Int. J. Environ. Res., 2(3): 279-284, Summer 2008
ISSN: 1735-6865

Metals Accumulation in Marine Bivalves and Seawater from the Lagoon of Boughrara in Tunisia (North Africa)

Mensi, Gh. S. [1, 2], Moukha, S. [3], Creppy, E. E. [2] and Maaroufi, K.[1*]

[1]Research Unit: Mycotoxins, Phycotoxins and Associated Pathologies,Faculty of Pharmacy, Monastir 5000 Tunisia.
[2]University Victor Segalen Bordeaux, Laboratory of Toxicology and Applied Hygiene, 146, rue Léo-Saignat, 33076 Bordeaux, France.
[3] INRA, Centre de Recherche de Bordeaux Aquitaine,UPR 1264 MycSA, 71 avenue Edouart Bourleaux, BP 81, 33883 Villenave d'Ornon Cedex. France.

Received 5 March 2008; Revised 29 April 2008; Accepted 5 May 2008

ABSTRACT: Since 1994 the lagoon of Boughrara located in the south western coast of Tunisia was a seat of repeated episodes of harmful algal proliferation. Due to intoxications by shellfish harvested in this region the area was banned. In order to estimate the metal contribution to this phenomenon, seawater and bivalves collected from Boughrara during the algal bloom period were analysed for Cd, Cr, Hg, Pb, Cu and Al content using the atomic absorption spectrometry.The average of the metals and heavy metals concentrations in clams flesh and shells were lower or in the range of the European Union regularity limits and exhibited the following decreasing order: Al>Cu>Cd>Cr>Pb>Hg. Levels were ranging between 11.541 mg/kg and 0.025 mg/kg wet weights. For seawater and inter-shell water the order was approximately the same. The lagoon of Boughrara might be considered relatively unpolluted with metals and repeated toxic algal proliferations remained unexplained.

Key words: Algal bloom, Bivalves, Heavy metal, Marine pollution

INTRODUCTION

In the marine ecosystem, the microscopic planktonic algae and mussels are intimately related since they are dependent on each other for their survival (Harris, 1990). In fact phytoplankton is the critical food for filter-feeding bivalve shellfish, and their proliferation is beneficial and necessary for aquaculture (Okay *et al.*, 2000).Unfortunately, in some cases phytoplankton can cause economic and environmental damages and public health problems because they produce several harmful toxins for humans and animals.Marine algal bloom is a natural process which can be caused by several factors including wind, sea water temperature and salinity (Lassus, 1994). It could also be caused by stress on ecological systems such the excess of dissolved metal pollutants (Gentien and Arzul, 1990).Heavy metals represent one of the major environmental problems causing long-term effects on marine ecosystems; these contaminants are incorporated by bivalve shellfish (oysters, mussels, scallops, clams) (Phillips, 1976).The bioaccumulation of heavy metals in the bivalve's tissues can be useful for the detection of polluted area and can be used as environmental bio-indicator (Langston and Spence, 1995). Because of their high sensibility, ubiquity, high filtration rates and high bio-concentration factor for most sea pollutants, clams have been selected as indicator organisms.

In Tunisia, metal elements are widely distributed in the Gulf of Gabès situated on the south western coast of Tunisia (Smaoui-Damak *et al.*, 2004). Since 1994, the same area was a seat of recurring proliferations of algal bloom accompanied by the co-proliferation of toxic phytoplankton *Gymnodinium sp* identified as *Karenia selliformis* (Hamza and Elabed, 1994; Hamza *et al.*, 1999; Biré *et al.*, 2002).The lagoon of Boughrara, located in the Gulf of Gabes, was

*Corresponding author:Email- souid_tn@yahoo.fr

the most affected region by the algal contamination (Hamza *et al.,* 1999), and clams harvested in this area were found to be toxic to mice according to the Yasumoto bioassay (Yasumoto *et al.,* 1978). Since then the area was banned from the commercial circuit and consequently the aquacultural activities were also blocked.In order to tentatively explain the repeated episodes of harmful algal bloom, and to conclude if toxic phytoplankton proliferation in the lagoon of Boughrara are correlated to a metal pollution, the metal contamination in this area have been investigated with the aim to install a survey in a long term setting. The clams from Boughrara were used as a bio-indicator of heavy metal pollution; they were collected in the period of algal bloom (usually in March) and analysed for Cd, Cr, Pb, Al, Hg and Cu. With the intention to assess the extent of metal contamination in this area, heavy metal concentrations in samples from Boughrara were compared to levels set by the European community directives.

MATERIALS & METHODS

Heavy metal solutions (Cd, Cr, Pb, Al, Hg and Cu) used as atomic absorption standards were prepared from 1g single element/L solutions obtained from CPI international USA. Marine samples were collected from the Gulf of Gabès, precisely from the lagoon of Boughrara, situated at 27Km to the south of the island of Djerba (Fig. 1). Clams and seawater were collected weekly during the month of March within the period of algal bloom in this area. During the sampling of clams, the seawater temperature was ranging between 17 to 21°C and salinity between 36 and 42‰. The clams were immediately placed in coolers chilled with ice for transport and 1l of the sea water was kept in clean polypropylene bottles.

The clams were weighed, the whole soft tissues were then separated from the shell, the mixed fleshes fresh weight measured and the whole was stored at -20°C until analysis. The shells and the inter-shell water were also collected and stored at -20°C.The inter-shell water and the seawater were filtered through a 0.45µm porosity filter using a Millipore holding filter system (Millipore, France). The mixed tissue and shells (2g) were digested in 10ml of 25N HNO_3 and 4 mL of 20N H_2SO_4 (Merck, France) during 24 h,

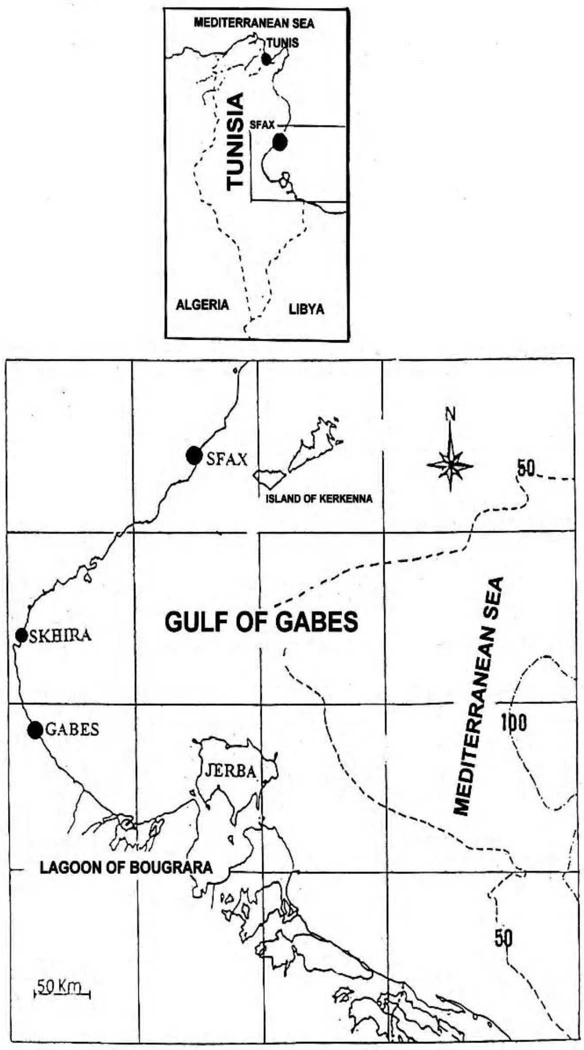

Fig. 1. Location of the lagoon of Boughrara (the sampling site) in the Gulf of Gabès

samples were then mineralised by using the Maxi-digested MX 350 equipment (Prolabo, France) following addition of H_2O_2 (10v, 3times, 2 mL). The mineralised samples were diluted to 25ml with ultra-pure water produced by a Millipore milli-Q plus equipment (Millipore, St Quentin, France). The filtered sea water, the filtered inter-shell water, the mineralised shells and the mineralised soft tissue (total flesh), were assayed for several metals including: copper, cadmium, chromium, lead and aluminium, separately by electro-thermal atomic absorption with Zeeman correction using the equipment and the automatic injector of a Zeeman atomic absorption spectrometer (Varian) and the quality control software (QC protocol Zeeman) according to the analytical method described by Amiard *et al.,* (1987). For Mercury, flameless atomic absorption has been used under the same operating conditions. To avoid possible contamination; all glassware and equipment used were washed with an acidic solution (HCl 10 % in ultra pure water) and rinsed with ultra-pure water (Milli-Q, Millipore, France). The data are expressed as means with standard deviation (SD) for three independent analyses and four different samples.

RESULTS & DISCUSSIONS

Heavies' metals and metals content from the whole soft tissues and from the shells of the clam collected from the lagoon of Boughrara are summarized in Table1. The average concentrations of heavy metals and metals analysed in the shells were significantly higher than those in the whole soft tissues. Our data showed that the amounts of heavy metals and metals in the soft tissues and in the shells of clams exhibited the following decreasing order: Al > Cu > Cd > Cr > Pb > Hg. We conclude that metals such as Al and Cu are the most abundant as compared to the tested heavy metals. In fact, the Al content in whole soft tissue is approximately 20 fold higher than Cd content, 130 fold higher than Cr, 500 fold higher than Pb and 1000 fold higher than Hg content. The Cu content in whole soft tissue is approximately 10 fold higher than Cd, 50 fold higher than Cr, 200 fold higher than Pb and 450 fold higher than Hg content. These proportions are approximately the same in shells. The results obtained from the clams of Boughrara were compared to the heavy metalsregulatory values of the European Union (Table1). The European directives set the maximal accepted content of heavy metals such as Cd, Pb and Hg in the bivalve whole soft tissues, but for other metals such as Zn, Cu, Cr, Al, the maximal accepted levels are qualitative and not fixed. The dispositions for bivalves are included in the decree 91-1283 just signifying that metals concentrations must be lower than harmful levels to the bivalve and its larva.Levels of tested metals and heavy metals in inter-shell water and in seawater from Boughrara are represented in the Table1. The results showed that copper has the highest concentration in both samples as compare to heavy metals. We also observe that inter-shell water contained significantly higher levels of heavy metals, except for Pb (0.020 mg/L in inter-shell water versus 0.173 mg/L in sea water) and Cu (3.452 mg/L in inter-shell water versus 7.080 mg/L in sea water). Similar to the physical conditions, such as the temperature and salinity, the chemical environment could be directly linked to harmful algal bloom events. The metal elements at high concentrations may influence water quality leading to harmful bloom development (Granéli and Moreira 1990).

The main goal of the present study was to investigate the metal and heavy metal contamination and its eventual implication in the harmful algal bloom in the marine ecosystem of Boughrara. The analysis of heavy metals in whole soft tissues of clams as bio-indicator provides someinformation about the degree of metal pollution in the environment. In the studied case, metal concentrations in clam tissues were ranged between 25.631 mg/kg and 0.025 mg/kg wet weights; these values are in the same range to the values described by Riba *et al.,* (2005) and Catsiki

Table 1. Concentrations of toxic metals in the seawater, inter-schell water, shells and the whole soft tissues of clams from Boughrara compared to reference European Regulation values

Metals	Cd	Cr	Pb	Al	Hg	Cu
inter-schell water (expressed as mg/L)	0.126±0.41	0.033±0.01	0.020±0.03	2.602±0.29	0.012±1.7	3.452±0.70
Seawater (expressed as mg/L)	0.026±0.23	0.018±0.02	0.173±0.23	0.368±0.69	0.011±0.65	7.080±0.4
Shells (expressed as mg/kg)	3.333±0.16	0.894±0.63	0.055±0.96	58.621±1.38	0.063±0.78	45.120±2.73
whole soft tissue (expressed as mg/kg wet weight)	1.09±0.17	0.196±0.1	0.05±0.01	25.631±0.53	0.025±0.43	11.541±0.63
Reference values of metals in total flesh of clams safe to eat (expressed inmg/kg wet weight)	1.0	Not fixed	1.5	Not fixed	0.5	Not fixed

operating conditions. To avoid possible contamination; all glassware and equipment used were washed with an acidic solution (HCl 10 % in ultra pure water) and rinsed with ultra-pure water (Milli-Q, Millipore, France). The data are expressed as means with standard deviation (SD) for three independent analyses and four different samples.

RESULTS & DISCUSSIONS

Heavies' metals and metals content from the whole soft tissues and from the shells of the clam collected from the lagoon of Boughrara are summarized in Table1. The average concentrations of heavy metals and metals analysed in the shells were significantly higher than those in the whole soft tissues. Our data showed that the amounts of heavy metals and metals in the soft tissues and in the shells of clams exhibited the following decreasing order: Al > Cu > Cd > Cr > Pb > Hg. We conclude that metals such as Al and Cu are the most abundant as compared to the tested heavy metals. In fact, the Al content in whole soft tissue is approximately 20 fold higher than Cd content, 130 fold higher than Cr, 500 fold higher than Pb and 1000 fold higher than Hg content. The Cu content in whole soft tissue is approximately 10 fold higher than Cd, 50 fold higher than Cr, 200 fold higher than Pb and 450 fold higher than Hg content. These proportions are approximately the same in shells. The results obtained from the clams of Boughrara were compared to the heavy metalsregulatory values of the European Union (Table1). The European directives set the maximal accepted content of heavy metals such as Cd, Pb and Hg in the bivalve whole soft tissues, but for other metals such as Zn, Cu, Cr, Al, the maximal accepted levels are qualitative and not fixed. The dispositions for bivalves are included in the decree 91-1283 just signifying that metals concentrations must be lower than harmful levels to the bivalve and its larva.Levels of tested metals and heavy metals in inter-shell water and in seawater from Boughrara are represented in the Table1. The results showed that copper has the highest concentration in both samples as compare to heavy metals. We also observe that inter-shell water contained significantly higher levels of heavy metals, except for Pb (0.020 mg/L in inter-shell water versus 0.173 mg/L in sea water) and Cu (3.452 mg/L in inter-shell water versus 7.080 mg/L in sea water). Similar to the physical conditions, such as the temperature and salinity, the chemical environment could be directly linked to harmful algal bloom events. The metal elements at high concentrations may influence water quality leading to harmful bloom development (Granéli and Moreira 1990).

The main goal of the present study was to investigate the metal and heavy metal contamination and its eventual implication in the harmful algal bloom in the marine ecosystem of Boughrara. The analysis of heavy metals in whole soft tissues of clams as bio-indicator provides someinformation about the degree of metal pollution in the environment. In the studied case, metal concentrations in clam tissues were ranged between 25.631 mg/kg and 0.025 mg/kg wet weights; these values are in the same range to the values described by Riba *et al.*, (2005) and Catsiki

Table 1. Concentrations of toxic metals in the seawater, inter-schell water, shells and the whole soft tissues of clams from Boughrara compared to reference European Regulation values

Metals	Cd	Cr	Pb	Al	Hg	Cu
inter-schell water (expressed as mg/L)	0.126±0.41	0.033±0.01	0.020±0.03	2.602±0.29	0.012±1.7	3.452±0.70
Seawater (expressed as mg/L)	0.026±0.23	0.018±0.02	0.173±0.23	0.368±0.69	0.011±0.65	7.080±0.4
Shells (expressed as mg/kg)	3.333±0.16	0.894±0.63	0.055±0.96	58.621±1.38	0.063±0.78	45.120±2.73
whole soft tissue (expressed as mg/kg wet weight)	1.09±0.17	0.196±0.1	0.05±0.01	25.631±0.53	0.025±0.43	11.541±0.63
Reference values of metals in total flesh of clams safe to eat (expressed inmg/kg wet weight)	1.0	Not fixed	1.5	Not fixed	0.5	Not fixed

and Florou (2006) for mussels. Those concentrations of metals could be theoretically considered as the highest concentrations accumulated by mussels during one year period (Riba *et al.,* 2005; Catsiki and Florou, 2006). The bioaccumulation of metals by bivalves varies according to the season and actually it is established that mussels bioaccumulate metals during the warm period in a lower degree than during the colder period. Considering that metal availability for bivalves is maximal at the cold season. Consequently we intentionally have chosen March as the most favourable month to perform the sampling of clams and water. This period is simultaneously the end of the cold period and the beginning of the algal bloom episode in the lagoon of Boughrara.

The results obtained in the whole soft tissue of clams from Boughrara, showed relatively high levels of Al (25.631 mg/kg wet weight) and Cu (11.541 mg/kg wet weight); these values could be explained by harbours and the ferry traffic between Ajim, Boughrara and the island of Djerba (27 Km distant from Boughrara) and by the sewage wastes, since the lagoon of Boughrara is a closed area having limited communication with the open sea. In fact the lagoon of Boughrara is in touch with the open sea just through the channel of Ajim (2.2 Km wide) and the channel of Borj-Kastil (5.5 Km wide). Aluminium concentrations obtained in the whole soft tissues were similar to those published by Gagné *et al.,* (2006).In their study, the clams flesh accumulate 21 mg/kg wet weight of Al and were collected from a referenced uncontaminated site (Baude). This value is close to the concentration of 25 mg/kg wet weight Al found in the clams flesh of bivalves harvested in Boughrara. In an area polluted by mining activities, levels of copper in mussels collected from the gulf of Cadiz in Spain ranged between 113 and 560 mg/kg wet weight. (Riba *et al.,* 2005). These values are much higher that the value of 11.5 mg/kg total flesh wet weight found in Boughrara, this value is approximately 10 times to 46 times lower to the values found in Cadiz. The copper concentration in clams from Boughrara are more closely related to those from non polluted area of Mediterranean (Italy) and Black sea (Ukraine) coast, where the copper contenant vary between 5.96 mg/kg wet weight and 8.18 mg/kg wet weight (Capelli *et al.,* 1978; Majori *et al.,* 1991; Giusti *et al.,* 1999). Accumulation of Cd in mussels from the Golf of Gabès in Tunisia has been reported by Hamza-Chaffai *et al.,* (2000), Smaoui-Damak et al (2003) and Smaoui-Damak *et al.* (2004). Cadmium concentration reported in these studies ranged between 0.046 and 0.31 mg/kg wet weight; these values are three times lower than those obtained from the clams of Boughrara. This could be explained by: (i) the seasonal variations of the heavy metal bioaccumulation by bivalves. In fact the sampling period in these previous studies was between April and August, and the temperature were ranging between 28°C and 32°C versus 17°C and 21°C in the present study. (ii) The differences in pH and salinity of these sampling sites could also be taken into account because the capacity of bivalves to accumulate Cd can be affected by these parameters, and (iii) the limited communication of the lagoon of Boughrara with the open sea lead to more important accumulation of Cd pending from the phosphor-gypsum rejects present in the south of Tunisia (Zaouali, 1993; Zairi and Rouiss 1999).

The Cd concentration in soft tissues (1.08 mg/kg wet weight) of the clams harvested in Boughrara reach the maximal accepted level of the European regulatory limit which is fixed to 1 mg/kg wet weight (Table 1). Therefore based on this regulatory limit the harvested clams flesh is slightly exceeding the regulatory limit. Although the level of contamination is low, the clams flesh from Boughara can not be recommended for frequent consumption by humans. This fact is based on the consideration that the value of the standard deviation that we found can allow to exceed the maximal regulatory limit (see Table1). Considering the chromium pollution in the area of Boughrara, results showed that the level of Cr in the bivalves is low when compared to those from non-polluted Mediterranean areas, 6.52 mg/kg wet weight against 0.19 mg/kg wet weight in our study. Our result were also ten fold lower then those reported by Gagné *et al.* (2006) using the clams from a non-polluted site in Canada (1.76 mg/kg). Therefore it is assumed that Cr level in bivalves from Boughrara is in the range of the accepted standard suitable for human consumption. Similarly, Pb showed a lower concentration when compared to the value reported by Riba *et al.* (2005) that was already reported as low level of Pb, 0.3 mg/kg wet weight versus 0.05 mg/kg wet weight in our study. Indeed, the Pb level in the clams from Boughrara is 3 fold lower than the maximal regulatory value authorized by the European Union (Table1).

Mercury is considered as the most toxic heavy metal; especially in its organic form; it affects dramatically phytoplankton and bivalves growth. In this study, Hg has the lowest concentration in clams total flesh 0.025 mg/kg wet weight; this concentration is 20 fold lower than the threshold safety value defined by the European Union (Table1). The levels of the heavy metals in shells were in average relatively higher than those in the whole soft tissue of bivalves. This could be the result of a detoxification mechanism realized cyclically by bivalves as reported by some authors, contrary to shells that strongly accumulate environmental metal elements (Coombs and Keller 1981, Nicholson and Szafer, 2003).

Concerning the seawater from Boughrara, except for Cu, metal and heavy metal levels are slightly lower than those found in the inter-shell water and the concentrations exhibited the same decreasing order as found in bivalves total flesh or shells. We can assume that the levels of metal elements in seawater are correlated with those in the soft tissues of bivalves (Szefer *et al.*, 2004 and 2006). In fact, both seawater and inter-shell water showed an elevated concentration of Cu when compared to other metal levels. These results can be explained by the industrial rejects, the sewage wastes, harbours and the ferry traffic in this area. In the present study, the levels of other analysed metals (Al, Cd, Cr, Pb and Hg) in inter-shell water and seawater from Boughrara should not exceed those causing deleterious effects on bivalves according to Anon (1979). In fact our results are lower than the LC50 values impairing the bivalve embryogenesis fixed by His *et al.* (2000).

CONCLUSION

Based on these results, some comments can be made as following, (i) except for copper, the area of Boughrara might be considered relatively unpolluted by metals (Al, Cr, Hg, Cd and Pb). (ii) In fact, the concentrations of metals in bivalves are also below the seafood safety limits for human consumption, and could not be considered as toxic from this point of view if metal are considered individually, by issuing a reservation on cadmium. But we can not predict the toxicity of mixture of heavy metals and metals at the concentration presently measured in Boughrara. (iii) Unfortunately, the lagoon is a closed area with limited exchange with open seawater; for that reason metal and organic pollutants might easily be periodically accumulated and their interaction with some physical and chemical factors could be one of the reasons favouring the proliferation of toxinogenic algae during algal bloom. (iv) From an ecological point of view, we do not know yet what can be the contribution of the mixture of such metals concentrations, principally Cd and Cu, on the ecosystem of the lagoon especially during the algae bloom period. Nevertheless the presence of metals has to be considered in association with other nutritional factors that are known to influence algal bloom such as the nutrient feeding in nitrogen, phosphorus and sulphur source of the lagoon. These factors probably vary with seasonal changes of the atmospheric conditions and marine currents. Although it can not be assumed that metals are important factors that contribute to toxic algae proliferation our finding are still in infancy but lead us to consider that metal and heavy metals accumulation in clams parallel the periodic proliferation of the harmful micro-alga *Karenia selliformis* in the Gulf of Gabès and especially in the lagoon of Boughrara.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Sea Sciences and Technologies (INSTM). Tunisia We would like to thank Dr. Asma Hamza, Dr. Najib Madhyoub and Dr. Amel Madhyoub for their scientific support and their help to prepare this work.

REFERENCES

Amiard , J.C., Pineau , A., Boiteau, H.L., Metayer, C., and Amiard-Triquet, C. (1987). Application de la spectrométrie d'absortption atomique Zeeman Aux dosage de huit éléments tracés (Ag, Cd, Cr, Cu, Mn, Ni, Pb et Se) dans des matrices biologiques solides. Wat. Res., **21**, 693-697.

Anon, J. (1979). Directiva del consejo de 30 de Octobre de 1979 relativa a la calidad exgida a las aguas para cria de moluscus. (79/923/CEE). Diario Official de las Comunidades Europeas **02**, 156-161.

Biré, R., Krys, S., Frémy, J.M., Dragacci, S., and Kharat, R., (2002). First Evidence on occurrence of gymnodimine in clams from tunisia. J. Nat. Tox., **11,** 252-258.

Capelli, R., Contardi, V., Fassone, B., and Zanicchi, G., (1978). Heavy metals in mussels (*Mytilus galloprovincialis*) from the gulf of La Spezia and from the promontory of Portofino, Italy. Mar. Chem., **6**, 179-185.

Catsiki, V.A., and Florou, H., (2006). Study on the behaviour of the heavy metals Cu, Cr, Ni, Zn, Fe, Mn and 137 Cs in an estuarine ecosystem using *Mytilus* *galloprovincialis* as a bioindicator species : the case of Thermaikos gulf, Greece. J. Environ. Rad., **86**, 31-44.

Coombs, T.L., and Keller, P.J., (1981). *Mytilus byssal* threads as an environmental marker for metals. Aquat. Toxicol., **l**, 291-300.

Gagné, F., Blaise, C., Pellerin, J., and Pelletier, E.J., (2006). Strand Health status of *Mya arenaria* bivalves collected from contaminated sites in Canada (Saguenay Fjord) and Denmark (Odense Fjord) during their reproductive period. J. Ecotox. Environ. Safe., **64**, 348-361.

Gentien, P., and Arzul, G., (1990). Exotoxin production by *Gyrodinium cf. aureolum* (Dinophyceae). J. Mar. Biol. Assoc., **70**, 571–581.

Giusti, A.C. Williamson, S., and Mistry, A., (1999). Biologically available trace metals in *Mytilus edulis* from the coast of Northeast England. Environ. Int., **25**, 969-981

Granéli, E., and Moreira, M.O., (1990). Effects of river water of different origin on the growth of marine dinoflagellates and diatoms in laboratory culture. J. Exp. Mar. Biol. Ecol., **136,** 153-156.

Hamza, A., and El Abed , A., (1994). Les eaux colorées dans le golfe de Gabès : bilan de six ans de surveillance (1989-1994). Bull . Inst. Nat. Scie. Tech. Mer ., **21**, 66-72.

Hamza, A., Bouain , A., and El Abed, A., (1999). Le *Gymnodium sp* phytoplancton toxique du golfe de Gabès. Bull . Inst. Nat. Scie. Tech. Mer., **4**, 49-51.

Hamza-Chaffai, A., Bouain, A ., and El Abed, A., (2000). Observation sur la floraison et la fructification de la phanérogame marine *Posidonia oceanica* (Linneaus) Dellile sur les côtés du golfe de Gabès (Tunisie). Bull. Inst. Nat. scie. Tech. Mer., **58**, 93-99.

Harris, V.A., (1990). Sessile animals of the seashore. London 1st ed. Chapman & Hall. 379.

His, E., Beiras, R., and Seaman, M.N.L., (2000). The assessment of marine pollution bioeassays with bivalve embryos and larvae. Adv. Mar. Biol., **37**, 1-178.

Langston, W. J., and Spence, S.K., (1995). Biological factors involved in metal concentrations observed in aquatic organisms. In Tessier, A., and Turner, D. R., Metal speciation and bioavailability . John Wiley and Sons Ltd. 407-478.

Lassus , P., (1994). Microalgues toxiques dans l'environnement côtier, mécanismes de proliférations. MST "gestion de l'environnement " .Comm. IFREMER : 9.

Majori , L., Nedoclan, G., Daris, F., and Modonutti, G.B., (1991). Metal distribution (Cd, Cr, Cu, Fe, Mn, Pb, Zn) in *Mytilus galloprovincialis Lmk* in coast-andlagoon- areas in Northern Adriactic. Rev. Int. Oceanogr. Med., **101**, 225-228.

Nicholson, S. P., and Szefer, P., (2003). Accumulatioin of metals in the soft tissues. Byssus and shell of the myltilid mussel *Perna viridis* (Bivalvia: Mytiliadae) from polluted and uncontaminated locations in Honk Kong. Honk Kong. Bull., **46**, 1040-1043.

Okay, O.S., Donkin, P., Peters, L.D., and Livingstone, D.R., (2000). The role of algae (*Isochrysis galbana*) enrichment on the bioaccumulation of benzo[a]pyrene and its effects on the blue mussel *Mytilus edulis*. Environ. Pollut., **110**,103-13.

Phillips, D. J. H., (1976). The commen mussel *Mytilus eludis* as indicator of pollution by zinc, cadmium, lead and copper. Relationship of metals in the mussel to those discharged by industry. Mar. Biol. **38**, 71-80.

Riba, I., Blasco, J., Jiménez-Tenorio, N., and Angel DelValls, T., (2005). Heavy metal bioavailability and effects: Bioaccumilation caused by mining activities in the Gulf of Cadiz (S.W) Spain.Chemosphere., **58**, 659-669.

Smaoui-Damak, W., Hamza-Chaffai, A., Berthet, B., and Amiard, J.C., (2003). Preliminary study of the clam *Ruditapes decussatus* exposed in situ to metal contamination and originating from the Gulf of Gabès, Tunisia. Bull. Environ. Contam. Toxicol., **71**, 961-970.

Smaoui-Damak, W., Hamza-Chaffai, A., Bebianno, M.J., and Amiard, J.C., (2004). Variation of metallothioneins in gills of the clam *Ruditapes decussates* from the Gulf of Gabès (Tunisia). J. Environ. Rad., **86**, 31-44.

Szefer, P., Kim, B. S., Kim, Ch-k., Kim, E.H., and Lee, C-B., (2004). Trace metals in *Mytilus edulis gallopovincialis* and the associated water and suspended matter of ,the southern part of Korea Peninsula. Environ. Pollut., **129**,209- 228.

Szefer, P., Fowler, S.W., Ikuta, K., Paez Osuma, F., Ali, A.A., Kim, B.S., Fernandes, H.M., Belzunce, M.J., Gustertam, B., Kunzendorf, H., Wolowiez, M., Hummel, H., and Eslous-Paoli, M., (2006). MDA comparative assessment of heavy metal accumulation in soft parts and byssus of mussels from subarctic , temperate subtropical and tropical marine environments. Environ Pollut., **139**,70-78.

Yasumoto, T., Oshima, Y., and Yamaguchi, M., (1978). Occurrence of a new type of shellfish poisning in the Tohoku district. Bull. Jpn. Soc. Sci. Fish., **44**, 1249-1255.

Zairi, M., and Rouis, M. J., (1999). Impacts environnementaux du stockage du phosphogypse à Sfax (Tunisie). Bull Lab., **219**: 29-40.

Zaouali, J., (1993). Les peuplements benthiques de la petite Syrte, golfe de Gabès Tunisie. Résultats de la compagne de prospection du mois de juillet 1990. Etude préliminaire: biocoenoses et thanatocoenoses récentes. Mar Life., **2** :53-56.