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Ulva species Blooms and its Biochemical Composition in Relation to Aquatic Environmental Properties at North Damietta- Egypt

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Abstract

The high trophic status of brackish water and sediments at the Deltaic coast, especially the high nitrogen, phosphorus contents, along with relatively low pH and water temperature resulted in overgrowth of Ulva pertusa at El Garabaa and Ulva lactuca at Damietta river Nile Estuary. This represents an environmental problem of aquatic environment and water quality. On the other hand, this massive growth of Ulva species can be used as economic source of different natural products. The biochemical analysis of Ulva species indicated that Ulva pertusa have higher contents of proteins, lipids, minerals, soluble and insoluble carbohydrates than those of Ulva lactuca. The qualitative studies of natural products indicated that Ulva pertusa contains alkaloids, saponins, flavonoids, phenols and quinones while Ulva lactuca contains alkaloids, steroids, flavonoids, cumarins and quinones. Further study to determine the quantitative contents of natural products and its bioactivity is needed.

Keywords: Green seaweeds, Chlorophyta blooms, Ulva species, Environmental factors, Biochemical contents.

Introduction

Macro-algae are renewable marine resources existing in large quantities along the Mediterranean coast of Egypt. Their widespread distribution is attributed to the favorable environmental factors such as light, temperature, trophic status and salinity **(Rashad and El-Chaghaby 2020)**. Macroalgal species occur primarily near the shores of coastal waters, where they grow attached to rocks or suitable substrates **(Fawcett *et al.* 2017).** Under these conditions, some species can form stable, multi-layered and perennial vegetation. These organisms have been recognized as essential components for preserving the biodiversity of marine ecosystems. Although algal overgrowth is reported to have a nuisance effect on the ecosystem **(Jorgensen *et al.* 2010)**, it is also commercially important as a source of food, fodder, fertilizer, cosmetic agent, and as a medicament **(Andrea and Prieto *et al.* 2019; Michalak and Mahrose2020).**

Ulva species as green macro-algae are widely distributed along the coastline in Nile delta (brackish Lakes and river Nile Estuaries) developing uncontrolled, rapid and colossal mass production termed as "green tides" **(Dorgham *et al.* 2019).** The free-floating fragments can act as a nucleus to form green tides in new locations thus posing significant ecological and economic hazards. This makes these species a low-cost and abundant source of biomass **(Lyons *et al.* 2014).** Algal outgrowth has been reported to produce foul odor and to block access to the beach and facilities for launching boats which affecting the fishing grounds **(Human *et al.* 2016)**. An enhanced supply of nitrate and phosphate, low salinity and relatively low pH (around 7.0) were found to be responsible for exceptionally high growth rates of *Ulv*a species **(Wang *et al*. 2019).**

Ulva is a very adaptable genus to environmental conditions. These adaptive characteristics of *Ulva* would mean that it can be easy to cultivate and harvest **(Mata 2016)**. The genus *Ulva* represents a large group of green macroalgae with 131 species **(Guiry 2014)**. *Ulva* species plays a key role in coastal ecosystems, contributing to nutrient cycling, providing food and habitat for a variety of marine animal species **(Human 2015).** *Ulva* species are among the most popular edible seaweeds worldwide, e.g. *ULVA lactuca* is known as "sea lettuce", with a high nutritional value due to its high levels of polysaccharides, proteins, vitamins and trace minerals **(Taboada and Millan *et al.* 2010).**

Ulva can grow in saline water, waste water and has a higher ability to sequester atmospheric CO_2 than terrestrial energy crops **(Raikova *et al*. 2017)**. In addition, the growth rate and productivity are high compared to those of land crops, and they can withstand harsh conditions **(Herminia Dominguez *et al*. 2019).**

The rate of accumulation of metals is high in macro-algae which make them efficient bio-sorbents in bioremediation processes. The biomass can be utilized either as fresh matter or after drying **(Hlihor 2017)**. Among others, characters such as wide distribution, availability of high surface area, and fast growth make *Ulva* a suitable candidate for bioremediation processes **(Adrianna Ianora *et al*. 2013).**

This study explores the formation dynamics of *Ulva* blooms with respect to habitat condition and biochemical contents.

Materials and Methods:

Study Area:

Two sites along the Nile delta were studied Fig (1).

The first site: El Garabaa at north of El-Manzala Lake, is characterize by very shallow brackish stagnant water (25-40 cm), soft muddy sediment and a supply of waste water discharge from domestic and gas plant activity. **The second site**: Damietta estuary shore of river Nile is characterized with brackish stagnant water, rocky sediment and receives waste water discharged from domestic and agricultural activities.

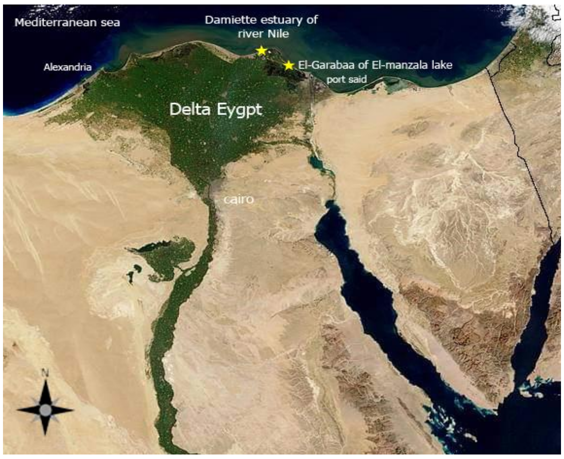


Fig (1): El Garabaa at North El-Manzala Lake and River Nile estuary at Damietta- Egypt.

Sampling of water and sediments

Water and sediment was sampled two times from each site (February and April, 2019), filtered and stored at 4°C in the dark to be used for chemical analysis. Oxygen determination was done in situ as described by (**Winkler 1962; Wood 1975)**.

The sediment was transferred to the laboratory in plastic bags, air-dried and Sieved in Mm mesh. The sieved sediment (<2.0 mm) was preserved in plastic bags and a 1:1 aqueous extract was used for chemical analysis as described by **(Radojevic 1999; Mussa and Hawaa 2009)**.

Physico-chemical analysis

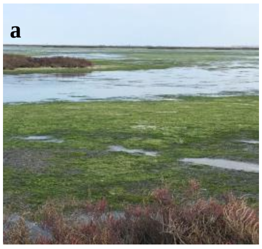
Water temperature was measured in situ using a Celsius Thermometer.

Hydrogen ion concentration (pH) determined by using a Horizon Ecology Co pH meter 5995. Salinity, Electrical Conductivity and total

dissolved salts (T.D.S) measured by using YSI Model 33 (yellow springs) S-C-T Meter % MHOS.
Dissolved oxygen measured according to (EPA 1983).
Biochemical oxygen demands (BOD) were assayed according to APHA (1989).
Total alkalinity determined by using the method of Kumar and Shailaja (1998).
Chlorides determined according to Ramteke and Moghe (1988).
Ammonia - nitrogen determined according to Dawes *et al.* (1971).
Nitrite – nitrogen determined according to (Adams 1990).
Nitrate – nitrogen determined according to Strickland and Parsons (1965).
Total nitrogen determined according to Kryskalla (2003).
Total phosphorus determined according to APHA (1989).
Orthophosphate determined according to APHA (1989).
Heavy metals determined according to Moore and Chapman (1986).

Sampling of Macroalgal materials (Ulva species)

Ulva species were collected from an area of 1 m^2 format the two sites Fig (2) during February and April 2019 and algal mass was recorded. Algal material was washed in the water of the study sites to remove any epiphytic organisms and debris.



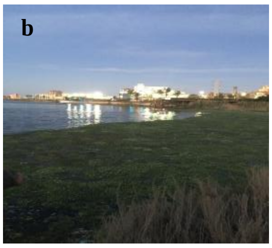


Fig (2): a- Blooms of *Ulva pertusa* at El-Garabaa North El-Manzala Lake-Egypt (Site1) , **b-** Blooms of *Ulva lactuca* at Damietta River Nile Estuary shore (Site2).

Macroscopic observation of the fresh macroalgae included the thallus color, length, texture, and the holdfast. Microscopic investigation includes shape and size of cells, thallus thickness, and arrangement of the chloroplast in surface view for identification of *Ulva* species according to **Kong *et al.* (2011)**.
In the laboratory, algal biomass was cleaned from epiphytes and non-living matrix in running taps water and rinsed many times in distilled water to remove all salts on the surface then weighed . The fresh matter was then spread on string nets and allowed to dry in air and air-dried weight was recorded. The dried matter was ground to fine powder using electric blender and stored in polyethylene bags in the refrigerator for further use as recorded by **Tran *et al.* (2018).**

Algal extract

Extraction of algal material was performed with methanol as stated by **Rosaline *et al.* (2012).** The dried powdered algae (500 g) were kept in 800 mL of methanol for 5 days in sealed container with occasional shaking . Then extracts were filtered through fresh cotton bed, the solvent was evaporated in a water bath at temperature of 40 ± 2ºC and the powder was stored at 4ºC until use.

Phytochemical analysis of Ulva species extracts:

Determination of proteins was done by Bradford (1976).
Determination of soluble and insoluble Sugars was done according to Schortemeyer *et al.* (1997).
Determination of lipids was done according to Egan *et al.* (1981).
Determination of K^+, Na^+ and Ca^{2+} ions was done by using method of (Hawk *et al.* 1947).
Determination of Heavy Metals was done according to Moore and Chapman (1986).

Qualitative analysis of natural products in Ulva species extracts:

The phytochemical screening of algal extracts was assessed by standard method as described by (**Savithramma *et al.* 2011)**. Phytochemical screening was carried out to identify the major natural products such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts.

1. **Alkaloids:** For Alkaloid identification, 2 mL of concentrated hydrochloric acid (HCl) was added to 2 mL algal extract. Then few drops Mayer's reagent was added. Presence of green color or white precipitate indicates the presence

of alkaloids.

2. **Terpenoids:** For terpenoid identification, 2 mL of chloroform along with 3 mL concentrated sulphuric acid were added to 0.5 ml of the algal extract. Formation of reddish brown color at the interface indicates the presence of terpenoids.
3. **Steroids:** For steroids identification, 2 mL of chloroform and 1 mL of sulphuric acid (H_2SO_4) were added to 0.5 mL of the algal extract. Formation of reddish brown ring at interface indicates the presence of steroids.
4. **Tannins:** For tannin identification, one mL of ferric chloride (5% $FeCl_3$) was added to 1 mL of the algal extract. Formation of dark blue or greenish black color indicates the presence of tannins.
5. **Saponins:** For saponin identification, 2 mL of distilled water was added to 2 mL algal extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.
6. **Flavonoids:** For flavonoids identification, 1 mL of (2 N) sodium hydroxide (NaOH) was added to 2 mL of algal extract. Formation of yellow color indicates the presence of flavonoids.
7. **Phenols:** For phenol identification, 2 mL of distilled water followed by few drops of 10 % ferric chloride were added to 1 mL of the algal extract. Formation of bluegreen color indicates the presence of phenols.
8. **Coumarins:** For coumarin identification, 1 mL of 10 % NaOH was added to 1 mL of algal extract. Formation of yellow color indicates the presence of coumarins.
9. **Quinones:** For Quinone identification, 1 mL of concentrated sulphuric acid (H_2SO_4) was added to 1 mL of the algal extract. Formation of red color indicates the presence of quinones.
10. **Glycosides:** For glycoside identification, 2 mL of chloroform and 2 ml sulphuric acid was added to 2 mL of the algal extract. Formation of pink color indicates the presence of glycosides.

Results and discussion:

The present study revealed massive growth of the macroalgae *Ulva lactuca* at site (2) and *Ulva pertusa* at site (1).

The diversity of *Ulva* species at the two study sites may be due to the significant variation of water salinity as well as the variation of substratum nature as suggested by **Chavez Sanchez *et al.* (2017)**.

Ulva Pertusa thallus is membranous, tough glossy bright to dark green blades up to 40 cm long. The blade is rounded when young but becomes lobed and more or less perforated at the base when old. The alga is found growing in the lower littoral and upper subtidal zones of a wide variety of habitats Fig (3).

Ulva lactuca thallus is bright green much broader (50–70 cm) flat, rounded, foliose, leafy, soft membranous with undulated margins and is normally wider at the top than at the base resembling a lettuce leaf Fig (3). The field observation recorded *Ulva lactuca* growth on Damietta Estuary shore along 9 km distance from Damietta city to the Mediterranean Sea Fig (2).

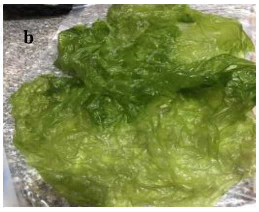


Fig (3): a- *Ulva pertusa* at North El-Manzala Lake , **b-** *Ulva lactuca* at River Nile Estuary, Damietta.

Table (1): Physicochemical properties of water at the study sites.

Physicochemical parameters	site (1)	site (2)
Temperature (˚C)	18	19
pH	7.4	7.3
salinity (g/L)	12	18
Total Alkalinity (meq/L)	2.3	3.2
DO (mg/L)	4.8	5.8
BOD (mg/L)	14.8	15.4
Ammonia-N (mg/L)	0.6	0.7
Nitrite-N (mg/L)	0.02	0.03
Nitrate-N (mg/L)	1.5	1.6
Total-nitrogen (mg/L)	3.1	3.3
Total-phosphorous (mg/L)	1.2	1.3
Ortho-phosphate (mg/L)	0.5	0.6
Chlorides (g/L)	19	14
Fe (µg/L)	30	50
Cu (µg/L)	10	20
Cd (µg/L)	7	7
Co (mg/L)	0.2	0.2

Fletcher (1996) concluded that *Ulva* belongs to a macroalgal group characterized by extensive tolerance to environmental conditions such as temperature, light intensity, oxygenation, salinity and nutrients. The temperature varied between 18-19 C□, pH between 7.4-7.3 and salinity between 12-18 g/L which stimulate the massive growth of *Ulva* species This ability to adapt rapidly to variable environmental conditions, including high resistance to metal contamination, makes *Ulva* "environmentally robust" **Hurd *et al.* (2014).**

The high concentration of nitrate (1.5-1.6

mg/L), and orthophosphate (0.5-0.6 mg/L), along with low concentrations of total iron (30-50 µg/L) were found especially in well-oxygenated habitats. Also, the high concentration of chloride (19-14 g/L) gives the optimal trophic condition for *Ulva* blooming. This highly eutrophic status of water is mainly due to the sewage and agriculture discharges into the study area. An enhanced supply of nitrate and phosphate, low salinities (10 and 20‰) and low pH (pH around 7.0) were found to be responsible for exceptionally high growth rates of *Ulv*a species **Wang *et al*. (2019).**

The impact of green tides on seawater manifests itself primarily by local limitation of nitrogen availability for photoautotrophic organisms, control of daily fluctuations in pH, reduced oxygenation in demersal waters and production of allelopathic compounds **Van Alstyne *et al.* (2015).**

Mass development of *Ulva* species in waters containing high concentrations of nitrogen (3.1-3.3 mg/L) and phosphorus (1.2-1.3 mg/L) recommends their use as bioindicators of water contamination with nutrients **(Morand and Briand 1996; Riddin and Adams 2010).**

Furthermore the low dissolved oxygen (4.8-5.8 mg/L), high biological oxygen demand (14.8-15.4 mg/L) **(Table 1)** and the relatively high content of ammonia (0.6-0.7 mg/L) resulted from the discharge of waste water especially domestic and agricultural sewage in the study area as suggested by **Mocuba Jeremias *et al.* (2010).**

On the other hand, the relative low content of heavy metals in the water may be related to low industrial waste water discharge at study sites according to **Ravindra *et al.* (2014).**

Table (2): Physicochemical properties of sediments at the study sites.

Physicochemical parameters	site (1)	site (2)
pH	7.3	7.1
Chlorides (g/L)	12.2	21.7
salinity (mg/L)	16	19
Nitrite-N (mg/L)	0.2	1.1
Nitrate-N (mg/L)	2.8	1.9
Total-nitrogen (mg/L)	4.2	3.4
Total-phosphorous (mg/L)	2.5	1.8
Ortho-phosphorous (mg/L)	0.5	0.6
Fe (µg/L)	5	5
Cu (µg/L)	13	10
Cd (µg/L)	4	1
Co (µg/L)	30	20

The present results indicate that, sediments have high content of salts, nitrogen, phosphorous and heavy metals relative to water at the same study sites. This is mainly due to the fact that salts, nutrients and the water contents in general increase gradually towards bottom **(Table 2).** Hence, the sediment of shallow water systems may contribute significant concentrations of dissolved inorganic nitrogen and phosphorous to the overlying water to stimulated the growth of *Ulva* species **Human *et al. (*2015)**

It is well-known that, dissolved oxygen is relatively low at the bottom compared with the water surface. This is mainly due to the very high consumption, both chemically and biologically, of dissolved oxygen at the bottom along with very low oxygen production. Dominance of opportunistic macroalgal species in areas undergoing eutrophication has been attributed to storage of inorganic nutrients, high growth rates and tolerance to temperature and salinity fluctuations (**Aveytua-Alcázar *et al.* 2008).**

Table (3): Vegetation analysis of *Ulva* species

Vegetative Parameter	*Ulva* species1	*Ulva* species2
Fresh weight (g/m^2)	4150	5815
Dry weight (g/m^2)	490	669
Covering (%)	91%	96%
Length (cm)	40	70
Height (km)	21	35
field covering area	1000 hectare	9 hectare

The significant difference in growth of the two *Ulva* species between two sites in favor of *Ulva lactuca* at site 2 can be attributed to the rocky nature of the substratum and the receiver of high nutrient supply through domestic sewage discharge **(Schmidt *et al.* 2013**).

The low algal growth at site 1 compared with site 2 can be attributed to the discharge of fish farms drainage water and gas plant wastes in addition to the soft muddy sediment which can affect the species of *Ulva* dominating this site. In this regard, **Zhang *et al.* (2011)** indicated that the polluted muddy sediment retards growth of *Ulva* species.

Although the growth of Ulva lactuca at site 2 was higher than that of *Ulva pertusa* at site 1, the concentration of biochemicals of *Ulva pertusa* at site 1 was higher than that of *Ulva lactuca* at site 2. This can be attributed mainly to the high contents of nutrients in the sediment of muddy substratum at site 1 relative to the rocky substratum at site 2. **Castañeda *et al.* (2006)** concluded that *Ulva* species chemical composition varies depending on the geographical distribution, the season and the principal environmental factors.

Although of the higher ferric content of water at site 2 (50 µg/L) at site 1 (30 µg/L), the ferric content of alga was higher in *Ulva pertusa* at site 1 than *Ulva lactuca* at site 2. This may be due to the higher Fe bioaccumulation capacity of *Ulva pertusa* compared with *Ulva lactuca*. A noticeable amount of Fe, Al and B were also found in the algal samples, this in accordance with **Guieu *et al.* (2002).**

Table (4): Biochemical composition of *Ulva* spp extracts.

Constituent (mg/g dry wt.)	*Ulva Pertusa*	*Ulva lactuca*
Protein	14.9	12.4
Soluble sugars	36.6	31.3
Insoluble sugars	44.8	40.2
Lipids	22	21
Total K	4.2	2.2
Total Na	3.3	1.2
Total Ca	1.3	3.9
Fe	5.4	1.3
Cu	0.15	0.06
Cd	0.3	0.14
Co	1.8	2.7

The generally higher cobalt content of algal tissues above that of water and the higher cobalt content in *Ulva lactuca* at site 2 above its content in *Ulva pertusa* at site 1 suggest bioaccumulation of Co by the algae which was more pronounced in *Ulva lactuca* at Damietta branch estuary of River Nile at site 2. This finding agrees with **Swanner *et al.* (2014).**

Table (5): Qualitative Estimation of *Ulva* species natural products.

Phytochemical	*Ulva Pertusa*	*Ulva lactuca*
Alkaloids	+	+
Terpenoids	-	-
Steroids	-	+
Tannins	-	-
Saponins	+	-
Flavanoids	+	+
Phenols	+	-
Cumarins	-	+
Quinones	+	+
Glycosides	-	-

Each *Ulva* species have five natural product groups, they share three products that is alkaloids, flavonoids, quinones but differ in the presence of saponins, cumarins and steroids. *Ulva pertusa* contained saponins and phenols which were absent in *Ulva lactuca*. On the other hand, *Ulva lactuca* contained steroids and cumarins which were absent in *Ulva pertusa*. This considerable variation maybe due to the remarkable variation in the physicochemical properties of water and sediments at both sites **(Table 5).** Normally, *Ulva* species contain 9–14% protein; 2–3.6% ether extract (n-3 and n-6 fatty acids 10.4 and 10.9 g/100 g of total fatty acid); 32–36% ash. Alkaloids, glycosides, saponins, and tannic acid are near to null according to **Rodriguez *et al.* (2016).**

Conclusions:

The semi optimal physicochemical properties and high trophic status of aquatic habitat at North delta Egypt resulted in massive growth of *Ulva pertusa* (and *Ulva lactuca* which dramatically affects the aquatic environment and water quality. The present study revealed high contents of proteins, lipids, minerals, soluble and insoluble carbohydrates in *Ulva* species with relatively high content in *Ulva pertusa* than *Ulva lactuca*. Moreover, determination the present of natural product contents of alkaloids, Terpenoids, steroids, tannins, saponins, flavonoids, phenols, cumarins, quinones and glycosides in both *Ulva* species consequently, this overgrowth of *Ulva* species can be economically important as source of food, fodder and various natural products.

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الملخص العربي

ترجع الحالة الغذائية العالية للمياه قليلة الملوحة والرواسب في الموقعين التي يتم دراستهم خاصة ارتفاع النيتروجين، الفوسفور، انخفاض درجة الحموضة، ودرجة الحرارة نسبيًا إلى النمو الهائل للأولفا بيرتوزا (حوالي ١٠٠٠ هكتار في منطقة الجرابعة) وأولفا لاكتوكا على شاطئ مصب نهر النيل بدمياط (بطول حوالي ٩ كيلو) حيث تؤدي الي مشاكل بيئية وتؤثر علي جودة المياة. من ناحية أخري فأن هذا النمو الهائل للاولفا لاكتيوكا يمكن استخدامه كمصدر اقتصادي لمركبات طبيعية مختلفة.
التحليل الكيميائي للاولفا بيرتوزوا يوضح انها تحتوي علي نسبة عالية من البروتين، دهون، معادن، والكربوهيدرات القابلة للذوبان والغير قابلة للذوبان مقارنة بالاولفا لاكتوكا. كما تشير الدراسات النوعية للمركبات الطبيعية للاولفا بيرتوزوا احتوائها علي الالكالويد، فلافينويد، فينولات، وكينوين بينما الاولفا لاكتوكا تحتوي علي الالكالويد، وستيرويد، وفلافنويد وكومارين، وكينون.
يتضح من الدراسة السابقة أن هناك حاجة للمزيد من الدراسات الكميه للمركبات الطبيعية للاولفا بنوعيها ودراسة الأنشطة الحيوية والتأثير الذي تحدثه في البيئة.