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Influence of Different Habitats on The Chemical Constituents of *Codium tomentosum*

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THE CURRENT study was conducted to assess the influence of different harvesting site (habitat) on the chemical constituents of *Codium tomentosum* Stack collected from two different sites on the coast of Egypt (Mediterranean [M] and Red [R] Seas). The current results showed that, salinity and temperature of the Red Sea is relatively higher than those of the Mediterranean one. Additionally, results indicated significant differences in the biochemical profiles of *Codium* between the two sampling sites. The total carbohydrate, proline, glycerol content together with total antioxidant capacity, reducing power as well as mineral content (Na^+, K^+ and Ca^{++}) and the activities of antioxidant enzymes (PPO, POX, ASO, APX and CAT) of *Codium* collected from site R were significantly higher than of those collected from site M. In contrast, total protein, total amino acids, flavonoids contents were predominated in *Codium* harvested from the Mediterranean Sea. No significant differences in ascorbic acid, glutathione, chlorophyll a content and total phenols collected from the two sites. The electrophoretic analysis of protein pattern revealed the appearance of of 7 and 8 polypeptide bands in *Codium* from the two sites (M and R, respectively). However polypeptides with molecular weights 93, 72 and 42kDa were recorded only for *Codium* of site (M), while that of 17 kDa was specific for *Codium* of site (R). In conclusion, this study shows that the different sampling sites influences on biochemical profiles of *Codium*. However, *Codium* in response to extreme temperatures and salinity, may alter its metabolism, building a strong protection system and producing compatible solutes in various ways to overcome these stress. This result is a step towards to study the algal diverse biochemical integrations involved in cellular adaptation of algae to different environmental factors and habitats.

Keywords: Seaweeds with seasonal and location variations, Biochemistry of macro-algae, *Codium tomentosum*, Physico-chemical characters of Mediterranean and Red Seas.

Introduction

Seaweeds are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods (Robledo & Pelegrin, 1997). Chemical composition of the macro-algae vary depending on geographical distribution, habitats, maturity, seasons and the principal environmental conditions, such as water temperature, salinity, light, and nutrients (Ortiz et al., 2006 and Messyasz & Rybak, 2010). Salinity and temperature represents a dominant factor affecting both the local distribution (Leland et al., 2001). Temperature has proved to be of paramount importance (Van den Hoek, 1984), however a certain stage of an algal life cycle usually is sensitive to temperature. Also salinity can act as a limiting factor in the distribution of macro-algal species. Algae have been attracted the attention of scientists specially those growing under salinity stress (Mansour et al., 2017). They can serve as model organisms for a better understanding of salt acclimation in the more complex physiological processes of higher plants (Kakinuma et al., 2006 and Ashraf & Foolad, 2006). Seasonal and locational changes of chemical composition of *Ulva rigida* were investigated by İrkin & Erduðan (2014). However, significant differences were recorded in protein content of the collected species in terms of season and stations.

The North (Mediterranean Sea) and west (Red Sea) coasts of Egypt are rich in macro-algae resources. The green macro-algae (*Codium* spp.) are the most abundant in these coastal areas (Aleem, 1993 and El-Said & El-Sikaily, 2013) and in Suez Canal (Farghaly & El-Shoubaky, 2015) yearly. However, the utilization of this macro

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alga in these regions is very restricted. *Codium tomentosum* is found in many parts of the world, it is also, native to the northeast Atlantic Ocean from the British Isles southwards to the Azores and Cape Verde. *Codium tomentosum* is used in products from the United States, Germany, Italy, and the UK. Some of these products are repair and restoration moisturizers, hydration serums, eyes and skin creams, anti-aging masks, lip balms, and lotions. It is a popular food in some parts of Asia (Loiseaux-de Goër & Noailles, 2008).

Numerous studies have demonstrated the biochemical contents of Egyptian seaweeds according to seasonality, but there is no data on the biochemical composition of the same species collected from two different localities. Hence, the current work aim to demonstrate the crucial influence of habitat variation on the biochemical composition of *Codium tomentosum* selected from the coasts of Mediterranean and Red Seas.

Materials and Methods

Algal sampling

Seaweed samples of *Codium tomentosum* (Chlorophyta: Codiaceae) were hand-picked from Alexandria (Mediterranean Sea) and Hurgada (Red Sea) during 2014. The Collected algal samples immediately washed with the surrounding water to remove extraneous matters, sand particles and epiphytes as much as possible. Then, they kept in ice box containing frozen gel cold packs to maintain the low temperature and moisture during the journey and immediately transported to the laboratory. On arrival samples were thoroughly washed with tap water and finally with distilled water, then spread on blotting paper to remove excess water. Finally, cleaned algal samples were divided into four groups prior to the chemical analysis. In first group, samples were preserved in freezer for fresh weight analysis, second group was shaded air-dried, cut into small pieces and grounded into fine powder using a dry grinder and preserved for dry weight analysis, third group was oven-dried at 80℃ to constant weight for mineral analysis and the final group was preserved in 4% formalin for identification (voucher specimens).

Some physico-chemical analysis of sampled water

Some physico-chemical charactersof the water samples that collected from Mediterranean and Red Seas were measured according to Jackson & Thomas (1960).

Biochemical analysis of algae

Soluble sugars and sugar-free residues were extracted following the method adopted by Homme et al. (1992) and Naguib (1963), respectively. Soluble sugars and those obtained after polysaccharides hydrolysis were estimated using the anthrone reagent as described by Blakeney & Mutton (1980). Soluble proteins were extracted according to the method described by Daughaday et al. (1952). Meanwhile, the water insoluble residue remaining after extraction of soluble proteins was extracted with 1N NaOH. Soluble proteins and those resulting after insoluble residue hydrolysis were measured using BIO-RAD protein assay dye reagent according to the method adopted by Bradford (1976). Total free amino acids were carried out according to Wasfi (1970). Free proline was determined according to the method described by Bates et al. (1973). Glycerol was extracted from algal tissues according to the method adopted by Kochert (1978). Moreover, glycerol content was estimated according to Lambert & Neish (1950). Sodium, potassium, and calcium were estimated according to the method described by Ranganna (1977) using atomic absorption spectrophotometer (Pekrin Elmer USA 3100). Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted in 80% acetone and determined spectrophotometrically as recommended by Metzner et al. (1965).

Antioxidants as glutathione and ascorbic acid were extracted and estimated following the methods described by Hissin & Hilf (1976) and Mukherjee & Choudhuri (1983), respectively. The amount of total phenol was determined with the Folin-Ciocalteu reagent using the method of Malik & Singh (1980) and the absorbance was measured at 765nm using spectrophotometer (Spectronic 601, Milton Roy Company). Total flavonoid content can be determined by Aluminum chloride method (Harborn, 1998) and the absorbance was measured at 415nm. Ferric reducing power (FRAP) was estimated according to Dorman et al. (2003). Moreover, total antioxidant capacity was determined according to the method of Oyaizu (1986). Polyphenol oxidase activity (PPO), peroxidase activity (POX), ascorbate oxidase activity (ASO), ascorbate peroxidase activity (APX) and catalase activity (CAT) were evaluated according to Kar & Mishra (1976), Diallinas et al. (1997), Koricheva et al. (1997) and Chen et al. (2000), respectively.

Moreover, the determination, identification and characterization of different protein fractions were obtained using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis. Polyacrylamide slab gel (12.5%) was prepared according to Laemmli (1970). The destining gel was analyzed by Gel Documentation System (GDS), model UVP"s GDS 8000 from UVP Inc. (California 91786 USA), to analyze the band pattern, molecular weights and band percentages.

N.B. The biochemical results of *Codium tomentosum* collected from Mediterranean Sea were cited in M. Sc. Thesis of Mostafa, N.H. (2016) and didn't yet published previously. This thesis was under the supervision of A.M. Shaaban, M.M. Emam and H.A. Mansour.

Statistic analysis

All determinations were made in triplicate for all assays. Data were subjected to an analysis of variance (ANOVA) with statistical significance at $P < 0.05$ being tested using the Duncan's Test and Pearson correlation.

Results

The results of some physico-chemical characters of sampled water in Table 1 showed no major differences in the chemistry of water between the two studied localities except for salinity and temperature. However salinity and temperature of the Red Sea is relatively higher than those of the Mediterranean one.

TABLE 1. Some physico-chemical characters of sampled water.

Parameters	Red Sea		Mediterranean Sea	
	Min.	Max.	Min.	Max.
Temperature (°C)	28	29.5	16.9	18.5
Salinity (%)	41.7	42.2	38.4	38.9
pH	8.5	8.7	8.2	8.6
Dissolved oxygen (mg/L)	4.9	5.8	5	5.5

Phytochemical evaluation results of *Codium tomentosum* of Mediterranean Sea (M) and Red Sea (R) coasts were represented in Table 2. There are significant differences in the biochemical profiles between the two samples. It is clearly obvious that, the total carbohydrate content of *Codium* collected from R site (359.3mg.g^{-1}fw) was significantly higher than of those collected from site M (224.9mg.g^{-1} fw).

Concerning to protein, the highest content was recorded for *Codium* collected from Mediterranean Sea (5.9mg.g^{-1} fw) where as *Codium* from the Red Sea has the total protein content of (5.4mg.g^{-1} fw). In addition the total amino acids showed significant differences between the two sampling sites, 1.8mg.g^{-1} fw for samples harvested from site M and (0.32mg.g^{-1} fw) for samples of site R.

Moreover, the mineral compositions (presented by Na^{+}, K^{+} and Ca^{+2}) data were strongly different between the two sampling sites. For R samples, values were 7.1, 31.6 and 14.2mg.g^{-1} dw, respectively while, values for M sample were 2.2, 4.9 and 2.48mg.g^{-1} dw, respectively. Thereby the mineral composition of *Codium* collected from Red Sea has a higher value than those collected from Mediterranean Sea.

In respect to the pigments, the results showed that there was no significant difference in chlorophyll a content of *Codium* collected from both sites while chlorophyll b and carotenoids were slightly higher in *Codium* collected from the Mediterranean Sea (5.66 and 9.39µg.g^{-1} fw, respectively) than the same species collected from the Red sea (4.5 and 8.5µg.g^{-1} fw, respectively).

Moreover, it is clearly shown that, glycerol and proline (Table 2) recorded a marked increase in *Codium* collected from the Red Sea (64.5µM.g^{-1} fw and 2.5µg.g^{-1} fw, respectively) compared to that from the Mediterranean Sea (50.8µM.g^{-1} fw and 2.1µg.g^{-1} fw, respectively). Data in Table 3 indicated that the total phenol content in *Codium* collected from the R site has the same value (0.39 mg.g^{-1} fw) as *Codium* collected from the M site. However, total flavonoids were strongly different between the two sampling sites, where the maximum value was predominated with *Codium* harvested from the Mediterranean Sea (10.8mg.g^{-1} dw) and the minimum value was predominated with *Codium* harvested from the Red Sea (2.9mg.g^{-1} dw).

In contrast, ascorbic acid and glutathione have more or less significant difference between the two sampling sites. In M samples, ascorbic acid (0.35µM.g^{-1} fw) was lower than those of R

samples (0.54µM.g^{-1} fw). Also, the glutathione content of M samples (9.5mM.g^{-1} fw) was lower in comparison to R samples (9.8mM.g^{-1} fw). These associated with a greater value in the total antioxidant capacity (Table 3) of samples collected from the Red Sea than that collected from the Mediterranean Sea (230.1 and 126.6ig.g^{-1} dw, respectively). Moreover, the reducing power (Table 3) of R samples (0.9µg.g^{-1} dw) recorded a pronounced higher value than of M samples (0.3µg.g^{-1} dw).

TABLE 2. Changes in the biochemical composition (carbohydrate, proteins, total amino acids, some minerals and pigments) of *Codium tomentosum* collected from Mediterranean Sea (M) and Red Sea (R).

Carbohydrate (mg.g^{-1} fw)	Biochemical parameters of *Codium tomentosum*					
	Total soluble sugars		Total insoluble sugars		Total sugars	
	M	R	M	R	M	R
	0.87±0.02	1.22±0.04	224.05±5.3	358.06±16.6	224.89±5.3	359.34±16.6
Proteins (mg.g^{-1} fw)	Total soluble proteins		Total insoluble proteins		Total proteins		Total amino acids	
	M	R	M	R	M	R	M	R
	3.54±0.04	3.03±0.05	2.33±0.06	2.39±0.14	5.86±0.1	5.42±0.14	1.83±.2	0.32±0.05
Minerals (µg.g^{-1} dw)	Sodium		Potassium		Calcium	
	M	R	M	R	M	R
	2.20±0.03	7.10.035 ±	4.97±0.18	31.6±0.15	2.48±0.01	14.24±0.09
Pigments (µg.g^{-1} fw)	Chl. a		Chl. b		Carotenoids	
	M	R	M	R	M	R
	3.73±0.15	3.97±0.29	5.66±0.3	4.54±0.05	9.39±0.5	8.52±0.1

Values (means ± standard deviation of data for duplicated groups) for each site in the same column are significantly different at 5% level.

TABLE 3. Changes in the biochemical compositions (proline, glycerol, antioxidants compounds, antioxidants enzymes and antioxidants activity) of *Codium tomentosum* collected from Mediterranean Sea (M) and Red Sea (R).

	Biochemical parameters	*Codium tomentosum*	
		Mediterranean sea	Red sea
Antioxidants compounds	Glycerol (µM.g^{-1} fw)	50.76 3 ±	64.54 0.6 ±
	Proline (µg.g^{-1} fw)	2.07 0.02 ±	2.49 0.02 ±
	Total phenols (mg.g^{-1} fw)	0.39 ± 0.01	0.39 0.03 ±
	Total flavonoids (mg.g^{-1} dw)	10.83 ± 0.05	2.93 ± 0.1
	Ascorbic acid (iM.g^{-1} fw)	0.35 ± 0.06	0.54 ± 0.01
	Glutathione (mM.g^{-1} fw)	9.46 ± 0.4	9.78 0.6 ±
Antioxidants enzymes	(PPO) Amount of quinon.g^{-1} fw.min^{-1}	0.69 0.039 ±	1.244 0.06 ±
	(POX) Amount of quinon.g^{-1} fw.min^{-1}	0.67 0.004 ±	1.31 0.12 ±
	(ASO) mM of ascorbate oxidized.g^{-1} fw.min^{-1}	18.27 0.063 ±	18.76 0.01 ±
	(APX) mM of ascorbate oxidized.g^{-1} fw.min^{-1}	20.24 0.115 ±	20.99 042 ±
	(CAT) µM of H_2O_2 destroyed g^{-1} fw.min^{-1}	0.40 0.003 ±	0.454 0.2.7 ±
Antioxidants activity	Reducing power µg.g^{-1} dw	0.33 ± 0.02	0.95 ± 0.06
	Total antioxidant capacity (mg.g^{-1} dw)	126.62 5.2 ±	230.07 12.3 ±

Values (means ± standard deviation of data for duplicated groups) for each site in the same column are significantly different at 5% level

Results presented in Table 3 also showed variation in the antioxidant enzyme activities of *Codium* collected from both sites. The sample collected from Red Sea showed the greatest activity. However, (PPO) and (POX) recorded the maximum activities in *Codium* from site R (1.2 and 1.3 Amount of quinon. g $fw^{-1}.min^{-1}$, respectively) compared to the activities of both enzymes from site M (0.69 and 0.67 Amount of quinon. g $fw^{-1}.min^{-1}$, respectively). In addition, (ASO) and (APX) showed higher activities in R samples (18.8 and 20.9 ascorbate oxidized. g $fw^{-1}.min^{-1}$, respectively) compared to M samples (18.27 and 20.24 ascorbate oxidized. g $fw^{-1}.min^{-1}$ respectively). Catalse (CAT) recorded also the maximum activity in *Codium* collected from the Red Sea (0.45µM of H_2O_2 destroyed.g $fw^{-1}.min^{-1}$) in comparison with those from the Mediterranean Sea (0.40µM of H_2O_2 destroyed. g $fw^{-1}.min^{-1}$).

Electrophoretic profiles of total soluble protein of the two algal samples were shown in Plate 1. Gel scanning revealed to the appearance of 7 and 8 polypeptide bands in *Codium* from the two sites (M and R, respectively). Moreover, five common protein bands covering molecular weights ranged from 125 to 15kDa were detected in the two samples (125, 24, 18, 15 and 8kDa). However polypeptides with molecular weights 93, 72 and 42kDa were appeared only in *Codium* of site (M), whereas that of 17kDa was specific for *Codium* of site (R).

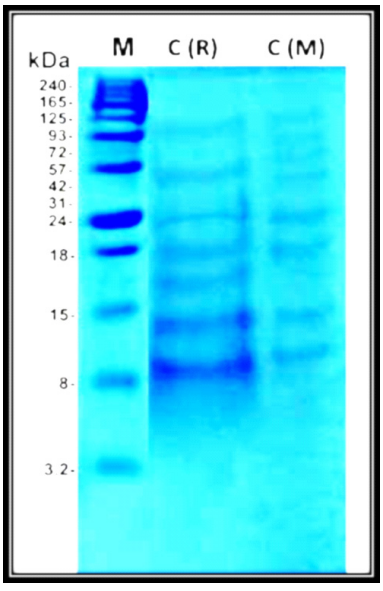

Plate 1. Changes in protein profiles of *Codium tomentosum* from two sampling sites; Red Sea (CR) and Mediterranean Sea (CM).

The electrophoretic analysis of protein pattern showed reduction in the relative concentration of certain polypeptides particularly those with molecular weights 15 and 8kDa in the *Codium* sample collected from site M.

Discussion

Physico-chemical characteristics of the seawater with different climate, geology, and hydrology affect in great extent on the algal chemical composition and density of species in the surveyed area (Ibraheem et al., 2014). According to the current results, salinity and temperature of the Red Sea is relatively higher than those of the Mediterranean one. This high temperature may increases evaporation of water and consequently increases salinity of the water of Red Sea (Felis et al., 2000 and Gertman & Brenner, 2004). However, salinity and temperature represents the dominant factors affecting both the local distribution (Leland et al., 2001) and growth of algae (Rao et al., 2007). Seaweeds alter their metabolism in extreme temperatures and salinity by producing a compatible solutes to organize proteins and cellular structures, maintain cell turgor by osmotic adjustment, and modify the antioxidant system to re-establish the cellular redox balance. (Janská et al., 2010 and Dennis, 2009). Strong difference in the carbohydrate content of both algal samples was observed in our study to elucidate the effect of site distribution on the chemical composition of *Codium tomentosum*. Sugars serve as osmo-adjustment, protect-ants and play a role in protecting cellular membranes from damage (Shao et al., 2006 and Yuanyuan et al., 2009). Soluble sugars are known to affect signal transduction and gene regulation mechanisms (Couée et al., 2005).

Soluble sugars are the main carbon skeletons of amino acids; the increase in soluble sugar was concomitant with sharp decrease in amino acids of *Codium* collected from the site R. Subsequently, amino acid metabolism might be shifted toward synthesis of sugars (Iraqi & Tremblay, 2001). Meanwhile, the reduction in free amino acid contents in the sample of site R might be also attributed to the observed increase in glutathione. Hildebrandt et al. (2015) were reported that, the production of glutathione depends on the availability of amino acids.

Results revealed also that, the highest glycerol

content was detected in the samples collected from site R of high salinity. This may be due to the protective effect of glycerol on the stability of PSII protein complexes as reported by Allakhverdiev et al. (2000). The observed increase in proline content of the sample collected from site R might be resulted from the enhancement of proline biosynthesis and/or decrease in its degradation (Kavikishore et al., 2005). Proline can act as a compatible osmolyte, thereby protecting cells from relative high salinity of Red Sea (Verbruggen & Hermans, 2008 and Krasensky & Jonak, 2012). Accumulation of proline has been implicated in the establishment and maintenance of thermo-tolerance against relative high temperature of the Red Sea compared to the Mediterranean one (Kakinuma et al., 2006 and D'souza & Devaraj, 2013). Similar results on the thalli of *Gracilaria corticata* were obtained by Kumar et al. (2010), where salinity has accounted to an increase of almost two fold from the initial proline contents.

The observed increase in the mineral contents in the sample of Red Sea sit with relatively elevated salinity, greatly affects the homeostasis of the cell. This could be explained due to the antagonistic behavior of Na^+ and K^+ at the uptake sites which in turn impaired the ion selectivity and integrity of the cell membrane and permit the passive and excess accumulation of Na^+ in the thalli. The osmotic potential generated by high internal potassium concentrations can alleviate sodium toxicity (Maathuis, 2014).

Results showed more or less similar content of chlorophyll a value in *Codium* collected from the two sampling sites. Regardless to the site distribution, the total chlorophyll content was closely similar between the different regions. Similar results were obtained by Moustafa & Saeed (2014) on *Ulva fasciata* collected from Ras Al-Tin and El-Muntazah from Alexandria shore at Egypt.

Fluctuations in salinity between the two sampling sites induce noteworthy alterations in antioxidant enzyme activities as well as stress metabolites in algae. According to the early literature data, marine algae, like other photosynthesizing plants, are exposed to a combination of environmental conditions that leads to the formation of free radicals. However, the absence of oxidative damage in the structural components of macro-algae suggests that their cells have protective anti-oxidative defense systems (Kelman et al., 2012 and Silva et al., 2017). All antioxidant enzymes of *Codium* from the two sites significantly increased in the sample of the site R. Some anti-oxidant enzymes were selected by Kumar et al. (2010) to evaluate the biochemical responses of the red alga *Gracilaria corticata* to salinity induced oxidative stress.

The current study also indicated that, there were no differences in the phenolic contents estimated in *Codium* collected from the two studied sites. This was concomitant by increased activity of PPO in the site R. Phenol is a substrate for PPO enzyme. Accordingly, the high activities of PPO have to reduce the accumulation of phenols which might be consumed as substrates (Taranto et al., 2017). Glutathione usually showed antioxidant property (scavenge the singles of oxygen, peroxide and hydroxyl radicals). However, it plays an important role in expression of defense genes and may be involved in the control of cell division (Shao et al., 2008). The observed increase in glutathione might be attributed to the accumulation of soluble sugars in *Codium* of sample R. Soluble sugars were hypothesized to act transcriptionally on the induction of cytosolic glucose-6-phosphate dehydrogenase (Hauschild & Von Schaewer, 2003) which in turn provide NADPH required for GSH production. The central role of GSH in anti-oxidative defense is due to its ability to regenerate another powerful-water soluble antioxidant ascorbic acid (Foyer & Noctor, 2001) which significantly increased in samples of R site. The relative high temperature and salinity of the Red Sea may stimulate the accumulation of ASO. Ascorbate oxidase can directly scavenge superoxide, hydroxyl radicals and singlet oxygen as well as reduce hydrogen peroxide to water via ascorbate peroxidase reduction (Caputo et al., 2010). According to the early literature data, the antioxidant capacity of algae (Ismail, 2017 and Srikong et al., 2017) is highly positively correlated with phenolics (Zubia et al., 2008) and flavonoids content (Sarojini et al., 2012). However, our study indicated that the increase in antioxidant capacity and reducing power (samples collected from the site M) is not attributed to the redox properties of either phenols or the antioxidant enzymes but to the enhancement of flavonoid accumulation.

Scanning of the gel showed the *de novo* synthesize, over expression of some polypeptide chains in *Codium* from the two sites. However,

data showed the appearance of polypeptides with molecular weights 17kDa was specific for samples collected from site R concomitant with the disappearance of it from samples collected from the site M. The 17kDa protein functions as a molecular chaperone preventing protein denaturation under relative high temperature (Kim & Ahn, 2009). However, the disappearance of protein bands of 93, 72 and 42kDa in R site were in accordance with Kaur et al. (2009) who reported that heat acclimatized showed *de novo* synthesis of some low molecular proteins and the disappearance of some of existing proteins which failed to fold correctly and are generally degraded.

Conclusion

Codium is a source of many biologically functional substances which deserve attention due to their many health benefits. In this study, measurable differences in chemical composition were recorded between the two investigated strains of *Codium* in response to several environmental factors as temperature and salinity. Most of the environmental parameters vary according to season. Moreover, the changes in ecological conditions can stimulate or inhibit the biosynthesis of several nutrients.

Future work is needed to investigate the 'omic' profiling (integration of genomics and proteomics tools) to elucidate and understand diverse biochemical networks involved in cellular adaptation to different environmental factors according to different habitats sites.

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تأثيرالبيئات المختلفة على المحتوى الكميائى للكوديوم تومينتوسوم

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إن اختلاف درجة الملوحة ومايرتبط بها من مقاومة اسموزية تلك التي تتعرض لها الطحالب تكون مرتبطة بصورة وثيقة بالبيئة التي تعيش فيها. ويعتبر البحر الأحمر أكثر المياه سخونة وملوحة في العالم مقارنة بالبحر الأبيض المتوسط.

ولذلك تهدف هذه الدراسة إلى تقييم التركيب الكيميائي والقدرة الكلية لمضادات الأكسدة لطحلب الكوديم (*Codium tomentosum*) الذى تم جمعه من بيئات مختلفة مثل البحر الأبيض المتوسط (M) والبحر الأحمر (R) بجمهورية مصر العربية. فقد أظهرت النتائج الحالية أن الملوحة ودرجة الحرارة في البحر الأحمر أعلى نسبيا من سطح البحر المتوسط. ولقد أثبتت الدراسة أيضا وجود ارتفاع واضح في المحتوى الكلي للكربوهيدرات، البرولين، الجلسرين مع القدرة الكلية لمضادات الأكسدة، والقدرة الإختزالية وكذلك المحتوى الأيوني (الصوديوم، البوتاسيوم و الكالسيوم) وأنشطة الأنزيمات المضادة للأكسدة (PPO, POX, ASO, APX and CAT) للكوديم الذى جمع من الموقع (R) مقارنه بنظيره الدي يعيش في الموقع (M). وعلى النقيض من ذلك، فقد ساد كلا من البروتين الكلي، والأحماض الأمينية الكلية، ومحتويات الفلافونويد في الكوديوم من الموقع (M). كما أسفرت النتائج بعدم وجود اختلافات معنوية ملحوظة في محتوى حمض الأسكوربيك، الجلوتاثيون، الكلوروفيل والفينولات الكلية لعينات الكوديم المجموعة من الموقعين قيد الدراسة. وبالإضافة إلى ذلك فقد أظهر تحليل أنماط البروتين باستخدام طريقة التفريد الكهربي إلى تباين ملحوظ بين الموقعين (M و R). حيث ان كلا من الببتيدات ذات الأوزان الجزيئية 93 و 72 و 42 كيلو دالتون قد سجلت فقط فى ال*Codium*، من الموقع (M)، في حين أن الببتيد ذات ال 17 كيلو دالتون ظهر فقط فى ال *Codium* من الموقع (R).

وفي هذا المقام يمكننا القول أن الأرتفاع النسبي للملوحة وكذلك درجة الحرارة في الموقع R مقارنه بالموقع M ادى إلى الإختلاف في المحتوى الكيميائي للكوديم حيث قام هذا الطحلب بتغير عملية التمثيل الغذائي وبناء جهاز حمايه قوي (بإنتاج مذيبات متوافقة) استجابة لإرتفاع درجة الحرارة والملوحة الناتجة عن إختلاف مواقع أخذ العينات. وهذا الأمر الذي يلعب دورا فى كبح التأثير الضارالناجم عن الإجهاد التأكسدى. و هذه النتيجة هي خطوة لدراسة التكامل البيوكيميائي المتنوع فى الطحالب الذى ينطوي عليه التكيف الخلوي للعوامل و للمواقع البيئية المختلفة.