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Evaluation of metallic pollution of north eastern Algerian coasts using the demosponge *Chondrilla nucula* Schmidt, 1862

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ABSTRACT

In the last couple of years, a “Sponge Watch” Programme was launched in the Gulf of Annaba (North-eastern Algeria) with a goal of involving for the first time the sponges as bioindicators of heavy metals contamination in the biomonitoring programmes of the Algerian coasts. This study aimed to investigate the potential of a sponge’s species (*Chondrilla nucula* Schmidt, 1862) in the assessment of trace metal elements in the Gulf. In this context, the seasonal variation in the concentration of four trace metals (Cupper, Zinc, Lead and Cadmium) was monitored in *C. nucula* from two different areas during a one-year period in 2017-2018. Furthermore, a complementary approach based on the analysis of three oxidative stress biomarkers (glutathione, glutathione S-transferase, and malondialdehyde) was performed on the same samples to better characterize the biological response of the sponge to this kind of environmental stress. Similar environmental conditions in both sites of the Gulf were detected. The highest concentrations of the studied elements were recorded in the humid season (winter and spring), while the lowest were in the dry season (late summer to autumn). Otherwise, significant ($P \leq 0.05$) variations were recorded in the accumulation of all metals, with the exception of lead, which seemed regulated at a certain level. On the other hand, the sponge’s biochemical parameters showed significant temporal fluctuation, and seemed to follow those of metals. Therefore, the sponge *C. nucula* seems to be a suitable sentinel species to be considered in the biomonitoring programmes although it shows less tolerance to lead.

INTRODUCTION

The marine medium has long been used as a waste dumping ground of all types. The marine environment is exposed to all toxicant releases that may appear as sewage or spills, such as insecticides and biocides (**Cebrian, 2007**). The contamination by trace metals is one of the most serious problems growing all along the Mediterranean coastal areas (**Puig *et al*., 1999, Guendouzi *et al*., 2021**).

These highly toxic pollutants can accumulate in the environment for a very long time because of their low biodegradability, which makes them among the most dangerous pollutants (**Glorennec, 2006**).

Metals are usually present at low-lying concentrations in the seawater. Their detection represents one of the principal methodological constraints to assess that kind of pollution in the water column due to the extremely low concentrations and the potential interference of the salt matrix (**Cebrian, 2007; Sondergaard *et al.* 2015; Ranjbar Jafarabadi *et al.*, 2018**). On the other hand, sediment analysis is much easier to execute thanks to the accumulation of the metals in this compartment, which allows a better measuring of trace metals concentrations. However, there are some disadvantages as well, because the quantity of captivated metals depends on the sediment quality, and takes into account only the total and not the metal which is bioavailable **(Rainbow, 1995)**, offering a low representativeness of the overall contamination of the ecosystem. Thus, the biota is now used for marine environment biomonitoring programmes to obtain more informations about the ecosystem health status **(Amoozadeh *et al.*, 2013; Abi-Ghanem *et al.*, 2014; Caricato *et al.*, 2019; Pereira *et al.*, 2019 Cappello *et al.*, 2021)**.
Therefore, considerable efforts have been achieved in order to ameliorate integrative tools for the assessment of chemical impacts on the environment **(Batista *et al.*, 2013)**. This simultaneously includes the biotic and abiotic compartments **(Borja *et al.*, 2009)**. Consequently, organisms were suggested to be used as 'sentinels' of metals contamination through an extended and a very successful coastal areas monitoring programme namely 'US and Europe Mussel Watch'**(Goldberg, 1975; O'Connor, 1996)** since most environmental studies are based on the use of organisms mainly to evaluate different chemicals bioavailability in coastal ecosystems (**Maisano *et al.*, 2017; Cappello *et al.*, 2019**).
Most sessile and abundant filter-feeders invertebrates seem to be adequate monitoring tools to evaluate the biological impact of different contaminants such as trace metal's **(de Mestre *et al.*, 2012, Batista *et al.*, 2014, Cappello *et al.*, 2017)**. Among these, some sponges have proven their ability as good bioindicators of ecosystem **(Perez *et al.*, 2004)** and to be used in the biomonitoring of different kind of marine pollutants, such as hydrocarbons **(Gentric *et al.*, 2015)**, organ-chlorinated compounds **(Srikanth & RAO, 2017)**, metals **(Roveta *et al*,. 2020)** and even microplastics **(Celis-Hernández *et al.*, 2021)**.

In addition to bioindicators, the biomarkers response is the second main complementary approach that can be used for the assessment of the health status of an ecosystem **(Valavanidis *et al.*, 2006)**. In fact, some organisms are more sensitive to ecological changes that may affect their habitats, in particular, the presence of harmful substances of any kind **(Almamoori *et al.*, 2013)**. However, the data offered by this second approach could be affected by too many natural variables, making it difficult to determine what really stressed the organism's biological processes during the study period **(Gonzalez-Fernandez *et al.*, 2015)**. Therefore, some authors tested the use of sponges as sentinels of coastal environment since they are primitive organisms with more simple tissue differentiation, thus less physiological processes and less variables to be considered comparing to upper metazoa **(Abed, 2011; Khati *et al.*, 2018)**.

Sponges were used as biomonitoring tools for the first time for the evaluation of ecotoxicological risks in the Algerian coasts in the last couple of years **(Bensafia & Khati 2018; Khati *et al.*, 2018)**. The current study is complementary and aimed to continue the same investigation through a new group of demosponges (Chondrillidae). Hence, two groups of *C. nucula* were herein used to measure the seasonal variation of four trace metals concentrations (cadmium, copper, lead and zinc) in the Gulf of Annaba (north-eastern Algeria). Moreover, the analysis of three oxidative stress biomarkers; namely, glutathione (GSH), glutathione *S*-transferase (GST) malondialdehyde (MDA) was used aligned with this metals biomonitoring approach to better evaluate the impact of this kind of pollution on biota.

MATERIALS AND METHODS

Biological material

Chondrilla nucula (Schmidt, 1862) is a demosponge commonly found in the Mediterranean **(Ferantte *et al.*, 2018)**. It inhabits the hard substrata of shallow waters in the form of splashes **(Brümmer *et al.*, 2002)**. The two sexes are separated; sexual reproduction takes place in the late of summer **(Gaino, 1980)**. However, the species can reproduce asexually too **(Gaino & Pronzato, 1983)**. Thus, *C. nucula* shows an intense competition for space **(Alcolado, 1994)** by forming large discontinued flattened colonies with high growth rates **(Pronzato, 2002)**.

Chondrilla nucula has a great capacity to remove bacteria, in particular *Escherichia coli*. One square meter patch of this sponge can filter up to 14 L/h of seawater, retaining up to $7x10^{10}$ bacterial cells/h **(Milanese *et al.*, 2003)**.

Study area and measurement of environmental parameters

This study was performed in the Gulf of Annaba (Fig.1), one of the most important economical and touristic zones in Algeria. This wide bay is opened on the Mediterranean Sea to the north and bounded by two headlands: Cape Rosa

(8°15'E and 36°58'N) in the East and Cape de Garde (7°47'E and 36°58'N) in the West.

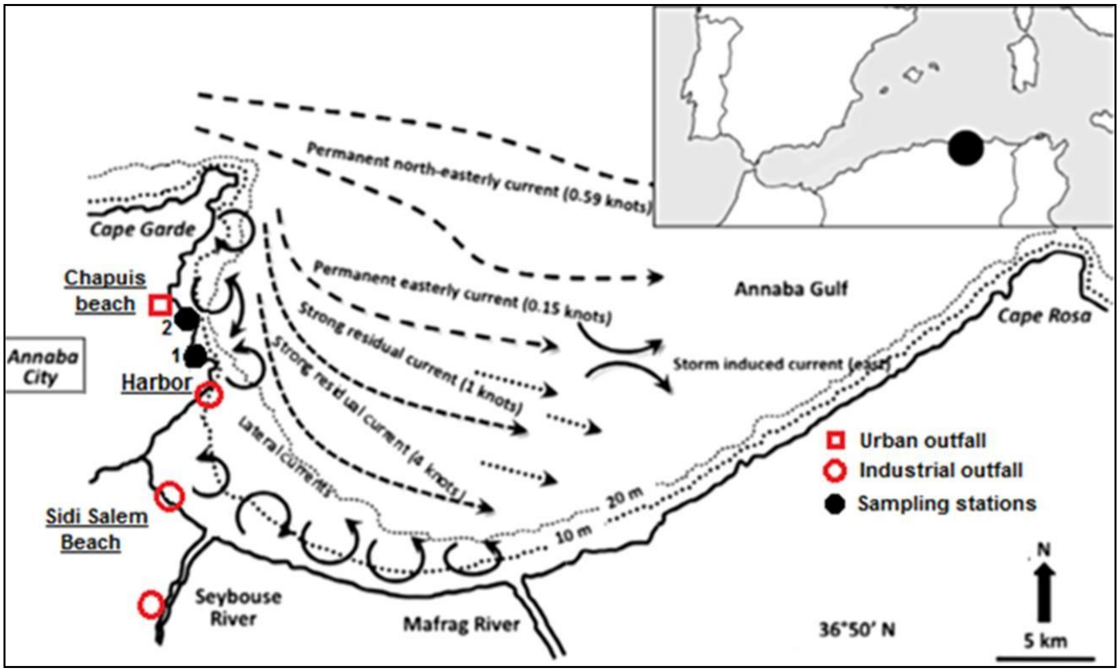

Fig.1. Study area and sampling of *Chondrilla nucula* (in **Belabed *et al.*, 2013**, amended).

After three months of free diving prospection along the coast, two monitoring sites were selected for this study, where the demosponge *C. nucula* was abundant and accessible. Lever de l'aurore station 1 (36°54'57.8''N - 7°46.14.5''E) is a public beach located about 2 km west of the port of Annaba and Fertilizer Company of Algeria (FERTIAL) at Sidi Salem. This beach is marked by the presence of a remarkable benthic biodiversity including sponges. La Caravelle station 2 (36°54'33.3'N - 7°46.26'.3''E) is a small rocky beach bordering the port of Annaba (36°54'07.3"N 7°46'22.9"E), which is marked as highly polluted by heavy metals coming mainly from fuels, antifouling paints, boats repair and refit materials (**Ouali *et al.*, 2018**). Therefore, the harbor basin shares permanently and directly its polluted water with this beach.

Measurements of water temperature (T°), potentiel hydrogen (pH), salinity (Sal) and dissolved oxygen (DO) were recorded in both stations three times per season all over the sampling year using a field multiparameter (HANNA- HI9829).

Sampling and storage

Six samples of *C. nucula* (5 - 8 cm² each) were collected per station and per season (January 2017*winter, April 2017*spring, August 2017*summer and October 2017*autumn) by free diving between 0-3 meters depth using a stainless steel small knife. After being transported rapidly to the laboratory in sea water bags, the sponges were cleaned up from sediment, separately placed in labeled eppendorfs tubes and conserved until processing. The samples for trace metals analysis were conserved at -20°C, while those for biomarker analysis were kept at - 40°C.

Trace metal elements (TME) analysis

One gram of each seasonal sample was freeze-dried at 80°C for at least 24 hours until constant weight was obtained. Then, every sample was mineralized with 4 mL of nitric acid (NHO_3) for at least 48 hours to achieve a maximum digestion. After completing digestion, every solution was filtered through a Whatman 41 filter paper then brought to a final volume of 18 mL with demineralized water **(Amiard *et al*., 1987)**. Afterwards, the concentrations of four metal elements (copper, cadmium, zinc and lead) were determined in all samples by flame atomic absorption spectrometry (AAS), using a Shimadzu AA6500 device connected to a microcomputer equipped with an integrated calculator.

Biomarkers analysis

One gram of each sample was homogenized in 20mL. Tris buffer (pH 7.6) contained 1 mL EDTA, 0.5 M sucrose, 0.15 M of KCl and 1 mL of DTT. The homogenate was centrifuged at 14000 rpm at 4°C for 30min. The obtained supernatant (S9) was recovered and aliquoted into eppendorfs and stored immediately at -40°C until the analysis of the oxidative stress parameters.

The GSH level was estimated as described by **Weckbecker and Cory (1988)**. The method is based on the absorbance of 2- nitro-5-mercapturic acid resulting from the reduction of 5-50- dithio-bis-2-nitrobenzoic acid (DTNB) by the thiol group (SH) of glutathione. The absorbance was measured at 412 nm. GSH concentration was expressed as nanomoles per milligram of protein. The GST was analyzed using the method of **Habig *et al.*, (1974)**, where the molecule results of the conjugation reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with the reduced glutathione is able to absorb light at a wavelength of 340 nm. The obtained results were expressed in µmol/min/mg of proteins.

Finally, MDA was analyzed using the methods as described by **Esterbauer *et al.* (1984)** and **Uchiyama and Mihara (1978)**. The chromogen (pink pigment) results of the condensation of MDA in an acidic and hot medium with thiobarbituric acid was measured at 530 nm by absorption spectrophotometry. The obtained values of MDA content were expressed in µmol/mg of protein.

The content of proteins was determined at 595 nm according to the Bradford colorimetric method **(Bradford, 1976)** using bovine serum albumin (BSA) as the standard.

Statistical analyses

The statistical analyses were performed using R program, version 3.6.2. The data were presented as averages plus or minus standard error (m ± se). After checking the data distribution symmetry, it was opted for non-parametric tests for the physicochemical parameters and parametric tests for the chemical (trace metals) and biochemical parameters (biomarkers). The effect of seasons and stations on the concentration of trace metals and biomarkers in sponges' tissues were statistically verified using one-way ANOVA and Tukey-post test. While, the effect of the interaction between stations and seasons was verified through two-way ANOVA and Tukey-post test. Furthermore, the relationship between the trace metals, environmental stress biomarkers and water physicochemical parameters were checked at once by a Pearson correlation coefficient analysis. The significance level for all tests was set at $P < 0.05$. Furthermore, the spatiotemporal variations results were illustrated by a principal component analysis (PCA) graph.

RESULTS

Environmental characterization

The seasonal variations of the environmental parameters in the two study stations are summarized in Table (1). The ANOVA Kruskal-Wallis test was applied to check the station's and the season's effect on the variation of environmental parameters. Overall, all mean values of environmental parameters (temperature, pH, salinity and dissolved oxygen) were similar in the two study sites. However, a significant seasonal fluctuation of all parameters during the sampling period was recorded. Water temperature, pH and salinity mean values ranged from 14.20°C, 7.18 and 35.67 PSU recorded in winter to 27.24°C, 8.51 and 37.61 PSU, measured in summer, respectively, while the dissolved oxygen ranged from 5.73 $mg.L^{-1}$ in autumn to 7.12 $mg.L^{-1}$ in spring.

TME in sponges' tissues

The seasonal variations of trace metals concentrations (Cu, Zn, Pb and Cd) in *C. nucula* in both stations are summarized in Table (2).

Table 1. Seasonal and spatial variations of the physicochemical parameters at the two sampling stations of Annaba Gulf (Mean values ± SE, n= 3)

Parameters	**Station 1**				**Station 2**			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
T°	14.31 ± 0.00	22.31 ± 0.00	27.15 ± 0.00	15.82 ± 0.00	14.20 ± 0.00	22.22 ± 0.00	27.24 ± 0.00	15.71 ± 0.00
pH	7.32 ± 0.00	8.13 ± 0.00	8.40 ± 0.00	7.56 ± 0.00	7.18 ± 0.00	8.20 ± 0.00	8.51 ± 0.00	7.49 ± 0.00
Sal	35.67 ± 0.00	37.08 ± 0.00	37.58 ± 0.00	36.56 ± 0.00	35.78 ± 0.00	37.12 ± 0.00	37.61 ± 0.00	36.21 ± 0.00
DO	6.61 ± 0.00	7.06 ± 0.00	6.71 ± 0.00	5.73 ± 0.00	6.55 ± 0.00	7.129 ± 0.00	6.52 ± 0.00	5.66 ± 0.00

Table 2. Seasonal and spatial variations in the concentration of trace metals (µg g^{-1}) and biomarkers (µmol mg^{-1}) in *Chondrilla nucula* tissues at the two sampling stations of Annaba Gulf (Mean values ± SE. n= 6)

Element	**Station 1**				**Station 2**			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
Cu	16.02 ± 0.653	15.42 ± 1.09	13.87 ± 0.443	13.30 ± 0.589	15.48 ± 0.413	13.09 ± 0.483	11.826 ± 0.353	12.75 ± 0.847
Zn	112.21 ± 2.66	126.93 ± 2.73	96.09 ± 1.21	78.30 ± 2.57	111.60 ± 3.59	115.56 ± 2.78	104.77 ± 3.41	77.42 ± 6.94
Pb	41.98 ± 2.89	55.3 ± 11.4	33.89 ± 3.96	43.83 ± 2.88	71.5 ± 27.1	34.62 ± 1.24	33.89 ± 3.96	46.29 ± 4.44 ±
Cd	7.10 ± 1.44	3.69 ± 0.645	2.21 ± 0.617	0.24 ± 0.152	23.3 ± 13.3	1.39 ± 0.373	1.10 ± 0.381	0.40 ± 0.129
GST	568 ± 134	578.1 ± 54.3	716.8 ± 93.5	736 ± 121	635 ± 105	721.3 ± 70.0	612.0 ± 33.7	643.2 ± 6.4
GSH	5.486 ± 0.326	5.838 ± 0.128	9.747 ± 0.373	5.451 ± 0.268	6.463 ± 0.216	8.225 ± 0.130	7.83 ±1.53	8.008 ± 0.387
MDA	9.51 ± 1.21	4.083 ± 0.52	22.19 ± 1.56	6.116 ± 0.953	20.80 ± 1.23	4.46 ± 1.25	23.77 ± 2.54	8,356 ± 0.939

For the Cu, the highest concentrations were recorded in winter for both station 1 and 2, with values of 16.02± 0.65 µg g^{-1} and 15.48 ± 0.41 µg g^{-1} dry weight (DW), respectively. The lowest value was recorded in autumn with 13.30 ± 0.58 µg g^{-1} DW, while in summer: 11.82 ± 0.35 µg g^{-1} DW for station 1 and 2 respectively.

The maximum levels of Zn concentrations were observed in spring for the two stations 1 and 2 (126.93 ± 2.73 µg g^{-1} DW and 115.56 ± 2.78 µg g^{-1} DW respectively) and the minimum concentrations in autumn (78.30 ± 2.57 µg g^{-1} DW and 77.42 ± 6.94 µg g^{-1} DW respectively). Pb concentrations reached their maximum level in spring (55.3 ± 11.4µg g^{-1} DW) and winter (71.5 ± 2.1 µg g^{-1} DW) for station 1 and 2, respectively, while their minimal levels (33.89 ±

3.96 µg g^{-1} DW and 33.89 ± 3.96 µg g^{-1} DW, respectively) were observed during summer for both stations.

In the case of Cd, the highest values were detected in winter (7.10 ± 1.44 µg g^{-1} DW and 23.3± 3.3 µg g^{-1} DW, respectively), and the lowest in autumn (0.24 ± 0.15 µg g^{-1} DW and 0.40 ±0.12 µg g^{-1} DW) for both stations 1 and 2, respectively.

According to the ANOVA results (Table 3), there was no significant station's effect on the variation of the studied elements, with the exception of Cu which showed a high significant variation between the two study sites ($P \leq 0.01$).

Table 3. The results of ANOVA tests on the effects of station and season on the variation of physicochemical parameters and the concentration of trace metals and biomarkers in *Chondrilla nucula* soft body tissues

Variable	Factors					
	Stations (DF = 1)			**Seasons (DF = 3)**		
	***F*- value**	***P*- value**	**Observation**	***F*- value**	***P*- value**	**Observation**
T°	0	1	ns	6.358	0.000	***
pH	0	1	ns	2.845	0.000	***
Sal	0	1	ns	9.062	0.000	***
O2	0	1	ns	2.425	0.000	***
Cu	5.891	0.0192	**	7.159	0.000	***
Zn	0.037	0.848	ns	49.87	0.000	***
Pb	0.128	0.722	ns	1.443	0.243	ns
Cd	0.742	0.394	ns	4.063	0.0124	**
GST	0.002	0.963	ns	0.349	0.001	***
GSH	3.136	0.0832	ns	5.465	0.00279	**
MDA	2.555	0.117	ns	42.96	0.000	***

NB: * ($P \leq 0.05$). ** ($P \leq 0.01$). * ($P \leq 0.001$). ns ($P > 0.05$)**

Whereas, the season's effect was very significant ($P \leq 0.01$) to very highly significant ($P \leq 0.001$) on the variation of all elements, with the exception of Pb which did not exhibit any variations for neither stations' effect nor seasons'.

On the other hand, the two-way ANOVA results (Table 4) demonstrate that the interaction between seasons and stations had significant effects on Cu ($P \leq 0.001$), Zn ($P \leq 0.001$) and Cd ($P \leq 0.05$) but no effect was detected on Pb.

Table 4. Results of two-way ANOVA testing the effect of the interaction between station and season on the variation of the concentration of trace metals and biomarkers in *C.nucula* soft body tissues

Variable	Factor		
	Interaction (DF = 3)		
	***F*- value**	***P*- value**	**Observation**
Cu	5.331	0.0002302	***
Zn	24.457	1.423e-12	***
Pb	1.4406	0.2165	ns
Cd	2.6741	0.02278	*
GST	0.537	0.8012	ns
GSH	6.7319	2.735e-05	***
MDA	36.318	2.181e-15	***

NB : * ($P \leq 0.05$); ** ($P \leq 0.01$); * ($P \leq 0.001$); ns ($P > 0.05$).**

The ANOVA results are in line with those of the Tukey-post test, which illustrates the different groups precisely (Figs. 2, 3).
According to these results, winter is the season in which trace metals achieve their maximum level of concentration in sponges' body, with the exception of Zn where the maximum level was not reached until spring.

Biomarkers response

The seasonal variations of biomarkers concentrations (GST, GSH and MDA) in *C. nucula* in both stations are summarized in **Table 2**.
For the GST, the highest concentrations were recorded in summer for station 1 and in spring for station 2, with 736 ± 121 and 721.3 ± 70.0 µmol mg^{-1} protein, respectively. The lowest concentrations were recorded in spring for both stations with 568 ± 134 and 612.0 ± 33.7 µmol mg^{-1} protein, respectively.

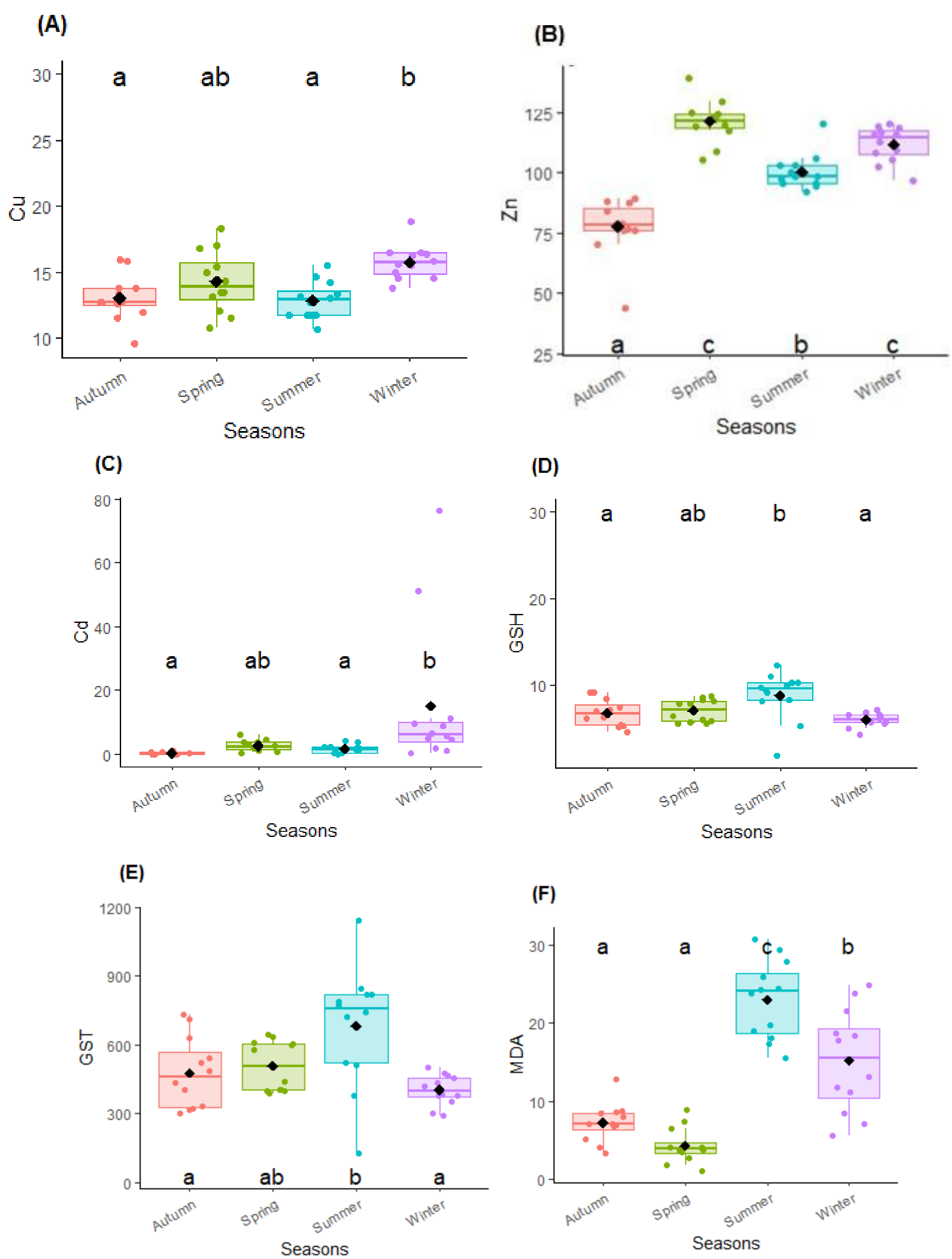

Fig. 2. Temporal variations in metals and biomarker response in the tissues of *Chondrilla nucula*

Cu (A), Zn (B), Cd (C), GSH (D), GST (E) and MDA (F). a, b and c indicates that interseason variation is significant at $P < 0.05$, using the Tukey's test. Boxplots labeled with the same letter are not significantly different at $P > 0.05$ (Mean values ± SE, n = 6).

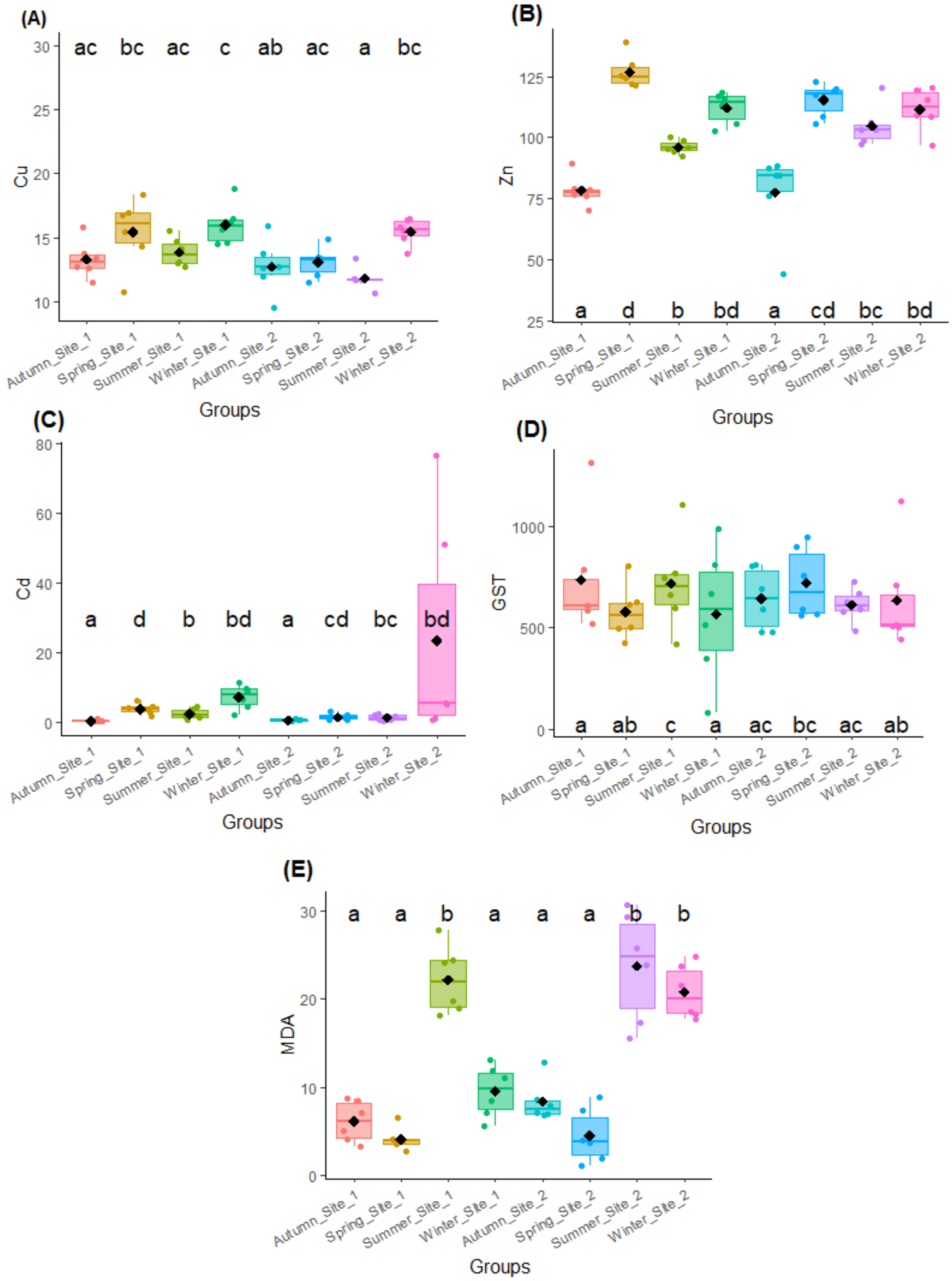

Fig. 3. Spatio-temporal variations in trace metals and biomarker response in the tissues of *Chondrilla nucula*

Cu (A), Zn (B), Cd (C), GST (D) and MDA (E). a, b and c indicates that intergroup variation is significant at $P < 0.05$, using the Tukey's test. Boxplots labeled with the same letter are not significantly different at $p > 0.05$ (Mean values ± SE, n = 6).

The maximum levels of GSH concentration were observed in summer (9.747 ± 0.373 µmol mg^{-1} protein) for station 1 and spring (8.225 ± 0.130 µmol mg^{-1} protein) for station 2. The minimum values were observed in autumn (5.451 ± 0.268 µmol mg^{-1} protein) for station 1 and in winter (6.463 ± 0.216 µmol mg^{-1} protein) for station 2.

MDA concentrations reached their maximum levels (22.19 ± 1.56 and 23.77 ± 2.54 µmol mg^{-1} protein) in summer and their minimum (4.083 ± 0.520 and 4.46 ± 1.25 µmol mg^{-1} protein) in spring for both stations 1 and 2, respectively.

According to the ANOVA results (Table 3), the stations had no effect on the variation of the biomarkers concentration. However, they varied significantly from one season to another (GSH $P \leq 0.01$; GST and MDA $P \leq 0.001$).

The two-way ANOVA results (Table 4) demonstrated that the interaction between seasons and stations had significant effects on GSH and MDA ($P \leq 0.001$) but not on GST.

The ANOVA results are in line with those of the Tukey-post test, which illustrates the different groups precisely (Figs. 2, 3)

Pearson correlation

Fig. (4) presents the Pearson's correlation coefficients between all the variables: the metal concentrations (Cu, Zn, Pb and Cd) in *C. nucula* soft tissues, the water characteristics (T°, pH, Sal and Oxy) and the stress biomarkers (GSH, GST and MDA).

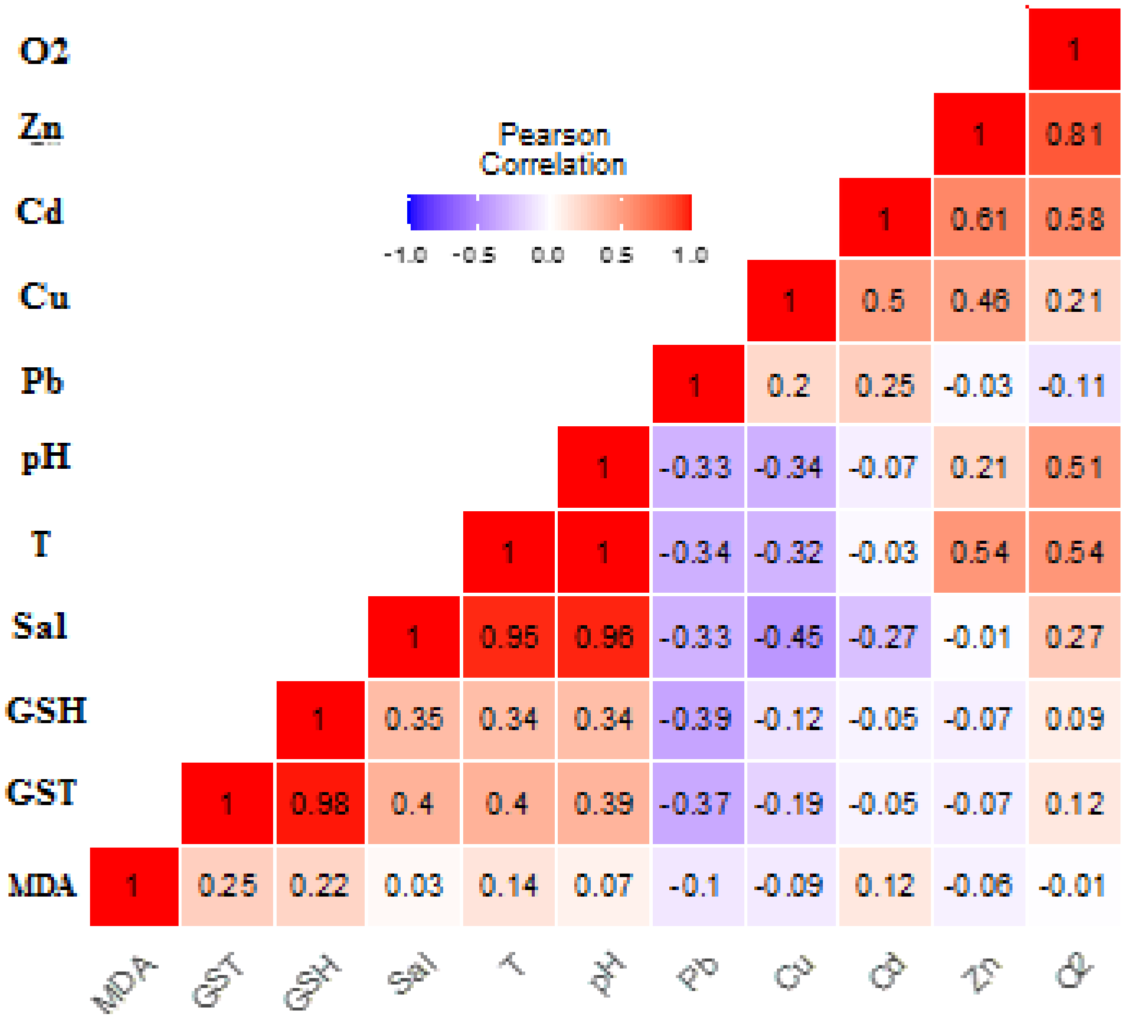

Fig. 4. Pearson's correlation coefficient analysis for the physicochemical parameters with the trace metals and oxidative stress biomarker concentrations in *Chondrilla nucula*

The present results revealed some noticeable links between the concentration of metals and the physicochemical parameters on one hand. On the other hand, findings showed positive correlation of Zn with T° and d. oxy (r = 0.54 and r = 0.58 relatively), negative correlation of Cu with the T°, salinity and pH (r = -0.32, r = -0.45 and -0.34, respectively) with the stress biomarker concentrations, and negative correlation of Pb with the GSH and GST (r = -0.39; r = 0.37 respectively). The significance levels of all correlations are demonstrated in Table (5).

Table 5. The significance levels of Pearson's correlation coefficient analysis for the physicochemical parameters with the trace metals and oxidative stress biomarker concentrations in *Chondrilla nucula*

	Cu	**Zn**	**Pb**	**Cd**	**T**	**pH**	**Sal**	**O2**	**GST**	**GSH**
Cu		0.0108	0.3898	0.0002	0.0018	0.0018	0.0018	0.8702	0.0451	0.0956
Zn	0.0108		0.6090	0.0000	0.8064	0.8064	0.8064	0.0000	0.5499	0.4813
Pb	0.3898	0.6090		0.2518	0.0091	0.0091	0.0091	0.0553	0.0007	0.0003
Cd	0.0002	0.0000	0.2518		0.1591	0.1591	0.1591	0.0177	0.1819	0.1788
T	0.0018	0.8064	0.0091	0.1591		0.0000	0.0000	0.0000	0.0001	0.0004
pH	0.0018	0.8064	0.0091	0.1591	0.0000		0.0000	0.0000	0.0001	0.0004
Sal	0.0018	0.8064	0.0091	0.1591	0.0000	0.0000		0.0000	0.0001	0.0004
O2	0.8702	0.0000	0.0553	0.0177	0.0000	0.0000	0.0000		0.0748	0.1295
GST	0.0451	0.5499	0.0007	0.1819	0.0001	0.0001	0.0001	0.0748		0.0000
GSH	0.0956	0.4813	0.0003	0.1788	0.0004	0.0004	0.0004	0.1295	0.0000	
MDA	0.3654	0.2948	0.2961	0.5001	0.2457	0.2457	0.2457	0.4072	0.0139	0.0083

NB: * ($P \leq 0.05$); ** ($P \leq 0.01$); * ($P \leq 0.001$); ns ($P > 0.05$)**

Principal components analysis (PCA)

The spatiotemporal overlap of all the studied parameters; namely, sea water characteristics (T°, pH, salinity and dissolved oxygen), the accumulated TME in sponges tissues (Cu, Zn, Pb and Cd) and the oxidative stress biomarkers (GST, GSH and MDA) was explored through a principal components analysis (PCA) as depicted in Fig. (5). The first two factorial axes present 71.9 % of the total components of the information.

The PCA results (Fig. 5) clearly illustrate the absence of sites' effect on the contamination by the trace metals, and thus indicates similar environmental conditions at the two stations under investigation. However, Fig (5) highlights a clear effect of seasons on the different variables.

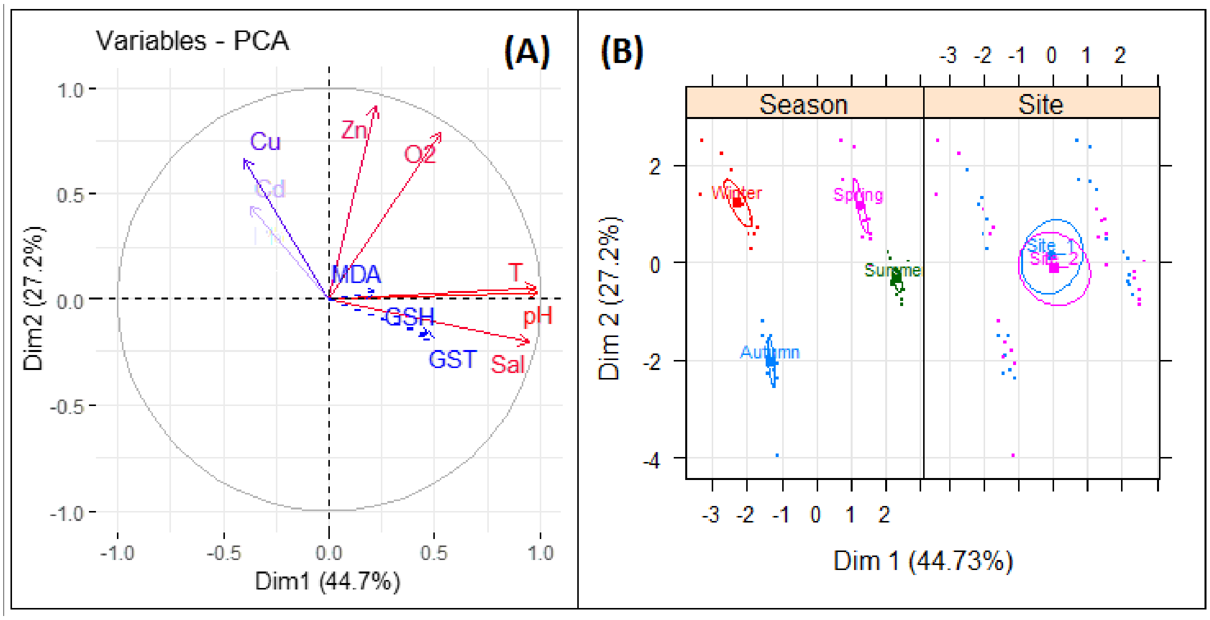

Fig. 5. Principal component A=analysis based on the spatiotemporal variation.

Factorial plane: D1: 44.73%, D2: 27.2. (A): Correlation circle of variables assayed with the first two principal axes. (B): Projection of seasons and sites on the first two principal axes.

Axis 1 obviously explains a difference between the group consisting of spring and summer and that of winter and autumn which is characterized by high rates of Cu, Cd and Pb, and lower rates of T°, pH, salinity, dissolved oxygen, Zn, GSH, GST and MDA. Axis 2 explains a difference between the winter-spring group and the summer and autumn group, which is characterized by high levels of Sal, GST and GSH.

To summarize, the axis 1 explains mostly the relationship between the metals bioaccumulation by the sponges and the variation in water characteristics, while axis 2 explains more the metals concentration compared to that of the detoxification components (biomarkers).

DISCUSSION

Among the different approaches for assessing and monitoring the degree of pollution in coastal and estuarine ecosystems, those that use marine sponges as bioindicators remain promising **(Abed, 2011)**. However, these organisms are still slightly used as sentinels for the evaluation of the health state of the marine environment. The present work carried out in the Gulf of Annaba (north-eastern Algeria) evaluated the potentialities of *C. nucula*, a demosponge widely found in the rocky community of Mediterranean shallow waters as a bioindicator or even bioremediator of the metallic contamination in coastal areas **(Ferrante *et al.*, 2018**).

Water characteristics did not show any significant differences between the two selected study sites. Therefore, the two sponge populations under insvestigation were probably living under the same abiotic environmental conditions throughout the study cycle. However, these conditions showed considerable fluctuations throughout the year, presenting the same typical seasonal cycle of the Gulf of Annaba as reported in previous studies **(Amri *et al.*, 2017; Bouzahouan *et al.*, 2018)**.

Among the four physicochemical parameters monitored in this study, water temperature is the most sensitive because it can be affected by too many variables such as air temperature, water depth, precipitations, soil natureand even anthropogenic impacts on the environment like industrial wastewater as the case in the Gulf of Annaba which ceaselessly receives cooling discharge waters from the Fertilizer Company of Algeria "FERTIAL" into Sidi Salem Beach (36°52'02.0''N and 07°46'26.6''E). The pH, salinity and dissolved oxygen mean values are usually linked to those of the temperature. This can be observed in results herein presented as well as in some other studies conducted on the gulf of Annaba **(Belabed *et al.*, 2013b; Boucetta *et al.*, 2016a, b; Bensafia & Khati, 2018; Bouzahouan *et al.*, 2018; Ouali *et al.*, 2018; Laouati *et al*., 2021)**.

Marine sponges are sessile filter-feeders with a well-known capability to filter thousands of liters of water per day, with volumes equivalent to up once or twice the equivalent of their own body volume in 3.7 – 20 seconds to satisfy their physiological and biological requirements **(Weisz *et al.*, 2008 as cited in Morganti *et al.*, 2019)**. Therefore, they can absorb and accumulate different contaminants in their tissues such as heavy metals **(Perez *et al.*, 2004)**. The sponges capacity to bioacumulate these contaminants in their tissues differs from one species to another, depending on three main factors ; (i) the metal element (some metals are intolerable for some species) , (ii) its bioavailability in the water, and (iii) the relative volume of the sponge's aquafarious system which affects mainly the filtration and clearance rates **(De Mestre *et al.*, 2012)**.

Compared to data documented by **Belabed *et al.* (2017)** and **Bensafia and Khati (2018)**, the results herein reported were similar, with the highest concentrations of heavy metals in sponges bodies detected in winter (Cu and Cd) and early spring (Zn and Pb) and the lowest in summer (Cu and Pb) and early autumn (Zn and Cd). The monitoring of TME concentrations in the tissue of *C. nucula* demonstrated identical seasonal fluctuation in both study sites with the following decreasing sequence of Zn > Pb > Cu > Cd. This similarity in results may be attributed to the relative distance between the two stations, or their position in the middle of the Gulf, which makes them relatively exposed to the same anthropogenic activities or even due to the stable bioaccumulation strategy of the species under any conditions.

The enrichment by Cu, Zn and Cd in the tissue of sponges sampled during winter could be a result of the inputs of urban and industrial sewage of Annaba city, which discharges much more in this season (when there is no swimming) at three principal outfalls: the Chpuis Beach's outfall (36°55'30.4"N 7°45'46.5"E), Annaba harbour's and Sidi Salem Beach's. In fact, the two study stations are located near the center of the gulf buckling, and thus surrounded by these three emissaries of polluted waters spilled straight in the bay without any preliminary treatment. Moreover, both study sites are in line with the main passage corridor for all small motorized boats, particularly those of leisure (e.g. jets ski) and artisanal fishing, which prefer the tight coastal navigation. Thus, their fuel might be the origin of seawater enrichment with TME **(Bensafia & Khati, 2018)**, especially considering that Annaba city witnesses an important increase in the number of these small boats in the last six years.

Cu is strongly present in the sediment of the Gulf of Annaba **(Belabed *et al.*, 2008, 2013b; Laouati *et al*., 2021)** in their study. The enrichment of the biotope by this element is probably linked to telluric and soil geology of this area, in which the runoffs and erosion are the major reasons for the transfer of pesticides based on Cu to coastal waters as suggested by the same authors. In detail, **Bouzahouan *et al.* (2018)** who monitored the annual variation of TME in the surface waters of the same area, suggested soil dust, plants decomposition, forest fires and the discharges of FERTIAL industrial zone to be the main sources of this enrichment by Cu.

The highest concentration levels of Zn were observed in spring, which correspond usually to a period of release of TME in water after the process of degradation of the organic matters that starts with the increase of temperature as proposed in the study of **Belabed *et al*. (2017)**. This could probably be the case of our study due to the highly significant correlation between Zn concentration in the sponges tissues and the levels of dissolved oxygen ($P \leq 0{,}001$; r = 0,81) and temperature ($P \leq 0.001$; r = 0.54). In addition, this bioaccumulation of Zn may be related to the energy reserves storage, which happens usually a few months before the reproduction cycle as suggested by **Bouzahouan *et al.* (2018)**. **Bensafia and Khati (2018)** declared the observation of the gametogenesis activity in the sponge *Sarcotragus spinosulus* just after the maximum of Zn bioaccumulation in the sponge's tissues. Remarkably, the sexual reproduction of *C. nucula* is also known to be in the summer **(Sidri *et al.*, 2005)**.

The Cd mean concentration values in sponges tissues showed greater difference between winter and the other seasons, which could be mainly related to its concentration in the water. Actually, this element is almost present in all kind of chemical products used for industrial, urban and even agricultural activities. As a result, sediment of coastal waters of civil zones are always exposed to the pollution by this heavy metal as it is the case for the Gulf of Annaba, in which the presence of considerable amounts of Cd in the sediment was reported by **Belabed *et al*. (2013)**. This metal might be kept in the sediment, but only until the wet season. The strong winter storms act as remobilization and resuspension major forces for the sediment, significantly increasing the bioavailability of metals in the water including Cd **(Boutiba-Trea *et al.*, 2017)**.

On the other hand, the demosponge *C. nucula* seems able to regulate the levels of Pb and not able to accumulate it in its tissues because the results showed no significant differences for the annual variations of this metal. The same results were observed by

Ferantte *et al.* (2018) who tested the capacity of this species to accumulate three TMEs (Cu, Cd, and Pb) through an *in vivo* experience, in which the sponge *C. nucula* was exposed to concentrations progressively higher than the afore-mentioned metals. At the third level of that experience, the sponges were still able to absorb and accumulate more Cu and Cd (Constant bioaccumulation factor) from the artificial environment water, but no more Pb. Therefore, *C. nucula* is probably less tolerant to Pb comparing to other metals. In the frame of the same investigation, the results of **Cebrian *et al.* (2007)** on some sponges sampled from north-western Mediterranean were comparable to ours. In detail, three species (*Chondrosia reniformis, Phorbastenacior tenacior* and *Dysidea avara*) out of four investigated were more efficient in accumulating Cu and were less tolerant to Pb. Whereas, the demosponge *Sarcotragus spinosulus* collected from the Gulf of Annaba seems able to accumulate this metal **(Bensafia & Khati, 2018)**.

Chemical pollution resulting from different human activities has a major impact on coastal ecosystems **(Giarratano *et al.*, 2010)**. The presence of harmful substances in an environment may cause some interactive effects on the biota **(Khati *et al.*, 2018)**. The biomarkers can be defined as any measurable changes of organisms biochemical, biological or even behavioral parameters **(Kaiser, 2001)** in an ecosystem from cellular to populations level. The monitoring of some of these parameters, especially the organisms' enzymatic responses to xenobiotics may provide early signals of any environmental disturbance **(Viarengo *et al.*, 2007)**. Recently, this approach has become highly recommended as complementary study to chemical analyses of environmental diagnosis **(ICES, 2008)**.

The ANOVA results (Table 3) did not show any significant differences in all the sponges' biomarkers from the two populations, which is in accordance with the previous results that revealed identical environmental conditions in both the study sites including metals contamination. However, the results demonstrated that the sponges' biological response to environmental stress varied significantly among seasons.

GSH is a tripeptide (g-L-glutamyl-L-cysteinylglycine) responsible of the protection of intracellular environment from ROSs generated by xenobiotics **(Beldi, 2006)**. It has the ability to neutralize radical species and deactivate their abilities to harm the cell different components **(Jozefczak *et al.*, 2012)**. Therefore, the antioxidant activity of the GSH content is used to estimate whether or not the organism is under environmental stress as suggested by **Gorbi *et al.* (2008)**. Our results on the glutathione rates indicated high significant variation ($P \leq 0.01$) over seasons, and thus causing changes of the environmental conditions where the two sponges' populations live along the year. The GSH annual variation trends in *C. nucula* seems to follow those of metals. Similar results reporting close interconnection between GSH activity and exposure to some metals contamination (mainly Cu, Pb and Cd) were observed in other marine invertebrates mostly filter-feeders: *Crassostrea virginica* **(Ringwood *et al.*, 2004)**, *Ruditapes decussates* **(Khebbeb *et al.*, 2010)**, *Perna perna* digestive gland **(Khati *et al.*,**

2012), *Donax trunchulus* **(Sifi *et al.,* 2013)** and the sponge *Sarcotragus spinosulus* **(Khati *et al.,* 2018)**.

The GST is a phase II enzyme in charge of the detoxification process of xenobiotics in the organisms by contributing in making them more water-soluble, and thus much easier to be eliminated out of the system **(Amiard- Triquet *et al.,* 2009; Schmidt *et al.,* 2012)**. The defence activity of this enzyme makes it one of the most important biomarkers of the oxidative stress defence system **(Lamn, 2009)**, and it is able to provide insights on the health status of an organism **(Mebarki *et al.,* 2015)**, which is particularly effected by the environmental conditions. In the case of our study, the significant seasonal variation induced marked changes in the response of the GST in the two populations. The considerable fluctuations in the environmental factors and the significant variation in metals bioconcentration recorded throughout the year could be the reasons for the variation in the sponges' biological response. Some authors have already suggested that the GST activity in an animal is linked to some biotic factors (age and reproductive cycle) and abiotic factors (T°, pH, sal., DO) **(Chouahda, 2006; Giarratano *et al.,* 2011)**, while others have documented a strict correlation between GST activity and pollutants level in the organism (**Saenz *et al.,* 2010; Barhoumi *et al.,* 2014; Bensouda & Soltani-Mazouni, 2014 ; Bouzenda *et al.,* 2017; Mejdoub *et al.,* 2017; Bouzahouan *et al.,* 2018)**. Accordingly, our results revealed a significant increasing concentration of GST activity with the level of metals, and high positive correlation coefficient with the GSH ($P \leq 0.001$; r = 0.98). **Khati *et al.* (2018)** also reported a highly significant variation of GST activity over seasons in the sponge *S. spinosulus* collected from a polluted site selected in the Gulf of Annaba, which is so close to our stations. These authors related the activity of the GST in the sponge to the detoxification process against the accumulated pollutants. The Gulf of Annaba was already signaled many times as receptacle of different chemical pollutants introduced mostly via rivers and valleys (wadis) **(Bougherira *et al.,* 2015; Keblouti *et al.,* 2015; Ziouch *et al.,* 2020)**. It was detected particularly for the presence of heavy metals pollution **(Beldi *et al.,* 2006; Belabed *et al.,* 2008 ; Abdennour *et al.,* 2010; Belabed *et al.,* 2010, 2013b ; Bouzahouan *et al.,* 2018; Ouali *et al.,* 2018)**. Similar results highlighting the strong links between stimulation of GST activity and exposure to a source of heavy metal contamination have been reported in other marine organisms such as Senegalese sole *Solea senegalensis* **(Funes *et al.,* 2006)**, *Donax trunculus* **(Amira *et al.,* 2011; Soltani *et al.,* 2012)** and the bivalves *Mytilus galloprovincialis* **(Cappello *et al.,* 2013)**.

The formation of the MDA is an early signal of the assaultment of radical species on cell membrane. Furthermore, the concentration of the MDA content in the tissues can be used to estimate the degree of lipid peroxidation and thus the level of oxidative stress in an organism **(Lykkesfeldt, 2007)**. Our results revealed the same highly significant seasonal effect on this parameter in both stations. This variation in MDA content in the sponge's tissues over seasons can be related to the contamination level with TMEs, and therefore the imbalance of antioxidant system. The ROSs generation becomes more important in aquatic organisms when exposed to TME **(Giarratano *et al.*, 2013)**. This happens because these pollutants can exhaust the antioxidant defence, which allows more lipids peroxidation, and hence more cells degradation **(Soltani *et al.*, 2012)**. Several studies reported similar results about the increase in MDA content in the molluscs tissues, exposed to a contamination of heavy metals in their habitat, viz. *Cerastoderma glaucum* **(Machreki-Ajmi *et al.*, 2008)**, *C. edule* (**Bergayou *et al.*, 2009)**, *D. trunculus* **(Tlili *et al.*, 2010)**, *P. perna* **(Khati *et al.*, 2012)**, *M. galloprovincialis* **(Abbassi *et al.*, 2015)** and *S. haemastoma* **(Bouzahouan *et al.*, 2018)**.

CONCLUSION

According to many authors, marine sponges are excellent sentinel organisms widely used in the biomonitoring and bioremediation programmes of coastal and estuarine environments. Many species have already proved their abilities in accumulating different kind of contaminants, particularly the heavy metals. The efficiency of *C. nucula* in accumulating some metals (Cu, Zn and Cd) was investigated under laboratory conditions, and therefore suggested to test this species in real biomonitoring fieldwork. Our study based on a global analysis of trace metals concentration and the use of a battery of oxidative stress biomarkers applied in parallel in the tissues of *C. nucula* provides further information on the bioaccumulation strategy of this sponge in natural environment as well as its response to the environmental conditions' fluctuations. In term of efficiency, *C. nucula* showed a strong accumulation pattern for Cu, Zn and Cd and less tolerance for Pb which seems to be regulated. While in terms of spatial and temporal variation of the pollution level, the species showed similar seasonal variation trends to those revealed in several studies performed on other organisms in the Gulf of Annaba. Thus, our results support the suitability of this species as bioindicator in the biomonitoring programmes of heavy metals contamination, and specifically for the "Sponge Watch" programme.

REFERENCES

Abbassi, M.; Banaoui, A.; Kaaya, A.; Elkhou, A.; Nadir, M. and Lefrere, L. (2015). Biomarker approach to the assessment of the health status of Moroccan marine ecosystems: preliminary study in Sidi Ifni coast (South of Morocco). J. Mater. Environ. Sci. 6: 3086- 3093.

Abdennour, C.; Drif, F.; Boulakoud, M.S. and Ounissi, M. (2010)**.** Trace metals in the mussel *Donax trunculus* of Annaba estuaries, Algeria. Oceanogr. 1: 15-20.

Almamoori, A.M.J.; Salman, J.M.; Hughes, R. and Al-Saadi, A.A.H. (2013). Biochemical changes in two species of molluscs as environmental biomarkers of pollution in Hilla river. Iraq IJSN 4: 40-43.

Abed, C. (2011). Spongiaires Ircinidae de Méditerranée: chimiotaxonomie, métabolites volatils et bio-indicateurs de pollution par les éléments traces métalliques. Thèse de Doctorat en Océanographie. Université de la Méditerranée Aix-Marseille II. Ecole Doctorale Science de l'environnement, 301 pp.

Abi-Ghanem, C.; Khalaf, G. and Najjar, E. (2014). Distribution of Lead, Cadmium, and Vanadium in Lebanese Coastal Sediments and Mussels. J. Coast. Res. 297:1074–1080.

Alcolado, P.M. (1994). General trends in coral reef sponge communities of Cuba. In: R.M.V. Van Soest, T.M.G. Van Kempen, J. Braekman (Eds) Sponges in time and space. AA Balkema, Rotterdam, pp 251-255.

Amiard, J.; Amiard-Triquet, C .; Berthet, B. and Metayer, C. (1987). Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. J. Exp. Mar. Biol. Ecol. 106(1): 73-89.

Amiard-Triquet, C. (2009). Behavioural disturbances: the missing link between suborg anismal and supra-organismal responses to stress? Prospects based on aquatic research. Hum. Ecol. Risk Assess. 15: 87-110.

Amiard-Triquet, C., Berthe, T., Creach, A., Denis, F., Dourou, C. G.,_Evaert, F. Mouneyrac, C., Ramond, J.B. and Petit, F. (2009). Tolerance in organisms chronically exposed to estuarine pollution. In: Amiard-Triquet, C., Rainbow, P.S. (Eds.). Environmental Assessment of Estuarine Ecosystems, a Case Study. CRC Press/Taylor and Francis Group, pp 135-157.

Amira, A.; Merad, I.; Almeida, C.M.R.; Guimarães, L. and Soltani, N. (2018). Seasonal variation in biomarker responses of *Donax trunculus* from the Gulf of Annaba (Algeria): Implication of metal accumulation in sediments. Cr. Geosci. 350(4): 173–179.

Amira, A.; Sifi, K. and Soltani, N. (2011). Measure of environmental stress biomarkers in *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba (Algeria). Eur. J. Exp. Biol. 1: 7- 16.

Amoozadeh, E.; Malek, M.; Rashidinejad, R.; Nabavi, S.; Karbassi, M. and Ghayoumi, R. (2013). Marine organisms as heavy metal bioindicators in the Persian Gulf and the Gulf of Oman. Environ. Sci. Pollut. Res. Int. 213: 2386–2395.

Amri, S.; Samar, M.F.; Sellem, F. and Ouali, K. (2017b). Seasonal antioxidant responses in the sea urchin *Paracentrotus lividus* (Lamarck 1816) used as a bioindicator of the environmental contamination in the South-East Mediterranean. Mar. Poll. Bull. 122: 392-402.

Banni, M.; Bouraoui, Z.; Ghedira, J.; Clearandeau, C.; Jebali, J. and Boussetta, H. (2009). Seasonal variation of oxidative stress biomarkers in clams *Ruditapes decussatus* sampled from Tunisian coastal areas. Environ. Monit. Assess. 155: 119-128.

Barhoumi, B.; Le Menach, K.; Clerandeau, C.; Ben Ameur, W.; Budzinski, H.; Driss, M.R. and Cachot, J. (2014). Assessment of pollution in the Bizerte lagoon (Tunisia) by the combined use of chemical and biochemical markers in mussels, *Mytilus galloprovincialis*. Mar. Poll. Bull 84: 379-390.

Batista, D.; Muricy, G.; Rocha, R.C. and Miekeley, N.F. (2014). Marine sponges with contrasting life histories can be complementary biomonitors of heavy metal pollution in coastal ecosystems. Environ. Sci. Pollut. Res. Int. 21(9): 5785–5794.

Belabed, B.E.; Djabourabi, A. and Bensouilah, M. (2008). Teneurs en Plomb, Cadmium, Mercure et Zinc relevées dans la chair de la moule *Perna perna*, dans le littoral d'Annaba. Revue Synthèse des sciences et de la technologie de l'Université Badji Mokhtar, Annaba, 18: 12-22.

Belabed, B.E., Laffray, X., Dhib, A., Fertouna-Belakhal, M., Turki, S. and Aleya, L. (2013). Factors contributing to heavy metal accumulation in sediments and in the intertidal mussel *Perna perna* in the Gulf of Annaba (Algeria). Mar. Poll. Bull. 74: 477-489.

Belabed, B.E.; Meddour, A.; Samraoui, B. and Chenchouni, H. (2017). Modeling seasonal and spatial contamination of surface waters and upper sediments with trace metal elements across industrialized urban areas of the Seybouse watershed in North Africa. Environ. Monit. Assess. 189, 265.

Beldi, H.; Gimbert, F.; Maas, S.; Scheifler, R. and Soltani, N. (2006). Seasonal variations of Cd, Cu, Pb and Zn in the edible mollusc *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba, Algeria. Afr. J. Agric. Res. 1(4): 85-90.

Bensafia N, Khati W. (2018) Seasonal variations in four trace metals (Cu, Zn, Pb, Cd) in sponges Sarcotragusspinosulusof the Gulf of Annaba, Northeast Algeria. AACL Bioflux 11(3) http://www. bioflux.com.ro/aacl

Bensouda, L. and Soltani-Mazouni, N. (2014). Measure of oxidative stress and neurotoxicity biomarkers in *Donax trunculus* from the Gulf of Annaba (Algeria): case of the year 2012. Annu. Res. Rev. 4: 1902-1914.

Bergayou, H.; Mouneyrac, C.; Pellerin, J. and Moukrim, A. (2009). Oxidative stress responses in bivalves (*Scrobicularia plana*, *Cerastoderma edule*) from the Oued Souss estuary (Morocco). Saf. Ecotox. Environ. Safe. 72: 765-769.

Borja, A.; Ranasinghe, A. and Weisberg, S.B. (2009). Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: Challenges for the future. Mar. Poll. Bull. 59(1-3): 1–4.

Boucetta, S.; Beldi, H. and Draredja, B. (2016a). Effects of metal pollution on the activities of Acetylcholinesterase and glutathione-S-transferase in *Phorcus (Osilinus) turbinatus* (Gastropoda, Trochidae) of the coast East-Algerian. Adv. Environ. Res. 10(5): 46-61.

Boucetta, S.; Beldi, H. and Draredja, B. (2016b). Seasonal variation of heavy metals in *Phorcus (Osilinus) turbinatus* (gastropod, Trochidae) in the eastern Algerian coast. Glob. Vet. 17: 25-41.

Bougherira, N.; Hani, A.; Djabri, L. and Chouchane, S. (2015). Assessment of contaminant migration in groundwater from an industrial development area, Annaba District NE

Algeria. Eng. Geol. for Soc. and Terr. 3: 383-387.

Boutabia-Trea, S.; Habachi, W. and Bensouilah. M. (2017). Assessment of metallic trace elements using the Seagrass *Posidonia oceanica* and the surface sediment from North Eastern of Algeria. Asian J. Biol. Sci. 10: 17-26.

Bouzahouane, H.; Barour, C.; Sleimi, N. and Ouali, K. (2018). Multi-biomarkers approach to the assessment of the southeastern Mediterranean Sea health status: Preliminary study on *Stramonita haemastoma* used as a bioindicator for metal contamination. Chemosphere 207: 725–741.

Bouzenda, R.; Soltani, N. and Khebbeb, M.E.H. (2017). Assessment of pollution in the Gulf of Annaba (Algeria) by monthly measurements of two biomarkers in a fish species *Liza aurata*. J. Entomol. Zool. Stud. 5: 366-372.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemestry 72(1-2):248–254.

Brümmer, F.; Gotz, M.; Leitermann, F.; Nickel, M.; Sidri, M. and Zucht, W. (2002). Maintenance and growth of sponges in aquariums: fundamentals for in vitro cultivation approaches far from the sea. Bollettino dei Musei e degli Istituti Biologici. 37, 66-67.

Cappello, T.; De Marco, G.; Oliveri Conti, G.; Giannetto, A.; Ferrante, M.; Mauceri, A. and Maisano, M. (2021). Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. Ecotoxicol. Environ. Saf. 209: 111780.

Cappello, T.; Maisano, M.; Giannetto, A.; Natalotto, A.; Parrino, V.; Mauceri, A. and Spanò, N. (2019). Pen shell *Pinna nobilis* L. (Mollusca, Bivalvia) from different peculiar environments: adaptive mechanisms of osmoregulation and neurotransmission. Eur. Zool. J. 86: 333-342.

Cappello, T.; Maisano, M.; Mauceri, A. and Fasulo, S. (2017). ^{1}H NMR-based metabolomics investigation on the effects of petrochemical contamination in posterior adductor muscles of caged mussel *Mytilus galloprovincialis.* Ecotoxicol. Environ. Saf. 142: 417-422.

Cappello, T.; Maisano, M.; D'Agata, A.; Natalotto, A.; Mauceri, A. and Fasulo, S. (2013). Effects of environmental pollution in caged mussels (*Mytilus galloprovincialis*). Mar. Environ. Res. 91: 52-60.

Caricato, R.; Giordano, M.E.; Schettino, T.; Maisano, M.; Mauceri, A.; Giannetto, A.; Cappello, T.; Parrino, V.; Ancora, S.; Caliani, I.; Bianchi, N.; Leonzio, C.; Mancini, G.; Cappello, S.; Fasulo, S. and Lionetto, M.G. (2019). Carbonic anhydrase integrated into a multimarker approach for the detection of the stress status induced by pollution exposure in *Mytilus galloprovincialis*: A field case study. Sci. Total Environ. Sci. Total. Environ. 690: 140-150.

Cebrian, E.; Uriz, M.J. and Turon, X. (2007). Sponges as biomonitors of heavy metals in spatial and temporal surveys in north-western mediterranean: multispecies comparison.

Environ. Toxicol. Chem. 26(11): 2430.

Celis-Hernández, O.; Ávila, E.; Ward, R.D.; Rodríguez-Santiago, M.A. and Aguirre-Téllez, J.A. (2021). Microplastic distribution in urban vs pristine mangroves: Using marine sponges as bioindicators of environmental pollution. Environ. Pollut. 284 : 117391.

Chouahda, S. (2006). Impact de deux xénobiotiques (cadmium et halofenozide) sur Gambusia affinis et évaluation du stress environnemental dans le golfe d'Annaba par l'utilisation de *Donax trunculus.* Thèse de Magistère en Biologie et physiologie Animale, Université de Annaba, 76 p.

De Mestre, C.; Maher, W.; Roberts, D.; Broad, A.; Krikowa, F. and Davis, A.R. (2012). Sponges as sentinels: Patterns of spatial and intra-individual variation in trace metal concentration. Mar. Poll. Bull. 64(1): 80–89.

Esterbauer, H.; Lang, J. and Zadravec, S. (1984). Slater TV. Detection of malonaldehyde by high performance liquid chromatography. Methods Enzymol. 105: 319–329.

Ferrante, M.; Vassallo, M.; Mazzola, A.; Brundo, M.V.; Pecoraro, R.; Grasso, A. and Copat, C. (2018). In vivo exposure of the marine sponge *Chondrilla nucula* Schmidt, 1862 to cadmium (Cd), copper (Cu) and lead (Pb) and its potential use for bioremediation purposes. Chemosphere 193: 1049–1057.

Funes, V.; Asensio, E.; Ponce, M.; Infante, C.; Cañavate, J.P. and Manchado, M. (2006). Insulin-like growth factors I and II in the sole *Solea senegalensis*: cDNA cloning and quantitation of gene expression in tissues and during larval development. Gen. Comp. Endocrinol. 149: 166-172.

Gaino, E. (1980). Indagine ultrastrutturale sugli ovociti maturi di *Chondrilla nucula* Schmidt (Porifera, Demospongiae). Cah. Biol. Mar. 21: 11-22.

Gentric, C.; Rehel, K.; Dufour, A. and Sauleau, P. (2015). Bioaccumulation of metallic trace elements and organic pollutants in marine sponges from the South Brittany Coast, France. J. Environ. Sci. Health - Toxic/Hazard. Subst. Environ. Eng. Part A. 513: 213–219.

Giarratano, E.; Duarte, C.A. and Amin, O.A. (2010). Biomarkers and heavy metal bioaccumulation in mussels transplanted to coastal waters of the Beagle Channel. Ecotoxicol. Environ. Saf. 73 : 270 - 279.

Giarratano, E., Gil, M.N. and Malanga, G. (2011). Seasonal and pollution-induced variations in biomarkers of transplanted mussels within the Beagle Channel. Mar. Poll. Bull. 62: 1337-1344.

Giarratano, E., Gil, M. N., and Malanga, G. (2013). Assessment of antioxidant responses and trace metal accumulation by digestive gland of ribbed mussel *Aulacomya atra atra* from Northern Patagonia. Ecotoxicol. Environ. Saf. 92: 39-50.

Glorennec, P.; Lucas, J.P.; Mandin, C. and Le Bot, B. (2012). French children's exposure to metals via ingestion of indoor dust, outdoor playground dust and soil: Contamination data. Environ. Int. 45:129 - 134.

Goldberg, E. D. (1975). The Mussel Watch-A first step in global marine pollution monitoring. Mar. Poll. Bull. 6: 111.

Gonzalez-Fernández, C.; Albentosa, M.; Campillo, J.A.; Viñas, L.; Romero, D.; Franco, A. and Bellas, J. (2015). Effect of nutritive status on *Mytilus galloprovincialis* pollution biomarkers: Implications for large-scale monitoring programmes. Aquat. Toxicol. 167: 90–105.

Gorbi, S.; Virno Lamberti, C.; Notti, A.; Benedetti, M.; Fattorini, D.; Moltedo, G., and Regoli, F. (2008). An ecotoxicological protocol with caged mussels, *Mytilus galloprovincialis*, for monitoring the impact of an offshore platform in the Adriatic sea. Mar. Environ. Res. 65(1): 34-49.

Guendouzi, Y.; Boulahdid, M.; Rouane Hacene, O.; Inal, A.; Boudjellal, B. and Fowler, S.W. (2021). Contamination level and ecological risk assessment of particulate trace metals in Southwestern Mediterranean Sea. Reg. Stud. Mar. Sci. 46 : 101876.

Habig, W.H.; Pabst, M.J. and Jacobi, W.B. (1974). The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

ICES. (2008). Report of the Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMONIV). ICES, Copenhagen, 49: 71 pp.

Jozefczak, M.; Remans, T.; Vangronsveld J. and Cuypers A. (2012). Glutathione Is a Key Player in Metal-Induced Oxidative Stress Defenses. Int. J. Mol. Sci.13(3), 3145–3175.

Kaiser, J. (2001**).** Bioindicators and biomarkers of environmental pollution and risk assessment. Science publishers Inc., Enfield, 204 pp.

Keblouti, K.; Ouerdachi, K. and Berhail, S. (2015). The use of weather radar for rainfall runoff modeling, case of Seybouse watershed (Algeria). Arab. J. Geosci. 8 : 1-11.

Khati, W.; Bensafia, N.; Ouali, K and Derbal, F. (2018). Sponges (Porifera) as bioindicator species of environmental stress from de Gulf of Annaba (Algeria). Biodiv. J. 2018, 9 (4): 319-324

Khati, W.; Ouali, K.; Mouneyrac, C. and Banaoui, A. (2012). Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use in biomonitoring. Energy Procedia. 18: 784-794.

Khebbeb, M.E.H.; Nadji, S. and Amrani, A. (2010). The effect of cadmium exposure on malonedialdehyde and reduced glutathione concentrations in several tissues of a bivalve mollusc (*Ruditapes decussatus*) fished from Mellah lagoon (North East of Algeria). Ann. Biol. Res. 1: 166-173.

Lam, P.K.S. (2009). Use of biomarkers in environmental monitoring. Ocean. Coast. Manag. 52: 348-354.

Laouati, I.; Rouane-Hacene, O.; Derbal, F. and Ouali, K. (2021). The mussel caging approach in the assessment of trace metal contamination in southern Mediterranean coastal waters: a multi-biomarker study. Environ. Sci. Pollut. Res. Int. doi:10.1007/s11356-021-15203-8

Larsen, P.S. and Riisgård, H.U. (1994). The sponge pump. J. Theor. Biol. 168: 53-63.

Lykkesfeldt, J. (2007). Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clinica. Chimica. Acta. 380: 50-58.

Machreki-Ajmi, M.; Ketata, I.; Ladhar-Chaabouni, R. and Hamza-Chaffai, A. (2008). The effect of in situ cadmium contamination on some biomarkers in *Cerastoderma glaucum*. Ecotoxicology. 17: 1-11.

Maisano, M.; Cappello, T.; Natalotto, A.; Vitale, V.; Parrino, V.; Giannetto, A.; Oliva, S.; Mancini, G.; Cappello, S.; Mauceri, A. and Fasulo, S. (2017). Effects of petrochemical contamination on caged marine mussels using a multi-biomarker approach: Histological changes, neurotoxicity and hypoxic stress. Mar. Environ. Res. 128: 114-123.

Mebarki, R.; Khebbeb, M.E.H. and Soltani, N. (2015). Biomonitoring of El Mellah lagoon (Northeast, Algeria): seasonal variation of biomarkers in *Cerastoderma glaucum* (mollusc, Bivalvia). J. Entomol. Zool. Stud. 3: 408-413.

Mejdoub, Z.; Fahd, 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 Coast. Manag. 136: 95-103.

Milanese, M.; Chelossi, E.; Manconi, R.; Sara, A.; Sidri, M. and Pronzato, R. (2003). The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. Biomol. Eng. 20: 363-368.

Morganti, T.M.; Ribes, M.; Yahel, G. and Coma, R. (2019). Size Is the Major Determinant of Pumping Rates in Marine Sponges. Front. Physiol. 10.

O'Connor, T.P. (1996). Trends in chemical concentrations in mussels and oysters collected along the US coast from 1986 to 1993. Mar. Environ. Res. 41(2): 183-200.

Ouali, N.; Belabed, B.E. and Chenchouni, H. (2018). Modelling environment contamination with heavy metals in flathead grey mullet *Mugil cephalus* and upper sediments from north African coasts of the Mediterranean Sea. Environ. Sci. Total. Environ. 639: 156-174.

Pereira, P.; Korbas, M.; Pereira, V.; Cappello, T. ; Maisano, M.; Canario, J.; Almeida, A. and Pacheco, M. (2019). A multidimensional concept for mercury neuronal and sensory toxicity in fish – From toxicokinetics and biochemistry to morphometry and behavior. Biochim. Biophys. Acta - Gen. Subj. 1863: 129298.

Perez, T.; Vacelet, J. and Rebouillon, P. (2004). In situ comparative study of several Mediterranean sponges as potential biomonitors of heavy metals. In Pansini, M.; Pronzato, R.; Bavestrello, R. and Manconi, R. (Eds.). Sponge Science in the New Millennium. Officine Grafiche Canessa Rapallo, Genova, Italy, pp 517-525.

Pronzato, R. (2002). A climber sponge. Boll. Mus. Ist Biol. Univ. Genova, 159, 66-67.

Puig, P.; Palanques, A.; Sanchez-Cabeza, J.A. and Masqué, P. (1999). Heavy metals in particulate matter and sediments in the southern Barcelona sedimentation system (North-western Mediterranean). Mar. Chem. 63(3-4): 311-329.

Rainbow, P.S. (1995). Biomonitoring of heavy metal availability in the marine environment.

Mar. Poll. Bull. 31: 183-192.

Ranjbar Jafarabadi, A.; Riyahi Bakhtiari, A.; Spanò, N. and Cappello, T. (2018). First report of geochemical fractionation distribution, bioavailability and risk assessment of potentially toxic inorganic elements in sediments of coral reef Islands of the Persian Gulf, Iran. Mar. Poll. Bull. 137: 185-197.

Ringwood, A.H.; Hoguet, J.; Keppler, C. and Gielazyn, M. (2004). Linkages between cellular biomarker responses and reproductive success in oysters *Crassostrea virginica*. Mar. Environ. Res. 58: 151-155.

Roveta, C.; Pica, D.; Calcinai, B.; Girolametti, F.; Truzzi, C.; Illuminati, S. and Puce, S. (2020). Hg Levels in Marine Porifera of Montecristo and Giglio Islands (Tuscan Archipelago, Italy). Appl. Sci. 10(12): 4342.

Saenz, L.A.; Seibert, E.L.; Zanette, J.; Fiedler, H.D.; Curtius, A.J.; Ferreira, J.F.; De Almeida, E.A.; Marques, M.R.F. and Bainy, A.C.D. (2010). Biochemicals biomarkers and metals in *Perna perna* mussels from mariculture zones of Santa Catarina, Brazil. Ecotoxicol. Environ. Saf. Ecotox. Safe. 73: 796-804.

Schmidt, W.; O'Shea, T. and Quinn, B. (2012). The effect of shore location on biomarker expression in wild *Mytilus spp*. and its comparison with long line cultivated mussels. Mar. Environ. Res. 80: 70-76.

Sidri, M.; Milanese, M. and Bümmer, F. (2005). First observations on egg release in the oviparous sponge *Chondrilla nucula* (Demospongiae, Chondrosida, Chondrillidae) in the Mediterranean Sea. Invertebr. Biol. 124(2), 91-97.

Sifi, K. ; Amira, A. and Soltani, N. (2013). Oxidative stress and biochemical composition in *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba (Algeria). Adv. Environ. Res. 7 : 595-604.

Soltani, N.; Amira, A.; Sifi, K. and Beldi, H. (2012). Environmental monitoring of the Annaba gulf (Algeria): measurement of biomarkers in *Donax trunculus* and metallic pollution. Bull. de la Soc. Zoo. 137: 47-56.

Sondergaard, J.; Asmund, G. and Larsen, M.M. (2015). Trace elements determination in seawater by ICP-MS with on-line pre-concentration on a Chelex-100 column using a "standard" instrument setup. MethodsX 2: 323–330.

Srikanth, K. and Rao, J.V. (2017). Potential toxic elements determined by ICP-MS in tissues of *Callyspongia fibrosa*, *Faciospongia cavernosa* and *Dysidia fragilis* from Tuticorin coast. J. Aqua. Poll. Toxico. 1(1): 4.

Tlili, S.M.; Etais, I.; Boussetta, H. and Mouneyrac, C. (2010). Linking changes at subindividual and population levels in *Donax trunculus*: assessment of marine stress. Chemosphere 81: 692-700.

Uchiyama, M. and Mihara, M. (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86 : 271-280.

Valavanidis, A.; Vlahogianni, T.; Dassenakis, M. and Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. Ecotox. Environ. Safe. 64(2): 178-189.

Viarengo, A.; Lowe, D.; Bolognesi, C.; Fabbri, E. and Koehler, A. (2007). The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comp. Comp. Biochem. Physiol. 3: 281-300.

Weckbecker, G. and Cory, J.G. (1988). Ribonucleotide reductase activity and growth of glutathione- depleted mouse leukemia L 1210 cells in vitro. Cancer Lett. 40: 257-264.

Weisz, J.B.; Lindquist, N. and Martens, C.S. (2008). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia. 155 : 367-376.

Ziouch, O.R.; Laskri, H.; Chenak, H.; N.; Daifallah, T. and Ounissi, M. (2020). Transport of nutrients from the Seybouse River to Annaba Bay (Algeria, SW Mediterranean). Mar. Pollut. Bull. 156: 111-231.