Website: jsci.utq.edu.iq

Volume 7, Number 1, June 2019

Email: jsci@utq.edu.iq

Effect of interactions between nitrogen fertilizer (urea) and time periods on the growth of algae *Chroococcus sp*.

Wesan Fadhel Khalef

Department of Biology - College of Sciences - University of Thi-Qar, Iraq

Email:- Karrairaq92@gmail.com

Mobile:- 07807910551

Abstract:-

This study included isolating, diagnosing and puriflying algae *Chroococcus sp.* (Blue - green algae) was collected from Al-Garraf River and cultured on (Ch-10) media (3,5and10)% urea was added in three concentration in addation to control treatment. This study showed the effect of urea fertilizer on the growth rate of algae the use of urea has led to variability in the number of cell in algae.

An increase in the numbers was observed in the first, second and third stage of growth.where recorded low numbers in the fourth and fifth stage of growth. The results showed that the lowest value recorded of *Chroococcus* was 30 (cells / 100 ml) in the ninth day while largest value was 650 (cells / 100 ml) on the fifth day. **Keyword:-** *Chroococcus*, Cyanophyta, Fertilizer urea.

Introduction:-

God created the seas and oceans and rivers and make them a source of many treasures and benefits that the modern world still reveals the benefits and secrets of this strange world, algae and seaweed one of the secrets of this wonderful world, which must be used is the semi-vegetative organisms and algae exist in all ecosystems special in aquatic ecosystems and their different forms range from small cells that do not see the eye diameter (1) micrometer called Microalgae to large multi-size seen in the naked eve called Macroalgae (Barsanti and Gualtieri, 2006), and therefore known algae thaleous plants have no roots or leg or real leaves living in seawater, fresh water and high humidity are growing thanks to nutrients available in the aquatic environment. Algae are known as simple autotrophic plants, lacking vascular tissue and containing chlorophyll (A) and have simple, nongrafted reproductive structures. Algae are classified according to the phenotypic properties, chemical structure of the cell wall and the quality of the pigments present Chlorophyllates, zanthophyllates and carotenoids, including blue-green algae, also called Myxophyta, because they are surrounded by a gelatine sheath. Some scientists call it Cyanobacteria, because

there is some similarity between this division and the bacteria these are found in the modern classification of animal kingdoms with bacteria in the Monera kingdom. examples are the genus *Chroococcus*, unicellar that exists either adherent to the clay or in a stray form. It may exist in clusters of two or more cells surrounded by a gelatine sheath, These cells within the sheath take a specific shape spherical or regular flat or irregular (Saadi and Salman, 2002) figure1.



Figure1: Chroococcus sp. (Saadi and Salman. 2002)

change particular species or groups of dominant organisms (Palmer, 1969). Chemical fertilizers (nitrogen and phosphates) are factors that promote algal growth

Website: jsci.utq.edu.iq

Volume 7, Number 1, June 2019

(Postgate and Hill, 1979), Howarth et al. (2000) indicates that the nutrients that cause Eutrophication in the water system are nitrogen and silica. Liquid nitrogen fertilizer is used for all agricultural crops with good success (Abidi, 2011). Agricultural land is the main source of nitrogen in natural water due to the use of nitrogen fertilizers (Hughes, 1989), The final result of household nitrogen waste is the main source of nitrogen in waste water (Nasrallah, 1997). The importance of nitrogen as a specific agent depends on what is present in the medium and what the algeal consumes. Urea is an important nitrogen fertilizer because it contains a high percentage nitrogen and its chemical form NH2CONH2, The importance of organic fertilization comes from the fact that organic fertilizers are not a major source of organic matter in the soil and are direct and indirect sources of many nutrients required by the plant. They are also improved for the total physical, chemical and biological properties of the soil (Murawska et al. 1995; Tisdal. et al. 1985; Finck, 1982). In addition organic fertilization improves the physical properties of soil and ultimately reflects the activity of organisms in the environment (Brady, 1996). Organic matter in organic fertilizer manure increases benthic algae, especially after the decomposition of organic fertilizer (Stirling, 1985). The aim of study is know the effect of adding different concentrations of urea fertilizer on the growth rate of algae at different periods of growth.

Materials and methods:-

Washing and sterilizing tools used :-

Wash the tools used in the water of the faucet and then with distilled water and then with diluted hydrolic acid 20% and then washed with distilled water and dried in the oven at 105 $^{\circ}$ C.

Collection of samples:-

Samples were collected from the surface water of the Gharaf River at a depth of 10 cm from different areas with 500 cm3 clean plastic bottles and brought to the laboratory, Part of the sample was confirmed using formalin at a concentration of 4% for microscopic examination, while the other part was left without installation for the purpose of the culture.

The culture medium:-

Using Al-Arajy (1996) the Chu-10 medium to develop the isolates (Table 1),attended the culture

medium was incubated in Stock Solution and stored in the refrigerator at a temperature of 4 m without sterilization until use. When preparing the culture medium, complete the desired size and adjust the pH between (7 - 7.4) when cultivation the algae by adding drops of sodium hydroxide solution (0.2 N). The strelization the culture medium using the autoclave (Germany),model Karl at pressure1.5 pound / ang) and temperture 121m for 20 minutes.

Email: jsci@utq.edu.iq

Concentrate 100ml / g distilled water	Salts
2.5	MgSo ₄ .7H ₂ o
5.76	Ca(NO ₃) ₂ .4H ₂ O
1.585	$NaHCO_3$
0.262	Na_2SIO_3
0.146	FeCL ₃
O.318	Na ₂ .EDTA
3.583	$CaCL_2.2H_20$
1	K ₂ HPO ₄
0.0045	MnCL ₂ .4H ₂ O
0.0007	$MoNa_2O_4$
0.0057	ZnSO ₄ .5H ₂ O
0.002	CuSo ₄ .5H ₂ O
0.072	H ₃ BO ₃
7.4	РН

Table (1) Components of Chu-10 Axis.

Isolation and purification of algae:-

For the purpose of obtaining Unialgal culture, the method of planning the petrdishes was used and after a series of dilution to obtain Unialgal culture (Stein, 1973), Unialgal culture were purified from germs according to the method (Weidman *et al.*, 1984) for the purpose of obtaining Axenic culture(Al-Mousawi, 1984).

Diagnosis of algae:-

The following sources were used to diagnose the type of moss used in the study: 1975;Desikachary,1959) ; Prescott, Bourrely,1980) Where the algae was isolated and indicated below Classifications:-

Cyanophyta (Blue green algae)

Class:Cyanophyceae

Order: Chroococcales

Family:Chroococcaceae

Genus: Chroococcus sp.

Website: jsci.utq.edu.iq

Volume 7, Number 1, June 2019

Email: jsci@utq.edu.iq

Preparation of urea fertilizer solutions:-

The standard solutions were obtained with concentrations of 1000 mg / L of urea fertilizer by dissolving the NH2CONH2 fertilizer in the ion-free water. Concentrations were attended by the necessary dilution, with concentrations of 3,5and10% of the fertilizer.

Add urea fertilizer:-

The isolates were added by adding (0.10) of the pure liquid plant as inoculums to 250 mL water bottles containing a medium of urea supported by (3.5, 10). Using three replicates for each concentration, incubated at 27 ± 2 ° C with Light period (12 hours light: 8 hours dark) with shaker daily and a sample was planted without the addition of urea fertilizer to be considered as a control sample.

Measuring growth rate:-

Used to calculate or count the algae method of counting count Counting Chamber and used in this way a slide called Petroff - hausser slide which is a piece of sliced into large squares and small cells are calculated in which the pilot after taking the size of a known sample and placed in the slide.

Statistical Analysis:-

The results were statistically analyzed using the variance analysis test (ANOVA test) using the Statistical Package for the Social Sciences (SPSS) using a Least Significant Difference Test (LSD) under the test level (P < 0.05).

Results and discussion:-

Nutrients are the most important environmental compounds affecting algae. Kamarianos et al. (1993) notes that nutrient concentration has a significant effect on the growth and reproduction of algae. Nitrogen and phosphorus compounds are involved in the synthesis of many cell components such as amino acids, proteins, nucleic acids, enzymes, et al., 1993). Nutrients in the algae environment, the most important of which are nitrogen and phosphorus, which are naturally added or human inputs, such as fertilization, waste water and plant waste, lead to algae blooming (Anderson et al., 2002). The results of the present study showed an increase in the number of chrysanthemums. This is due to the positive effect of urea fertilizer because it contains a high percentage of nitrates, which are a necessary source of food for algae (Hsieh and Wu, 2009). (Mulbry and Wlkie, 2001) found that the development of algae on the center of the animal

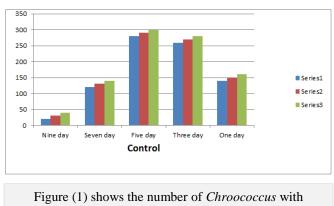
extract was good because it was rich in most of the nutrients necessary for its growth and was also consistent with (Husseini 2015). The results showed an increase in the number of moss cells on the first day, third and fifth due to the proportion of nitrogen in urea fertilizer, which is estimated at 46% (Abidi, 2011), but on the ninth day there was a decrease in the amount of consumption of nutrients in the middle, It is also noted from the results that as the concentration of fertilizer increases, there is a variation in the number of moss. The results showed that the lowest number of alfalfa records was (30 cells / 100 ml) on the ninth day while the highest number was 650 (cells / 100 ml) on the fifth day as shown in Table (2).

Table (2) shows the preparation of moss sp. Chroococcus with different concentrations of urea fertilizer

L	<u>S</u> .D	(9)days	(7)days	(5)days	(3)days	(1)days	Cell Preparation (Cell / 100 ml)
	60	30Ca	130Bd	290Ac	270Bc	150Ab	Control
	50	100Ad	250Cc	330Ab	300Bb	230Ba	3%
	70	160Ba	330Cb	400Bb	350Сь	300Bb	5%
	20	190Ba	420Dd	650Cb	500Da	450Cb	10%

The large similar characters in the same column between the coefficients indicate that there were no significant differences at the probability level (P < 0.05). The same small letters in the same row between the coefficients indicate significant differences at the probability level (P < 0.05).

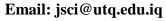
Small characters to compare transactions horizontally. Large characters to compare transactions vertically.



Control.

Website: jsci.utq.edu.iq

Volume 7, Number 1, June 2019



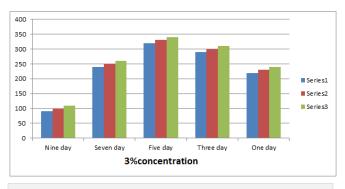


Figure (2) Effect of 3% urea fertilizer in the number of *Chroococcus*.

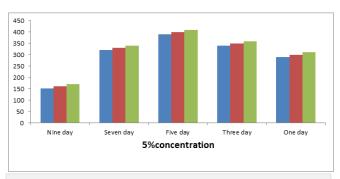


Figure (3) Effect of 5% urea fertilizer in the number of *Chroococcus*.

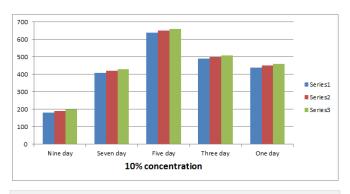


Figure (4) Effect of 10% urea fertilizer in the number of *Chroococcus*.

Conclusions and recommendations:-

- 1 good growth of algal cells on urea fertilizer because it contains a high proportion of nitrogen, which is a necessary source of food for algae.
- 2- Expand the study of the isolation, purification and culture of other types of algae available in our local environment.

- 3- The establishment of large open-air open spaces and space for the purpose of mass production of algae for research purposes.
- 4- Establishment of a special section within an institution interested in the development of alternative breeding plants for the development of algae.
- 5- Not to use the fertilizer urea extensively by the farmers even to the island to get the phenomenon of food enrichment Eutrophication, which results from an increase in the number and quantity of algae.

Source:-

- Al- Aarajy, M. (1996). Studies on the mass culture of some microalgae as food for fish larvae. Ph. D. Thesis, Univ. Basrah - Iraq, P : p 107.
- Abadi, Jalil Sabahi (2011). Directory of uses of chemical and organic fertilizers. Ministry of Agriculture, Iraq.
- Al-Husseini, Safaa Hussein Abdullah (2015). The chemical biochemical content of some micro algae and the effect of some environmental factors and pollutants in unsaturated fatty acids. Master Thesis, Faculty of Science, Dhi Qar University.
- Al- Mousawi, A. H. (1984). Biological studies on algae in rice field soil from the Iraqi marshes. Ph. D. Thesis Univ. Durham. England.
- Al-Rikabi, Hussain Yousef Khalaf (2003). The use of macro-farms for some types of micro-algae in poultry feed. College of Science - University of Basra.
- Anderson, D. M.; Glibert, P.olM. and Burkholder, J. M. (2002). Harmful algal blooms and eutrophication, nutrient sources, composition and consequences. *Estuaries*, 25(4B): 704 726.
- Barsanti,L .and Guatieri,P(2006).Algae Anatomy ,Biochemistry and Biotechnology. Tayor and Francis Group Boca Raton,London, New York.
- Bourrely, P. (1980). Les algaes deuu douce, initiation ala systematique, *Soc. Naur. Edit. Boubee*, *Paris*, (517, cited by Venkataraman and Becker, 1985).
- Brady ,N.C.(1996) The nature and properties of soils 11th ed. prenting , Hal , Inc , New Jersey, 740pp.
- Desikachary, T. (1959). Cyanophyta Edt. Indian Council of Agric. Res., New Delhi, 517.

Website: jsci.utq.edu.iq

Volume 7, Number 1, June 2019

- Finck, N, C, (1982) Fertilezers and Fertilization. Introduction and practical Guid to Crop Fertilization, Verlacg, Chemi, . Florida, Basel, 428p.
- Howarth , R.; Anderson, D.; Cloern. J.; Elfring, C.; Hopkinson, C.; Lapointe, B.; Malone, J; Marcus, N.A Mcglathry, K.; Sharply, and walker, D. (2000). Nutrient Pollution of coastal Rivers, Bays, and seas, Issues in Ecology, No.7.
- Hsieh, C. H. and Wu, W. T. (2009). Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. *Bioresour. Technol.*, 100: 3921 - 3926.
- Hughes, Lorent (1989) Environmental Pollution, University of Baghdad, Faculty of Science, Iraq
- Kamarianos, X.; Karamanlis,X.; Kousouris,T.; Fotis, G.; Delis, S. and Kilikidis,S. (1993).Ecological Studies on the Kerkini Reservoir(N Greece). II. Biological features. Geo.J., 29(4): 365-370.
- Lee, J.A.; Cho,K.J.; Known,O.S. and Chung, I.K. (1993). A study on the Environmental factors in Nekton estuarine ecosystem. J. Phycol., 8(1): 29-36.
- Mulbry, W. and Wlkie, A. (2001). Growth of benthic fresh water algae on dairy manures. J. APPL. Phycol., 13: 301 - 306.
- Murawska, B; Fabiasik, E .and Rejewski (1995) Effect of organic and mineral fertilization on the porprties of Podsolic soil, Zeszyty Naukawe techniczne Akademi RolniczeJ .W Bydgoszczy. ,183: 17 – 23.
- Nasrallah, Asraa Karim (1997). The ability of some types of green algae to remove phosphates and nitrates in wastewater. Master Thesis, Faculty of Education, University of Baghdad.
- Palmer ,C.M.(1969). Acomposite rating of algae tolerating orgnic pollution .Jour-phycology .5: 78-82.
- Postgate ,J.R.and Hill,S. . (1979). Nitrogen fixation . In "Microbial Ecology : Aconceptual Approach " J. M. Lynch and N.J. Poole (Editors). pp.191-213 . Blackwell Scl.Publ . Oxford.
- Prescott, G. (1975). Algae of the western Great lake areas Edt. Ellion C. Brown Co., pub. Dugugue. Iowa. 977p.
- Saadi, Hussein Ali, Sulaiman, Nidal Idris (2002). Dar Al Kutb for Printing and Publishing, University of Baghdad, p.647.

Stein, J. R. (1973). Hand book of phycological methods. Cambridge Unv. Press. Cambridge, U. K.

Email: jsci@utg.edu.ig

- Stirling,H.P.(1985).Chemical and Biological methods of water analusis for Aquaculture alists.Stirling Univ. Scotland(119)pp.
- Tisdal, S; Nelson, W.and Beaton, J. (1985) Soil fertility and fertilizers, Macmillan Publishing Company, New York. 754p.
- Weidman, V.; Walne, P. and Tainor, F. (1984). A new technique for obtaining axenic culture of algae *Can. J. Bot.*, *42*: 958 959.