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Detection of accumulated heavy metals in the shells and byssus of *Mytilus viridis* using energy dispersive X- ray analysis and atomic absorption spectrophotometry

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Abstract

Heavy metals viz. Cu, Ni, Pb, Cd, Cr and Fe were detected by Atomic Absorption Spectrophotometry (AAS) as well as Energy Dispersive X-Ray Analysis (EDX) in shells as well as byssus of *Mytilus viridis* from Mocha (21°20'N, 69°53'E) as well as Dwarka (22°25'N, 69°04'E). The accumulations of heavy metals in the shells of *Mytilus* from Mocha were 100 times more as compared to the ones collected from Dwarka. Mocha is a more polluted spotas compared to Dwarka. The animals from Dwarka were c.a. 10 times more in weight and very old, lack of pollution could be the probable reason for their longitivity. Fe is present in more amounts in the shells of *Mytilus* from Dwarka. This indicates that Fe is present in large amount in these areas. Dwarka being an ancient city has many remains of iron buried in the coastal areas. The byssus produced by the animals in their native environment i.e. in the sea showed large amounts of heavy metals in them. Cu, Fe and Zn are present as a structural component in byssus. Byssus is a very sensitive indicator of pollution in a particular area.

Keywords: *Mytilus viridis*, Atomic Absorption Spectrophotometry, Energy Dispersive X-Ray Analysis.

Introduction

Heavy metal accumulation in the body of organism is an indication of pollution in the particular locality where these animals thrive. Especially sedentary animals and in particularly byssate bivalves are paragons in these type of studies (Cantillo, 1998; Coimbra and Carraça, 1990; Lares and Orians, 2001). Numerous studies have been carried out with the seasonal variations as well as age and size related issues (Förstner and Wittmann, 1983; Páez-Osuna et al., 1995; Wong et al., 2000).

Pollution of coastal areas has been one of the most delicate subjects in most countries in the past few years (De Gregori et al., 1996). The ever-increasing number of urban settlements, the development of Intensive agricultural activities and the presence of industries along coastal areas have increased the polluting load of discharge waters flowing into these biologically productive coastal areas. Domestic sewage carries detergents, solids, various toxic substances and pathogens, which may directly affect the cultured organisms or indirectly affect man by contaminating the molluscs. Industrial activities give off a diverse variety of pollutants. In areas where the effluents are discharged untreated, as in many developing countries, the silting of culture farms near industrial sites could seriously affect production output as well as product quality. Due to the pollution problems and numerous health incidences related to the consumption of molluscs reared in these sites, the mollusc culture enterprises in some bays have suffered severe losses particularly in terms of reduced market demand (Kavun et al., 2002; Latouche and Mix, 1982; Michel and Zengel, 1998).

Heavy metal toxicity to aquatic organisms in association with the capacity to entry and to keep on the trophic chain for long time justifies metal determination studies on aquatic organisms. However, it is hard to work with environmental contamination by heavy metals due to the complex biogeochemistry of these elements on aquatic environments (Förstner and Witmann, 1983). Thus, it is necessary to monitor and to preserve the aquatic environments against the anthropogenic pollution by heavy metals through the knowledge of the base levels of these trace elements.

Use the soft tissues of the animals in majority of the studies carried out so far on this aspects have focused idea about pollution problems in the nearby time scale. The turnover rate i.e. accumulation and removal of heavy metal elements from the soft tissues is high, but hard and permanent parts of animals as shells are only known to accumulate these elements rendering them harmless to the animal. Hence an idea about the prevalence of pollution since long-time duration can be had by knowing the presence of heavy metals in these parts. As Mytilus is a long lived animal. (Sukhotin, A. et al, 2007). Also byssus which is a nonliving material and secreted outside the body of the animal serving many functions other than anchorage alone, can be used in this type of studies. More over certain metals as Fe, Cu, and Zn are present as a structural constituent and involved in the adhesive property of byssus (Szefer, 2004; Vaccaro and Waite 2001). A variation in the amount of these elements can throw more light on characteristics of byssus material in response to environmental variations and geographical distribution.

In pursuit of all the above-mentioned views, the present paper contains information about the presence of heavy metals like Pb, Cu, Cd, Zn, Co, etc. in the shells and byssus of *Mytilus viridis* detected through EDX and AAS.

Materials and Methods

1. Atomic Absorption Spectrophotometry of shells:

Shells of *Mytilus viridis* collected from two different localities *viz*. Dwarka and Mocha were cleaned by washing thoroughly with ample of running tap water for nearly two hours. These were then washed with alcohol and then with distilled water. Dried shells were weighed and subjected to digestion in royal mixture (*Aqua regia*, $HCl:HNO_3$ :: 3:1). When the shells were completely dissolved, the solution was used for AAS. The AAS was carried out at Chemical Testing Laboratory ATIRA, Ahmedabad, on GBC-906 AA Spectrophotometer (Australia made).

2. Atomic Absorption Spectrophotometry of byssus:

Byssus were collected from the animals in their native area as well as from the ones reaped from the animals maintained in aquarium tanks in the laboratory. Both these threads were washed with ample of running tap water for two hours and then with distilled water. After drying them, they were subjected to digestion in *aqua regia* for 5-6 hours. When completely solubilized the solution was subjected to AAS.

3. Energy Dispersive X-rayAnalysis of byssus:

The same procedure for cleaning of byssus threads was used and when the threads were completely dry these were subjected to EDX analysis on a Scanning Electron Microscope (LEO S-440i, Cambridge UK.) at Facilitation Centre Plasma Research Laboratory, Gandhinagar. Data were collected at various operating voltages and wavelengths of electron beam to get a spectrograph of the elements above sodium present in them.

Results and Discussion

1. AAS of shells

An idea about the presence and amount in mg/g dry weight (d.w.) of heavy metals in the shells from *Mytilus viridis* collected from different localities can be had from the table -1.

Table-1 Presence of heavy metals (mg/g d.w.) in the shells of *M. viridis* collected from Mocha and Dwarka.*

Metal	Shell from Mocha (mg/g) d.w.	Shell from Dwarka (mg/g) d.w.
Cu	1.600	0.0280
Ni	0.300	0.0033
Pb	0.420	0.0044
Cd	0.379	0.0041
Cr	0.257	0.0160
Fe	0.0016	0.0097
***Data collected from Chemical Testing Laboratory, ATIRA Ahmedabad.**		

It is observed that heavy metals like Cu, Ni, Pb, Cd, Cr and Fe are present in the shells of *Mytilus viridis* from both the localities viz. Mocha as well as Dwarka (Figure-1and 2). The content of heavy metals except Fe is more in the case of shells from Mocha (Figure-3). The probable reason for the difference may be due to more pollution in these areas. In addition, the animals thriving in this area are relatively smaller and younger as compared to the ones dwelling at Dwarka. The shells from the animals collected from Dwarka were around 75 grams where as the ones from Mocha were not more than 7.8 grams. Dwarka animals were more in age and size, lack of pollution in these areas could be the probable reason for their longitivity. The shells from Mocha were smooth and bright greenish in color. The shells from Dwarka were rough and bit brownish in color. Also some shells collected from these localities had rough inner surfaces as seen in the figure-4.

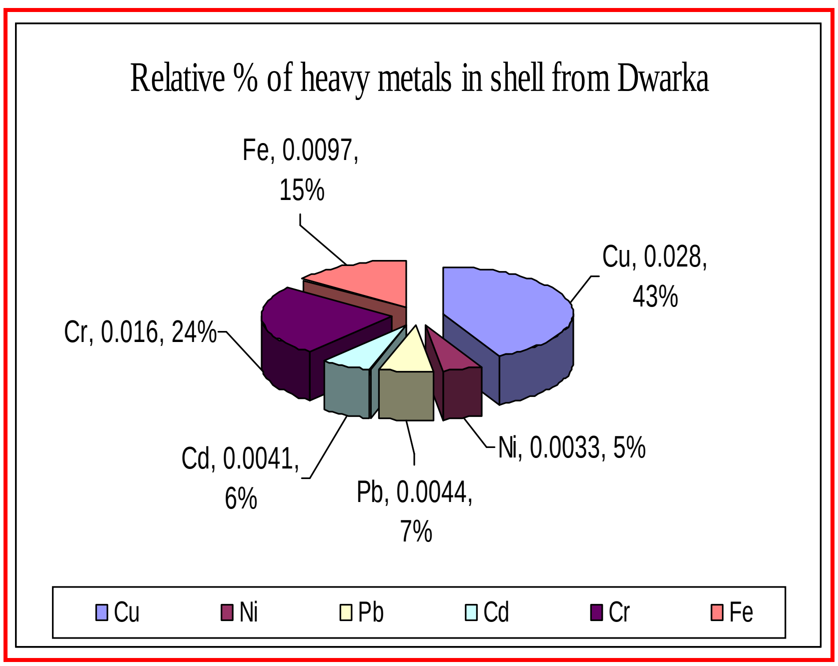

Figure-1 Graph showing relative percentage of heavy metals in the shell from Dwarka.

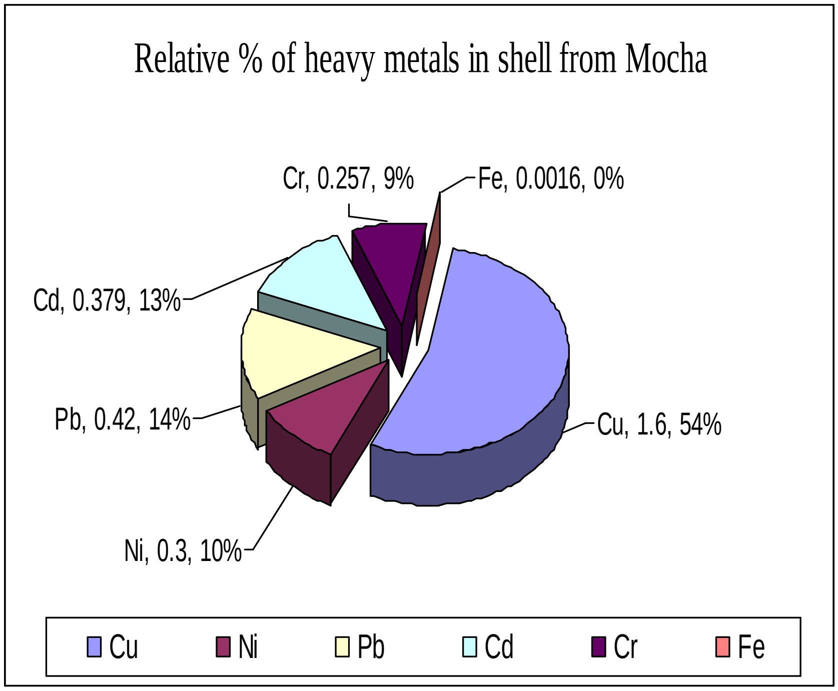

Figure-2 Graph showing relative percentage of heavy metals in the shell from Mocha

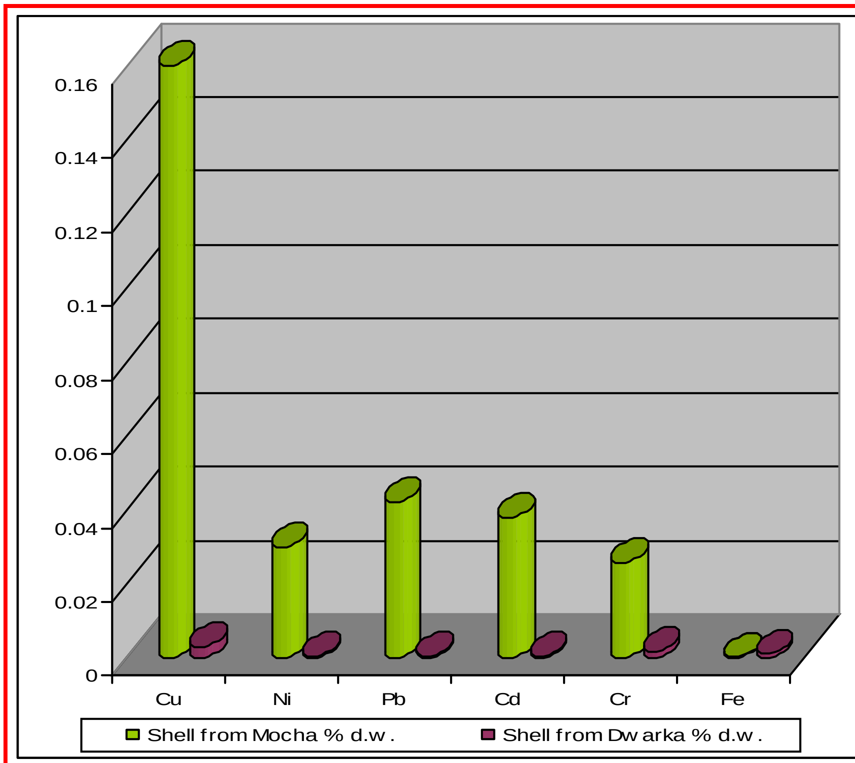

Figure-3 Graph showing comparison of heavy metals present in shells from *Mytilus*

Figure-4 Plate showing outer and inner surfaces of shells collected from Dwarka and Mocha.
1- outer surface of shell from Dwarka, 2- inner surface of shell from Dwarka, 3- outer surface of shell from Mocha, 4- inner surface of shell from Mocha.

It is a known fact that many mussel species produce pearls in order to protect themselves from the irritation caused due to intruding foreign particle in the mantle cavity. *Mytilus* is incapable of forming pearls but the inner surface of the shells i.e. the nacreous layer is known to cover some unknown mud like foreign substance frequently trapping large amount of air spaces (Figure-5). This phenomenon is not at all observed in the shells from *Mytilus* of Mocha.

Figure-5 Plate showing a shell of *Mytilus* from Dwarka showing nacreous layer covering mud like substance trapping large amount of air spaces

2. AAS of byssus

AAS of byssus threads produced in the natural environment i.e. at the sea show the presence of heavy metals like Cu, Ni, Pb, Cd, Cr and Fe (Table-2). Whereas the ones produced under laboratory condition show no heavy metals other than Cu. Cu which is present in the byssus, is not as a mere pollutant but as a structural component along with Fe and Zn (Szefer, 2004; Vaccaro and Waite, 2001; Goho 2004). Cu is present in very trace amount (0.0164 mg/gm d.w.) in the byssus from native place. Fe was not detected in the byssus procured from the one produced in laboratory. Fe may be present in amounts fairly less to be detected by AAS. Byssus is a more sensitive indicator of pollution in a particular locality (Szefer, 2004). This can be observed from the figure-6 showing relative percentage of heavy metal distribution in byssus and shell of *Mytilus*.

Table- 2 Presence of heavy metals (mg/g d.w.) in the byssus of *Mytilus viridis* collected from sea and laboratory.*

Metal	Byssus collected fromsea	Byssus collected from laboratory
Cu	7.3	3.22
Ni	3.336	0.0
Pb	2.336	0.0
Cd	1.744	0.0
Cr	2.664	0.0
Fe	0.0164	0.0

*Data collected from Chemical Testing Laboratory, ATIRA Ahmedabad.

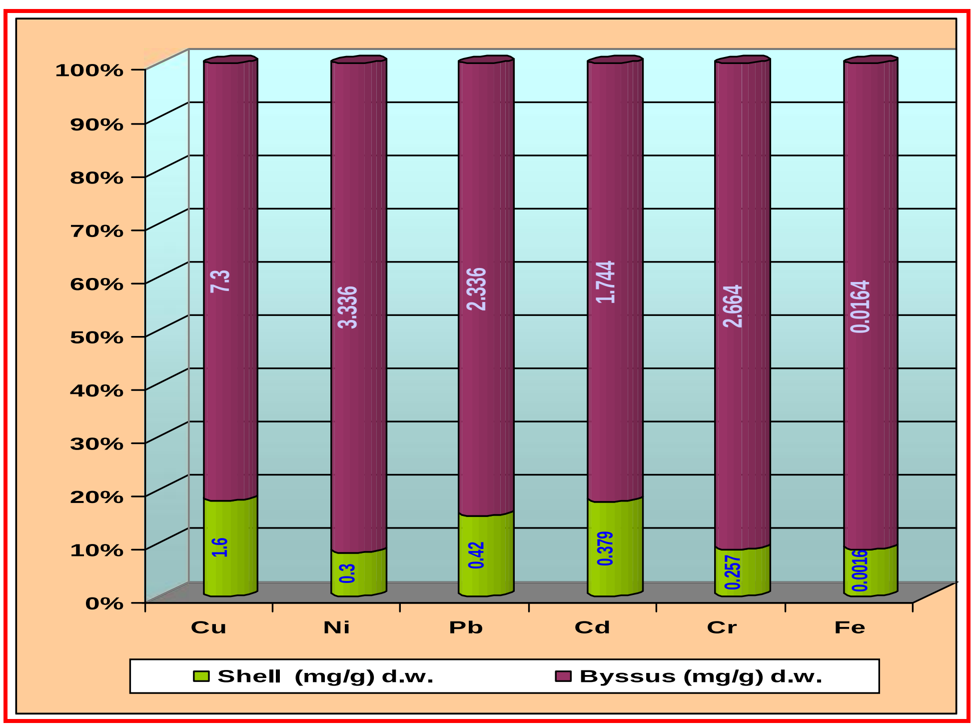

Figure-6 Graph showing comparisons of heavy metals present in Shells and Byssus of *Mytilus viridis*.

EDX of byssus

In order to detect elements other than heavy metals EDX of the byssus was done at SICART, Vallabh Vidyanagar and the following elements depicted in table-3 were detected. In addition, an idea about their relative weight percentage and atom percentage can be had from figure-7.

Table-3 Data of elements detected in the byssus threads by EDXspectroscopy.*

EDX ZAF Quantification (Standard less) Element Normalized						
Element	**Wt%**	**At%**	**K-Ratio**	**Z**	**A**	**F**
C K	73.15	79.88	0.3413	1.0066	0.4634	1.0002
O K	22.67	28.50	0.0315	0.9925	0.1401	1.0000
Zn L	0.74	0.15	0.0035	0.8507	0.5456	1.0003
Mg K	0.47	0.25	0.0022	0.9571	0.4850	1.0006
Si K	0.27	0.13	0.0020	0.9582	0.7881	1.0019
S K	1.24	0.01	0.0115	0.9525	0.9688	1.0026
Pb M	0.12	0.01	0.0015	0.7530	1.6343	1.0003
Cl K	1.34	0.49	0.0121	0.9126	0.9941	1.0000
Total	100.00	100.00				

Element	**NetInte.**	**BkgdInte.**	**Inte. Error**	**P/B**
C K	273.53	1.13	1.41	241.33
O K	79.23	2.16	2.65	36.70
Zn L	6.48	4.75	12.02	1.36
Mg K	11.66	6.53	8.50	1.79
Si K	10.85	12.47	10.34	0.87
S K	55.04	11.87	3.45	4.64
Pb M	1.84	11.87	46.87	0.15
Cl K	56.67	12.36	3.41	4.59

*Redrawn from original data of EDX of byssus done at SICART, VallabhVidyanagar. India.

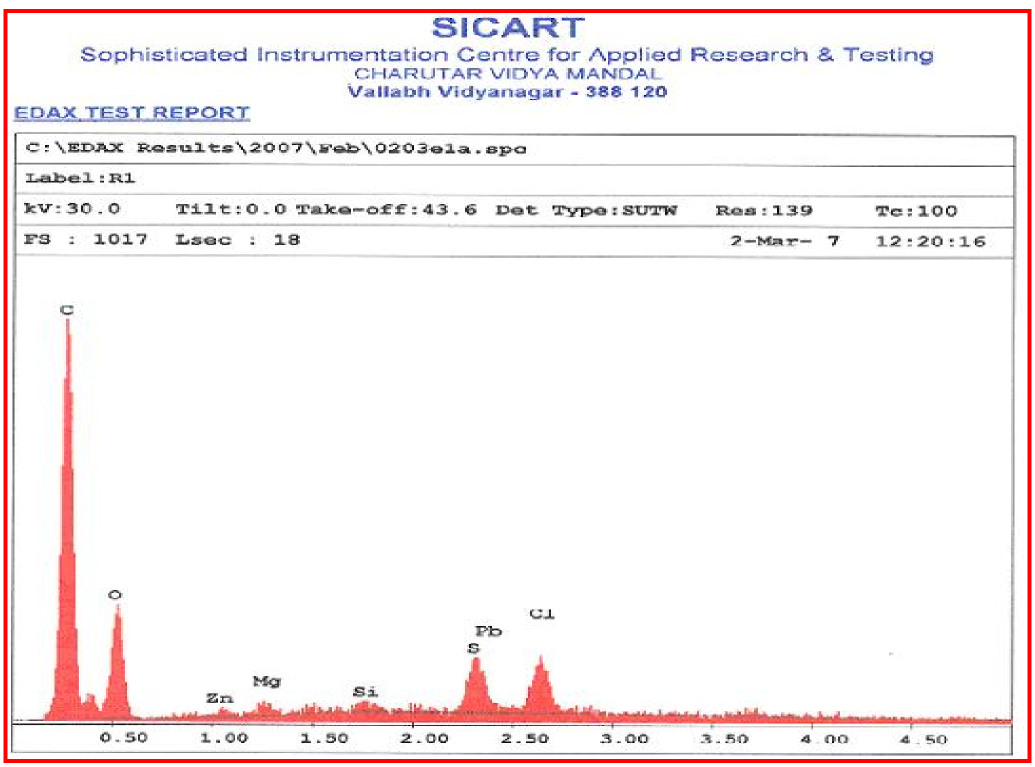

Figure-7 EDX Spectrograph of the byssus threads.

Conclusion

The byssus produced by the animals in their native environment i.e. in the sea showed large amounts of heavy metals viz. Cu, Ni, Pb, Cd, Cr and Fe. The accumulations of heavy metals in the shells of *Mytilus* from Mocha were 100 times more as compared to the ones collected from Dwarka, indicating that Mocha is a more polluted spot as compared to Dwarka. The animals from Dwarka were more in weight and very old, lack of pollution could be the probable reason for this. Fe is present in more amounts in the shells of *Mytilus* from Dwarka indicating that Fe is a major pollutant in these areas. Though Cu, Fe and Zn are presentas a structural component in byssus it is a very sensitive indicator of pollution in a particular area.

Acknowledgments

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References

1. **Cantillo, A. Y.**1998. *In*: Comparison of Results of Mussel Watch Programs of the United States and France with Worldwide Mussel Watch Studies. Mar. Pollut. Bullet. 36(9): 712-717.
2. **Coimbra, J. and Carraça, S.**1990. Accumulation of Fe, Zn, Cu and Cd during different stages of the reproductive cycle in *Mytilus edulis*. Comp. Biochem. Phisiol. **95C(2):** 265-270.
3. **De Gregori, I., Pinochet, H., Gras, N. and Munoz, L.** 1996. Variability of cadmium, copper and zinc levels in molluscs and associated sediments from Chile. Environ. Pollut. **92 (3):** 359-368.
4. **Förstner, U. and Wittmann, G. T. W.** 1983. *In:* Metal Pollution in the Aquatic Environment. 2nd ed. Springer-Verlag. pp271-321.
5. **Goho, A.**2004, Marine Superglue: Mussels get stickiness from iron in seawater. Science News. 165(3)**:** 36.
6. **Kavun, V. Y., Shulkin, V. M. and Khristforova, N. K.**2002. Metal Accumulation in Mussels of the Kuril Islands, North-West Pacific Ocean. Marine Environmental Research. **53:** 219-226.
7. **Lares, M. L. and Orians, K. J.**2001. Differences in Cdelimination from *Mytilus californianus* and *Mytilus trossulus* soft tissues. Environ. Pollut.**112(2):** 201-7.
8. **Latouche, Y. D. and Mix, M. C.**1982. The effectsof depuration, size and sex on trace metal levels in Bay Mussels. Mar. Pollut. Bull.**13(1):** 27-29.
9. **Michel, J. and Zengel, S.**1998. Monitoring of Oyster and Sediments in Acajutla, El Salvador. Mar. Pollut. Bull.**36(4):** 256-266.
10. **Páez-Osuna, P., Frias-Espericueta, M. G. and Osuna-López, J. I.**1995. Tracemetal concentrations in relation to season and gonadal maturation in the oyster *Crassostrea iridescens*. Mar. Environ. Res.**40 (1):** 19-31.
11. **Sukhotin,A.A , Strelkov P.P, Maximovich N.V and Hummel H. 2007,**Growth and Longitivity of *Mytilus edulus* (L) from North East Europe, Marine Biology Research. 3(3):155-167.
12. **Szefer, P., Kim, B. S., Kim, C. K., Kim, E. H. and Lee, C. B.**2004. Distribution and co-associations of trace elements in soft tissue and byssus of *Mytilus galloprovincialis* relative to the surrounding seawater and suspended matter of the southern part of the Korean Peninsula, Environmental Pollution.**129(2)**: 209-228.
13. **Vaccaro, E. and Waite, J. H.**2001. Yield and post-yield behavior of mussel byssal thread: A self-healing biomolecular material. Biomacromol.**2:** 906-911.
14. **Wong, C. K. C., Cheung, R. Y. H. and Wong, M. H.**2000. Heavy Metal Concentrations in Green-Lipped Mussels Collected from Tolo Harbour and Markets in Hong Kong and Shenzhen. Environmental Pollution. **109:** 165-171.

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