

Temporal and spatial assessment of iron in the soft tissues of six species of shellfish from Pulicat lake, Tamil Nadu, India

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Abstract

Marine organisms tend to accumulate heavy metals from the environment and are adapted to handle natural fluctuations in intake brought about by slight changes in their availability in water or food. Aquatic organisms are widely used to monitor environmental health due to anthropogenic impacts. In the present study, the bioaccumulation of iron 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 accumulation of iron 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 8.68µg/g iron during summer in the year 2012. As the concentration of the heavy metal in the shellfish tissue depends on the bioavailability of metals in the aquatic environment due to the urbanization and industrialization, proper steps should be taken to check the discharge of industrial wastes into the water bodies.

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

1. Introduction

Among the various ecosystems, estuaries are unique due to their continuous dynamic nature and higher productivity, because at the proximity of the mouth of the river, where it meets the sea, a dynamic and distinct environment prevails. At this juncture, river water mixes with the seawater, and the tides of the sea in regular recurring rhythm, pushes in and out the river waters. This transitional ecotone, which is the confluence of the river and the sea, is the estuary. A widely used definition of an estuary given by Pritchard ^[1] who states that “an estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage”. Estuaries rank along with tropical rainforests and coral reefs as the world's most productive ecosystems, more productive than both the rivers and the ocean that influence them from either side. Though they occupy only 0.5% of global marine areas, estuaries are responsible for 2.6% of primary marine production and potentially contribute 5.9% to the world fisheries harvest. They act as gigantic mixing vessels for waters of various biological, thermal, hydrochemical, and suspended matter characteristics that undergo daily, seasonal and long-term changes, defying generalization ^[2]. Estuaries provide a nursery for the juvenile 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 ^[3].

The name trace metals signifies, those metals that are present in extremely small quantities in a given environment with a distinct property of being toxic above a specific threshold of bioavailable level and is significant in determining the overall health of the ecosystem ^[4, 5]. In the natural environment, organisms are exposed chronically to sublethal concentrations of several contaminants simultaneously, and concentrations of metals present within the organisms result from the relative rates of metal accumulation and turnover ^[6]. The common feature of these metals is that they are all relatively toxic even at fairly low concentrations and are readily concentrated by aquatic organisms and plants ^[6]. Metals viz., Fe, Cu, Zn and Mn are essential, since they play important roles in biological systems; whereas Hg, Pb and Cd are toxic even in trace amounts ^[7]. 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 water, and crude oil spills replete with most common heavy metals in our environment ^[8].

Marine organisms tend to accumulate heavy metals from the environment and are adapted to handle natural fluctuations in intake brought about by slight changes in their availability in water or food. Such a process of accumulation of pollutants by organisms is termed as bioconcentration or bioaccumulation ^[9]. Aquatic organisms are widely used to monitor environmental health due to anthropogenic impacts ^[10-12]. The concentration of heavy metals in various parts of the

organisms is primarily indicative of the level of pollution in the environment [13]. The bioaccumulation of heavy metals by marine molluscs and other marine organisms may reach many orders of magnitude above background concentrations of certain locality. This phenomenon may demonstrate the potential of these species as a biomonitor of heavy metal pollution [14, 15]. The environmental quality standards rely on the concentrations of contaminants as quality objectives for comparing the sites. The ecological integrity is judged using water or sediment in toxicity tests [16]. The United States Environmental Protection Agency (USEPA) recommends the use of bioassays, biological and habitat data in addition to chemical data for water quality assessments [17, 18]. Therefore, the objective of the present study was to estimate the concentration of iron in the gills and muscles of six species of shellfish from Pulicat lake, Tamil Nadu, India.

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 species 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 six shellfish species were used to estimate iron content. The analysis was carried out using the method suggested by Watling and Emmerson [23]. Analytical grade reagents were used. For analysing iron, 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

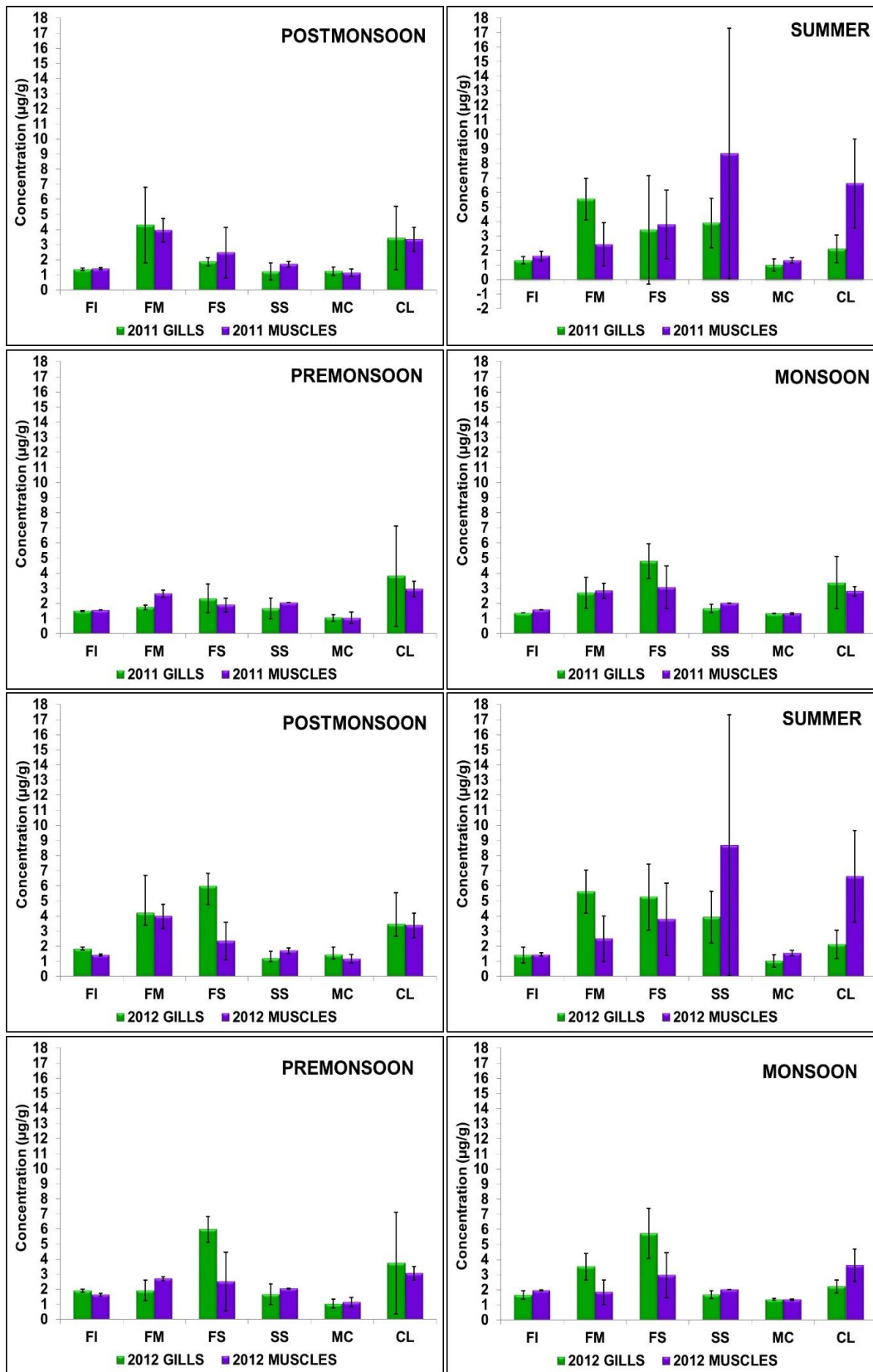
was dissolved in 10mL of distilled water and then filtered. The filtered sample was aspirated into the atomic absorption spectrophotometer and the reading was recorded. The solution was then diluted and filtered through a 0.45µm nitrocellulose membrane filter. Determination of iron in samples was carried out by inductively coupled plasma atomic emission spectroscopy (Optima 2100 DV, Perkin-Elmer, USA).

3. Results

The accumulation of iron in the gill and muscle tissues of the six shellfish species are exhibited as seasonal as well as species specific variations. The order of maximum accumulation of iron in the gill tissues of the six shellfish species for the year 2011 are as follows: *Fenneropenaeus monodon* (5.55µg/g) during summer; *Fenneropenaeus semisulcatus* (4.79µg/g) during monsoon; *Scylla serrata* (3.89µg/g) during summer; *Clibanarius longitarsus* (3.8µg/g) and *Fenneropenaeus indicus* (1.49µg/g) during premonsoon; and *Meretrix casta* (1.33µg/g) during monsoon. In the case of muscle tissues it was, *Scylla serrata* (8.66µg/g) and *Clibanarius longitarsus* (6.61µg/g) during summer; *Fenneropenaeus monodon* (3.94µg/g) during postmonsoon; *Fenneropenaeus semisulcatus* (3.78µg/g) and *Fenneropenaeus indicus* (1.62µg/g) during summer followed by *Meretrix casta* (1.32µg/g) during summer and monsoon. Whereas, in the year 2012, it was, the gill tissues of *Fenneropenaeus semisulcatus* (5.98µg/g) during postmonsoon; *Fenneropenaeus monodon* (5.62µg/g) and *Scylla serrata* (3.92µg/g) during summer; *Clibanarius longitarsus* (3.74µg/g) and *Fenneropenaeus indicus* (1.91µg/g) during premonsoon; and *Meretrix casta* (1.43µg/g) during premonsoon which exhibited the maximum accumulation of iron. For the year 2012, maximum iron accumulation was observed in the muscles of *Scylla serrata* (8.68µg/g) and *Clibanarius longitarsus* (6.62µg/g) during summer; *Fenneropenaeus monodon* (3.97µg/g) during postmonsoon; *Fenneropenaeus semisulcatus* (3.79µg/g) during summer; *Fenneropenaeus indicus* (1.95µg/g) during monsoon; and *Meretrix casta* (1.55µg/g) during summer. The overall results indicate the muscle tissues of *Scylla serrata* to have accumulated the maximum amount of 8.68µg/g iron during summer in the year 2012 (Figure 1).

4. Discussion

Although, heavy metals like cadmium, copper, lead, zinc and iron are normal constituents of marine and estuarine environments, they may be introduced into the biogeochemical cycle through industrial wastes and sewage and thereby create adverse impact on the biotic community apart from polluting sources like streams and rivers [24,25]. Heavy metals in the aquatic environment bioaccumulate in biota through absorption or ingestion. The higher concentrations of heavy metals in organisms prove that the bioavailability of these heavy metals has been increased during the last century due to urbanization and industrialization as suggested by Cheung *et al.* [26]. These heavy metals influence intrinsic biological factors of these organisms as well as contaminate them as human foods. Heavy metal estimation in coastal zone is of great importance to determine the metal contamination in the marine



FI: *Fenneropenaeus indicus*
 FM: *Fenneropenaeus monodon*
 FS: *Fenneropenaeus semisulcatus*

SS: *Scylla serrata*
 MC: *Meretrix casta*
 CL: *Clibanarius longitarsus*

Fig 1: Accumulation of iron in shellfish species from Pulicat lake

environment. In the aquatic environment, conventional methods for monitoring heavy metals were basically the determination of the heavy metal in water, sediment and biota. A study by Biswas *et al.* [27] showed that the concentration of cadmium, copper, lead, zinc and iron concentrations are found higher in the tissue of *Saccostrea cucullata* during premonsoon and postmonsoon seasons. According to Mitra and Choudhury [28] and Mitra [29], the fast industrialization and urbanization of the Kolkata, Howrah and Haldia complex in the state of West Bengal, India has created considerable ecological imbalance in the adjacent coastal areas. The lower part of this estuary has different industries *viz.*, paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tires and cycle rims [30]. Biswas *et al.* [27] reported that the concentration of metals like cadmium, copper, lead, zinc and iron in oyster tissue was higher during premonsoon and postmonsoon and this higher concentration of heavy metals in oysters was due to anthropogenic activities. In the same study, the downward order of the concentration of heavy metals present in the oyster tissue were iron, zinc, copper, cadmium and lead in Shankarpur and iron, zinc, copper, lead, cadmium in Satgelia during both seasons. Bryan [31] has reported that zinc and iron were present in large quantities in the tissues of both *Penaeus semisulcatus* and *Metapenaeus monoceros* in his study. These metals play a role in the enzymatic and respiratory processes. Heavy metals and their ions usually sink to their bottom sediments which constitute the food of the benthic organisms. The high concentration of iron in the shellfish could be due to its habitat effect since the shellfish is a bottom dwelling organism. It is suggested that iron, zinc and copper were preferentially accumulated by the oysters. It is to be mentioned that the pH, salinity and temperature may have affected the concentration and rate of uptake of the metals in oysters. Accumulation of the metals by mussels is affected by salinity, temperature and concentration of the trace metals in the water [32]. 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. Trace metals are taken up and accumulated by molluscs and many other marine invertebrates to tissue and body concentrations usually much higher on a wet weight basis than concentrations in the surrounding seawater [33]. As they are sessile, they reflect local

contaminant concentrations more accurately than crustaceans and free-swimming finfish. Marine bivalves *viz.*, oysters and mussels have been extensively used as model organisms in environmental studies of water quality [34, 35].

Iron, one of the most abundant metals on earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health [36]. In humans, iron is an essential component of proteins involved in oxygen transport [37]. It is also essential for the regulation of cell growth and differentiation [38, 39]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance and decreased immunity [36, 40]. On the other hand, excess amounts of iron in man can result in toxicity and even death [41]. Ingestion of iron accounts for most of the toxic effects because it is absorbed rapidly in the gastrointestinal tract. The corrosive nature of iron seems to further increase the absorption. Chief sources of iron include drinking water, iron pipes, cookware and welding. Foods rich in iron are: bone meal, bran, clams, heart, kidney, leafy vegetables, legumes, liver, meat, nuts, organ meats, oysters and other shellfish. Iron targets the liver, cardiovascular system and kidneys [42]. The symptoms of iron toxicity include amenorrhea, anger, rheumatoid arthritis, birth defects, bleeding gums, cancer, constipation, diabetes, dizziness, emotional problems, fatigue, headache, heart damage, heart failure, hepatitis, high blood pressure, hostility, hyperactivity, infections, insomnia, irritability, joint pain, liver disease, loss of weight, mental problems, metallic taste in mouth, myasthenia gravis, nausea, pancreas damage, premature aging, schizophrenia, scurvy, shortness of breath and stubbornness [43]. There is considerable potential for iron toxicity because very little iron is excreted from the body. Thus, iron can accumulate in body tissues and organs when normal storage sites are full. Symptoms of Alzheimer's and Parkinson's disease may also be iron-related [41]. As the concentration of the heavy metal in the shellfish tissue depends on the bioavailability of metals in the aquatic environment due to the urbanization and industrialization, proper steps should be taken to check the discharge of industrial wastes into the water bodies of these coastal regions [27]. Similar means are to be adopted to prevent the Pulicat lake from further contamination and to protect it for posterity.

5. References

1. Pritchard DW. What is an estuary? Physical viewpoint. In: Lauff G.H., (Ed.), Estuaries, Publ. A.A.A.S. Washington D.C. 1967; 83:3-5.
2. Zwolsman JGG. North sea estuaries as filters for contaminants. Delft hydraulics, The Netherlands. 1994, 1233.
3. 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.
4. Kucuksezgin F, Kontas A, Altay O, Uluturhan E, Darilmaz E. Assessment of marine pollution in Izmir Bay: Nutrient, heavy metal and total hydrocarbon concentration. Environment International. 2006; 32:41-51.
5. Lafabrie C, Pergent G, Kantin R, Pergent-Martini C, Gonzalez JL. Trace metals assessment in water, sediment, mussel and seagrass species-Validation of the use of *Posidonia oceanica* as a metal biomonitor, Chemosphere. 2007; 68:2033-2039.
6. Nammalwar P. Heavy metals pollution in the marine environment. Science Reporter. 1983, 158-160.
7. Chitrarasu P, Ali JA, Babuthangadurai T. Study on the bioaccumulation of heavy metals in commercially valuable and edible marine species of Ennore creek, South India. International Journal of Pharma and Bio Sciences. 2013; 4(2)(B):1063-1069.
8. Wills J. A survey of offshore oil field drilling wastes and disposal techniques to reduce the ecological impact of sea dumping. Sakhalin Environment Watch. 2000; 13:23-29.

9. Lyla PS, Khan AS. Pattern of accumulation of heavy metals (copper and zinc) in the estuarine hermit crab *Clibanarius longitarsus* (De Hann). Indian Journal of Geo-Marine Sciences. 2011; 40(1):117-120.
10. Hellowell JM. Biological indicators of freshwater pollution and environmental management. Amsterdam: Elsevier Publications. 1986, 546-554.
11. Evans DW, Dodoo DK, Hanson DJ. Trace elements concentrations in fish livers: implication of variations with fish size in pollution monitoring. Marine Pollution Bulletin. 1993; 26(6):329-334.
12. Rashed MN. Monitoring of environmental heavy metals in fish from Nasser lake. Environment International. 2001; 27:27-33.
13. Mediha C, Demir TA, Uyanoglu M, Bayramoglu, GO Emiroglu Z, Arslan N, *et al.* Preliminary assessment of heavy metals in water and some cyprinidae species from the Porsuk river. Turkey Journal of Applied Biological Sciences. 2007; 1(3):91-95.
14. Chan HM. Temporal and spatial fluctuations in the trace metal concentration in transplanted mussels in Hong Kong. Marine Pollution Bulletin. 1989; 20:82-86.
15. Hamed MA, Emara AM. Marine molluscs as biomonitors for heavy metal levels in the Gulf of Suez, Red sea. Journal of Marine System. 2006; 60:220-34.
16. Tueros I, Larreta BA, Rodriguez J, Valencia JGV, Millan E. Integrating long-term water and sediment pollution data, in assessing chemical status within the European water framework directive. Marine Pollution Bulletin. 2009; 58:1389-1400.
17. USEPA. Consolidated assessment and listing methodology: Toward a compendium of best practices. US Environmental Protection Agency, Office of wetlands, oceans, and watersheds. <http://www.epa.gov/owow/monitoring/calm.html>, 2002.
18. USEPA. Methods for measuring acute toxicity of effluent and receiving waters for fresh water and marine organisms, 5th Oct 2002. United States environmental protection agency, office of water (4303T), Washington DC, EPA 821-R02-012. 275. http://water.epa.gov/scitech/methods/cwa/wet/upload/2007_07_10_methods_wet_disk2_atx1-6.pdf, 2002.
19. Batvari BP, Kamala-Kannan S, Shanthi K, Krishnamoorthy R, Lee KJ, Jayaprakash M. Heavy metals in two fish species (*Carangoides malabaricus* and *Belone strongylurus*) from Pulicat lake, north of Chennai, southeast coast of India. Environmental Monitoring and Assessment. 2008; 145:167-75.
20. Ramadevi K, Indra TJ, Ragunathan MB. Fishes of Pulicat lake. Records of Zoological Survey of India. 2004; 102(3-4):33-42.
21. Periakali P, Padma S. Mercury in Pulicat lake sediments, east coast of India. Journal of Indian Association of Sedimentology. 1998; 17: 239-244.
22. 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.
23. Watling RJ, Emmerson WD. A preliminary pollution survey of the Papenkuils river, Port Elizabeth. Water Science Africa. 1981; 7:211-215.
24. Njoku PC, Keke LR. Evaluation of safe water pollution in Dilimi river, Jos due to mine effluents. Need for quality control measures. Paper delivered at the Sixteenth Annual Conference and International workshop of Nigerian Association of Aquatic Sciences, 2001.
25. Nsofor CI, Aguigwo JN. The bioaccumulation of zinc, iron, copper, lead and cadmium by some fish species from the river Niger, Anambra state, Nigeria. Environmental Studies Research Journal. 2005; 5:122-129.
26. 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.
27. Biswas T, Bandyopadhyay PK, Chatterjee SN. Accumulation of cadmium, copper, lead, zinc and iron in the edible oyster, *Saccostrea cucullata* in coastal areas of West Bengal. African Journal of Biotechnology. 2013; 12(24):3872-3877.
28. Mitra A, Choudhury A. Trace metals in macrobenthic mollusks of the Hooghly estuary, India. Marine Pollution Bulletin. 1992; 26(9):521-522.
29. 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.
30. UNEP. Pollution and the marine environment in the Indian Ocean. UNEP regional seas programme activity centre, Geneva, Switzerland, 1982.
31. Bryan GW. Concentrations of zinc and copper in the tissues of decapod crustacean. Journal of the Marine Biological Association of the United Kingdom. 1968; 48:303-321.
32. Boyden CR, Romerill MG. A trace metal problem in pond oyster culture. Marine Pollution Bulletin. 1974; 5:74-78.
33. Rainbow PS. The significance of trace metal concentrations in marine invertebrates: Ecotoxicology of metals in invertebrates. Proceedings of a session at the First Society of Environmental Toxicology and Chemistry-Europe Conference; Sheffield, England. 7-10 April 1991; Boca Raton, Florida: Lewis Publishers, 1993, 3-23.
34. Wang WX, Fisher NS, Luoma SN. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Marine Ecology Progress Series. 1996; 140:91-113.
35. Griscom SB, Fisher NS, Luoma SN. Geochemical Influences on assimilation of sediment bound metals in clams and mussels. Environmental Science and Technology. 2000; 34:91-99.
36. Institute of Medicine (US) Panel on micronutrients. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington (DC): National Academies Press (US), 2001.
37. Dallman PR. Biochemical basis for the manifestations of iron deficiency. Annual Review of Nutrition. 1986; 6:13-40.
38. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron

- metabolism in Man. St. Louis: Oxford: Blackwell Scientific. Oxford, England, 1979.
39. Andrews NC. Disorders of iron metabolism. *New England Journal of Medicine*. 1986; 341:1986-1995.
 40. Bhaskaram P. Immunobiology of mild micronutrient deficiencies. *British Journal of Nutrition*. 2001; 85:75-80.
 41. Corbett JV. Accidental poisoning with iron supplements. *MCN. American Journal of Maternal and Child Nursing*. 1995; 20:234.
 42. Roberts JR. Metal toxicity in children. In: *Training manual on pediatric environmental health: Putting it into practice*. Emeryville, CA: Children's Environmental Health Network, 1999.
 43. Anderson K. Excess iron and brain degeneration: The little-known link, <http://www.lifeextension.com/Magazine/2012/3/Excess-Iron-Brain-Degeneration/>, 2012, 1.