Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

ECOTOXICITY ASSESSMENT OF INDUSTRIAL WASTEWATER: EVOLUTION OF CATALASE ACTIVITY AS A BIOMARKER OF OXIDATIVE STRESS IN THE MUSSELS *MYTILUS GALLOPROVINCIALIS* AND *PERNA PERNA*

MEKNACHI Abdellah[1,2], DJELLALI Mustapha[2] and BADIS Abdelmalek[1,*]

[1] Laboratory of Natural Products Chemistry and Biomolecules (LNPC-BioM), Faculty of Sciences, University of Blida 1, Road of Soumaâ, P.O. Box 270, 09000 Blida, Algeria
[2] National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA) 11, Colonel Amirouche Street, P.O. Box 67, Bou-Ismaïl, 42415, Tipaza, Algeria

Reçu le 17/11/2019, Révisé le 29/05/2020, Accepté le 09/06/2020

Abstract

Description of the subject: Biomonitoring is being adopted by many research organizations to describe, explain, and predict the effects of pollutants and the degree of pollution on marine ecosystems.
Objective: The present research aims to evaluate the toxicological aspect of an industrial wastewater discharging in the Bou-Ismail bay area, based on catalase (CAT) as biomarker resulting under oxidative stress, measured in two sentinel species of mussels (*Mytilus galloprovincialis* and *Perna perna*), represented by three size classes.
Methods: Mussels are exposed to different concentrations of effluent for 4 days and then detoxified for 10 days. At the end of each cycle, we measure proteins and the antioxidant activities CAT.
Results: A preliminary analysis of this effluent showed a considerable polluting load (COD / BOD = 10 and [MES] very high) and presence of trace metallic elements in particular mercury (Hg). Positive correlations between CAT activity and the different reject concentrations were obtained under acute or chronic exposure. CAT responses were significantly increased in the two mussel species in two size classes: Large (Myt: 545.88-688.08; Per: 242.32-1443.60) and Small [Myt: 737.59-1469.98; Per: 585.90-2563.42). Concerning the medium size class, the CAT responses in the *Perna* mussel revealed a more increased sensitivity to pollution than that in *Mytilus*. The mussels detoxification, led to a decrease in the rates of CAT mainly in the mussels *Mytilus* of small sizes.
Conclusion: The present research allows to qualify catalase as a relevant defense biomarker, sensitive, fast and efficient in assessing the impact of pollution and the health of the surrounding environment.
Keywords: Ecotoxicity assessment ; Industrial wastewater ; Catalase biomarker ; Mussels ; Size class.

ÉVALUATION DE L'ÉCOTOXICITE DES EAUX USÉES INDUSTRIELLES : EVOLUTION DE L'ACTIVITE CATALASE EN TANT QUE BIOMARQUEUR DU STRESS OXYDATIF CHEZ LES MOULES *MYTILUS GALLOPROVINCIALIS* ET *PERNA PERNA*

Résumé

Description du sujet: La biosurveillance est adoptée par de nombreux organismes de recherche pour décrire, expliquer et prévoir les effets des polluants et le degré de pollution sur les écosystèmes marins.
Objectif: La présente recherche vise à évaluer l'aspect toxicologique d'un rejet d'eaux usées industrielles dans la baie de Bou-Ismail, basé sur la catalase (CAT) comme biomarqueur résultant du stress oxydatif, mesuré dans deux espèces sentinelles de moules *Mytilus galloprovincialis* et *Perna perna*, représentées par trois classes de taille.
Méthodes: Les moules sont exposées à différentes concentrations d'effluents pendant 4 jours puis détoxifiées pendant 10 jours. A la fin de chaque cycle, nous avons mesuré les protéines et les activités antioxydantes CAT.
Résultats: Une analyse préliminaire de cet effluent a montré une charge polluante considérable (DCO / DBO = 10 et [MES] très élevée) et la présence des éléments de traces métalliques notamment le mercure (Hg). Des corrélations positives entre l'activité CAT et les différentes concentrations du rejet ont été obtenues sous exposition aiguë ou chronique. Les réponses CAT ont été significativement augmentées dans les deux espèces de moules dans deux classes de taille: Large (Myt: 545,88-688,08; Per: 242,32-1443.60) et Petite [Myt: 737,59-1469,98; Per: 585,90-2563,42). Concernant la classe de taille moyenne, les réponses CAT chez la moule *Perna* ont révélé une sensibilité à la pollution plus élevée que celle de *Mytilus*. La détoxification des moules, a conduit à une diminution des taux de CAT principalement dans les moules *Mytilus* de petites tailles.
Conclusion: La présente recherche permet de qualifier la catalase en tant que biomarqueur de défense pertinent, sensible, rapide et efficace pour évaluer l'impact de la pollution et la santé du milieu environnant.
Mots-clés: Evaluation de l'écotoxicité ; Eaux usées industrielles ; Biomarqueur de catalase; Moules ; Classe de taille.

* Corresponding author: BADIS Abdelmalek. E-mail: badisabdelmalek@yahoo.fr

INTRODUCTION

For centuries, coastal environments have been considered as being areas of major ecological and socioeconomic interests. It is also subject to pollution, which is the outcome of urbanization, demographic process and the development of industrial and agricultural activities [1-5]. In order to address marine pollution which endangers living organisms, threatens human health and affects the quality of waters, several national and international monitoring programs have set targets to permanently estimate the degree of pollution mainly in the Mediterranean region to ensure an early prevention of risks caused by different pollutants, hence to protect species and to preserve the quality of marine waters [6-9]. Traditionally, the contamination level in marine environment is introduced in terms of the concentrations of chemical pollutants present in the environment, however, these measures rely on advanced and expensive analytical techniques and which neither provide an estimate nor make a prediction of the impact of these substances on living organisms, reaching thus their limits as environmental management tools.

Therefore, other channels have been explored by means of bioindicators and/or biointegrators or ecotoxicological bioassays carried on living matters such as shells, fishes and plants [10-13]. Scientists and international monitoring organizations of marine pollution also claim the use of biological responses or biomarkers to enhance chemical analyses in monitoring programs.

Indeed, their use allows assessing, on the one hand, the concentrations of contaminants and their bioavailability in the sediments, water columns and organisms and the effects of these pollutants on the biological component of ecosystems, on the other hand [4, 5, 10, 14-20]. Biomarkers are used to assess the effects of the analyzed levels by means of conventional chemistry tools on biological responses of test organisms. Mussels such as *Mytilus galloprovincialis*, *Perna perna* and other marine bivalves are commonly used as sentinel species for biomonitoring coastal environments throughout the world, for their inherent characteristics which make them outstanding bioindicators [1, 4, 9, 14, 21-32]. With the biomonitoring objective in mind, the National Center for Research and Development of Fisheries and Aquaculture (CNRDPA, Bou-Ismail) is currently developing a program for aquatic ecosystems management and environmental monitoring and thus contributing to the research on pollution indicators. This work seeks to study the catalase biomarker as a biological response to induced stress by acutely exposing the Mussel *Mytilus galloprovincialis* and *Perna perna* in industrial effluent, in order to adopt a fast technique which is likely to provide integrated information about the health of the Bou-Ismail bay area at Tipaza (Algeria).

MATERIALS AND METHODS

1. Sampling and physicochemical characterization of the industrial effluent

The liquid discharge collection of the industrial effluent has been undertaken at the final spillage point in the sea as part of the sanitation network of the industrial area of Bou-Ismail (paper and carton industry, kraft paper recycling, and ceramic industry).

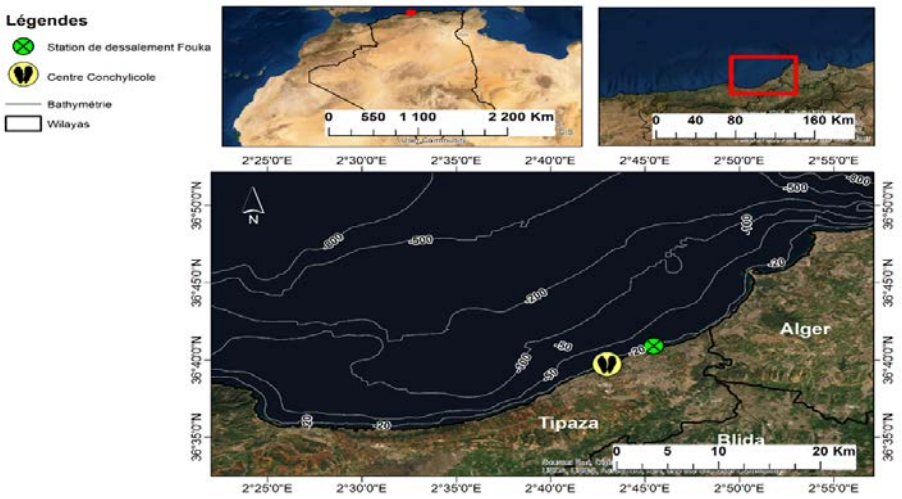

Figure 1: Map of Bou-Ismail bay with point of discharge location, Shellfish Center and Fouka Marine Desalination Plant.

Table 1: Physicochemical parameters of the industrial effluent.

Parameters	Units	Standards of analysis	Results	Standards JORA 2006
Temperature	°C		23,7	<30
pH	/	Multi parameters	6,89	5,5 – 8,5
Electrical conductivity	ms/cm		2,06	/
SPM		ISO 11923 : 1997	5150	35
Kjeldahl nitrogen		ISO 5663 : 1984	59,5	30
Total phosphorus		ISO 6878 : 2004	3,35	10
Ortho phosphates			< 0,05	/
COD		ISO 6060 : 1989	5250	120
BOD_5		ISO 5815-1 : 2003	525	35
chlorides		Rodier	390	/
Ammonium	mg/L		3,5	/
Nitrates		ISO 7890-11 :1986	9,75	/
Al		ISO 12020 : 1997	18	3
Cd		ISO 8288 : 1986	< 0,03	0,2
Cr		ISO 9174 : 1998	< 0,2	0,5
Co		ISO 8288 : 1986	< 0,2	/
Cu		ISO 8288 : 1986	0,4	0,5
Fe		FD T90-112	8,1	3
Hg	µg/L	ISO 5666 : 1999	2,615	0,01
Ni		ISO 8288 : 1986	< 0,2	0,5
Pb	mg/L	ISO 8288 : 1986	< 0,2	0,5
Zn		ISO 8288 : 1986	0,95	3
Sulfates		Rodier	170	/

JORA: Journal Officiel de la République Algérienne; Official Newspaper of the Algerian Republic

The varied origins of liquid discharges samples (industrial effluents, household effluents, final effluents of process and sewage treatment, leachate waters coming from landfills, unknown or accidental spill such as pipe breaks, etc.) frequently show significant variations of the level of pollutants equally from one sampling point to another, as well as from one time to another. It is crucial, to make the sampling more representative, to apply rigorous sampling protocols and to know the behavior of substances which will have influence on the choice of sampling methods. To this end, we drew on the protocols of Quebec Center of Expertise in Environmental Analysis [33, 34].

2. Mussels harvesting

Wild mussels used as a biological model are harvested from the sea buoyage signals in the sea water desalination plant in Fouka Marine (Fig. 1). The two mussel species *Mytilus galloprovincialis* and *Perna perna* co-exist and sampling has targeted the three size classes [2,2 ; 2,8], [4,3 ; 5,2] et [7,1 ; 8,6]. After harvesting the mussels, they are sorted, cleaned, their epibiont is removed, and measured by means of a vernier caliper gauge before launching adaptation phase.

3. Experimental procedure: Contamination / decontamination test

The different experimentations have been conducted and realized at the shellfish farming center of CNRDPA-Bou-Ismail (Fig. 1). The strategy of ecotoxicity tests consists of contaminating two mussel species *Perna* and *Mytilus*, of different size classes (**Large, Medium, Small**), by industrial discharge waters in the sea, and this, at different concentrations by dilution in breeding tanks of 70 liters. Mussels remain one week to adapt to experimental (acclimatization) conditions before the contamination test. All the mussels are kept in identical environmental conditions, and the use of air pumps ensures water aeration in tanks. Water used is daily renewed during the adaptation period and throughout the whole experimental cycle. Adaptation is a crucial step in implementing an experimentation as it should cover a sufficient period of time to allow organisms to adapt to laboratory conditions. In order to reduce other factors which might represent a source of disturbance or results interference, we measure the physicochemical parameters of seawater used for cultivating mussels on a daily basis during the experimental cycle (temperature, salinity, pH, dissolved oxygen) before and after changing water by means of YSI 556 multiparameter.

Following the acclimatization step, mussels are divided into five test groups in rectangular aquariums, and each group contain the both studied mussel species of different size classes. All the mussels are incubated in identical environmental conditions for a period of 4 days as a contamination cycle and 10 days as a decontamination cycle (0% pollutants). Thus, for 4 days mussels are exposed to four increasing concentrations (C1= 0.01%, C2= 0.15%, C3= 0.3% and C4= 0.5% (v/v)) of the effluent. The handling is compared to a control aquarium (0 mg/L pollutant). The four selected concentrations have been assessed in order to establish doses/responses relations between chemical pressure represented by the effluent's pollutants and the biological responses observed in mussels (CAT). At the end of each cycle, the digestive gland of the mussels is collected to be the object of biochemical assays of proteins and the antioxidant enzyme CAT.

4. Analytical methods and biochemical analyses

In our practice, the catalase activity is determined according to the method described by Atli et al. [35]. Total protein levels are estimated with the method of Lowry et al. [36], using bovine serum albumin (BSA) as a standard. In ice-cold, tissues are homogenized at the ratio of 1/10 (w/v) in Tris buffer (tris-hydroxylmethyl-aminomethane) (20 mM; pH 7.8) for three minutes using a blender. The centrifugation of the homogenate is conducted at 10 000 g for 30 minutes between 4 and 5 °C. The obtained supernatant (S9 Fraction) is subsequently used to measure proteins and the catalase activity. 2.5 mL of substrate solution (25 mM H_2O_2 in the phosphate buffer 75 mM at pH 7) is placed in spectrophotometer cuvettes already seted in kinetic mode. After that 50 µL of the fraction S9 (enzyme source) is added to the mixture and followed by the decomposition of hydrogen peroxide measured by spectrophotometer at 240 nm within 60s-time span.

5. Statistical analyses

The data was tested for normality and homoscedasticity using the Shapiro–Wilk and Levene tests, respectively. After, the statistical analysis was performed, using a bilateral two-way analysis of variance (ANOVA) for independent samples, followed by Tukey post-hoc analysis. Significance was set at $P< 0.05$.

RÉSULTATS

1. Industrial effluent characterization.

The results of the analysis of the main parameters of the industrial effluent are mentioned in the table (1). In general, the values recorded in this table are far beyond (considerably) discharge spill limit values of the industrial effluent liquids recommended by the Algerian standards (JORA, 2006). Also, some parameters stand even higher than maximum limit values (JORA, 2009) for harmful substances in non-domestic wastewater at the time of discharge in a public sewerage system or in a wastewater treatment plant. Thus, the values of SPM and that of COD exceed 8 times and 5 times respectively the Algerian standards of untreated liquid discharges (JORA, 2009). In addition, the industrial effluent appears to be rich in dissolved salts (when conductivity > 2 [37]) and highly concentrated in chlorides (390 mg/L) and some metallic elements like iron (8,1 mg/L), aluminum (18 mg/L) and mainly mercury (2,615 mg/L). The particularity of the latter is that it is not removed by living organisms and thus it generates bioaccumulation all along the food chain with remarkable adverse effects including: neurotoxicity, immunotoxicity, stress and oxidative damage, behavior modification, inhibition of growth and alteration of tissues [38, 39]. The discussed values, are alarming if we consider the direct impact in the long and short terms in the receiving environment. The industrial effluent is not readily biodegradable when we noticed the ratio values COD/BOD5 in the range 10. The presence of toxic substances in the discharges inhibits the development of some aquatic organisms or causes their mortality [40]. High level of SPM in the natural aquatic environment (lagoon, watercourse, estuary etc.) might have two types of adverse effects: physical at first, by the formation of light-blocking screen (photosynthesis decrease and consequences along the food chain, until reaching the eutrophication of the environment), or even clogging fish gills; Secondly, chemically, the sedimentation SPM generates a potential pollution/pollutants reserve [41, 42].

If silting is not a problem, for the time being, given the seawater large absorptive capacity, the decrease of clarity and high turbidity of water, visible from a great distance, indicating an evident pollution in the water where animal and plant life is compromised [43]. The values of the other parameters recorded in the table (1), lie close or in the normative interval of the industrial effluent discharges. Notwithstanding the fact that in the current study the discharge is directly released at sea. Hence, taking into account the continuous flow of the liquid discharge, the levels of harmful substances will certainly contribute to enriching the receiving environments. For instance, 30 mg/L kjeldhal nitrogen are potentially a source producing about 130 mg/L of nitrates in water [43]. According to Yadav et al. [37], the presence of odor and color as in the case of the liquid discharge in the current study is due to the presence of ammonium (3.5 mg/L> 0,5 WHO) and transition elements and/or their complexes respectively. They attribute the toxicity of the effluent (Fertilizer industry effluent), on the fish *Channastriatus*, to the impact of some metallic elements and to ammonium also present in the discharge water. Moreover, while the levels of metallic elements (Cd, Cr, Co, Cu, Ni, Pb et Zn), in the effluent of this study, are below the detection limit or even below the standard, there is always a risk of permanent pollution of the environment along terrible ecological consequences. An increased mortality of the urchin *Paracentrotus lividus* was observed in the distant area of the leakage point of the liquid effluent. Damaging an equally important element of the trophic level will show the disturbance of biodiversity in the receiving environment. The harmful substances present in the industrial discharges, more specifically trace metals (TMs), are released in the aquatic environment in a bioavailable form, then bioassimilable by fauna and flora [44-47]. The presence of TMs in the aquatic compartment can have a detrimental effect (toxic) on the initial stage of the embryonic development of several marine species. Thus, Nadella et al. [48], demonstrated the highly toxic effect of some dissolved metals on embryos of *Mytilus trossolus*. They classified the toxicity level of dissolved metals as follows: toxicity Cu > Zn > Ni > Cd. According to Martin et al. [49], toxicity level of TMs on the embryonic stage of the mussel *Mytilus edilus* is as follows: Hg and Cu >Ag > Zn > Pb > Ni > Cd > Ar > Cr > Se. According to Roccheri et al. [50], even in the decontamination cycle, following urchin *Paracentrotus lividus* embryo's exposure mainly to cadmium, stress proteins continue to be synthesized by eventually concluding the irreversibility of stress mechanisms. According to Boukadida et al. [51], hypersensitivity of the embryo-larval stage of *Mytilus galloprovincialis*, to pollution by metals (Cu, Ag and the mixture) combined with increasing temperature will certainly lead to reproduction impairment of *Mytilus galloprovincialis* in the Mediterranean Sea due to global warming. Coppola et al. [52] confirm, once again, the acute risk of the combined effect of arsenic and the temperature on the mussel *Mytilus galloprovincialis*.

Also, negative effects on the immune system with histopathological effects and on fertilization (eg: late embryos of irregular pluteus) of several aquatic species are described under the effect of TMs and other chemical pollutants while activating the different stress and defense mechanisms, mainly the HSP synthesis and the apparition of apoptosis and / or autophagy.

Once again, the effects are associated with temperature increase [1, 2, 28, 53-58].

2. CAT evolution during the contamination cycle

The will to develop more sensitive and methods faster than the classical mortality or reproduction tests has allowed the concept of biomarkers to be implemented as an early response of the health of indicator species. Hence, the objectives of this new approach are often the search of concentrations that induce the triggering of biological mechanisms (eg: defence mechanism CAT). Mortality is therefore only the final stage indicating the ineffectiveness to maintain homeostasis. Results pertaining to catalase measurement (CAT) in the mussels (*Mytilus galloprovincialis)* contaminated by

the industrial discharge are shown in the figure (2) below.

Regarding the medium-size class mussel *Mytilus*, statistical analyses have shown no significant effect (p<0.05), by the industrial effluent, on the responses of the defense enzyme catalase, and that is compared to control group and to assessed concentration gradient.

The four assessed pollutant concentrations do not seem to have a significant effect on the induction of the antioxidant defensive mechanism where no significant effect is measured between concentrations. A significant impact (p<0.01) of contamination by the industrial effluent is noticed on the large-size class mussels *Mytilus* exposed to the concentrations C2 and C3. Also compared to control individuals (CAT: 307.89± 23.86 U/mg Protein/min), a proportional increase of catalase activity is measured in specimens of mussels exposed to the concentrations C1, C2 and C3. The activities reached are respectively 545.89±106.65, 623.71±81.48 and 688.09±35.33 U/mg protein/min.

The concentration C4 (CAT: 551.08±90.46 U/mg Protein/min) had a non-significant effect (p<0.05) and identical to the concentration C1 on the induction of the antioxidant enzyme CAT. In the small-sized class mussels, the individuals of the latter have shown hyperactivity in the induction of the antioxidant mechanism marked by CAT. Thus, and compared to control group, the highest induction of catalase is measured under the effect of the concentration C4. Also, the trend was almost proportional with the four assessed pollutant concentrations. At this time we have noticed no significant effect (p<0.05) between the concentration C1 (CAT: 737.59±4.33U/mg Protein/min) and C3 (CAT: 760.33±64.27U/mg Protein/min), while a very significant effect (p<0.01) of concentration is recorded compared to control group (416.13±65.25U/mg Protein/min). The study of the independent effect (ANOVA-2, p<0.05) of each concentration on the CAT response in the three size classes reveal significant and non-significant effects depending on the case under consideration. Hence, the measured values of the catalase activity in the control individuals (no contaminant) have shown no significant difference between the three studied classes. Under the effect of the concentration C2, the statistical difference is significant irrespective of the studied size class. Moreover, and compared to the small-sized class, the difference is always significant between the compared size classes. However, the statistical analysis reveals a non-significant effect between the medium and large size classes and this under the effects of the concentrations C1, C3 and C4. A cording to the obtained results, the statistical analysis (ANOVA-2) of the effects of the contaminant concentration and the interaction with the size class highlighted the fact that the medium-sized class mussels *Mytilus* (juvenile) have been the most resistant to contamination. The large-sized individuals *Mytilus* reveal an average sensitivity, while the smaller ones *Mytilus* seemed more sensitive to the concentration effect. Figure (3), below, represents the catalase activities measured in the mussel *Perna perna*.

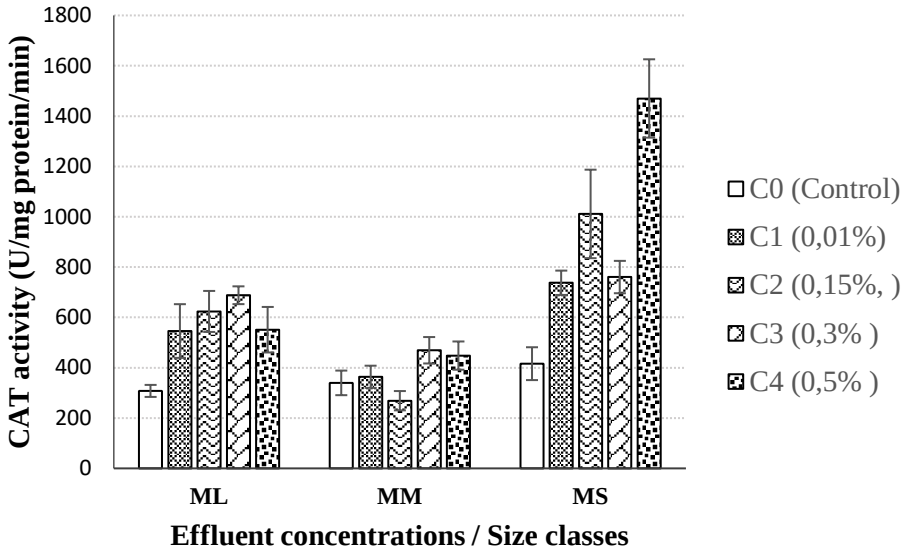

Figure 2: Catalase activity variation in the mussel *Mytilus galloprovincialis* contaminated by the industrial wastewater effluents.
ML: *Mytilus* Large, MM: *Mytilus* Medium, MS: *Mytilus* Small.

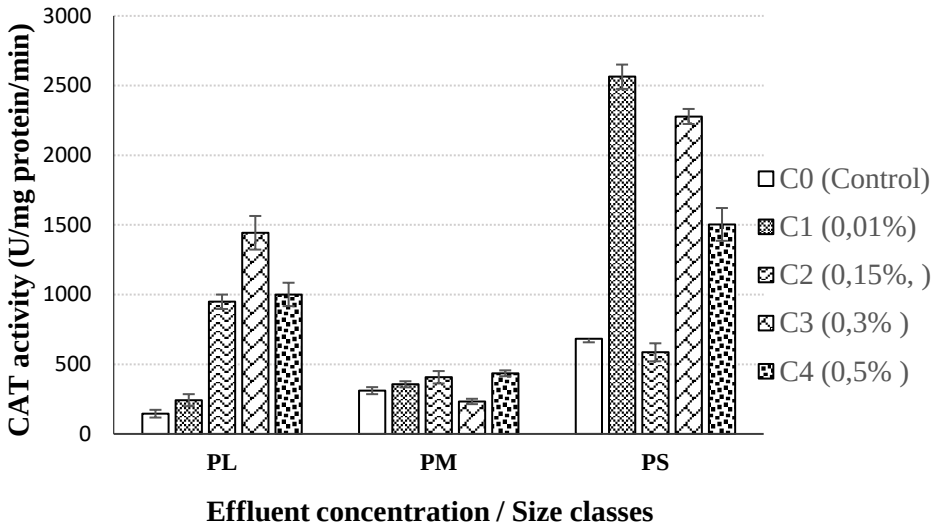

Figure 3: Catalase activity variation in the mussel *Perna pena* contaminated by the industrial wastewater effluents.
PL: *Perna* Large, PM: *Perna* Medium, PS: *Perna* Small.

As was the case in the mussel *Mytilus*, the medium-sized class mussel *Perna* (juvenile) has again shown no sensitivity to contamination by the mixture of xenobiotics present in the industrial effluent. Therefore, the catalase activities measured in the individuals of this size class, under the effect of four concentrations, have shown no significant difference (P<0.05) from the measured enzymatic response (CAT). In the large-sized class individuals *Perna*, the concentration C1 had no significant effect (p<0.05) on the induction of the antioxidant enzyme CAT. Compared to the control group (CAT : 145.24±27.68 U/mg Protein/min), and under the effect of the concentrations C2, C3 and C4 we measured a very significant induction (P<0.001) of CAT along with a maximum activity (893.95%) (CAT : 1443.61±120.57U/mg protein/min) measured under the effect of the concentration C3. The concentrations C2 and C4 have revealed an identical effect on the activation of the defense enzyme CAT (984.45±51.69 and 1001.07±83.95 U/mg protein/min; 553% and 589.26%, respectively). The most important inductions of the CAT activity in the mussel *Perna* are also measured in the small-sized class specimens. Furthermore, and compared to the controls (CAT: 684.14±26.60 U/mg protein/min), we noticed very significant levels of induction (p<0.001) in the range of 274.69%,233.01% and 119.76% in the individuals exposed to the concentrations C1, C3 and C4 respectively.

In contrast, the CAT activity measured under the effect of the concentration C2 (CAT: 585.91±64.54 U/mg protein/min) can only show an inactivation of the anti-oxidative stress activity, despite the difference compared to the controls was not significant (P<0.05). The measurements taken were reproducible which allowed us to say that the enzyme might be inhibited. In aquatic organisms, antioxidant system enzymes are induced or inhibited by the pollutant and this according to the tested dose, to the specie and mainly to the exposure time [11, 59]. Compared to the small-sized class (CAT: 684.14±26.60 U/mg protein/min) and in the absence of contaminants, we measure the significant differences (ANOVA-2, p<0.01) in the rate of CAT activity between the three studied size classes. Nevertheless, no difference (P<0.05) has been measured between the two medium and large-sized classes. The effects of concentrations C3 and C4, on the rate of the catalase induction, have shown significant differences (ANOVA-2, p<0.001) regardless of size class with marked effect of the concentration C3 in the small-sized individuals *Perna*. The concentration C1 has shown the highest activity of CAT and this in small-sized *Perna*. But the same concentration has shown no significant effect of contamination in the two medium and large-sized classes. The difference is not significant (p<0.05) between species and even in the same size class and this compared to the controls.

We have observed no significant difference (ANOVA-2, $p<0.05$) of the effect of the concentration C2 between the small and medium-sized classes. However, the difference of the effect of the same concentration is very significant (ANOVA-2, $p<0.05$) between small *Perna*/large *Perna* on the one hand and medium *Perna*/large *Perna* on the other hand. In addition, based on the obtained results, the medium-sized mussel *Perna* shows up the most resistance to contamination by the industrial wastewater. Large-sized class revealed sensitivity more or less significant towards the effect exerted by the xenobiotics of the real effluent. The hyperactivity of anti-oxidative stress defense mechanism is still marked in the small-sized class mussels *Perna*.

3. CAT evolution during the decontamination cycle

The figures (4 and 5), illustrate the CAT activities achieved at the end of the decontamination cycle in the control groups and those previously contaminated by the concentrations C3 and C4 of the species *Mytilus* and *Perna*, respectively.

The CAT activities measured at the end of the contaminations cycle are introduced in the diagram for a comparative purpose in order to study the reversibility of the anti-oxidative stress mechanism and the curability of the sentinel specie. In the individual medium-sized class mussels *Mytilus*, contamination has no outstanding effect on the increase of the rate of antioxidant activity CAT. Moreover, detoxification of the medium-sized mussels led to a decrease of the level of activity CAT in the range of 8.68% (C3Detox) and 3.85% (C4Detox) to reach activities close to those measured in the control group. The statistical analysis has highlighted no significant difference ($P<0.05$) between the measured CAT activities. The large-sized mussel *Mytilus* detoxification led to a decrease (7.53%) of the CAT activity in the mussels previously exposed to the concentration C3. However, and compared to the control group, the statistical analysis revealed no significant difference ($P<0.05$) between the two CAT activities reached at the end of the detoxification cycle.

Furthermore, and by referring to the measured catalase activity at the end of the contamination cycle (CAT: 551.08± 90.49 U/mg protein/min) in the large-sized class individuals under the concentration effect C4, an increase of 31% is measured at the end of the decontamination cycle (C4Detox). Also, the statistical difference was significant ($p<0.05$) compared to the controls group. So, the degree of the enzyme activation can be considered as a sign of mussel's exposure to a past contamination caused by the industrial effluent. As a first assumption, the detoxification seems to have a curative effect on the specimen mussels previously exposed to the concentration C3 with a reversibility of the antioxidant mechanism. Nevertheless, the 10 days' time does not seem to be sufficient for mussels to recover their initial physiological mechanism, mainly in individuals previously contaminated by the concentration C4. However, the reversibility of the enzymatic process is much clearer in the small-sized class mussels *Mytilus*. Thus, and compared to CAT activities attained at the end of the contamination cycle, we measured decrease in CAT activity in the range of 40.01% and 66.60% in the mussels previously contaminated by the concentration C3 and C4 respectively. The statistical differences are very significant ($p<0.01$). Also, the catalase activities reached at the end of the detoxification cycle are close to those measured in the controls and no significant difference ($P<0.05$) is noticed between the measured activities. The decrease of the induction of the CAT activity might be considered as a sign of reversibility of the health of the specimens. Biomarkers of antioxidant defense like CAT contribute to maintaining organism homeostasis and are usefully used in the environmental biomonitoring [11, 59-65]. Except large-sized mussels previously contaminated by the concentration C4, the study of the independent effect (ANOVA-2, $p<0.05$) of the detoxification has highlighted no significant difference between the CAT activities measured and this regardless of the studied class size. Moreover, the measured activities of the anti-oxidative defense enzyme can only be translated in the curability of the individuals and the efficiency of the detoxification cycle so that the mussels *Mytilus* return to their initial homeostasis state.

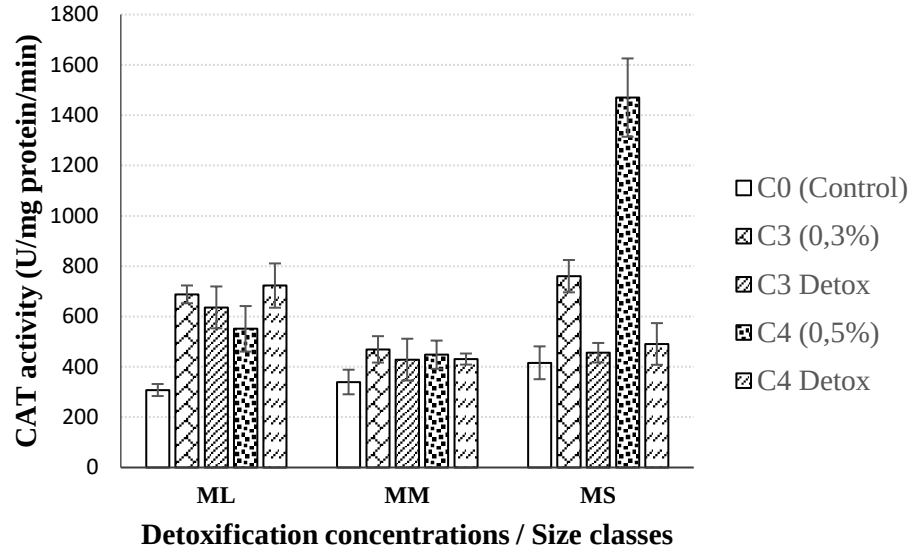

Figure 4: Catalase activity variation in the mussel *Mytilus* after the decontamination cycle.
ML: *Mytilus* Large, MM: *Mytilus* Medium, MS: *Mytilus* small.

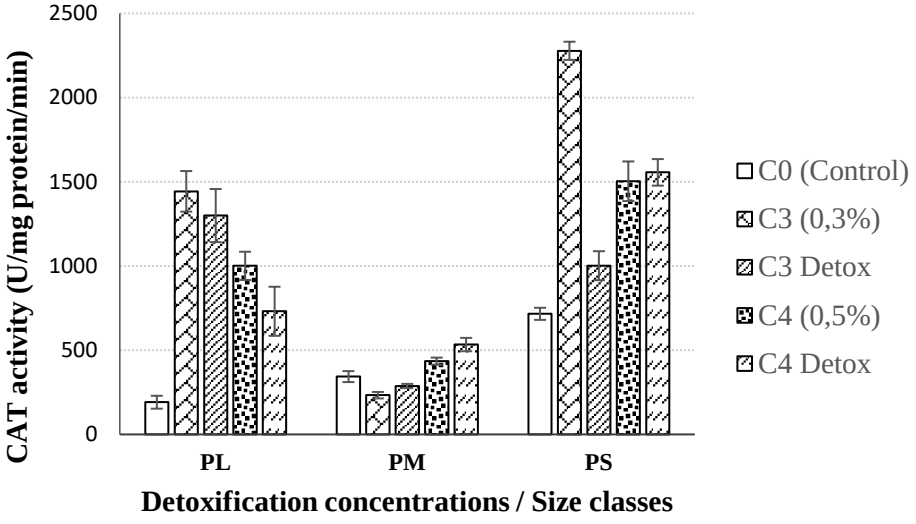

Figure 5: Catalase activity variation in the mussels *Perna* after the decontamination cycle.
PL: *Perna* Large, PM: *Perna* Medium, PS: *Perna* small.

Compared to control group, the medium-sized class mussels *Perna* have shown no significant difference ($p<0.05$) between the CAT activities measured at the end of the decontamination cycle. Nevertheless, increases in the range of 2.34% and 2.25% are measured in the previously contaminated specimens by the concentrations C3 and C4, respectively. This non-significant difference translates the long-term stability of the physiological activity (biochemical metabolisms) of juvenile mussels. We measured a decrease in CAT activity at the end of the detoxification cycle in the large-sized mussel *Perna*. The decrease being significant (26.95%) in the individuals previously contaminated by the concentration C4 while it is not significant (9.95%) in mussels previously contaminated by the concentration C3. By contrast, the attained activities were higher than those measured in the control group. Hence, the mussels *Perna* seem that they did not find sufficient detoxification duration so that the homeostasis of specimens recovers. In the small-sized class mussel *Perna*, we measured a very significant decrease of activities in the range of 56% in the individuals previously contaminated by the concentration C3.

Compared to the CAT activity (CAT: 1503.53±117.67 U/mg protein/min) measured at the end of the contamination cycle, the detoxified individuals from the concentration C4 have shown a slight increase (3.51%) of the CAT activity. Nevertheless, the difference was not significant (p<0.05). Moreover, it seems that the detoxification duration remains insufficient so that the CAT activity of the previously contaminated specimens (CAT C3D: 1001.87±86.11 and CAT C4D: 1556.33±78.88 U/mg protein/min) becomes close to that of the controls (CAT: 716.22±35.94 U/mg protein/min). As was the case in the contamination cycle, the specie *Perna* kept the same difference measured between the controls of the different size classes. Also, two-way ANOVA test show a significant difference (p<0.01) irrespective of the size class in the detoxified mussels of the la concentration C3. Furthermore, the mussels detoxification of the concentration C4 revealed significant differences (p<0.001) between small *Perna* / medium *Perna* on the one hand and small *Perna* / large *Perna* on the other hand. Between medium *Perna* / large *Perna* the independent effect of the detoxification, being non-significant (P<0.05), expresses a similar trend to the CAT response.

DISCUSSION

The industrial wastewaters vary from one industry to another, they might contain toxic products, trace metals, organic pollutants, hydrocarbons, etc.

The mussel is an accumulating filtering bivalve of micropollutants. It can concentrate trace elements into concentrations higher than those found in its environment [61, 62, 64, 66-68]. During every physiological exchange process with the surrounding environment.

The exogenous molecules penetrate through biological barriers separating the internal environment from the external environment of the organism [12]. The accumulation of chemical substances in the organisms is the reason behind the unsteadiness of the redox balance [64]. The origin of the oxidative stress in the aerobic organisms comes from the intracellular intake of the oxygen molecule which is essential for several physiological functions but it generates, at the same time, reactive oxygen species (ROS) which are potentially toxic to the cell.

The main origins of ROS production come from the oxidative phosphorylation of the ADP, microsomal electron transport chains, phagocytic activity and the activity of several enzymes that produce ROS such as intermediary molecules. The xenobiotics might increase the intercellular production of ROS, for instance, during their reduction, where they can be converted into their free radical which will quickly give its electron to an oxygen molecule and produce superoxide radical anion. The latter, in turn, will respond in a chain reaction and hence amplify the initial phenomenon [4, 5, 64, 69-71].

In order to control the formation of these reactive species, cells have an antioxidant defense complex composed of enzymes (super oxide dismutase, catalase, glutathione peroxidase) and of molecules that trap the free radical species at the level of membranes (vitamin E, β-carotene) or of the aqueous phase (ascorbic acid, uric acid and glutathione).

The antioxidant defense enzyme CAT is known for being induced to deal with oxidative stress [5, 59, 64, 71-73]. The catalase is an haemoprotein present at the level of peroxisomes which contributes to the defense against the ROS. It catalyzes the dismutation of the hydrogen peroxide into oxygen and water ($2H_2O_2 \rightarrow 2H_2O + O_2$) [35, 69, 70, 74-76]. Thus, the induction of the CAT activity is a sign of mussels' exposure, of the current study, to the oxidative stress caused by the xenobiotics present in the industrial effluent. The major importance of the CAT lies in its role to eliminate hydrogen peroxide [3, 5, 11, 62, 77, 78]. Excess of the latter diffuses into the cell causing oxidative damage [64]. H_2O_2 might alter the cellular physiology as a result of the formation of $OH^{\cdot}$ radical by the Fenton reaction (1) [61, 64].

The $OH^{\cdot}$ radical is the most reactive and the most toxic of the ROS species to the cell. **Fe (II) / Cu (I) + H_2O_2 → $OH^{\cdot}$ + OH^- + Fe (III) / Cu (II). (1)**

At the cell level, the damage brings about alteration and more precisely the oxydation of compenents such as the DNA, the proteins and the lipides with a widespread disturbance of the redox balance (ratios GSH/GSSG et NADH/NAD^+) [11, 61, 64, 78, 79].

Ideally, the energy metabolism is associated with the energy allocated to the organism's defense functions and to the maintenance of the basic metabolism, of growth and reproduction [12, 69, 80-82].

According to Jean-Claude and Claude [69], the defense biomarkers allow organisms to fight and survive in the presence of pollutants at reasonable levels but this at an energy cost for theindividual. Thus, several studies [76, 83-86] confirm, once again, the increase of metabolic rates in the organisms exposed to various chemical stresses generating different defense biomarkers. In our study, the medium-sized class individuals (young) of the two mussel species *Perna* and *Mytilus*, are found to be the most resisting to contamination, and the assessed pollutant concentrations have shown no significant effect (P<0.05) on the CAT responses. However, the small-sized ones have shown greater sensitivity to contamination. The results obtained show a highly important defense enzyme CAT increases. This increase of energy spending to deal with the endured stress might lead to a reduction of energy investment in growth and/or reproduction with possibly an impact in maintaining the population. Toxic molecules interact with biological molecules. Therefore, the exposed organisms develop various defense mechanisms: avoidance and/or isolation, active removal, neutralization by complexation with proteins, etc. [69]. These defense costs are added to maintenance costs. Therefore, there is a quantitative correlation between the organism's defense capacity (survival) and its production capacity of biomass (growth and reproduction). Furthermore, the energy allocation for defense, maintenance and cells regeneration is privileged than growth or reproduction [82]. In mussels, the avoidance or isolation strategy is not possible, the strategy of the antioxidant enzymes seems to be the best resort to deal with xenobiotics. The excretion and sequestration in tissues might also be another resort. However, it has been demonstrated that the first responses to the presence of contaminants are those linked to an antioxidant system [11, 61-64].

In the present work, we have measured increases in the CAT activity under the effect of contamination and also decreases of the CAT activity have been noticed at the end of the detoxification cycle. Hence, it is easy to highlight the existence of a direct link between the level of pollution and the degree of the CAT stimulation. Furthermore, the decrease of the induction of the CAT activity might be considered as a sign of the reversibility of the specimens' health.

The defense biomarkers like the CAT contribute to maintaining the homeostasis of the organism [61-64]. In the absence of contaminants in the environment, the reverse processes to the accumulation quickly intervene, especially when achieving the biological target was not acute, and this in order to increase the efficiency to excrete trace elements concentrated within the organism. According to Geffard et al. [61-64], the elimination of metals might be demonstrated by moving organisms from a contaminated to a clean environment. Indeed, the curability of the health of the specimens is the result of an expected efficiency of the different decontamination processes and the elimination of the accumulated xenobiotics translated into a decrease of the quantity of a bioaccumulated pollutant. In parallel to measuring the CAT activity, Box et al. [62] have measured the concentration of the malondialdehyde MDA (the oxidation marker of the phospholipid membrane) in mussels (*Mytilus galloprovincialis*) transplanted in different estuaries characterized by a different pollution gradient. However, no significant change of MDA has been observed along with an increase of the induction of the CAT activity, thus justifying the protecting role of the CAT against the oxidation of the components of the cellular membrane. According to the same authors, the glutathione peroxidase activity (GP_x) appeared unchangeable (The GP_x breaks down H_2O_2 like the CAT) hence concluding the major importance of the CAT response to oxidative stress. According to Jean-Claude et Claude [69], because of stress, organisms implement an energy strategy in favour of low-energy consumption enzymes. In fact, the catalase neither requires a co-factor nor energy for its activity, whereas glutathione peroxidase consumes reduced glutathione and NADPH. Under the effect of a concentration of 2 µg/L of copper, Xu et al. [87], also observed a significant response of the CAT along with non-significant changes of SOD activity and concentrations in MDA measured in the mussels *Mytilus coruscus*. However, significant levels of MDA with reduced CAT activity has been demonstrated in the case of a persistent high pollution. According to Mejdoub et al. [11], the low activity of antioxidant enzymes such as the CAT, will increase the content of superoxide radical and H_2O_2.

They will be reacting together to produce hydroxyl radical that can attack all biomolecules and disturb the cell metabolism. Moreover, and according to Vlahogianni et al. [64], many scientists consider the CAT as a biomarker, sensitive and important of the oxidative stress, better than the SOD, revealing biological effects in the redox status of marine organisms. Although some authors [4, 88-92] measured late answers of CAT activity with contradictory responses or again non-significant correlations between the CAT and the nature of the pollutant, several other scientists confirm the relevance of the catalase as a biomarker of oxidative stress caused by chemical pollutants and even biological [5, 11, 93-95]. Richir and Gobert [96], in their study of the correlations of bioaccumulation of 19 TMs, by the mussel *Mytilus galloprovincialis*, on the basis of size, weight, sex and stage of maturity, have noticed that the medium-sized class showed the best correlation with some metabolic stability. Furthermore, small-sized class mussels presented inter-individual variations and that bioaccumulation modelling was not easy, given the hyperactivity of this size class. Notwithstanding, the highest accumulation of almost all of the 19 TMs was in the small-sized mussels. Thus, the authors recommended, not using extreme size classes (small or large) in the monitoring programs. The medium-sized class is the most resisting with a more stable metabolism, thus responding to the choice of a bioindicator of the level of pollution. Therefore, the results obtained by Richir and Gobert [96], can only confirm our experimental results and conclude for the effect and/or the impact of the wastewater effluents, of the current study, on the sentinel species of the mussels *Mytilus* and *Perna*. Hence, the environmental impact affects the small individuals and prevents them from having the opportunity to survive through to mature stage and reproduce. Also, large individuals will not be able to produce a new generation under stressful conditions. As stated, an increase of energy spending for the basic metabolism to combat endured stress might lead to the reduction of energy investment for growth and/or reproduction. In this way, the medium-sized class cannot by itself ensure preserving the generation and the results will be the extinction of the specie as was the case in the Algerian coasts, hence leading to considerable effects on the wealth and the biodiversity of the coastal environments which represent practically almost all (80%) of the marine biodiversity according to several scientists [97].

CONCLUSION

The findings of mussels exposure to wastewater have demonstrated positive correlations between the induced enzymatic activity (CAT) and the assessed concentrations. Thus, significant increases of CAT have been identified in both mussel species of large-sized classes but mainly in small-sized classes. No significant difference of CAT activity has been found in the medium-sized class of the two species, which showed resistance and/or adaptation to the effects of the assessed concentrations. The mussels detoxification, led to a decrease in the rates of CAT mainly in the mussels *Mytilus* of small sizes. The present research allows to qualify catalase as a relevant defense biomarker, sensitive, fast and efficient in assessing the impact of pollution and the health of the surrounding environment, especially in aquatic ecosystems.

Acknowledgements

The authors wish to express their gratitude to the financial support of DGRSDT. This work could not have happened without the support of a large number of people. I particularly want to thank Mr Abassi Djamal Eddine for his contribution to the English translation. Also, I am very grateful to three other people who counted enormously in the outcome of this work Mr Zenati Bilal, Mr Kordali Sid Ali and Mr Dilmi Amar. An immense thanks also to Mrs Belmaskine Hayate for her efforts to correct the manuscript and her pertinent remarks. I am also very grateful to the intern Mr Kritli Nabil.

REFERENCES

[1]. Akaishi, F. M., St-Jean, S. D., Bishay, F., Clarke, J., da, S. R. I. and de Oliveira Ribeiro, C. A. (2007). Immunological responses, histopathological finding and disease resistance of blue mussel (*Mytilus edulis*) exposed to treated and untreated municipal wastewater. *Aquat Toxicol*, **82**(1): 1-14.

[2]. Borgia, VJF., Thatheyus, AJ., Murugesan, AG., Alexander, SCP. and Geetha, I. (2018). Effects of effluent from electoplating industry on the immune response in the freshwater fish, Cyprinus carpio. *Fish & shellfish immunology*, 79:86-92.

[3]. Kopecka, J., Lehtonen, KK., Barsiene, J., Broeg, K., Vuorinen, PJ. and Gercken, J. (2006). Measurements of biomarker levels in flounder (*Platichthys flesus*) and blue mussel (*Mytilus trossulus*) from the Gulf of Gdansk (southern Baltic). *Marine pollution bulletin*, 53(8-9):406-21.

[4]. Peric, L., Nerlovic, V., Zurga, P., Zilic, L. and Ramsak, A. (2019). Variations of biomarkers response in mussels *Mytilus galloprovincialis* to low, moderate and high concentrations of organic chemicals and metals. *Chemosphere*, 174:554-62.

[5]. Sparks, C., Marnewick, J., Toefy, R., Snyman, R. and Odendaal, J. (2019). Baseline levels of antioxidant activities in *Mytilus galloprovincialis* along the coast of Cape Town, South Africa. *Marine pollution bulletin*, 140:287-93.

[6]. Viarengo, A., Burlando, B., Giordana, A., Bolognesi, C. and Gabrielides, G. (2000). Networking and expert-system analysis: next frontier in biomonitoring. *Marine environmental research*, 49(5):483-6.

[7]. Viarengo, A., Lafaurie, M., Gabrielides, G., Fabbri, R., Marro, A. and Romeo, M. (2000). Critical evaluation of an intercalibration exercise undertaken in the framework of the MED POL biomonitoring program. *Marine environmental research*, 49(1):1-18.

[8]. Green, N., Ruus, A., Bjerkeng, B., Brevik, E., Håvardstun, J. and Mills, A. (2008). Coordinated Environmental Monitoring Programme (CEMP). Levels, trends and effects of hazardous substances in fjords and coastal waters-2007.

[9]. Taleb, ZM., Benali, I., Gherras, H., Ykhlef-Allal, A., Bachir-Bouiadjra, B. and Amiard, J-C. **(2009).** Biomonitoring of environmental pollution on the Algerian west coast using caged mussels *Mytilus galloprovincialis*. *Oceanologia*, 51(1):63-84.

[10]. Brooks, SJ., Farmen, E., Heier, LS., Blanco-Rayon, E. and Izagirre, U. (2015). Differences in copper bioaccumulation and biological responses in three *Mytilus* species. *Aquat Toxicol*, 160:1-12.

[11]. Mejdoub, Z., Fahde, A., Loutfi, M. and Kabine, M. (2017). Oxidative stress responses of the mussel *Mytilus galloprovincialis* exposed to emissary's pollution in coastal areas of Casablanca. *Ocean & Coastal Management*, 136:95-103.

[12]. Casas, S. and Bacher, C. (2006). Modelling trace metal (Hg and Pb) bioaccumulation in the Mediterranean mussel, *Mytilus galloprovincialis*, applied to environmental monitoring. *Journal of Sea Research*, 56(2):168-81.

[13]. Flammarion., P, Devaux, A. et Garric, J. (2001). Marqueurs biochimiques de pollution dans les écosystèmes aquatiques continentaux. Exemples d'utilisation et perspectives pour le gestionnaire. *Bulletin Français de la Pêche et de la Pisciculture*, (357-360):209-26.

[14]. Aslan, E., Görgün, AU., Katalay, S., Filizok, I., Becerik, S. and Aydemir, T. (2018). An investigation on the seasonal variations of the biomarkers of oxidative stress response and their correlations to Polonium-210 in mussel (*Mytilus galloprovincialis*) and common sole (*Solea solea*) from Izmir Bay, Turkey. *Journal of environmental radioactivity*, 189:103-8.

[15]. van der Oost, R., Beyer, J. and Vermeulen, NPE. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, 13(2):57-149.

[16]. Fossi, M., Marsili, L., Junin, M., Castello, H., Lorenzani, J.and Casini, S. (1997). Use of nondestructive biomarkers and residue analysis to assess the health status of endangered species of pinnipeds in the south-west Atlantic. *Marine pollution bulletin*, 34(3):157-62.

[17]. Ruiz, P., Ortiz-Zarragoitia, M., Orbea, A., Theron, M., Le Floch, S. and Cajaraville, MP. (2012). Responses of conventional and molecular biomarkers in turbot Scophthalmus maximus exposed to heavy fuel oil no. 6 and styrene. *Aquatic toxicology*, 116:116-28.

[18]. Orbea, A., Ortiz-Zarragoitia, M., Solé, M., Porte, C. and Cajaraville, MP. (2002). Antioxidant enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuaries (Bay of Biscay). *Aquatic toxicology*, 58(1-2):75-98.

[19]. Bessi, H. et El Alami, M. (2009). Les bio-essais dans l'évaluation d'impact des polluants sur les écosystèmes dulçaquicoles. Les technologies de laboratoire, 4(15).

[20]. Forbes, V.E., Palmqvist, A. and Bach L. (2006). The use and misuse of biomarkers in ecotoxicology. Environmental Toxicology and Chemistry: *An International Journal*, 25(1): 272-280.

[21]. 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. *Marine Chemistry*, 6(2):179-85.

[22]. Raspor, B., Pavičić, J. and Branica M. (1989). Cadmium-induced proteins from *Mytilus galloprovincialis*-polarographic characterization and study of their interaction with cadmium. *Marine Chemistry*, 28(1-3):199-214.

[23]. Odžak, N., Martinčić, D., Zvonarić, T. and Branica, M. (1994). Bioaccumulation rate of Cd and Pb in *Mytilus galloprovincialis* foot and gills. *Marine Chemistry*, 46(1-2):119-31.

[24]. Kurelec, B. and Pivčević, B. (1991). Evidence for a multixenobiotic resistance mechanism in the mussel *Mytilus galloprovincialis*. *Aquatic toxicology*, 19(4):291-301.

[25]. Regoli, F. and Orlando, E. (1993). *Mytilus galloprovincialis* as a bioindicator of lead pollution: biological variables and cellular responses. *Science of the total environment*, 134:1283-92.

[26]. Giandomenico, S., Spada, L., Annicchiarico, C., Assennato, G., Cardellicchio, N. and Ungaro, N. (2013). Chlorinated compounds and polybrominated diphenyl ethers (PBDEs) in mussels (*Mytilus galloprovincialis*) collected from Apulia Region coasts. *Marine pollution bulletin*, 73(1):243-51.

[27]. Barut, IF., Meric, E. and Yokeş, MB. (2016). Assessment of recent and chalcolithic period environmental pollution using *Mytilus galloprovincialis* Lamarck, 1819 from Yarimburgaz Cave, the northern Marmara Sea and Bosphorus coasts. *Oceanologia*, 58(2):135-49.

[28]. Balbi, T., Franzellitti, S., Fabbri, R., Montagna, M., Fabbri, E. and Canesi, L. (2016). Impact of bisphenol A (BPA) on early embryo development in the marine mussel *Mytilus galloprovincialis*: Effects on gene transcription. *Environ Pollut*, 218:996-1004.

[29]. Danellakis, D., Ntaikou, I., Kornaros, M. and Dailianis, S. (2011). Olive oil mill wastewater toxicity in the marine environment: alterations of stress indices in tissues of mussel *Mytilus galloprovincialis*. *Aquatic toxicology*, 101(2):358-66.

[30]. Vidal-Liñán, L., Bellas, J., Campillo, JA. and Beiras, R. (2010). Integrated use of antioxidant enzymes in mussels, *Mytilus galloprovincialis*, for monitoring pollution in highly productive coastal areas of Galicia (NW Spain). *Chemosphere*, 78(3):265-72.

[31]. Torre, A., Trischitta, F. and Faggio, C. (2013). Effect of CdCl2 on regulatory volume decrease (RVD) in *Mytilus galloprovincialis* digestive cells. *Toxicology in Vitro*,27(4):1260-6.

[32]. Giandomenico, S., Spada, L., Annicchiarico, C., Assennato, G., Cardellicchio, N. and Ungaro N. (2013). Chlorinated compounds and polybrominated diphenyl ethers (PBDEs) in mussels (*Mytilus galloprovincialis*) collected from Apulia Region coasts. *Marine pollution bulletin*, 73(1):243-51.

[33]. Walkley, A. et Black, C. (2003). Centre d'expertise en analyse environnementale du Québec et Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec. Détermination de la matière organique par dosage du carbone organique dans les sols agricoles: méthode Walkley-Black modifiée, MA.

[34]. du Québec G. (2008). Ministère du développement durable, de l'environnement et des parcs du Québec. Sampling Guide for Environmental Analysis: Booklet.

[35]. Atli, G., Alptekin, Ö., Tükel, S. and Canli, M. (2006). Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish *Oreochromis niloticus*. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 143(2):218-24.

[36]. Lowry, OH., Rosebrough, NJ., Farr, AL. and Randall, RJ. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193:265-75.

[37]. Yadav, A., Gopesh, A., Pandey, RS., Rai, DK. and Sharma, B. (2007). Fertilizer industry effluent induced biochemical changes in fresh water teleost, *Channa striatus* (Bloch). *Bulletin of environmental contamination and toxicology*, 79(6):588-95.

[38]. Coppola, F., Almeida, Â., Henriques, B., Soares, AM., Figueira, E. and Pereira, E. (2017). Biochemical impacts of Hg in *Mytilus galloprovincialis* under present and predicted warming scenarios. *Science of the total environment*, 601:1129-38.

[39]. Oliveira, P., Barboza, LGA., Branco, V., Figueiredo, N., Carvalho, C. and Guilhermino, L. (2018). Effects of microplastics and mercury in the freshwater bivalve *Corbicula fluminea* (Muller, 1774): Filtration rate, biochemical biomarkers and mercury bioconcentration. *Ecotoxicology and environmental safety*, 164:155-63.

[40]. Rodier, J. et Legube, B. (2009). L'analyse de l'eau: Dunod.

[41]. Dongo, KR., Niamke, BF., Adje, AF., Britton, BGH., Nama, LA. et Anoh, KP. (2013). Impacts des effluents liquides industriels sur l'environnement urbain d'Abidjan-Côte D'Ivoire. International *Journal of Biological and Chemical Sciences*, 7(1):404-20.

[42]. Hazourli, S., Boudiba, L. et Ziati, M. (2007). Caractérisation de la pollution des eaux résiduaires de la zone industrielle d'El-Hadjar, Annaba. *LARHYSS Journal* P-ISSN 1112-3680/E-ISSN 2602-7828. (6).

[43]. Bawa, ML., Djaneye-Boundjou, G. et Boukari, Y. (2006). Caractérisation de deux effluents industriels au Togo: étude d'impact sur l'environnement. *Afrique Science*: Revue Internationale des Sciences et Technologie, 2(1).

[44]. Chalghmi, H. (2015). Etude de la pollution marine par les hydrocarbures et caractérisation de leurs effets biochimiques et moléculaires sur la palourde de Ruditapes sp.

[45]. Salim, B. and Driss, AT. (2014). Bio-monitoring of Trace Mercury Using Seabirds' Dejections from Arzew Gulf: A Potential Risk for Human Health. *Journal of Environment Pollution and Human Health*, 2(1):7-11.

[46]. Ayah, M., Grybos, M., Tampo, L., Bawa, LM., Bril, H. et Djaneye-Boundjou, G. (2015). Qualité et pollution des eaux d'un hydrosystéme littoral tropical: cas du systéme lagunaire de Lomé, Togo. *European Scientific Journal*, 11(15).

[47]. Boucheseiche, C., Cremille, E., Pelte, T. et Pojer, K. (2002). Bassin Rhône–Méditerranée–Corse. Guide technique (7).

[48]. Nadella, SR., Fitzpatrick, JL., Franklin, N., Bucking, C., Smith, S. and Wood, CM. (2009). Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 149(3):340-8.

[49]. Martin, M., Osborn, KE., Billig, P. and Glickstein, N. (1981). Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and Cancer magister larvae. *Marine pollution bulletin*, 12(9):305-8.

[50]. Roccheri, MC., Agnello, M., Bonaventura, R. and Matranga, V. (2004). Cadmium induces the expression of specific stress proteins in sea urchin embryos. *Biochemical and Biophysical Research Communications*, 321(1):80-7.

[51]. Boukadida, K., Banni, M., Gourves, PY. and Cachot, J. (2016). High sensitivity of embryo-larval stage of the Mediterranean mussel, *Mytilus galloprovincialis* to metal pollution in combination with temperature increase. *Marine environmental research*, 122:59-66.

[52]. Coppola, F., Almeida, Â., Henriques, B., Soares, AM., Figueira, E. and Pereira, E. (2018). Biochemical responses and accumulation patterns of *Mytilus galloprovincialis* exposed to thermal stress and Arsenic contamination. *Ecotoxicology and environmental safety*, 147:954-62.

[53]. Arienzo, M., Toscanesi, M., Trifuoggi, M., Ferrara, L., Stanislao, C. and Donadio, C. (2019). Contaminants bioaccumulation and pathological assessment in *Mytilus galloprovincialis* in coastal waters facing the brownfield site of Bagnoli, Italy. *Marine pollution bulletin*, 140:341-52.

[54]. Boukadida, K., Cachot, J., Morin, B., Clerandeau, C. and Banni, M. (2019). Moderate temperature elevation increase susceptibility of early-life stage of the Mediterranean mussel, *Mytilus galloprovincialis* to metal-induced genotoxicity. *The Science of the total environment*, 663:351-60.

[55]. Yavasoglu, A., Ozkan, D., Guner, A., Katalay, S., Oltulu, F. and Yavasoglu, NUK. (2016). Histopathological and apoptotic changes on marine mussels *Mytilus galloprovincialis* (Lamark, 1819) following exposure to environmental pollutants. *Marine pollution bulletin*, 109(1):184-91.

[56]. Annamaria, VG., Chiara, L., Alessandra, AN., Alvise, B., His, E. and Francesco, GP. (2005). *Mytilus galloprovincialis* as bioindicator in embryotoxicity testing to evaluate sediment quality in the lagoon of Venice (Italy). *Chemistry and Ecology*, 21(6):455-63.

[57]. Radenac, G., Fichet, D. and Miramand, P. (2001). Bioaccumulation and toxicity of four dissolved metals in *Paracentrotus lividus* sea-urchin embryo. *Marine environmental research*, 51(2):151-66.

[58]. Wepener, V., van Vuren, JHJ., Chatiza, FP., Mbizi, Z., Slabbert, L. and Masola, B. (2005). Active biomonitoring in freshwater environments: early warning signals from biomarkers in assessing biological effects of diffuse sources of pollutants. *Physics and Chemistry of the Earth*, Parts A/B/C, 30(11-16):751-61.

[59]. Dong, S., Yang, Y., Cheng, B., Ren, C., Zhang, H. and Xu, H. (2019). Responses of antioxidant defenses in the clam *Mactra veneriformis* to 2, 2′, 4, 4′‑tetrabromodiphenyl ether exposure. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 217:98-105.

[60]. Bao, M., Huo, L., Wu, J., Ge, D., Lv, Z. and Chi, C. (2018). A novel biomarker for marine environmental pollution of CAT from *Mytilus coruscus*. *Marine pollution bulletin*, 127:717-25.

[61]. Verlecar, X., Jena, K. and Chainy, G. (2008). Seasonal variation of oxidative biomarkers in gills and digestive gland of green-lipped mussel *Perna viridis* from Arabian Sea. *Estuarine, Coastal and Shelf Science*, 76(4):745-52.

[62]. Box, A., Sureda, A., Galgani, F., Pons, A. and Deudero, S. (2007). Assessment of environmental pollution at Balearic Islands applying oxidative stress biomarkers in the mussel *Mytilus galloprovincialis*. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 146(4):531-9.

[63]. Damiens, G., His, E., Gnassia-Barelli, M., Quiniou, F. and Roméo, M. (2004). Evaluation of biomarkers in oyster larvae in natural and polluted conditions. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 138(2):121-8.

[64]. Vlahogianni, T., Dassenakis, M., Scoullos, MJ. and Valavanidis, A. (2007). Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Marine pollution bulletin*, 54(9):1361-71.

[65]. Sureda, A., Box, A., Tejada, S., Blanco, A., Caixach, J. and Deudero, S. (2011). Biochemical responses of *Mytilus galloprovincialis* as biomarkers of acute environmental pollution caused by the Don Pedro oil spill (Eivissa Island, Spain). *Aquat Toxicol*, 101(3-4):540-9.

[66]. Perošević, A., Joksimović, D., Đurović, D., Milašević, I., Radomirović, M. and Stanković, S. (2018). Human exposure to trace elements via consumption of mussels *Mytilus galloprovincialis* from Boka Kotorska Bay, Montenegro. *Journal of Trace Elements in Medicine and Biology*, 50:554-9.

[67]. Maanan, M. (2008). Heavy metal concentrations in marine molluscs from the Moroccan coastal region. *Environmental Pollution*, 153(1):176-83.

[68]. Schintu, M., Durante, L., Maccioni, A., Meloni, P., Degetto, S. and Contu, A. (2008). Measurement of environmental trace-metal levels in Mediterranean coastal areas with transplanted mussels and DGT techniques. *Marine pollution bulletin*, 57(6-12):832-7.

[69]. Jean-Claude, A. et Claude, A-T. (2008). Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques: Lavoisier.

[70]. Jing, G., Li ,Y., Xie, L. and Zhang, R. (2006). Metal accumulation and enzyme activities in gills and digestive gland of pearl oyster (*Pinctada fucata*) exposed to copper. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 144(2):184-90.

[71]. Kraak, MH., Wink, YA., Stuijfzand, SC., Buckert-de Jong MC., de Groot, CJ. and Admiraal, W. (1994). Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*. *Aquatic toxicology*, 30(1):77-89.

[72]. Borković, SS., Šaponjić, JS., Pavlović, SZ., Blagojević, DP., Milošević, SM. and Kovačević, TB. (2005). The activity of antioxidant defence enzymes in the mussel *Mytilus galloprovincialis* from the Adriatic Sea. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 141(4):366-74.

[73]. Dellali, M., Romeo, M. et Aissa, P. (2001). Suivi annuel de l'activité catalase chez des moules et des palourdes originaires de la lagune de Bizerte. *Oceanologica acta*, 24(3):263-71.

[74]. Fournier, E. (2005). Bioaccumulation du sélénium et effets biologiques induits chez le bivalve filtreur *Corbicula fluminea*: prise en compte de l'activité ventilatoire, de la spéciation du sélénium et de la voie de contamination: Bordeaux 1.

[75]. Figueiredo-Fernandes, A., Fontaínhas-Fernandes, A., Peixoto, F., Rocha, E. and Reis-Henriques, M. (2006). Effects of gender and temperature on oxidative stress enzymes in Nile tilapia *Oreochromis niloticus* exposed to paraquat. *Pesticide biochemistry and physiology*, 85(2):97-103.

[76]. Atli, G. and Canli, M. (2007). Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 145(2):282-7.

[77]. Vlahogiannij, T., Dassenakisj, M., Scoullosj, MJ. and Valavanidisj, A. (2007). Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Marine pollution bulletin*, 54(9):1361-71.

[78]. Akcha, F., Izuel, C., Venier, P., Budzinski, H., Burgeot, T. and Narbonne, J. (2000). Enzymatic biomarker measurement and study of DNA adduct formation in benzo [a] pyrene-contaminated mussels, *Mytilus galloprovincialis*. *Aquatic toxicology*, 49(4):269-87.

[79]. Barillet, S., Adam-Guillermin, C., Palluel, O., Porcher, JM. and Devaux, A. (2011). Uranium bioaccumulation and biological disorders induced in zebrafish (*Danio rerio*) after a depleted uranium waterborne exposure. *Environ Pollut*, 159(2):495-502.

[80]. Lagadic, L. (1997). Biomarqueurs en écotoxicologie.

[81]. Amiard, J-C., Caquet, T. and Lagadic, L. (2000). Use of biomarkers for environmental quality assessment: CRC Press.

[82]. Ducrot, V. (2005). Compréhension et modélisation des relations entre les caractéristiques biologiques et écologiques et la sensibilité aux contaminants des communautés d'invertébrés benthiques: perspectives pour l'évaluation des effets des substances chimiques: Université Paul Verlaine-Metz.

[83]. Almeida, J., Diniz, Y., Marques, S., Faine, L., Ribas, B and, Burneiko, R. (2002). The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. *Environment International*, 27(8):673-9.

[84]. Linde-Arias, AR., Inácio, AF., de Alburquerque, C., Freire, MM. and Moreira, JC. (2008). Biomarkers in an invasive fish species, *Oreochromis niloticus*, to assess the effects of pollution in a highly

degraded Brazilian River. *Science of the total environment*, 399(1-3):186-92.

[85]. ROCHE, H., BUET, A. et RAMADE, F. (2003). Caractéristiques écophysiologiques d'une population d'anguilles de Camargue exposée à une pollution clandestine par des polluants organiques persistants. *Revue d'écologie*.

[86]. Almeida, J., Novelli, E., Silva, MDP. and Júnior, RA. (2001). Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environmental Pollution*, 114(2):169-75.

[87]. Xu, K., Tang, Z., Liu, S., Xia, H., Liu, L. and Wang, Z. (2018). Effects of low concentrations copper on antioxidant responses, DNA damage and genotoxicity in thick shell mussel *Mytilus coruscus*. *Fish & shellfish immunology*, 82:77-83.

[88]. Gomes, T., Pereira, CG., Cardoso, C., Pinheiro, JP., Cancio, I. and Bebianno, MJ. (2012). Accumulation and toxicity of copper oxide nanoparticles in the digestive gland of *Mytilus galloprovincialis*. *Aquatic toxicology*, 118:72-9.

[89]. Almeida, A., Freitas, R., Calisto, V., Esteves, VI., Schneider, RJ. and Soares, AM. (2015). Chronic toxicity of the antiepileptic carbamazepine on the clam *Ruditapes philippinarum*. *Comparative biochemistry and physiology Toxicology & pharmacology* : CBP.;172-173:26-35.

[90]. Lima, I., Moreira, SM., Osten, JR., Soares, AM. and Guilhermino, L. (2007). Biochemical responses of the marine mussel *Mytilus galloprovincialis* to petrochemical environmental contamination along the North-western coast of Portugal. *Chemosphere*, 66(7):1230-42.

[91]. Livingstone, D. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine pollution bulletin*, 42(8):656-66.

[92]. Gravato, C., Oliveira, M. and Santos, M. (2005). Oxidative stress and genotoxic responses to resin acids in Mediterranean mussels. *Ecotoxicology and environmental safety*, 61(2):221-9.

[93]. Boukadida, K., Cachot, J., Clerandeaux, C., Gourves, PY. and Banni, M. (2017). Early and efficient induction of antioxidant defense system in *Mytilus galloprovincialis* embryos exposed to metals and heat stress. *Ecotoxicology and environmental safety*, 138:105-12.

[94]. Prego-Faraldo, M., Vieira, L., Eirin-Lopez, J., Méndez, J. and Guilhermino, L. (2017). Transcriptional and biochemical analysis of antioxidant enzymes in the mussel *Mytilus galloprovincialis* during experimental exposures to the toxic dinoflagellate Prorocentrum lima. *Marine environmental research*, 129:304-15.

[95]. Gorbi, S., Avio, GC., Benedetti, M., Totti, C., Accoroni, S. and Pichierri, S. (2013). Effects of harmful dinoflagellate *Ostreopsis cf. ovata* exposure on immunological, histological and oxidative responses of mussels *Mytilus galloprovincialis*. *Fish & shellfish immunology*, 35(3):941-50.

[96]. Richir, J. and Gobert, S. (2014). The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*. *Ecological Indicators*, 36:33-47.

[97]. Dauvin, J-C., Grimes, S. and Bakalem, A. (2013). Marine biodiversity on the Algerian continental shelf (Mediterranean Sea). *Journal of natural history*, 47(25-28):1745-65.

[98]. Peixoto, F., Alves-Fernandes, D., Santos, D. and Fontaínhas-Fernandes, A. (2006). Toxicological effects of oxyfluorfen on oxidative stress enzymes in tilapia *Oreochromis niloticus*. *Pesticide biochemistry and physiology*, 85(2):91-6.

[99]. Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, J-M., and Ait-Aissa, S. (2005). Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology*, 19(1):177-83.

[100]. Sun, Y., Yu, H., Zhang, J., Yin, Y., Shi, H. and Wang, X. (2006). Bioaccumulation, depuration and oxidative stress in fish *Carassius auratus* under phenanthrene exposure. *Chemosphere*, 63(8):1319-27.

[101]. Koutsogiannaki, S., Franzellitti, S., Fabbri, E. and Kaloyianni, M. (2014). Oxidative stress parameters induced by exposure to either cadmium or 17beta-estradiol on *Mytilus galloprovincialis* hemocytes. The role of signaling molecules. *Aquat Toxicol*, 146:186-95.