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The quantity determination of total carbohydrates and monosaccharides from some green algae (Chlorophyta)

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Abstract

The present study was employed the determination of total carbohydrates and monosaccharides from three green algal species are : *Chlorella vulgaris*, *Cladophora crispata and Ulothrix cylindricum* isolated from (Al-Ashar,Abul Khaseeb and Garmat –Ali) rivers in Basra.

The results revealed that carbohydrate concentrations reach about (280, 200,120) µg/g in *Cl. crispata* , *U. cylindricum* and *C. vulgaris* Respectively. Gas chromatography technique (GC) the identification of the monosaccharides glucose , rhaminoes, galactose, xyloes & ribose was done, which were differs in their concentration and existence together with different of the algal species. *C. vulgaris* contain glucose ,ribose , xyloes , rhaminoes ,& galactose in concentrations are (11.5, 8.6 , 8.7 , 8.8 , 8.6) % respectively , while *Cl. crispata* contain rhaminoes, galactose, xyloes, & ribose in concentrations (11.49, 12.3, 10.5, 13.3)% respectively , and *U. cylindricum* contain a galagtose , xyloes, ribose in concentrations (12.5 , 11.4, 10.5) % respectively .

Key words : Green algae , Carbohydrates, Monosaccharides .

1- Introduction

Algae consist of different categories of living organisms that able for photosynthesis , their shapes ranged between unicellular to multicellular and there were a large differences in their sizes , some of them are very small (1) micron , but the others are very large (200) meter as in *macrocystis sp.* (Lee 2008).

Algae have beneficial for human in nutrition, industry of alginate, carraginin, agar, and pharmaceutical industries (Cardozo *et al.*, 2007). Natural products are those biochemical compounds wich are derived from living organisms including algae, bacteria, fungi, plants, and animals. Algae produce awide variety of chemically active metabolites potentially as an aid to protect themselves against the other settling organisms. The active metabolites also known as biogenic compounds, such as polysaccharides, alkaloids, lipo proteins, aldehyds, alcohols and terpenoids. (Bhadury and Wright, 2004; Smith 2004; Athbi 2009). Algae contain high percentages of polysaccharide which are found on the cell wall, in addition of storage starch at the form of mexophycean on the blue green algae, floridians on the red algae and true starch in green algae (Percival and Mc Dowell,1985).

The alga *Spirulina platensis* contains high concentration of proteins reached to 70% , the carbohydrates consist of the principal parts of the algal cell component, it was found that 11% of water soluble polysaccharide contains mannose , galactose , glucose xylose, rhamanose and arabinose (Venkatarman and Bealer ,1985). It was found that, the alga *Oscillatoria hildebrandti* contains some of monosaccharide sugar especially rhamanose 47%, glucose 9%, galactose 4% , mannose 4% (Mikheiskaya *et al.*,1985). The sugar found in the alga *Porphridium sp*. were manose , glucose , galactose, arabinose, rhamanose and xylose (Salced-Olvarrieta and Ortega,1978).

The cell wall of the alga *Enteromorph aintestinalis* consist (63%) of sugar which were rhamanose , galactose , glucose and glucouronic (Dodson and Aronson, 1978).

Chlorophyta also have the beneficial for human in nutrition such as the alga *Cladophora crispate* used us food for human which is contain a high percentage 40.3 % of protein (Athbi *et al*., 2009). Where is the alga *Ulothrix cylindricum* contain different fatty acids such as oleic acids 15.7 % and palmatic acid 23.6 % (Athbi *et al*., 2011), also the alga of *C. vulgalis* contain protein in concentration 50.5 % and different amino acids aspartic, leucin, isoleucin, alanin and tryptophan. (Athbi *et al*., 2009).

The alga *Ulva sp*. contains rhamanose, glucose , xylose and d-glucuronicacid (Percival and McDoweel,1985).

The aim of this study is reveled the determination of total carbohydrates and monosaccharides in some green algae.

2- Material and Methods

- **Cleaning and Sterilization of glass ware:**

Glasses were washed before uses to the preparation of media and growth of algal isolation, by hydrochloric acid (20%) , then washed by distilled water and sterilized in oven (TIE-Unitemp) in 180 C^o for two hours. Conical flask at volumes 250 ml were used for the experiments, cleaned and sterilized cotton was used to close the conical flasks , also sterilized glass Petridishes were used for inflexible culture media.

- **Preparation and sterilization of growth media:**

The modified culture medium from (A1- Aarajy, 1996) named (Chu-10) was used for the growth of isolated algae. The growth media prepared as stock solution and stored at temperature 4 C^o in refrigerator with out sterilization since its used .

- **Collection of water samples :**

Water samples were collected from different rivers of Basrah represented by (Al-Ashaar, Abul Khaseeb and Garmat-Ali). Samples were brought directly to the laboratory 2 diagnosis of algal species.

- **Isolation and classification of algae:**

The procedure of (Stein,1975) was applied to get the unialgal cultures . Streaking and spreading methods were used on the inflexible culture medium , and dilution method used on the unialgal cultures , then the algae were classified according to the (Desikachary,1959 ; Prescot,1975 ; Bourrly,1980).

Division: Chlorophyta
Class : Chlorophyceae
Order : Chlorococcales
Family : Chlorococcaceae
Genus : *Chlorella*
Species : *Chlorella vulgaris* Beinjerink
Division : Chlorophyta
Class : Chlorophyceae
Order : Ulothricales
Family : Ulothricaceae
Genus : *Ulothrix*
Species : *Ulothrix cylindricum* Prescott
Division : Chlorophyta
Class : Chlorophyceae

Order : Chaetophorales

Family : Cladophoraceae

Genus : *Cladophora*

Species : *Cladophora crispata* (Roth) Kuetizing

- **The culturing of algae :**

After the process of isolation and diagnosis of the algae, each species was transformed separately by loop to liquid culture media.

- **Purification of isolated algae :**

The purification of algal isolated from microorganisms, the procedure described by (Weidman *et al.*, 1984).

- **Estimation of growth rate :**

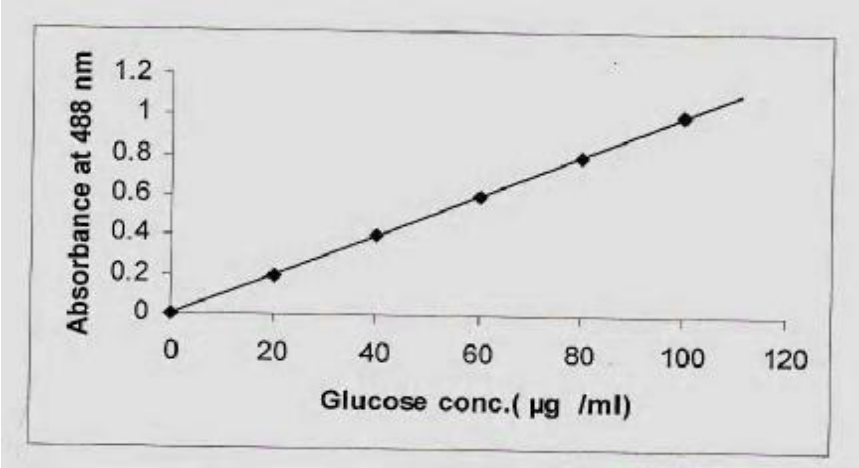
The growth was estimated by measuring optical density of cells by use the spectrophotometer (Spectro Sc. USA) at the wave length 650 nm (Stein 1973).

- **Estimation of total carbohydrates :**

The procedure described by (Herbert *et al*., 1971) was followed to the estimated of total carbohydrates. The standard curve of glucose solution was prepared by dissolved 100 mg from glucose sugar in (1) liter of distill water, then the dilution had done to obtained the concentrations (20 , 40 , 60 , 80, 100) µg / ml , then treated according the methods above as its demonstrated in (figure -1-).

- **Estimation of monosaccharide:**

The procedure described by (Naviner *et al* ., 1999) was followed for the extraction of algal cell components. Gas chromatography technique was used to estimated the monosaccharide.



(Figure -1-) Calibration curve of glucose in (µg/ml)

3- Results

- **Isolation and diagnosis :**

In the present study , three species of green algae were isolated and purificated as axenic culture which belonged to the division (Chlorophyta) . There were a variation observed between algae in their shapes and sizes. One of the algal isolate represented unicellular *Chlorella vulgaris*, whereas the others from filamentous multicellular algae, was true branched *Cladophora crispata* while the other was unbranched *Ulothrix cylindricum*.

Description of the isolated algae species :

***1- Chlorella vulgaris* Beinjerink .**

Unicellular green algae , the cell was small in size (5.0 – 8.5) micrometer , the shape was ovate , cup shape plastid occupy all the space of the cell , this algal was found in the fresh water in rivers, becks and polluted water contains high concentrations of nutrients.(picture -1-).

***2- Cladophora crispata* (Roth) Kuetizing .**

The filamentous multicellular green algae and had true branched , it was found in shallow fresh water conglutination or free floating, cells were cylindrical in shapes , the diameter ranged between 40 – 75 micrometer , while the lateral branches was a small 20 -35 micrometer , thick cell wall composed of a number cellulose layers , each layer composed of a small fibrils arranged on the spindle shapes , the green plastid reticulate and had a number of pyrenoid. (picture -2-).

***3- Ulothrix cylindricum* Prescot .**

Green algae, filamentous multicellular, unbranched cells, cylindrical in shapes , the diameter ranged between 11.0 – 12.5 micrometer . Cells length exceed their width. The cells had green plastid (wide girdle) contains a number of pyrenoid 2 – 5 . The algal was found in shallow fresh water demonstrated. (picture -3-).

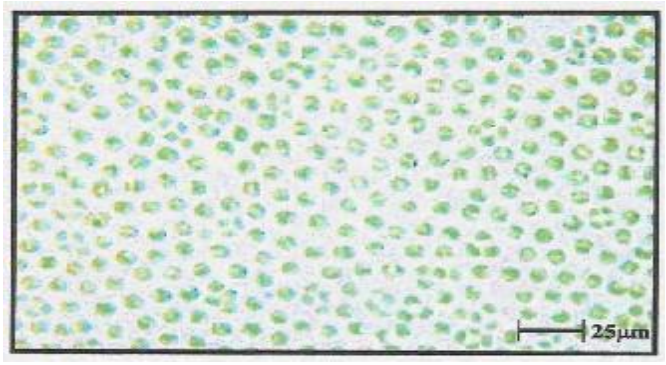
- **Growth rate :**

The growth rate of algal species were measured. Alga *C. vulgaris* was revealed that the growth constant (K=1.1) while the value of generation time was (G=0.28) as in (figure-2) (table-10. The growth constant of algal *Cl. Crispate* was estimated (K=1.3) and the generation time (G=0.23) (figure-3) while the algal U. cylindricum was observed that the growth constant (K=0.82) whereas the value of generation time was (G=0.36)(figure-4).

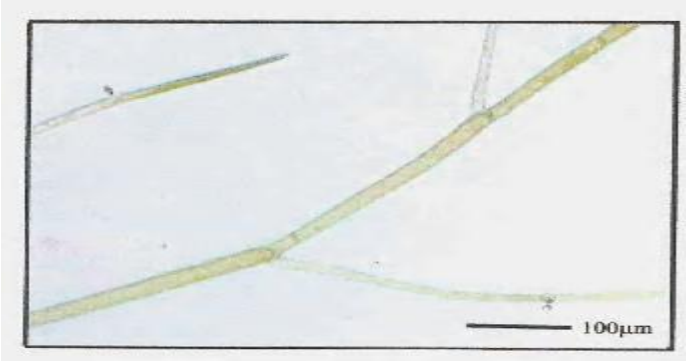
Total carbohydrates :

The contents of total carbohydrates were varied in the algal species . The high content of carbohydrates was found on the algal *Cl. crispata* 280 µg / gm from the dry weight , the second *U. cylindricum* 200 µg / gm , whereas the *C. vulgaris* on the third position 120 µg / gm . The contents of monosaccharide of the studied algal species were divers (table -2-). The alga *U.*

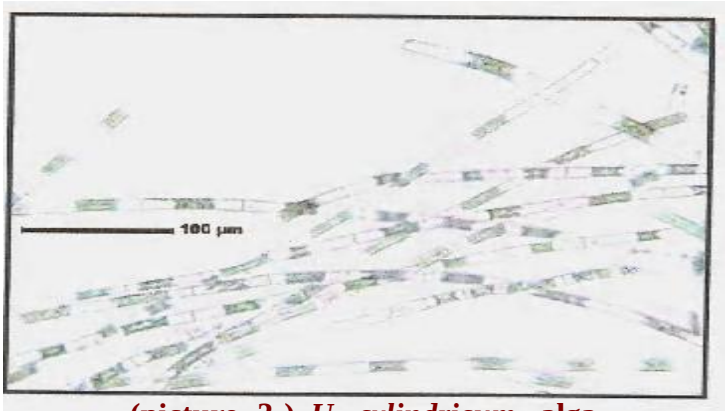
cylindricum was contained galactose 12.5% , xylose 11.4% and ribose 10.5% (figure-6-), whereas the *C. vulgaris* contained five kinds of monosaccharides represented by glucose 11.5% , rhamanose 8.6% , galactose 8.7% , xylose 8.8% and ribose 8.6% (figure -7-) . While the algal *Cl. crispata* contains four kinds of sugar , there were rhamanose 11.49% , galactose 12.3% , xylose 10.5% and ribose 13.3% (figure –8-).



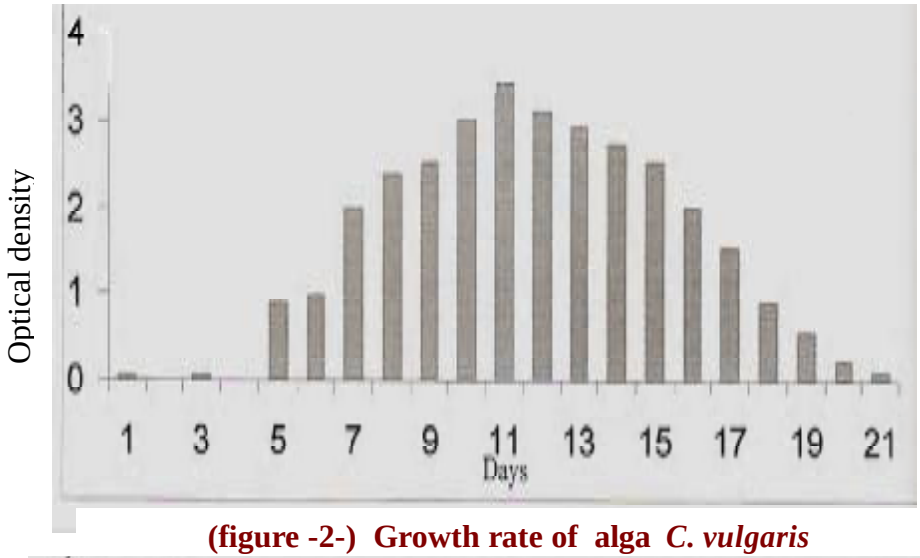
(picture -1-) *C. Vulgaris* alga



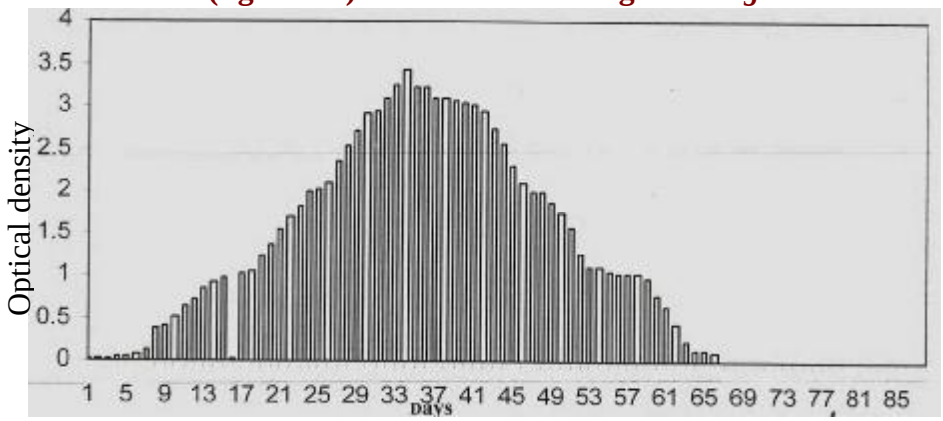
(picture -2-) *Cl. Crispata* alga



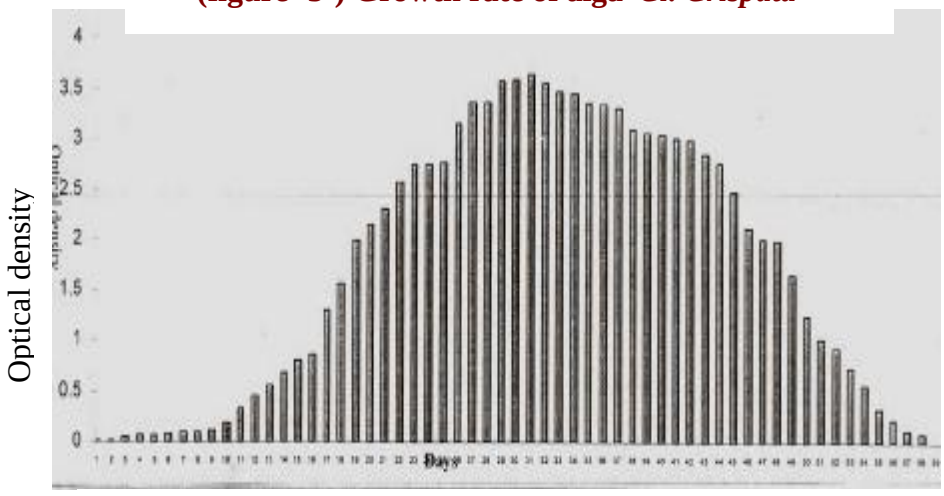
(picture -3-) *U. cylindricum* alga



(figure -2-) Growth rate of alga *C. vulgaris*



(figure -3-) Growth rate of alga *Cl. Crispata*



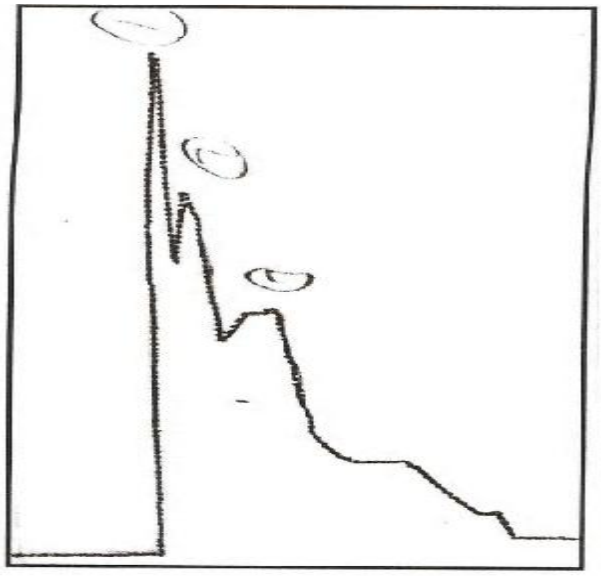
(figure-4-) Growth rate of alga *U. cylindricum*

(Table -1-) Growth constant (K) and generation time (G) and harvesting time of algae

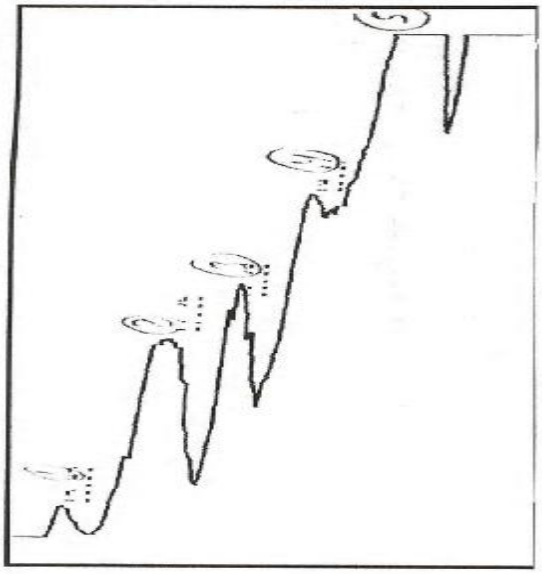
Algal species	Lag phase	Log phase	Stationary phase	Harvesting phase	Growth constant (K)	Generation time
C. vulgaris	0 - 4	4 – 10	10 - 15	12 - 13	1.1	0.28
Cl. Crispata	0 - 8	8 – 30	30 – 42	36 - 37	1.3	0.23
U. cylindricum	0 - 12	12 – 29	29 – 45	36 - 37	0.82	0.36

(Table -2-) Concentration of total monosaccharides isolated from algae (%)

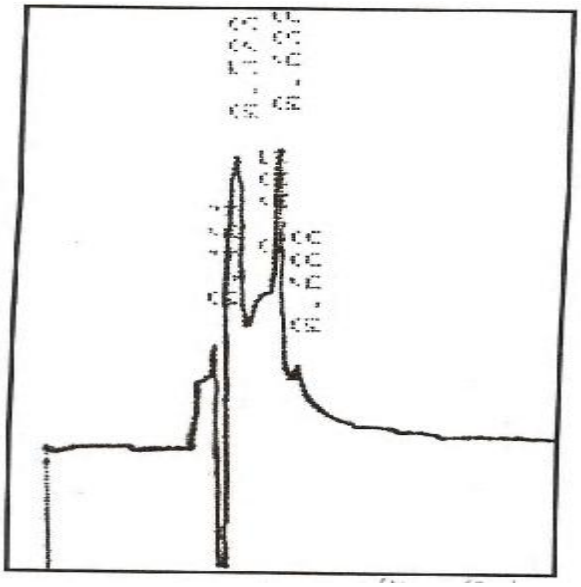
Algal type	Ribose	Xylose	Galactose	Rhamanose	Glucose
C. vulgaris	8.6	8.8	8.7	8.6	11.5
Cl. Crispata	13.3	10.5	12.3	11.49	—
U. cylindricum	10.5	11.4	12.5	—	—



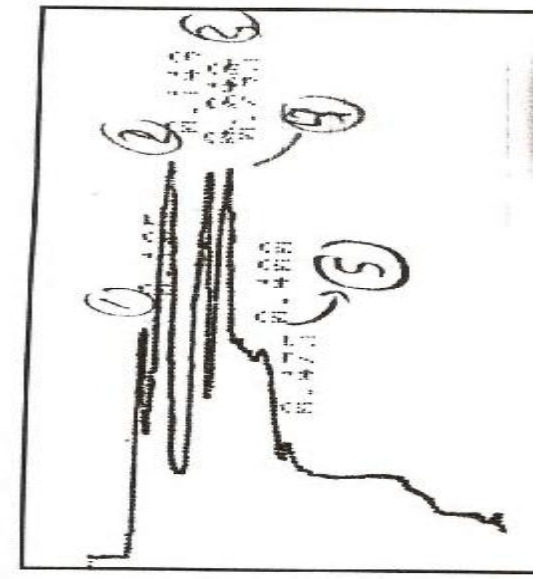
(fig.-6) Monosaccharide in *U. cylindricum*



(fig. -5) Stander curve of monosacchaides



(figure-8) Monosaccharides in *Cl. Crispata* alga



(fig.-7) Monosaccharides in *C. vulgaris* alga

4- Discussion

The algae *U. cylindricum* and *Cl. crispata* had contain high percentages of carbohydrates as compared with *C. vulgaris*. The studies referred that , the carbohydrates content of the small algae were different according to the growth phase (between log and stationary phase) , but the difference here according to the species (Brown *et al.*, 1997). The high percentages of carbohydrates in *U. cylindricum* and *Cl. crispata* perhaps due to the layers of cellulose in the cell wall was composed of cellulose and considered an important of carbohydrates component on the algae. The monosaccharide represented by manose , galactose , xylose , rhamanose and ribose which were found in the soluble state in water. (Venkatarman and Becker ,1985) were demonstrated that, 11% of monosaccharides in algae represented by rhamanose , xylose , ribose , glucose and galactose . The results of the present study are in accordance with (Bourne *et al.*,1970) , who referrers to that , the algal *Cl. rupesirs* contains ribose , galactose , xylose , rhamanose and glucose . The algal *Cl. crispata* belong to the same genus , and the results are in accordance with (Chekoi *et al.*,1970) , who demonstrate that on their study , the algal *Cl. vagabunda* contain galactose , glucose , xylose and rhamanose.

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التحديد الكمي للكاربوهيدرات الكلية والسكريات الاحادية في بعض الطحالب الخضر Chlorophyta

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الخلاصة

تناولت الدراسة تحديد محتوى الكاربوهيدرات الكلية والسكريات الاحادية في ثلاثة انواع من الطحالب الخضر *Chlorella vulgaris* و *Cladophora crispata* و *Ulothrix cylindricum* المعزولة من مياه أنهار (العشار وأبي الخصيب والكرمة) في محافظة البصرة .

بينت النتائج أن محتوى الطحالب المدروسة من الكاربوهيدرات الكلية تمثلت بتراكيز قدرها (280 و 200 و 120) مايكروغرام / غرام في الطحالب *Cl. Crispata* و *U. cylindricum* و *C. vulgaris* على التوالي . تم تشخيص السكريات الاحادية الكلوكوز glucose والرايبوز ribose والزايلوز xylose والرامينوز rhamnose والكالكتوز galactose بتقنية كروماتوغرافيا الغاز والتي اختلفت في وجودها وتراكيزها معاً باختلاف نوع الطحالب المدروسة إذ احتوى طحلب *C. vulgaris* على الكلوكوز والرامينوز والكالكتوز والزايلوز والرايبوز وبتراكيز (11.5 ، 8.6 ، 8.7 ، 8.8 ، 8.6) % على التوالي ، أما طحلب *Cl. crispata* فقد احتوى على الرامينوز والكالكتوز والزايلوز والرايبوز وكان تراكيزها (11.49 , 12.3 , 10.5 , 13.3) % على التوالي ، أما الطحلب *U. cylindricum* فقد احتوى على الكالكتوز والزايلوز والرايبوز وتراكيزها (12.5 ، 11.4 ، 10.5) % على التوالي .