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Concentration of copper in gills and muscles of six species of shellfish from Pulicat Lake, Tamil Nadu, India

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Abstract
Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystem due to their toxicity and can enter and contaminate estuarine waters. The contamination by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns. In the present study, the concentration of copper in the gills and muscles of six species of shellfish *viz*., *Fenneropenaeus indicus, Fenneropenaeus monodon, Fenneropenaeus semisulcatus*, *Scylla serrata*, *Meretrix casta* and *Clibanarius longitarsus* from Pulicat lake, Tamil Nadu, India on a monthly basis for a period of two years from January 2011 to December 2012 during four seasons *viz*., postmonsoon, summer, premonsoon and monsoon were analysed. The concentration of copper in the gills and muscles of the six shellfish species exhibited seasonal as well as species specific variations. The overall results indicate the muscle tissues of *Scylla serrata* to accumulate the maximum amount of copper (9.36µg/g) during postmonsoon in 2011. In conclusion, the results of the present study has revealed that the concentration of copper in the gills and muscles of the six shellfish species studies were within the safe limits, further contamination of this important water body should be prevented by continued biomonitoring studies to protect the shellfish and its consumers.

Keywords: heavy metals, copper, pollution, shellfish, Pulicat lake

1. Introduction

Estuaries have been the focal point of maritime studies and activities. As they are semi-enclosed, they provide natural harbour for trade and commerce. They are also effective nutrient traps and provide a vital source of natural resources to man and are used for commercial, industrial and recreational purposes. Biodiversity in this ecosystem is very impressive. They are the best settling places for clams and oysters besides acting as nursery ground for a variety of shrimps and finfishes. Estuaries are in a state of constant flux and such a dynamic environment provides many ecological niches for diverse biota [1]. Pollution of marine environment by heavy metals has become a national and international problem [2]. Most living organisms need small amount of essential metals *viz*., iron, manganese, copper and zinc for essential processes especially for growth [3, 4]. However, all these metals will produce harmful effects when certain limits are exceeded [5]. Metals are classified as pollutants only when added by man in quantities sufficient to produce deleterious effects [2]. Normally, such contaminants are transported from its sources through river system and deposited downstream [6]. Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystem due to their toxicity [7]. Metals can enter and contaminate estuarine waters from feeder rivers and from direct discharges, and once there, they can be trapped and accumulated in sediments [8] or be directly captured by living organisms [9]. Therefore, an estuary is a potential sink for these pollutants for a long period of time [6].

Estuaries often act as efficient reservoirs of river-borne and marine-derived pollutants [10]. Water bodies contaminated by heavy metals may lead to bioaccumulation in the food chain of an estuarine environment [6]. Metals may be present in the estuarine system as dissolved species, as free ions or forming organic complexes with humic and fulvic acids [11]. Contaminants can persist for many years in sediments in both freshwater and marine systems where they hold the potential to affect human health and the environment [12]. The contamination of natural waters by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns [13, 14]. The green revolution in India has increased the usage of pesticides, fungicides and weedicides to the maximum and these chemicals containing heavy metals are profoundly leached from the agricultural fields and drain into the sea through river and estuary [15]. Different animals in the same community at the same trophic level could accumulate pollutants differently due to differences in habitat/ niche's physical and chemical properties [16]. Factors known to influence metal concentrations and accumulation in these organisms include metal bioavailability, season of sampling, hydrodynamics of the environment, size, sex and changes in tissue composition and reproductive cycle [17]. Due to their potentially detrimental effects on human health, the presence of heavy metals can limit the quantity of bivalves humans can consume [16]. Monitoring programmes and research on heavy metals in aquatic environment samples have become widely important due to concerns over accumulation and toxic effects in aquatic organisms and to humans through the food chain [18]. Therefore, in the present study, the concentration of copper in the gills and muscles of six species of shellfish from Pulicat Lake, Tamil Nadu, India were analysed.

2. Materials and methods

2.1 Study area

Pulicat lake (13°24'–13°47' N, 80°03'–80°18' E) is the second largest brackish water body of India with an area of 18,440 hectares and is located 40km north of Chennai. The length of this lake is about 60km and varies in breadth (0.2 to 17.5km). Pulicat lake is drained by four rivers, the Swarnamukhi, the Kalangi, the Araniar and the Royyala Kalava apart from many minor inflows. Industrial and domestic waste are brought into this lake by the Buckingham canal and finally to the Bay of Bengal [19]. Local climate, riverine inflow and the neritic waters from the Bay of Bengal influence the hydrological characters of Pulicat lake. Many euryhaline species are present in this lake which act as breeding grounds for many organisms and certain fishes [20]. Untreated effluents from industries and urban areas are considered to be point sources of pollution [19, 21, 22].

2.2 Collection of specimens

Six shellfish spe*cies viz.*, *Fenneropenaeus indicus*, *Fenneropenaeus monodon*, *Fenneropenaeus semisulcatus*, *Scylla serrata*, *Meretrix casta* and *Clibanarius longitarsus* were collected from Pulicat lake, Tamil Nadu, India on a monthly basis for a period of two years from January 2011 to December 2012 during four seasons *viz*., postmonsoon, summer, premonsoon and monsoon. The collected organisms were brought to the laboratory in an ice box and were stored at 4°C until analyses. The organisms were thoroughly washed with running tap water to eliminate mud and other debris and were subsequently rinsed with double-distilled water. Rust free stainless steel kit was used to dissect the animal. Care was taken to avoid external contamination of the samples.

2.3 Determination of metals in animals

The gills and muscles of the shellfish species were used to estimate copper content. The analysis was carried out using the method suggested by Watling and Emmerson [23]. Analytical grade reagents were used. For analysing copper, the samples were oven dried at 60°C for 24 hours. The dried sample (0.5g) was taken and ground with a mortar and pestle. Using nitric and perchloric acid (3:1), the ground samples were digested. After adding the acids, the samples were kept in a hot plate at 120°C until white residues were formed. Finally the residue was dissolved in 10mL of distilled water and then filtered. The filtered sample was aspirated into the atomic absorption spectrophotometer and the reading was recorded. The solution was then diluted and filtered through a 0.45µm nitrocellulose membrane filter. Determination of copper in samples was carried out by inductively coupled plasma atomic emission spectroscopy (Optima 2100 DV, Perkin-Elmer, USA).

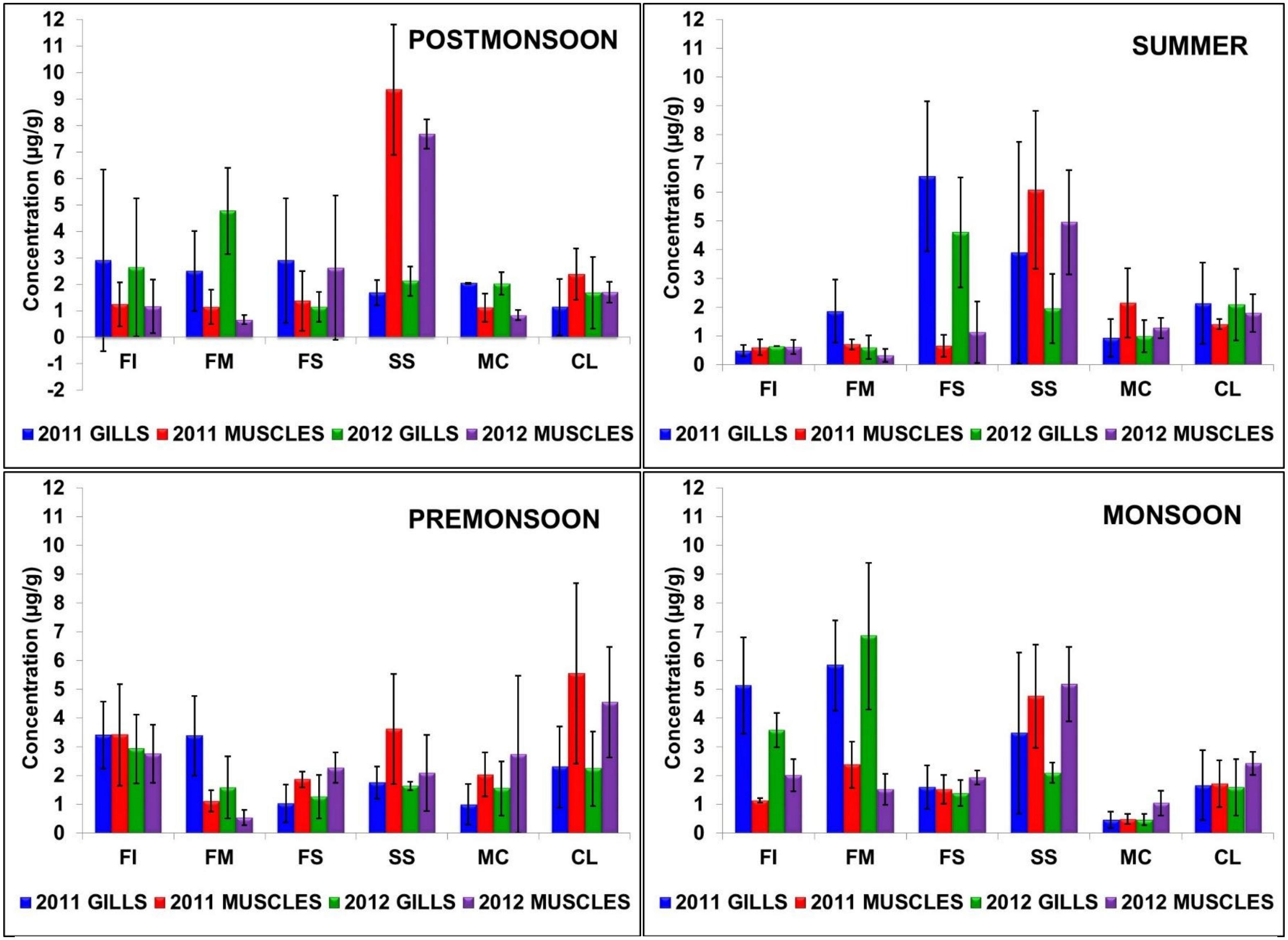
3. Results

During postmonsoon, amongst the six shellfish species studied, *Fenneropenaeus monodon* accumulated the highest amount of copper in their gills in 2012 followed by *Fenneropenaeus indicus* in 2011 and their respective values were 4.78 and 2.90µg/g. On the other hand, *Scylla serrata* accumulated the highest amount of copper in their muscles in the year 2011 and 2012 and the values were 9.36 and 7.68µg/g respectively. In summer, *Fenneropenaeus semisulcatus* had the highest accumulation of copper in their gills in 2011 and 2012 with 6.55 and 4.61µg/g as respective values. Whereas, *Scylla serrata* accumulated 60.8 and 4.96µg/g copper respectively in its muscles during the two years. In premonsoon, *Fenneropenaeus indicus* accumulated the highest amount of copper of 3.40 and 2.93µg/g in 2011 and 2012 respectively and *Clibanarius longitarsus* accumulated the highest copper amount of 5.55 and 4.50µg/g in 2011 and 2012 respectively. In monsoon, accumulation of copper was maximum in *Fenneropenaeus monodon* with 6.85µg/g in 2012 and 5.83µg/g in 2011, and *Scylla serrata* exhibited maximum accumulation of copper in 2012 and 2011 with values of 5.18 and 4.76µg/g respectively. The overall results indicate the muscle tissues of *Scylla serrata* to accumulate the maximum amount of copper (9.36µg/g) during postmonsoon in 2011 (Figure 1).

4. Discussion

Copper is present abundantly in the environment and is an essential micronutrient for the normal growth and metabolism of all living organisms. The United States is the major world producer and consumer of copper and its compounds. Copper release to the global biosphere may approach 1.8 million metric tons per year mostly from anthropogenic activities [24]. Heavy metals mainly accumulate in metabolically active tissues like the hepatopancreas and gill tissues resulting in the uptake and storage of heavy metals [25]. Inputs of copper into aquatic ecosystems have increased sharply during the past century due to a number of reasons including atmospheric fallout from industrial activities, waste and industrial discharges, and of antifouling marine paints and wood preservatives. Copper is elevated in sediments of many marinas, probably as a result of copper containing antifouling paints used on boats. Copper is among the most toxic of the heavy metals in freshwater and marine biota. Excess copper can cause cellular damage by generating oxygen free radicals and inactivating biological thiols into disulfides. Copper and zinc function as micronutrients, but when in excess even these essential elements can cause ecotoxicological effects [24].

Among marine organisms, the highest accumulations are generally found in molluscan tissues and soft parts, especially those of cephalopods and oysters. The average concentrations of the heavy metals exhibited the following decreasing order: Zn > Cu > As > Cd > Pb > Hg as determined in soft tissues of different shellfish from Zhejiang coastal waters, East China sea [25]. The hepatopancreas contained higher levels of Zn, Cu, Fe and Cd followed by the gills in both species in every season. However, the highest metal levels were observed in the gills in both species. Compared with the other tissues examined, the muscle tissues of both species contained the



FI: *Fenneropenaeus indicus*
FM: *Fenneropenaeus monodon*
FS: *Fenneropenaeus semisulcatus*
SS: *Scylla serrata*
MC: *Meretrix casta*
CL: *Clibanarius longitarsus*

Fig 1: Accumulation of copper in the gills and muscles of shellfish species in Pulicat lake lowest levels of all metals. The high levels of this metal may be related with essential role of this metal in the production of the respiratory protein, haemocyanin [26-28]. Essential metals like zinc, copper and manganese are regulated in crustaceans whereas non-essential metals like cadmium appears not to be regulated [29]. The studies on copper and zinc in *Crongon crongon* [30], *Palaemonetes varians* [31] and *Homarus americanus* [32] indicate a marked ability on the part of crustaceans to regulate internal copper and zinc concentrations. Crustaceans respond to heavy metal exposure by producing metallothionein, particularly in hepatopancreas [33-35]. High level of metal found in the hepatopancreas of *Penaeus semisulcatus* and *Metapenaeus monoceros* is possibly due to binding of the metals to metallothionein proteins. The gill tissue is where active and passive exchanges occurs between the animal and aquatic environment. High levels of metals accumulate in the gill tissues by absorption and adsorption [36]. The lowest levels of metals was observed in the muscle tissues of both species in every season. Similar results were reported for a number of shrimp species [33, 37, 38]. There were significant differences in metal levels in the tissues of *Penaeus semiculatus* and *Metapenaeus monoceros* in every season which may be attributable to many factors like feeding habits, metabolic activity and behaviour of the two species. Similar findings were reported by Darmono and Denton [38] in the tissues of *Penaeus merguiensis* and *Penaeus monodon*.

Marine bivalves *viz*., oysters and mussels have been extensively used as model organisms in environmental studies of water quality. Marine bivalves are filter feeders that take up and accumulate metals and other pollutants from the water column or via ingestion of contaminants adsorbed to phytoplankton, detritus and sediment particles. As they are sessile, they reflect local contaminant concentrations more accurately than crustaceans and free-swimming finfish. Trace metals are taken up and accumulated by oysters and many other marine invertebrates to tissue and body concentrations usually much higher on a wet weight basis than concentrations in the surrounding seawater [24]. Huang *et al*. [25] have observed that the levels of all metals were significantly higher in tissues in the summer when compared with other seasons in clams and oysters. This is the probably due to the use of synthetic fertilizers, pesticides and the greater human activity in the gulf during summer. Alliot and Frenet-Piron [39] found that metal levels increased in water during summer and also in shrimps. Each metal presents a peak of pollution in water every year in July and August, after which the metal level decreased during winter, going back to the normal level.

Copper is one of the several heavy metals that are essential to life despite being as inherently toxic as non-essential heavy metals exemplified by lead and mercury [40]. Plants and animals rapidly accumulate it. It is toxic at very low concentration in water and is known to cause brain damage in mammals [41]. The sources of copper includes copper cookware, copper IUDs, copper pipes, dental alloys, fungicides, industrial emissions, insecticides, welding, lobster, oysters, perch, shellfish, etc. The natural inputs of copper to the marine environment are from erosion of mineralized rocks. Anthropogenic inputs of copper are from the production of electrical equipments, as chemical catalysts, antifouling agents in paints, algicides, in alloys, and as wood preservatives. Copper dissolved in seawater is chiefly in the form of $CuCO_3$ or in reduced salinity as $CuOH^+$. It also forms complexes with organic molecules. Molluscs have a tremendous capacity to accumulate copper from contaminated waters. Reports revealed that the copper concentration factor for oysters growing in contaminated waters was 7500 and may accumulate 2000ppm of copper in their blood [42]. Acute symptoms of copper poisoning in man include vomiting, hematemesis, hypotension, melena, coma, jaundice and gastrointestinal distress. Chronic effects of copper exposure can damage the liver and kidneys [43]. In conclusion, the results of the present study has revealed that the accumulation of copper in the gills and muscles of the six shellfish species studies were within the safe limits, further contamination of this important water body should be prevented by continued biomonitoring studies to protect the shellfish and its consumers. Stringent control measures are required to prevent further pollution of Pulicat lake.

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