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Zinc accumulation in gills and muscles of shellfish species from Pulicat lake, Tamil Nadu, India

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Abstract
Estuaries play a crucial role in the socio-economic development of mankind in many aspects. Estuaries provide a nursery for the larval forms of some marine fish species, and provide shelter and food for many young and adult fish and shellfish. Introduction of untreated municipal waste water and industrial effluents into these water bodies leads to serious water pollution and majority of them with heavy metal pollution, which gets biomagnified and even reaches man through the food chain. In the present study, six species of shellfish, *Fenneropenaeus indicus*, *Fenneropenaeus monodon, Fenneropenaeus semisulcatus*, *Scylla serrata*, *Meretrix casta* and *Clibanarius longitarsus* in Pulicat lake, Tamil Nadu, India were analysed for the presence of zinc in its gills and muscles from January 2011 to December 2012. The results showed seasonal variations in the uptake of zinc. The maximum accumulation of zinc was observed during premonsoon in the gills of *Fenneropenaeus indicus* (8.56µg/g) in 2011 followed by *Clibanarius longitarsus* (8.44µg/g) in 2012. In muscles it was 8.23µg/g during monsoon and 7.85µg/g during premonsoon respectively in 2011. The overall conclusions indicated that the Pulicat lake is highly vulnerable to the threats of heavy metal pollution and the consumption of contaminated meat may lead to toxicity. Stringent and adequate control measures are required to save this important lake from heavy metal and other types of pollution.

Keywords: heavy metals, zinc, pollution, pulicat lake

1. Introduction

The coastal zone has different biotopes *viz*., estuaries, mangroves, coral reefs and lagoons endowed with splendid beauty and high productivity. Although these biotopes present only 10% of the open ocean, 90% of the human needs are obtained from this zone. Among the different biotopes, estuaries play a vital role as they serve as areas of interaction between fresh and salt water. Human race and civilization are always associated with the coastal systems and rivers. The basic requirement of water was not the sole reason for this, but the enormous life promoting and protecting resources man receives from these aquatic bodies is the coercion. The terrestrial resources are becoming extinct and are not capable to serve human needs. It was predicted in the beginning of this century that aquatic resources are the only solace for the survival of mankind [1].

Historically, the term estuary has been applied to the lower tidal reaches of a river. According to Pritchard [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". Estuaries play a crucial role in the socio-economic development of mankind in many aspects. Most of the great cities of the world have developed around the estuaries. In India, the coastal population density has been quite high since many centuries and the metropolitan cities like Mumbai, Kolkata and Chennai are developed around the estuaries. Plants and animals have adapted specially for the different habitats of this unique ecosystem. Thousands of birds, mammals, fish and other wildlife use estuaries as places to live, feed and reproduce [3]. Estuaries provide a nursery for the larval forms of some marine fish species, and provide shelter and food for many young and adult fish and shellfish. These in turn provide food for other levels of the food chain including shore birds, waterfowl, larger fish and marine mammals [4]. The health status and the biological diversity of the Indian estuarine ecosystem are deteriorating day by day through multivarious man-made activities including dumping of enormous quantities of sewage into the estuary, resulting in drastic reduction of shallow water fish population. It has also caused considerable ecological imbalance and resulted in large scale disappearance of their flora and fauna. Further, introduction of untreated municipal waste water and industrial effluents into these water bodies leads to serious water pollution and majority of them with heavy metal pollution, which gets biomagnified and even reaches man through the food chain [5].

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 [6]. 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 [7]. Untreated effluents from industries and urban areas are considered to be point sources of pollution [6, 8, 9].

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. 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 six shellfish species were used to estimate zinc content. The analysis was carried out using the method suggested by Watling and Emmerson [10]. Analytical grade reagents were used. For analysing zinc, 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 zinc in samples was carried out by inductively coupled plasma atomic emission spectroscopy (Optima 2100 DV, Perkin-Elmer, USA).

3. Results

The accumulation of zinc in the gills and muscles of shellfish are exhibited as seasonal as well as species specific variations. The maximum accumulation of zinc in the gills of shellfish species are as follows: *Fenneropenaeus indicus* (8.56µg/g) during premonsoon in 2011; *Fenneropenaeus monodon* (7.09µg/g) during premonsoon in 2012; *Fenneropenaeus semisulcatus* (4.70µg/g) during summer in 2011; *Scylla serrata* (6.05µg/g) during pre-monsoon in 2012; *Meretrix casta* (3.42µg/g) during post monsoon in 2012 and *Clibanarius longitarsus* (8.44µg/g) during premonsoon in 2012 (Figure 1). Whereas, in the case of muscles of the shellfish species, it was *Fenneropenaeus indicus* (7.25µg/g) during premonsoon in 2011; *Fenneropenaeus monodon* (6,56µg/g) during monsoon in 2011; *Fenneropenaeus semisulcatus* (7.34µg/g) during premonsoon in 2011; *Scylla serrata* (4.24µg/g) during summer in 2012; *Meretrix casta* (5.64µg/g) during summer in 2012 and *Clibanarius longitarsus* (7.85µg/g) during premonsoon in 2011 which exhibited a high level of zinc accumulation (Figure 2). Overall results conclude that the maximum accumulation of zinc was observed during premonsoon in the gills of *Fenneropenaeus indicus* (8.56µg/g) in 2011 followed by *Clibanarius longitarsus* (8.44µg/g) in 2012 and for muscles it was 8.23µg/g during monsoon and 7.85µg/g during premonsoon respectively in 2011.

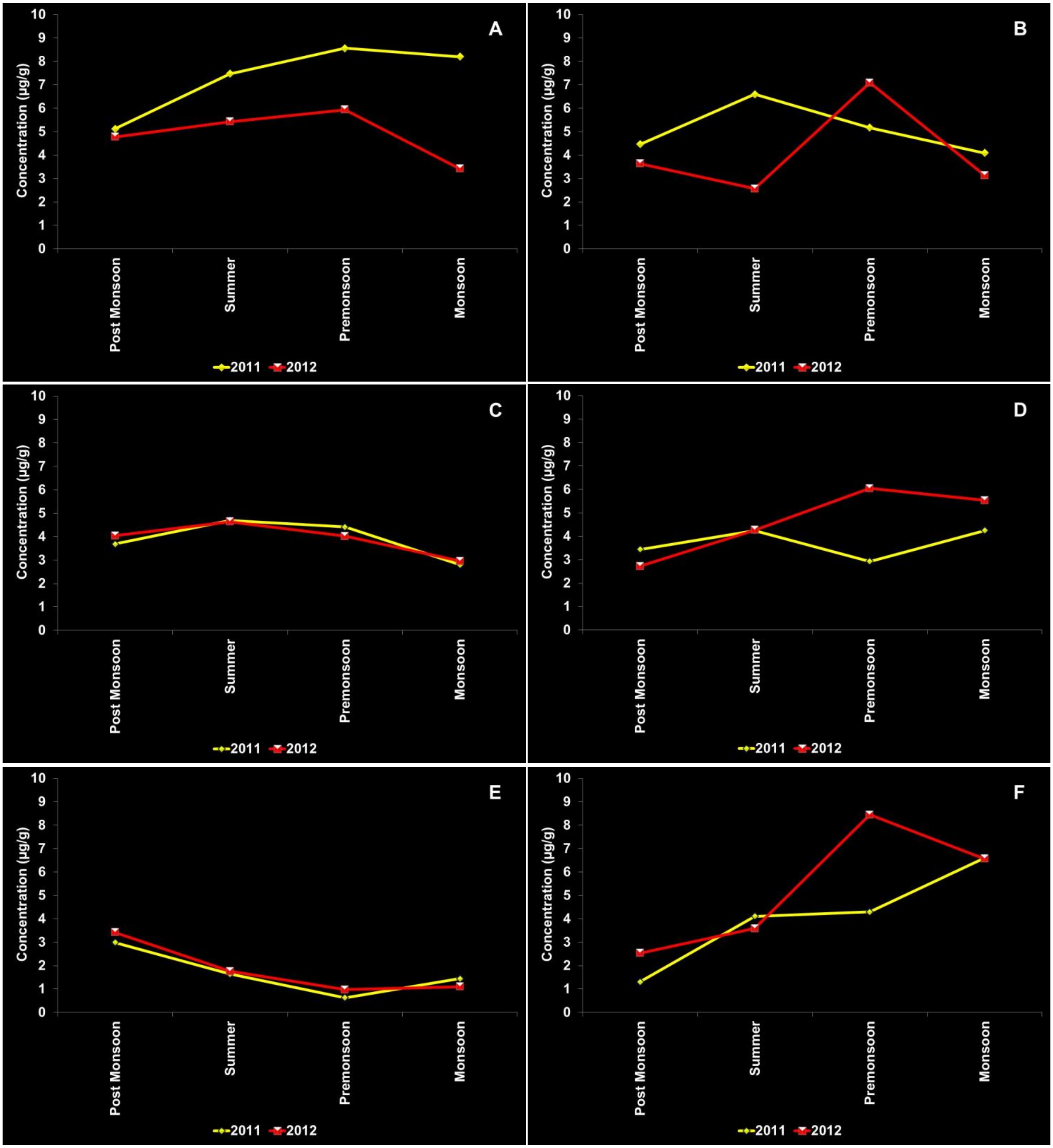

Fig 1: Presence of zinc in the gills of shellfish species

A: *Fenneropenaeus indicus*
B: *Fenneropenaeus monodon*
C: *Fenneropenaeus semisulcatus*
D: *Scylla serrata*
E: *Meretrix casta*
F: *Clibanarius longitarsus*

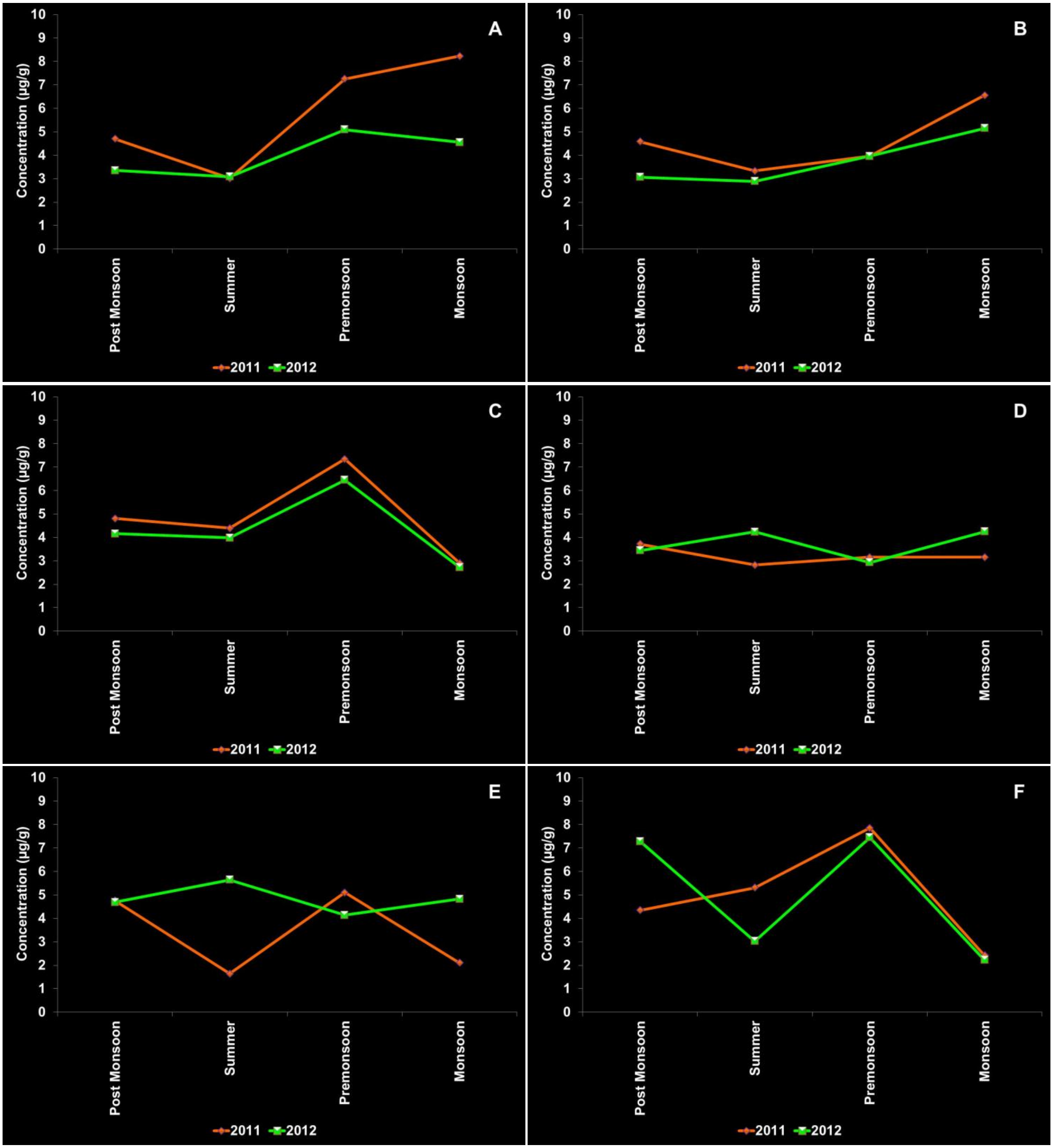

Fig 2: Presence of zinc in the muscles of shellfish species

A: *Fenneropenaeus indicus*
B: *Fenneropenaeus monodon*
C: *Fenneropenaeus semisulcatus*
D: *Scylla serrata*
E: *Meretrix casta*
F: *Clibanarius longitarsus*

4. Discussion

A river is a sink of environmental pollution bringing in untreated sewage, industrial effluents, indiscriminate dumping of wastes, residues and run offs from agricultural farms characterised by indiscriminate applications of inorganic fertilizers which has received great importance in developed countries [11, 12]. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms [13]. Heavy metal is among one of the pollutants, which cause severe threats to humans and the environment especially in the coastal areas which face great challenges due to rapid urbanization and industrialization [14, 15].

Zinc is an essential element for many marine organisms for normal growth, reproduction and longevity [12, 16], the other biological essential heavy metals include copper, nickel and iron [17]. Generally, the effluent discharges from metallurgic, electroplating and petrochemical industry, run offs from agricultural areas and dredging of harbour and municipal wastes are the sources of zinc in coastal waters [18]. Zinc is one of the most ubiquitous and mobile among the heavy metals and is transported in natural waters in both dissolved forms and is associated with suspended particles [19] (Mance *et al*., 1984). In river water, zinc is predominantly present in the dissolved form. In estuaries, where concentrations of suspended particles are higher, a greater proportion of zinc is adsorbed to suspended particles [20]. In low salinity areas of estuaries, zinc can be mobilised from particles by microbial degradation of organic matter and displacement by calcium and magnesium. Zinc in aquatic environment predominantly binds to suspended material before settling in sediments [18]. These sediments act as the most important reservoir or sink of zinc and other pollutants in the aquatic environment [21]. Heavy metal contamination in sediment can affect the water quality and bioaccumulation of metals in aquatic organisms, resulting in potential long term implications on human health and ecosystem [22]. In seawater, much of the zinc is found to be in the dissolved form as inorganic and organic complexes [16].

The occurrence of extensive upwelling in areas which carries zinc rich subsurface waters to surface might explain why zinc concentrations are elevated in the muscle tissue of various fishes [23]. Sasikumar *et al*. [18] have observed that zinc concentrations in suspended particulate matter exceeded typical estuarine values and were much higher than the homogeneously distributed concentrations in different depth ranges of the basin sediment along southern Karnataka coast. The extent of accumulation of trace metals by organisms in different tissues is dependent on the route of entry, that is, either from surrounding medium or in the form of food or chemical form of material available in the media [24, 25]. However, Mihajlo *et al*. [26] differed by stating that zinc is not biomagnified, rather the absorption of zinc by aquatic organisms tends to be from water not food; only dissolved zinc tends to be bioavailable and the bioavailability depends on the physical and chemical characteristics of the environment, *viz*., pH, dissolved organics, water hardness, competing ions and biological processes.

Various studies have shown that aquatic invertebrates tend to accumulate high level of heavy metals. Thus, determination of harmful and toxic substances in water sediments and biota gives direct information on the significance of pollution in the aquatic environment [27]. Romeo *et al*. [28] pointed out that the metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food and sediment, as well as other factors like salinity, temperature and interacting agents. Carbonell and Tarazona [29] concluded that different tissues of aquatic animals provide and/or synthesize nonexchangeable binding sites resulting in different accumulation levels. The results of a study conducted by Soegianto *et al*. [30] for the presence of zinc in aquatic animals caught from Gresik coastal waters, Indonesia showed that all animals contained zinc levels higher than the level of other metals (As, Cd, Cr, Ni, Cu, Se and Pb). The concentration of zinc in whole body of animals was relatively higher than those recorded in muscle tissues alone. Its accumulation in the organs was very high when compared with the other essential and non-essential metals [12]. It is noteworthy that generally, higher ranges of zinc concentrations have also been reported for species from relatively polluted areas of the world [31-34], which in turn infers that fish body regulates zinc concentrations at an elevated level when exposed to high ambient zinc concentrations.

Metal accumulation in shellfish, can be realized via two routes; either via hepatopancreas during feeding or via gills. Accumulation often occurs in the digestive gland, which plays a role in assimilation, excretion and detoxification of contaminants [16]. Reports on metal concentration in shrimps and crabs under natural conditions for coastal waters of India are limited [35-37]. Meshram *et al*. [27] have reported that heavy metals accumulated in *Metapenaeus dobsoni* is in the order Zn >Pb> Ni >Cd. The concentrations of zinc were relatively higher, compared to the concentrations of other metals in same shrimp samples. They infer that this phenomenon is because zinc is an essential element required by animals for metabolic process. The higher the metal concentration in shellfish was suggestive of the ability of the shellfish to bio-accumulate these metals above their concentrations in water [11]. Several species of crustaceans are able to regulate the uptake of zinc but, at higher concentrations, this process appears to breakdown leading to an influx of zinc [16]. Bioaccumulation patterns of metals in shellfish muscle can be utilized as effective indicators of environmental metal contamination [38, 39]. Thus, shellfish and shellfish products can be used for monitoring potential risk to humans because these are directly consumed by a large population [40].

Vazquez *et al*. [41] reported elevated zinc concentrations in oysters collected from a highly polluted lagoon in Mexico. However, mussels are known to regulate zinc uptake and are therefore reported as a variable indicator for zinc contamination [42]. Significant positive correlations between zinc in seawater and tissue concentrations imply that zinc is accumulated in soft tissue and shell in green mussels in a proportion similar to its availability in ambient waters. Although, zinc uptake is regulated, it was observed that the incorporated zinc remained in the soft tissue, suggesting the use of *Perna viridis* as a potential biomonitoring agent for assessing zinc contamination over extended periods [18]. Zinc like other metals can be toxic in high concentrations to human [43]. Although uncommon, gastrointestinal distress and diarrhoea have been reported following ingestion of zinc through food and beverage [44]. Other symptoms of zinc toxicity are slow reflexes, shakes, paralysation of extremities, anaemia, metabolic disorder, teratogenic effects and increased mortality [45].

Results of the present study coincided with the findings of earlier works reported by Nsofor [11] and Olawusi-Peters [16]. The suggestion of Subramanian and Sukumar [40] to use shellfish and shellfish products to monitor the potential risk to humans caused by heavy metals holds good as per this study. In conclusion, the present study has shown that the accumulation of zinc in the gills and muscles of crustaceans *viz*., *Fenneropenaeus indicus*, *Fenneropenaeus monodon*, *Fenneropenaeus semisulcatus* and *Scylla serrata* was moderate to high when compared to the other shellfish spceies. Thus the consumption of these shellfish is safe, but do not exclude the risk of bioaccumulation in crustacean meat. This study highlights the needs for estuarine bio-monitoring to avoid contamination of shellfish and their consumers. The overall conclusions indicated that the Pulicat lake is highly vulnerable to the threats of heavy metal pollution and consumption of contaminated meat may lead to toxicity. Stringent and adequate control measures are required to save this important lake from heavy metal and other types of pollution.

5. References

1. Balasubramanian T. Estuaries of India, state of the art report. Balasubramanian T (Ed), Environmental Information System Centre, Annamalai University. 2002, 195.
2. Pritchard DW. What is an estuary: a physical viewpoint. American Association for the Advancement of Science. 1967; 83:3-5.
3. Zwolsman JJG. North sea estuaries as filters for contaminants. Delft hydraulics, Report No T1233, The Netherlands, 1994.
4. Rajendran N, Sanjeevi SB, Khan SA, Balasubramanian T. Ecology and biodiversity of Eastern Ghats estuaries of India. EPTRI-ENVIS Newsletters. 2004; 10(4):1-12.
5. Reddy S, Reddy KN, Prasad PRC, Raju VS. Threatened endemic plants from Eastern Ghats, India, The Eastern Ghats, EPTRI ENVIS Newsletter. 2003; 9(2):2-6..
6. Batvari BP, Kamala-Kannan S, Shanthi K, Krishnamoorthy R, Lee KJ, Jayaprakash M. Heavy metals in two fish species *Carangoidel malabaricus* and *Belone stronglurus* from Pulicat lake, north of Chennai, southeast coast of India. Environmental Monitoring and Assessment. 2008; 145:167-175.
7. Ramadevi K, Indra TJ, Ragunathan MB. Fishes of Pulicat lake. Records of Zoological Survey of India. 2004; 102(3-4):33-42.
8. Periakali P, Padma S. Mercury in Pulicat lake sediments, east coast of India. Journal of Indian Association of Sedimentology. 1998; 17:239-244.
9. Padma S, Periakali P. Physico-chemical and geochemical studies in Pulicat lake, east coast of India. Indian Journal of Marine Science. 1999; 28:434-437.
10. Watling RJ, Emmerson WD. A preliminary pollution survey of the Papenkuils river, Port Elizabeth. Water Science Africa. 1981; 7:211-215.
11. Nsofor CI, Igwilo IO, Ikpeze OO, Ikeogu CF, Umeoguagu FO, Okonkwo CJ. Bioaccumulation of heavy metals in shellfish *Macrobrachium rosenbergi* in Niger river at Onitsha, Anambra state, Nigeria. International Journal of Agriculture and Biosciences. 2014; 3(1):38-40.
12. Chaitanya I, Satyaprakash M, Reddy TB. Bioaccumulation of heavy metals in marine fish samples at Visakhapatnam and Bheemili region, North east coast of Andhra Pradesh, India. International Journal of Science, Environment and Technology. 2016; 5(3):1718-1729.
13. Storelli MM, Storelli A, D'ddabbo R, Marano C, Bruno R, Marcotrigiano GO. Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: Overview and evaluation. Environmental Pollution. 2005; 135:163-170.
14. Fang ZQ, Cheung RY, Wong MH. Heavy metals in oysters, mussels and clams collected from coastal sites along the Pearl river delta, South China. Journal of Environmental Sciences. 2003; 15(1): 924.
15. Wang SL, Xu XR, Sun YX, Liu JL, Li HB. Heavy metal pollution in coastal areas of South China: a review. Marine Pollution Bulletin. 2013; 76:7-15.
16. Olawusi-Peters OO, Aguda OE, Okoye FO. Heavy metals (Pb, Cu, Fe, and Zn) level in shellfish (*Etheria elliptica*), water and sediments of river Ogbese, Ondo state, Nigeria. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering. 2015; 9(3):331-334.
17. Pooter DD. Heavy metals. http://www.coastalwiki.org/wiki/Heavy_metals, 2013.
18. Sasikumar G, Krishnamoorthy M, Krishnakumar PK, Bhat GS. Accumulation of trace metals in green mussel *Perna viridis* in the shellfish harvesting environment along southern Karnataka coast. Indian Journal of Fisheries. 2011; 58(1):53-58.
19. Mance G, Brown VM, Yates J. Proposed environmental quality standards for List II substances in water. Copper. Water Research Centre Technical Report TR210, 1984.
20. CCREM (Canadian Council of Resource and Environment Ministers). Canadian water quality guidelines. Prepared by the Task Force on Water Quality Guidelines, 1987.
21. Gupta A, Rai DK, Pandey RS, Sharma B. Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environmental Monitoring and Assessment. 2009; 157:449-458.
22. Fernandes C, Fontainhas-Fernandes A, Peixoto F, Salgado MA. Bioaccumulation of heavy metals in *Liza saliens* from the Esomriz-Paramos coastal lagoon, Portugal. Ecotoxicology and Environmental Safety. 2007; 66:426-431.
23. Rejomon G. Biogeochemistry of trace metals in the Indian Exclusive Economic Zone (EEZ) of the Arabian Sea and Bay of Bengal. Doctoral Thesis, Cochin University of Science and Technology, India, 2005.
24. Ghosh TK, Kshirsagar DG. Selected heavy metals in

seven species of fishes from Bombay offshore areas. Proceedings of the National Academy of Sciences, India. 1973; 63(B III):350-311.
25. Maddock BG, Taylor D. The acute toxicity and bioaccumulation of some lead alkyl compounds in marine animals presented at the internet. Expert's discussion meeting on: Lead occurrence, fate and pollution in the marine environment. Rovinz, Yugoslavia. 1977, 18-22.
26. Mihajlo J, Ana S, Slavković-Beskoski L, Ilija T, Sandro D, Slavka S. Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro). Journal of the Serbian Chemical Society. 2011; 76(6):933-946.
27. Meshram LN, Udawant SM, Pawar SS, Mishra PS. Bioaccumulation of heavy metals (Zn, Pb, Cd and Ni) in tissues of *Penaeus monodon* (Fabricius, 1798) from India. International Journal of Advanced Research. 2014; 2(3): 548-555.
28. Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. Heavy metal distribution in different fish species from the Mauritania coast. Science of Total Environment. 1999; 232: 169-175.
29. Carbonell G, Tarazona JV. Toxicokinetics of Cu. Aquatic Toxicology. 1994; 29:213-221.
30. Soegianto A, Irawan B, Hamami. Bioaccumulation of heavy metals in aquatic animals collected from coastal waters of Gresik, Indonesia. Asian Journal of Water, Environment and Pollution. 2009; 6(2):95-100.
31. Halcrow W, Mackay DW, Thornton I. The distribution of trace metals and fauna in the firth of Clyde in relation to the disposal of sewage sludge. Journal of the Marine Biological Association of the United Kingdom. 1973; 53:721-739.
32. Eustace IJ. Zinc, cadmium, copper, and manganese in species of finfish and shellfish caught in the Derwant estuary, Tasmania. Australian Journal of Marine and Freshwater Research. 1974; 25: 209-220.
33. Roth I, Hornung H. Heavy metal concentrations in water, sediments and fish from Mediterranean coastal area. Israel. Environmental Science and Technology. 1977; 11:265-269.
34. Plaskett D, Potter IC. Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. Australian Journal of Marine and Fresh Water Research. 1979; 30:607-616.
35. Zingde MD, Singbal SYS, Moraes CF, Reddy CVG. Arsenic, copper, zinc and manganese in the marine flora and fauna of coastal estuarine waters around Goa. Indian Journal of Marine Science. 1976; 5:212.
36. Matkar V, Ganpathy S, Pillai KC. Distribution of zinc, Cu, Mn and Fe in Bombay harbour bay. Indian Journal of Marine Science. 1981; 10:35-40.
37. Qasim SZ, Gupta SR. Some problems of coastal pollution in India. Marine Pollution Bulletin. 1988; 19:100.
38. Atchinson GJ, Murphy BR, Bishop WE, McIntosh AW, Mayes RA. Trace metal concentration of bluegill *Lepomis macrochirus* from the Indiana lakes. Transactions of the American Fisheries Society. 1977; 106:637-640.
39. Larsson A, Haux C, Sjobeck M. Fish physiology and metal pollution: Results and experience from laboratory and field studies. Ecotoxicology and Environmental Safety. 1985; 9:250-281.
40. Subramanian R, Sukumar A. Biological reference materials and analysis of toxic elements. Fresenius Journal of Analytical Chemistry. 1988; 332:623-629.
41. Vazquez FG1, Sharma VK, Alexander VH, Frausto CA. Metals in some lagoons of Mexico. Environmental Health Perspectives. 1995; 103(1):33-34.
42. Lobel PB, Mogie P, Wright DA, Wu BL. Metal accumulation in four molluscs. Marine Pollution Bulletin. 1982; 13:170-174.
43. ANZECC. National water quality management strategy, Guidelines for water quality monitoring and reporting. Australian and New Zealand Environment and Conservation Council and Agriculture Resource Management Council of Australia and New Zealand, Canberra, 2000.
44. WHO. Environmental Health Criteria: Zinc, World Health Organization, Geneva. 2001, 221.
45. Klaassen CD. Toxic effects of metals: mercury. Casarett and Doull's Toxicology: The Basic Science of Poisons, New York: McGraw-Hill. 1996, 5.