SEASONAL VARIATION ON BIOMASS AND AGAR QUALITY EXTRACTED FROM THE MARINE RED ALGAE *PTEROCLADIA CAPILLACEA* AND *HYPNEA MUSCIFORMIS* GROWING ALONG MEDITERRANEAN SEASHORE OF ALEXANDRIA, EGYPT

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Abstract

Seasonal biomass variation, agar yield and some agar properties of *Pterocladia capillacea* and *Hypnea musciformis* were examined during the period from July 2003 to June 2004. The maximum biomass values of both studied algae were recorded during winter and the minimum values were obtained during spring and summer. Agar yield of *P. capillacea* varied from a minimum of 26.6% (dry weight) in August, to a maximum value of 35.6% in October, while that of *H. musciformis* varied from a minimum of 15.3% at June, to a maximum of 24% at December. The maximum agar gel strengths were recorded at July (being 225 and 158 gcm^{-2} of *P. capillacea* and *H. musciformis*, respectively). Gelling and melting temperatures showed significant seasonal variation for both studied algal species. Agar yield and quality of *P. capillacea* and *H. musciformis* were within the range of accepted commercial values. Yield and gel properties of agar extracted from *H. musciformis* were comparable with those obtained from *P. capillacea*. Results also indicated that *H. musciformis* can be considered as a good candidate for commercial agar use.
Keywords: Agar, *Hypnea musciformis, Pterocladia capillacea,* Red seaweeds.

Introduction

Most of agar production in the world is derived from the red seaweed genera *Gracilaria* (53%) and *Gelidium* (44%). Only a small quantity (3%) is produced from other agarophytes like *Gelidiella* and *Pterocladia* (McHugh, 1991; Marinho-Soriano; Bourret, 2003). It has a wide variety of use as human and animal food in addition of numerous industrial, biological and medical applications for its excellent thickening and gelling properties (Armisén, 1995; Mtolera and Buriyo, 2004; Meena *et al*., 2006; Pereira-Pacheco *et al*., 2007). Biochemistry of agar is known to change in response to several factors. Thus, agar yield and quality not only depend on its specific characteristics, but are closely related to species and time of collection (Marinho-Soriano *et al*., 1999). Although the quality of agar is usually judged in terms of its technical performance as a gelling agent (gel strength), other parameters are also important

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to determine its use in food industry, such as gelling and melting temperatures (Pereira-Pacheco *et al*., 2007).

The poor quality of agar obtained from species of *Gracilaria* make them useful in other applications such as low fat and soft-texture products. Thus, researchers concentrated their work on the extraction of agar from other red seaweeds (Armisén, 1995; Pereira-Pacheco *et al*., 2007). *Pterocladia* is one of the most genera that comprise the greatest number of the rhodophytes distributed along the seashore of Alexandria (Egypt) (Nasr *et al*., 1966; Rao and Bekheet, 1976). It has been observed that the quality of agar extracted from *Pterocladia*, collected from Alexandria is higher than a lot of other known agarophyte species at different countries (Rao and Bekheet, 1976). Although there are many studies on taxonomy and distribution of other species of red seaweeds grown along the seashore of Alexandria but a little is known on their agar content and gel properties. *Hypnea musciformis* is one of the red algae inhabiting most shallow seashores of Alexandria all over the year (Nasr *et al*., 1966).

This study investigates the effect of seasonal variation on agar yield and quality of the native agarophyte *Pterocladia capillacea* as well as *Hypnea musciformis* as a promising agar source.

Materials and Methods

Study site

Abu-Qir Bay is a semi-enclosed basin located about 36 km East of Alexandria (Fig. 1). It lies between 30°05'- 30°22' E and 31°16'- 31°21' N. This western inshore area of the bay is a shallow region (with average depth of 3.8 m), interrupted with several rocky islets.

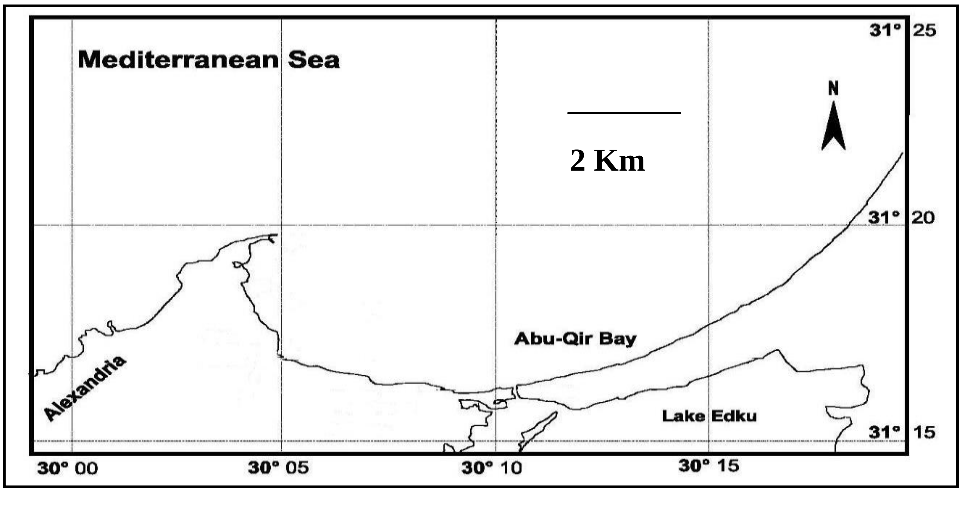

Figure (1): Sampling site in the Southwestern part of Abu-Qir Bay, Alexandria, Egypt.

Sampling and biomass estimation

Healthy individuals of *Pterocladia capillacea* (Gmelin) Bornet and Thuret and *Hypnea musciformis* (Wulfen) Lamouroux were collected monthly from July 2003 to June 2004 (in triplicates) using a square metallic frame (0.25 m^2). Algal samples were brought to the laboratory in dark plastic bags, where they were identified, washed with tap water to remove epiphytes and impurities, weighed and then oven dried at 60^0C till a constant weight, prior to agar extraction.

Agar extraction and quality determination

The native agar extraction was performed according to the method described by Marinho-Soriano *et al*. (1999). Each dried sample was ground and well mixed before use. Extraction of agar was carried out by boiling a sub-sample (10g) of the dried algae in 600 mL distilled water, adjusted to pH 6.5 in an Erlenmeyer flask and heated for 1 h at 110^0C in an autoclave, after which the extract was filtered through a cotton tissue. The residue was re-extracted under the same conditions. The filtrate was allowed to gel at the room temperature and then placed in a freezer overnight. The frozen gel was thawed, washed with distilled water to remove impurities and dried for 24 h at 60^0C. All extractions were carried out using triplicate samples. The agar yield (%) was calculated as the percentage of the dry matter.

Gel strength (g cm^{-2}) was performed on aliquots of 4mL of 1.5% w/v agar solution in 0.1 M KCL in plastic vials (Hurtado-Ponce and Umezaki, 1988), using a cylindrical plunger 10 mm in diameter**,** by increasing the weight load on the gel surface until the point of gel breakage.

The gelling and melting temperatures were measured according to the method described by Craigie and Leigh (1978) and Murano *et al*. (1992). For gelling temperature measurement, 10 mL agar solution was allowed to cool gradually and a thermometer was emerged in the solution. The temperature at which the thermometer was fixed to the gel was noted. Melting temperature was determined with glass beads suspended on the gel. The gels were heated stepwise till the glass beads sank into the gel**.**

Statistical analyses

Data were analyzed statistically using one-way analysis of variance (ANOVA, $P<0.05$) using a computer statistical analysis software program (COSTAT 2.0).

Results

Maximum biomass values of *P. capillaceae* were recorded during December, January and February while the minimum values were recorded during April, May, June, July and August (Fig. 2). The major peak was observed in December (486 gm^{-2}), while the lowest value was recorded in June (156 gm^{-2}). Biomass production of *P. capillaceae* was significantly decreased from January to

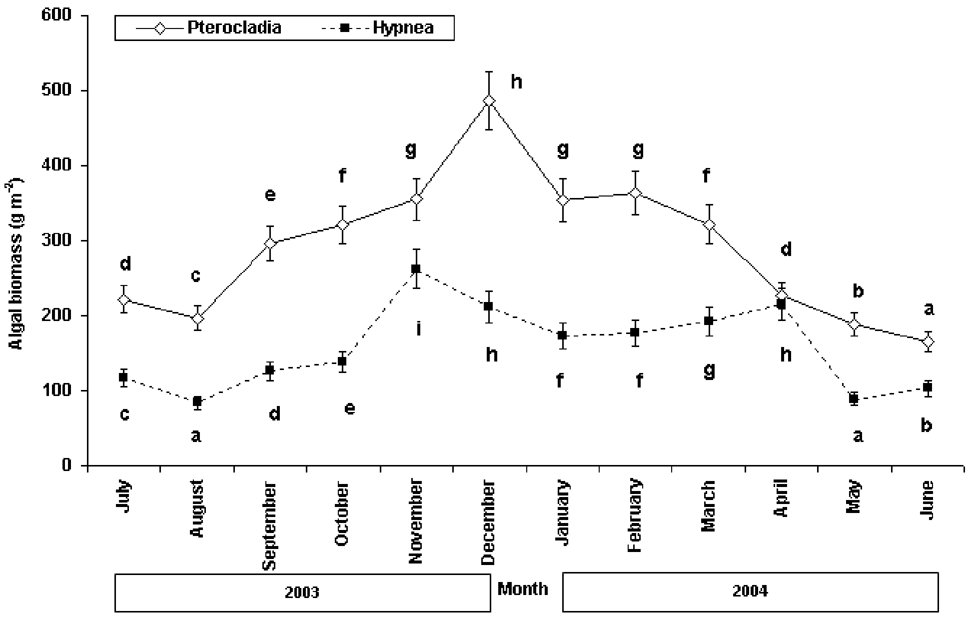

Figure (2): Variation in biomass (gm^{-2}) of *Pterocladia capillacea* and *Hypnea musciformis*. Vertical bars represent the standard deviation, different letters for each species means significantly different at $p < 0.05$

June, and then gradually increased till December (Fig. 2). A significant fluctuation on the biomass of *H. musciformois*, (with minimum values of 89 and 84 gm^{-2}) was observed in May and August, respectively. However, the maximum value (355 gm^{-2}) was observed in November (Fig. 2). Results showed that the biomass of the studied species varied significantly along the study period, $p < 0.05$ (Fig. 2 and Table 1).

Table (1): One way ANOVA calculated to test monthly variations in biomass and agar yield and properties for *Pterocladia capillacea* and *Hypnea musciformis* along the sampling period.

	Pterocladia capillacea		***Hypnea musciformis***	
Source of variation	**$LSD_{0.5}$**	**P**	**$LSD_{0.5}$**	**P**
Biomass	**8.224838065**	***	**8.320478931**	***
Agar Yield	**1.666471373**	***	**2.090317041**	***
Gel strength	**3.66533051**	***	**5.620569977**	***
Gelling temperature	**2.232175763**	***	**3.043196585**	***
Melting temperature	**0.839149476**	***	**3.680678509**	***

***: Means highly significant at $P < 0.05$.

Agar yield of *P. capillacea* and *H. musciformis* showed a well-defined variation along the study period. The agar yield of *P. capillacea* varied from the minimum value of 26.6 % in August, to the maximum value of 35.6% in October. While the values of *H. musciformis* were always lower than *P. capillacea* since they ranged from a minimum of 15.3% at June to a maximum of 24% at December (Fig. 3). Variation in agar yield of both studied seaweeds was statistically significant along the study period as shown by ANOVA test (Table1).

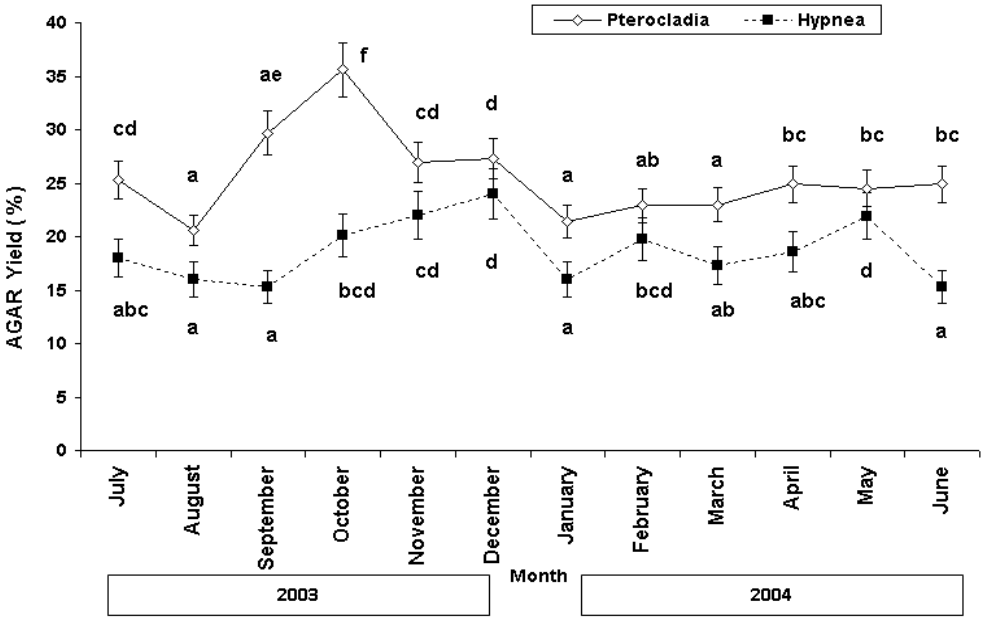

Figure (3): Variation in agar yield (%) of *Pterocladia capillacea* and *Hypnea musciformis*. Vertical bars represent the standard deviation, different letters for each species means significantly different at $p < 0.05$.

Statistical analysis for gel strength of the considered algae showed significant differences along the study period. The maximum strength values of *P. capillacea* and *H. musciformis* ($225 gcm^{-2}$ and $158 gcm^{-2}$ respectively) were recorded at July (Fig. 4A). The gelling and melting temperatures varied significantly among the seasons in both algal samples. Agar extracted from *P. capillacea* had a maximum gelling and melting temperatures (62.9 and 90.7˚C) at July and February, respectively. For *H. musciformis*, the maximum gelling and melting temperatures (62 and 97˚C) were recorded at February and July, correspondingly. Although the variation was significant along the study period but there was no a regular trend, where several gelling and melting temperature peaks were observed for both studied species at different months.

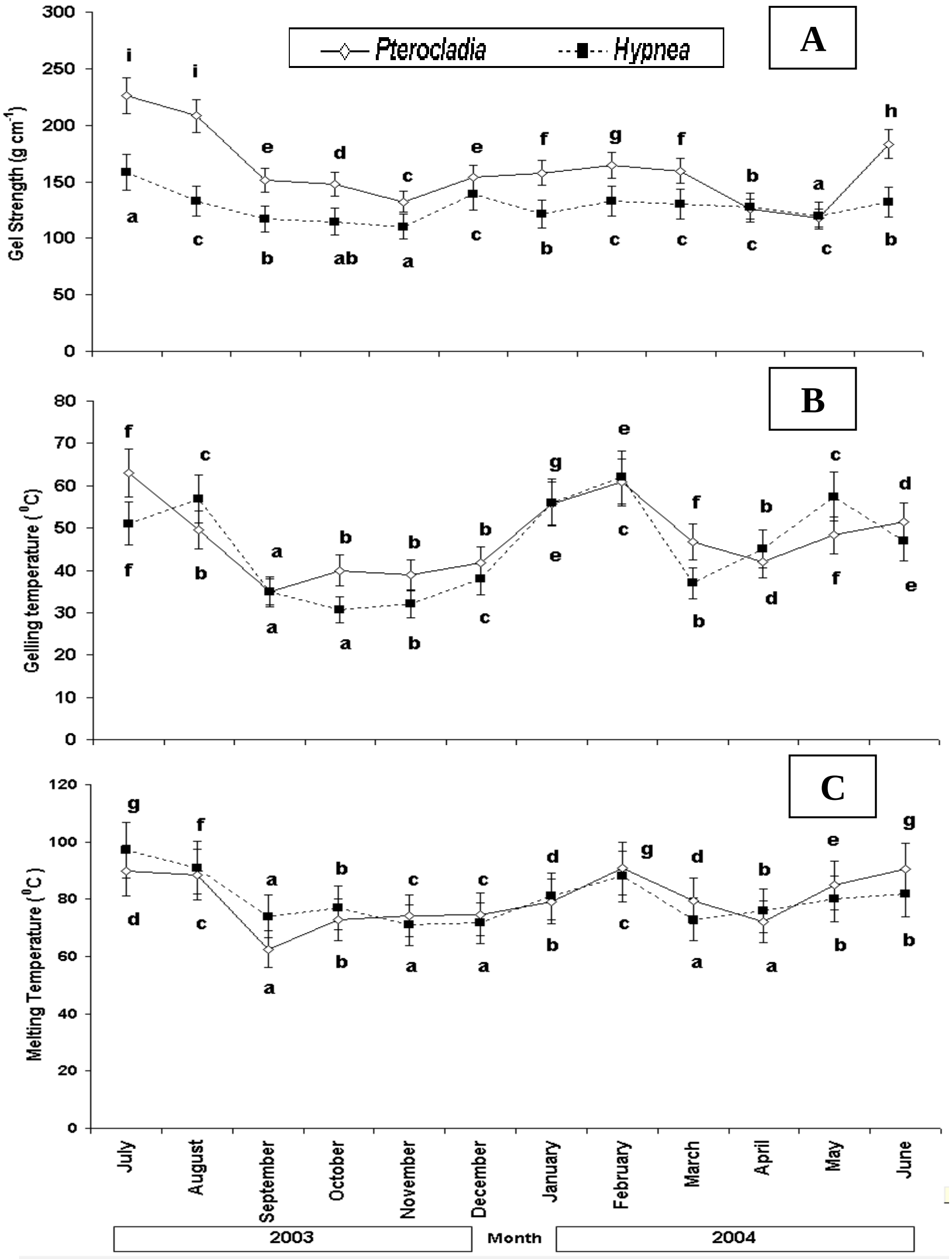

Figure 4: Variations in (A) Gel Strength (gcm^{-2}), (B) Gelling Temperature ^{o}C and (C) Melting Temperature ^{o}C of agar from *Pterocladia capillacea* and *Hypnea musciformis*. Vertical bars represent the standard deviation, different letters for each species means significantly different at $p < 0.05$

Discussion

The algal biomass in this study showed a different seasonal variation. *P. capillacea* showed their minimum biomass values from May to August and maximum values from December to January. The peak (486 gm^{-2}) recorded in December, coincides with high rainfall season (December, January, February) which was in contrast with the results of Luhan (1996), who working on a rhodophycean alga *Gracilaria heteroclada*, and the work of Pondevida and Hurtado-Ponce (1996) on *Gracilaria bailinae*.

The high biomass values recorded during winter season are probably due to improved nutrient availability (Buriyo and Kivaisi, 2003) and/or low light intensity. Where high light intensity accompanied with high temperature (in summer season) may lead to reduce algal productivity partialy due to photoinhibition (Krause and Weis, 1991) and desiccation especially for intertidal algae (Moorjani, 1982). A gradual decrease in algal biomass values were observed between May and August. This period was associated with high light intensities and high temperature. Furthermore, this period normally has relatively longer hours of sunshine, which may affect benthic intertidal seaweeds (Moorjani, 1982). The detrimental combination effects of light stress, desiccation, higher salinity and nutrient limitation probably conducted to the decline of algal biomass.

Agar yield showed fairly significant variation along the study period, and this was true for both *P. capillacea* and *H. musciformis*. There was clear relationship between agar yield and biomass. Where, their peaks were observed nearly at the same period from October to December, this is in contrast with the work on other red algae revealed by (Freile-Pelegrin, 2000; Marinho-Soriano and Bourret, 2003). The agar yield of *P. capillacea* during the whole study period was slightly higher than that of *H. musciformis*. However, the range of agar yield in this study for both algae species was comparable to values found for the other agarophytes species (Durairatnam *et al*., 1990; Matsuhiro and Urzua, 1990). Previous studies on agarophytes have shown that the seasonal variation of biomass and agar yield can or cannot exhibit a similar pattern. As in the study of Marinho-Soriano (1999) who observed that the peak of biomass and agar yield of *Gracilaria bursa-pastoris* were during the summer**;** while Whyte *et al*. (1981) found inverse relationship between these two parameters in *Gracilaria gracilis.*

The process responsible for agar degradation at different seasons could be of bacteriological or enzymatic origin (Armisén, 1995; Kapraun, 1999). In fact, this loss in gel parameters may be due to hydrolysis (Armisén, 1995). Although these parameters were fluctuated within seasons, but at the end of this study, agar quality characteristics were as good as those recorded for other red species achieved by (Rebello *et al.*, 1996; Zemke-White and Ohno, 1999; Zemke-White *et al*., 1999; Freile-Pelegrin, 2000).

It is evident from this study that time of harvesting algal biomass for agar production is a significant factor. Furthermore, high-quality of agar extracted production is a significant factor. Furthermore, high-quality of agar extracted from *H. musciformis,* encourage the usage of this alga as a good source for commercial agar production.

References

Armisén, R. (1995). World-wide use and importance of *Gracilaria*. *J. Appl. Phycol*., **7:231-243**.

Buriyo, A. S. and Kivaisi, A. K. (2003). Standing Stock, agar yield and properties of *Gracilaria salicornia* harvested along the Tanzanian coast. Western Indian Ocean J. Mar. Sci., 2(2): 171-178.

Craigie, J. S. and Leigh, C. (1978). Carrageenans and agars. In: Hellebust J A; Craigie J S (Eds.), Hand book of phycological Methods. Cambridge Univ. Press, Cambridge. **Pp: 109-131**.

Durairatnam, M.; Medeiros, T. M. B. and Sena, A. M. (1990). Studies on the yield and gel strength of agar from *Gracilaria domingensis* Sonder ex Kuetzing (Gracilariales, Rhodophyta) following the addition of calcium. *Hydrobiologia*, **204/205: 551-553**.

Freile-Pelegrin, Y. (2000). Does storage time influence yield and agar properties in the tropical agarophyte *Gracilaria cornea*. *J. Appl. Phycol*., **12:153-158**.

Hurtado-Ponce, A. Q. and Umezaki, I. (1998). Physical properties of agar gel from *Gracilaria* (Rhodophyta) of the Philippines. *Bot. Mar*., **31:171-174**.

Kapraun, D. F. (1999). Red algal polysaccharide industry: economics and research status at the turn of the century. *Hydrobiologia*, **398/399:7-14**.

Krause, G. H. and Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., **42:313-149**.

Luhan, M. R. J. (1996). Biomass and reproductive status of *Gracilaria heteroclada* Zhang et Xia collected from Jaro, Central Philippines. *Bot. Mar*., **39:207-211**.

Marinho-Soriano, E. (1999). Biomass and agar yield of *Gracilaria bursa-pastoris* in a Mediterranean lagoon. *Seaweed Res. Utili*., **21(1&2):1-8**.

Marinho-Soriano, M. and Bourret, E. (2003). Effect of season on the yield and quality of agar from *Gracilaria* species (Gracilariaceae, Rhodophyta). *Bioresource Technol.*, **90:329-333**.

Marinho-Soriano, E.; Bourret, E.; De Casabianca, M. L. and Maury, L. (1999). Agar from the reproductive and vegetative stages of *Gracilaria bursa-pastoris*. *Bioresour. Technol*., **67:1-5**.

Matsuhiro, B. and Urzua, C. (1990). Agars from *Gracilaria chilensis* (Gracilariales). *J. Appl. phycol*., **2:273-279**.

Mc Hugh, D. J. (1991). Worldwide distribution of commercial sources of seaweeds including *Gelidium*. *Hydrobiologia*, **221:19-21**.

Meena, R.; Prasad, K. and Siddhanta, A. K. (2006). Studies on “Sugar-reactivity” of agars extracted from some Indian agarophytes. Food *Hydrocolloids*, **20:1206-1215**.

Moorjani , S. A. (1982). Rocky shores zonation in Kenya: horizontal and vertical distribution patterns in the marine flora. Proc. Symp. Coast. & Mar. Envir. Red Sea, Gulf of Aden and Trop. West.Indian Ocean, Vol.2 AESCO/UNESCO, Khartoum.

Mtolera, M. S. P. and Buriyo, A. S. (2004). Studies on Tanzanian Hypneaceae: Seasonal variation in content and quality of Kappa-Carrageenan from *Hypnea musciformis* (Gigartinales: Rhodophyta). Western Indian Ocean. *J. Mar. Sci*., **3(1):43-49**.

Murano, E.; Toffarin, R.; Zanetti, F.; Knutsen, S. H.; Paolettis, S. and Rizzo, R. (1992). Chemical and macromolecular characterization of agar polymers from *Gracilaria dura* (C. Agardh) J. Agardh (Gracilariaceae, Rhodophyta). *Carbohyd. Polym*., **18:171-178**.

Nasr, A. H.; Mohsen, A. F. and Bekheet, I. A. (1966). Effect of salinity and temperature variation on *Pterocladia capillacea*. *Hydrobiologia*, **27:395-400**.

Pereira-Pacheco, F.; Robeldo, D.; Rodríguez-Carvajal, L. and Freile-Pelegrín, Y. (2007). Optimization of native agar extraction from Hydropuntia cornea from Yucatán, México. *Bioresources Technol*., **98:1278-1284**.

Pondevida, H. B. and Hurtado-Ponce, A. Q. (1996). Assessment of some agarophyte from the coastal areas of Tloilo, Philippines I. Seasonal variations in the biomass of *Gracilaria changii*, *Gracilaria manilaensis* and *Gracilariopsis bailinae* (Gracilariales, Rhodophyta). *Bot. Mar*., **39:117-122**.

Rao, A. V., Bekheet, I. A. (1976). Preparation of agar-agar from the red seaweed *Pterocladia capillacea* of the coast of Alexandria, Egypt. *Appl. Environ. Microbiol*., **32(4):479-482**.

Rebello, J.; Ohno, M.; Critchley, A. T. and Sawamura, M. (1996). Growth rates and agar quality of Gracilaria gracilis (Stackhouse) Steentoft from Namibia, Southern Africa. *Bot. Mar*., **39:273-279**.

Whyte, J. N. C.; Englar, J. R.; Saunders, R. G. and Lindsay, J. C. (1981). Seasonal variations in the biomass, quantity and quality of agar from the reproductive and vegetative stages of *Gracilaria (Verrucosa type). Bot. Mar*., **24:493-501**.

Zemke-White, W. L. and Ohno, M. (1999). World seaweed utilization: An end-of-century summary. *J. Appl. Phycol*., **11:369-376**.

Zemke-White, W. L.; Bremner, G. and Hurd, C. L. (1999). The status of commercial algal utilization in New Zealand. *Hydrobiologia*, **398/399:487-494**.

التغيرات الموسمية للكتلة الحيوية و نوعية الآجار المستخرج من الطحالب البحرية الحمراء *تيروكلاديا كابيليشيا و هبنيا ميوسيفورمز*، النامية على ساحل البحر المتوسط ,الإسكندرية, مصر

علاء أحمد فتحي و نجوى جمال الدين محمدي

قسم النبات - كلية العلوم - جامعة الإسكندرية

تمت دراسة التغيرات الموسمية للكتلة الحيوية، و إنتاجية الآجار و بعض صفات الآجار المستخلص من *تيروكلاديا كابيليشيا* و *هبنيا ميوسيفورمز* النامية على امتداد سواحل الإسكندرية بمصر. و قد سجلت أكبر قيمة للكتلة الحيوية لكلا الطحلبين محل الدراسة خلال أشهر الشتاء، في حين سجلت أقل القيم في أشهر الربيع و الصيف. وقد تباينت إنتاجية الآجار المستخلص من طحلب *تيروكلاديا*، حيث تراوحت من قيمتها الدنيا 26.6 % (وزن جاف) في شهر أغسطس، و سجلت أعلى قيمة 35.6% في شهر أكتوبر. في حين تباينت قيم الآجار المستخلص من *هبنيا ميوسيفورمز* من أدنى قيمها 15.3% في شهر يونيو إلى أعلى قيمها 24% في شهر ديسمبر. وقد سجلت أعلى قيمة مقاومة للجيلاتين المستخرج من كلا الطحلبين في شهر يوليو. وقد كانت قيم إنتاجية الآجار و نوعيته لكلا الطحلبين محل الدراسة مقبولة بالمعايير التجارية. وقد لوحظ أن إنتاجية الآجار و نوعية الجيلاتين المستخرج من *هبنيا ميوسيفورمز* مشابهة لحد كبير لتلك القيم الخاصة للطحلب *تيروكلاديا كابيليشيا* و المعروف بكونه من المصادر الهامة لاستخراج الآجار. ونتائج هذه الدراسة من شأنها تعضيد فكرة استخدام الطحلب الأحمر *هبنيا ميوسيفورمز* كمصدر لاستخراج الآجار بجانب الطحلب المعروف *تيروكلاديا كابيليشيا*.