that result in the release of heavy metals in the ecosystem. These metals exert considerable biological effects even at low levels because of their pervasiveness and persistence nature [3]. They transfer to the next tropic level through the food chain [4]. If the toxic metal accumulated shell-fish are ingested by the consumer regularly, these toxic metals are gradually accumulated in some tissues and organs of the human being also, which may cause serious health hazards by dysfunction of the enzymatic functions, impairment of the biochemical and metabolic mechanisms of the body thereby total dysfunction of the body [5]. These prawns come into the markets daily from different cultured and captured water bodies to fulfill the demand of the fish consumers of the state where about 90% of the population are the fish eater. In spite of knowing the possible hazard, very few studies were carried out in India on human health risk assessment due to metals uptake through shell-fish consumption [6,7]. So, we did this experiment to assess the levels of weekly intake of Cu by the average population in Kolkata of West Bengal through consumption of a commonly edible prawn (*Macrobrachium rosenbergii*). With this background, the three fish markets (Sealdah, Garia and Sonarpur) were chosen as a large quantity of prawn 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 January 2016 to June 2016. The present study was designed to quantify the bio accumulative toxic metal (Cu) in the prawn marketed in Greater Kolkata of West Bengal and also to assess the health risk of the shell-fish consumers at these areas.

2. Materials and methods

2.1 Selection of species

Macrobrachium rosenbergii (Fig 1) is an important, highly demandable and consumable and extensively cultured shell-fish species popularly called giant freshwater prawn. It is eaten on a daily basis and also on different occasions or ceremonies. It is consumed by the major portion of the population of the West Bengal.

Fig 1: *Macrobrachium rosenbergii* (Giant freshwater prawn)

2.2 Selection of markets for sampling

The samples were 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 fish 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 Kolkata of West Bengal.

2.3 Collection and preservation of shell-fish samples

The prawn was sampled every month from each market randomly from different retailers. The freshly sampled shell-fish were carried out to the laboratory for analysis of metals. The muscle, shell, and head of the sampled fish were dissected out. The shell and head of the sampled shell-fish were merged to form a sample of offal. The muscle and offal of the shell-fish were weighted in electronic balance and dried in a hot air oven at 103ºC for 24 hours. Then these samples were kept at room temperature for further analysis.

2.4 Digestion of samples

A modified dry-weight method of Churn off (1975) was followed to prepare the shell-fish tissue samples for the determination of Cu [8]. The dried muscle and offal of the sampled shell-fish were crushed with mortars and pestles to form a composite sample of each shell-fish species by adding three shell-fish in each species. The dry weight of each composite sample (5.0 g for muscle and 3.0g for offal) in triplicate was kept in a 100 ml beaker. Then 5 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 color. The 1.0-2.0 ml of $HClO_4$ was added drop-wise to the sample to make a transparent solution. After complete digestion, the solution would be cool down at room temperature, diluted with ion-free double distilled water and filtered in Whatmann filter paper No.1 (110mm) and kept in sample bottles (Tarson ®) with a volume of 30 ml of each.

2.5 Detection of Metals by Atomic Absorption Spectrophotometer (AAS)

The metal content of the sample was detected in Atomic Absorption Spectrophotometer (Varian AA 240) using hollow cathode lamps of Cu. Three standard solutions (0.5mg/l, 1.0mg/l, and 1.5mg/l) of Cu were prepared from stock solutions (1,000 mg/l) of Cu 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 of sample.

2.6 Calculation 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 [9]:

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

Where,

IR = The daily shell-fish intake rate or meal size of shell-fish (i.e., 30g/person/day)

C= he metals concentration (µg/kg of shell-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 (shell-fish intake for 5 days/week/person in West Bengal)

In this calculation, 8.2 kg/person/year of fish and fish products consumption rate in West Bengal was considered based on the national report (Needham *et al*., 2014). An average 57 kg weight of an adult male (>18 years) and 50 kg of an adult female (>18 years) were considered ((Shukla *et al*., 2002) for the calculation for estimation of human health hazard. 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. Therefore, the daily shell-fish intake rate in West Bengal was 31.54 g/day considering the intake frequency 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 difference of the data both as month-wise and market-wise were done using the statistical software like Microsoft office Excel 2007 and Med Calc Statistical Software (Med Calc Software bvba and version 14.8.1, 2014, Ostend, Belgium).

3. Results

3.1 Status of copper (Cu) accumulation in meat

The mean value of the Cu content in the meat of giant

freshwater prawn (*Macrobrachium rosenbergii*) collected from Garia, Sealdah and Sonarpur fish markets in Kolkata during January 2016 to June 2016 was 17.74 ± 9.00 µg/g. The maximum Cu contents in meat (in dry weight) was 33.12 µg/g and the minimum level was 3.23 µg/g (Fig 2). The pair-wise comparison of the accumulated Cu contents in the meat of shell-fish between the different markets (Garia, Sealdah and Sonarpur) as well between the months of study (January-June 2016) were varied significantly except February vs June and March vs April (*P<0.05*).

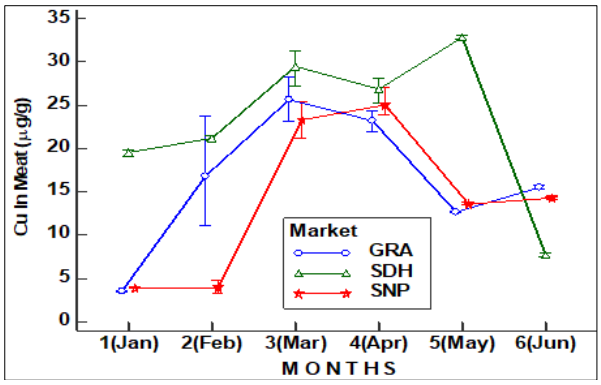

Fig 2: Mean Cu contents in the meat of *M. rosenbergii* in Garia, Sealdah and Sonarpur fish markets in Kolkata during January-June 2016

3.2 Status of copper (Cu) accumulation in offal

The mean value of the Cu content in offal (in dry weight) was 32.70 ± 19.26 µg/g. The maximum Cu contents were 63.43 µg/g and the minimum was 2.99 µg/g during the study period (Fig 3). The pair-wise comparison among the different markets and among the month of the accumulated Cu contents in the offal of all shell-fish species collected from Garia, Sealdah and Sonarpur fish markets in Kolkata during January-June 2016 were varied significantly (*P<0.05*).

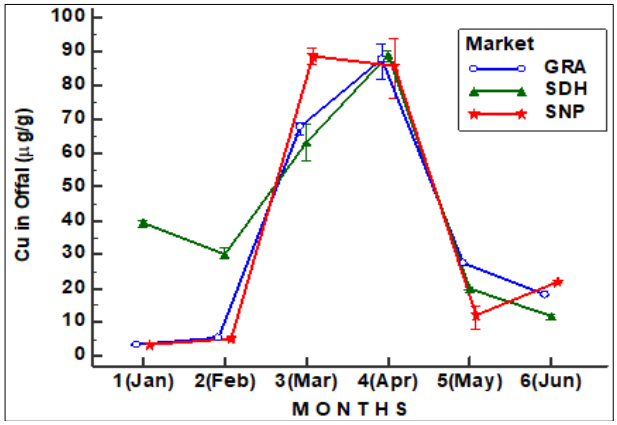

Fig 3: Mean Cu contents in the offal of *M. rosenbergii* collected from Garia, Sealdah and Sonarpur fish markets in Kolkata during January-June 2016

3.3 Status of Copper (Cu) Accumulation in the whole body

The mean values of the Cu contents in the whole body were 50.44 ± 27.00 µg/g. The maximum Cu contents in the whole body were 93.01 µg/g, and the minimum was 7.74 µg/g, 7.19 µg/g and 5.02 µg/g during the study period (Fig 4). The pair-wise comparison of the whole-body Cu contents accumulated in during the January 2016 to June 2016 was varied significantly except January vs February and March vs April (*P<0.05*).

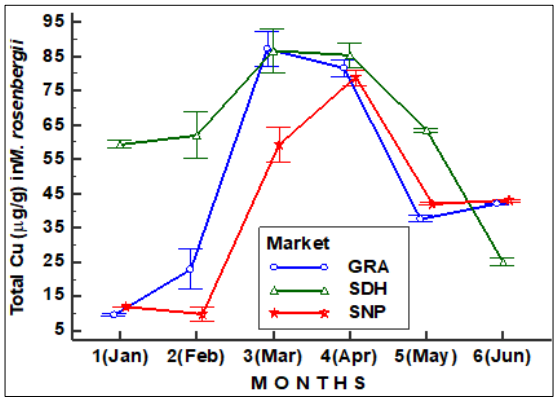

Fig 4: Mean whole body Cu contents in *M. rosenbergii* collected from Garia, Sealdah and Sonarpur fish markets in Kolkata from January 2016 to June 2016

3.4 Estimated Weekly Intake (EWI) (µg/kg/w)

We also calculated the Estimated Weekly Intake (EWI) to assess the possible human health risk. In West Bengal, the mean of estimated weekly intake (EWI) of Cu through female and male was 2.39 ± 1.27 µg/kg/w and 2.09 ± 1.11 µg /kg/w respectively. The maximum EWI of Cu in females was 4.61 µg/kg/w and the minimum value was 0.42 µg/kg/w (Fig 5). Whereas, in males, the maximum EWI of Cu was 4.05 µg/kg/w and the minimum were 0.37 µg/kg/w (Fig 6). Overall EWI of Cu from all the shell-fishes were varied significantly (*P<0.05*) in both females and males of West Bengal. The average EWI of Cu in females and males of West Bengal significantly varied among the months and markets (*P<0.05*).

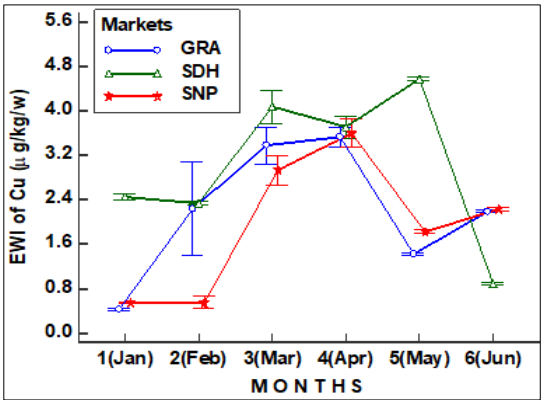

Fig 5: Mean of EWI in Cu in females of West Bengal from *M. rosenbergii* collected from Garia, Sealdah and Sonarpur fish markets in Kolkata during January-June 2016

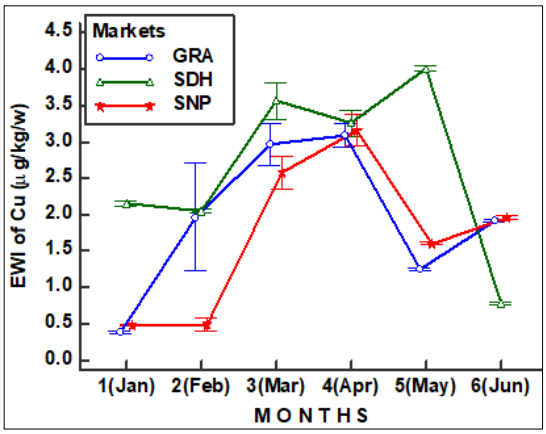

Fig 6: Mean of EWI of Cu in males of West Bengal from *M. rosenbergii* collected from Garia, Sealdah and Sonarpur fish markets in Kolkata during January-June 2016

4. Discussion

4.1 Accumulation of copper in tissues

It is clear from the above discussion that Cu accumulates in the tissues of prawn and the levels vary depending upon markets and months. It was found that the Cu contamination free shell-fishes were more common in a small market (Sonarpur) as compared to the large and medium market (Sealdah and Garia). As per FAO and WHO guidelines the maximum permissible limit of Cu in food fishes including shrimps/prawns meat is 30 µg/g [10]. The present investigation showed a higher level of Cu in *M. rosenbergii* meats collected from Sealdah fish market and near to the permissible limit from other markets. It indicates that Cu contents are in danger levels in shrimps/prawns marketed in urban and big fish markets. Whole body Cu contents of the present investigation collected from Sealdah, Garia and Sonarpur markets particularly during March and April were crossed the maximum permissible limits prescribed by FAO and WHO [10]. The Cu contents of 10.3-145.2 µg/g were recorded in *P. indicus* collected from Subarnarekha River of Ranchi in India, which were closely resembled of the present results [11]. Olgunoglu and coworkers reported the maximum Cu content (3.43±0.02 µg/g) in the muscle (meat) of Giant Red shrimp collected from Mediterranean Sea of Turkey during summer and minimum (1.99±0.01 µg/g) during winter [12]. In contrast, the Cu content in the liver of those shrimps was maximum (126.0±1.11 µg/g) in summer and minimum (162.24±7.51 µg/g) in winter. These results are much higher than the present levels of Cu in all the shrimps/prawns collected from Kolkata fish markers. However, the Cu contents in the muscle of *P. semisulcatus* (6.19 µg/g) and in *L. vannamei* (30.37 µg/g) collected from the Gulf of Antalya/Turkey and Zhanjiang harbor Bay respectively were comparable with the present results [12]. The Cu contents in the muscle (dry weight) of *L. vannamei* was 923.2-63.4 µg/g collected from Jaguaribe estuarine basin in Northeastern Brazil were very high as compared to the present levels of Cu in the muscles (dry weight) of shrimps/prawns marketed in West Bengal [13]. But, the Cu concentration in the muscle of *L. vannamei* (24.1 to 38.5 µg/g) collected from NE Brazil as reported by Lopes and Lacarda was close proximity to the present results other species of shrimp/prawns [14]. It was observed that a large amount of Cu was accumulated in offal (head and shell) of all the shell-fish species. A similar result was observed by other researchers in Kolkata also [7]. Therefore, head and shell-less shrimps/prawns are more hygienic with respect to Cu accumulation and may be recommended for regular consumption to avoid the maximum Cu entry in the human body. However, still, the levels of Cu accumulation in the meat of shell-fish species marketed in West Bengal fish markets are in danger levels.

4.2 Estimated Weekly Intake (EWI) (µg/kg/w)

The maximum EWI of Cu in females was 4.61 µg/kg/w and the minimum value was 0.42 µg/kg/w. Whereas, in males, the maximum EWI of Cu was 4.05 µg/kg/w and the minimum were 0.37 µg/kg/w. 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 marketed in greater Kolkata are safe for consumption. But, the results also aware us about the possible threats of metal pollution in the near future.

5. Conclusion

The mean concentration of Cu in the meat, offal and whole body of giant freshwater prawn (*Macrobrachium rosenbergii*) collected from Garia, Sealdah and Sonarpur fish markets in Kolkata from January 2016 to June 2016 was 17.74 ± 9.00 µg/g, 32.70 ± 19.26 µg/g and 50.44 ± 27.00 µg/g respectively. The maximum Cu contents were 33.12 µg/g, 63.43 µg/g and 93.01 µg/g. The maximum EWI reported in female and male were 4.61 µg/kg/w and 4.05 µg/kg/w. All these values are below the permissible limits or sometimes close to it. But we can't ignore the ever-increasing problem of heavy metal pollution in our environment. The more we are approaching towards modernization, the more we are becoming susceptible towards pollution hazards. We can reduce the hazard in two ways; firstly being careful during the preparation of fish meal secondly and most importantly reducing the release of contaminants into the ecosystem. Unless and until we take necessary steps towards a clean and green environment we can't offer a habitable World for the next generation.

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