Usefulness of Bioindicators and Biomarkers in Pollution Biomonitoring

Amel Hamza-Chaffai*

ES11ES90 Marine Ecotoxicology, IPEIS, Sfax University-Tunisia, BP1172-3018-SFAX, Tunisia

Abstract: We have different possibilities and tools to assess the impact of pollution on marine ecosystems. The ecotoxicological approaches are based on the use of biomonitors and biomarkers. They aim to study the effect of toxic chemicals on the biological organisms especially at the population, community and ecosystem levels. The ultimate goal of ecotoxicology is to be able to predict the effects of pollution so that the most efficient and effective action to prevent or remediate any detrimental effect.

In order to assess the impact of anthropogenic activities on the aquatic ecosystem and to insure compliance with regulation or guidelines, we use biomonitoring. This kind of approach is based on the use of biological responses in order to assess anthropogenic changes in the environment. Biomonitoring involves the use of indicator species such as filter feeding mollusk bivalves. These organisms tend to accumulate pollutants in their tissues without showing any apparent detrimental effect. Moreover, they could reflect the real bio available fraction of the pollutant. In order to have an early warning system predicting the pollution effects even at low levels, biomarkers were extensively studied. Some of them were validated in both field and *in vivo* conditions.

In the present paper, the usefulness of bioindicators and biomarkers in pollution monitoring are discussed. An overview of results from case studies dealing with *in situ, in vivo* and transplantation experiments is presented.

Keywords: Marine pollution, bivalves, *Ruditapes decussates*, *Cerastoderma glaucum,* field validation.

INTODUCTION

The marine environment is exposed to different anthropogenic pollutants generated by industrial, domestic and agricultural activities. In marine organisms, many pollutants are susceptible to interact with the physiological processes such as growth and reproduction. In fact pollutants can alter their life and lead to serious disruptions such as reduction of the animal populations, changes of the reproductive functions.

In order to provide an optimal use of marine resources, one of the major preoccupations of governments and researchers consists to distinguish between “clean” and polluted ecosystems. For this reason, monitoring programs are used to evaluate pollution state of coastal zones and implement short term and long term strategies for marine resources protection. Different compartments could be involved: sea water, sediment and marine organisms.

Measuring pollutant concentrations in sea water presents some disadvantages such as the low concentrations and the random spatial and temporal variations. The sediment is a long-term integrator of pollution where concentrations are higher than in seawater, but contaminants are not always bio-available for organisms owing to their physico-chemical forms. Moreover, heterogeneity of sediment (particle size and organic matter) could make comparison between sites difficult. That is why the use of living organisms called biomonitors is preferable for pollutant quantification. In fact, these organisms accumulate contaminants usually from water and food, a fact reflecting only the bio-available fraction that is of potential ecotoxicological significance and could therefore interest environmental managers. The use of biomonitors to evaluate pollution impact is called biomonitoring.

Biomonitoring programs based on measuring contaminants in marine organisms are interesting from a human health point of view. However, they could not give information about the toxicological significance of pollutants accumulated and do not indicate the health status of the organisms [1]. Consequently, recent biomonitoring programs are now involving biomarkers, which are are measurable parameters at different levels of biological organisation (molecular, cellular or physiological). Biomarkers traduce changes in the metabolic regulatory processes resulting from the effect of anthropogenic stressors [2].

In this paper we will focus on biomonitors and biomarkers and discuss their usefulness in biomonitoring pollution.

*Address correspondence to this author at the ES11ES90 Marine Ecotoxicology, IPEIS, Sfax University-Tunisia, BP1172-3018-SFAX, Tunisia; Tel: +21620333622; E-mail: amel.chaffai@tunet.tn

ISSN: 1927-3037/14

BIOINDICATORS OR BIOMONITORS

The first definition was proposed by Phillips and Rainbow [3] who considered biomonitors as animal (s) or plant(s) which accumulate contaminants in their tissues and organs from their surroundings. The quantification of such contaminants (in fish, crustaceans, bivalves, etc…) can therefore reflect the concentration of contaminants in the surrounding environment.

In 1999, Gerhardt [4] proposed another definition; bioindicators are defined as species or group of species that readily reflects the abiotic or biotic state of an environment represents the impact of environmental change on a habitat, community or ecosystem or is indicative of the diversity of a subset of taxa or the whole diversity within an area. The author has considered that bioindicators are useful in three situations: (i) where the indicated environmental factor cannot be measured, (ii) where the indicated factor is difficult to measure, e.g. pesticides and their residues or complex toxic effluent (iii) where the environmental factor is easy to measure but difficult to interpret, e.g. whether the observed changes have ecological significance.

According to the aim of bioincation, three types of biomonitors were proposed, (i) compliance indicators, (ii) diagnostic indicators (iii) early warning indicator.

According to various authors, bioindicators are organisms that indicate the long-term interaction of several environmental conditions, but also react to a sudden change of important combinations of factors. Other authors consider only those organisms that react to changes in the environment by alterations in their metabolism, activity, or other aspects of their biology or that accumulate toxic substances [5].

In order to monitor pollution, bio-monitors or bio-indicators are organisms that can be used to provide information on the variation of pollutants over time and space.

Taking the case of metal pollution, each bioindicator shows the special merits for the biomonitoring aquatic ecosystem when compared to the others [6].

In the practice, the organisms living in the aquatic systems are sampled for the analysis of various biological responses to chemical exposures. A “perfect” bioindicator is expected to have several characters. The most important are : (a) it can accumulate high levels of pollutants without death ; (b) it has enough abundance and wide distribution for the repetitious sampling and comparison ; (c) its life is long enough for the comparison between various ages ; (d) it can afford suitable target tissue or cell for the further research at microcosmic level ; (e) easy sampling and easy raising in the lab ; (f) well dose-effect relationship can be observed in it [6].

For aquatic pollution, the common used bioindicators mainly contained organisms including mollusks bivalves, mollusks gastropods and fishes. Zooplankton species can accumulate and metabolize pollutants, offering the possibility to be used as bioindicators of water quality and show their special advantages in biomonitoring [6]. Aquatic algae, considered as the important elementary producers in marine water plays key role to the whole ecosystem, and can directly reflect the water quality. As an example of bioindicators algae we can note *Chlorella ellipsoidea*, *C. ellipsoidea* [7], in addition to the algae *Cystoseira stricta* and the famous *posidonia oceanica* which are very sensible and disappear in polluted surroundings. Moreover they are used to outline the history of pollution [7].

Bivalve mollusks, such as mussels and oysters are characterized by their aptitude to concentrate both metals and organic contaminants, their immobility, their limited ability to metabolize accumulated contaminants, their abundance, their persistence, and their ease of collection They have become recognized bio-monitors of pollutants in coastal waters and have been used in different international monitoring programs such as the Mussel Watch (USA) and the RNO (France) [8-10].

In the case of Tunisia, Mussels and oysters are available only in the northern coast. As a substitute, we have proposed and validate the use of *Ruditapes decussatus or Cerastodema glaucum.* In fact these two species are sedentary filter feeding marine bivalves that are widely available along the Tunisian coast and satisfying criteria required for good bio-monitors of pollution. The first species *Ruditapes decussatus* represents an important economic endpoint since it is a natural resource in the gulf of Gabès, the stock being mostly exported to Europe. The second bivalve is the cockle *Cerastoderma glaucum* is also a filter feeding organism living in the superficial sediment and available in many sites along the Tunisian coast.

Nevertheless, mussel and oysters are not available everywhere. In such circumstances, each country should adapt its monitoring program according to available species.

Before considering these two bivalve species as Biomonitors, we need to check if the conditions above cited are satisfied.

Since many years we have undertaken both *in vivo* [11-13], *in situ* studies [14-16] as well as transplantation experiments [17]. Our choice was guided by their availability along the Tunisian coasts, their sedentary filter feeding habits. While *Ruditapes decussates* presents a long siphon and lives inside the sediments, *Cerastoderma glaucum* has a short siphon and lives in the superficial sediment. Together these two bivalve species could reflect the pollution of their surrounding water. We have demonstrated both *in vivo* and *in situ*, the relationship between trace metal concentration in the studied sites and in clam and cockle tissues. We also identify target organs for metal accumulation. Biotic variations linked to the reproductive cycle, size, sex were also studied [18,19].

BIOMAKERS

The term biomarker has been defined as a xenobiotically induced variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample [20]. Biomarkers were originally developed in the medical and veterinarian sciences and there has been an increasing emphasis on the use of invertebrate and particularly bivalve biomarkers to assess marine pollution [21].

Biomarkers occurs at different levels of organization, from subcellular to whole organisms and ecosystem. Effect at molecular level trend occurs first, followed by responses at the cellular (biochemical), tissue/organ and whole-body levels. Responses that occur at individual, population and ecosystem level are generally accepted to have ecological relevance and tend to be less reversible and more detrimental than effects at lower levels. In fact, much attention is given towards identifying and understanding toxic effects initiated at the sub-organism level (molecular, bio-chemical or physiological changes) and towards developing biomarkers at this level to be incorporated into routine biomonitoring programs [22,23].

Biomarkers can be generally, broadly categorized as markers of exposure and effect.

1. Biomarkers of exposure are the product of integration between a xenobiotic and some target molecule or cell that is measured within a compartment of an organism. In general, biomarkers of exposure are used to predict the dose received by an individual, which can be related to change resulting in a disease state.
2. Biomarkers of effect are defined as measurable biochemical, physiological, behavioral, or other alterations within an organism that, according to their magnitude, can be recognized as established or potential health impairment or disease.

Some biomarkers are highly specific for individual chemicals; such biomarkers include inhibition of cholinesterase by organophosphate or carbamate [24] metallothioneines by toxic trace metals [14,25-27] and ethoxyresorufin-o-deethylase (EROD) respond to organic chemicals, particularly PAHs and PCBs. Others biomarkers are also well validated, but they have wider application and tend to respond to broader classes of chemicals. Example of these biomarkers are the induction of the multiplexenobiotic Resistance (MXR) protein [28,29], Stress on stress [30], the formation of DNA adduct and other DNA alteration [31-33], and also lysosomal alteration in molluscan digestive gland cell [34,35], etc… These assays required either additional biomarker studies or chemical residue analysis in order to link causative agent to adverse effect.

An important application of biomarkers is their ability to integrate multiple chemical exposure across an area with variety of chemical contaminants; the CYP A1 responses to sediment contaminated with dioxin, polychlorited biphenyl (PCBs), or polynuclear aromatic hydrocarbons (PAHs) can provide insight to the status of the contaminants on site, their bioavailability, and overall risk that they pose [36-38]. Similarly, metallothionein content and immune function can provide insight to the combined effect of metals found on metal contaminated sites [39].

Some Examples

We below present some biomarkers which were studied in our laboratory: metallothioneins (MTs), malonedialdehyde (MDA), acetylcholinesterase (AchE) glycogen and stress on stress test.

Metallothioneins (MTs): Most scientists agree that biochemical and physiological mechanisms allowing mollusk bivalve species to accumulate and tolerate high amounts of heavy metals are based on their metal handling by metallothioneins (MTs). These are low molecular weight, cysteine-rich, cytosolic proteins of ubiquitous occurrence which are suggested to inactivate toxic metal ions by binding them to sulfur atoms of the peptide cysteine residues [40]. In fact, it has repeatedly been shown that concentrations levels of MT can be correlated to accumulated fractions of toxic metal ions such as copper or cadmium in animals' tissues [41]. In addition, metallothionein concentrations in molluscs may also vary due to influence by non-metallic pollutants. Hence, the idea was raised that MTs might be used as biomarkers for environmental pollution by measuring their concentrations in bivalves from contaminated habitats.

Earlier work in our laboratory has shown the presence of metallothioneins in the gills and digestive gland of *Ruditapes decussatus* and *Cerastoderma glaucumin* field conditions and after an experimental contamination.

Malonedialdehyde (MDA), is a product of lipid peroxidation due to over production of oxyradicals in cells, following contaminant exposure or stress due to natural conditions [42]. Lipid peroxidation is considered an important feature in cellular injury. It results from free radical reactions in biological membranes, which are rich in polyunsaturated fatty acids. MDA has been used extensively to assess detrimental effects of various pollutants.

Glycogen, is the fuel for different metabolic and physiological processes. Glycogen has been shown to respond quite well to complex and diffuse contamination situations [43]. Decreased levels of glycogen related to an altered growth were observed in mussels *Mytilus edulis* and clams (*Mya arenaria*) after an exposure to pulp and paper mills effluents as well as in a mesocosms where bivalves were exposed to oil and silicon based polymer [44].

Acetylcholinesterase activity (AchE) is an enzyme essential to the correct transmission of nerve impulse. A reduction or inhibition of this enzymatic activity has been used to detect and measure the biological effect of organophosphorus and carbamates in the marine environment [45].

The **stress on stress** test is considered as a non-specific biomarker allowing the evaluation of the general health status of bivalves. It consists in exposing animals to anoxia by air and to evaluate survival time. Measuring the lethal time for 50% or the organisms (LT50) provides information about the health status [46].

According to our research work, the assessment of both environmental and biological variables that may affect the biomarker responses should be investigated

Table 1: Overview of Research Work about Bioindicators and Biomarker Validation

Approach	Bioindicator	Studied metals (pollutant)	Biomarkers	Biotic factors	Reference
In vivo *In situ* Transplantation	*R. decussatus*	Cd, Cu, Zn	MTs, AchE, MDA, glycogen	Sex, size, reproductive state	[14] [26]
In vivo	*R. decussatus*	Cu, Lindane	AchE, MTs, Stress on stress		[46]
In situ	*R. decussatus*	Cd, Cu, Zn	MTs	Sex, size, reproductive state	[58]
In vivo *In situ* Transplantation	*C. glaucum*	Cd	MTs, AchE, MDA	Sex, Reproductive cycle, size	[11] [15] [17]
In vivo *In situ*	*C. glaucum* *R decussates* and *C. glaucum* *C. glaucum*	Cd Cd Cd	MTs, MDA MTs gene expression MTs, MDA, AchE, Stress on stress	Sex	[12] [59] [60]
In situ	*R decussatus*	Hg			[61]
In vivo *In situ*	*C glaucum*	Cd, Cu, Zn, Ni, Mn, Cr	MTs, MXR, CuZnSOD, MnSOD, COI, CAT (gene expression)	Sex, size	[62]

R decussatus: Ruditapes decussates; C glaucum: Cerastoderma glaucum.

prior using in pollution monitoring. It was necessary to combine; *in vivo* experiments based on pure contaminants and industrial effluents, *in vivo* and filed transplantations, and field studies approaches. The combination of these approaches helped us to identify the most reliable biomarkers in relation to pollution exposure and taking into account some biotic factors (size, age, reproductive state). Table **1** summarizes the main used approaches, bioindicators, biomarkers as well as the biotic factors studied.

Biomarker Use

The use of biomarkers to evaluate pollution has noticeably increased in the past few years. Indeed, the biomarker approach has now attracted the attention of international regulatory agencies as a new and potentially powerful tool for detecting exposure to and the effect of environmental contamination [47].

This approach should be multiparametric, using different and complementary biomarkers to reflect the effects of different contaminants. However, we also need to consider variations linked to biotic and abiotic factors. In fact, the physiological state of an organism within an ecosystem is the result of equilibrium between the influences of anthropogenic, abiotic and biotic factors [14,48,49].

The use of biomarkers measured at molecular or cellular level have been proposed as sensitive ‘early warning’ tools for biologic effect measurement in environmental quality assessment [47]. The selected biomarkers should indicate that organism has been exposed to pollutants (exposure biomarkers) and / or the magnitude of the organism’s response to the pollutant (effect biomarkers). These early warning biomarkers can be used in a predictive way, allowing to initiation of bioremediation strategies before irreversible environmental damage of ecological consequence occurs. These biomarkers are then defined as short-term indicators of long-term biological effects [34,50].

A practical and successful biomarker should satisfy a number of criteria:

1. The Biomarker response should be sensitive enough to detect early stage of the process of toxicity and should precede the effect at high levels of biological organization.

2. The Biomarker should be specific to a particular contaminant or for a class of contaminants.

3. The Biomarker should respond in a concentration-dependant manner to change in ambient levels of the contaminant.

4. Identification of the non toxicological variability identified in particular variations linked to biotic factors.

In practice, all of these characteristics are not satisfied; most biomarkers have limited specificity, because of the variety of pollutants present in an environment. That is why, a pool of biomarkers at different levels of biological organization is required to be effectively applicable in a biomonitoring program [47,51,52]. Non-specific biomarkers provide information that may indicate environmental perturbations but not the causal agent. To identify causal relationship, analytical chemistry and specific biomarkers are needed. However, it is not cost-effective to use a suite of specific biomarkers and measure a wide range of contaminants in all monitoring programs. It is, therefore recommended that the effects of pollutants might be, initially, detected by relatively non-specific biomarkers, usually high in the hierarchy (e.g., behavioral and physiological biomarkers). Detection of abnormalities with these non-specific biomarkers at a site at risk from pollution might then justify the measurements of more costly, lower hierarchy, specific biochemical and cellular biomarkers (e.g., MFO activity, metallothioneins, genotoxic marker, and tissue lesions) to identify the class of pollutant responsible for the exposure [50]. Another compelling reason to use general biomarkers in the initial stages of monitoring is that organism will undoubtedly be exposed to range of contaminants in the environment which have the potential to act antagonistically or synergistically. Specific biomarkers may miss the effect of certain chemical whereas non specific biomarkers will respond to the complex mixture of chemical present in the environment.

By the application of biomarker measurement the use of expensive and complex analytical chemical equipment can be reduced, as these analyses are relatively quick to perform. Furthermore, since biomarkers are a part of the detoxification mechanism. This provides not only early warning system about degradation in environmental quality, but also specific measures of the toxic, carcinogenic and mutagenic compound in the biological material. Many studies about biomarker response in organisms are being carried out in most of the developed biomonitoring program in Europe and USA. Moreover, different methods for biological effect measurement have been evaluated in a series of practical workshops organized by the International council for the Exploration of the Sea (ICES) and the Intergovernmental Oceanographic Commission (IOC), such as those in the North Sea.

In the Mediterranean Sea, the United Environment Program has funded a biomonitoring program including a variety of biomarkers [53]. Biomarkers have also been included in the joint monitoring program of the OSPAR convention where Portugal, Spain and others European countries are the members [34]. Several of biomarkers have been applied in a pollution monitoring program of the Mediterranean Sea [54]. The program involved use of both biomarkers of effects (micronuclei frequency and lysosomal membrane stability) and biomarkers of exposure (cytochrome P450, EROD and metallothioneins).

The BEEP (Biological Effect of Environmental Pollution in Marine Coastal Ecosystems) project, involving 30 institutions from 12 countries in Europe and Scandinavia including the North Atlantic region, has focused on development of new biomarkers [55].

Finally, there is a long list of biomarkers that are under development or have been used with varying degrees or success but required further validation before can be used in hazard evaluation.

In natural ecosystem, this is very likely as changes in physicochemical and biological characteristics often vary over very small distances. Such heterogeneity will then be reflected in small-scale differences of the bioavailability of pollutants. However, an equally important source of variability may be inherent differences in the morphology and biochemical/ physiological status of exposure organisms [51]. Variability in most of the biochemical and physiological biomarker responses may be, thus, attributed to abiotic factors (Temperature, salinity, dissolved oxygen, ect) or biotic factors (Size, age, genotype, ect.) All these factors can vary in both time and space [52]. These sources of variability render the biomarker responses insensitive compared to traditional chemical monitoring techniques.

In order to integrate biomarkers in modern pollution problem, taking into account temporal and spatial variability in biomarker responses, and with various pollutant mixtures, Handy *et al*. [52] proposed several priorities in future researches. These include the following:

1. Funding of long term biomarker studies (minimum 5 year) as part of validation of chronic biomarkers

2. Primary research to mathematically quantify and model temporal and spatial variables that influence biomarker responses. These should include age, sex and nutritional status corrections for biomarkers in specific organism exposed to known contaminants in the laboratory, following by field validation.

3. More fundamental research in to chronic dose-response relationship.

CONCLUSION

Aquatic ecosystems are under constant pressure of anthropogenic pollutants originating from various sources. In the recent decades, there have increasing concerns about pollutants entering the aquatic environment. A need to discover simple and reliable ways to monitor the level of particular chemicals such as heavy metals or other pollutants in the aquatic environment, and to elucidate the mechanisms of pollutants uptake and storage in organisms, has resulted in a proliferation of studies into the use of biomonitor organisms. The success of some Mollusc Bivalves, is due to some factors including their wide geographical distribution, abundance, sedentary, tolerance to environmental changes, high bioconcentration factors, population stability, and size, adaptability for field, cage and laboratory experiments.

This was completed by the biomarker approach, based on early warning systems for pollution monitoring. A detailed knowledge of natural variation in biomarkers responses must, therefore, be ascertained before field application in order to prevent confounded interpretation when they are applied in environmental monitoring programs.

REFERENCES

[1] Murray TB, Michael HD. Determinant of trace metal concentrations in marine organisms. In: Langston B, Bebianno MJ, editors. Metal metabolism in aquatic environments. London: Chapman & Hall 1998; p. 185-217.

[2] Lagadic L, Caquet T, Amiard JC. Biomarqueurs en écotoxicologie: principes et définitions (introduction). In: Lagadic L, Caquet T, Amiard JC, editors. Biomarqueurs en écotoxicologie, aspects fondamentaux. Paris: Masson 1997; pp. 1-7.

[3] Phillips DJH, Rainbow PS. Biomonitoring of Trace Aquatic Contaminants. 2nd. ed. London: Chapman and Hall 1994.

[4] Gerhardt A. Biomonitoring of Polluted Water. Reviews on Actual Topics. Trans Tech Publ, Zürich, Switzerland 1999; p. 301.

[5] Fureder L, Reynolds JD. Is austropotamobius pallipes a good bioindicators? Bull Fr Pêche Piscic 2003; 157-63. http://dx.doi.org/10.1051/kmae:2003011

[6] Zhoua Q, Zhanga J, Fua J, Shi J, Jiang G. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 2008; 606(2): 135-50. http://dx.doi.org/10.1016/j.aca.2007.11.018

[7] Marbà N, Santiago R, Díaz-Almela E, Álvarez E, Duarte CM. Seagrass (*Posidonia oceanica*) vertical growth as an early indicator of fish farm-derived stress. Estuarine, Coastal and Shelf Sci 2006; 67: 475-83. http://dx.doi.org/10.1016/j.ecss.2005.11.034

[8] Stellio C, Cédric B. Modelling trace metal (Hg and Pb) bioaccumulation in the Mediterranean mussel, *Mytilus galloprovincialis*, applied to environmental monitoring. J Sea Res 2006; 56(2): 168-81. http://dx.doi.org/10.1016/j.seares.2006.03.006

[9] Yungkul K, Eric NP, Terry LW, Bobby JP. Relationship of parasites and pathologies to contaminant body burden in sentinel bivalves: NOAA Status and Trends 'Mussel Watch' Program. Marine Environ Res 2008; 65(2): 101-27. http://dx.doi.org/10.1016/j.marenvres.2007.09.003

[10] Rocher B, Le Goff J, Peluhet L, *et al*. Genotoxicant accumulation and cellular defence activation in bivalves chronically exposed to waterborne contaminants from the Seine River. Aquat Toxicol 2006; 79 (1): 65-77. http://dx.doi.org/10.1016/j.aquatox.2006.05.005

[11] Macherki-Ajimi M, Rebai T, Hamza-Chaffai A. Variation of metallothionein-like protein and metal concentration during the reproductive cycle of the cockle *Cerastoderma glaucum* from uncontaminated site: a one year study in the gulf of Gabès area. Marine Biol Res 2010; 7(3): 261-71. http://dx.doi.org/10.1080/17451000.2010.497187

[12] Ladhar-Chaabouni R, Smaoui-Damak W, Hamza-Chaffai A. *In vivo* variation of some biomarkers with time and cadmium concentration in the cockle *Cerastoderma glaucum*. Marine Biol Res 2009; 5: 487-95. http://dx.doi.org/10.1080/17451000802534881

[13] Damak-Smaoui W, Mathieu M, Rebai T, Hamza-Chaffai A. Histology of the reproductive tissue of the clam *Ruditapes decussates*. Inv Repr Dev 2007; 50(3): 117-26. http://dx.doi.org/10.1080/07924259.2007.9652235

[14] Hamza-Chaffai A, Amirad JC, Pellerin J, Joux L, Berthet B. The potential use of metallothionein in the clam *Ruditapes decussatus* as a biomarker of in situ metal exposure. Comp Biochem Physiol 2000; 127(Pt C): 185-97.

[15] Macherki-Ajimi M, Ketata I, Ladhar-Chaabouni R, Hamza-Chaffai. The effect of in situ cadmium contamination on some biomarkers in *Cerastoderma glaucum*. Ecotoxicol 2008; 17: 1-11. http://dx.doi.org/10.1007/s10646-007-0166-9

[16] Damak-Smaoui W, Hamza-Chaffai A, Berthet B, Amirad JC. Preliminary study of the clam *Ruditapes decussatus* exposed in situ to metal contamination and originating from the gulf of gabès. Bull Environ Contam Toxicol 2003; 71(5): 961-70. http://dx.doi.org/10.1007/s00128-003-8899-5

[17] Macherki-Ajimi M, Hamza-Chaffai A. Assessment of sediment/water contamination by *in vivo* transplantation of the cockles *Cerastoderma glaucum* from a non-contaminated to a contaminated area by cadmium. Ecotoxicol 2008; 17: 802-10. http://dx.doi.org/10.1007/s10646-008-0238-5

[18] Damak-Smaoui W, Guebsi F, Kostil C, Rebai T, Hamza-Chaffai A. Storage and reproductive strategy of the carpet-shell clam, *Ruditapes decussatus* in the Gulf of Gabès (Tunisia). Inv Reprod Dev 2011; 1-11. http://dx.doi.org/10.1080/07924259.2011.607518

[19] Macherki-Ajimi M, Rebai T, Hamza-Chaffai A. Reproductive strategy in a littoral population of the cockle *Cersatodertma glaucum* from the gulf of Gabès. J Shellfish Res 2013; 32(3): 733-38. http://dx.doi.org/10.2983/035.032.0315

[20] National Research Council. Committee on biological markers. Env Health Perspective 1987; 74: 3-9. http://dx.doi.org/10.2307/3430428

[21] Nicholson S. The mytilid mussel *Perna viridis* (Mytilidae: Bivalvia) as a pollution monitor in Hong Kong. In: Shin PKS, editors. Turning the tides, Marine Biological Association of Hong Kong. Hong Kong: University Press 2003; pp. 201-28.

[22] Forbes VE, Forbes TL. Ecotoxicology in Theory and Practice. London: Chapman and Hall 1994.

[23] Shugart LR, Theodorakis CW. Genetic ecotoxicology: The genotypic diversity approach. Comp Biochem Physiol 1996; 113(Pt C): 273-76.

[24] Thompson JAJ, Cosson RP. An improved electrochemical method for the quantification of metallothionein in marine organisms. Mar Environ Res 1984; 11: 137-52. http://dx.doi.org/10.1016/0141-1136(84)90027-8

[25] Isani G, Andreani G, Kindt M, Carpène E. Metallothioneins (MTs) in marine molluscs. Cell Mol Biol 2000; 46(2): 311-30.

[26] Hamza-Chaffai A, Pellerin J, Amirad JC. Health assessment of *Ruditapes decussatus* from the Gulf of Gabes (Tunisia). Environ Int 2003; 28: 609-17. http://dx.doi.org/10.1016/S0160-4120(02)00102-2

[27] Ivanković D, Pavičić J, Erk M, Filipović-Marijić V, Raspor B. Evaluation of the *Mytilus galloprovincialis* Lam. digestive gland metallothionein as a biomarker in a long-term field study: Seasonal and spatial variability. Marine Pollution Bull 2005; 50(11): 1303-13. http://dx.doi.org/10.1016/j.marpolbul.2005.04.039

[28] Legeay A, Achard-Joris M, Baudrimont M, Massabuau JC, Bourdineaud JP. Impact of cadmium contamination and oxygenation levels on biochemical responses in the Asiatic clam *Corbicula fluminea*. Aquat Toxicol 2005; 74(3): 242-53. http://dx.doi.org/10.1016/j.aquatox.2005.05.015

[29] Sandrine P, Marc P. Identification of multixenobiotic defence mechanism (MXR) background activities in the freshwater bivalve *Dreissena polymorpha* as reference values for its use as biomarker in contaminated ecosystems. Chemosphere 2007; 67 1258-63. http://dx.doi.org/10.1016/j.chemosphere.2006.11.017

[30] Hellou J, Law RJ. Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. Environ Pollut 2003; 126 (3): 407-16. http://dx.doi.org/10.1016/S0269-7491(03)00231-8

[31] Dixon DR, Pruski AM, Dixon LRJ, Jha AN. Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis 2002; 17: 495-507. http://dx.doi.org/10.1093/mutage/17.6.495

[32] Siu WHL, Hung CLH, Wong HL, Richardson BJ, Lam PKS. Exposure and time dependent DNA strand breakage in hepatopancreas of green-lipped mussels (*Perna viridis*) exposed to Aroclor 1254, and mixtures of B[a]P and Aroclor 1254. Marine Pollution Bull 2003; 46: 1285-93. http://dx.doi.org/10.1016/S0025-326X(03)00234-0

[33] Magni P, De Falco G, Falugi C, *et al*. Genotoxicity biomarkers and acetylcholinesterase activity in natural populations of *Mytilus galloprovincialis* along a pollution gradient in the Gulf of Oristano (Sardinia, western Mediterranean). Environ Pollution 2006; 142: 65-72. http://dx.doi.org/10.1016/j.envpol.2005.09.018

[34] Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C, Viarengo A. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci Total Environ 2000; 247: 295-311. http://dx.doi.org/10.1016/S0048-9697(99)00499-4

[35] Domouhtsidou GP, Dailianis S, Kaloyianni M, Dimitriadis VK. Lysosomal membrane stability and metallothionein content in

Mytilus galloprovincialis (L.), as biomarkers Combination with trace metal concentrations. Marine Pollution Bull 2004; 48(5-6): 572-86. http://dx.doi.org/10.1016/j.marpolbul.2004.01.013

[36] Nebert DW. The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. Crit Rev Toxicol 1989; 20:153-74. http://dx.doi.org/10.3109/10408448909017908

[37] Stegeman JJ, Hahn ME. Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. In: Malins DC, Ostrander GK, editors. Boca Raton: Aquatic Publishers 1994; pp. 87-203.

[38] Parkinson A. Biotransformation of xenobiotics. In: Klaassen CD, editor. Casarett and Doull's toxicology. 3rd ed. New York: McGraw-Hill 1995; pp. 113-86.

[39] Auffret M, Rousseau S, Boutet I, *et al.* A multiparametric approach for monitoring immunotoxic responses in mussels from contaminated sites in Western Mediterranean. Ecotoxicol Environ Safety 2006; 63: 393-405. http://dx.doi.org/10.1016/j.ecoenv.2005.10.016

[40] Amiard JC, Cosson RP. Les métallothionéins. In: Lagadic L, Caquet Th, Amiard JC, Ramade F, editors. Biomarqueurs en Ecotoxicologie: Aspects Fondamantaux. Paris: Masson 1997; pp. 53-66.

[41] Roesijadi G. Metal transfer as a mechanism for metallothionein-mediated metal detoxification. Cell Mol Biol 2000; 46: 293-405.

[42] Pellerin- Massicotte J. Influence of elevated temperature and air exposure on MDA levels and catalase activities in digestive glands of the blue mussel (*Mytilus edulis*). J Rech Océanogr 1997; 22: 91-8.

[43] Gauthier-Clerc S, Pellerin J, Blaise C, Gagné F. Delayed gametogenesis of *Mya arenaria* in the Saguenay fjord (Canada): a consequence of endocrine disruptors? Comp Biochem Physiol 2002; 131(Pt C): 457-67. PII: S1532-0456Ž0 2.00041-8

[44] Pellerin J, Vincent B, Pelletier E. Evaluation écotoxicologique de la qualité de la baie des Anglais (Québec). Water Pollut Res J Can 1993; 28: 665-89.

[45] Hamza-Chaffai A, Amiard-Triquet C, El Abed A. Metallothionein-like protein, is it an efficient biomarker of metal contamination? A case study based on fish from the Tunisian coast. Arch Environ Contam Toxicol 1997; 33: 53-62. http://dx.doi.org/10.1007/s002449900223

[46] Hamza-Chaffai A, Romeo M, Gnassia-Barelli M, El Abed A. Effect of copper and lindane on some biomarkers measured in the clam *Ruditapes decussatus*. Bull Environ Contam Toxicol 1998; 61: 397-404. http://dx.doi.org/10.1007/s001289900776

[47] Mc Carty JF, Shugart LR. Biomarkers of Environmental Contamination. Chelsea: Lewis Publishers 1990.

[48] Pellerin-Massicotte J. Oxidative processes as indicators of chemical stress in marine bivalves. J Aquat Ecosystem Health 1994; 3: 101-11. http://dx.doi.org/10.1007/BF00042940

[49] Ladhar-Chaabouni R, Macherki-Ajimi M, Hamza-Chaffai A. Use of metallothioneins as biomarkers for environmental quality assessment in the Gulf of Gabès (Tunisia). Environ Monit Assessment 2012; 184: 2177-92. http://dx.doi.org/10.1007/s10661-011-2108-5

[50] Nicholson S, Lam PK. Pollution monitoring in South East Asia using biomarkers in the mytilid mussel *Perna viridis* (mytilidae: Bivalvia). Environ Int 2005; 31: 121-32. http://dx.doi.org/10.1016/j.envint.2004.05.007

[51] Depledge MH, Fossi MC. The role of biomarkers in environmental assessment (2). Invertebrates. Ecotoxicol 1994; 3: 161-72. http://dx.doi.org/10.1007/BF00117081

[52] Handy RD, Galloway TS, Depledge MH. A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. Ecotoxicol 2003; 1-2: 331-43. http://dx.doi.org/10.1023/A:1022527432252

[53] UNEP. Report of the meeting of experts to review the MED POL biomonitoring programme. Athens, Greece: UNEP-OCA/MED WG. 132/7, 1997.

[54] Med Pol, UNEP. Guidelines for river (including estuaries) pollution monitoring programme for the mediterranean region. Athens: UNEP/MAP; 2004.

[55] Verlecar XN, Desai SR, Sarkar A, Dalal SG. Biological indicators in relation to coastal pollution along Karnataka coast, India. Water Res 2006; 40(17): 3304-12. http://dx.doi.org/10.1016/j.watres.2006.06.022

[56] Nicholson S. Ecocytological and toxicological responses to copper in *Perna viridis* (L.) (Bivalvia: Mytilidae) haemocyte lysosomal membranes. Chemosphere 2001; 45: 399-407. http://dx.doi.org/10.1016/S0045-6535(01)00039-X

[57] Nicholson S. Ecophysiological aspects of cardiac activity in the subtropical mussel *Perna viridis* (L.) (Bivalvia: Mytilidae). Journal of Experimental Marine Biol Ecol 2002; 267: 207-22. http://dx.doi.org/10.1016/S0022-0981(01)00362-8

[58] Smaoui-Damak W, Rebai T, Berthet B, Hamza-Chaffai A. Does cadmium pollution affect reproduction in the clam *Ruditapes decussatus*? A one-year case study. Comp Biochem Physiol 2006; 143 (Pt C): 252–61. http://dx.doi.org/10.1016/j.cbpc.2006.02.009

[59] Ladhar-Chaabouni R, Gargouri- Mokdad R, Denis F, Hamza-Chaffai A. Cloning and characterization of cDNA probes for the analysis of metallothionein gene expression in the Mediterranean bivalves: *Ruditapes decussatus* and *Cerastoderma glaucum*. Mol Biol Rep 2009; 36:1007-14. http://dx.doi.org/10.1007/s11033-008-9274-8

[60] Ladhar-Chaabouni R, Macherki-Ajimi M, Hamza-Chaffai A. Spatial distribution of cadmium and some biomarkers in *Cerastoderma glaucum* living in a polluted area. Marine Biol Res 2009; 5: 478-86. http://dx.doi.org/10.1080/17451000802683985

[61] Mezghani-Chaari S, Hamza A, Hamza-Chaffai A. Mercury contamination in human hair and some marine species from Sfax coasts of Tunisia: levels and risk assessment. Environ Monit Assess 2011; 180: 477-87. http://dx.doi.org/10.1007/s10661-010-1800-1

[62] Karray S, Denis F, Moreau B, Chaffai A, Chénais B, Marchand J. Transcriptional responses of stress genes in experimental and natural populations of the cockle *Cerastoderma glaucum* originating from the gulf of Gabes, Sfax, Tunisia. PRIMO 17th, Faro, Portugal 2013.

Received on 05-03-2014 Accepted on 18-03-2014 Published on 15-04-2014

DOI: http://dx.doi.org/10.6000/1927-3037.2014.03.01.4