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Isolation and identification of marine algae contaminated crude oil from Al-Hariga port, Tobruk in Libya

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Abstract

The present work was mainly intended to study the marine algae from Al-Hariga port. The study was carried out from spring to autumn (2020). Sea water samples were collected from the coast of the city of Al-Hariga port which were microalgae, and macro-algae (seaweeds) and identified. A total of 47 algal species (36 genera) that were recorded in the study area are able to grow on crude oil. Seven species of them (14.89%) were belonging to Cyanobacteria (4 families), eleven species (23.40%) belonged to Chlorophyta (9 families), twenty-four species (51.64%) belonged to Bacillariophyta (11 families), three species (6.38%) belonged to Phaeophyta (3families) and two species (2.26%) belonged to Rhodophyta (1family). The obtained results showed that the most common *Nitzschia* was found with four species and *Licmophora* was found with three species. However, diatoms were the most dominant species in this area during three seasons. The study area had three brown algal species, The absence of this species in the port is due to oil pollutants, A total of two species of red marine algae (2.26%) was recorded in the current Al-Hariga port area with a special reference to the family of the Corallinaceae. Although some good indicators of algae were recorded, one could conclude that the study area is relatively poor in marine algae and polluted.

Keywords: Isolation, identification, marine algae.

Introduction

Algae comprise a diverse group of typically autotrophic organisms, ranging from unicellular to multicellular forms. The largest and most complex marine forms are generally termed seaweeds. Algae convert solar energy to biomass using photosynthesis, like plants, although they lack the many distinct organs found in most terrestrial vegetation. The organisms considered in this report are photosynthetic macro-algae or microalgae growing in aquatic environments (1).

Macro-algae or “seaweeds” are multicellular plants growing in salt or fresh water. They are often fast growing and can reach sizes of up to 60 m in length (2), their natural environment, macro-algae grow on rocky substrates and form stable, multi-layered, perennial vegetation capturing almost all available photons (3). They are classified into three broad groups based on their pigmentation: i) brown seaweed (Phaeophyceae), ii) red seaweed (Rhodophyceae) and iii) green seaweed (Chlorophyceae). Seaweeds are mainly utilized for the production of food and the extraction of hydrocolloids. Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified (3).

Microalgae are microscopic organisms that grow in salt or fresh water. The three most important classes of micro-algae in terms of abundance are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae(Chrysophyceae) (2).

Marine algae are considered as one of the largest producers of biomass in the marine environment. They produce a great variety of structurally unique secondary metabolites with pharmacological and biological activities (4) . Algae in the process of bioremediation of water use their photosynthetic capacity, allowing them to convert solar energy into biomass, and then incorporate nutrients such as nitrogen and phosphorus which are responsible for eutrophication (5). Crude oil is a natural, heterogeneous mixture of hydrocarbons, with potentially 20,000 chemical components (6). Oil spill is the accidental release of petroleum crude oil into the environment by vehicle, vessel or pipeline, it happens due to human negligence and is considered a major form of pollution (6)

The substance is so toxic that it can cause massive loss of species that live in the sea. The contamination can result from leaking

Underground Storage Tanks (UST), petroleum refineries and bulk storage facilities, broken oil pipelines, spills of petroleum products in chemical plants and transportation processes (7). Therefore this study was aimed to isolation and identification of marine algae contaminated crude oil from Al-Hariga port, Tobruk in Libya.

Materials and Methods:

The Study area

The study area is located in the southeastern part of the Gulf of Tobruk, which is located in the north-east of Libya. Marsa Al Hariga is located at East Mediterranean in Libya at coordinates N 32° 03' 41.84" - E 023° 59' 18.05". Marsa Al Hariga is situated 1nm SSW of Tobruk Point. The study area also contains many oil installations, which are located in the southeastern side of the Gulf, represented by the anchorage Al Hariga oil refinery for the export of crude oil.

Isolation and Cultivation and Identification of algae

The algae were collected seasonally from three locations represent to the Al- Hariga port, Tobruk - Libya.

Harvesting of microalgae

Bring samples to the laboratory in plastic Gallons 5 liter during 4 hours and perform deposition sampling process Sedimentation by putting it in the laboratories listed capacity of 1 liter and left for three days until the precipitate and then pull the upper part of the sample until the volume up to 50 ml, reservation samples in flasks conical for the purpose of examination and counting agriculture, and deposition is part of the samples by adding material Lugol's Iodine and consists of 1 gram of Iodine and 2 grams of Potassium Iodide in order to differentiate between algae that store starch and those that do not store it as it is done within the taxonomic characteristics.

Isolation and Cultivation of microalgae

The marine microalgae were isolated from water samples collected from Al-Hariga port, Tobruk - Libya. The algae were grown in three different media namely:

1. Rippka (BG11) was used to isolate the blue- green algae (8).

2.(Chu-10) was used to isolate green algae (9)

3. **Bolds Basal Medium (BBM)** was used to isolate bacillus algae or green algae yellowed (10) .

Isolation of microalgae:

The Cultivation and isolation of the microalgae the might be persisting in for spores, hormogonia, akinetes or any other parenting stages were carried out using the moist plate method recommended by (11) isolated and inoculated into liquid medium at $25\text{ }^{\circ}\text{C}\pm 1$ (12).

Culture conditions:

Microalgae were grown in conical Erlenmeyer pyrex-glass flasks (capacity 250 ml). Each flask contained 50 ml culture medium. The cultures were grown under controlled laboratory conditions (temperature at $25\text{ }^{\circ}\text{C}\pm 1\text{ }^{\circ}\text{C}$ and light was provided by cool-white fluorescent lamps at 4000 Lux.) in a controlled culture chamber. Temperature inside the growth chamber was kept as possible within $25\text{ }^{\circ}\text{C}$ through periodical ventilation.

Culture experiments were conducted under a regime of 16 hour light/ 8 hour dark. Each experimental culture flask was regularly swirled daily by hand to detach adhered algal cells from the walls of the flask. After each mixing, the flasks were returned back to a different position on the glass shelves inside the controlled chamber to remove any bias due to the illumination or temperature gradient on the shelves. The culture period lasted for 18 days.

Identification of Microalgae

Microalgae were identified in the laboratory with the aid of a microscope. And will adopt a scientific basis in the classification, which in algae form and nature of the cell wall and dyes and flagella and the nature saver food in addition to the volume of moss cell-volume and on these grounds were divided algae to the ranks, ranks, families, genera and the types. Morphological features of the algae were compared with the algal compendium and identification keys found in the of special keys to the definition of algae listed in the references (13) .

Harvesting of macroalgae

Manual harvesting of seaweed has been practiced for centuries and it is still common for species naturally growing in coastal areas and epiphytes, impurities and salts are carefully and quickly removed in the laboratory with tap and distilled waters (14).

Identification of macroalgae

Sea weeds were examined by mean naked eye or a binocular microscope (Zeiss, with camera M35 W) and identified according to the following references (15-17). Macroalgae were defined as algae were divided into rows, arranged, families, species and species using special keys to define marine algae (18) . The collected samples were identified in Botany Department, Faculty of Science, Omar Al Mokhtar University.

Results:

Isolation of marine microalgae

The marine microalgae were isolated from water samples collected from Al-Hariga port, Tobruk-Libya. Each algal was isolated and inoculated into liquid medium (Rippka (BG11)), (Chu-10) and (Bolds Basal Medium) at $25^{\circ}\text{C}\pm 1$. The purity of cultures was ensured by repeated plating and regular observation under the microscope.

Identification of marine algae from Al-Hariga port, Tobruk-Libya:

Table 1 listed the collected and identified of the marine algae of Al-Hariga port, Tobruk-Libya. Sea water samples have been collected from the coast of the Al-Hariga port, which were microalgae, and macro algae (seaweeds) and have been identified. The percentage of algal populations calculated based on the number of species was calculated by *Bacillariophyta*, *Chlorophyta*, followed by *Cyanophyta*, *Phaeophyta* and *Rhodophyta* was the least concerned species. Thirty six genera (47 species) of algae have been identified from the entire study period on the Al-Hariga port; Seven species of them (14.89%) were belonging to *Cyanobacteria* (4 families), eleven species (23.40%) belonging to *Chlorophyta* (9 families), twenty four species (51.06%) belonging to *Bacillariophyta* (11families), three species (6.38%) belonging to *Phaeophyta* (3families) and two species (4.26%) belonging to *Rhodophyta* (1family). The obtained results showed that the most common *Nitzschia* were found four species and *Licmophora* were found three species. However, diatoms were the most dominant

species in this area during this study. The results showed that Phaeophyta and Rhodophyta were lower the algae isolated from Hariga port. A total of 47 marine algal species (36genera) was recorded from along the coasts and Al-Hariga port, Tobruk- Libya to cover the study area Table 1.

Table 1 : List of marine algae isolated from Al-Hariga port, Tobruk

Division	Family	Algae
Cyanobacteria Cyanophyta (4Families & 7 species)	Oscillatoriaceae	<i>Oscillatoria erythraea</i> <i>Lyngbya majuscula</i> sp <i>Phormidium</i>
	Chroococcaceae	<i>Chroococcus turgidus</i>
	Nostocaceae	<i>Nostoc punctiforme</i> <i>Anabaena circinalis</i>
	Nostochopsidaceae	<i>Nostochopsis lobatus</i>
Chlorophyta Green algae (9 Families & 11 species)	Chlorellaceae	<i>Chlorella vulgaris</i>
	Cladophoraceae	<i>Cladophora rupestris</i>
	Monodopsidaceae	<i>Nannochloropsis gaditana</i>
	Chlamydomonadaceae	<i>Chlamydomonas reinhardtii</i>
	Haematococcaceae	<i>Haematococcus pluvalis</i>
	Pithophoraceae	<i>Pithophora oedogonia</i>
	<u>Ulotrichaceae</u>	<i>Ulothrix</i> sp
	Scenedesmaceae	<i>Scenedesmus quadricauda</i>
Bacillariophyta (Diatoms) (11 Families & 24 species)	Naviculaceae	<i>Navicula</i> sp <i>Navicula lanceolata</i>
	Fragilariaceae	<i>Diatoma vulgare</i> <i>Fragilaria Capucina</i> <i>Fragilaria</i> sp
	Bacillariaceae	<i>Nitzschia</i> sp <i>Nitzschia acicularis</i> <i>Nitzschia longissima</i> <i>Nitzschia</i> <i>reversa</i> <i>Cylindrotheca closterium</i>

		<i>Bacillaria paradoxa</i> <i>Bacillaria paxillifer</i>
	Surirellaceae	<i>Surirella gemma</i>
	Gomphonemataceae	<i>Gomphonema</i> sp <i>Gomphonema Gracile</i>
	Licmophoraceae	<i>Licmophora</i> sp <i>Licmophora abbreviata</i> <i>Licmophora gracilis</i>
	Catenulaceae	<i>Amphora</i> sp
	Striatellaceae	<i>Striatella</i> sp
	Pinnulariaceae	<i>pinnularia</i> sp
	Coscinodiscaceae	<i>Coscinodiscus wailesii</i>
	Stephanodiscaceae	<i>Stephanodiscus Astraea</i> <i>Stephanodiscus</i> sp
Phaeophyta (Brown algae) (3 Families & 3 species)	Laminariaceae	<i>Laminaria japonica</i>
	Scytosiphonaceae	<i>Colpomenia peregrine</i>
	Dictyotaceae	<i>Padina pavonica</i>
Rhodophyta (Red algae) (1 Family & 2 species)	Corallinaceae	<i>Corallina granifera</i> <i>Jania rubens</i>
Total	Families28	47 algal species

Dissection

In the present study, the algae were isolated from water sample collected from Al-Hariga port, Tobruk-Libya, were conducted for the purpose to obtain the best isolated microalgae have the ability to use for bioremediation of crude oil. In previous studies recorded many new species of marine algae on the east Libyan coast (19-23) . It had 168 species belonging to Chlorophyta, Phaeophyta and Rhodophyta (5, 24, 25).

In these study these algae are found in five groups including Cyanobacteria, Chlorophyta, Bacillariophyta, Phaeophyta, Rhodophyta .

Table 1 illustrated identified microalgae like cyanobacteria (7 genera) and also had diatoms(15 genera) of the present study area of Al-Hariga port with dominancy of *Nitzschia* specie. Regarding to Chlorophyta in these study which had(9 families)12 species It is rich of green algae as like to all eastern coasts like Ghazala coasts which had 14 species (26) .

Phaeophyta and Rhodophyta were lower than the algae isolated from Hariga port, Tobruk - Libya in this study. Generally, Tobruk coasts which had 11 brown algal species(26).The study area was less had 3 brown algal species .The study area were more or less similar to Tukra coasts which had 7 brown algal species (27) while it was richer than Ain-Ghazala coasts which had just one brown species (26) . Benghazi coasts were richer than the nearest eastern coasts of Libya like Derna and Tolmeta which had just 14 brown algal species of each (28), whereas Tukra coasts also had only 7 brown algal species (29) .

A total of two species of red marine algae (4.26%) was recorded in the current Al-Hariga port area and were tabulated in Table 1 with a special reference to the family of the Corallinaceae. The registered red algae species were quite different from the western Libyan coasts such as the coast of Tokra (38 species) and the Tolmeta coasts (13 species) recorded before (27, 29), in Darna (5 species) by (27) , and those of the Tobruk coast (16 species) recorded by (26). The relative decline of some species especially Phaeophyta and Rhodophyta, is due to pollution by petroleum-derived pollutants in the port. The result is a focus of some pollution and human disturbance that leads to reduce the presence of macroalgae (seaweeds). Although some good indicators algae was recorded, one could conclude that the study area is relatively poor of marine algae and polluted according to (28), who reported that the decrease in the number of species and increase in number of individuals is a characteristic feature of polluted water.

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عزل وتحديد الطحالب البحرية الملوثة بالنفط الخام من ميناء الحريقة بطبرق - ليبيا

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قسم النبات بكلية العلوم جامعة عمر المختار البيضاء - ليبيا

المستخلص العربي

يهدف العمل الحالي بشكل أساسي إلى دراسة الطحالب البحرية من ميناء الحريقة. أجريت الدراسة من الربيع إلى الخريف (2020). تم جمع عينات مياه البحر من ساحل مدينة الحريقة وتم تحديدها من الطحالب الدقيقة والطحالب البحرية. تم تسجيل 47 نوعاً من الطحالب (36 جنساً) في منطقة الدراسة قادرة على النمو على النفط الخام. سبعة أنواع منها (14.89%) تنتمي إلى البكتيريا الزرقاء (4 عائلات) ، 11 نوعاً (23.40%) تنتمي إلى الطحالب الخضراء (9 عائلات) ، أربعة وعشرون نوعاً (51.64%) تنتمي إلى الطحالب الدياتومية (11 عائلة) ، ثلاثة أنواع (6.38%) تنتمي إلى الطحالب البنية (3 عائلات) ونوعين (2.26%) ينتمون إلى الطحالب الحمراء (عائلة واحدة). أظهرت النتائج التي تم الحصول عليها أن أكثر أنواع شيوغاً *Nitzschia* تم العثور عليها مع أربعة أنواع و *Licmophora* تم العثور عليها مع ثلاثة أنواع. ومع ذلك ، كانت الدياتومات هي أكثر الأنواع انتشاراً في هذه المنطقة خلال ثلاثة مواسم. كانت منطقة الدراسة تحتوي على ثلاثة أنواع من الطحالب البنية ، وعدم وجود هذا النوع في الميناء بسبب الملوثات الزيتية ، تم تسجيل نوعين من الطحالب البحرية الحمراء (2.26%) في منطقة ميناء الحريقة الحالية مع مرجع خاص. لعائلة *Corallinaceae*. على الرغم من تسجيل بعض المؤشرات الجيدة للطحالب ، يمكن للمرء أن يستنتج أن منطقة الدراسة فقيرة نسبياً من الطحالب البحرية وملوثة.

الكلمات المفتاحية : عزل وتعريف , الطحالب البحرية