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Investigation of Some Ecological Factors and Isolation Techniques for Some Local Algae in Iraq

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Abstract
Biological diversitydue to contrast environmental andheterogeneousregions in Iraq made them a very interesting for study. Some local algae were collected in this study from three stations along Tigres river in Baghdad city.Algae were isolated by specific threelab techniques.The dominant algae groups wereChlorophyta and Cyanophyta in three seasons (for all months), whileChrysophyta and other groups were seen insome months more than others.Results were taken weekly for nine month of the year 2020. Study effects shows a significant difference among most verified data for each factor, the correlation was investigatedbetween temperature and pH, in other hand EC and TDS were linked according to the outcomes of the work.
The study aimed forunderstanding the relation among abiotic issues and their effects on the algae distribution in Iraqi aquasystem and gathering some technique for algae isolating additionally picking a decent segregation methodology can prompt a quick isolating algal strain to be utilized in numerous applications.
Key wards: dominant algae, isolating techniques, biological diversity, abiotic factor.

Introduction
Algae are essential makers at the base of most oceanic natural pecking orders [1, 2]. Algae that give us air we inhale, food we eat, and the fuel for our vehicles, they are additionally a wellspring of dynamic drug intensifies that can be utilized against drug-safe bacterial strains, infections and tumors [3]. Also they have been broadly utilized in hydroponics as feedstuff and feed added substance, in fish cultivating. Subsequently algaegroups give us such a great amount from past till now and maybe in future[4, 5, 6]. Unquestionably it was imperative to continue considering these splendid living beings.Confining algae with numerous procedures has been grown step by step, actuality the separation of alga from common water samples required the utilization of classic and non-classictype of isolation and different media types containing different groupings of many key supplements so as to guarantee the growth of all algal species present in the testedsamples[7].
The procedures like streaking, spraying, serial dilution, and single-cell isolations, these methods have been used by researchers. A few strategies are noted as time taking and dubious towards snappy segregation[8, 9]. It has been remarked that Natural and engineered systemsalgae can be presented to an assortment of natural conditions that influence development rate and cell organization[10]. Natural factors, for example, temperature, light, pH, and nutrients or supplements not just influence photosynthesis and development pace of the algae, yet in addition impact the action of cell digestion and structure. These impacts have been recognized independently by scientists nonetheless, no combined audit on the impact of these variables on algae is accessible[11, 12].

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Materials and methods

I-collecting of samples

Algae used in present experiments were obtained from three stations (sta.)in Baghdad Tigris river at north of Baghdad (sta1), Tigris river at middle of Baghdad (sta2) and Tigris river at south of Baghdad (sta3)

Algae samples were gathered to be disengaged from the water collections of Tigris river these examples were kept up. Micro algae that developed effectively were moved into independent cylinders for routine maintenance. After this immediate separation the rest of the cell suspension was infused into tubes containing Chu-10 medium with a photoperiod of 16 hours light/8 hours dark, light intensity was (25-30°C, 125 µE/m/s, 16:8 light:dark).[13, 14].

II- measuring the environmental factors

Some ecological factors temperature, pH, total dissolved salts (TDS) and electrical conductivity (EC) were estimated all the while by utilizing portable digital devices. An amount of water that is available with algaesamples in fixed plastic cooled jugs to protect their properties from change and afterward move to the lab.[15]

III- isolation of algae cultures

The algae were isolated by using gatheredtechniques to get uni algae cells from the mixed algae

1. Using serial dilution with centrifuge

Explicit techniques were utilized to detach algae, samples were exposed to serial dilution to be isolated then incubated for 14 days and examed by microscope, this activity were accomplished for a few time till disengaged culture showed up, at that point tests were brooded at dull at24 C^0 for 3 hr. respectively, at that point centrifuged (2000 r\m for 3min) 5-10 time, the hasten was washed with distilled water at that point moved to flasks with new medium.[16]

2. Using Phototaxis withplatting

The method includes a focused light source toward one side of the containers or plats, and, contingent upon intensity, either sure or negative phototaxis can be utilized. [17]

3. Using Phototaxis with pipettes(pasture pipettes).

figuring out single algal cells byplacing cotton plugs and closing the wide end of the tubes, then sterilizing and cooled down, a quantity of the medium was drawn the algae is added, then the tube is wrapped and placing a light source, then after a period of 6-24 hours the microalgae moved towards the source of the light, after which the sealed part is removed and the sample is poured onto suitable media and is examined. This cycle is rehashed till we get a solitary algal cell, can be confidently positioned into culture medium. [17].

The incubation for algae cultures wascultured using Chu-10 media at (25C°, 125µE/m^2/s, light 16 : dark 8 hr.) algae were allowed to grow for about 14 days.

IV-Statical analysis.

In this experimental plan all information measurements of the investigation were taken week after week and set oftriplicates and they were analyzed by using statistical analysis least significant difference (LSD) at P≤ o.o5 and P≤0.01in other side the correlation (r) used to study the effect of some environmental factors on algae[18].

Results

I- Measuring the environmental factors

Four abiotic environmental factors were measured, namely temperature, pH, and total dissolved salt(TDS) concentrations, which might consider as concentrations of available nutrients in the aquatic environment, and finally the values of electrical conductivity(EC) of the water samples of the sites from which the algae samples were taken to study them and

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study the effect of these factors on the presence and spread of algae in the water, so the data were recorded. Weekly every month, starting from January until September, and the data were trip

There were significant differences among monthly readings for temperature, pH value, TDS and EC, and it was noticed that there was relation between temperature and PH value, on the other hand there was a connection between TDS and EC, correlation (r) ratio calculated between first two factors was 0.97 while other two factors was 0.41 (table 1),The results of environmental factor measurements for the nine months of the year, shown in (table 1), indicated that the monthly average values of temperatures ranged between (5-33)C^0 while the pH values were (8.0- 6.6), TDSconcentrations were recorded and ranged between (305.2 - 607.1) mg/l. finally it was noticed that the values of electrical conductivity varied between (590 -1233)Ms/cm.

Table 1. Mean of monthly recordings for some abiotic issues during nine month and their correlation value.

Month	Temperature C^o	PH	Correlation (r)	TDS mg/l	EC Ms/cm	Correlation (r)
1	10	7.5		388.5	900	
2	5	8.0		305.2	590	
3	14	7.6		370.6	820	
4	17	7.4		475.7	957	
5	26	7.1	**r= 0.97 ****	391.4	799	r= 0.41 *
6	28	7.0	**Highly-**	530.9	1055	Significant.
7	33	6.6	**Sig.**	607.1	1233	
8	30	6.9		500	1000	
9	26	7.0		410.1	870	
LSD	**5.381 ***	**1.073 ***	**---**	**126.093 ***	**362.59 ***	**---**

* ($P \leq 0.05$), ** ($P \leq 0.01$).

II- Collections of algae present during the study months

The algae groups most present in the water system from which samples were collected were determined. Green and blue-green algae were the most common, while diatoms and the rest of the other groups were not present in some of the months of the study and the results are shown in (Table 2)

Table 2. Common algae groups found in tested sample for nine month

n.	**Month**	**Chlorophyta**	**Cyanophyta**	**Chrysophyta**	**Other groups**
1	January	+	+	-	+
2	February	+	+	-	+
3	March	+	+	-	+
4	April	+	+	+	+
5	May	+	+	+	+
6	June	+	+	+	-
7	July	+	+	+	-
8	August	+	+	+	+
9	September	+	+	+	+

III – algaeidentification and isolationtechniques

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The algae species were detected and a delegation used a classification method based on the morphological characteristics as well as microscopic examinations to determine the algae species that were isolated [27] table (3).

Table 3. Classification of tested green algae isolated from the Iraqi aquasystem under study.

n.	genus of algae	Family	Order	class	(Phylum)
1	Chlorella sp.	Chlorococcaceae	Chlorococcales	Chlorophyceae	Chlorophyta
4	Scenedesmus sp.	Scenedesmaceae	Chlorococcales	Chlorophyceae	Chlorophyta
5	Chlamydomonas sp.	Chlamydomonaceae	Volvocales	Chlorophyceae	Chlorophyta

The utilization of various detachment strategies in this study energized the getting of isolated kinds of algae from mixed growth by following another arrangements, which is the utilization of a blend of classic separation techniques as a portion of the new methodologies to get certain species without others as shown in table(4).

Table4. Isolated green algae by different tested isolation techniques

n.	Green algae genus	Technique (tec 1)	Technique (tec2)	Technique (tec3)
2	Chlorella sp.	+	-	-
4	Scenedesmus sp.	+	-	-
5	Chlamedomonas sp.	-	+	+

Discussion

There was an inverse relationship between the temperature and the pH values. The higher the temperature, the lower the pH values, while the higher the concentration of TDS we find that led to an increase in electrical conductivity. In the cold first year months it was found that the pH values were relatively high and vice versa in the warmer months of the year. The temperature would affect the hydrogen bonds of the water. The degree of acidity of the water environment, many studies confirming these results [19, 20]. The concentrations of dissolved salts in the water environment are also affected by the temperature. The higher the temperature, the evaporation rates will increase, and consequently the concentrations of salts, nutrients and compounds present in the water will increase, and this will lead to an increase in the electrical conductivity values as a result of the increase in the concentrations of salts charges [21].It was noted that the increasing concentrations of dissolved salts is linked to an increase in urban human activities, and this is confirmed by [22]. These results can also be related to the difference in climatic conditions between the months of the studied year, and this is what was mentioned by [23].

The reason for algae distribution and The reason for the prevalence and dominance of a specific population of algae in habitats over different months of the year can be attributed to the ability of some algae to adaptation because they are among the organisms that tolerate stress and environmental changes, and there are types of them that bear the change in temperature, pH and other environmental factors, and the results of the study were in agreement with a study by Nayaka, S (2017) that insure that algae produce specialized spores, defensive chemical substances organic osmolytes that protect them from desiccation, high irradiation and UV light. Algae likewise have de novo biosynthesis system to manage the

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damage occurred due to desiccation [24], other research by Holzinger A and Pichrtova M (2016) [25] consider that formation of dormant spores is a commonplace system of freshwater classes, , high photo physiological plasticity and amassing of UV-screening constituents are significant defensive in conditions with high light. Scientists Karsten U and Holzinger A (2012) [26] found that some algae develop a strategy in which fragmentation of filaments into more modest units would develop a self-security systems for surviving at stress conditions.

A number of isolates belonging to Chlorophyta (green algae), which is the group under study, were obtained. In this manner, samples were gathered from stations and algae cultures put in growth media (Chu-10) [28] and become under suitable conditions each algal successfully isolated as in (table 4).*Chlorella sp.* and *Scenedesmus sp*. microalgae were isolated from studied stations. found to be tolerant for various range of environmental conditions, without flagella, multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce.[29, 30].

Results of dilution process for confinement *Chlorella sp.* was completed to got algal culture with using (tec 1) this was nearly in agree with an examination by NailaGhani*et al* 2020 [31] so we found that *Chlorella sp.* was effectively isolated through dilution technique many times until a single cell on slide. Some studies consistent with the results of the current research carried out by a number of scientists, which also included the cultivation of microalgae using nitrogen sources as food media, clearly some types of microalgae were isolated by using decimal dilution methods to obtain successful single isolates and produce good biomass.[32]. Isolated *Scenedesmus sp.* was done by using (Tec1) that result settle with a study by Jasmin, K.A. *et al.,* 2018, [33] that isolate possible uni algal cultures under controlled conditions by using partially the same technique. Add to that a method from a research by Lee, K. *et al.,* 2014 insure the study technique for isolating algal strain.[7].

Phototaxis with platting or with pasture pipate tec2 and tec3 in this study were modified for faster and better isolate. Glass micropipettes were obtained for manipulation of single living cell because of its specific end tips, this was possible to capture a single algae species, and that was very helpful for isolating [34].Setting up an isolated culture with this pipette washing strategy is a traditional/standard technique and is the most dependable strategy to use to detach the ideal cells for an investigation. A study by Tsuchikane, Y. *et al.,* 2018 concluded that the isolating by this technique encouraged of getting a single or un isolate algal, most reliable method to use to isolate the desired cells for a study. [35]. *Chlamydomonas sp.* isolation with Pasteur tubes developed in the microalgae isolation laboratory tec2 and tec3. Some micro algae grow towards the source of the light, thus *Chlamydomonas sp.* had the ability to move after which the sealed part is removed and the sample is poured onto suitable media and is examined, so the method succeeded Isolate them and a study bySchaller and other researchers (Schaller *et al.,* 1997)reached for conclusions that support the results of this research, they found that Chlamydomonas had ability to motile or grow towards light direction and switches its movement from direction to other[36]. Other study by Choudhary, S.K. *et al.,* 2019 [37] reached for that some algae such as *Chlamydomonas spp.* show phenomenology of Phototactic response enables them to be at some points sensors for light effect, that can be confirmed our study results.

Conclusion

Local environment factors of the air and water could be the significant restricting components for choosing which kind of algal strains can be filled rapidly in a set up region. Local species became adapted for the abiotic and biotic factors, and thus these organisms which had a specific compounds and systems made them successful for fruitful for developing and

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spreading. Also choosing a good isolation strategy can led to successful and rapid isolating algal strain to be used in many biological applications.

Reference

[1] Xu, Y., Lu, A., Chen, X., Su, D., Wang, W.and Zhang, Y..(2018). Isolation, Identification and Growth Optimisation of Freshwater Microalgae.*Turkish Journal of Fisheries and Aquatic Sciences* 18,1293-1302. https://doi: 10.4194/1303-2712-v18_11_06.

[2] Moreno, G.I. (2008). Microalgae immobilization: Current techniques and uses. *Bioresource Technology*, 99 (10), 3949-3964. https://doi.org/10.1016/j.biortech.2007.05.040.

[3]Fayyad, RaghadJ.. Mohammed Ali, Alaa N., Dwaish ,Ahmed S. and Al- Abboodi, Ahmed Khayoon Abed. (2019).Anticancer Activity of SpirulinaplatensisMethanolic Extracts Against L20B and MCF7 Human Cancer Cell Lines. *Plant Archives*. 19(1), 1419-1426.

[4] Spolaore, P., Cassan, J.C., Duran, E., andIsambert, A. (2006). Commercial applications of microalgae.*Journal of Bioscience & Bioengineering*, 101(2), 87–96.https://doi.org/10.1263/jbb.101.87

[5] Zhu, M., Yan, W.L., and Chen, X.K. (2012). The primary study of the initial feed and f Journal of Aquacultureeeding methods for the larvae of Misgurnusanguillicaudatus (Cantor).*Journal of Aquaculture*, 33(01), 17-19. https://doi.org/10.3969/j.issn.1004-2091.2012.01.005.

[6] Ma, A.J., Zou. J., Sun, J.H., Wang, T., Wang, G.N., Cui, W.X., andGuo, Z.L. (2014). Research progress and outlook of Takifugurubripes culture techniques. *Marine Sciences*, 38(02), 116-121. https://doi.org/10.11759//hykx20150917002.

[7] Lee, K.Eisterhold, M.L., Rindi, F., Palanisami, P. and Nam, P.K..(2014). Isolation and screening of microalgae from natural habitats in the Midwestern United States of America for biomass and biodiesel sources.*Journal of Natural Science, Biology and Medicine*. 5(2), 333-339.

[8] Gerloff, G.C., Fitzgerald, G.P. and Skoog, F.. (1950). The Isolation, Purification, and Culture of Blue Green Algae. *AM J BOT*,37, 216-218.

[9] Skinner, C.E.. (1932). Isolation in Pure Culture of Green Algae From Soil By a Simple Technique. *Plant Physiol*. 7(3), 533–537.https://dio:10.1104/pp.7.3.533.

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[10] Juneja, A.,Ceballos, R.M. and Murthy, G.S. (2013). Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: *A Review*. *Energies*, 6, 4607-4638.https:// doi:10.3390/en6094607.

[11]Morris, I., Glover, H. andYentsch, C.. (1974). Products of photosynthesis by marine phytoplankton: The effect of environmental factors on the relative rates of protein synthesis. *Mar. Biol.* 27, 1–9.

[12] Visviki, I. andSantikul, D.. (2000).The pH tolerance of Chlamydomonasapplanata (Volvocales, Chlorophyta). *Arch. Environ. Contam.Toxicol*.38, 147–151.

[13] Yang, X., Lu, P., Hao, X., Shi, X. and Zhang, S..(2012). Characterization and identification of freshwater microalgal strains toward biofuel production.*Bioresources.* 7: 686-695.

[14] Sathya.S and Srisudha.S. (2013). Isolation and Identification of FreshwaterMicroalgal Strains-Potentialfor Biofuel Production. *International Journal of Recent Scientific Research*,4(9),1432- 1437.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 18395- 18402
Received 25 April 2021; Accepted 08 May 2021.

[15] Jaafar, F.A. and Abdulwahhab, A.S. (2021). Impacts of the Physico-chemical Properties of Al-Chibayish Water Marshes on The Biodiversity of Phytoplankton.*Iraqi Journal of Science*, 62(2), 402-414.https://dio: 10.24996/ijs.2021.62.2.6

[16] Cullen, J. J., and MacIntyre, H. L. (2016). On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment. *Journal of applied phycology*, 28(1), 279–298. https://doi.org/10.1007/s10811-015-0601-x.

[17] Robert A. Andersen. (2005). Algal Culturing Techniques. Elsevier Academic Press, .pp:90-95.

[18] SAS. (2012). Statistical Analysis System, User's Guide.Statistical.Version 9.1th ed. SAS.Inst. Inc. Cary.N.C. USA.

[19] Hiba .K.S.(2018). Environmental study on algae species and water quality in Lake of Mosul Dam /Mosul.*International Journal of Environment & Water*. 7(4),13-24.

[20] Talling, J.F. (1980)," Chapter 6 in Euphrates and Tigris Mesopotamia Ecology and Destiny by Julian Rzoska", *MonographiaeBiologicae*. 38: 63-80.

[21] Thirumulini, S. and Joseph, K. (2009)," Correlation between Electrical Conductivity and total dissolved solids in natural water", *Malaysian Journal of Science*. 28(1), 55-61.

[22] Alobaidy, A.H.M.J., Maulood, B.K. and Kadhem, A.J.. (2010). Evaluating raw and treated water quality of Tigris River with in Baghdad by index analysis, *Jurnal of Water Resour. Protect*, 2, 629–635.

[23] Al-Janabi, Z.Z., Al-Kubaisi, A.and Al-Obaidy, A.H.M.J.. (2012). Assessment of water quality of Tigris River by using water quality index (CCME WQI), *Jurnal of Al-Nahrain Uni*. 15,119–126.

[24] Nayaka, S., Toppo, K. and Verma, S..(2017). Adaptation in Algae to Environmental Stress and Ecological Conditions. In book: Plant Adaptation Strategies in Changing Environment.https://doi: 10.1007/978-981-10-6744-0_4.

[25]Holzinger, A. andPichrtova, M.. (2016). Abiotic stress tolerance of Charophyte green algae: New Challenges for Omics Techniques. *Front.Plant Sci*. 7(678),1–17.https://doi: 0.3389/fpls.2016.00678

[26]Karsten, U.,Holzinger,A.. (2012) Light temperature and desiccation effects on photosynthetic activity and drought-induced ultra-structural changes in the green alga Klebsormidiumdissectum (Streptophyta) from a high alpine soil crust. *Microb Ecol*. 63:51–63. https://doi: 10.1007/s00248-011-9924-6

[27] Prescott, G.W. (1982).Algae of the Western Great Lakes Areas. Willam, C.; Brown, C.O .Pub. Dubuque. I. Iowa,16th printing.

[28] Atlas, Ronald M. (2010) Handbook of Microbiological Media (4th edn), CRC press.

[29] KaiRu, L.T., Sung, Y.Y., Jusoh, M., Abdul Wahid, M.E. andNagappan, T. (2020). Chlorella vulgaris: a perspective on its potential for combining high biomass with high value bioproducts. *Applied Phycology*, 1(1),2-11.https://doi.org/10.1080/26388081.2020.1715256

[30]Sarwa, P. and Verma, S.K. (2017). Identification and Characterization of Green Microalgae, Scenedesmus sp. MCC26 and Acutodesmusobliquus MCC33 Isolated from Industrial Polluted Site Using Morphological and Molecular Markers. *Int. J. Appl. Sci. Biotechnol*. 5(4), 415-422. https://doi: 10.3126/ijasbt.v5i4.18083

[31] Ghani, N., Shahzadi, N., Sadaf, S., Ullah, I., Ali, E., Iqbal, J, Rafique, T.andMaqboo, M. (2020) . Isolation of Several Indigenous Microalgae from KallarKahar Lake, Chakwal Pakistan.*Iranian Jurnal of Biotech*. 18(3), 70-79.https://doi: 10.30498/IJB.2020.122025.2214.

[32] AL-Shandah, B.T.I., Abd Al-Jabar1, R.A., Farkha, T.K.J.,(2018). Isolation, Purification and Identification of Seven species of Algae in three drinking water plants supply

springs in Sulaimani province and growth control of isolated algae by using some plant extracts.*Tikrit Journal of Pure Science* . 23 (1),26-37.https://dx.doi.org/10.25130/tjps.23.2018.005

[33]Jasmin, K.A., Jabir, T., JesmiYousuf, Saramma A.V. and Mohamed Hatha A.A. (2018). Isolation and Identification of Vaious Species of Scenedesmus from Tropical (Cochin).Conference: International Biodiversity Congress (IBC-2018) 60 Forest Research Institute, Dehradun, India.4, 55-57. https://doi: 10.13140/RG.2.2.18556.59520.

[34]Bretag, A.H..(2017). The glass micropipette electrode: A history of its inventors and users to 1950. March 2017. *The Journal of General Physiology*, 149(4): 417-430.https://doi: 10.1085/jgp.201611634.

[35] Tsuchikane, Y., Hamaji, T., Ota, K. and Kato, S.. (2018).Establishment of a Clonal Culture of Unicellular Conjugating Algae.*Journal of visualized experiments*. 37(57761),1-7.https://doi: 10.3791/57761.

[36] Schaller, K., David, R. andUhl, R..(1997). How Chlamydomonas Keeps Track of the Light Once It Has Reached the Right Phototactic Orientation.*Biophysical Journal*, 73(September), 1562-1572.

[37]Choudhary, S. K., Baskaran, A. and Sharma, P. (2019). Reentrant Efficiency of Phototaxis in Chlamydomonasreinhardtii Cells.*Biophysical Journal*, 117(150),: 1508–1513.