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POLLUTION BIOMONITORING IN THE NADOR LAGOON USING ALGAE AND PHYSICO-CHEMICAL PARAMETERS

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ABSTRACT

To study the biomonitoring capacity of some seaweeds from the Nador lagoon “Marchica”, we have analyzed the physico-chemical parameters of lagoon water (3 sites) including temperature, dissolved oxygen (DO), pH, salinity, turbidity, NO_3^-, NO_2^- and PO_4^{2-}, and also we determined some biochemical parameters such as chlorophyll, protein, glucose and proline concentrations of 4 seaweeds (*Gracilariagracilis, Alsidiumcorallinium* and *Ulva rigida*and *Caulerpaprolifera*).Waters and algae samples were collected in July 2015, from three sampling sites (S1, S2 and S3). Variations in the physical and chemical parameters were observed between sampling stations. The site 2 was the most polluted area characterized by high levels of turbidity, NO_3^-, NO_2^- and PO_4^{2-}) followed by the site 1 with high NO_3^- and NO_2^- concentrations. In relation to biochemical parameters in different algal species, the *Alsidiumcorallinium* showed a higher level of chlorophyll a and b in sites 1 and 2 comparing to the site 3. Finally, *Gracilariagracilis* collected from the site 2,accumulated more proteins, glucose and proline. From these results, we can conclude that algal species found in the Nador lagoon “Marchica” can be used in the biomonitoring of pollution in a marine ecosystem.

1. Introduction

Algae are very simple chlorophyll-containing organisms [1], and are found everywhere on earth: in the sea, rivers and lakes, on soil and walls. They also exist in all wetlands and tropical regions; in fact just about everywhere where there is a light to carry out photosynthesis [2].

Climate change especially global warming and anthropogenic pollution are affecting negatively marine environment and causing the disappearance of some aquatic organisms from their habitats. For this reason, the determination of gphysico-chemical properties of the marine environment, such as salinity and temperature, are of particular interest [3].

In this study, we have mainly focused on marine algae, which are limited to tidal zones and photic or benthic zone; they contribute to 10% of total marine productivity [4]. In addition, they are among the oldest members of the plant kingdom and are vital parts of the ecosystem of marine life. Algae

play a significant role in maintaining the balance of aquatic environments as primary producers and an important source of oxygen for the links in the ecosystem food chain. Nevertheless, the excessive N and P in aquatic systems cause eutrophication and algae blooms and could have negative impacts on both the stability of the natural lagoon system and on human health [5,6]. Thereby, regular assessments aimed at monitoring and controlling the development of these macroalgae is extremely necessary.

Macroalgae have received remarkable interest for its potential in assessing the environment, as well as in biomonitoring of aquatic pollution either by heavy metals or by organic pollutants and production of the green energy [7-10].

Due to their ability to bioaccumulate pollutants, these algae are often consideredbioindicators of pollution and *Chaetomorphaaerea*could be used in phytoremediation [11].

The objective of this study is to evaluate the effects of the physicochemical parameters of the lagoon water on the biochemical and physiological parameters of some seaweed species collected along the shoreline of the Nador lagoon, in order to select the good algal species that can be used as indicator of pollution of the marine ecosystem.

2. Materials and methods

1.1. Study area

The samples were collected in July 2015 at the Nador Lagoon (Figure 1). The climate of the region is characterized by a low rainfall which varies between 116 mm / year and 430 mm / year, and average temperatures which vary between 22.9 °C in January at 39.17 °C in August.The lagoon is fed by the waters of the Mediterranean by means of a new pass, by the fresh water courses, the discharges of human activities agriculture4. , industries and urban waters) and the groundwater of two aquifers : Gareb and Bou-Areg[12].

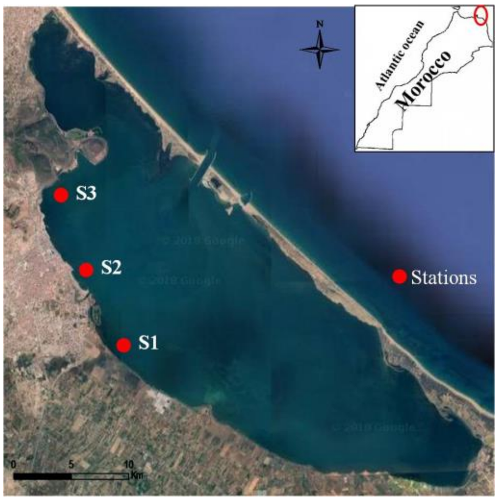

Figure 1. Map showing the study area and the locations of the sample sites of surface seawater and algae

1.2. Physico-chemical parameters of lagoon water

As shown in the figure 1, this study was conducted at three lagoon sites (S1, S2 and S3). Surface water samples from each site were collected directly in 500 ml bottles polyethylene from about 20 cm below the surface seawater.

The temperature (°C) and the salinity of water are measured using a typical conductivity meter 197i. A pH-meter (SUNTEXTS) was used to assess the pH of the water samples. The oxygen dissolved by Eutech DO 6+ Meter and the turbidimeter to identify the turbidity of the water are measured *in Situ*.

The samples were filtered through 0.45 µm pore size millipore filters, and stored in coolers (4°C) for transport to the laboratory.

In laboratory, water samples were used to analyze of the concentration of nitrates (NO3), nitrites (NO2), phosphates (PO4) are determined by the method of Aminot and Chaussepied[13]., to make these measurements, and we used a spectrophotometer type «Sp-2000 UV».

1.3. Biochemical parameters of algae

Algae sampling: Algal materials were collected from the sites shown in the Figure 1, during July 2015. Algae sampling was conducted at a depth of 0 to 1.5 meter depending on the species collected. The collected samples were washed with seawater and stored in polyethylene bags. The samples were taken from the laboratory and washed with tap water and distilled water. The harvested species are sorted into two groups : (i) Red algae (Rhodophyta) ; Alsidiumcorallinum and Gracilariagracilis ((Stackhouse) Steentoft, Irvine &Farnham) (ii) Green algae (Chlorophyta); Caulerpaprolifera ((Forsskal) J.V. Lamouroux) and Ulva rigida (C. Agardh). The collected algal material was used to analyze bio-chemical and physiological parameters.

Algaeanalyzes:Chlorophyll a and b concentration was determined in seaweeds using the method described by Lichtenthaler[14]and Ben Chekroun et al. 15].Fresh algae samples were used to

determine protein content in different algal species using the Bradford method [16]. Glucose and proline levels were determined by using the methods described by Irigoyen et al. [17] and Paquin and Lechasseur [18], respectively.

Statistical analyses. Data were analyzed using ANOVA. The Duncan's multiple range test was used to compare treat¬ment means

3. Results

1.1. Physico-chemical parameters of the lagoon water

Table 1 shows the values of the physicochemical parameters measured from water sample of Nador Lagoon. The Temperature and pH were higher in site 3 and lower in the sites 1 and 2. The station 1 is characterized by maximum levels of salinity, dissolved oxygen and NO2-. Finally, at the station 2, we found higher levels of turbidity, NO3- , NO2- and PO42

Table 2. Physico-chemical parameters of the lagoon water

Stations		T (°C)	pH	Sal (g/l)		DO (mg/l)		Turb (NTU)	NO3-
(mg/l)	NO2-(mg/l)		PO42- (mg/l)						
1	28.9	8.2	38.18	7.32	1.2	0.038	0.02	0	
2	28.32	8.19	37.7	6.86	9.79	0.18	0.016	0.018	
3	29.9	8.41	38.04	6.15	1.21	0.02	0.011	0	

T: Temperature; DO: Dissolved Oxygen; Sal: Salinity; Turb: Turbidity

1.2. Photosynthetic pigments

The comparison of chlorophyll a and b content among the four studied algae collected from the three stations of the lagoon shows that red algae Alsidiumcorallinium, which was found in the three sites studied here, had higher level of these pigments in sites 1 and 2, while the lowest value was found in site 3 (Table 2). In relation to the green alga Caulerpaproliferasampled from the sites 1 and 3, we did not find a significant difference between these two sites. Finally, from the site 2 we collected green alga Ulva rigidaand the red alga Gracilariagracilis.

Table 3. Chlorophyll (a) and Chlorophyll (b) levels in different algae species (mg/100g) (mean ± SE).

Stations						
Species		Chlorophyll a		Chlorophyll b		
1	Alsidiumcorallinium			16.78±3.38		5.85±2.17
Caulerpaprolifera			110±35.85		135±32.01	

2	Gracilaria gracilis	22.82±7.93	8.34±0.39
	Alsidiumcorallinium	15.04±1.01	5.32±1.27
	Ulvarigida	42.65±1.38	69.51±10.65
3	Alsidiumcorallinium	9.57±1.22	2.44±0.37
	Caulerpaprolifera	114±53.65	107±60.85

1.3. Protein, glucose and proline

With regard to the soluble proteins (Table 3), both Alsidiumand Gracilaria accumulated higher concentrations at the station 2, reaching the values of 4.12±0.43 and 3.58±0.54, respectively. However, at the station 1, we found lower levels of proteins in Alsidium and Caulerpa.

In relation to glucose content (Table 3), Gracilariagracilis accumulated maximum concentration at the station 2 (0.159±0.018 mg/g f.w.) andCaulerpaprolifera reached high value at the station 1 (0.106±0.006 mg/g f.w.) and station 3 (0.110±0.010 f.w.). However, the glucose content in Alsidium did not show significant differences among three sites.

Table 4. Proteins, glucose and proline content in different algae collected from the lagoon(mean±SE)

Stations	Species	Proteins (mg/g)	Glucose (mg/g)	Proline (µg/g)
1	Alsidiumcorallinium	1.32±0.53	0.063±0.005	1.58±0.44
	Caulerpaprolifera	0.46±0.04	0.106±0.006	1.86±0.23
2	Gracilaria gracilis	3.58±0.54	0.159±0.018	19.85±8.15
	Alsidiumcorallinium	4.12±0.43	0.055±0.031	0.72±0.11
	Ulvarigida	1.16±0.28	0.056±0.015	1.74±0.08
3	Alsidiumcorallinium	1.60±0.04	0.055±0.002	0.74±0.23
	Caulerpaprolifera	0.53±0.02	0.110±0.010	1.35±0.15

The proline concentration was significantly higher in Gracilariagracilis at the station 2 (19.85µg/l f.w.). Moreover, both Alsidiumcorallinium and Caulerpaprolifera collected from the station 1 accumulated higher levels of proline than those found at the station 3 (Table 3). Finally, the lowest value of proline was detected in Alsidium species at the site 2.

4. Discussion

The results of physico-chemical parameters showed that the temperature, pH, salinity and dissolved oxygen in surface waters of the lagoon were practically similar between different sites analyzed in this study (Table 1); however the turbidity and PO42 at the station 2, and NO3- and NO2- at the station 1 and 2 were relatively higher comparing to the site 3. These results can be explained by the trigenous inputs to the sites 1 and 2, these stations receive pollutants from OuedSelouan and OuedCaballo, respectively. OuedCaballo transport urban wastewater of Zeghanghane municipality and partly of Nador city and OuedSelouan (Selouan stream) carries the urban and industrial wastes of the Selouane village into the lagoon [12].

It is eventually evident from the results found that red and green algae show significant variation in pigment levels and the accumulation of protein, carbohydrate and proline in response to current environmental conditions. Moreover, the variation was not only between the two different groups, but also between the species of each group.

Chlorophyll measurements are import for monitoring environmental degrada¬tion of aquatic ecosystems. In our previous work, we found that chlorophyll levels were directly correlated with those of nitrogen [19].

At stations 1 and 2, the high levels of chlorophyll pigments found in Alsidiumcorallinium(Table 2), is a consequence of a strong assimilation of nitrate and nitrite found in these two stations [20]. In fact, these areas are experiencing agricultural pollution that is caused by excessive fertilization and intensive use of fertilizers and pesticides, which leads to the contamination of water with a high concentration of nitrates, phosphates and organic pollutants precursors of eutrophication the lagoon [15]. Shen [21] reported that the growth of algae is closely related to the concentration of phosphorus and nitrogen in the water. Previously, Lopez-Cantarero et al. [22] demonstrated that exogenous concentrations of P and N are directly correlated with total chlorophyll concentration. These results are in agreement with those found in Ulva rigida at the Venice Lagoon [23]

and in Lake of Tunis [24], and in Ulva lactuca and Chaetomorphalinuim at the Roskilde Fjord in Danish estuary [25]. Dere et al. [26] usedUlva as a biofilter and bio-accumulator because of its high efficiency in the removal of inorganic nitrogen compounds. According to Khan and Ansari [27], Gracilariagracilisis considered as biological filter for phosphate in lagoon effluents and has a capacity to accumulate nitrogen and phosphorus more efficiently than any other macroalgae[28]. As a result, variation in chlorophyll content is a response to environmental variation that allows an organism to adapt to a particular habitat, namely, temperature rise and nutrient accumulation.

In algae, the accumulation of osmolytes such as proline, glucose and proteins confers greater tolerance to stressful conditions of the marine ecosystem (Ben Chekroun et al., 2013; Baghour, 2017). It is well established that the proteins could be accumulated in some algae such as Caulerpa, Ulva, Gracilaria and Alsidiumas a consequence of an increase in anthropogenic activities causing the enrichment of the environment by nutrients, in particular nitrogen and phosphate [15,29].

The maximum concentrations of proteins found in our studies correspond to the zones with inputs from Selouane River and the areas with high agricultural activities (Bou-Areg). This agricultural activity is characterized by an intensive use of fertilizers and pesticides.

Comparing protein levels in different groups of algae, we observe that rhodophyceae accumulated more protein than chlorophycea (Table 4), the same result was found by Ben Chekroun et al. [15], and Khan et al. [30]. This indicates that this group is more protected against abiotic stress. In addition, a relatively higher accumulation of proteins in rhodophycea may thus contribute to an improvement in resistance to different stresses by synthesizing antioxidant enzymes [30]

Furthermore, carbohydrates are the most important components for metabolism because they provide energy needed for respiration. They intervene in other metabolic processes [31], but they are very sensitive to environmental constraints such as the high levels of N or P in the medium (Table 4) and the increase of the water temperature [32]. In this sense, similar observations have been made on other species [29,33]. In addition, an accumulation of glucose in the algae is subject to intense evaporation, as the main cause of their supersalinization, which induces the synthesis of glucose in Ulva.

Finally proline is an important osmoprotectant synthesized in stress conditions acting as a tolerance mechanism and adaptive form. They appear to be molecules involved in defense mechanisms and resistance to pollution, cold and salinity.In algae, biochemical compounds such as proline can be considered biomarkers and indicators of pollution to monitor the quality of aquatic systems [34].

In this study, the increase in proline levels in algae (Caulerpa and Gracilaria) appears to be induced by the increase in of pollution in the sites 2 and 3 (Table 4). By comparing with the results found by de Aknaf et al 11], that the value of proline in the Chaetomorpha during the same period does not exceed 1.76 and 1.17µg/l whereas in our present work the value of proline in the Caulerpa is 1.86 µg/l, so we can conclude that the Caulerpa have the ability to adapt in a contaminated environment more than the Chaetomorpha. The results show that rhodophyceae, mainly Gracilariagracilis, accumulate more proline to protect cells against osmotic and oxidative

stress more effectively than chlorophyceae and allow an organism to adapt to a particular habitat. They can act as an osmotic-adjusting compatible solute, a metal chelator, a pH buffer [30].

5. Conclusion

The study of the biochemical parameters of the algae harvested at the Nador lagoon allowed us to conclude that the chlorophyll a and b, especially in Alsidiumcorrallinium,are correlated with nitrate and phosphate in the medium. Therefore, Alsidiumcorralliniumcould be used in biomonitoring as an indicator of pollution in the aquatic systems. In relation to protein, glucose and proline content, Gracilariagracilis can biosynthesize more of these substances in comparison with the other algal species, which allows them better resistance to different types of stress such as increase in temperature, high levels of nutrients and high salinity. The two algal species, Alsidiumcorralliniumand Gracilariagracilisare considered as very good indicators of the pollution of the lagoon and possess a higher level of protection against changing climatic conditions than other two species of algae

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