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An Assessment of the Bivalve *Perna viridis*, as an Indicator of Heavy Metal Contamination in Paradise Point of Karachi, Pakistan

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Abstract. The edible bivalves *Perna viridis* (green mussel), (n = 100) were analysed for their total Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn concentrations to indicate heavy metal contamination in Paradise Point of Karachi coast using atomic absorption spectrophotometer. There are large seasonal variations in the metal concentrations of Mn (0.025-0.67 µg/g), Fe (0.055-7.740 µg/g), Ni (0.004-0.52 µg/g), Hg (0.0001-0.004 µg/g), Zn (0.04-3.32 µg/g), Cu (0.008-1.66 µg/g), Pb (0.022-2.43 µg/g), Co (0.01-0.044 µg/g), Cd (0.04-0.88 µg/g) and Cr (0.13-1.20 µg/g) recorded in bodies/soft tissues of *P. viridis* obtained in the samples of the year 1993 and 2012 at the Paradise Point of Karachi coast. The results of heavy metals are in the following descending order of concentration in the samples collected in the year 1993: Fe>Cr>Zn>Mn>Pb>Cd>Cu>Ni>Co>Hg, while Fe>Zn>Pb>Cu>Cr>Cd>Mn>Ni>Co>Hg order was recorded in samples collected in the year 2012. The high accumulation of metals was found mostly in the samples collected in the year 2012 when compared with the samples of the year 1993. This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. The concentrations of these heavy metals were lower than the permissible limits for human consumption. However, if this pollution persists, it can prove to be very detrimental in future.

Keywords: Paradise Point, heavy metals, pollutants, industrial wastes, domestic wastes

Introduction

Marine pollution is a major threat to the health of millions of people, marine animals and plants. The pollution, especially of the metals (either heavy or trace) poses a direct threat to marine life and ultimately the human health (Qari and Siddiqui, 2004). The main sources of pollution in Karachi coastal water includes domestic and industrial waste, tanneries effluents, rainfall and associated pollutant from runoff, shipping and agricultural sources (Qari and Siddiqui, 2008). The rapid industrialisation and urbanisation of the city has altered the quality of the environment and created ecological disturbances and associated problems for the local community (Siddiqui *et al*., 2009).

Pollution in the Karachi coastal region is mainly attributed to the Lyari and Malir rivers, which are served by various channels of untreated domestic and industrial waste, carrying more than 300 million gallons per day (MGD) (1,125,000 m^3/day) of the untreated effluent of more than 6,000 industries. These rivers ultimately drain into the beaches of the Arabian sea (Hasnie and Qureshi, 2002; Rizvi *et al*., 1988). It is estimated that about 300 MGD of wastewater is generated in Karachi out of which only 40 MGD is treated, rest of the water waste and the treated effluents are discharged into the sea and creating harm to marine environment (Khattak *et al*., 2012). Thus, it has become important to estimate the heavy metal concentration in organisms which act as bio-accumulators in sediments, in overlying water, in fresh water, estuarine and marine environment because many of these organisms are indicator of metal contamination. Bio-monitoring by employing living organisms such as mussels, which is an economically important bivalve with outstanding potential as sentinel organism (Qari *et al*., 2015) and as sensor plays a vital role in governmental and industrial strategies to identify, assess, control, and reduce pollution problems (Krishnakumar *et al*. 1995; 1994). The intertidal portion of the exposed rocky shore at Paradise Point, the present study site, harbours a large population of *P. viridis*. The present

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investigation provides first-hand basic information about many aspects of bioecology of green mussel (*P. viridis*) from northern Arabian Sea. The aim of this present study is to determine the levels of contaminants in *P. viridis* from exposed shore of Paradise Point, Karachi coast at low tide and possible health risks to the consumers. The study also examines an increase of metal concentration levels with time in Paradise Point of Karachi coast.

Materials and Methods

Sampling. The green mussel, *P. viridis* (n = 100) were sampled from exposed shore of Paradise Point, Karachi coast (Fig. 1) at low tide in the year 1993 and 2012 for comparison of the data of two different periods. All the samples collected were carefully cleaned from mud debris and other epiphytes with filtered seawater, in the laboratory. Digestion of dried samples was carried out as described by Denton and Burdon-Jones (1986).

Sample preparation. *Perna viridis* samples were dried at 70 °C for 24 h till a constant weight was achieved. The samples were then homogenised with a porcelain pestle and mortar to a powder form, sieved and stored in plastic bottle until further analysis. Digestion of *P. viridis* samples was carried out as described by Denton and Burdon-Jones (1986). Triplicate samples of *P. viridis* (1 g) was digested with concentrated nitric acid (4 mL) and concentrated perchloric acid (2 mL) in 50 mL Teflon beaker (prewashed with nitric acid solution) covered with lid at 80 °C on a hot plate.

Analysis. After digestion and evaporation of acid, metal salts were re-dissolved in metal free deionised water and the final volume was made up to 100 mL in volumetric flask. Standards were prepared in deionized water from stock standard AA solution (May and Baker Ltd., Dagenham, England). Reagents blank were treated similarly as samples using same volume of acid and deionised water. In digested samples of *P. viridis*, concentrations of Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd and Mn were measured by Atomic Absorption Spectrophotometer.

Results and Discussion

There are large seasonal variations in metal concentrations of Mn (0.025-0.67 µg/g), Fe (0.055-7.740 µg/g), Ni (0.004-0.52 µg/g), Hg (0.0001-0.004 µg/g), Zn (0.04-3.32 µg/g), Cu (0.008-1.66 µg/g), Pb (0.022-2.43 µg/g), Co (0.01-0.044 µg/g), Cd (0.04-0.88 µg/g) and Cr (0.13-1.20 µg/g) recorded in bodies/soft tissues of *P. viridis* collected as samples in the year 1993 and 2012 at the Paradise Point of Karachi coast (Fig. 2-11). The values of Fe, Zn, Cu, Cd and Pb concentrations in *P. viridis* collected from the exposed shore of Paradise Point of Karachi coast were compared to permissible limits from established guidelines for food safety (FDA, 2001;

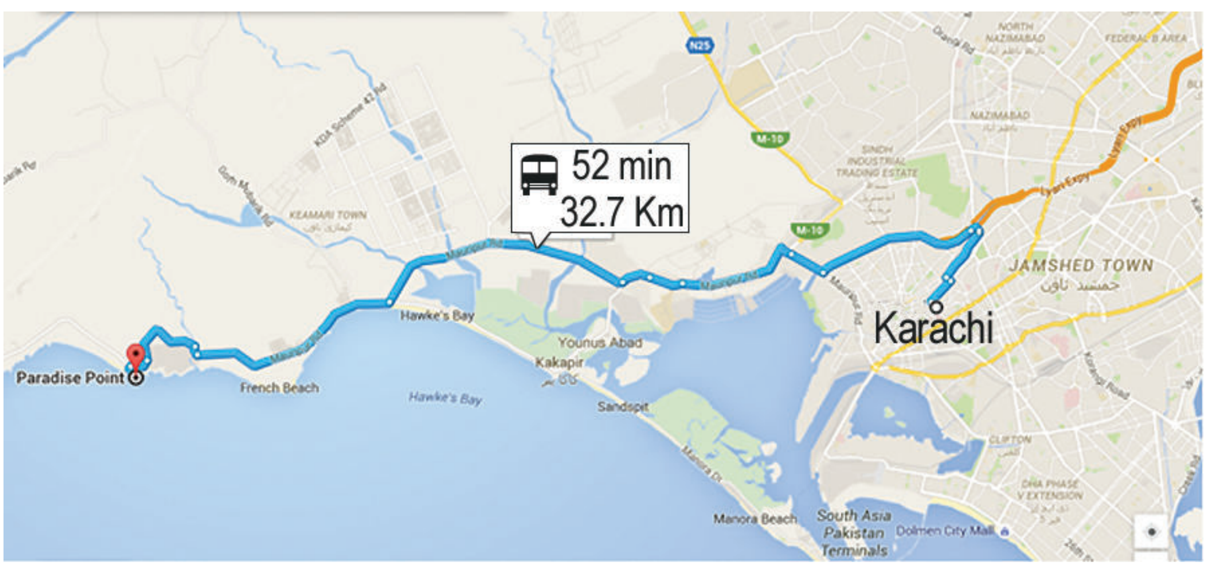

Fig. 1. Map of the Karachi coast showing location of the sampling beach (Paradise Point), source: www. google.com

WHO, 1982) and maximum permissible limits established by Malaysian Food Regulations (MFR, 1985) (Table 1). The observed concentrations of these metals were within the maximum permissible level (MPL) as shown in Table 1. However, if this pollution persists, it can prove to be very detrimental in future.

All the studied metal levels (Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn) found in the green mussels, *P. viridis* collected from exposed shore of Paradise Point, Karachi coast at low tide in the year 2012 were higher than those detected in mussels collected in 1993. The results are shown in (Fig. 2-11). This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. The results of heavy metals concentrations in the following descending order in the samples collected in the year 1993 were Fe>Cr>Zn>Mn>Pb>Cd>Cu>Ni>Co>Hg, while Fe>Zn>Pb>Cu>Cr>Cd>Mn>Ni>Co>Hg were recorded in samples collected in the year 2012. The results of the present study have therefore, confirmed that the green mussels (*P. viridis*) have greater capacity for accumulation of metals.

The large seasonal variation recorded in the metal concentrations from the exposed shore of Paradise Point, Karachi coast in the year 1993 and 2012 could be as a result of upwelling on the composition of coastal water. High concentrations of metals also indicate anthropogenic influences (Qari and Siddiqui, 2004). The present ranges of concentration of metals (Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn) were higher when compared with the baseline composition of seawater (Riley and Skirrow, 1975; Horne, 1969). This could be due to the direct discharge of industrial and domestic wastes (along the coast) by Lyari river and the riverborne trace metals. The erosion of the shores at the bottom, diffusion from shelf sediments and deposition of atmospheric particulate also increased the metal concentration levels. Another source of pollution is Karachi atomic nuclear power plant located near the Paradise Point. This heavy water reactor has a generating capacity of 137 megawatt. It uses 0.15 million gallons of seawater per minute for cooling purpose. Chlorine is used for the control of bio-fouling problems in this plant, which discharge stack of gases in the atmosphere and liquid wastes, radioactive substances, a number of heavy metals and heated water sub-tidally on the nearby rock shore.

The trends of accumulation of heavy metals were also worked out with respect to seasons/months at low tide in the year of 1993 and 2012 for comparison of the data of two different periods. For the present probe, the metal variations are recorded in two seasons i.e., summer (starting from April-September) and winter (from October-March). The study showed that the concentration levels of most of the metals recorded in the soft tissues of *P. viridis* varied from season to season, year to year and even month to month. The highest concentration of Mn (0.67 µg/g) was measured in the month of October 2012 and lowest (0.025 µg/g) in the month of May 1993 as shown in (Fig. 2). The maximum concentration of Fe (7.740 µg/g) was measured in August 2012 and minimum level (0.055 µg/g) in July 1993 as shown in (Fig. 3). The highest Ni concentration (0.52 µg/g) was recorded in August 2012 while the least (0.004 µg/g) was recorded in July 1993 as shown in (Fig. 4). The maximum concentration of Hg (0.004 µg/g) was measured in February 2012 and the minimum level (0.0001 µg/g) in October 1993 as shown in (Fig. 5). The highest concentration of Zn (3.32 µg/g) was measured in June 2012 while the least concentration (0.04 µg/g) were observed in May 1993 as shown in (Fig. 6). The maximum concentration of Cu (1.66 µg/g) was recorded in November 2012 and

Table 1. Comparison of different heavy metals (Fe, Zn, Cu, Cd and Pb) concentrations in soft tissue of *P. viridis* sampled from Paradise Point of Karachi with other sampling locations from previous studies and International regulation value for food

Sampling area	Cu	Cd	Zn	Pb	Fe
			(µg/g)		
Present study (Paradise Point, Karachi, Pakistan)	0.008-1.66	0.04-0.88	0.04-3.32	0.022-2.43	0.055-7.740
Certified reference material (CRM)	2.34±0.16	0.043±0.008	25.6±2.3	0.065±0.007	142±10
Maximum permissible levels (MPL) (WHO, 1982)	10	2	100	5	-
Maximum permissible levels (MPL) (FDA, 2001)	100	0.2	150	1.5	-
Maximum permissible levels (MPL) (MFR, 1985)	30	1	100	2	-

the least (0.008 µg/g) in May-July 1993 as shown in (Fig. 7). The highest Pb (2.43 µg/g) concentration was detected in September 2012 and the lowest (0.022 µg/g) in May 1993 as shown in (Fig. 8). Co and Cd had the highest levels (0.044 µg/g, 0.88 µg/g) in May and July 2012, respectively, and lowest (0.01 mg/L, 0.04 mg/L) in January1993 as shown in (Fig. 9) and (Fig. 10). The highest mean of Cr (1.20 µg/g) was detected in February 2012 and the lowest (0.13 µg/g) in February 1993 as shown in (Fig. 11). Mostly, the high concentration of metals was found in summer and low in winter (Figs. 2-11).

Higher concentrations of heavy metals like Fe, Pb, Zn, and Cu in the green mussel (*P. viridis*) had been reported by Qari *et al*. (2015); Ali *et al*. (2014); Sasikumar *et al*. (2006); Krishnakumar *et al*. (1998) and Sankaranarayanan *et al*. (1976), while Fe accumulation was highest among the metals studied. Similarly, higher concentrations of heavy metals like Pb, Cd, Cr and Cu were also observed in *P. viridis* as reported by Blackmore and Wang (2003) and Shin *et al*. (2002). The port and several industries are located in the Karachi city. Untreated or partially treated industrial and domestic waste waters which are discharged directly or carried by several streams into the Karachi coastal water could be attributed to the higher concentrations of heavy metals such as Fe, Zn, Pb, Cd, Cr, Cu and other heavy metals recorded in the soft tissues of *P. viridis* in Paradise Point of Karachi. Studies conducted by Qari and Siddiqui (2004) showed that concentrations of Mn, Cu, Zn, Cr and Pb and other metals in coastal areas are believed to be elevated due to discharge of considerable quantities of untreated and partially treated domestic and industrial wastes.

The concentration of Fe was the highest as compared to other heavy metals at low tides in both years i.e., 1993 and 2012 (Fig. 3). It is assumed that high input of metals and other inorganic and organic substances are coming in the form of industrial and domestic wastes in Paradise Point, Karachi coast. These anthropogenic sources play a major role in increasing the concentration of Fe in Paradise Point, Karachi coast. The main sources of Fe are mostly from the industries situated in the coastal area. The pollutants from the industries are directly poured into the stream from where it has been mixed with the sea water. The low residence time in oxidizing environment favours the rapid precipitations of iron (Khattak *et al*., 2012). The observed highest concentration of Fe in the soft tissue of *P. viridis* in the two periods (1993 and 2012) when compared to other heavy metals detected in the soft tissues of the green mussels was clearly indicating the tendency of organism in accumulating higher concentration of Fe in soft tissue. This might also be due to the major role played by this essential metal in catalysing various enzymatic activities (Kamaruzzaman *et al*., 2011).

High concentrations of Cu, Zn, Cr, Pb and other metals detected in *P. viridis* sampled at the exposed shore of Paradise Point, Karachi coast in the two periods (1993 and 2012) are also due to harbour activities such as dredging and cargo handling, the dumping of ship waste and other coastal activities (Qari and Siddiqui, 2008). It was also reported by Ansari *et al*. (2001) that the concentration of Cu, Ni, Zn, Pb, Cr and Cd were high in sludge samples of industrial area. Considering these facts, the present study asserted the research findings by those authors (Ansari *et al*., 2001). According to Qari and Siddiqui (2008), coastal areas with heavy metal pollution needs special attention because continuous pollution inventories will have adverse effect in terms of increase in toxicity levels of the marine food chain, stress on marine plants and animals, adverse effect on the health of inhabitants such as fisher men and bathing tourist. Urgent attention is needed to combat pollution in marine environment of Karachi coast.

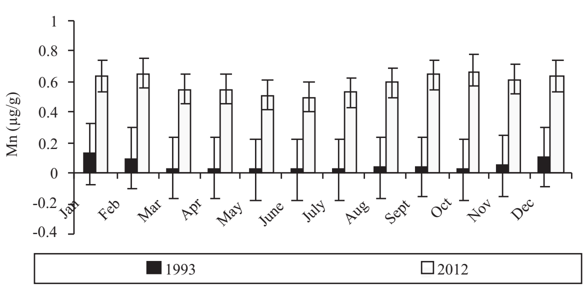

Fig. 2. Mn concentration in *P. viridus* in two different periods.

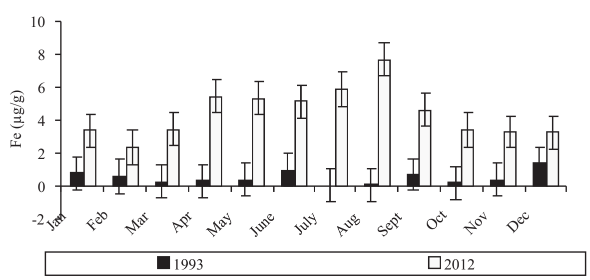

Fig. 3. Fe concentration in *P. viridus* in two different periods.

The highest concentration in cadmium (Cd) causes several health problems in human. Cadmium and its compounds along with mercury and some other dangerous metals are, however, included in the blacklist. It is being used routinely in different industrial processes and its potential hazard to life form is predominant. Eating food or drinking water with very high cadmium levels severely irritates the stomach, leading to vomiting and diarrhoea, and sometimes death. Eating lower levels of cadmium over a long period of time can lead to a build-up of cadmium in the kidneys. If it reaches a high enough level in the kidney it will cause kidney damage, and also causes bones to become fragile and break easily. Cadmium (Cd) is widely distributed at low level in the environment and most foods have an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level (FDA, 2001). Ololade *et al*. (2008) reported that Cd level is almost 10 times higher in shell fishes than in fin fishes. The transport of fertilizer in nearby areas to the estuaries by leaching and erosion as a result of agricultural activities apart from fishing by the village folks could be responsible for the higher level of Cd in shell fishes than in fin fishes. On the other hand, it has been observed that bivalves do not regulate Cd in their body tissues. According to Li *et al*. (2006), bivalves do not regulate Cd therefore, accumulate this element in their body. In view of this reason, it is plausible that bivalves such as *P. viridis* might not be able to regulate Cd in their body.

Mercury (Hg) is one of the heavy metals in the marine ecosystem that are of great concern if present at an elevated level as it could have hazardous impacts due to its toxicity (Yap *et al*., 2007). Evidence of this concern to public health was given by other researchers reporting on Hg contamination in the biota related to human beings (Zhou and Wong, 2000; Moraes *et al*., 1997; Leah *et al*., 1982). However, the concentration of Hg was the lowest as compared to other heavy metals analysed in present investigation in the two periods (1993 and 2012). The low concentrations of Hg detected in the soft tissues of *P. viridis* in the present study, could be due to the fact that metallothionein might play a role in regulating the Hg excretion as well as storage (Roesijadi, 1980). The lowest Hg levels observed in the present study could also be due to the metal-binding proteins being present at much lower concentrations in *P. viridis* body tissues. The observed low concentration of Hg in the soft tissue of the organism was clearly

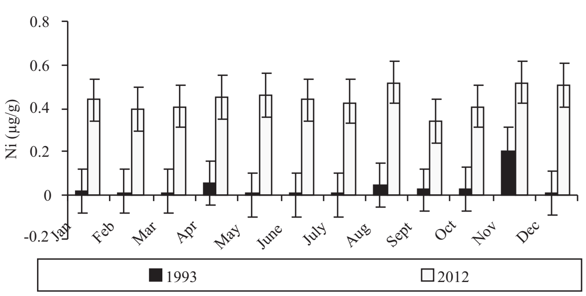

Fig. 4. Ni concentration in *P. viridus* in two different periods.

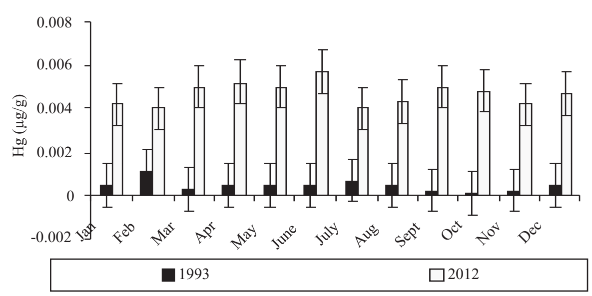

Fig. 5. Hg concentration in *P. viridus* in two different periods.

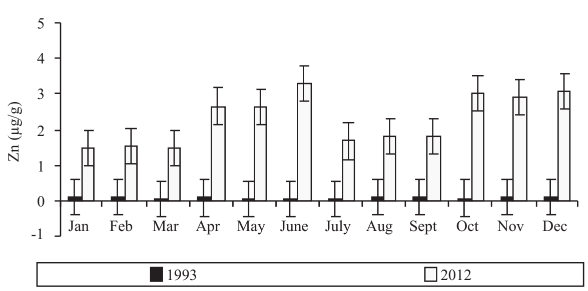

Fig. 6. Zn concentration in *P. viridus* in two different periods.

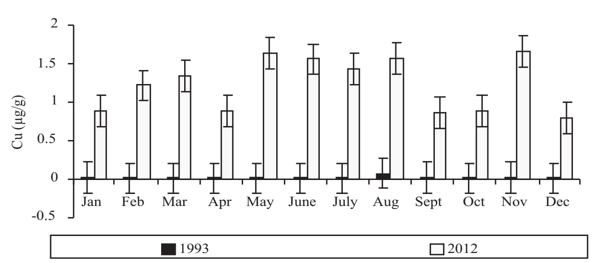

Fig. 7. Cu concentration in *P. viridus* in two different periods.

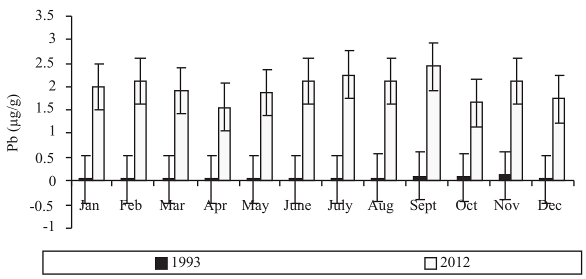

Fig. 8. Pb concentration in *P. viridus* in two different periods.

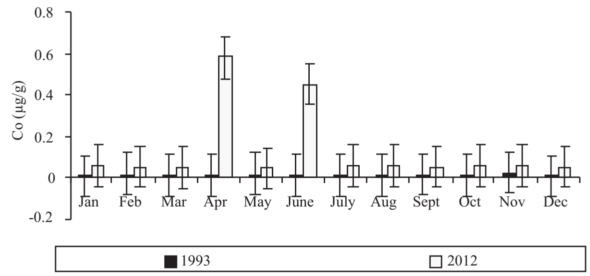

Fig. 9. Co concentration in *P. viridus* in two different periods.

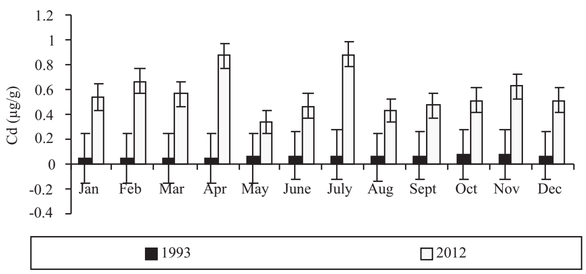

Fig. 10. Cd concentration in *P. viridus* in two different periods.

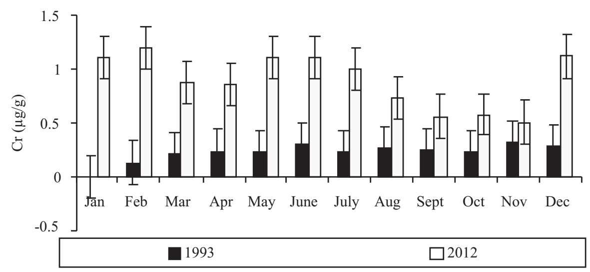

Fig. 11. Cr concentration in *P. viridus* in two different periods.

indicating the tendency of organism in accumulating lower concentration of Hg in soft tissue. In a study conducted by Usero *et al*. (2005), Hg showed the lowest values among all the metals studied on heavy metal concentrations in molluscs from the Atlantic coast of southern Spain.

The data generated from this study, showed that the concentrations of metals in the mussels collected from the Paradise Point are increasing with time. Obviously, the present investigation recorded highest concentrations of heavy metals in the year 2012 when compared to the year 1993. On a normal basis, it was expected that the levels of the heavy metals in 2012 would have drop drastically after the first detection levels of the heavy metals in the year 1993 but the reverse was the case. This is an indication that the Paradise Point of the Karachi coastal areas is subjected to intense industrial and anthropogenic activities and that coastal population encroachment and sewerage system plays an important role in the increase of metal concentration levels. Besides, there is an accelerating accumulation of toxic metals and gases in atmosphere, irrigation water and agricultural soils while the industrial estates of Karachi are discharging large quantities of effluents of organic matter, heavy metals, oil, greases, liquid and solid wastes into Malir and Lyari rivers, which are causing serious environmental degradation (Khattak *et al*., 2012) to various ecosystems of the city.

Conclusion

The concentrations of these heavy metals were within the maximum permissible level (MPL) and should result in no acute toxicities of the metals since they were lower than the permissible limits for human consumption. In addition, these metal concentrations were also considered to be low when compared with regional data based on *P. viridis* as a biomonitoring agent. However, the data generated from this study, showed that the concentrations of metals in the mussels collected from the Paradise Point are increasing with time. Obviously, the higher accumulations of metals were found mostly in the samples collected at the Paradise Point of Karachi coast in the year 2012 when compared with the samples collected in the year 1993 at the same sampled site. This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. In view of this, continued monitoring of heavy metals from Paradise Point of Karachi coast should be undertaken to avoid reduction in the valuable export of bivalves and shrimps as well as the reduction of marine life in the coastal waters.

The most important large industries in Karachi involve metal and non-metal manufacturing textiles, tobacco, food and beverages, chemicals, paints, rubber, paper and paper product, pharmaceutical and product of coals and oil that contributes approximately 99% of the total industrial pollution (Khan and Saleem, 1988; Haq, 1976) which resulted to a considerable trace metal contamination of the marine environment. Immediate measures to control the indiscriminate discharge of effluent and domestic sewage directly or indirectly in to the sea need to be addressed. In addition, regular monitoring programmes of marine pollution are essentially required and need to be well implemented. If this pollution persists, it can prove to be very detrimental in future. The use of green mussel (*P. viridis*) as a suitable biomonitor for heavy metals concentration could be employ in programmes designed to identify, assess, control, and reduce pollution problems in the coast of Paradise Point of Karachi.

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