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GRACILARIA BURSA-PASTORIS OF BIZERTE LAGOON (NORTH OF TUNISIA): A SPATIO –TEMPORAL STUDY OF SOME HYDRO BIOLOGICAL PARAMETERS, AGAR YIELD AND QUALITY

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ملخص

الطحلب الأحمرغراسيلاريا بورسا باستوريس ببحيرة بنزرت (الشمال التونسي): دراسة في الزمان والمكان لبعض العوامل الهيدروبيولوجية ومردود الأغرة وجودتها : تعتبر الدراسات الايكوبيولوجية للطحالب البحرية وتثمينها بتونس حديثة نسبيا. وفي هذا الإطار قمنا بدراسة حول الطحلب الأحمر غراسيلاريا بورسا باستوريس طيلة سنة كاملة في ثلاثة مواقع مختلفة من بحيرة بنزرت وهي منزل الجميل ومنزل عبد الرحمان ومنزل بورقيبة. وتمت متابعة عدة عوامل هيدرولوجية كالحرارة والملوحة والأكسيجين ودرجة الحموضة والأملاح المعدنية.و إضافة إلى ذلك تمت متابعة الكتلة الحيوية والوزن والطول وعدد الفروع الواقعة على بعد 0.5 صم من القمم وذلك لدى النباتات المشيجية والبوغية. كما تمت متابعة التغيرات الموسمية لمردود الأغرة وجودتها . وقد بينت النتائج وجود تغيرات في هذه العوامل في الزمان والمكان. وفي هذا الإطار بلغت الحرارة القصوى 32.2 درجة مئوية في شهر أوت في منزل بورقيبة. وتراوحت الملوحة بين33 غ/ل في شهر أفريل في منزل عبد الرحمان و41.2غ/ل في نوفمبر في منزل بورقيبة. وتأرجحت درجة الحموضة بين 8 و8.8، في حين أن الأكسيجين قد بلغ 11.2 مغ/ل في أكتوبر في منزل الجميل. أما العناصر الأزوتية والفسفورية فقد تغيرت حسب الفصول والأماكن. وقد بلغ الوزن الأقصى للطحالب 134.58غ في جوبلية عند النباتات البوغية. بينما وصل الطول الأقصى لدى نفس النباتات ولكن في شهر جانفي في منزل بورقيبة. وقد بلغ العدد الأقصى للفروع الذي تم تسجيله لدى الطور الأنثوي البالغ والحامل للأكياس الثمرية في شهر أفريل وفي منزل عبد الرحمان 54 فرعا. ويجدر بنا الذكر أن الكتلة الحيوية القصوى(56.23 غ/م^2) من الوزن الجاف تم الحصول عليها في منزل عبد الرحمان في فصل الصيف. وأما المردود الأقصى للأغرة (27.48٪) فقد تم الحصول عليها في منزل الجميل في فصل الربيع. وبلغت قوة الجمد 148.33 غ/صم^2 أما نقطة الجمد والانصهار القصوى فتم الحصول عليها أيضا في الربيع وهي على التوالي40.16 درجة مئوية و 83 درجة مئوية. وقد بينت كل النتائج المتحصل عليها أن الطحلب غراسيلاريا بورسا باتوريس يمكن أن يكون مصدرا لصناعة تونسية للأغرة و استعمالها في عديد المجالات كالصناعات الغذائية والتجميل.

كلمات مفاتيح : غراسيلاريا بورسا باتوريس، نمو،أغرة، تغيرات موسمية

RESUME

***Gracilaria bursa-pastoris* de la lagune de Bizerte (Nord de la Tunisie) : étude spatio-temporelle de quelques paramètres hydro-biologiques, du rendement en agar-agar et de sa qualité:** Les études éco-biologiques des algues et leur valorisation sont relativement récentes en Tunisie. Dans ce contexte, une étude sur la rhodophycée *Gracilaria bursa-pastoris* a été entreprise dans la lagune de Bizerte durant une année dans trois localités différentes: Menzel Jemil (MJ), Menzel Abderrahmane (MA) et Menzel Bourguiba (MB). Différents facteurs hydrologiques tels que la température, la salinité, l'oxygène dissous, le pH et les sels nutritifs ont été étudiés. Par ailleurs, la biomasse, le poids individuel des thalles gamétophytiques et tétrasporophytiques, la longueur et le nombre de ramifications à 0.5 cm des apex ont été suivis. En outre, la variation saisonnière du rendement et de la qualité de l'agar-agar ont été investiguées. Les résultats ont montré une variation spatio-temporelle de tous les paramètres étudiés. Ainsi, la température maximale (32,2 °C) a été enregistrée au mois d'août à MB. La salinité a varié entre 33 psu en avril à MA et 41,2 psu en novembre à MB. Le pH a varié entre 8 et 8,8. L'oxygène dissous a atteint 11,2 mg L^{-1} à MJ en Octobre. Les éléments azotés et phosphorés ont fluctué selon la saison et le site d'échantillonnage. Le maximum de poids individuel (134,58 g) a été enregistré en juillet chez les tétrasporophytes à MJ. La longueur maximale (30,5 cm) a été aussi atteinte chez les tétrasporophytes à MB en janvier. Le nombre maximum de ramifications (54) a été enregistré en avril à MA chez les thalles femelles mâtures à cystocarpes. La biomasse maximale (56,23 g m-^{2}, en poids sec) a été obtenue en été à MA. Concernant l'agar, le rendement le plus élevé (27,48 %) a été enregistré au printemps à MJ. La force de gel, le point de gélification et le point de fusion maximum ont également été obtenus au printemps, soit respectivement de 148,33 g cm^{-2}, 40,16 °C et 83 °C. Tous les résultats obtenus indiquent que *Gracilaria bursa-pastoris* pourrait être source potentielle pour une industrie tunisienne d'agar-agar. Ce dernier pourrait être utilisé dans différents domaines tels que les industries agro-alimentaires et en cosmétique.

Mot-clés : *Gracilaria bursa-pastoris*; croissance, agar; saisonnalité

ABSTRACT

In Tunisia, seaweed eco-biological and valorization studies are relatively recent. In this context, the red seaweed *Gracilaria bursa-pastoris* has been studied during a year at three different locations in the Bizerte Lagoon (North of Tunisia): Menzel Jemil (MJ), Menzel Abderrahmane (MA) and Menzel Bourguiba (MB). Environmental parameters such as temperature, salinity and nutrients were measured. The weight, length, number of ramifications at 0.5 cm from the apices in gametophyte and terasporophyte thalli and biomass were investigated. Besides, the seasonal variation of the agar yield and quality was studied. Results showed seasonal and temporal fluctuations of all parameters studied. Thus, the maximum of temperature (32.2 °C) was measured in August at MB. The salinity ranged from 33 psu in April at MA to 41.2 psu at MB in November. The pH varied from 8 to 8.8. The dissolved oxygen reached 11.2 mg L^{-1} at MJ in October. The nitrogen and the phosphorus elements varied also during the study period according to the season and the sampling site. The maximum weight of *Gracilaria bursa pastoris* occurred in summer (134.58g) in the tetrasporophyte thalli at MJ. The highest length was recorded in January (30.5 cm) in tetrasporophytes at MB. The maximum ramification number occurred in April (54) in mature females (cystocarpic thalli) at MA. The highest biomass was obtained in summer (56.23 g m^{-2} dry weight) at MJ. The maximum agar yield (27.48 %) was obtained in spring at MJ. The highest gel strength, gelling and melting temperatures were also recorded in spring (148.33 g cm^{-2}, 40.16 °C and 83 °C, respectively). All of the results indicated that *Gracilaria bursa-pastoris* may be considered as a potential resource for agar industry in Tunisia. This hydrocolloid (agar) may be used in different fields, namely in food and cosmetic industries.

Keywords: *Gracilaria bursa-pastoris*; growth; agar; seasonality

INTRODUCTION

Seas and oceans are considered as an important source of natural substances which are used in different fields. Among marine organisms, interest in seaweeds is increasing worldwide. Several species are used as food; feed, in pharmacy and cosmetic industries, etc. The production of aquatic plants, mostly seaweeds reached 27.3 million tons in 2014 (FAO, 2016). Accordingly, seaweed cultivation is actually practised in several countries such as China, Japan, Chile, Argentina, Philippines, Indonesia and Malaysia to supply hydrocolloid factories with substantial biomass of agarophyte and carragenophyte macroalgae (Zuldin *et al*. 2016). Species of the genus *Gracilaria* are widely distributed and have been reported to be harvested or cultivated for commercial production of agar (Rebello *et al.*1996; Bixler & Porse 2011).These species contribute to around 80% of global agar production (Bixler & Porse, 2011; Araujo *et al.*2014). On the other hand, 100 species of red algae, including *Gracilaria* spp are cultured worldwide for the agar industry. In fact, *Gracilaria* is well known for its high quality of both agar and agarose which are mainly used for food, pharmaceutical and biotechnology applications (Hurtado *et al*.2014). Several studies were reported on G bursa pastoris (Marinho-Soriano, 2012, Korzen *et al.*, 2016). In Tunisia, *Gracilaria* was studied especially in Tunis Lake (Ksouri *et al*.1996; 1997) and in Bizerte Lagoon (Ksouri & Ben Said, 1998; Ben Said & Ksouri, 1999; Ben Said *et al*.2015) to assess the biomass, establish the cartography of *Gracilaria gracilis* (previously known as *G.verrucosa*) in these two sites and study the agar yield and quality of the product. Considering that there is a lack of information on *Gracilaria bursa-pastoris*, this study aims to investigate the spatio-temporal variation of some hydro-biological parameters of this species in Bizerte Lagoon. Agar yield and quality were also examined.

MATERIAL AND METHODS

Sampling sites and environmental parameters

Seaweeds were collected monthly at three locations in the Bizerte Lagoon, in the North of Tunisia: Menzel Jemil (MJ), Menzel Abderrahmane (MA) and Menzel Bourguiba (MB) (9°48'-9°56'E; 37°8'-37°14'N) (Fig.1)

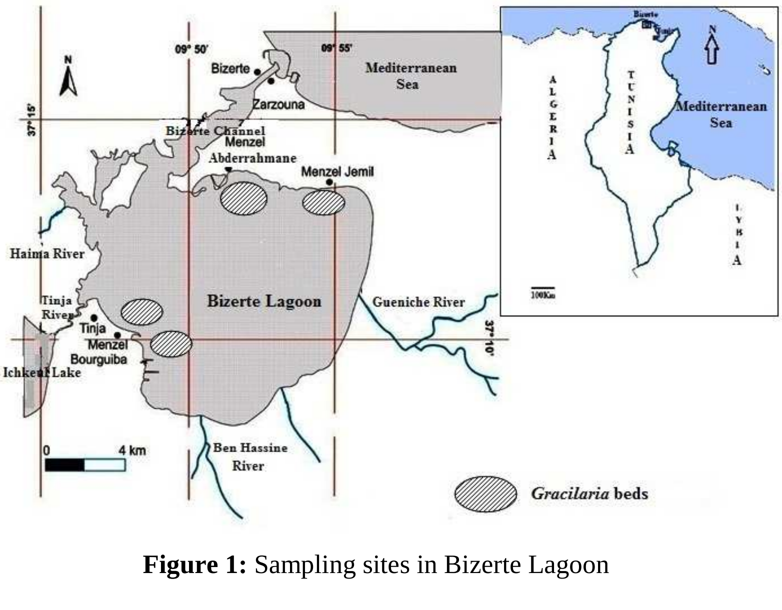


Figure 1: Sampling sites in Bizerte Lagoon

from November 2008 to October 2009. Environmental parameters: water temperature (°C), salinity (psu), dissolved oxygen (mgL-1), pH and nutrients (µM) were also measured during the sampling surveys. The water temperature was recorded "*in situ*", using an electronic thermometer SA880SSXH (Huger type). Salinity, pH, and dissolved oxygen (DO) were recorded with a multiparameter apparatus (HACH, HQ40d). Nutrients: dissolved inorganic nitrogen (DIN) composed of nitrates (NO_3^-), nitrites NO_2^- and ammonium (NH_4^+), total nitrogen (TN), orthophosphates(PO_4^{3-}) and total phosphates (TP) were determined with an auto analyzer 3 Bran Luebbe.

Biotic parameters

The variation in weight, length and number of ramifications of *Gracilaria bursa-pastoris* at 0.5 cm from the apices of seaweed specimens were examined in male gametophytes, mature females (cystocarpic specimens) and tetrasporophytes (Fig.2). The number of samples varied monthly from 15 to 30 according to the availability of seaweeds. The biomass was also estimated using a 50 cm x 50 cm quadrate. In each sampling site, the quadrate was randomly launched 5 times and the seaweeds found in the quadrate were collected, placed in plastic bags and brought to the laboratory. After which, sand, mud and undesirable epiphytes were removed. Seaweeds were then wrung and weighed to determine wet weight and then oven dried overnight at 60 °C until dry weight was obtained. The results of dry and wet weights were converted to the square meter to estimate the average biomass.

Figure 2: Thallus of *Gracilaria bursa-pastoris*

Agar yield and quality

Seaweed samples consisted of a mixture of different life history stages. Spatio-temporal variations in agar yield (% dry weight) and quality: gel strength (g cm^{-2}), gelling and melting temperatures (°C) were determined according to the method described by Ben Said et *al.* (2011).

Statistical analysis

All data are given as mean ± standard deviation (SD) and the two-way analysis of variance (ANOVA) was performed, using the SPSS 17.0 statistical software to determine the effect of season and sampling site on the agar yield, gel strength, gelling and melting temperatures. Level of confidence was set at 5 %. The multi comparison Tukey test was also used to detect differences between means.

RESULTS

Environmental parameters

Results showed that the highest temperature was recorded in July (32.5 ± 0.8 °C) at MB (Fig.3), while the minimum was obtained in January at MJ (9°C).

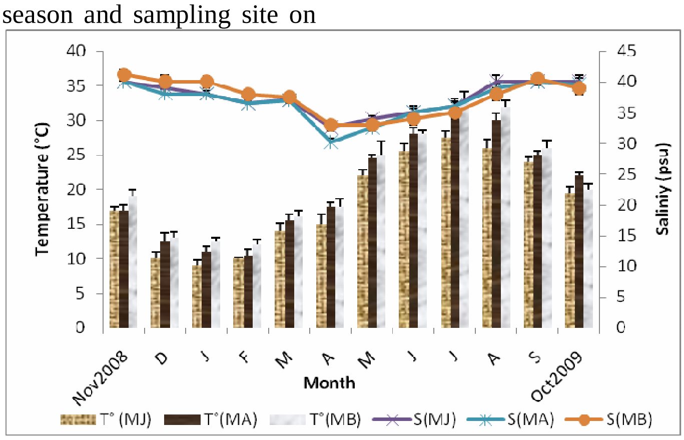


Figure 3: Variation of temperature and salinity. Results are presented as means ± SD, n=3

The highest salinity was 41.2 ± 0.5 psu at MB in November (Fig.3), while the minimum was 30.2 psu at MA in April. The pH varied from 8 (at MJ and MA) to 8.8 (at MJ and MB) (Fig.4). The dissolved oxygen reached 11.2 ± 0.92 mg L^{-1} at MJ in October, while the minimum was 2.82 ± 0.6 mg L^{-1} at the same sampling site in November (Fig. 4). The nitrogen and the phosphorus nutrients varied also depending on the sampling site and the season (Fig. 5). Thus, the nitrate concentration at MJ ranged from 1.69 µM in September to 23.57 µM, in January. At MA, the nitrate concentration ranged from 0.98 µM in October to 9.78 µM in June. At MB, the maximum of nitrate concentration was 0.98 µM in October, while the maximum was 12.72 µM in December. The nitrite concentration varied slightly and did not exceed 1.26 µM, except one value (5.72 µM) which was recorded at MJ in November. The variation in ammonium concentration was great during the study. Thus, at MJ, the minimum was 1.02 µM in December, while the maximum was 8.64 µM in August. At MA, the lowest value was 0.46 µM in December, while the highest one was 9.1 in June. At MB, the ammonium concentration ranged from 0.68 µM in February to 7.49 µM in June. The total nitrogen ranged from16.75 µM to 49.26 µM at MJ. It varied from 16.24 µM to 27.59 µM at MA and from 15.98 µM to 26.23 µM at MB. The orthophosphate ranged from 0.11 µM in February at MJ to 2.43 µM in January at MJ. It varied from 0.17 to 0.74 µM at MA and from 0.08 (February) to 0.42 µM (May) at MB. Regarding the total phosphate, the latter varied from 1.98 µM (November) to 12.35 µM (March) at MJ. The highest value was 4.47 µM (June), while the lowest was 1.96 µM (October) at MA. It ranged from 1.57 µM (November) to 4.68 µM (July) at MB.

Variation in biotic parameters: weight, length, number of ramifications and biomass of *Gracilaria bursa-pastoris*

A spatio-temporal fluctuation of the growth in *Gracilaria bursa-pastoris* was observed throughout the study. Generally, all life –cycle stages did not exist contemporaneously in the same sampling site. In the current study , the relevant results were reported. Thus, the highest average weight was 205.98 ±15.6 g in June for mature female thalli (cystocarpic thalli) at MJ (Fig.6 a). For the males, the weight did not exceed 60g in June at MJ. For the tetrasporophytes, the weight reached around 135 g in July at MJ. The maximum of thalli length (30.5 ± 4.23 cm) was obtained at MB in January for tetrasporophyte specimens (Fig.6 b). The highest length for the female specimens was 28.1 ± 3.2 cm at MJ, while for the males was 24.83±4.2 cm in February at MJ, too. The highest number (54 ±6.45) of ramifications was recorded for mature female thalli in May at MA (Fig.6 c). For the males, the highest number of ramifications was obtained at MJ in May (28±4.487). For the tetrasporophyte thalli, the maximum was 40.2± 6.23 at MA. The maximum biomass in wet weight (405.81 ± 5.01g.m^{-2}) and in dry weight (56.23 g.m^{-2}) was recorded at MJ in summer (June). *Gracilaria bursa-pastoris* was absent in during several months mainly at MJ and MA (Fig.7). The statistical analysis showed in general significant effects of sampling site and season ($p<0.05$) except during some months for the thalli length, number of ramifications and biomass ($p>0.05$).

Agar yield and quality

The highest agar yield (27.48 ± 16.1 % of dry weight) was recorded in spring at MJ (Fig.8 a). The gel strength ranged from 100 ± 10.11 to 148.33 ± 25.20 g cm^{-2} (Fig. 8 b). The highest value was recorded in spring at MA. The gelling temperature varied from 20.98 ± 1.20 to 40.16 ± 6.20 °C (Fig.8c). The melting temperature ranged from 33.00 ± 4.2 to 83.23 ± 8.80°C (Fig.8 d). The statistical analysis showed a highly significant ($p<0.05$) effect of season on agar yield, but there was no significant effect of sampling site. Regarding the agar properties, the statistical analysis showed a discrepancy in results.

DISCUSSION AND CONCLUSION

This study evidenced that *Gracilaria bursa-pastoris* grew differently at the three sampling sites (MJ, MA and MB) in relation with seasonal environmental variations. Nevertheless, growth occurred in spring and summer in the three sampling sites. The different stages of life history did not all occur at the same month and at the same sampling site. In fact, in several months, males, mature females (cystocarpic thalli) and tetrasporophytes din not exist from November to January at MJ and MA. On the contrary, at MB and during the same period, mature females did not exist, but males and tetrasporophytes occurred. This statement suggested the presence of a micro zonation in the lagoon. The environmental parameters in the three sampling sites in the lagoon may influence the maturity, the emission and the elongation of the thalli in concomitance with the seasonal variation of several other factors such as irradiance, photoperiod, water motion, etc (Lobban &Harrison, 1997). This results in the increase of weight, length and number of ramifications and consequently the biomass. Among the abiotic factors, salinity and pH were found the main ones to influence biotic factors. Salinity was always negatively correlated to them. On the contrary, pH was always positively correlated to them. Temperature was inversely correlated with length in male thalli at MB(r=-0.614). Dissolved oxygen was not correlated with biotic factors at three sampling sites. Among nitrogen elements, nitrites were the

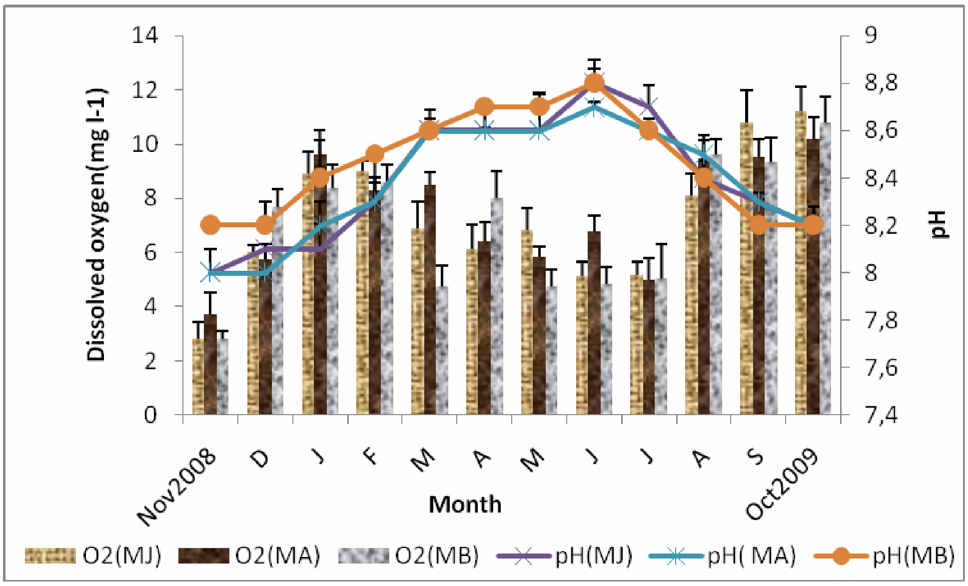
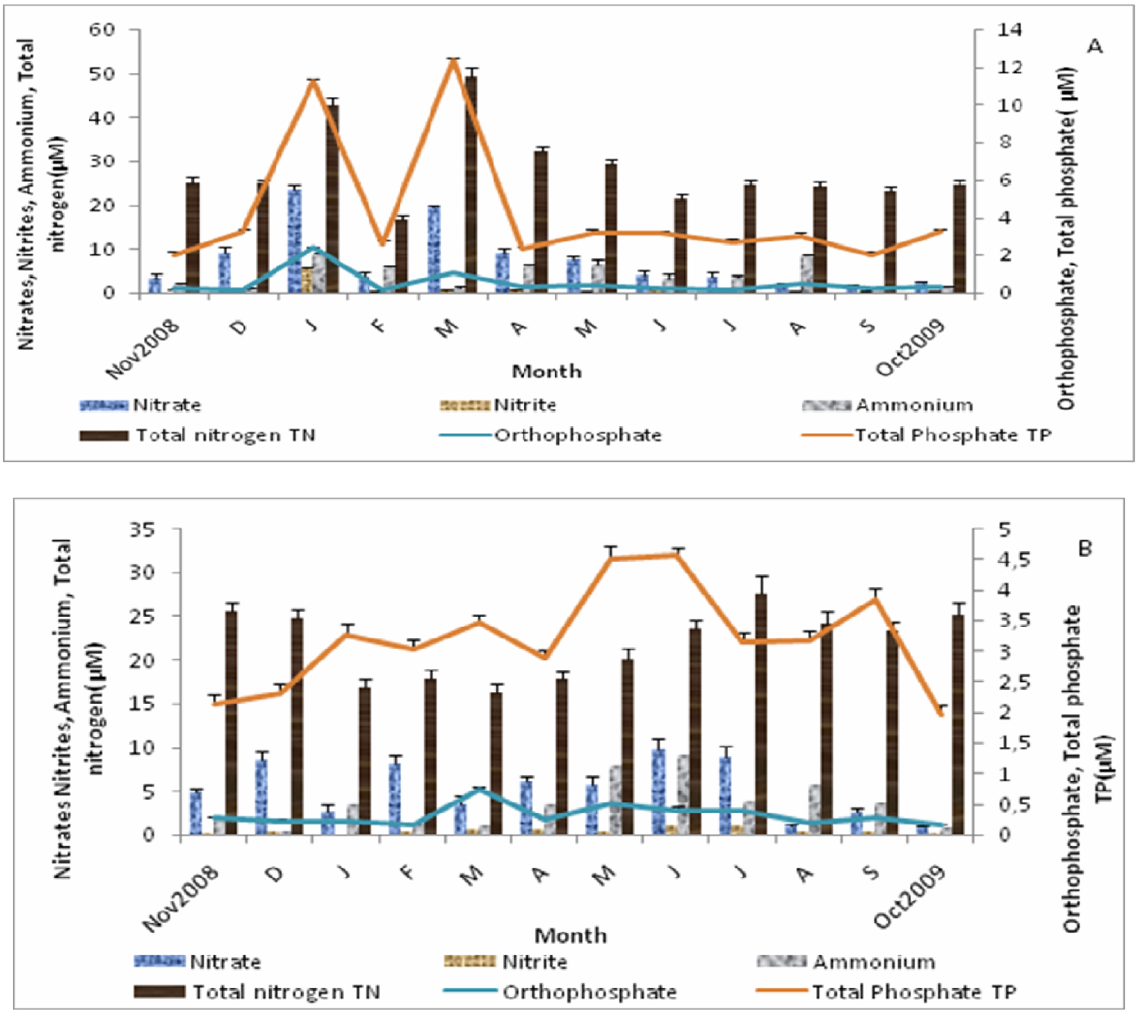


Figure 4: Variation of pH and dissolved oxygen. Results are presented as means ± SD, n=3



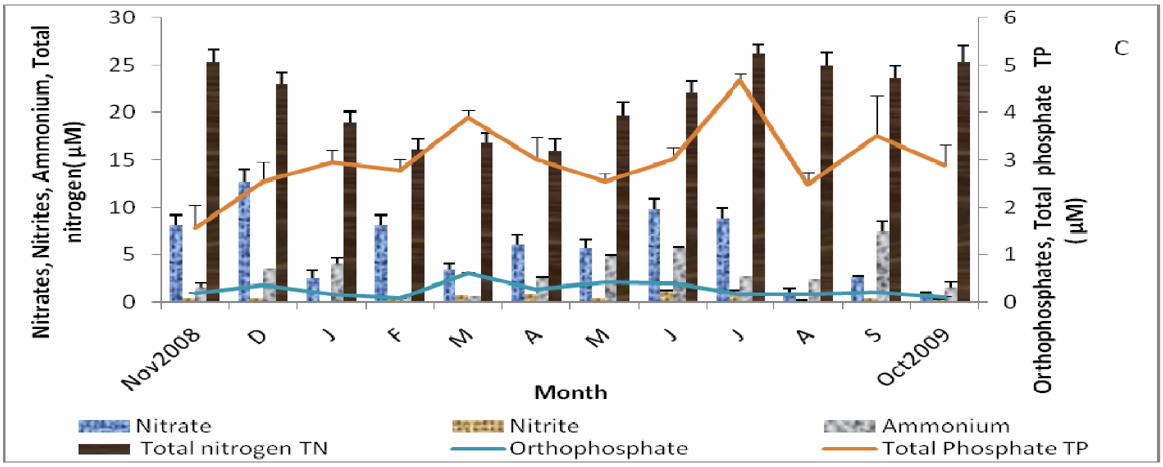
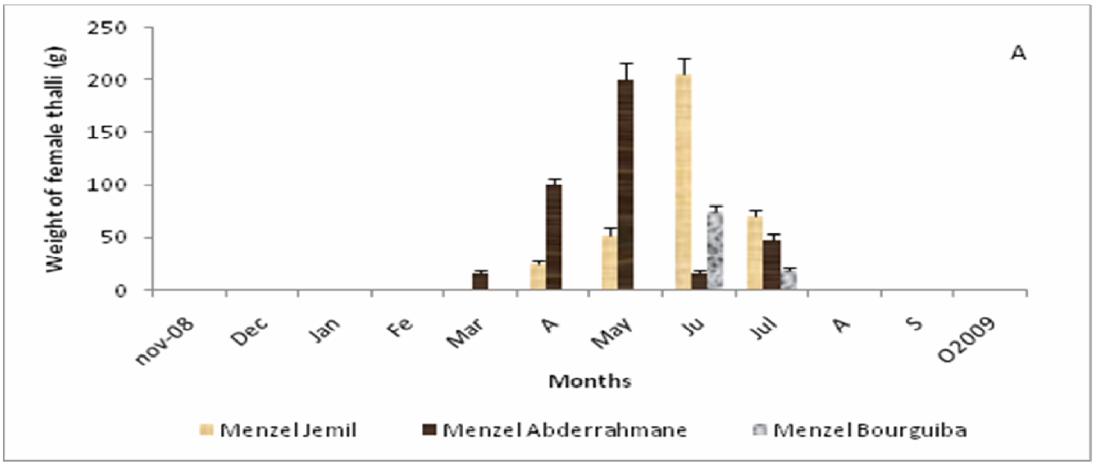
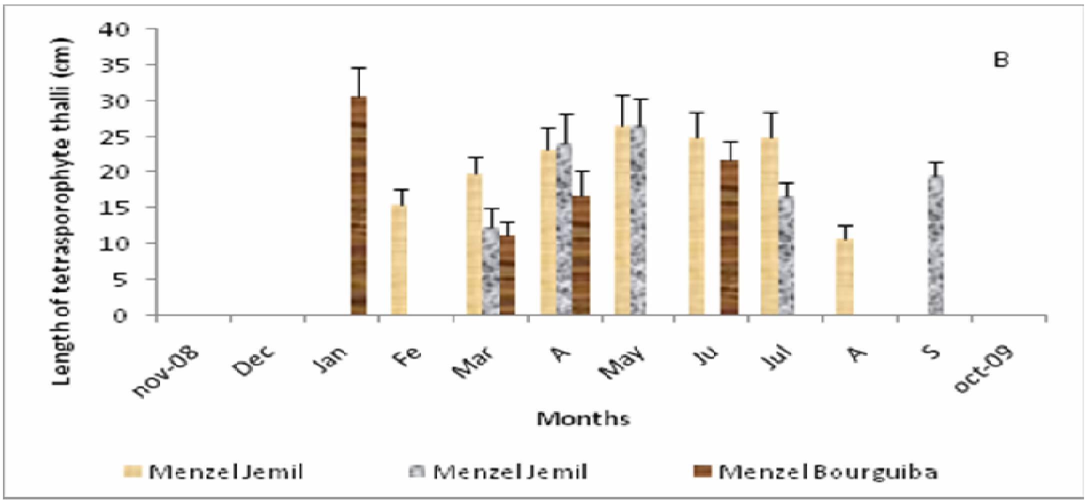


Figure 5: Variation of nitrogen and phosphorus elements (A: MJ; B: MA; C: MB). Results are presented as means ± SD, n=3





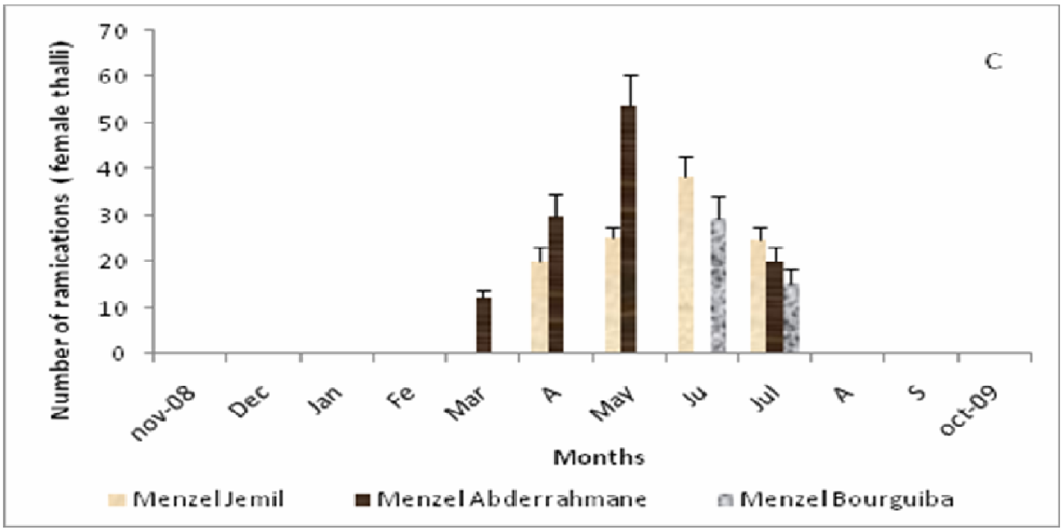


Figure 6: Variation of weight (A), length (B) and number of ramifications (C). Results are presented as means ± SD, n is variable according to the sampling site and month

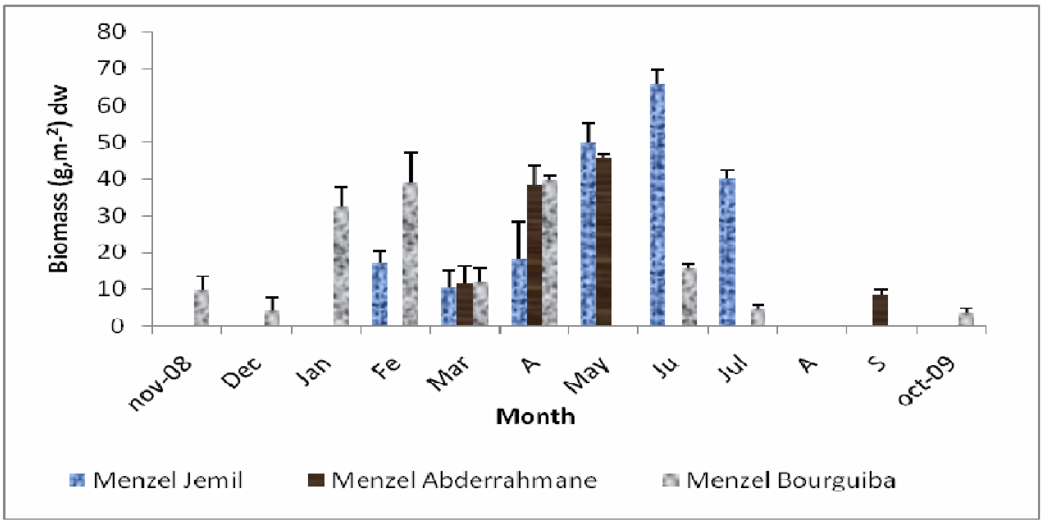
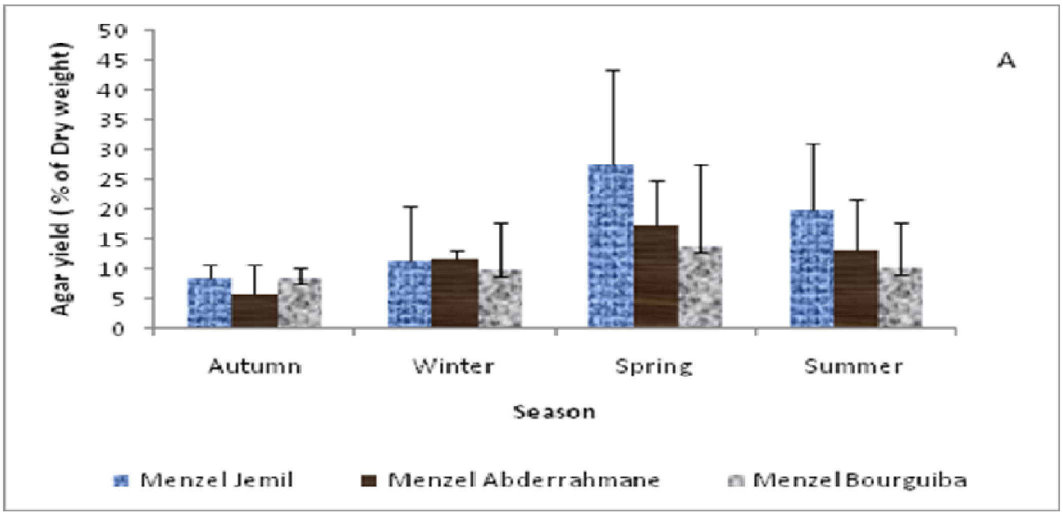


Figure 7: Variation of biomass. Results are presented as means ± SD, n=5



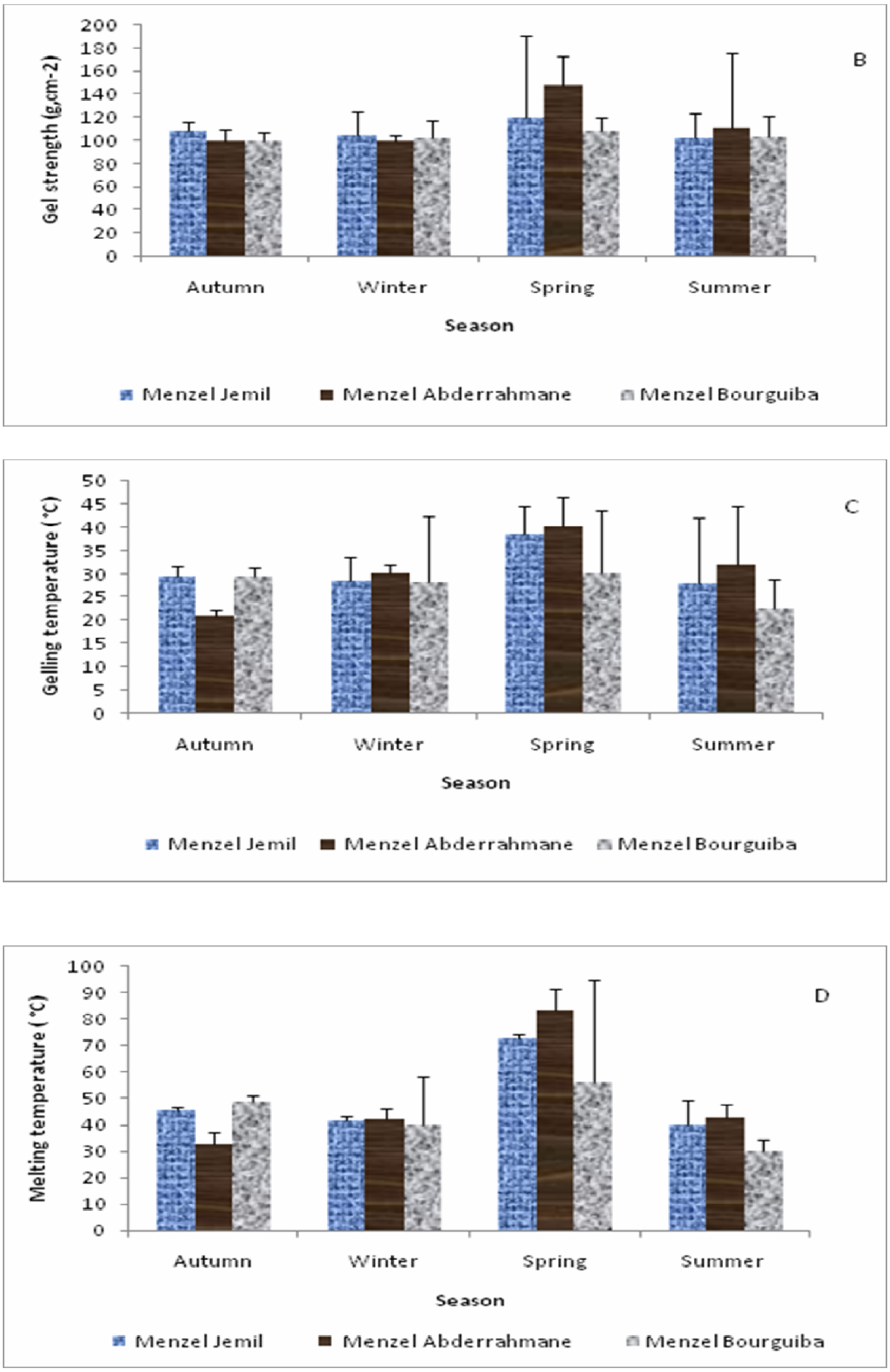


Figure 8: Seasonal variation of agar yield (A), gel strength (B), gelling temperature (C) and melting temperature (D). Results are presented as means ± SD, n=3

most components which influenced growth of different life history stages of *G. bursa-pastoris*, in weight, length and number of ramifications. They were positively correlated, mainly with the weigh, length and number of ramifications in mature females bearing cystocarps at MB(r=+0.692, r= +0.814, r=+0.779, respectively). Phosphates were not strongly correlated with biotic parameters studied. The absence of *G. bursa-pastoris* during several months indicated that this species is fragile. In fact, it tolerates environmental variations less than *G. gracilis* which lives in the same lagoon as observed during the study. In July and August, the thalli of *G. bursa-pastoris* became yellow colored and the branches were easily detachable. This stage is known as the senescence. On the other hand, the biomass was lower than that reported by Ben Said & Ksouri (1999) for *G. verrucosa* (350±98g m^{-2} dry weight) at the same period (June). This statement led us develop seaweed farming in order to increase the biomass.

Concerning the seasonal variation in agar yield, our results indicated that the highest values were recorded in spring regardless of the sampling site. This result is in conformity with previous studies on *G. verrucosa* (Ben said & Ksouri, 1999). In Thau lagoon in France, Marinho-Soriano & Bourret (2003) found that the greatest agar yield of *Gracilaria –bursa-pastoris* was recorded in summer (36%) and the lowest in winter (23%). Regarding the agar properties, namely gel strength, our findings are higher than those reported by Marinho-Soriano *et al*.(1999) for all of the life history stages of *G. bursa-pastoris*, but lower than the one reported by Marinho-Soriano & Bourret (2003) for the same species. Compared to the Gelidiales, the rheological properties of agar extracted from *G. bursa-pastoris* are generally lower. Thus, in *Gelidium spinosum*, the gel strength reached more than 700 g cm^{-2}. In addition, the highest gelling and the melting temperatures reached more than 34 °C and 94 °C, respectively (Ben Said *et al*. 2011). The results obtained in this study suggest that geographical location and seasonal environmental factors influence very likely the agar synthesis and rheological properties of extracted hydrocolloids, besides the life history stages of the species. In this context, Rebello *et al.* (1996) reported that *Gracilaria gracilis* had the highest agar yield and gel strength in early summer and spring, which is in conformity with the results obtained by Martin *et al.* (2013). On the other hand, in several studies, light, nutrient levels, especially nitrogen level have been reported to be important factors affecting growth, agar yield and quality of *Gracilaria* (Bird, 1980; Bird & Ryther, 1990; Molloy & Bolton, 1996; Arano *et al*.2000).In the current study, a strong correlation was found between the orthophosphate concentration on the one hand and the agar yield at MB ($r = +0.993$) and gel strength at both MA and MB($r =+0.979$, $r =+ 0.990$, respectively), on the other hand. No correlation was found between nitrogen concentration and agar yield and rheological properties.

In fact, the gel strength, gelling and melting temperatures are the result of the agar composition. The low gel strength is the result of the lowly methylated and high sulfated galactan. This agarocolloid is mainly composed of the galactosyl-6 sulfate residues considered as the putative precursor of the 3,6-anhydrogalcatose (Mollet *et al.* 1998), whereas the methoxyl groups influence the gelling temperature (Guiseley,1970), the sulfate groups influence the gel strength and melting temperature (Duckworth & Yaphe,1971). On the contrary, Lee *et al*. (2014) reported that both the 3, 6 anhydrogalactose and total sulfate esters content were found to have no relationship with the agar gel strength. In the current study, the extracted agar may have lowly methylated and high sulfated galactans. Therefore, the polysaccharide composition should be analyzed to have a clearer idea on the agar from *G. bursa-pastoris.*

In conclusion, the presence and growth of *Gracilaria bursa-pastoris* in Bizerte lagoon depends on both abiotic factors and biotic ones. The highest agar yield and quality parameters were always obtained in spring regardless of the sampling site. Hence, seaweeds should be harvested in spring and early summer, which coincided with the highest biomass. The obtained agar, despite the low gel strength, could be valorized in some fields such as cosmetic and food industries. The cultivation is recommended to supply potentially agar industry in Tunisia in future.

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