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Biomonitoring of heavy metals availability in the marine environment of Karachi, Pakistan, using oysters (*Crassostrea* sp)

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Abstract

The Concentrations and distributions levels of six heavy metals (Cu, Zn Ni, Mn, Fe and Pb) were recorded in bodies/soft tissues of oysters obtained, between November 2005 and October 2006 from three polluted areas along Karachi, Pakistan coastal zone namely; Gizri, Korangi creek and Manora. The accumulation patterns of different metals with reference to seasons/months were also followed. According to the results, the oysters accumulated trace metals over a wide range of varying concentrations, most probably in reflection of their metal bioavailability. The oyster inhabiting the Manora coast (Navigation Channel) carried the highest load of Cu followed by Korangi creek and Gizri. All the study areas carried low concentrations of Mn at all the concerned locations. Highest concentrations of Mn were recorded in Korangi creek followed by Gizri and Manora. The highest concentrations of Zn were confirmed in Gizri and Korangi creek while the lowest concentrations observed in Manora. The study site Manora avowed to be the most polluted area having the highest concentrations of Cu, Fe and Pb. All the concentration levels of heavy metals were obtained using Atomic Absorption Spectrophotometer.

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Introduction

Coastal areas are commonly situated at the end point of the discharge of toxic and environ-mentally harmful chemicals. These toxic materials get released through direct and indirect discharge of industrial and anthropological activities. The indiscriminate discharge of these materials can disrupts and destroys the fragile coastal ecology (Blackmore, 1998; Ferreira *et al.*, 2005). Anthropogenic inputs of pollutants, such as heavy metals, to the marine environment have become a global problem (Irabien and Velasco, 1999).

The term "heavy metal" is used synonymously with trace metals and includes both essential and non-essential trace metals. All aquatic organisms need very low quantities of these metals however in cases where these exceed certain thresholds, deleterious effects are imposed upon the ecosystem (Maanan, 2008). The toxicological impacts vary between taxa, probably even at the specific level (Zhou *et al.*, 2008) Consequently these metals must be defined with reference to chemical criteria, typically in terms of their properties as Lewis acids and bases (Kucuksezgin *et al.,* 2006). Thus heavy metals, as defined by Nieboer and Richardson, are normal constituents of the marine environment (Nieboer & Richardon, 1980).

The present investigation aimed to determine the ecotoxicology of heavy metals in the marine environment of Pakistan through biomonitoring of heavy metal bioavailability.

Pakistan has a coastline of 990km, of which 960km are comparatively free of contamination. At Karachi, 30 km of coastline harbors the biggest industrial & commercial hub and receives a very heavy load of contaminants (Monawwar *et al.*, 1999). Karachi is situated on the southwestern part of Indus delta and northern border of the Arabian Sea. It is the most thickly populated city of Pakistan with a population of over thirteen million and 60% of discharge from Pakistan's industries amounting to 292 million gallons/day of untreated effluents is introduced to the coastal waters through the Lyari and Malir rivers (Beg *et al.*, 1975). The city is the biggest hub of industrial activity having more than 7000 industries at four different sites, Sindh Industrial Trading Estate (SITE); Landhi Industrial Trading Estate (LITE); Korangi Industrial Area (KIA); and the West Wharf Industries (WWI) (Monawwar *et al.*, 1999). These industrial sites produce oil, steel, paints, chemicals, fabrics, pharmaceutical and food products (Quraishee 1975; Naqvi *et al.*, 1993; Zehra *et al.*, 1996). Apart from few well-established industries, which have installed preliminary treatment facilities, all units discharge their wastes directly or indirectly into the Karachi coastal areas.

In the present study, bioaccumulation pattern of Cu, Fe, Mn, Ni, Pb and Zn, were targeted in oysters inhabiting the estuarine and coastal waters around Karachi coast. The investigation pertains to three sampling locations namely: Gizri (24°47'54.53"N, 67°4'58.30"E), Korangi creek (24°46'41.53"N, 67°9'53.80"E) and Manora (24°48'10.81"N, 66°57'57.74"E) (Fig.1).

Material and methods

Sample collection

For sampling, each site was regularly visited, during low tide, at monthly intervals from November 2005 to October 2006. Samples of oysters were obtained from the rocky habitats of Gizri, Korangi Creek and Manora (Fig.1). Samples were collected, by hand picking, using hammer and chisel, with respect to tidemarks at each location from the littoral and sub-tidal zone, where necessary by snorkeling from similar depths. Surface sediment and epibiota were removed from the shells and the samples were immediately placed in labeled polythene bags, which were then stored in an insulated box with ice cubes and transported to the laboratory.

Laboratory treatment

In the laboratory, the samples were sorted, identified and transferred to deep freezer (–10°C) until the analysis was carried out. The samples were thawed and shucked using stainless steel instruments. The soft tissues were taken out and the fresh weight was determined with an electronic balance. Ten individual oysters from each sampling site were homogenized using a high speed electric blender and 3 replicates (10g each) were made, digested with 10 ml concentrated HNO_3, made up to 50 ml in a volumetric flask with de-ionized water (Zehra, *et al.*, 1996). Digests (or dilutions of digests) were analyzed for Cu, Fe, Mn, Ni, Pb and, Zn, using Atomic Absorption Spectrophotometer (Perkin-Elmer A-Analyst 700, USA) (Naqvi, 1993).

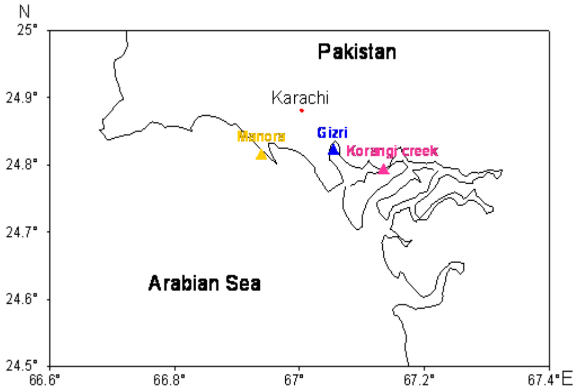

Fig. 1. Map showing the sampling sites and their locations on the coast of Karachi

Statistical analysis

A one way analysis of variance (ANOVA) was used to compare metal concentration. All statistical computations were carried out using MINITAB software version 11.13.

Results

Heavy metals concentrations

The concentrations and distribution levels of six heavy metals Cu, Fe, Mn, Ni, Pb and Zn were recorded in bodies/soft tissues of oysters from Gizri, Korangi creek and Manora. The results are shown in Tables 1-3. The trends of accumulation of heavy metals were also worked out with respect to seasons/months (Figure 2).

Apparent differences were noted in the pattern of accumulation within species, further to that it was also noted that different metals respond differently as described, for instance, in Gizri there were significant differences ($P<0.01$) in the accumulation patterns of Fe, Mn, Cu, Zn and Pb. However no significant differences ($P>0.01$) were noted in the accumulation patterns of Cu and Pb. The study area Korangi creek was also behaved in the similar fashion as far as the accumulation pattern is seen but here no significant difference ($P>0.01$) was observed between Fe and Zn. Pattern of accumulation followed a similar pattern in Manora, but here all metals responded equally.

Table 1. Variation in metal concentrations (μg g^{-1}± SD) in Gizri (Nov. 2005- Oct. 2006).

Sampling months	Fe	Mn	Cu	Zn	Pb
November	11.05±3.4	0.39±0.4	0.12±0.08	12.95±0.45	0.05±0.06
December	18.56±0.8	0.48±0.4	0.25±0.19	13.65±0.39	0.04±0.05
January	20.51±0.9	0.68±0.4	0.33±0.18	13.30±0.65	0.02±0.02
February	15.93±4.5	0.35±0.6	0.23±0.11	9.25±3.80	0.03±0.02
March	14.86±5.9	0.27±0.07	0.16±0.08	12.05±0.84	0.06±0.01
April	14.37±6.2	0.35±0.09	0.20±0.07	12.19±1.67	0.05±0.01
May	13.36±0.5	0.34±0.01	0.29±0.09	12.22±1.79	0.48±0.18
June	12.15±0.5	0.40±0.01	0.32±0.05	12.26±1.80	0.04±0.02
July	15.86±0.6	0.20±0.08	0.29±0.06	12.02±0.10	0.05±0.02
September	17.31±0.9	0.02±0.1	0.09±0.05	11.05±0.14	0.05±0.03
October	23.78±0.8	0.98±0.4	0.02±0.08	6.96±2.26	0.37±0.10

Discussions

Present investigation projects that the oysters all study areas composed of trace metals varying over a wide range of concentrations, most probably in reflection of their metal bioavailability. The probe was targeted to assess the concentration level of six metals (Cu, Fe, Mn, Ni, Pb, and Zn) in oysters. The data presented herein reveals a consistent picture of metal contamination in these organisms with the possible exception of nickel concentration for which no strong contamination gradient is recorded. During the present investigations three species of oysters were monitored to assess their metal load. The results show that *C. glomerata* inhabiting Manora coast (Navigation Channel) carried the highest load of Cu followed by *C. rivularis* from Korangi creek and *C. belcheri* of Gizri (Fig. 2c). The highest concentration of Fe was observed at Manora (Fig. 2a).

Table 2. Variation in metal concentrations (μg g^{-1}± SD) in Korangi creek (Nov. 2005- Oct. 2006).

Sampling months	Fe	Mn	Cu	Zn	Pb
November	13.86±4.28	0.44±0.42	0.05±0.04	8.52±0.27	0.07±0.007
December	10.90±3.98	0.19±0.01	0.10±0.03	7.97±0.30	0.03±0.006
January	7.77 ±1.60	0.22±0.09	0.18±0.02	8.70±7.37	0.01±0.007
February	10.04±0.49	0.12±0.008	0.09±0.04	12.68±0.72	0.05±0.002
March	12.10±3.56	0.48±0.63	0.16±0.06	10.65±2.40	0.04±0.02
April	29.13±0.48	0.59±0.49	0.55±0.38	13.98±0.16	0.18±0.03
May	16.47±3.44	0.77±0.08	0.43±0.03	15.29±0.17	0.21±0.061
June	14.51±2.96	0.29±0.11	0.13±0.08	11.97±0.16	0.05±0.003
July	19.96±1.07	0.27±0.19	0.13±0.07	10.81±0.15	0.06±0.003
September	16.68±1.12	0.21±0.19	0.09±0.01	9.85±0.008	0.06±0.01
October	15.75±3.98	0.38±0.09	0.06±0.01	8.09±0.007	0.07±0.008

Table 3. Variation in metal concentrations (μg g^{-1}± SD) in Manora (Nov. 2005- Oct. 2006).

Sampling Months	Fe	Mn	Cu	Zn	Pb
November	12.81±1.68	0.28±0.009	0.79±0.39	11.99±1.98	0.02±0.004
December	13.36±4.13	0.29±0.018	0.81±0.46	12.49±2.14	0.01±0.004
January	26.95±4.50	0.49±0.008	0.69±0.19	11.95±1.76	0.03±0.003
February	30.92±3.70	0.61±0.008	0.56±0.18	11.75±1.66	0.04±0.027
March	21.33±6.45	0.49±0.29	0.34±0.16	12.70±0.48	0.05±0.028
April	22.51±5.58	0.45±0.30	0.36±0.19	10.69±0.39	0.52±0.021
May	23.80±4.16	0.42±0.17	0.39±0.13	9.071±3.03	0.05±0.029
June	25.86±5.06	0.41±0.18	0.45±0.40	9.00±5.91	0.06±0.03
July	22.92±6.45	0.43±0.20	0.65±0.29	8.70±0.48	0.05±0.028
September	19.38±1.69	0.29±0.18	0.62±0.19	9.01±3.005	0.06±0.029
October	15.29±0.28	0.30±0.20	0.75±0.47	10.79±2.05	0.01±0.002

Oysters of the genera *Ostrea* and *Crassostrea* have been used on a global scale as biomonitors of heavy metals (Rainbow, 1995): for example in the UK, *O. edulis* and *C. gigas* (Bryan *et al.*, 1985; Boyden and Phillips, 1981); in the United states, *C. virginica* and *C. gigas* (Zaroogian *et al.*, 1979; Okazaki and Panietz, 1981; Phelps *et al.*, 1985) ; in Australia *C. gigas* (Ayling, 1974; Thomson 1982) and *C. margaritacea* (Watling and Watling 1976).The accumulation patterns of Cd and Zn have also been recorded from Clear Water Bay, Kowloon, Hong Kong using *C. rivularis* the estuarine oyster as a biomonitor (Wang, 2001).

The present study suggests that the area receives effluents, loaded with these metallic pollutants, particularly from oil, that gets discharged while loading and uploading of ships (Munishi *et al.*, 2004). It becomes obvious that the area of the Lyari outfall is the most polluted followed by the beaches of the Manora channel and then the creeks. Further to that, the Lyari River flows out at Karachi Harbor, through the Manora Channel, its run-off increases in the monsoon season and water is highly polluted (Beg *et al.*, 1975).

Mn concentration

All the species of oyster carried low concentrations of Mn in all the concerned locations. Highest concentrations of Mn were recorded in Korangi creek followed by Gizri and Manora (Fig. 2b). The results suggest that Korangi creek; the worst pollution affected section of Karachi coast receives effluents from domestic, industrial and Export Processing Zone. Further to that, industrial units 2500 in number, including 170 tanneries and two oil refineries, heated effluents by thermal power plant at Ibrahim Haidri, dispose untreated waste into the coastal waters at this site (Annon, 1989; Ahmed, 1977).

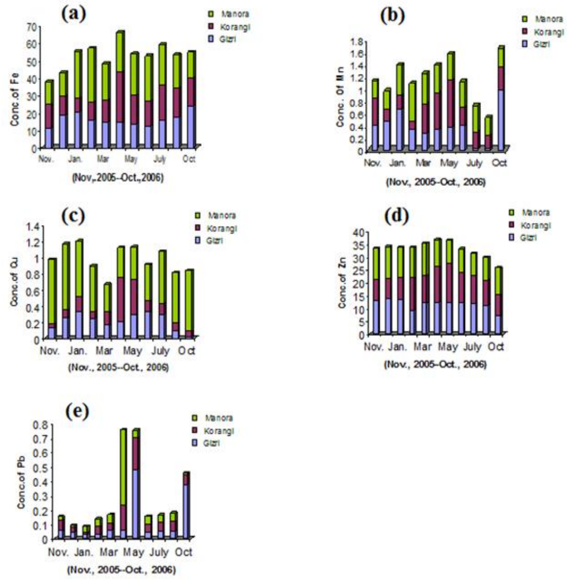

Fig. 2. Variations pattern in metal Concentrations (µg g^{-1}± SD) at Gizri, Korangi creek and Manora from (Nov., 2005—Oct., 2006).

Ni concetration

At least 40 times lower concentration of Ni was found in our coastal areas in comparison with the recommended marine water quality standard for UK, for the protection of marine life (Mance *et al.,* 1984). In the current study, the concentrations of Ni were not detected due to unknown reasons. The highest concentration of Ni was recorded in *Perna viridis* at Karachi Harbour and lower concentrations at Korangi creek (Monawwar *et al.*, 1999). This data is comparable to other parts of the world such as Bombay Harbour (Patel *et al.*, 1985), with higher concentrations of nickel reported in sediment compared to the Karachi and Gwadar coastal areas (Monawwar *et al.*, 1999). In contrast, lower concentrations of Ni were recorded, while using the oyster *C. rhizophorae* in the Potengi estuary Natal, Brazil (Silva *et al.*, 2001). The results suggest that Ni concentrations are almost lower as compared to other metals. Thus nickel concentrations are not apparently above a range that might be considered normal. Such comparisons are predictably somewhat general for it is important to be aware of the potential influence of size, age, sex, reproductive stage and physiological condition on the concentrations of trace metals in oysters (Silva *et al.*, 2001; Phelps *et al.*, 1985).

Pb concetration

The highest concentrations of Pb were recorded in Manora and Gizri whereas lower concentrations were observed in Korangi creek (Fig.2e). Lead is not an essential element and sub-lethal effects of low lead concentration include a depression of the growth (Bryan *et al.,* 1985). However, accumulation of high concentrations of Pb does not apparently harm the animals. A rare instance of lead poisoning was reported among shore birds at Mersey estuary where a large number of birds died. These birds were found having toxicities with Pb, 30-70% in the form of trialkyl Pb that originated from a factory manufacturing tetraethyl Pb additives for petrol (Maddock *et al.,* 1979).

Zn concetration

Since Zn is one of the essential metals in the marine organisms; it increases the enzymatic activity (Vallee, 1978). It has been discussed that the concentration level of Zn in plants and animals is actually higher than those of other trace elements. A few examples point to its toxicity to some species at concentrations commonly observed in seawater and sediment in estuaries (Stauber *et al.*, 1990). According to the present investigation, the highest concentrations of Zn were recorded in Gizri and Korangi creek while the concentration was lower in Manora (Fig.2d). Due to the flushing of wastes in the Karachi Harbour at Gizri and Korangi creeks the highly toxic wastes make their way into the coastal waters of Karachi (Annon, 1989),resulting in the increased levels of pollution and thus our results confirm this trend. The oysters smaller in size have the capacity of higher accumulation of metals and it is important to be aware of the potential influence of size, age, sex, reproductive stage and physiological condition on the concentrations of trace metals in oysters (Páez-Osuna and Marmolejo,1990; Silva *et al.*, 2001). It should be remembered that the oyster (*C. belcheri*) collected from Gizri was smallest in size than those from other study sites and the results highly subscribe to this observation.

Seasonal variations

A number of explanations for seasonal variations of trace metals have been proposed while using bivalve molluscs for heavy metal prone study, for instance, biological or biochemical activity or change in the environmental concentrations, chemical species/forms of the elements, filtration rate, gonadal stages, age and size of the oysters (Ferreira *et al.*, 2005; Páez-Osuna and Marmolejo, 1990). The seasonal variations are also associated with local phytoplankton productivity, an increase in phytoplankton efficiency an increase in bivalve nutritional status, which in turn leads to an increase in metal concentrations in organisms observed (Ferreira *et al.*, 2005). Phillips (1976a) also recognized seasonal fluctuations in heavy metals concentrations while comparing the dry weights with wet weights of common mussel *Mytilus edulis*. Similarly, seasonal variations of the metal concentrations at a given site may often be due to seasonal changes of the organism's tissue weight rather than to any variability in the total metal content of the organism (Ansari *et al.*, 2004).

According to the present investigation, highest concentrations of Cu were observed in Manora during the month of November followed by Korangi creek in April and Gizri in January. The Fe concentrations were highest at Manora during February followed by Korangi in April, and Gizri in October. The highest concentrations of Mn were recorded at Gizri in October followed by Korangi in May and Manora in February. Highest concentrations of Pb were found at Manora during the month of April followed by Gizri and Korangi in May. The Zn concentrations were highest at Korangi during May followed by Gizri in December and Manora in March.

For the present probe, the metal variations are recorded for two seasons i.e. summer (starting from April-September) and winter (from October- March). Our results present the situation wherein concentrations of the metals have been discovered to be high in winter and drop in the concentrations of most metals reported for summer. It is confirmed that Manora, the navigation channel is the most polluted area having highest concentrations of Cu, Fe and Pb. According to Annon (1989), about 2,500 ships visit Karachi Port (Manora Channel) every year and some 15 million tons of cargo are handled, consequently contamination of inshore waters, creeks and bays occur. Several scientists reported that seasonal variations of metals might be observed, during phytoplanktonic blooms, reproductive cycle and weight fluctuations of the organisms (Regoli and Orlando 1994a and b; Cain and Luoma 1986; Boyden and Phillips 1981). In addition to that physiological state of the gonads, due to the penetration of gonadic tissues into the digestive gland during gametogenesis, may biologically dilute metal concentrations in mussels from both of the polluted and unpolluted areas of organisms. The present investigation also suggests that most of the metal concentrations observe winter maximum and summer pre-spawning minimum. In comparison to Manora, the salt water creeks, also called tidal creek areas Ahmed (1977), such as Gizri and Korangi reflect low concentration levels of metals. Seasonal variations are also attributed to various issues like salinity changes, growth and somatic tissue and food storage products (Phelps *et al.*, 1985; Wang, 2001; Silva *et al.*, 2006). A decrease in salinity results in a higher metal uptake, as predicted by the free ion activity model due to the less competition with the chloride ion. Our results also observe this trend.

This research demonstrates that regions subjected to intense industrial and anthropogenic activities are more contaminated regions of the Karachi coastal areas, and that coastal population encroachment and sewerage system plays an important role in the increase of metal concentration levels.

Finally, given the geographical distribution and their ability to put together the seasonal variations in bioavailability, it can be concluded that all the three oyster species: *C. belcheri C. rivularis* and *C. glomerata* could be served as appropriate tools for biomonitoring research. These species could be employed as a resource for investigation, directed towards monitoring changes in the marine environment.

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