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Bioaccumulation of lead in gills and muscles of shellfish species from Pulicat lake, Tamil Nadu, India

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Abstract
Heavy metals are one of the most serious pollutants in the environment due to their toxicity, persistence and ability to concentrate along the food chain. Water bodies contaminated by heavy metals may lead to bioaccumulation in the food chain of an estuarine environment. Contamination of natural waters by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns. In the present study, six species of shellfish, *Fenneropenaeus indicus, Fenneropenaeus monodon, Fenneropenaeus semisulcatus, Scylla serrata, Clibanarius longitarsus* and *Meretrix casta* in Pulicat lake, Tamil Nadu, India were analysed for the presence of lead in its gills and muscles from January 2011 to December 2012. The results showed seasonal variations in the uptake of lead. The highest concentration of lead was recorded in the gills of *Meretrix casta* (3.12µg/g) in the summer of 2012 and in the muscles of *Fenneropenaeus indicus* (2.15µg/g) during the premonsoon of 2011. In conclusion, the results of the present study indicated a mild to moderate accumulation of lead in the gills and muscles of shellfish species. *Meretrix casta* and *Fenneropenaeus indicus* showed a tendency to accumulate higher levels of lead than other shellfish species studied. At this rate, consumption of shellfish meat from Pulicat lake may lead to toxicity of man and other animals. Control measures are required to reduce lead pollution in Pulicat lake.

Keywords: heavy metals, lead, pollution, Pulicat Lake

1. Introduction
The coastal zone constitutes about ten per cent of the oceanic area on the earth's surface and it not only accounts for more than half of ocean's biological productivity, but also for the most vulnerable and abused zone. In Southeast Asia, which includes the Indian subcontinent, about seventy five per cent of the population live along the coastline with population density reaching upto 500 per sq.km. An estuary is an integral part of the coastal environment and is the outfall region of the river, making the transitional zone between the fluvial and marine environs [1]. According to Prichard [2], "an estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which the sea water is measurably diluted with the fresh water derived from land drainage". An estuary is a primary habitat and spawning area for many species since it is highly productive [3]. They are the best settling places for clams and oysters besides acting as a nursery ground for a variety of shrimps, finfishes and other marine organisms. Advancement in technology as well as increase in population have led to environmental concerns relating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste and crude oil spills replete with most common heavy metals in our environment. Industrial activity has led to very high heavy metal concentrations in the environment, which are in general 100–1000 fold higher than those in the earth's crust, and locally living organisms can be exposed to even higher levels. The health status and the biological diversity of the Indian estuarine ecosystems are deteriorating day by day through anthropogenic activities and, dumping of enormous quantities of sewage into the estuary has drastically reduced the population of the aquatic biota. It has also caused considerable ecological imbalance and resulted in the large-scale disappearance of their flora and fauna. Further, introduction of untreated municipal waste-water and industrial effluents into these aquatic bodies has led to serious water pollution including heavy metal pollution, which gets biomagnified and reaches man through food chain implications.

Many metals have a wide range of uses, but these have come at a significant environmental price, having serious negative environmental consequences, yet human dependence on them continues to result in large inputs into the environment [4]. The contamination of natural waters by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns [5, 6]. Heavy metal may form organic complexes with fulvic and humic acids. They may also occur as free ions or as dissolved species. The phenomenon of co-precipitation or adsorption of lead with sulphides, carbonates, oxyhydroxides and minerals in clay may also occur [7]. The organic and inorganic chemicals present in the estuary are either essential or nonessential. The nonessential metals are considered highly toxic to the biota at high concentrations. Along these lines, aquatic ecosystems and organisms are directly exposed to a great variety of these metals whose chemical forms and concentrations are governed by different processes, whether natural or not [8]. Their negative effect is evident, not only by decreasing aquatic species diversity but by also exposing human beings to these xenobiotics directly through the food chain, with a potential danger to human health. Heavy metals are one of the most serious pollutants in the environment due to their toxicity, persistence and ability to concentrate along the food chain. Water bodies contaminated by heavy metals may lead to bioaccumulation in the food chain of an estuarine environment. Normally, such contaminants are transported from its sources through river system and deposited downstream. Since most of the pollutants gets mixed and becomes suspended as solid and bottom sediment through sedimentation, the estuary becomes a potential sink for these pollutants for a long period of time [9]. Therefore, in the present study, the concentration of the trace metal lead was evaluated in the muscle and gill tissues of six species of shellfishes from the estuarine Pulicat lake, Tamil Nadu, India.

2. Materials and methods

2.1. Study area

Pulicat lake (latitude 13˚24' and 13˚43'N and longitude 80˚03' and 80˚18'E) is the second largest estuarine lake present in the east coast of India situated about forty kilometre from the metropolitan city of Chennai. The length of this lake is about 60 km and its breadth varies from 0.2 to 17.5 km. The Sriharikota island is present as an inland split which separates this lake from the Bay of Bengal. The main source of freshwater is land runoff through four rivers, the Swarnamukhi, the Kalangi, the Araniar and the Royyala Kalava apart from many minor inflows. The Buckingham canal, which runs parallel to the Bay of Bengal, brings in the industrial and domestic wastes to the lake and eventually to the Bay of Bengal [10]. The hydrology of the Pulicat lake is influenced by local climate, the regime of the inflowing rivers, the Buckingham canal that enters the lake, in addition to the effect of the neritic waters of the Bay of Bengal. Pulicat lake, harbours many euryhaline species and serves as a nursery for several marine species and very few secondary freshwater fishes [11]. The point sources of pollution mainly come from nearby industries and untreated urban wastes from Chennai metropolitan city [10, 12, 13].

2.2. Collection of specimens

Six shellfish species *viz*., *Fenneropenaeus indicus, Fenneropenaeus monodon, Fenneropenaeus semisulcatus, Scylla serrata, Clibanarius longitarsus* and *Meretrix casta* were collected from Pulicat lake, Tamil Nadu, India on a monthly basis for a period of two years from January 2011 to December 2012. The collected organisms were brought to the laboratory in an ice box and were stored at 4˚C until analyses. In the laboratory, the animals were cleansed to remove mud and debris and were subsequently washed 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 were used to estimate lead content. The analysis was carried out using the method suggested by Watling and Emmerson [14]. The reagents used were of analytical grade. For analysing lead, 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. The ground samples were digested with nitric acid and perchloric acid in the ratio of 3:1. After adding the acids, the samples were kept in the 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 (AAS) and the reading was recorded. The solution was then diluted and filtered through a 0.45µm nitrocellulose membrane filter. Determination of lead in samples was carried out by ICP-AES (Optima 2100 DV, Perkin-Elmer, USA).

3. Results

Highest concentration of lead was recorded in the gills of *Meretrix casta* in the post monsoon (2.04 and 2.09µg/g) and summer (1.50 and 3.12µg/g) of 2011 and 2012 respectively. During premonsoon, high concentration was recorded in *Fenneropenaeus indicus* in 2011 (2.06µg/g) and in *Meretrix casta* in 2012 (2.30µg/g). Whereas in 2011 and 2012 monsoon, *Fenneropenaeus indicus* indicated high content of nickel with values of 2.41 and 2.09µg/g respectively (Figure 1). In the case of muscles of shellfish species studied, high concentration of lead was recorded in *Fenneropenaeus monodon* (1.48µg/g) in 2011 and in *Meretrix casta* (1.52µg/g) during post monsoon. In summer, high content was exhibited by *Fenneropenaeus indicus* (0.86µg/g) in 2011 and *Fenneropenaeus semisulcatus* (1.17µg/g) in 2012. During premonsoon and monsoon, *Fenneropenaeus indicus* indicated high lead content of 2.15 and 1.22µg/g in 2011, and 1.70 and 1.96µg/g in 2012 respectively (Figure 2).

4. Discussion

Ecotoxicologists and environmental scientists use the term "heavy metals" to refer to metals that have caused environmental problems [15]. Ecotoxicologists classify heavy metals as essential, non-essential and borderline. Essential metals include Calcium (Ca), Magnesium (Mg), Manganese (Mn), Potassium (K), Sodium (Na) and Strontium (Sr). Cadmium (Cd), Copper (Cu), Mercury (Hg) and Silver (Ag) are non-essential whereas, the borderline metals are Zinc (Zn), Lead (Pb), Iron (Fe), Chromium (Cr), Cobalt (Co) Nickel (Ni), Arsenic (As), Vanadium (V) and Tin (Sn) [16]. The world-wide emissions of metals to the atmosphere (thousands of tons per year) by natural sources is estimated as: Ni: 26, Pb: 19, Cu: 19, As: 7.8, Zn: 4, Cd: 1.0, Se: 0.4. Whereas, from anthropogenic sources it was: Pb: 450, Zn: 320, Ni: 47, Cu: 56, As: 24, Cd: 7.5, Se: 1.1 It is obvious from these numbers that Pb, Zn, Ni and Cu are the most important metal pollutants from human activities [17]. Heavy metals are non-biodegradable and undergo a global eco-biological cycle in which natural waters serve as the main pathways [18]. Atmospheric and river inputs, dredging, direct discharges, industrial dumping and sewage sludge are some of the important contributors to metal pollution, which lead to the release of metals to the marine environment [19]. Some metals enter the sea from the atmosphere, e.g. natural inputs of metals, such as aluminium in wind-blowing dust of rocks and shales, and mercury from volcanic activity. Lead inputs in the atmosphere from industrial and vehicular exhaust are much greater than natural inputs. Some metals are deposited by gas exchange at the sea surface, by fallout of particles (dry deposition) or are scavenged from the air column by precipitation (rain) which is called wet deposition [20]. The metals which have been studied extensively in the last decades are: Cd, Hg, Zn, Cu, Ni, Cr, Pb, Co, V, Ti, Fe, Mn, Ag and Sn. Some metals that have received more attention are Hg, Cd and Pb because of their highly toxic properties and effects on the environment and living organisms [17].

The extent of accumulation of trace metals by organisms in different tissues is dependent on the route of entry, i.e., either from the surrounding medium, or in the form of food or chemical form of material available in the media [21, 22]. The effect of heavy metals on aquatic organisms can be divided into direct and indirect effects. The latter is effected through the effect on food chain organisms and ecological stress. The direct effects are seen in behaviour, migration, physiology, metabolism, reproduction, development and growth of aquatic animals [23]. Metal concentrations in organisms at the same location differ between different species and individuals due to species specific ability/capacity to regulate or accumulate metals [24, 25]. Factors known to influence metal concentrations and accumulation in these organisms include metal bioavailability, season of sampling, hydrodynamics of the environment, size, sex, changes in tissue composition and reproductive cycle [26]. Heavy metal accumulation in bivalves is influenced by several factors [27]. Some of these include seasonality [28], location [29], salinity [30], organic matter [31], sex [32], food acquisition capability [33], stage of gonadal development [34] and size-weight relationships [35, 36]. Studies assume that a simple linear relation exists between metal concentration in water and in marine organisms. This may not necessarily be true. The net accumulation of any metals taken up will depend for example on the relative rates on metal excretion and storage of metal in detoxified form. It is, therefore, inappropriate to assume that a given species will accurately reflect the ambient concentrations of pollutants in its environment without regulating the amounts accumulated [37]. 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 [25].

Invertebrates are considered excellent indicator organisms because of their ability in concentrating metals, among other pollutants [23]. Organism can accumulate heavy metals in their tissues at concentrations in excess of the ambient water [38]. In comparison to fish and crustacea, bivalves have a very low level of activity of enzyme systems capable of metabolizing Persistent Organic Pollutants (POPs), such as aromatic hydrocarbons and polychlorinated biphenyls. Therefore, contaminants concentrations in the tissues of bivalves more accurately reflect the magnitude of environmental contamination [39, 40]. Heavy metals may be accumulated by shrimps either through food or water. The more important route of heavy metal concentration in the marine biota is through water [22]. Bivalves can accumulate these heavy metals in their tissues at concentrations greater than the ambient water and pose a health threat to humans who consume them [41]. The importance of marine shrimp for environmental monitoring studies as bio-indicators of heavy metal pollution has been emphasized by several investigators [42-46]. Shellfish and shellfish products can be used for monitoring potential risk to humans since they are directly consumed by a large population [47]. Of all the possible biomonitors available for monitoring aquatic environments, bivalves fulfil most of the above-mentioned characteristics. This is because they are widely distributed globally, easy to handle and sessile [7]. The hepatopancreas and gills are the two main routes of metal accumulation in crustaceans [48-50]. Since crustaceans are filter feeders, they tend to accumulate high amounts of metals without appreciable metabolism [51-53]. Major amount of metals enter through the gills by its mucous layer which has carrier in their cell wall [54]. The amount of metals present in the gills generally represents the metal content of the ambient water [55]. The concentration of metals in muscle is important because it constitutes the greatest mass of the crab and shrimp that is consumed. It was also possible that periodic moulting could have helped the shrimp in eliminating lead and cadmium levels from the exoskeleton or less availability of food sometimes might have led to uptake of heavy metal through drinking of water [56]. In general, lead was found to be the highly accumulated metal and copper was the least accumulated metal in both crab and shrimps [10]. Previous studies, indicated high concentration of lead in fish, mullet and oyster collected from the Pulicat lake, Tamil Nadu, India [10, 57, 58]. The adjusted Average Daily Intake (ADI) of lead through crustacean consumption for Asian population is 12.86μg/person/day [59]. Accumulation of lead in the gills and muscles of shellfish were observed throughout the year and during all the four seasons in the present study. Low accumulation of lead was observed in the summer for both the years. However, there was an increase during premonsoon and monsoon. Lead accumulation during post monsoon was high for all the shellfish species studied. Random fluctuation in the accumulation of lead in the gills and muscles of shellfish was observed during both the years with no set pattern. This may be due to the varying accumulation capacity of the shellfish or due to the fluctuation in the inflow of the heavy metal or it may also be a combination of both these factors.

In natural life, some trace metals are essential at low levels but are toxic at higher concentrations. They enter the human body through food chain causing different diseases and damages to humans [55, 60, 61]. In the human body, the metallic toxicants attack the proteins, notably the enzymes [62] and their toxic effects are cumulative and cause slow poisoning of the system over a period of time [18, 63]. Heavy metals have been implicated in the upsurge of liver and kidney diseases, and is believed to be responsible for a high proportion of mortality caused by kidney and liver morbidity [64-66], pain in bones [67], mutagenic, carcinogenic and teratogenic effects [64, 68-70], neurological disorders, especially in foetus and in children which can lead to behavioral changes and impaired performance in IQ tests [71, 72]. Therefore, the human health aspect linked to the consumption of heavy metal contaminated fish is of great concern [73].

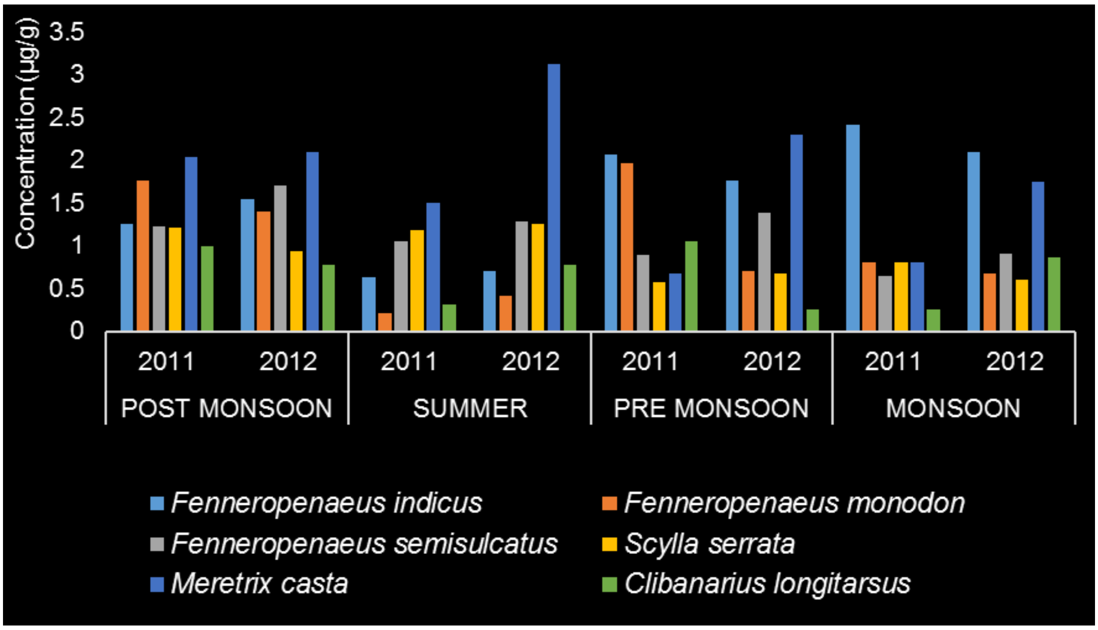

Fig 1: Presence of lead in gills of shellfish species

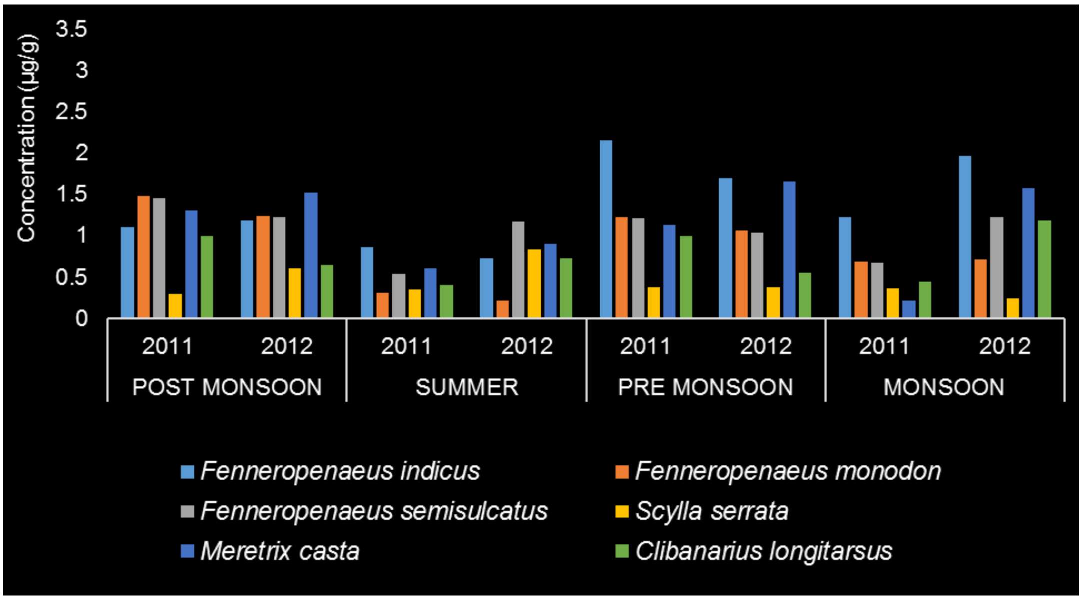

Fig 2: Presence of lead in muscles of shellfish species

5. Conclusion

In conclusion, the results of the present study indicated a mild to moderate accumulation of lead in the gills and muscles of shellfish species. *Meretrix casta* and *Fenneropenaeus indicus* showed a tendency to accumulate higher levels of lead than other shellfish species studied. At this rate, consumption of shellfish meat from Pulicat lake may lead to toxicity of man and other animals. Control measures are required to reduce lead pollution in Pulicat lake.

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