ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Role of macroalgae in biomonitoring of pollution in «Marchica», the Nador lagoon

Rol de las macroalgas en el biomonitoreo de la contaminación en «Marchica», la laguna de Nador

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Abstract. The Aquatic system contamination by organic pollutants and heavy metals is one of the most serious problems that might face the environment and organisms as a whole. Interestingly, the accumulation of these pollutants is due to the anthropogenic activity, namely the intensive use of fertilizers to obtain high yields in crop production. In this paper, we compared the levels of chlorophylls, proteins, dry weight and sucrose in some red (*Alsidium* sp. and *Gracilaria* sp.) and green algae (*Ulva* sp.) collected from Mohandis, near Kariat and Bou Areg. Our results indicate that the algae collected nearby Bou Areg, an area well-known for its high agricultural activity, contained high levels of pigments, proteins and sugars. From the chlorophyll results, we conclude that *Ulva* sp. is the best indicator for eutrophication biomonitoring. Nevertheless, the *Gracilaria* sp. can be used in phytoremediation due to its high carbohydrate synthesis that makes it much more pollutant resistant.

Keywords: Algae; Biomonitoring; Carbohydrates; Nador lagoon; Phytoremediation; Pigments; Pollution; Proteins.

Resumen. La contaminación de los sistemas acuáticos por los contaminantes orgánicos y metales pesados es uno de los problemas más graves a los que están expuestos el medio ambiente y los organismos. La acumulación de estos contaminantes se debe a la actividad antropogénica, principalmente al uso intensivo de fertilizantes para la obtención de altos rendimientos en los cultivos. En este trabajo, hemos comparado los niveles de clorofila, proteínas y carbohidratos (glucosa y sacarosa) en algunas algas rojas (*Alsidium* sp. y *Gracilaria* sp.) y verdes (*Ulva* sp.) recogidas en Mohandis, cerca de Kariat y Bou Areg. Nuestros resultados indican que las algas recogidas cerca de Bou Areg, una zona bien conocida por su alta actividad agrícola, contienen altos niveles de pigmentos, proteínas y azúcares. A partir de los resultados de clorofila podemos concluir que *Ulva* sp. es un buen indicador para el registro de la eutroficación. No obstante, *Gracilaria* sp. puede ser utilizada en la fitorremediación debido a la alta tasa de síntesis de carbohidratos lo que la hace mucho más resistente a la contaminación.

Palabras clave: Algas; Biomonitoreo; Carbohidratos; Laguna de Nador; Fitorremediación; Pigmentos; Contaminación; Proteínas.

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Recibido / Received 20.III.2012. Aceptado / Accepted 30.V.2012.

INTRODUCTION

The lagoon of Nador, also known as Marchica, is located in north-eastern Morocco. Over time, this lagoon has been subjected to an increasing anthropogenic pressure owing to the economic activities in the adjacent zones and the growing population. The normal water quality parameters (salinity, pH, nutrients) of the dominant marine flows are altered by local fecal water effluents, urban discharges, sewages derived from a water treatment station, and residues originated in a slaughterhouse (Ruiz et al., 2006).

Recently, there has been a growing interest in using algae for biomonitoring eutrophication, organic and inorganic pollutants. By using the chlorophyll formation of the alga, for example, it was possible to estimate spectrophotometrically the total nitrogen content in water collected from aquatic systems (Abe et al., 2004), giving us an idea on eutrophication levels.

The brown algae *Cystoseira* sp., the green algae *Ulva* sp. and *Enteromorpha* sp. have high potential as cosmopolitan biomonitors for trace metals in the Aegean Sea (Akcali & Kucuksezgin, 2011).

In the present work, we analysed some physiological parameters in green and red algae, and discussed the effect of using these parameters for biomonitoring the organic and inorganic pollution in the lagoon of Nador, comparing contaminated (Bou Areg) and non-contaminated (Mohandis) areas.

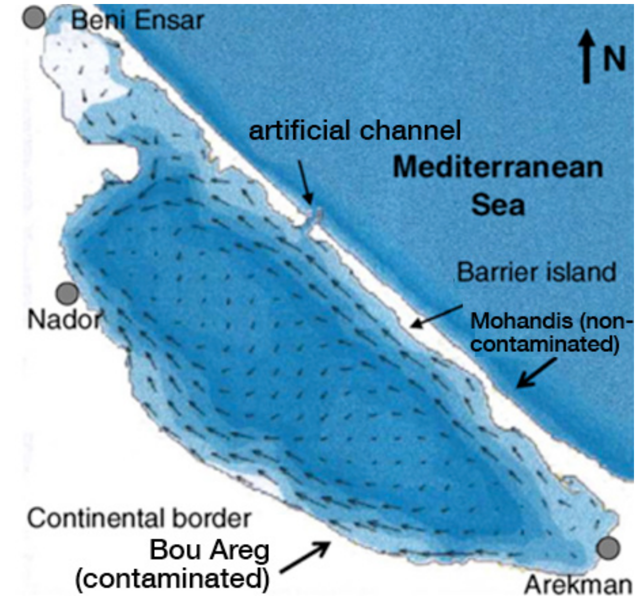

Fig. 1. Location of the study area, and currents according to Hilmi (2005).

Fig. 1. Ubicación del área de estudio y de las corrientes de acuerdo a Hilmi (2005).

MATERIALS AND METHODS

Study area. Geographically, the lagoon of Nador is located on the eastern coast of Morocco along the side of the Mediterranean sea, with a surface area of about 115 km^2 (25 km by 7.5 km) and with a depth not exceeding 8 m. Intrinsically, this lagoon is separated from the sea by a belt of dunes (approx. 24 km in length). The lagoon still connects with the Mediterranean Sea through a new artificial channel of 300 meters in width and 6.5 meters in depth (Fig. 1).

Algae Sampling. This study was conducted at two lagoon sites (Bou Areg and Mohandis, near Kariat). Algal materials were collected from these two sites (Fig. 1) in April 2011. We collected different species of green and red algae (*Ulva* sp., *Gracilaria* sp. and *Alsidium* sp.).

Algae sampling was conducted at a depth of 0 to 1.5 meter depending on the species collected. The samples were collected by hand and placed in plastic bags, and transported to the laboratory for further analysis. The algal materials were rinsed, and then blotted on filter paper. At each sampling, we used four to five replicates to determine pigments (chlorophylls), proteins, glucose and sucrose.

Sample analysis

Chlorophyll Determination. The algal materials used for the measurement of *A*max were extracted in 5 mL of pure methanol for spectrophotometric determination of the concentration of chlorophylls (total chl, chl *a* and chl *b*) according to Lichtenthaler (1987).

Soluble-protein determination. Fresh algae samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH_2PO_4, pH 7.0) and centrifuged at 12 000 × *g* for 15 min. The resulting supernatant was used for the determination of soluble proteins using Bradford G-250 reagent (Bradford, 1976) and expressed as mg bovine serum albumin/g of fresh weight.

Glucose and sucrose determination. Samples of 0.5 g of algal tissues were crushed in 5 mL 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 mL of 70% ethanol. All soluble fractions were centrifuged at 3500 × *g* for 10 min. The supernatants were collected and stored at 4 °C for glucose and sucrose determinations (Irigoyen et al., 1992). Glucose and sucrose concentrations were determined spectrophotometrically at *A*650 using the colorimetric assay with the anthrone reagent (Irigoyen et al., 1992).

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

RESULTS

The algae samples collected from Bou Areg were characterized by higher levels of total chlorophyll compared with those collected from Mohandis (e.g. values for *Ulva* sp. and *Gracilaria* sp. were two-fold higher in Bou Areg; Table 1).

Protein concentrations were significantly higher ($p<0.001$) in the red (*Alsiudium* sp. and *Gracilaria* sp.) than in the green algae (*Ulva* sp.). The three algal species (*Ulva* sp., *Alsidium* sp., and *Gracilaria* sp.) showed a higher protein content in Bou Areg than in Mohandis; increases were 30% ($p<0.001$), 80% ($p<0.001$) and 7% ($p<0.05$), respectively.

Soluble carbohydrate (glucose and sucrose) concentrations varied significantly between species ($p<0.001$). The highest values were found in *Gracilaria* sp. (Table 2), while those of *Alsidium* sp. were lowest at both sites. Concentrations of glucose and sucrose increased markedly in Mohandis compared to Bou Areg for both *Ulva* sp. and *Alsidium* sp. *Gracilaria* sp., on the other hand, followed an opposite trend, reaching higher concentrations of glucose and sucrose in Bou Areg than in Mohandis (Table 2).

Table 1. Total chlorophyll and protein levels in different algae collected from Bou Areg and Mohandis (mean ± SE, n = 4 to 5).
Tabla 1. Niveles totales de clorofila y proteína en diferentes algas recolectadas en Bou Areg y Mohandis (promedio ± error estándar, n = 4 a 5).

Species	Total Chlorophyll mg/100g fresh weight		Proteins mg/g fresh weight	
	Bou Areg	Mohandis	Bou Areg	Mohandis
Ulva sp.	70.71 ± 5.75 a	35.82 ± 7.56 a	1.14 ± 0.06 c	0.88 ± 0.05 c
Alsidium sp.	20.63 ± 1.47 b	15.56 ± 1.58 b	2.62 ± 0.20 b	1.45 ± 0.07 b
Gracilaria sp.	15.26 ± 1.25 c	6.23 ± 1.34 c	3.37 ± 0.10 a	3.16 ± 0.06 a

Mean values followed by the same letter within a column are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

Table 2. Soluble carbohydrates (glucose and sucrose) in *Ulva* sp., *Alsidium* sp. and *Gracilaria* sp. collected from Bou Areg and Mohandis (mean ± SE, n = 4 to 5).
Tabla 2. Carbohidratos solubles (glucosa y sacarosa) en *Ulva* sp., *Alsidium* sp y *Gracilaria* sp recolectadas en Bou Areg y Mohandis (promedio ± error estándar, n = 4 a 5).

Species	Glucose mg/g fresh weight		Sucrose mg/g fresh weight	
	Bou Areg	Mohandis	Bou Areg	Mohandis
Ulva sp.	0.61 ± 0.18 b	0.94 ± 0.16 b	0.49 ± 0.14 b	0.76 ± 0.13 b
Alsidium sp.	0.21 ± 0.02 b	11.15 ± 0.13 b	0.17 ± 0.02 b	0.94 ± 0.11 b
Gracilaria sp.	5.52 ± 0.15 a	3. 43 ± 0.17 a	4.46 ± 0.57 a	2.76 ± 0.14 a

Mean values followed by the same letter within a column are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

DISCUSSION

The lagoon of Nador has been recently subjected to industrial, domestic and agricultural pollution. Organic and inorganic contamination, such as pesticides and heavy metals, has been produced as a result. The agricultural activity in the Bou Areg area has led to its widespread water contamination at the surface and groundwater. González et al. (2007) suggested that Cd is the most important pollutant near the Bou Areg station as a result of anthropogenic activities (pollution from *i*ndustry and intensive agriculture). The continental input comes from the Salouane River, Bou Areg and the channel that drains the Bou Areg plain. There are also inputs from used waters and sewage treatment plants at Nador, Beni Enzar and Kariat Arkmane (Piazza et al., 2009).

Some plant parameters such as chlorophyll content, photosynthesis, nutrient uptake and metabolism may be used as biomarkers in the monitoring of organic and inorganic pollution in terrestrial and aquatic ecosystems (Baghour et al., 2001; Ayeni et al., 2010).

Conrad et al. (1993) suggested that chlorophyll measurements are important for monitoring environmental degradation of aquatic ecosystems. In our previous work, we found that chlorophyll levels were directly correlated with those of nitrogen (Baghour et al., 2000). In this experiment (Table 1) the higher levels of total chlorophylls in the algae of the Bou Areg area were must likely due to the increased amounts of fertilizers (i.e., eutrophication) employed in that area as a result of the agricultural activity. However, in Mohandis (Fig. 1), no eutrophication phenomenon was noted.

The proteins are among the final products of nitrogen assimilation by algae. In some algae, like *Palmaria palmata* (Rhodophyta), protein is the major N pool and constitutes a large fraction of the dry weight of the algae (Morgan et al., 1980). The protein levels in the green and red algal species (Table 1) were higher in Bou Areg (contaminated) than in the Mohandis (less contaminated) area. This was probably due to the high levels of heavy metals and organic pollutants in Bou Areg (Bloundi et al., 2008), which may have determined a greater protein synthesis as a mechanism of algal resistance to pollution. Similar results were found by Torres et al. (2008), who suggested that the presence of metals in algae induced the synthesis of several proteins.

Ulva sp. and *Alsidium* sp. accumulated less glucose and sucrose in contaminated than in non-contaminated areas (Table 2). Similar findings were reported by Tzvetkova & Kolarov

(1996) in trees. These authors found that trees in the polluted regions had lower concentrations of starch, and total and soluble sugars than trees in the unpolluted regions. The decrease in total sugar content of damaged leaves probably was the result of photosynthetic inhibition or stimulation of respiration rates (Tzvetkova & Kolarov, 1996). However, we found an opposite trend in *Gracilaria* sp., with higher concentrations of both sugars (glucose and sucrose) in contaminated than in non-contaminated areas. Ashraf & Harris (2004) suggested that carbohydrates might be used as indicators of plant stress tolerance in specific cases. It might be that proteins and carbohydrates sequestrate heavy metals and other pollutants in the study algae species as a mechanism of heavy metal resistance.

CONCLUSIONS

Based on the chlorophyll results in *Ulva* sp., and the carbohydrate concentrations in *Gracilaria* sp., we conclude that these two species could be used as indicators for biomonitoring eutrophication in "Marchica", the lagoon of Nador.

ACKNOWLEDGMENTS

We thank Soumya Essayeh and Fatiha Bousfia for technical assistance. We also thank Kamal Baghour for his comments and corrections.

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