Assessment of Water Quality in Coastal Environments of Mohammedia Applying Responses of Biochemical Biomarkers in the Brown Mussel *Perna perna*

Laila El Jourmi*, Abdessamad Amine*, Meryem Mrani Alaoui, Said Lazar, Abdelaziz Hmyene and Said El Antri
***These authors contributed equally to this work**

Laboratory of Biochemistry Environment and Agroalimentary, Biology Department, University Hassan II, Faculty of Sciences and Techniques Mohammedia, Morocco

Abstract

The present work aims to assess the marine environment quality in Mohammedia, using the response of the biochemical biomarkers in the brown mussel *Perna perna*. The biomarkers selected in this work are : glutathione S-transferase (GST) as phase II enzyme and the acetylcholinesterase (AChE) activity as neurotoxicity marker. The Oxidative stress is evaluated using catalase (CAT), a well-known anti-oxidant enzyme, and malondialdehyde (MDA) accumulation as marker of oxidation of membrane phospholipids through lipid peroxidation. And finally the metallothioneine (MT) as stress proteins.

Our data indicated that CAT, GST activity and MDA, MT concentration in whole mussel bodies, are a higher and significant ($p < 0.05$) in mussels collected at polluted site when compared to specimen sampled from control one. In contrary the response of AChE activity was significantly ($p<0.05$) inhibited in mussels from polluted site when compared to control value. The multi-marker results confirm that mussels from Mohammedia have been submitted to polluted environment.

Keywords: *Biomarkers; Catalase; Malondialdehyde; Glutathione S-transferase; Acetylcholinesterase; Metallothioneine; Mussels; Perna perna; Marine Pollution.*

1. Introduction

Bivalves, in particular mussels, are widely used in biomonitoring programs, mainly due to their biological characteristics; they are sessile, filter-feeding, widely distributed and abundant in coastal and estuarine areas, able to accumulate several classes of pollutants, thus providing a time-integrated picture of their bioavailability. For such characteristics, these organisms are widely used in Mussel Watch monitoring programs, in which chemical analyses are integrated with the use of biomarkers, to evaluate molecular, biochemical and cellular effects induced by pollutants [1].

The measurement of the biological effects of chemical pollutants has become of major importance for the assessment of the quality of the coastal environment. It has been reported that the use of biomarkers is very informative about the organism's stress response to individual toxicants and mixtures [2-4]. Measuring the same biomarkers in different localities simultaneously gives us information about the pollution status and provides a better comprehension of the mechanistic mode of action of environmental pollutants.

Among these biomarkers are CAT, a well-known antioxidant enzyme, which converts H2O2 into water. The biological importance of CAT is more evident from various studies due to the fact that H2O2 is the main cellular precursor of the hydroxyl radical ($HO^{\cdot}$) which is a highly reactive and toxic form of ROS (Reactive oxygen species) leading to oxidative damage to basic biological molecules.

Toxicity biomarkers, such as MDA, well-known lipid peroxidation products, have been also proposed to reflect the oxidative status of exposed species [5]. MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation [6]. GST which is a phase II enzyme involved in the metabolism of lipophilic organic contaminants. GST catalyzes the conjugation of various electrophilic compounds (e.g. epoxides of PAHs) with the tripeptide glutathione, the resulting conjugates being water soluble and thus more easily excretable.

AChE is an enzyme essential to the correct transmission of nerve impulses. Its inhibition is directly linked with the mechanisms of toxic action of anticholinesterase compounds [7-9].

MT are useful metal-pollution biomarkers [10], it constitutes a family of low molecular weight, cysteine-rich, and metal binding proteins that occurs throughout the animal kingdom. Biological functions of metallothioneins include homeostasis and sequestration of both essential and nonessential metals, detoxification of metals and scavenging of free radicals [11,10].

The present work aims to assess the marine environment quality in Mohammedia, using the response of the biochemical biomarkers in the brown mussel *Perna perna*.

2. Material and Methods

2.1 Reagents

Hydrogen peroxide (H2O2), Thiobarbituric Acid (TBA), Acetylthiocholine (AtChl) and Tetramethoxypropane (TMP) were obtained from Sigma (Saint Quentin Fallavier, France). 1-chloro-2,4-dinitrobenzene (CDNB), 5,5' dithio-bis 2 nitrobenzoic acid (DTNB), Reduced Glutathione (GSH), and Bovine serum albumin (BSA) were purchased from Genome Biotechnologies (Casablanca, Morocco).

2.2 Studied Areas

For this study, two stations (Fig. 1) are selected attending to various degree of human impact. Site 1 (S1) constitutes the site furthest away from the polluting industrial activities established on the coastal fringe Casablanca-Mohammedia. The clean reference area, which is selected due to the absence of contamination sources, located in beach of the south, Skhirat which is characterised by a total prohibition of human activities. S1 is characterized by an important density of mussels and the high faunistic and floristic richnesses of the site are well marked. On the contrary, Site 2 (S2) is located approximately 7 km in South of Mohammedia beach. Due to intense human activities, the S2 is constantly subjected to contamination. In addition, S2 has low biodiversity of intertidal organisms, indicating high levels of pollution pressure.

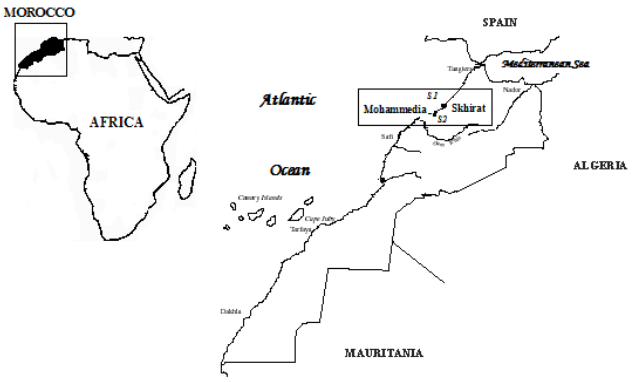


Fig 1. A map showing mussel sampling locations.

2.3 Sampling

Ten mussels from each site were sampled during May 2010. Following collection, the adult mussels were placed in thermally insulated boxes previously filled with water from the sampling site and immediately transported to the laboratory and stored at -80 °C until analysis.

2.4 Biochemical Analyses

Whole soft tissues from each specimen (n=5 for each station) were dissected out and immediately homogenized (1:3) in phosphate buffer 100 mM, pH 7.4. Homogenates were then centrifuged at 9000×g at 4 °C for 30 min. After centrifugation, supernatants were collected and immediately used for the determination of enzymatic activity, MDA and MT concentration.

CAT activity was measured following the decrease of absorbance at 240 nm due to H2O2 consumption [12]. The reaction takes place in 100 mM phosphate buffer, pH 7.4 containing 500 mM H_2O_2. GST activity was assayed by the method described by Habig et al. (1974) [13] using the CDNB as substrate, and 1 mM GSH, in 100 mM sodium phosphate buffer, pH 7.4. GST activity was determined by kinetic measurement at 340 nm. AChE activity was determined according to the method described by Ellman et al. (1961) [14] using 8 mM DTNB, and 45 mM AtChl as substrate in 100 mM sodium phosphate buffer, pH 7.4. AChE activity was determined by kinetic measurement at 412 nm. Results of enzymatic activities were expressed as nmol /min/mg proteins. MDA was estimated according to the method described by Sunderman (1985) [15] with use of TMP as a standard. The reaction was determined at 532 nm, using TBA as reagent. MDA content was expressed as nmol /mg proteins.

MT content was evaluated in whole soft tissues according to a spectrophotometric method described by Viarengo et al. (1997) [16]. Tissues (n=5 for each station) were homogenised (1:3) in Tris Buffer (Tris 20 mM, 0.5 M sucrose, pH 8.6) containing 0.5 mM phenylmethylsulphonyl fluoride and 0.01% β-mercaptoethanol. The soluble fractions containing MT were obtained by centrifuging the homogenate at 10 000 g for 30 min. The supernatant was then treated with cold absolute ethanol and chloroform. Finally, MT content was spectrophotometrically determined at 412 nm using Ellman_s reagent (DTNB) and GSH as standard. The results were expressed as µg/mg proteins. Protein concentrations were measured according to the Bradford (1976) [17] method, at 595 using BSA as standard.

2.5 Statistical Analyses

The results for biomarker measurements were investigated by the use of a parametric one-way analysis of variance (ANOVA) and level of significance was set at $p < 0.05$.

3. Results

The marine quality in Mohammedia, is checked in May 2010 at two sites (Fig. 1), by analyzing a battery of biochemical biomarkers of pollution in whole mussel

bodies. For each biomarker, the mean of measurements obtained for each site studied is shown in Fig. 2.

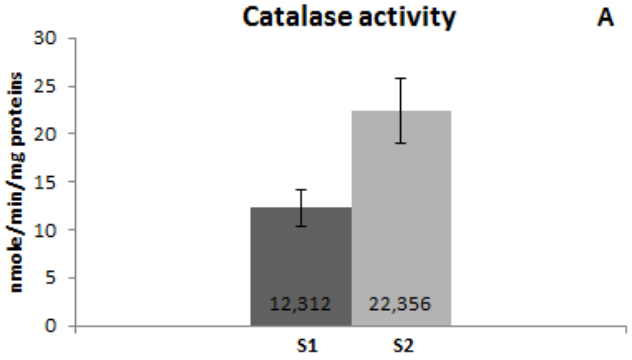
The results relative to the CAT activity are reported in Fig. 2.A. Our results showed that CAT activity presented a higher and significant differences ($p<0.05$) between polluted and non-polluted stations. The highest CAT activity was registered in mussels from polluted area (S2) with value up to 22,356 nmol min^{-1} mg^{-1} protein, and the lowest activity was reported in mussels from the clean one (S1), with 12,312 nmol $min^{-1}mg^{-1}$ protein.

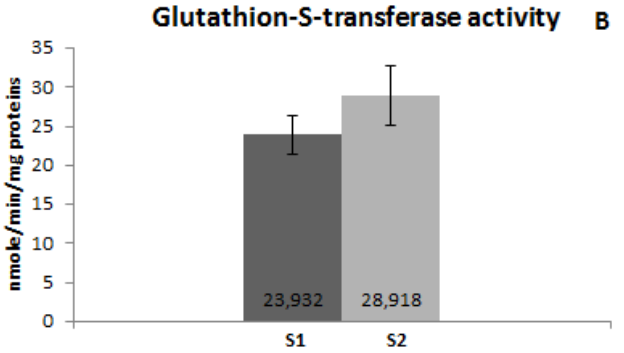
The response of the phase II enzyme GST activity is shown in Fig. 2.B. A significant differences ($p<0.05$) values of GST activity were found in mussels collected at the studied stations. It can be seen from Fig. 2.B that The phase II enzyme GST activity values recorded in mussels were significantly lower in animals from S1 (23,932 nmol min^{-1} mg^{-1} protein), while those from S2, which is considered as a polluted station, presented the higher significant values (28,918 nmol min−1 mg−1 protein).

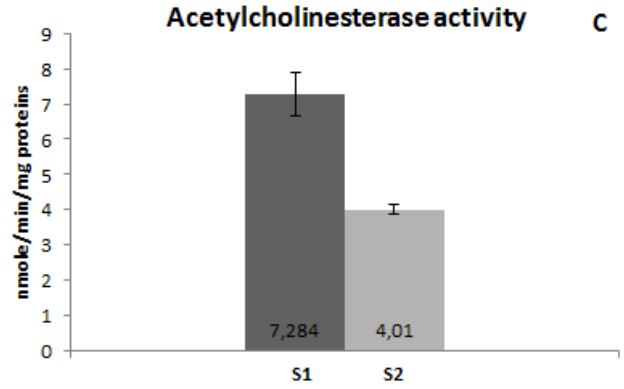
The response of AChE activity is reported in Fig. 2.C. The results indicate that AChE activity was significantly ($p<0.05$) inhibited in mussels from S2 with a value reaching to 4,01 nmol $min^{-1}mg^{-1}$ protein when compared to control value (7,284 nmol $min^{-1}mg^{-1}$ protein).

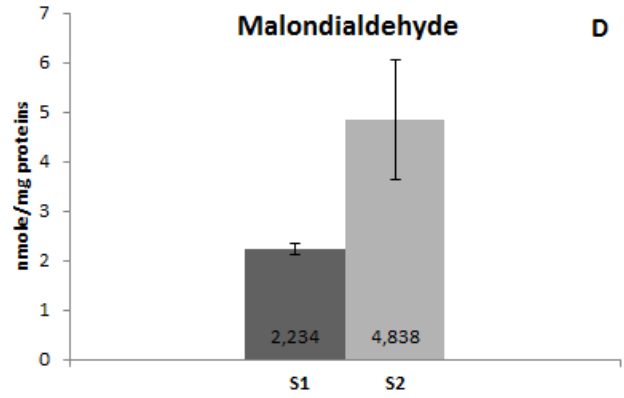
As shown in Fig. 2.D, a higher and significant ($p < 0.05$) accumulation of MDA was registered in *Perna perna* collected at S2 with an amount of 4,838 nmole mg^{-1} proteins when compared to specimen sampled from control site (2,234 nmole mg^{-1} proteins).

Our data indicated that the levels of MT showed a higher and significant increase ($P < 0.05$) in mussels from S2 (4,682 µg mg^{-1} proteins) in comparison to S1 (2,19 µg mg^{-1} proteins), selected as a suitable reference site (Fig. 2.E).









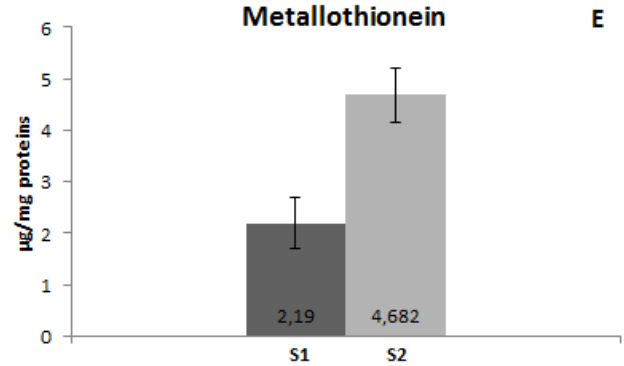


Fig 2. Activities of CAT (A), GST (B), AChE (C), and the levels of MDA (D) and MT (E) in Perna perna collected from the studied areas (S1 and S2).

4. Discussion

Marine bivalve, especially the mollusks because of their sedentary habitat, are exposed to a variety of environmental stressors, such as heavy metals, pesticides, petroleum products and thermal releases especially in area where cluster of industries are located.

Under normal physiological condition, animals maintain a balance between generation and neutralization of ROS. However when organisms are subjected to xenobiotic compounds, rate of production of ROS, exceeds the scavenging capacity [18] of antioxidant defence systems comprising both antioxidant enzymes, such as CAT, and small molecular weight free radical scavengers.

Exposure of organisms to pro-oxidant attack can increase antioxidant defences by increasing synthesis of antioxidant enzymes [18,19] to counteract oxidative stress. If antioxidant defences are effective in detoxifying ROS, then no harmful consequence results to the tissues. However, if the ROS attack is severe, then antioxidant defence systems may be overwhelmed. Such processes may in turn provoke alterations in molecular and membrane structures and functions leading to cell and tissue damage [20]. The increased activity of CAT in mussels from the investigated site compared to control value indicates that these animals are facing an oxidative challenge, associated with the presence of contaminants in the environment. But, these induced antioxidant defence enzyme increases were not enough to reduce lipid peroxidation levels in the polluted sites. Although CAT removes most of the H2O2 by increasing its activity levels, it cannot compete with the excess presence of H2O2 which diffuses into the cells, thereby causing increased concentration of MDA in S2. According to Charissou et al., (2004) [21], an increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality. Increased concentration of MDA was observed in mussels exposed in polluted areas when compared to less polluted sites [22,23].

Upon exposure to pollutants, organisms usually attempt to metabolize and depurate directly, minimizing any cellular damages they cause. Such protective mechanisms often include the intervention of enzymes such as GST, a family of multi-functional enzymes [24] involved in Phase II of biotransformation. GST catalyzes the conjugation of various electrophilic compounds (e.g. epoxides of PAHs) with the tripeptide glutathione, the resulting conjugates being water soluble and thus more easily excretable. The toxicity of many exogenous compounds can be modulated by induction of GST. Indeed GST activities were found to be modulated by metals or organic contaminants under both field conditions [25,28] and laboratory exposure [29]. This study demonstrated that mussels collected at site S2 presented significant higher levels of GST activity than those collected at site S1. So their induction can be regarded as an adaptive response to an altered environment. Several other field studies have demonstrated a similar relationship between environmental contamination and GST activity in mussels [30,31].

The main physiological function of AChE is splitting of acetylcholine, a mediator of cholinergic synapses, during transduction of nerve impulses [32]. The inhibition of AChE activity has been widely used to diagnose exposure to anticholinesterase compounds [7-9]. Many studies indicate that Cholinesterase activities are inhibited in the presence of some pesticides [33,34]. In fact, in addition to anticholinesterase pesticides, a few other contaminants such as heavy metals, detergents, some pyrethroids compounds like cypermethrin and deltamethrin and complex mixtures of pollutants can also affect the AChE activity [35,39]. In our study, we demonstrate that mussels inhabiting the site 2 (S2) present a higher inhibition of AChE activities compared to the site 1 (S1). The observed inhibition of AChE activities may be attributed to the presence of contaminants in the environment.

MT constitutes a family of low molecular weight, cysteine-rich, and metal binding proteins that occurs throughout the animal kingdom. Although the expression of MTs may be influenced by a number of natural factors [40] such as water temperature, they are also clearly induced by some of the environmentally most important heavy metals, making MT a potential biomarker for metal exposure in the aquatic environment [41]. Indeed the induction of MTs as a measure of response to metal exposure in aquatic organisms has been widely investigated in laboratory and field conditions [42-44].

It have been reported that MTs are known to be involved in heavy metal homeostasis and overexpressed in organisms experiencing high metal concentrations in their environment [45,46]. In this work, we have found that MT accumulation level is higher in mussels from S2 in comparison to S1. Concerning the role of MT in the homeostasis of trace metals and the elevated concentrations of these elements in mussels from S2, might compensate the metal bioavailability. So the elevated MT level at the site S2 may be considered as the result of higher overall level of metals pollution.

5. Conclusions

In conclusion, our data confirm the pollution status of site S2, which subjected to various types of pollution sources. The main sources of pollution are industrial, due to the presence of S2 in industrial park, and urban activities. Thus agricultural activities developed in the area, generate important quantities of toxic substances able to

contaminate the surface and deep waters whose general flow is directed towards the Ocean. So the geographical distribution of site 2 can explain the alteration of oxidative parameters.

References

[1] D.R., Livingstone. Biotechnology and pollution monitoring: use of molecular biomarker in the aquatic environment. J. Chem. Technol. Biotechnol., 1993, 57, 195–211.

[2] C., Svendsen , D.J., Spurgeon, P.K., Hankard, J.M., Weeks. A review of lysosomal membrane stability measured by neutral red retention: is it a workable earthworm biomarker? Ecotoxicol. Environ. Saf., 2004, 54, 20–29.

[3] L., Gastaldi, E., Ranzato, F., Caprì, P., Hankard, G., Pérès, L., Canesi, A., Viarengo, G., Pons. Application of a biomarker battery for the evaluation of the sublethal effects of pollutants in the earthworm Eisenia Andrei. Comp. Biochem. Physiol Part C, 2007, 146, 398–405.

[4] O., Adam, P.M., Badot, F., Degiorgi, G.,Crini. Mixture toxicity assessment of wood preservative pesticides in the freshwater amphipod Gammarus pulex (L.). Ecotoxicol. Environ. Saf., 2009, 72, 441–449.

[5] M., Sole, C., Porte, X., Biosca, C.L., Mitchelmore, J.K., Chipman, D.R., Livingstone, J., Albaiges, Effects of the "Aegean Sea" oil spill on biotransformation enzymes, oxidative stress and DNA-Adducts in digestive gland of the mussel (Mytilus edulis L). Comp. Biochem. Physiol. Part C Pharmacol. Toxicol.,1996, 113, 257–265.

[6] M.L., Alexandrova, P.G., Bochev. Oxidative stress during the chronic phase of stroke. Free Radical Biol. Med., 2005, 39, 297–316.

[7] G., Rodriguez-Fuentes, & G., Gold-Bouchot. Characterization of cholinesterase activity from different tissues of Nile tilapia (Oreochromis niloticus). Marine Environmental Research, 2004, 58, 505–509.

[8] E., Ozcan-Oruc, & D., Usta,. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of Cyprinus carpio. Environmental Toxicology and Pharmacology, 2007, 23, 48–55.

[9] F. R., De la Torre, L., Ferrari, & A., Salibian,. Freshwater pollution biomarker: Response of brain acetylcholinesterase activity in two fish species. Comparative Biochemistry and Physiology Part C, 2002, 131, 271–280.

[10] A.,Viarengo, B., Burlando, N., Ceratto, I., Panfoli. Antioxidant role of metallothioneins: a comparative overview. Cellular and Molecular Biology, 2000, 46, 40-417.

[11] I., Bremner, J.H., Beattie. Metallothionein and the trace minerals. Annual Review of Nutrition, 1990, 10, 63-83.

[12] H., Aebi,. Catalase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, vol. 3. Academic Press, New York, 1983, pp. 237–286.

[13] W. H., Habig, M. J., Pabst, & W. B., Jakoby. Glutathione S-transferases, the first step in mercapturic acid formation. The Journal of Biological Chemistry, 1974, 249(22), 7130–7139.

[14] G.L., Ellman, D., Courtneyk, V., Andres, R.M.,. Featherstone. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol.,1961, 7, 88–95.

[15] F.W., Sunderman .Annals of clinical and laboratory science., 1985, 13 (3) ; 229.

[16] A., Viarengo, E., Ponzano, F., Dondero, & R.. Fabbri. A simple spectrophotometric method for metallothionein evaluation in marine organisms: An application to Mediterranean and Antarctic molluscs. Marine Environmental Research, 1997, 44, 69–84.

[17] M., Bradford. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976, 72, 248–254.

[18] B., Halliwell and J.M.C., Gutteridge. Free Radicals in Biology and Medicine. Oxford Univ. Pres, Oxford. Hodgson EK Fridovich I. (1975): The Interaction of Bovine Erytrocyte Superoxide Dismutase with Hydrogen Peroxide: Inactivation of the Enzyme. Biochemistry, 1999, 14, 5294–5298.

[19] M.J., Bebianno, R., Company, A., Serafim, R.P., Cosson, A., Fiala-Medoni. Antioxidant systems and lipid peroxidation in Bathymodiolusazoricus from Mid-Atlantic Ridge hydrothermal vent fields. Aquat. Toxicol, 2005, 75, 354–373.

[20] A.Z., Reznick, L. Packer and C.K., Sen. Strategies to Assess Oxidative Stress. In: A.Z., Reznick, L., Packer, C.K., Sen, J.O., Holloszy and M.J., Jackson (eds.): Oxidative Stress in Skeletal Muscle. Birkhauser Verlag, Basel, Switzerland, 1998, 43–48.

[21] A.M., Charissou, C., Cossu-Leguille, P., Vasseur, Relationship between two oxidative stress biomarkers, malondialdehyde and 8-oxo-7,8-dihydro- 2'-deoxyguanosine, in the freshwater bivalve Unio tumidus. Sci. Tot. Environ, 2004, 322, 109–122.

[22] P.S., Lau, H.L.,Wong, Effect of size, tissue parts and location on six biochemical markers in the green-lipped mussel, Perna viridis. Mar. Pollut. Bull., 2003, 46, 1563–1572.

[23] D.M., Pampanin, L., Camus, A., Gomiero, I., Marangon, E., Volpato, C., Nasci. Susceptibility to oxidative stress of mussels (Mytilus galloprovincialis) in the Venice Lagoon (Italy). Mar. Pollut. Bull., 2005, 50, 1548–1557.

[24] A., Pathirante, & S. G., George. Oxicity of malathion to Nile tilapia, Oreochromis niloticus and modulation by other environmental contaminants. Aquatic Toxicology, 1998, 43, 261–271.

[25] J. A., Knight, R. K., Pieper, & L., McClellan. Specificity of the thiobarbituric acid reaction: Its use in studies of lipid peroxidation. Clinical Chemistry, 1988, 34, 2433–2438.

[26] P. J., Fitzpatrick, J., O'Halloran, D., Sheehan, & A. R.,Walsh. Assessment of a glutathione-S-transferase and related proteins in the gill and digestive gland of Mytilus edulis (L.) as potential organic pollution biomarkers. Biomarkers, 1997, 2, 51–56.

[27] P., Hoareau, M., Gnassia-Barelli, M., Roméo, & J. P., Girard. Differential induction of GST in the clam Ruditapes decussatus. European Journal of Biochemistry, 2001, 269, 4359–4366.

[28] C., Durou, L., Poirier, J.C., Amiard, H., Budzinski, M., Gnassia-Barelli, K., Lemenach, L., Peluhet, C., Mouneyrac, M., Romeo, C., Amiard-Triquet. Biomonitoring in a clean and a multi-contaminated estuary based on biomarkers and

chemical analyses in the endobenthic worm Nereis diversicolor. Environ. Pollut., 2007, 148, 445–458.

[29] D. R., Livingstone. Organic xenobiotic metabolism in marine invertebrates. Advanced Comparative Environmental and Physiology, 1991, 7, 145–187.

[30] C.C.C., Cheung, G.J., Zheng, A.M.Y., Li, B.J., Richardson, P.K.S., Lam. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, Perna viridis. Aquat. Toxicol, 2001, 52, 189–203.

[31] S.M., Moreira, M., Moreira dos Santos, R., Ribeiro, L., Guilhermino. The 'Coral Bulker' fuel oil spill on the North coast of Portugal: spatial and temporal biomarker responses in Mytilus galloprovincialis. Ecotoxicology, 2004, 13, 619–630.

[32] C. H., Walker, & H. M., Thompson. Phylogenetic distribution of cholinesterases and related esterases. In P. Mineau (Ed.), Cholinesterase-inhibiting insecticides. Their impact on wildlife and the environment. Amsterdam: Elsevier, 1991, 3–19.

[33] F., Galgani, & G., Bocquené. A method for routine detection of organophosphates and carbamates in sea water. Environmental Technology Letters, 1989, 10, 311–322.

[34] P., Scaps, & O., Borot. Acetylcholinesterase activity of the polychaete Nereis diversicolor: Effects of temperature and salinity. Comparative Biochemistry and Physiology, (Part C), 2000, 125, 377–383.

[35] T., Balint, T., Szegletes, & H., Szegletes. Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and pyrethroid deltamethrin. Aquatic Toxicology, 1995, 33, 279–295.

[36] L., Guilhermino, M. N., Lacerda, A. J. A., Nogueira, & A. M. V. M., Soares. In vitro and in vivo inhibition of Daphnia magna acetylcholinesterase by surfactant agents: Possible implications for contamination biomonitoring. Science of the Total Environment, 2000, 247, 137–141.

[37] M. P., Cajaraville, M. J., Bebianno, J., Blasco, C., Porte, C., Sarasquete, & A., Viarengo. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: A practical approach. Science of the Total Environment, 2000, 247(2–3), 295–311.

[38] M., Banni, J., Jebali, M., Daubeze, C., Clerandau, H., Guerbej, J. F., Narbonne. Monitoring pollution in Tunisian coasts: Application of a classification scale based on biochemical markers. Biomarkers, 2006, 10(2–3), 105–116.

[39] J., Jebali, M., Banni, E. A., Almeida, A., Bannaoui, & H., Boussetta. Effects of malathion and cadmium on acetylcholinesterase activity and metal lothionein levels in the fish Seriola dumerilli. Fish Physiology and Biochemistry, 2006, 32, 93–98.

[40] D., Ivankovic´, J., Pavicic, M., Erk, V., Filipovic-Marijic, B., Raspor. Evaluation of the Mytilus galloprovincialis Lam. digestive gland metallothionein as a biomarker in a long-term field study: seasonal and spatial variability. Mar. Pollut. Bull., 2005, 50, 1303–1313.

[41] G., De Boeck, T. T., Huong Ngo, K., Van Campenhout, & R., Blust. Differentialmetallothionein induction patterns in three freshwater fish during sublethal copper exposure. Aquatic Toxicology, 2003, 65, 413–424.

[42] H., Reynders, L., Bervoets, M., Gelders, W. M., De Coen, & R., Blust. Accumulation and effects of metals in caged carp and resident roach along a metal pollution gradient. Science of the Total Environment, 2008, 391, 182–195.

[43] H., De Smet, B., De Wachter, R., Lobinski, & R.. Blust. Dynamics of (Cd, Zn)-metallothioneins in gills, liver and kidney of common carp Cyprinus carpio during cadmium exposure. Aquatic Toxicology, 2001, 52, 269–281.

[44] A., Viarengo, D., Lowe, C., Bolognesi, E., Fabbri, & A., Koehler. The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry and Physiology Part C, 2007, 146, 281–300.

[45] A. Viarengo. Heavy metals in marine invertebrates, mechanisms of regulation and toxicity at cell level. CRC Critical Review in Aquatic Science, 1989, 1, 295–317.

[46] A., Viarengo, B., Burlando, M., Cavaletto, B., Marchi, E., Ponsano, & J., Blasco. Role of metallothionein against oxidative stress in the mussel Mytillus galloprovincialis. American Physiological Society, 1999, 363, 1614–1617.