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Use of the mussel expert system to evaluate the syndrome stress level on the Moroccan Central Atlantic coast

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Abstract

The current study examined the effect of cadmium exposure in the laboratory on the number of selected biomarkers (Lysosomal membrane stability, Metallothionein concentration, Acetylcholinesterase activity, and micronucleus frequency) in the Mussel *Mytilus galloprovincialis* to evaluate their ecological relevance and their potential use as pollution indicators in the field. This study was achieved using the mussel expert system for identifying and quantifying pollution-induced stress syndrome in mussels sampled on the Moroccan Central Atlantic coast.

According to Cd concentration and exposure time, the laboratory analysis revealed a decrease in lysosomal membrane stability, an increase in metallothionein concentrations, and an increase in total nuclear abnormalities.

The integration of the data from the in situ study with the mussel expert system has shown that the organisms of the M'diq, Cap Beddouza, and Dar Bouazza sites can be considered in good health. Low stress is detected at the Souiria kdima and El Jadida sites, the other sites show moderate stress.

Keywords: Mussel expert system; Cadmium contamination; Field validation; Coastal zone; Metallothionein; Acetylcholinesterase; Micronucleus frequency; Lysosomal membrane stability

1. Introduction

Marine ecosystems are always subject to the impact of industrial and urban wastewater (Halpern et al., 2008), and many tools have been developed to assess the quality of this area. The Chemical analysis furnishes information on the degree of contamination of different matrices (water, sediment, and biota), but it does not indicate the impact of this contamination on the ecosystem. Biomarkers represent changes from the molecular to the organism level related to toxic effects and exposure to chemical contaminants.

In addition, biomarker responses occur before alterations at the population and community levels, they can be predictive and anticipatory. Thus, biomarkers can diagnose causes and act as early warning signals of ecosystem-level damage (Tsangaris et al., 2007).

Over the last few decades, many biomarkers have been developed to evaluate the impact of toxic chemical contaminants at different biological levels in marine organisms (Hylland et al., 2012; Viarengo et al., 2007; Rank et al., 2007; Moore et al., 200;, Amiard et al., 2006; Bolognesi et al., 2004 and 2006; Depledge et al., 1993 and Moore, 1976). Mussels (*Mytilus spp*.) are known as sentinel organisms employed in many programs of marine biomonitoring because of their accessibility, wide distribution, usefulness for caging methods (Viarengo et al., 2007), and the possibility to accumulate

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different toxic contaminants present in the medium (Taleb et al., 2009; Banni et al., 2007; Moore et al., 2006; Romeo et al., 2005; Boutiba et al., 2003; Mourgaud et al., 2002; Besada et al., 2002; Viarengo et al., 1987).

The present study used a battery of biomarkers to assess the toxic effects of environmental compounds. Stress on stress (SOS) (Viarengo *et al.*, 1995) and lysosomal membrane stability (LMS) (El haimeur et al., 2017; Moore, 1982; Moore, 1988; Brooks et al., 2011) are biomarkers of general stress, acetylcholinesterase (AChE) activity biomarker of neurotoxic effects through neurotoxic compounds specifically organophosphate and carbamate pesticides (Bocquene and Galgani, 1998, Rickwood and Galloway, 2004). Metallothionein (MT), a low molecular weight protein and cysteine-rich, represents a biomarker of mussel exposure to heavy metals such as Cd, Cu, Hg, and Zn (Viarengo and Nott, 1993).

Finally, the genotoxic impact was evaluated at the chromosomal level through the evaluation of nuclear abnormalities (the micronucleus test). The micronucleus (MN), the more stable anomaly, is a small intracytoplasmic chromatin mass resulting from chromosomal breakage or aneuploidy during cell division (Bolognesi et al., 2004).

First, we tried to examine the effects of exposition to Cd as a model of contaminant on the four different biomarkers in the Mussel *Mytilus galloprovincialis* to evaluate their ecological relevance and potential use as pollution biomarkers in the field. In addition, inorganic contaminants present in the exposed mussel tissues were analyzed. After validation in the laboratory, the study was conducted on the Moroccan Central Atlantic coast. Some sites in this region are known for their contamination by Cd. Furthermore, to assess the level of stress syndrome in the organisms studied, we integrated various responses into an index using the Mussel Expert System (MES) (Dagnino et al., 2007).

2. Material and methods

2.1. Mussel sampling

For the exposure to Cd, the mussels were sampled at Dar Bouazza station, which is far from pollution sources. Several laboratory studies have shown the good quality of this site (Bouhallaoui et al., 2011; El haimeur et al., 2013; El haimeur et al., 2017). Mussels of *Mytilus galloprovincialis* of similar shell length (4 to 5 cm) were manually collected, and the necessary seawater was taken in polyethylene cans. For the field study, 30 individuals of *M. galloprovincialis* were collected at different sampling sites on the Moroccan Central Atlantic coast (Figure 1). Following sampling, the organisms were transported to the laboratory in isotherm containers within 2 hours of collection.

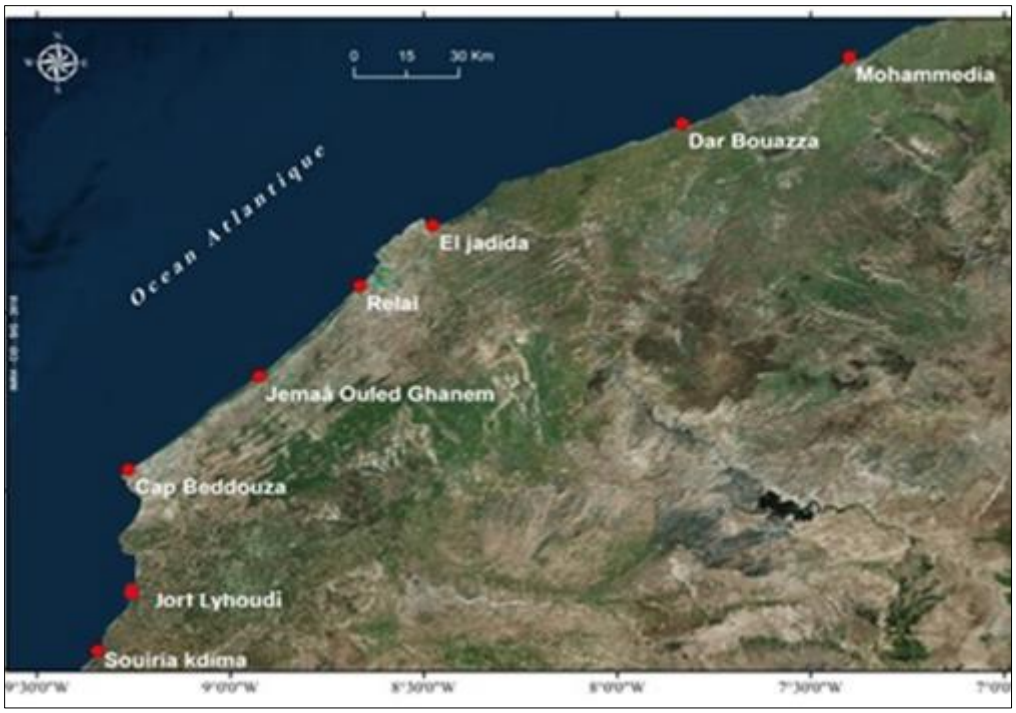

Figure 1 Map of the location of different mussel sampling sites on the central Atlantic Moroccan coast

2.2. Experimental exposure of mussels

In this part, we sought to validate the techniques of selected biomarkers: lysosomal membrane stability, micronucleus frequency, and metallothionein content. We used cadmium as a contaminant model and the mussel *Mytilus galloprovincialis* as a biological model. After removing the mussels and transporting them to the laboratory, the mussels were acclimated to laboratory conditions (T=20 °C) before Cd exposure, they were placed in tanks containing the seawater of the site (at a rate of 500 ml/individual). Oxygenation of the medium is ensured by permanent bubbling using a bubbler fitted with a diffuser. Cleaning of aquariums and seawater renewing were carried out every 48 hours. Two sub-lethal concentrations of Cd below health thresholds (order n° 1950–17, 2017) were tested: 100 µg/l and 500 µg/l. Individual mortality is monitored daily; dead individuals are removed from aquariums. The physicochemical parameters (salinity, oxygen, and pH) were monitored and noted daily at each time of medium renewal.

2.3. Pretreatment of samples

For all biomarkers except LMS, the mussels were dissected to remove the digestive gland which will be stored at -80 until analysis. For LMS, the treatment was done on the same day of sampling. However, for MN, the individuals were acclimated for 24 hours before analysis.

2.4. Evaluation of the behavior of mussels: Stress on stress

The protocol is inspired by Viarengo *et al.*, 1995. The test involves exposing mussels (*M. galloprovincialis,* 4-5 cm) (30 animals) to anoxia by air exposure at 20 °C in a humidified chamber. Survival individuals are monitored daily, and the number of dead individuals is noted. The criteria measured are the mortality rate of individuals. A median survival time (LT50), the time (days) when 50% of mussels are dead, must be calculated using IBM SPSS ($\alpha = 0.05$).

2.5. Evaluation of lysosomal membrane stability

The LMS evaluation was carried out according to the Lowe and Pipe method (1994) and the recommendations of UNEP/RAMOGE (1999). Haemolymph (0.5 ml) was collected from the posterior adductor muscle of a mussel. The haemocytes suspension was spread on slides and kept in a humid chamber for 30 min. Then, haemocytes were incubated in a neutral red (NR) solution. After 15 min, the slides were washed with filtered water and kept moist. After 1 h, the slides were observed under an optical microscope to take images which are analyzed by the "Scion Image" software and the leakage of lysosomal NR was quantified. Results were expressed as net percent change in color intensity in a sample.

2.6. Metallothionein content

The metallothionein (MT) content was analyzed using the method of Viarengo et al. (1997). The MT concentrations were determined using reduced glutathione (GSH) as a reference standard. Digestive glands were homogenized in 20 mM Tris-HCl buffer (pH 8.6) at a rate of 1g in 3 volumes (1 g/3 ml) using an Ultraturax homogenizer. The homogenate undergoes a series of centrifugations to prepare the fraction enriched in MTs. Pellets for the last centrifugation were resuspended in NaCl solution (0.25 M) and HCl solution (1 N) containing EDTA (4 mM). Before analysis, the solution of DTNB (0.43 mM) was prepared with 0.2 M phosphate buffer (pH 8) containing 2M NaCl. 4.2 ml of DTNB, and was added to different tubes (blank, standards, and samples). Then the samples were centrifuged (3000 g for 5 min) at room temperature, and the supernatant was analyzed at 412 nm. The results were expressed as micrograms of MT per gram of wet-weight tissue

2.7. Acetylcholinesterase (AChE) activity

The method of AChE activity analysis was inspired from Ellman et al. (1961) protocol and adapted to the microplate by Bocquené and Galgani (1998), the substrate used was acetylthiocoline (0.075M). The analysis was carried out in the S9 fraction of Mussel's digestive glands. The thiocholine reacts with DTNB (0.01M) and gives a yellow-colored product. The change in the optical density of this product per minute at 412 nm corresponds to the rate of AChE activity. The activity was expressed as nanomoles of thiocholine produced per minute per milligram of protein.

2.8. Micronucleus frequency

Micronucleus frequency was performed according to the method of Bolognesi and Fenech (2012). A volume of 0.5 ml of haemolymph is withdrawn using a syringe (0.22 G needle) containing 0.2 ml of filtered seawater of the sampling site in the posterior adductor muscle of the mussel. The haemocytes suspension is placed in an Eppendorf tube and left for a few minutes at room temperature, then centrifuged at 1000 g for 5 min. After centrifugation, a methanol-acetic Acid

solution (3:1) is added to the haemolymph suspension (pellet). This is the step of fixing the cells. Afterward, a sufficient volume of each suspension is spread on slides (5 slides per individual) previously kept at -20 °C.

After the distribution of the cell suspension, the slides are dried for a few minutes. They were stained with Giemsa solution (3%) (188 ml deionized water + 6 ml filtered Giemsa + 6 ml Sorensen solution), washed twice with deionized water, then dried and mounted with coverslips using DPX.

The slides are observed under an ordinary microscope with an x100 objective using immersion oil, and 500–1000 cells are counted. The anomalies are identified according to the criteria described by Bolognesi and Fenech (2012).

2.9. Chemical analyses

Samples of exposed mussels (whole animals) were lyophilized at -50 °C. 0.25 g of the sample and a volume of HNO_3 and H_2O_2 were mineralized in the microwave for 30 minutes. The product was completed to 50 ml by addition of Milli-Q water (AOAC, 1999). The concentration of Cd was determined using inductively coupled plasma mass spectrometry (ICP-MS) (Element II, Thermo). After that, a contamination index Ic is calculated following the formula of Quaouiyd et al., (2016).

Ic = Final metal content of the station x / Metal content of the reference station.

The IC value allows the distinction of four classes of contamination: Class 1: from 0 to 3 (**low contamination**); Class 2: from 4 to 10 (**medium contamination**); Class 3: from 10 to 20 (**significant contamination**); and Class 4: > 20 (**excessive contamination**).

2.10. Integration of biomarkers responses by MES

The expert system uses biomarkers responses at different levels of biological organization (from molecular to the whole organism) to screen for stress syndrome in its various phases of evolution.

The MES method is based on the method described by Dagnino et al. (2007). The number of altered biomarkers increases with the evolution of the stress syndrome, according to the sensitivity of each parameter in the battery and the progressive activation of the various biological stress response mechanisms. For each 20% increase in the percentage of the total number of parameters showing significant changes compared to controls, the classification shifts to the next higher stress level (Dagnino et al., 2007).

2.11. Statistical analysis

Statistical analyses were carried out using the ANOVA test using XLSTAT Version 2021.1.1.1096. The ANOVA test was used to compare the means of three replicates of biomarker responses. The pairwise multiple comparisons, if significant, were performed using the Fisher LSD test and Tukey HSD. In case of nonlinearity in the data, a nonparametric test (Kruskal-Wallis) is used for means comparison, and pairwise multiple comparisons are performed according to the Conover-Iman procedure.

3. Results

3.1. Validation of biomarkers

3.1.1 Physico-chemical parameters

In the case of mussels exposed to 100 ppb of Cd, the pH showed a slight decrease from the day 0 (D0) to the day 8 (D8), and the salinity showed a slight increase; on the other hand, the oxygen level in the medium did not change throughout the exposure period (Table 1). For mussels exposed to 500 ppb of Cd, a slight variation was recorded for parameters at the two lots (control and contaminated) (Table 2). The mortality rate was monitored throughout the experiment; no mortality was recorded in the control and 500 ppb concentration of Cd batches, and only one mortality was noted in the 100 ppb Cd batch on the 5th day of the exposition.

Table 1 Monitoring of the physicochemical parameters of the medium during the exposure of mussels to a concentration of 100 ppb of Cd

Exposure day	pH		Salinity		O_2	
	Control batch	Contaminated batch	Control batch	Contaminated batch	Control batch	Contaminated batch
D0	8.38	8.22	34.1	34.3	9.8	9.6
D3	8.27	8.32	34.9	35.3	9.6	9.5
D5	8.3	8.34	35.8	36.5	9.7	9.8
D7	7.99	7.76	35.4	35.3	9.6	8
D8	7.9	7.9	35.4	35.4	9.5	9.6

Table 2 Monitoring of the physicochemical parameters of the medium during the exposure of mussels to a concentration of 500 ppb of Cd

Exposure day	pH		Salinity		O2	
	Control batch	Contaminated batch	Control batch	Contaminated batch	Control batch	Contaminated batch
D0	7.94	8.22	34.2	34.3	9.5	9.5
D3	7.98	7.96	35.2	35	8.5	8.7
D5	8.02	8.04	35.4	35.3	8.5	8.7
D7	7.97	8.02	35.8	36	7.8	9.1
D8	7.98	7.94	35.7	35.3	8.6	8.6

3.1.2 Evaluation of lysosomal membrane stability

Cd-induced stress levels caused destabilization of the lysosomal membrane. The degree of LMS was expressed in lysosomal stability (Figure 2). For the control batch, the LMS level was the highest ; it remained constant until the 3rd day, then decreased slightly on the 8th day. For mussels contaminated with 100 ppb of Cd, we recorded a decrease in CD ranging from 100% to 40% on day 3. This rate continues to decrease until day 15. Statistical analyses showed a significant difference between exposure times, with p-values <0.0001.

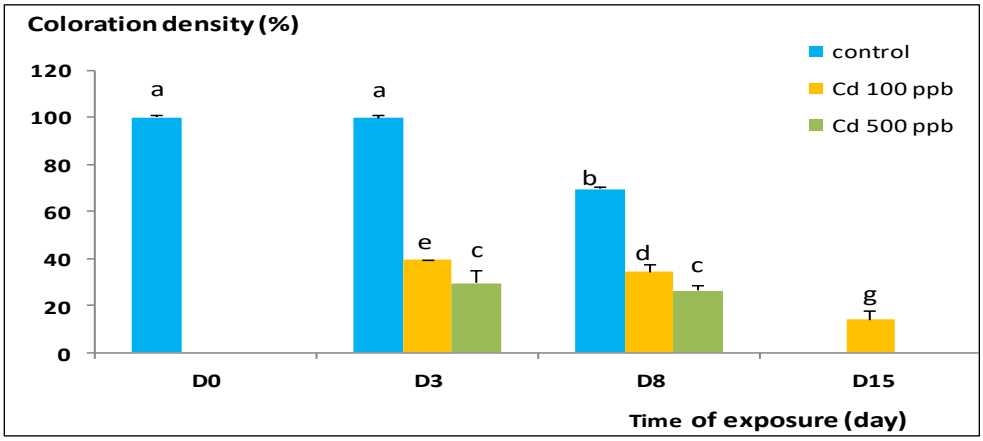

Figure 2 Variation of the degree of LMS as a function of the exposure time to a concentration of 100 ppb and 500 ppb of Cd

In the case of mussels contaminated with 500 ppb, the CD decreased after 3 days to register a value of 29.5% and decreased slightly on day 8. Statistical analyses showed a significant difference between control and exposed individuals, with p-values <0.0001. A statistically significant difference was recorded between 100 ppb and 500 ppb at D3 and D8, with p-values equal to 0.001 and 0.003, respectively.

3.1.3 Evaluation of the genotoxic impact of Cd

The results of total nuclear anomalies (TNA) in the haemocytes of mussels contaminated with Cd are presented in Figure 3. For the control batch, we noted a low frequency of nuclear anomalies (0.05–0.1). For the other batches, at 100 ppb of Cd, we have noted a low frequency of anomalies even after 15 days of contamination. But at 500 ppb, we recorded an increase in TNA, mainly in Micronucleus and Binuclear cell anomalies, as a function of time of exposure, with a maximum effect on day 15. For buds and necrotic cells, no increase in these anomalies was recorded. At day 15, all cells were in apoptosis in mussels contaminated with 500 ppb.

Statistical processing of the data showed that for the 100 ppb concentration, there was no significant difference between different exposure times. For the 500 ppb concentration, a statistically significant difference was recorded between individuals of the control batch and exposed individuals at D3 and D15 with p-values <0.0001. Significant differences were noted between 100 ppb and 500 ppb at D3 with a p-value equal to 0.01 and at D15 with a p-value <0.0001. No significant difference was noted for the control batch during exposure times.

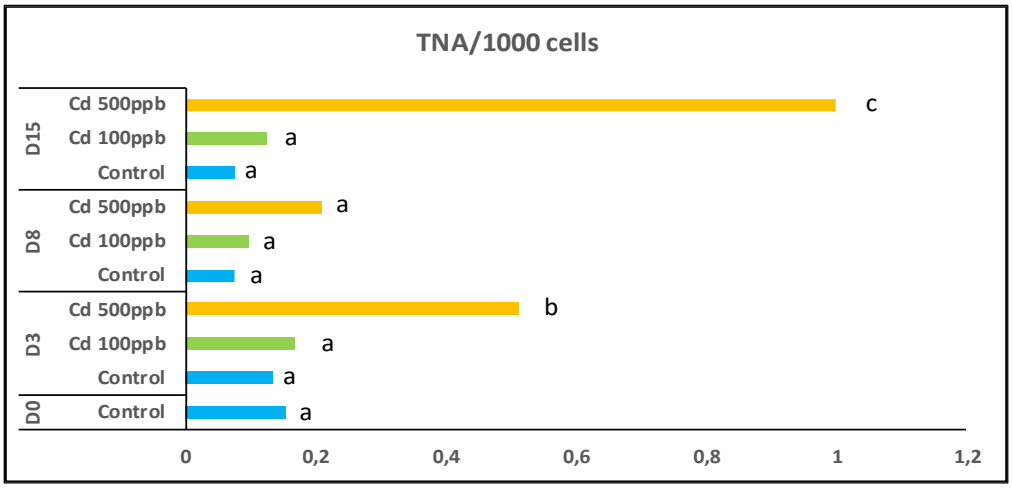

Figure 3 Frequency of total nuclear anomalies at different times of exposure to the two concentrations of Cd (100 and 500 ppb) (The bars with the different letters are significantly different at $p < 0.05$)

3.1.4 Evaluation of MT content

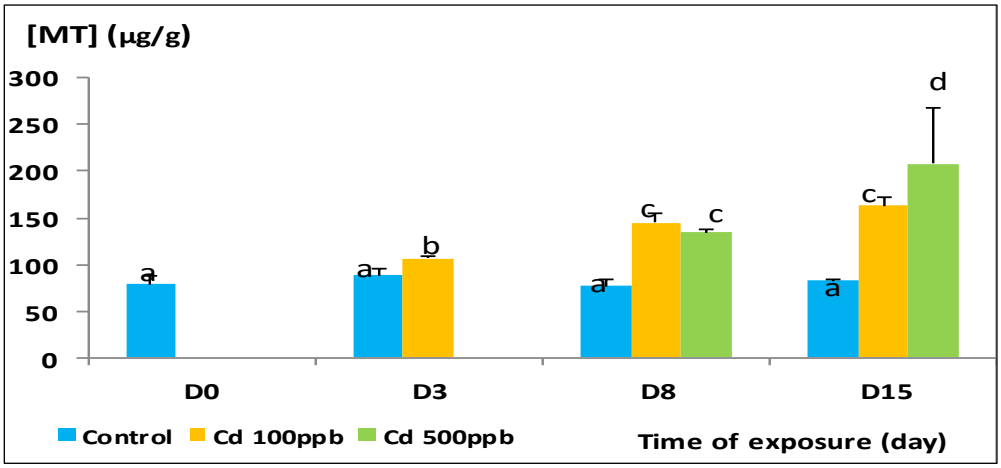

Figure 4 Metallothionein content in the digestive gland of *M. galloprovincialis* exposed to the two concentrations of Cd (100 ppb and 500 ppb)

Individuals in the control group showed no change in MT level during all exposure periods with a concentration not exceeding 88 µg / g pf. The concentration of 100 ppb of Cd induced an increase in the concentration of MT in the exposed mussels, we recorded concentrations ranging from 107 µg / g ww on D0 to 163 µg / g ww on D15. The MT variation profile for individuals exposed to 500 ppb of Cd was the same at D8 as that of the 100 ppb concentration. However, the MT level recorded was significantly higher at D15 for individuals exposed to 500ppb (Figure 4) with a p-value equal to 0.036.

3.1.5 Contamination Index of Cd

The calculation of Ic in mussels exposed to Cd showed an increase of the accumulation rate according to exposure concentrations and exposure time. The Ic ranged from 11 to 29 for 100 ppb and from 12 to 43 for 500 ppb. According to the classification grid, the level of contamination induced by 100 and 500 ppb of Cd was excessive (Figure 5).

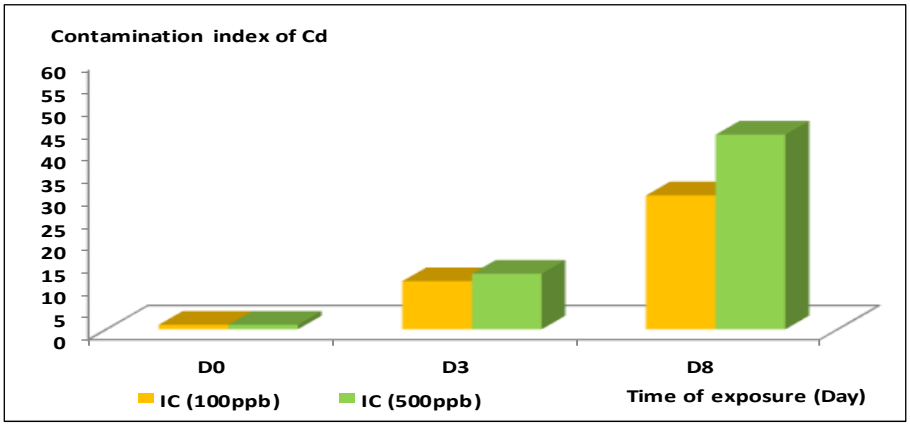

Figure 5 Contamination index in mussels exposed to concentrations of 100 ppb and 500 ppb of Cd

3.2. Evaluation of pollution impact in situ

3.2.1 Physico-chemical parameters

Table 3 presents the physicochemical parameters at the various studied sites. No significant change across sites was generally found; a slight variation was registered at temperature, which ranges between 17 and 22 °C, and at pH which ranges from 7.3 to 8.

Table 3 Variation of physicochemical parameters in different studied sites.

	Reference site	Mohammedia	Dar Bouazza	El Jadida	Relais	Jemaa Ouled Ghanem	Cap Beddouza	Jorf Lyhoudi	Souiria L'kdima
T	18	22	20	17	17	17	18	19	18
Salinity	35	35	35.1	35	35	34.8	35.2	35.1	35.2
pH	8	7.7	7.7	7.5	7.3	7.5	7.8	7.7	7.5

3.2.2 Evaluation of the mussel's behavior: Stress on stress

Figure 6 represents the percentage of survival mussels as a function of survivability time (days) in the studied samples. The mussels of the reference site, Dar Bouazza, Souiria Kdima, and Cap Beddouza had higher survivability (9–10 days), with LT50 ranging from 5 to 7 days on average. El Jadida, JOG, and Mohammedia sites demonstrated the lowest survivability (7 days) with an LT50 of 4.5 days on average. For Relais and Jorf Lyhoudi, despite their contamination and their proximity to pollution sources, they had the same survivability and LT50 as the reference site.

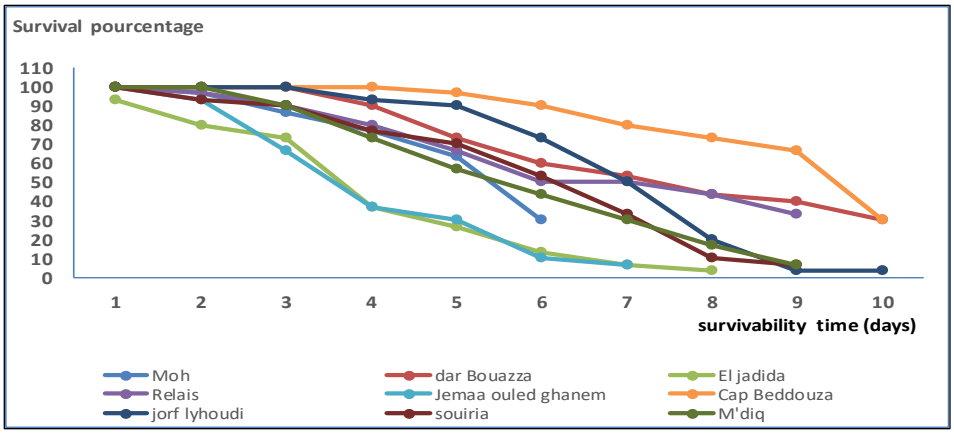

Figure 6 Survival curves obtained from mussel sampling at different sites

3.2.3 Evaluation of lysosomal membrane stability

The results show that mussels from the reference site and Cap Beddouza didn't record any stress in the medium. A moderate stress level was noted at the Dar Bouazza and Souiria Kdima sites. However, the other stations showed a high stress level, with a statistically significant difference from the reference site (Figure 7).

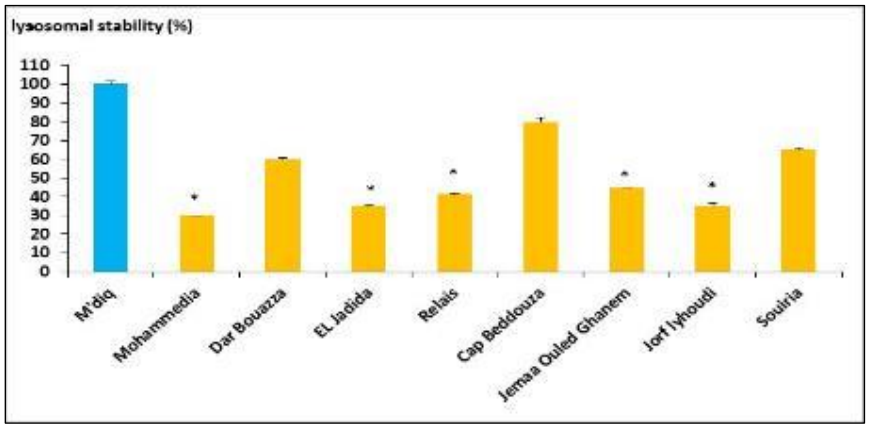

Figure 7 Variation of lysosomal stability Percentage in the haemolymph of the mussel sampled at various sites (The bars with * are significantly different, $p < 0.05$)

3.2.4 Evaluation of the genotoxic impact of pollution

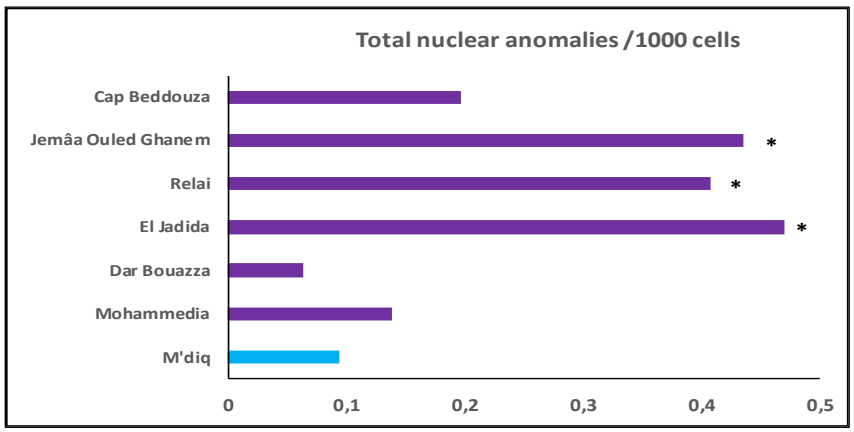

Figure 8 Frequency of total nuclear anomalies in mussels sampled at various sites (The bars with * are significantly different, $p < 0.05$)

The results of genotoxic impact show a low frequency of TNA and nuclear anomalies in the reference site and Dar Bouazza site. In the other sites, we recorded a slight increase in TNA in Cap Beddouza and Mohammedia, with frequencies that did not reach 0.22/1000 cells. In the other sites (El Jadida, Relais, and Jemâa Ouled Ghanem), we recorded a significant increase in TNA, mainly in the micronucleus, buds, and necrotic cell anomalies (Figure 8).

3.2.5 Evaluation of metallothionein content

The results of the metallothionein contents evaluation show reconciled and low values in all sites. The highest value was noted in Relais and Jorf Lyhoudi, with concentrations not exceeding 96 µg/g wet weight (figure 9). Moreover, statistical analysis confirmed no significant difference compared to the control for all sites.

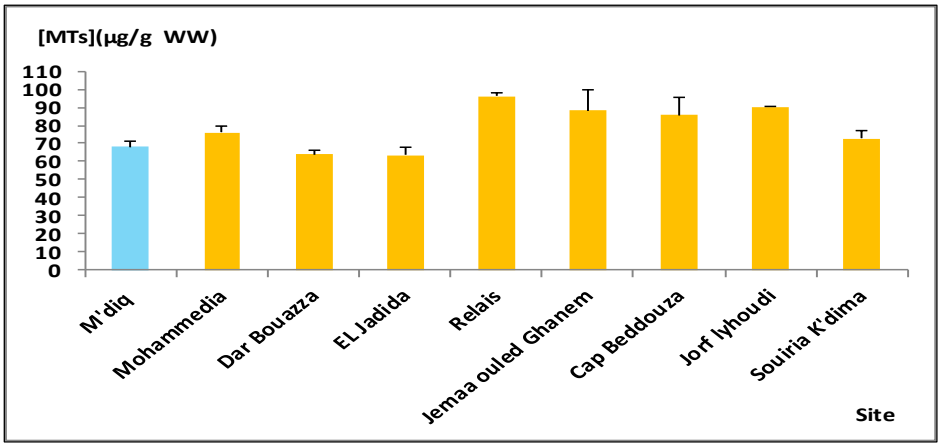

Figure 9 Concentrations of MT measured in the digestive gland of *Mytilus galloprovincialis* of different sites (The bars with * are significantly different, $p < 0.05$)

3.2.6 Evaluation of AChE activity

Figure 10 illustrates the variation in AChE activity at different sites on the Atlantic coast. The highest activity (16 nmol/min/mg protein) Was recorded in mussels from the reference site, Cap Beddouza, and Relais. The lowest value (10 nmol/min/mg protein) was noted in the mussels of Mohammedia. The other sites showed values between 12 and 14 nmol/min/mg protein. Statistical analysis showed no significant difference compared to the control for all sites.

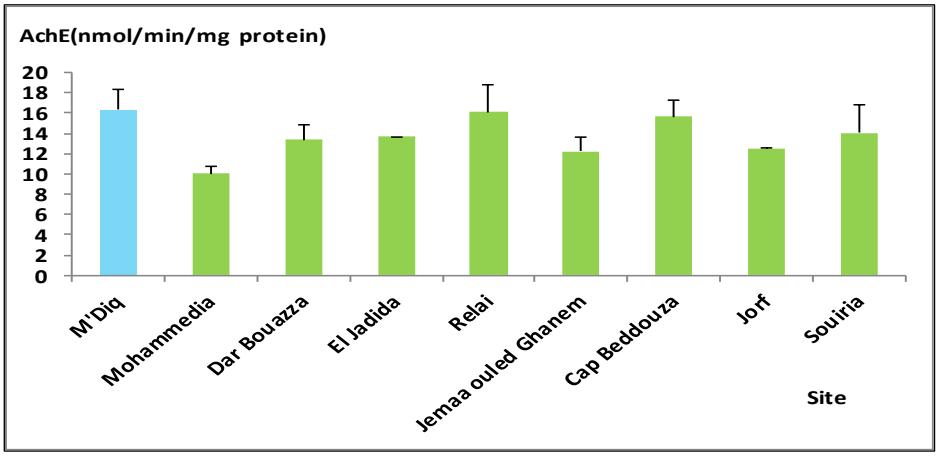

Figure 10 Concentrations of AChE activity measured in the digestive gland of *Mytilus galloprovincialis* of different stations (The bars with the * are significantly different, $p < 0.05$)

3.3. Data integration by MES

The results of integrating biomarker data are shown in Fig. 11. The MES data has shown that the organisms at the reference site, Cap Beddouza, and Dar Bouazza sites can be considered in good health (level A). Low stress is detected at the Souiria and El Jadida sites (level B); the other sites show moderate stress (level C).

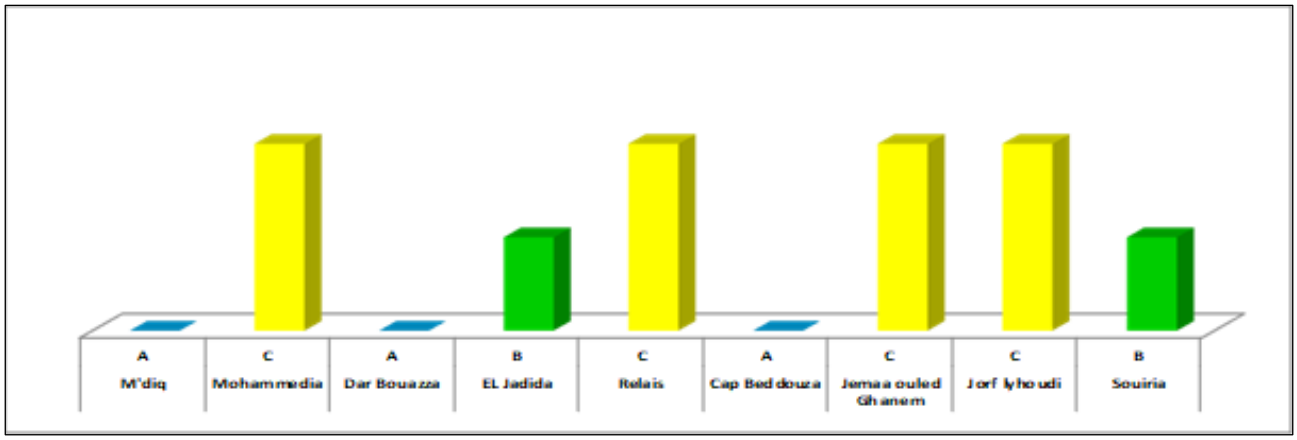

Figure 11 Variation of stress level syndrome using MES in studied mussel populations in Atlantic Moroccan areas

4. Discussion

The present study demonstrates that exposition to Cd (100 ppb and 500 ppb) induced a decrease in the degree of LMS proportionally to the concentration and the exposure time of Cd. According to the classification grid (El haimeur et al., 2017), a moderate stress level was registered at 100 ppb, and a stress level ranged from moderate to pathologic at 500 ppb. This verdict is consistent with the Ic values of exposed organisms. The same observation was evaluated in the mussel *Mytilus edulis* exposed to methylmercury (125 µg / l) (Pellerin-Massicote et al., 2004). Also, Brooks et al. (2011) demonstrated a decrease in LMS in mussel *Mytilus edulis* exposed to produced water containing trace metals among its constituents.

Exposure to genotoxic agents may cause damage beyond the individual level, being able to exert negative effects through several generations. Our experiments used a Cd as an aneugenic agent (Viarengo et al., 2007; Pruski and Dixon, 2002). The results show an increase in frequency anomalies as a function of time with 500 ppb with apoptosis at D15; this verdict confirms the dose-effect relationship of Cd and represents a time-integrated response to cumulative stress. According to Nigro et al. (2006), the frequency of micronuclei in translocated mussels doubled after 30 days of deployment at the Cecina estuary, where the metal concentrations were higher. The exposition of the mice for 28 days to the highest concentration of wastewater induced increasing in the level of micronuclei (Chaik et al., 2013).

Analysis of MT in mussels exposed to the Cd showed an induction of MT with a dose-effect relationship. The levels recorded were significantly different from those of the control batch, and significant differences were found between the two concentrations. Viarengo et al. (2007) and Viarengo and Nott (1993) confirmed the induction of MT in the mussel exposed to Cd and other heavy metals. A previous study on the Moroccan Atlantic coast showed that Metallothioneins concentrations were in line with heavy metal concentrations detected in mussel tissues (El haimeur et al., 2017).

The study was continued at the field level by assessment of the impact of all eventual contaminants present in the central Moroccan Atlantic area using a multi-biomarker approach. The mussels sampled at different sites were the subject of a study of organism behavior using SOS biomarker. The results showed that mussels from the reference site, Cap Beddouza, and Dar Bouazza have a high survival time. Mussels from Mohammedia, El Jadida, and JOG sites were affected by stress syndrome. This finding can be explained by a lack of energy in organisms previously subjected to environmental stress. Prior exposure to pollutants causes mussels to expend a large portion of their potential energy on detoxification, adaptation mechanisms, and maintaining homeostasis, which inhibits development and gonad production (Pampanin et al., 2005). Several studies have shown that exposure of mussels to pollutants increases their sensitivity to anoxia (Kamel et al., 2014; Thomas et al., 1999; Viarengo et al., 1995).

The assessment of stress level by LMS in mussels showed that, except for the reference site and Cap Beddouza site, the general stress level in all other sites ranges from moderate to high. The low rate of LMS detected in mussels from Jorf Lyhoudi could be linked to the contamination by Cr (El haimeur, 2019). Also, the relatively high degree of destabilization in the other sites could be explained, among other things, by certain metallic contamination measured in mussels (El haimeur, 2019).

The stress of Mohammedia mussels could be linked to the effects of several industrial wastes dumped near this area. This hypothesis is confirmed by previous studies evaluating the toxicity of some effluents spilled at the site (Bouhallaoui et al., 2011; 2017; El Haimeur et al., 2013; 2017). A study of the geographical distribution of metallic contamination (mercury, lead, and cadmium) on the Moroccan Atlantic coasts detected mercury contamination at Mohammedia (Benbrahim et al., 2006). Also, a measure of several biomarkers in mussels in the Mohammedia area confirmed the presence of pollution on the side (El Jourmi et al., 2014; 2015). According to studies by Benbrahim et al. (2006) and Merzouki et al. (2009), the amount of stress found in the El Jadida area may be related to cadmium poisoning, which was noted particularly in regions close to the discharges from Jorf Lasfar's phosphate processing industry.

For genotoxicity assessment, a negligible rate of nuclear aberrations was discovered in mussels from the reference site, Dar Bouazza, and Cap Beddouza sites. However, Relais and El Jadida two anthropogenically influenced sites had significantly higher TNA concentrations. Klobucar et al. (2003) documented similar outcomes in the haemocytes of zebra mussels after in situ exposure to freshwater conditions. A significant difference is demonstrated between control and mussel sampling in polluted areas (Bolognesi et al. (2012), Nigro et al. (2006), Klobucar et al. (2003), Izquiedro et al. (2003), Bolognesi et al. (2004), Magni et al. (2006)), and between control and exposed organisms to some contaminants (Giannapas et al. (2012), Brooks et al. (2012).

Despite this, when we compare the frequency of MN (stable nuclear anomaly) to the results of the literature, the genotoxicity impact on the Moroccan coast is lower, due to the low frequency of this anomaly, which didn't reach the baseline frequency identified by Bolognesi et al. 2012.

For MT dosage, the concentrations were low at different sites. Even if the Relais is the lowest contaminated site by Cd with a higher contamination index (El haimeur, 2019), the MT concentrations recorded in all sites were closer. This verdict will be explained by the fact that the level of contamination was insufficient for the induction of MT concentrations, which differentiates this area from the others (El haimeur et al., 2017). According to Giarattano et al. (2014), this situation means the presence of a low contamination gradient along the Moroccan Atlantic coast.

Concerning the AChE activity, similar to the MT variation, a non-significant difference was found between the reference site and other areas. Even at the Relais site, despite the higher metallic contamination that can exhibit AChE activity, the value recorded was closer to the reference one. The same verdict was observed by Dailianis et al. (2003).

Finally, we used the mussel expert system method to combine all biomarker data into an integrative index and draw a stress response profile. The MES showed a stress gradient and discrimination between the sites characterized by various pollution levels. The MES data showed that the organisms at the reference site, Cap Beddouza, and Dar Bouazza sites are in good health (level A). A low-stress level was detected at the Souiria and El Jadida sites (level B). The other sites indicated a moderate level of stress (level C), the effect at different stations was felt at the molecular and cellular levels.

5. Conclusion

The laboratory experiment showed how Cd might cause biological reactions in exposed mussels. We effectively recorded dose-effect relationship between the concentration of Cd and the lysosomal membrane stability, metallothioneins content, as well as a micronuclei frequency.

For the in-situ study, the MES integration revealed a stress gradient and discrimination across the sites with different pollution levels. However, the findings show that the Moroccan Atlantic region's maximum stress degree was mild. No stress was noted at the reference site, Dar Bouazza, and Cap Beddouzza sites. El Jadida and Souiria kdima's sites were classified to be in areas of low stress, whereas the other sites were in areas of moderate stress.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclose.

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