

The concentration of mercury in edible oysters *Saccostrea cucullata* within Indian Sundarbans estuarine region

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ABSTRACT

We analyzed mercury (Hg) concentrations in ambient water and muscle tissue of dominant edible oyster *Saccostrea cucullata* in 3 stations located in the western sector of Indian Sundarbans estuarine region for 3 seasons namely- premonsoon, monsoon and postmonsoon during 2017. Analyses were done by standard procedures using Inductively Coupled Plasma- Mass Spectrometer (ICP-MS). Significant Spatio-temporal variations of mercury content of the water and that of the muscle tissue were observed ($p < 0.01$). Significant positive value of correlation coefficient (r) indicates a strong direct relationship between dissolved and muscle metal. The reason for high concentrations of heavy metal in oyster muscle sampled from the stations in the western sector of Indian Sundarbans estuarine region may be the hyposaline aquatic media and growing trend of industrialization along the banks of River Hooghly.

Keywords: *Saccostrea cucullata*, mercury, Inductively Coupled Mass Spectrometer, Indian Sundarbans

INTRODUCTION

The coastal zone receives a large amount of heavy metal pollution from agricultural and industrial activities [1]. Due to dynamic nature of the marine and estuarine system these materials assimilate into the sediment matrix rapidly by several natural processes namely- dilution, dispersal, oxidation and sequestration. However, the capacity for such assimilation is limited. There is a general concern about the impact of metals in the aquatic environment [2]. The contamination of the aquatic environment, however, has been occurring for centuries, but its extent has increased markedly in the last fifty years due to technological developments and increased consumer use of materials containing these metals. Various routes are there for the entry of heavy metals into the aquatic environment including atmospheric deposition, erosion of geological matrix or anthropogenic activities caused by industrial effluents, domestic sewage, nuclear testing and mining wastes [3]. Oyster *S. cucullata* is one of the most important economically important shellfish in the Southeast Asia. Oysters are used in making very popular seafood products. The culture of oyster holds some good potential for aquaculture in India and it is used as a potential species in aquaculture practices.

Oysters have been discovered in the present sampling site (western sectors of the Indian Sundarbans). Their feeding behavior leads to a higher accumulation of toxic metals in their body parts which are biomagnified through the food chain. The present research was conducted to examine the bioaccumulation level of Hg, a very toxic heavy metal, in the muscle of dominant edible oyster (*S. cucullata*) sampled from 3 different stations of the deltaic Sundarbans (two in each of the western sector) which is of utmost importance in this sector of the biotic community.

MATERIALS AND METHODS

Selection of study site

A delta complex, Indian Sundarbans is situated at the confluence of the River Hooghly and the Bay of Bengal. Because of the presence of a rich gene pool, this deltaic complex has been declared as the Biosphere Reserve. The Sundarban Biosphere Reserve (SBR) holds an area of 9630 sq. km and houses some 102 islands. The western sector of this delta complex receives the snowmelt water coming from the Himalayan glaciers after being regulated through several barrages on its way. The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the downstream

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zone. On this background, three sampling stations (in and around western Indian Sundarbans) were selected (Table 1) to present the picture of selected heavy metals for three distinguished

seasons (premonsoon, monsoon and postmonsoon) in 2017 in the estuarine ambient water and muscle tissue of the edible oyster- *Saccostrea cucullata*.

Table1. Selected stations, their co-ordinates and major anthropogenic activities observed

Name of stations	Latitude	Longitude	Major anthropogenic activities
Sagar island (Station1)	21°39'04" N	88°01'47" E	1. Pilgrims come during 'Kumbh mela' every year 2. Unplanned tourism 3. Navigational channel
Frasergunj (Station2)	21° 33' 47.76" N	88°15' 33.98" E	1. Fish landing station 2. Unplanned tourism 3. Passenger vessel jetties 4. Repairing and conditioning of boats and fishing vessels 5. Marketing of fish and forestry related products
Kakdwp (Station 3)	21°52'06"N	88°11'12"E	1. Fish landing station 2. Passenger vessels and jetties 3. Aquacultural farms

Collection of ambient water and analysis of dissolved Hg

Surface water samples were collected by the help of 10-1 Teflon-lined GO-FLO bottles fitted with Teflon taps and employed on a rosette or on Kevlar line. Additional surface sampling was carried out by hand. Just after collection, the samples were filtered through Nuclepore filters (0.4 µm pore diameter) and brought to the laboratory. In the laboratory, aliquots of the filters were acidified by virtue of the sub-boiling distilled nitric acid to a pH of about 2. Then the treated sample was stored in cleaned low density polyethylene bottles. Heavy metals in the ambient water were separated and pre-concentrated from the sample water by virtue of dithiocarbamate complexation and subsequent extraction into Freon TF, followed by back extraction into HNO₃. Extracts were analyzed for mercury by Inductively Coupled Plasma Mass Spectrometer.

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of biological sample types [4]. A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for analysis of selected heavy metals in the crab muscle. A standard torch for this instrument was used provided with an outer argon gas flow at the rate of 15 L/min and with intermediate gas flow rate of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 mL/min.

For the digestion of biological samples collected, a Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 Watt maximum power Polytetrafluoroethylene (PTFE) reactor of 115 ml volume with wall thickness of about 1 cm additionally provided with hermetic screw caps, was used. Reagents used in this research, were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glassware were dipped in 10% (v/v) HNO₃ for 24 hours and then washed with deionised water before use.

Collection of oyster (*S. cucullata*) specimen and analysis of Hg in the muscle tissue

Oyster samples were collected manually carefully during high tide condition from the selected stations (Table 1) during premonsoon, monsoon and postmonsoon in 2017. From each station twenty specimens were collected and specimens of this species of similar size were sorted out. The collected and separated oysters were placed in polyethylene containers and transferred to the laboratory using ice boxes for further analysis. Before analysis these samples were cleaned with double distilled water. 2 gm soft muscle tissues from each specimen were scooped out for analysis of Hg in order to get a representative picture of bioaccumulation for the stations.

The analyses were carried out on composite samples of twenty specimens of oyster having uniform size. This is a measure to reduce possible variations in metal concentrations due

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to size and age. 20 mg composite sample from the collected oyster specimens were weighed after overnight oven drying at 60°C. Then it was treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H₂O₂ successively in a hermetically sealed PTFE reactor, inside a microwave oven, with power levels ranging between 330 – 550 Watt, for 12 min to obtain a clear solution. The use of microwave-assisted digestion appears to be very relevant for sample dissolution, especially because it is very fast [5-7]. After digestion, 4 ml H₂BO₃ was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. The blank sample was prepared replacing the biological samples with double distilled water and following all the treatment steps mentioned. The final volume was made up to 50 ml. Finally, the sample and blank solutions were analyzed by ICP-MS. All analyses were done in triplicate and the mean results were expressed.

Statistical analysis

Analysis of Variance (ANOVA) was done to assess the significance of variations in heavy metal concentration in ambient water and also in oyster muscle tissue. Analysis of Pearson's correlation coefficient (r) was also done to determine the degree and magnitude of relationship between oyster muscle metal and dissolved heavy metal. All statistical analyses were carried out by SPSS 16.0.

RESULTS

Mercury concentrations in 3 stations in ambient water and oyster muscle tissue in western sector of Indian Sundarbans are presented in Figures 1 and 2. A unique seasonal variation of dissolved Hg was observed with highest value in monsoon followed by postmonsoon and premonsoon. This trend is also same for oyster muscle metal.

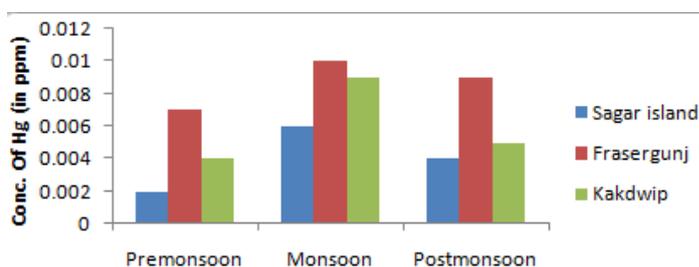


Fig1. Concentrations of Hg in oyster muscle in selected stations throughout the study period

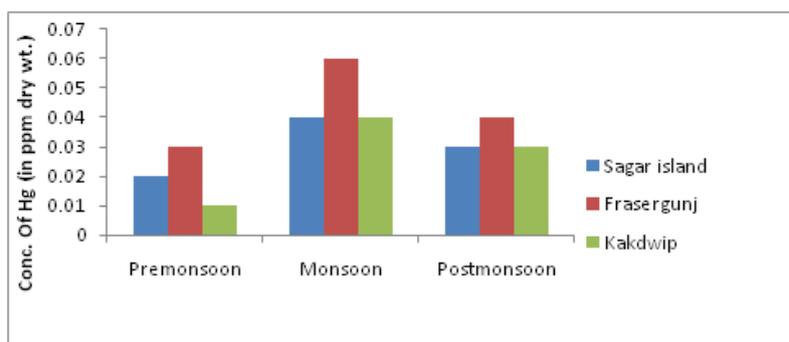


Fig2. Concentrations of Hg in oyster muscle in selected stations throughout the study period

ANOVA results shows significant variations ($p < 0.01$) of both dissolved Hg and oyster muscle Hg concentrations between seasons and also between the stations (Table 2). Strong correlation co-efficient (r) are also observed between oyster muscle metal and metal concentration in ambient media (Table 3).

Table3. ANOVA results showing variations of dissolved Hg and oyster muscle Hg between stations and between seasons

Factors	Combinations	F _{cal}	F _{crit}
Dissolved Hg	Between seasons	26.90909	6.944272
	Between stations	19.81818	6.944272
Oyster muscle tissue Hg	Between seasons	14.217721	6.944672
	Between stations	32.198229	6.944272

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Table 4. Inter-relationship between oyster muscle tissue Hg and Hg in ambient media

Season	Combination	r-value at selected stations			p-value (for 3 stations)
		Sagar island	Frasergunj	Kakdwip	
Premonsoon	Tissue Hg × Dissolved Hg	0.596	0.654	0.623	< 0.01
Monsoon	Tissue Hg × Dissolved Hg	0.693	0.765	0.588	< 0.01
Postmonsoon	Tissue Hg × Dissolved Hg	0.981	0.784	0.873	< 0.01

DISCUSSION

Mercury is one of the global pollutants and it cycles between air, water, sediments, soil and organisms in various forms [8]. Hg is a toxic metal causing serious human health problems like mental retardation, nervous breakdown, coronary heart disease and even cancer [9]. The presence of mercury in aquatic systems is of great concern due to its bioaccumulation and biomagnification property through all trophic levels of the aquatic food chain [10]. Riverine drainage is the main source of metal contamination in coastal areas [11].

Among the stations selected, station 2 showed the highest concentrations of Hg followed by station 1 and station 3 throughout the seasons. The higher concentration of heavy metals in oysters in station 2 was due to more anthropogenic activities leading to pollution. The higher concentrations of Hg in the oysters prove that the bioavailability of these heavy metals has been increased during the last century due to urbanization and industrialization [12]. The rapid trend of industrialization and urbanization in cities like Kolkata, Howrah and Haldia port-cum-industrial complex in the maritime state of West Bengal has caused considerable ecological

Table 4. Comparative analysis of maximum permissible limit (MPL) of Hg in seafood and concentration of Hg found in oyster muscle (in ppm dry wt.) in this study

MPL as per WHO [14]	MPL as per FSANZ [15]	MPL as per USEPA [16]	Concentration range of Hg found in this study
0.5	0.5	0.5	0.1-0.6

Oysters are one of the major ingredients in seafoods in various countries this observation is alarming as Hg if ingested, can cause serious physiological and mental problems to human after consumption via sea-food. Hence, proper monitoring and guidelines regarding dumping of electronic wastes and setting up of electronic industries near coastal cities are essentially needed. Culturing this biomonitor species in controlled inland conditions may be a solution

imbalance in the adjacent coastal areas [11]. In the lower part of the Hooghly estuary, different industries namely- paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tires and cycle rims industries are running with their full capacities [13]. These units are the principal sources of heavy metals found in the water.

The level of Hg found in this research work in oyster muscle is higher than the permissible limit in case of station 2 during monsoon season (Table 4). This may result from higher rate of precipitation induced change in pH of ambient media resulting in dissolution of sediment metal into the water compartment [11]. In premonsoon and postmonsoon season, the ambient water physico-chemical condition is reversed and results in lowering of Hg concentration in ambient water. In other stations, the concentrations of Hg were found below the permissible limit throughout the seasons. The significant variations of Hg concentrations between seasons may be attributed to dynamic nature of the selected ecosystem whereas, that between the stations may be the result of difference in physico-chemical parameters of environmental conditions like- surface water salinity, pH of ambient media, surface water temperature etc.

to this problem though long term monitoring on this idea is needed.

The Govt. and NGOs can further investigate upon this observation so that the detrimental effect of Hg on human health at the baseline can be checked.

REFERENCES

- [1] Usero J, Morillo J and Gracia I. Heavy metal concentrations in mollusks from the Atlantic coast

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- of southern Spain. *Chemosphere*.2005; 59: 1175-1181.
- [2] Grosell M and Brix KV. Introduction to the special issue on mechanisms in metal toxicology. *Aquatic Toxicology*. 2005; 72: 3-4.
- [3] Reddy M S, Mehta B, Dave S, Joshi M, Karthikeyan L, Sarma V K S, Basha S and Bhatt P. Bioaccumulation of heavy metals in some commercial fishes and crabs of the Gulf of Cambay, India. *Current Science*. 2007; 92: 1489-1491.
- [4] Date AR and Gray AL (Eds.). *Applications of Inductively Coupled Plasma Source Mass Spectrometry*, Blackie, Glasgow. 1988.
- [5] Nadkarni, R. A. Applications of microwave oven sample dissolution in analysis, *Analytical Chemistry*. 1984; 56: 22-33.
- [6] Matusiewicz, H. and Sturgeon, R. E. Present status of microwave sample dissolution and decomposition for elemental analysis. *Progresses in Analytical Atomic Spectroscopy*. 1989; 12: 21.
- [7] De la Guardia, M. (Ed.), *Empleo de los Hornos de Microondas en Química*, University of Valencia, Spain. Enoyer, E.R. Semi-quantitative analysis of environmental materials by lasersampling inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectroscopy*. 1990; 7: 11-87.
- [8] Moreno Fabio N, Anderson Chris WN, Stewart Robert B and Robinson Brett H. Mercury volatilization and phytoextraction from base-metal mine tailings. *Environmental Pollution*. 2005; 136: 341-352.
- [9] Horowitz Y, Greenberg D, Ling G and Lifshitz M. Acrodynia: a case report of two siblings. *Archives of diseases in Childhood*. 2002; 86 (6): 453. doi:10.1136/adc.86.6.453
- [10] Lindqvist Oliver, Johansson Kjell, Aastrup Mats, Andersson Arne, Bringmark Lage and Birgitta Tim. Mercury in the Swedish environment- Recent research on causes, consequences, and corrective methods: *Water Air Soil Pollution*. 1991: p. 55.
- [11] Mitra, A. Status of coastal pollution in West Bengal with special reference to heavy metals. *Journal of Indian Ocean Studies*. 1998; 5(2): 135-138.
- [12] Cheung YH, Wong MH. Trace metal contents of the Pacific oyster (*Crassostrea gigas*) purchased from markets in Hong Kong. *Environmental Management*.1992; 16:753-761.
- [13] United Nations Environment Programme (UNEP). *Pollution and the marine environment in the Indian Ocean*. UNEP Regional Seas Programme Activity Centre, Geneva, Switzerland, 1982.
- [14] World Health Organization (WHO) *Heavy metals environmental aspects*, Environmental Health Criteria. 1989. No. 85, Geneva, Switzerland.
- [15] Food Standard Code of Australia and New Zealand (FSANZ), Standard 1.4.1.: Contaminants and natural toxicants, 2011 (Website address: <https://www.legislation.gov.au/Details/F2011C00542>; Access date: 03/31/2013).
- [1] United States Environmental Protection Agency (USEPA). *Guidelines for ecological risk assessment*. EPA 630-R-95-002 F; 1998. Washington, D.C