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FULL PAPER TAM MAKALE

TRACE METAL CONCENTRATİONS İN THE GREENLİPPED MUSSEL *Perna viridis* (Linnaeus, 1758) COLLECTED FROM MAHESHKHALİ CHANNEL, COX'S BAZAR, BANGLADESH

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Abstract: The green-lipped mussel *Perna viridis* (L.) were collected from four sampling locations of the Maheshkhali channel, Cox's Bazar, Bangladesh to determine the concentration level of zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb). The concentrations (µg/g dry weight) of these trace metals ranged from 28.12 to 33.82 for Zn, 7.26 to 8.81 for Cu, 0.04 to 0.08 for Cd and 0.19 to 0.75 for Pb. The concentrations of trace metals in the mussel tissue were found at lower level than the permissible limits for human consumption. In addition, these metal concentrations are also considered to be low when compared with regional data using *P. viridis* as a bio-monitoring agent.

Keywords: *P. viridis*, Bio-monitoring, Trace metals, Maheshkhali channel, Bangladesh

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Öz:

Maheshkhali Kanalı, Cox's Bazar, Bangladeşten Toplanan Yeşil Dudaklı Midye (*Perna viridis*, Linnaeus, 1758)'de İz Metal Konsantrasyonları

Maheshkhali kanalı (Cox's Bazar, Bangladesh)'te bulunan dört örnekleme istasyonundan, çinko (Zn), bakır (Cu), kadmiyum (Cd) ve kurşun (Pb) konsantrasyon düzeylerinin belirlenmesi amacıyla yeşil dudaklı midye *Perna viridis* (L.) örnekleri toplanmıştır. Bu iz metallerin konsantrasyonları (µg/g kuru ağırlık) Zn için 28.12-33.82, Cu için 7.26-8.81, Cd için 0.04-0.08 ve Pb için 0.19-0.75 aralığında değişmektedir. Midye dokularındaki iz metal konsantrasyonları, insan tüketimi için izin kabul edilebilir limitlerin altında bulunmuştur. Buna ek olarak, bu metal konsantrasyonlarının, *P. viridis*'i biyogözlem ajanı olarak kullanan bölgesel veriler ile karşılaştırıldığında da düşük olduğu söylenebilir.

Anahtar Kelimeler: *P. viridis*, Biyogözlem, İz metaller, Maheshkhali kanalı, Bangladeş

Introduction

Most of the coastal area of the world has been reported to be threatened by different sources of contaminants and the major concerns are included persistent organic pollutants, nutrients, oils, radio-nuclides, trace metals, pathogens, sediments, litters and debris (Williams, 1996). Most of the contaminants are interrelated and jeopardize the environment and aquatic organisms at a same way (Phillips, 1985). Therefore, the control of aquatic pollution has been identified as an immediate need for sustainable management and conservation of the existing fisheries and aquatic resources.

The presence of trace metals in the aquatic ecosystem is of major concern because of their heavy toxicity, bio-accumulating tendency in the biota (Siddique and Aktar, 2012; Siddique et al., 2012). Trace metal pollution could be a threat to the aquatic ecosystems and as well as for human life (Liu and Kueh, 2005). Bio-monitoring of metallic pollution in the aquatic environment has been done by many scientists using different aquatic organisms that have higher metal accumulating tendency. Many intertidal species and benthic fauna have been used as bio-monitor agent of trace metal pollution in worldwide (Liu and Kueh, 2005). These were included the green mussel *Perna viridis* (Phillips, 1985; Chan, 1988; Chong and Wang, 2000; Blackmore and Wang, 2002; Nicholson and Szefer, 2003; Liu and Kueh, 2005), oysters *Saccostrea cucullata* and *Saccostrea glomerata* (Phillips, 1979) and barnacles *Balanus amphitrite* and *Tetraclita squamosa* (Rainbow and Smith, 1992; Blackmore, 1996; Blackmore and Chan, 1998). The green-lipped mussel *P. viridis* is widely distributed in the coastal waters of the Asia-Pacific region (Tanabe, 2000). A good number of studies have been used *P. viridis* as a bio-monitoring agent for trace metals investigation since *P. viridis* are sedentary long-lived organisms, easily identified and sampled, reasonably abundant, have good net accumulation capacities and tolerant ability in environmental fluctuations (Yap et al., 2004).

The determination of contaminant levels in mussel provides a means of assessing the possible toxicant risk to public health. *P. viridis* is widely consumed bivalve in the south Asian countries (Phillips, 1985; Farrington et al., 1987). The local indigenous people of the Maheshkhali Island collect this species for consumption from the inter-tidal zone of this channel system. The Maheshkhali channel is very important as a large fishing ground and a centre for recreation. On the other hand, the Bakkhali River opens into this channel, which brings much of the domestic, agricultural and industrial wastes. A good number of fishing trawler and mechanized boat and speed boats move through this channel every day. Moreover, many passenger boats from different places come to the jetty along the Maheshkhali channel. Therefore, oil spill and unused fuel from these vehicles finally goes into the water and continuously polluted the water which might result in increasing trace metals in the water column of the Maheshkhali Channel. Moreover, *P. viridis* can easily accumulate trace metals in their body from the ambient water and food. Some metals, such as Cd and Pb, have long been known to accumulate within the aquatic food chain. Since

Cd, Cu, Pb and Zn are widely distributed in the coastal environment, both from natural geological processes and anthropogenic activities. Mussels are well known to accumulate a wide range of contaminants in their soft tissues (Goldberg et al., 1978). The intertidal areas are the natural habitats of marine mussels, but they are usually close to estuaries. Therefore, the chance of exposure to several contaminants from the land-based activities is high through the riverine system as well as sea-based sources.

Marine mussels provide a cheap source of protein for human consumption. For *P. viridis*, it had been reported that there was about 60% protein in every 100 g (dry weight) of mussel soft tissues (Choo and Ng, 1990). From the nutritional point of view, the mussel is an important food source for supplying essential trace metals (e.g. Ca, Fe) and certain vitamins such as niacin, thiamine and riboflavin (Cheong and Lee, 1984). From the toxicological point of view, excessive consumption of metal-contaminated seafood may cause toxicity to humans. Since trace metals are inorganic chemicals that are non-biodegradable, cannot be metabolized and will not break down into harmless forms (Kromhout et al., 1985), the measurement of levels of metals in the soft tissues of *P. viridis* is becoming more significant. They can simply accumulate through time, becoming less and more of a toxic threat as their concentrations increase. Levels of metals above the permissible limits would certainly create a notorious food image to the consumers. Chronic exposure to trace metals such as Cu, Pb and Zn is associated with Parkinson's disease and the metals might act alone or together over time to cause the disease (Gorell et al., 1997). However, the major objective of this study was to determine the concentrations of trace metals (Cd, Cu, Pb and Zn) in the soft tissues of *P. viridis* collected from the Maheshkhali channel, Cox's Bazar and the present study was also aimed to investigate whether these metals are within the permissible limits for human consumption.

Materials and Methods

Study Site

The Maheshkhali channel is located at the south-eastern coast of the Bay of Bengal (Figure 1). Maheshkhali channel heavily influenced by monsoonal wind and the geographic location of the study area were 21° 27′ to 21° 31′ N and 91° 54′ to 91°56′ E. Four sampling sites were selected at Guruk Ghata area considering the abundance of green mussel *P. viridis* and due to trace load of domestic effluents and other pollutant sources. The samples were collected from January to February 2012. During the sampling period water temperature were recorded ranged from 21.0-21.5°C, salinity 29.20-30.10 ppt. and water transparency were observed 28.80-29.50 cm.

Sample collection

Temporal variations in the bioavailability of trace metals in the marine environment are affected over time of total ambient metal load. Therefore, trace metal bio-monitoring needs to conform to certain required characteristics, not least being metal accumulators. Sampling was conducted from a rented boat with local boatman to assist collecting green mussel (*P. viridis)*. Green mussels were randomly collected from each sampling sites at the low tide. Mussel samples were cleaned to rid of debris sessile flora and fauna with seawater in sampling area. Total 36 mussels were collected and kept cool in an ice-box in the field. Upon return to the laboratory, specimens were immediately frozen at -20° C prior to tissue preparation.

Sample preparation and analysis

In the laboratory, the whole soft parts of the mussel were separated carefully from the shell to avoid metal contamination. The soft parts of each mussel were homogenized in a glass blender with a stainless steel cutter and divided into two parts. One part was used for determine the moisture content by drying at 80-90^{0}C for 48 hours or until a constant weight was obtained to convert wet weight to dry weight and the other part was used for trace metal analysis by acid digestion.

Extraction of trace metals from mussels

For the digestion of mussel samples, some 10.0 g of dried and homogenized mussels were weighed and placed into an acid washed PTFE digestion vessel. The digestion of total mussel (fraction <63 µm) were performed with a mixture of HNO_3-HCl-HF (concentrated, Merck) at hot plate and cooling system (US EPA, 1999). Sample solutions were analyzed for trace metals following flameless atomic absorption spectrophotometer (Hitachi Z 9000).

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Preparation of working solutions

Dilute the standard solutions to a concentration about ten times that which was determined in samples (Table 1). Dispense measured aliquots of solution (say, 2, 6, 8 and 10) into small volumetric flasks (25 ml) in order to cover the working range for AAS, using a pipette and clean volumetric flask. Make up to the mark with 5% nitric acid as before. Record the concentrations of these in µg/L.

Table 1. Standard solution for each element

Element	Spectral line/nm	Concentration (ppm)	Method
Zn	248	0.2, 0.6, 1.0	Abs
Cu	279	0.5, 1.0, 1.5	Abs
Cd	229	0.5, 1.0, 1.5	Abs
Pb	325	0.5, 1.0, 1.5	Abs

Table 2. Detection limits for the selected trace elements by Atomic Spectroscopy

Element	Spectral line/nm	Flame	Method	Detection limit (ppm)
Zn	214	C_2H_2-air	Abs	0.008
Cu	325	C_2H_2-air	Abs	0.001
Cd	229	C_2H_2-air	Abs	0.0005
Pb	283	C_2H_2-air	Abs	0.01

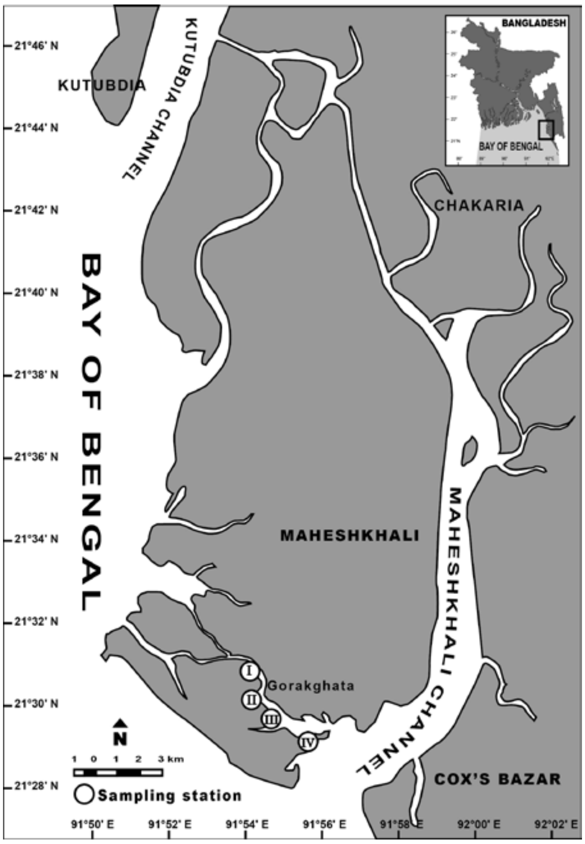

Figure 1. Sampling locations of the study area of Maheshkhali Channel, Cox's Bazar

Construction of calibration plots

For the determination of calibration plot of absorbance flame atomic absorption spectrophotometer (Hitachi Z 8230; the model SSC 300) for Zn and Cu and flameless atomic absorption spectrophotometer (Hitachi Z 9000) for Cd and Pb were used. The wavelengths of the most sensitive lines from the hollow cathode lamps (HCL) were 228.8, 217.0, 213.9 and 324.7 nm for Cd, Pb, Zn and Cu, respectively, and adjusted to the correct sensitivity according to the manufacturers' instructions.

Recovery percentage of analysis

About 2 g homogenized dry meat of green mussels were divided in to two parts. The first sample part (1 g dry weight) was added with 1000 µg of each Cd, Pb, Zn and Cu. The other parts of sample (1 g dry weight) were not added with Cd, Pb, Zn and Cu. Both of sample parts were digested in the process of trace metals extraction in green mussel and the filtrate solutions were measured by flame atomic absorption spectrophotometer (Hitachi Z 8230 the model SSC 300) for Zn, Cu and flameless atomic absorption spectrophotometer (Hitachi Z 9000) for Cd and Pb. The concentrations of each trace metal in both samples were compared to determine the percent recovery of the analysis.

Detection limits and accuracy

Detection limits for a number of common elements were determined by flame atomic absorption and compared them with those obtained with other atomic absorption methods (Table 2). Under usual conditions, the relative error of flame absorption analysis was 1-2%.

Data analysis

All calculations were based on dry weight of tissue. Mean concentration of the metals and standard deviation were estimated using the Statistical Package for Social Science (SPSS 16.0) program. All the statistical significance was tested at 95% confidence level.

Results and Discussion

The trace metal concentrations in the soft tissue of *P. viridis* were slightly varied among the stations. The concentrations of Zn, Cu, Cd and Pb in the mussels collected from different locations of Maheshkhali Channel are shown in table 3. The mean concentrations ranged from 28.12-33.82 µg/g (dry weight basis) for Zn, 7.26-8.81 µg/g (dry weight basis) for Cu, 0.04-0.08 µg/g (dry weight basis) for Cd and 0.19-0.75 µg/g (dry weight basis) for Pb (see table 3). The variation of mean concentrations of trace metals among the stations are shown in Figure 2.

The guideline on trace metals for food safety set by different countries are presented in Table 4. In comparison with the permissible limit set by the Ministry of Public Health of Thailand (MPHT, 1986), all the mean values of the present study (µg/g, dry weight basis) from all the sampling stations were lower than the limits. The concentration levels were also lower than the recommended guidelines for Cd, Pb, Cu and Zn set by the USFDA (1990), the Australian Legal Requirement for food safety (NHMRC, 1987) and the limits established by the Brazilian Ministry of Health (ABIA, 1991) (Table 4). As for the status of the 'increased contamination' reported by ICES (1988), the Cd levels of the present study were lower than the 'increased contamination' level (1.80 µg/g dry weight basis) for Cd and (3.00 µg/g dry weight basis) for Pb (Table 4).

Table 3. Concentrations of zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) in the soft tissues of *P. viridis* (dry weight basis) collected from the Maheshkhali Channel, Cox's Bazar, Bangladesh; Replicate, n= 4.

Sampling Location	Zn (µg/g)	Cu (µg/g)	Cd (µg/g)	Pb (µg/g)
Station I	32.19±0.80	8.81±0.67	0.08±0.01	0.68±0.25
Station II	33.82±0.93	7.92±0.49	0.07±0.01	0.75±0.24
Station III	31.43±0.78	7.26+0.31	0.04±0.01	0.31±0.18
Station IV	28.12±0.89	7.33±0.41	0.06±0.01	0.19±0.15
Mean concentration ± SD	31.39±2.29	7.83±0.77	0.07±0.02	0.48±0.31

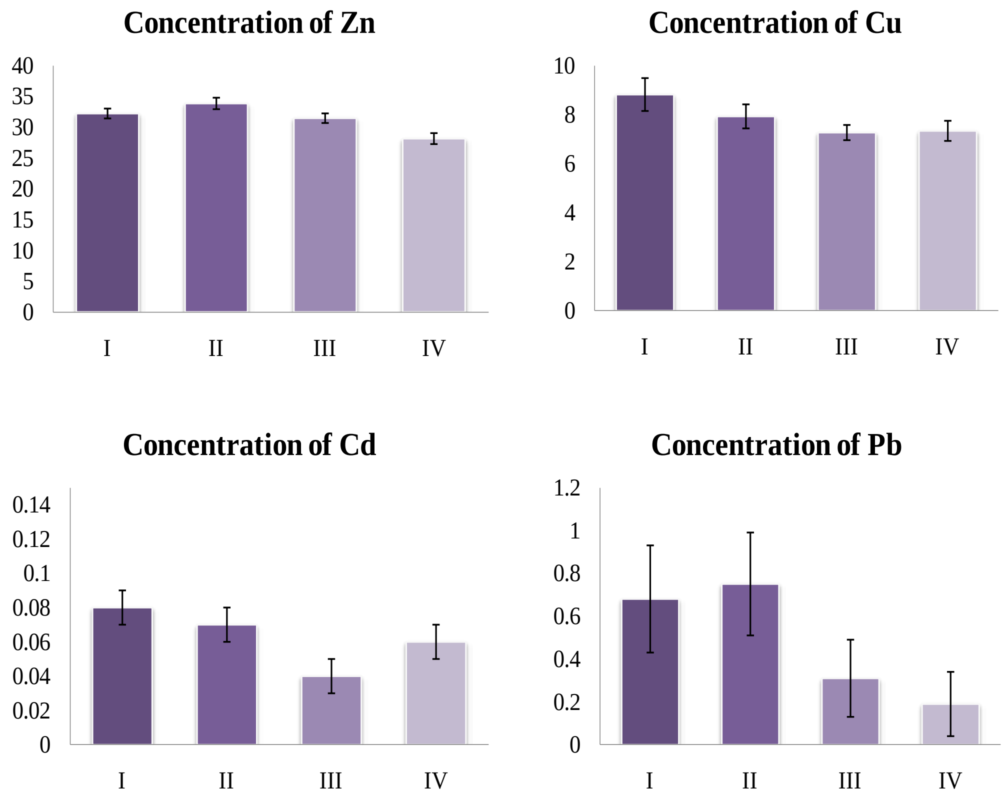

Figure 2. The variation of mean concentrations of the trace metals in *P. viridis* among the sampling stations of the Maheshkhali channel, Cox's Bazar

Table 4. Guidelines on trace metal concentrations for the food safety set by different countries (dry weight basis)

Location	Zn (µg/g)	Cu (µg/g)	Cd (µg/g)	Pb (µg/g)
Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	667.00	133.00	1.00	6.67
International Council for the Exploration of the Sea (ICES, 1988) for status: increased contamination	-	-	1.80	3.00
Food and Drug Administration of the United States (USFDA, 1990)	-	-	25.00	11.50
Australian Legal Requirements (NHMRC, 1987)	750.00	350.00	10.00	-
Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	250.00	150.00	5.00	10.00
Trace Metal levels of *P. viridis* from the Maheshkhali Channel, Cox's Bazar, Bangladesh (this study)	31.39±2.29	7.83±0.77	0.07±0.02	0.48±0.31

The mean concentrations of Zn (31.39±2.29 µg/g), Cd (0.07±0.02 µg/g) and Pb (0.48±0.31 µg/g) found in this study were lower than the previous investigations on *P. viridis* from Peninsular Malaysia, coastal water of Hong Kong, the gulf of Thailand, southeast coast of India and Tolo Harbour of Hong Kong (Phillips, 1985; Sukasem and Tabucanon, 1993; Senthilnathan et al., 1998; Wong et al., 2000; Yap et al., 2002, 2004). Although the mean concentration of Cu (7.83±0.77 µg/g) was higher than some other previous studies from different part of the world, but this concentration level is lower than Peninsular Malaysia (7.76-20.1 µg/g), coastal water of Hong Kong (16.0-27.90 µg/g) and southeast coast of India (33.6-49.2 µg/g) (Phillips, 1985; Senthilnathan et al., 1998; Yap et al., 2004). A comparison of reported concentration levels of zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) in *P. viridis* from regional studies with the present result are presented in Table 5.

According to Hutton (1987), Pb can be responsible for health problems in three major organ systems in human body, namely the haematological, nervous and renal systems. The potential hazards of metals transferred to humans are probably dependent on the amount (g weight) of mussels consumed by an individual. The effects of Pb on the central nervous system are generally seen in children and acute effects of Pb might cause coma and death to the affected person (Hutton, 1987). An adult who consume 10 g/day of *P. viridis* daily from the Maheshkhali channel would intake 4.8 (0.48×10=4.8) µg/g of Pb each day. If the consumer continue to consume this mussel for 7 days, then they would consume 33.6 µg/g Pb, which is still lower than the recommended limit for the provisional tolerable weekly intake of Pb (50.0 µg /adult) (FAO/WHO, 1984).

Similarly, if an adult consumes approximately 10 g of mussels per day, then the person who consumes mussels collected from Maheshkhali channel would intake 0.6 (0.07×10=0.7) µg/g of Cd each day. If the consumers continue to take the mussel for 7 consecutive days, then they would intake 4.9 µg Cd. Again, this consumption level is also lower than the recommended limit for the provisional tolerable weekly intake of Cd (6.70-8.30 µg/adult) (FAO/WHO, 1984). The elimination rate of Cd is very slow (an average 2.00 µg/day) in human body. Moreover, prolonged excessive Cd ingestion would cause Cd accumulation inside the human body (Filov et al., 1993). The acute toxic symptoms of higher concentration of Cd ingestion are nausea, vomiting, diarrhoea, headache, abdominal pain, muscular ache, salivation and shock (Patnaik, 1992).

Table 5. A comparison of reported concentrations of Zinc (Zn), Copper (Cu), Cadmium (Cd) and Lead (Pb) in *P. viridis* from regional studies with the present results (dry weight basis)

Location	**Zn (µg/g)**	**Cu (µg/g)**	**Cd (µg/g)**	**Pb (µg/g)**	**References**
Peninsular Malaysia	75.1-129	7.76-20.1	0.68-1.25	2.51-8.76	Yap et al. (2004)
Penang, Malaysia	76.0	8.0	-	7.0	Sivalingam and Bhaskaran (1980)
Coastal waters of Hong Kong	89.0-164	16.0-27.90	0.29-1.43	7.50-60.50	Phillips (1985)
The Gulf of Thailand	25.7-79.0	1.50-11.3	0.02-19.1	-	Sukasem and Tabucanon (1993)
Putai coast of Taiwan	14.4-25.7	1.78-5.41	-	-	Han et al. (1997)
Southeast coast of India	60.4-94.1	33.6-49.2	1.59-4.40	2.48-6.92	Senthilnathan et al. (1998)
The Gulf of Thailand	24.9-213	2.94-15.0	0.17-3.25	0.19-3.75	Ruangwises and Ruangwises (1998)
Tolo Harbour, Hong Kong	90-135	6.02-24	0.45-1.44	.02-4.36	Wong et al. (2000)
Maheshkhali Channel, Bangladesh	31.39±2.29	7.83±0.77	0.07±0.02	0.48±0.31	Present study

Conclusion

By using *P. viridis* as a bio-monitoring agent, the contamination of Cd, Cu, Pb and Zn in the Maheshkhali channel was found not to be serious. The results of the study revealed that the possibility of the occurrence of acute toxicities of Cd, Cu, Pb and Zn is unlikely. However, low-level and chronic toxicities to consumers may still pose an irreversible hazard. Since, *P. viridis* accumulates trace metals in the soft tissues and constitutes one of the important food-chain in the coastal environment; this information is therefore useful for predicting the metal contamination in this coastal communities. In addition, the trace metal concentrations in the mussels from the Maheshkhali channel could be attributed to natural or anthropogenic metal sources affecting their habitats. However, future studies should concentrate on the relative importance of water, sediment and food in the accumulation of metals by the mussel.

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