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Biochemical changes of some algae used in the remediation of some pollutant in municipal wastewater

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Abstract

The current study aim to investigate the susceptibility of three types of algae, including *Oscillatoriasp*, *Westiellopsisprolifica*, *Stigonemasp*, in reducing the values of some pollutants. These algae were used as unialgal culture and the waste water was used from the final settling ponds of central wastewater treatment plant (Almamierh) in Hilla city.as an growth culture for the development of these algae by cultivating each algae alone in waste water under constant laboratory conditions at 25 ± 20 ° C and in a light system 16: 8 hours Illumination: Darkness.

The results showed slightly increased in pH values, and low removal percent was recorded by both *Oscillatoria*sp and *Westiellopsisprolifica* ,while*Stigonemasp* record a role by reducing the values of total soluble solids , electrical conductivity and salinity .The results showed that the studied Algae have the ability to reduce the biological oxygen demand , total Alkalinity , total hardness , calcium and magnesium, chlorides , nitrite , nitrate and phosphates . As for the current algae physiological status, the chlorophyll content did not change in *Oscillatoria sp.* after treatment While chlorophyll content increased in *W. prolifica*, and decrease in*Stigonema sp*. after treatment. The effectiveness of the (SOD) enzyme did not change in all algae after treatment . The effectiveness of the catalase enzyme elevated in *Oscillatoria sp*. and *W. prolifica* but

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decreased in *StigonemaSp* after municipal wastewater treatment. The study also showed that Malondialdehyde (MDA) was increased in three studied species.

Introduction

Phycoremediation is used to express the role of microalgae and macroalgae in the removal or biological transformation of pollutants in waste water [1] .The accumulation of pollutant and heavy elements in algae is due to physical-chemical processes occurring in the cell surfaces where algae can assemble heavy metals in two phases, including the first stage rapid adsorption on the surface of the cell. The second stage involves slow regular and energy-efficient (efficient transport) called the Bioaccumulation [2]. At the same time, there are several mechanics to remove pollutant for example proteins Association of elements such as phytochetatins and Metallothionein and the process of rejection or exclusion of heavy elements of the cells by vectors of metals are optional or secreted out process or booked to reduce toxicity [3].Algae also has a mechanism to reduce the toxicity of certain elements by producing
organic bonds such as carbohydrates and sugary proteins that have the ability to bind and harvest copper ions in the cytoplasm and reduce their toxicity [4]. Large molecules in the cell wall for example Caetin and fat play a role in the removal of heavy metals by containing functional aggregates such as hydroxyl and carboxylic groups that play a role in the formation of complexes with heavy metals [5]

Material and Methods

Isolation and Culturing of Algae

The stock solutions of the BG11 medium was prepared by dissolving certain weight of salts as its mentioned in the table (1) with distal water to (1) liter. The pH value was adjusted to approximately (7.1) and then sterilized in autoclave for 15 min at 121 C° [6].Then added10 ml of pure algae to a flask contained 90ml of BG11 media and incubated at 25 ± 20 C° with a photo period of8 hour darks:16 hour lights for 14 days.

Table 1: BG11 medium Component

Compound	Stock Solution	ml/liter
$NaNO_3$	150 g/l	10ml
K_2HPO_4	40 g/l	1ml
$MgSO_4.7H_2O$	75 g/l	1ml
$CaCl_2.2H_2O$	36 g/l	1ml
Citric acid	6 g/l	1ml
Ferric ammonium citrate	6 g/l	1ml
EDTA-Na_2	1 g/l	1ml
Na_2CO_3	20 g/l	1ml
Trace metal mix A5	See blow	1ml

Treatment of Municipal wastewater with algae culture

The treatment was carried out by taking 1 liter glass flasks and placing 850 ml of municipal waste water thatCollected from the final settling ponds of central wastewater treatment plant (Almamierh) and adding 150 ml of the algae culture after it reached the Stationary phase with control containing 1 liter of municipal waste water only .the all flasksplaced the in the growth chamber Under previous incubation conditions.

Physical and Chemical analysis

The pH, total dissolved solids, electrical conductivity and salinity were measured using a HANA\ 211Multi-meter.BOD5 was estimated according to [7].The total Alkalinity,total Hardness,calcium and magnesium hardness were estimated according to [8].Chloride was estimated as reported in the [9]method. Nitrite and nitrate were estimated according to (Parson *et al*.,1984).Phosphate was estimated according to [10] and as described by [11].

Measurement of physiological and biochemical indicators

Total chlorophyll was estimated according to method [12]The effectiveness of SOD was measured by its ability to inhibit pyragallol oxidation by [13]. The efficiency of the CAT enzyme was estimated by [14]. and measured at a wavelength of 240 nanometers**.**Malondialdehyde content was estimated by [15].

Results and Discussion.

Hydrogen concentration is influenced by many ions such as carbonates, bicarbonates and dissolved gases such as CO2 and hydrogen sulfide [16].It represents the relationship between the OH ion produced by bicarbonate degradation and the concentration of hydrogen ion separated from carbonic acid [17]. The results show that the pH values rose slightly after treatment but remained within normal limits Figure (1).This may be due to the balance that occur by algae, since the CO2 that produced by the respiration of organisms reduces the pH values while the CO2 and bicarbonate ions that removed by algae increase the pH values [18]. TDS is a materials that produced by the solubility of compounds that produce positive and negative ions [19].which represent the total dissolved solids and salts and are an important indicator in water treatment plants[20]. The current results showed the algae have ability in the reduction of TDS value Figure(2). The possibility of algae in reducing soluble solids values is related to their ability to reduce positive and negative ions in treated water.

Electrical conductivity refers to the water's ability to carry electricity and an indicator of water soluble salts, which is closely associated with total solids and increases in areas under the influence of agricultural and industrial activity [21]. The current study showed that the algae recorded a slight reduction in the values of electrical conductivity and salinity Figure(3,4). This may be due to the release of ions from the outer surfaces of the cell wall by acidic materials that released from algae causing increased electrical conductivity [22]. BOD_5 refers to the amount of oxygen consumed from microorganisms in aerobic oxidation of organic matter [23], And consider as a measure of the amount of organic matter oxidized by microorganisms [24].The results indicate the efficiency of the algae under study in the reduction of BOD5 values throughout the experiment Figure(5).The reduction of BOD5 values occurs when the dissolved organic compounds and their derivatives are reduced from water [25].

Alkalinity is an indicator of the water content of bicarbonate, carbonate, hydroxides and other ions [8]. The studied algae recorded a role in reducing Alkalinity values with a clear superiority of *W. prolifica* Figure(6). This reduction can be attributed to degradation processes ,or may be related to fact that the CO_2 resulting from the breakdown and degradation of organic compounds by microorganisms is then consumed by algae [24].

Hard water is the water that contains high concentrations of multivalent ions, especially calcium and magnesium ions, and causes many problems in factories such as cracking boilers and cooling towers, as well as causing problems in households, as they do not form foam with soap [26]. Algae under study recorded higher efficiency in the total hardness reduction during the treatment period Figure(7). This reduction can be related to The process of photosynthesis that leads to high pH values which in turn helps in precipitation of calcium carbonate.

Calcium plays a role in maintaining the functional and structural integration of plant membranes and cell wall stabilization [27]. Magnesium is incorporated into the synthesis of the chlorophyll molecule [28]. The blue-green algae recorded a reduction in calcium and magnesiumFigure(8,9). The reduction of calcium is higher than magnesium, which means the consumption of the this element by algae. where In the presence of carbon dioxide, the later reacts with calcium ion more than magnesium ion, which convert large amounts of it into easily digestible bicarbonate [29]. Chloride ion is a negative ion in water that is obtained by saline taste, especially in relation to sodium ions to form sodium chloride [30]. Algae showed a significant role in reducing chloride valuesFigure(10) .The efficiency of chloride reduction by algae was recorded in previous studies such as[31]. Nitrogen is present in the environment in several oxidative states and is transformed from one form to another depending on the availability of oxygen. Ammonia is biologically oxidized by nitrification by two types of self-feeding bacteria that are very sensitive to pH, heat,heavy metals and chemical compounds [32].The results showed that the algae under study were not efficient in reducing the nitrate values during the treatment period But reduced the values of nitrite within a specified period of time and then returned values to rise Figure(11,12). The low nitrate reduction rates of algae may be due to the high concentration of ammonia that inhibits the removal of nitrates [33]. Ammonia is the preferred form of microscopic algae. Algae consume nitrates occur only when the ammonia has been carried out from the center[34].

Phosphorus is an essential nutrient for the growth and cellular functions of algae and its preferred form is orthophosphate. Phosphorus is a determinant of growth due to its easily combined with other ions such as carbonate and iron [35].algae showed a significant role in reducing phosphates Figure(13), where Algae can withdraw phosphates faster and in more quantities than they need to grow and then store them in cells in polyphosphate compounds [36].

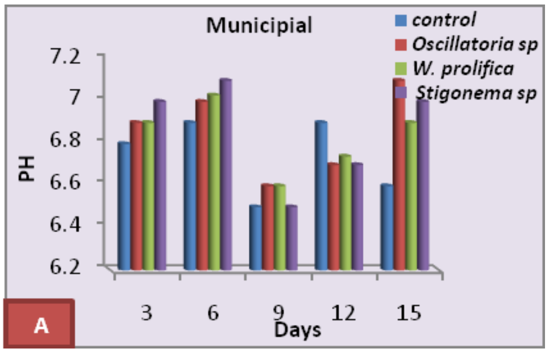

Figure 1: concentrations of PH

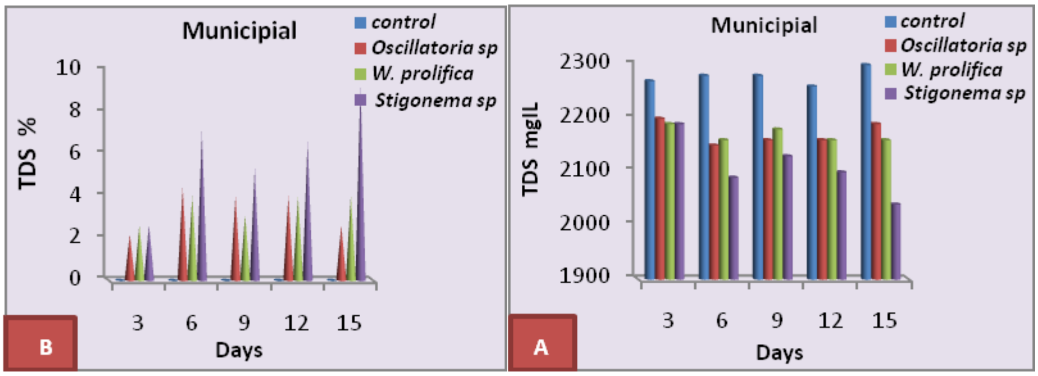

Figure 2: concentrations and Percent bio removal of TDS

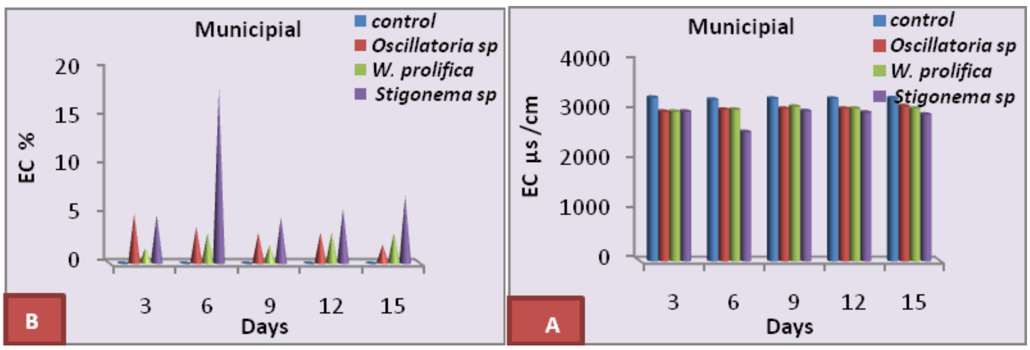

Figure 3: concentrations and Percent bio removal of electrical conductivity

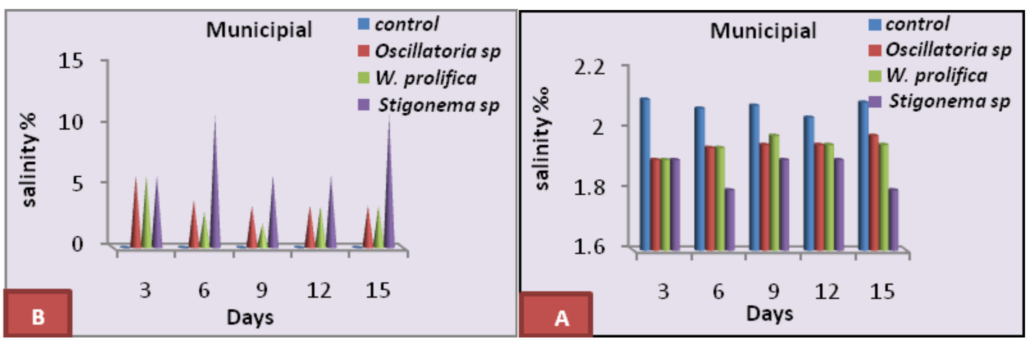

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Figure 4: concentrations and Percent bio removal of Salinity

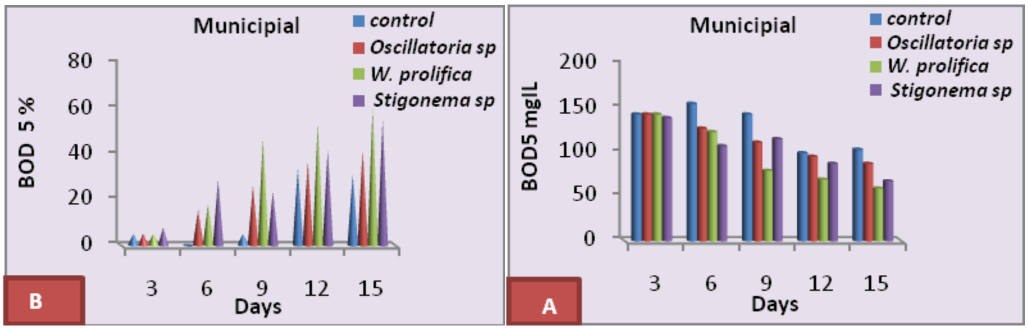

Figure 5: concentrations and Percent bio removal of BOD$_5$

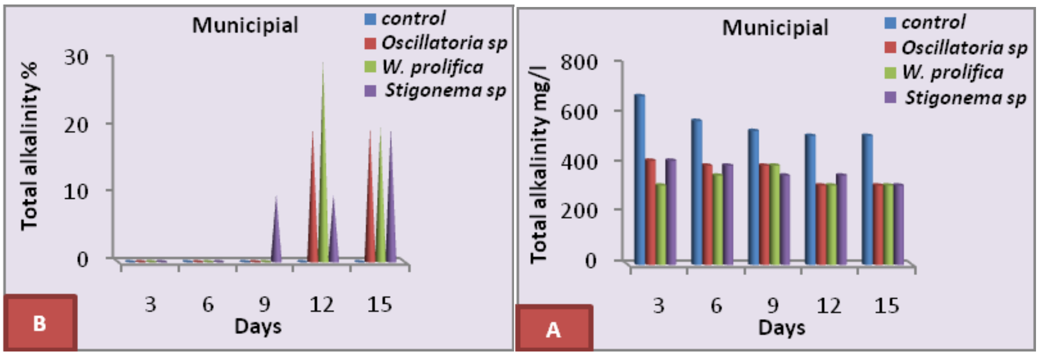

Figure 6: concentrations and Percent bio removal of Total alkalinity mg/l

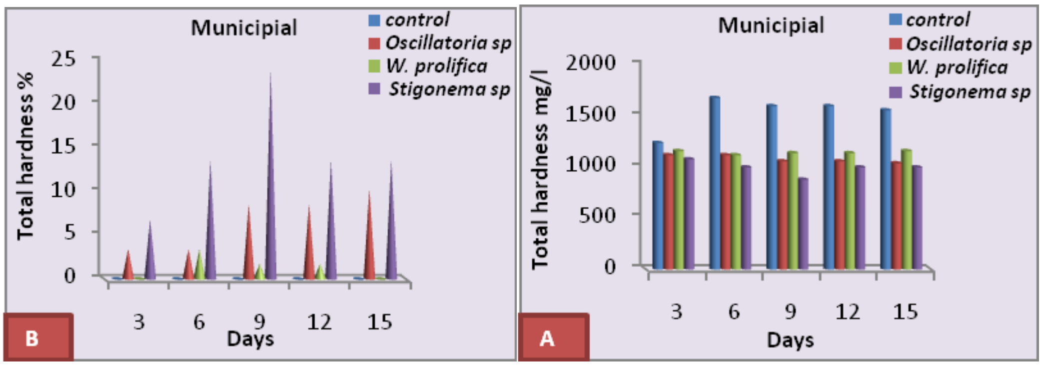

Figure 7: concentrations and Percent bio removal of Total hardness mg/l

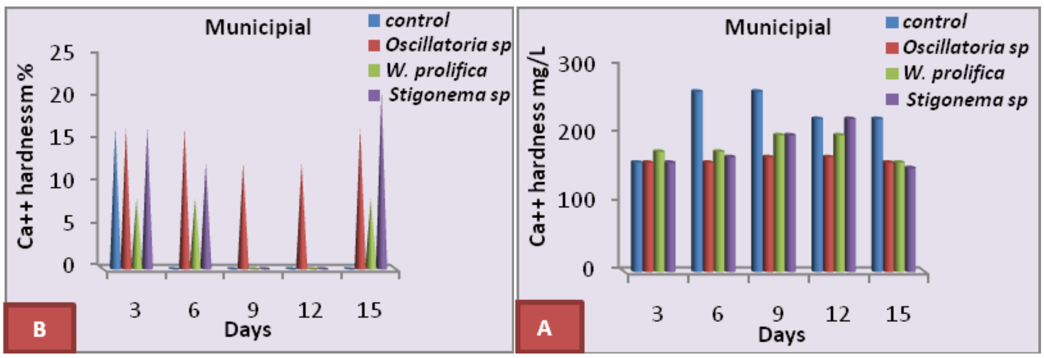

Figure 8: concentrations and Percent bio removal of Ca^{++} hardness mg/L

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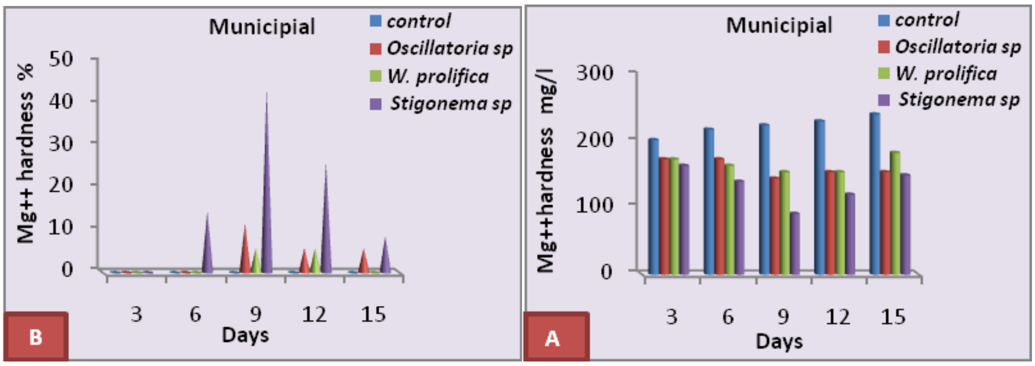

Figure 9: concentrations and Percent bio removal of Mg++hardness mg/l

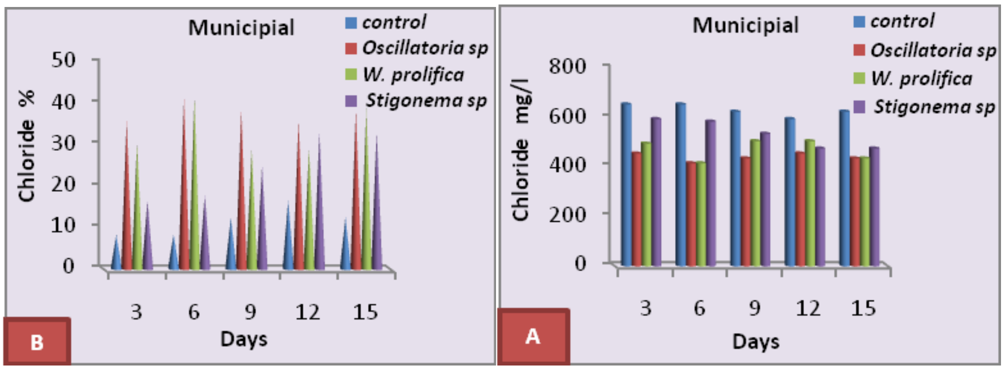

Figure 10: concentrations and Percent bio removal of Chloride mg/l

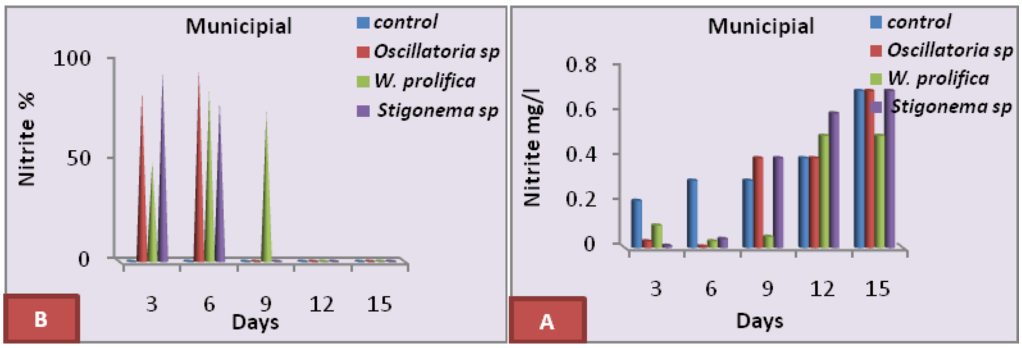

Figure 11: concentrations and Percent bio removal of Nitrite mg/l

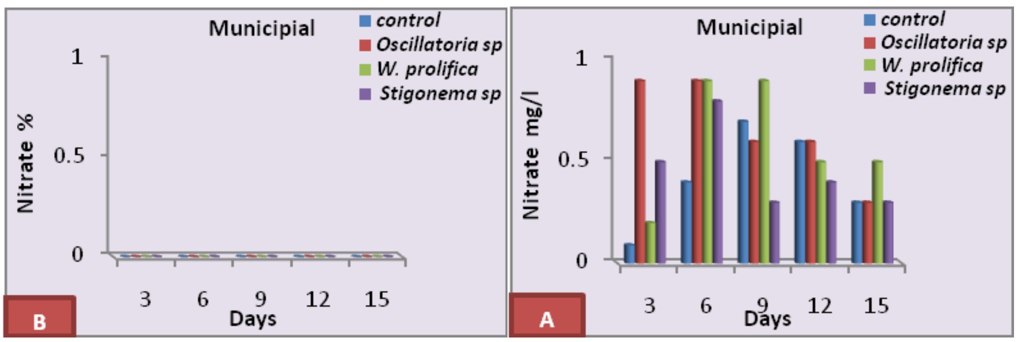

Figure 12: concentrations and Percent bio removal of Nitrate mg/l

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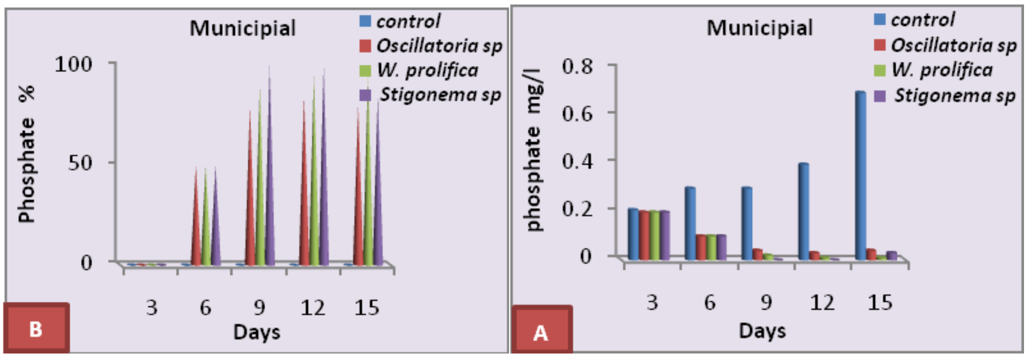

Figure 13: concentrations and Percent bio removal of phosphate mg/l

Biochemical indicators of organisms under study

Chlorophyll is type of pigments that responsible for photosynthesis and found in the plastids [37]. These pigments need many essential elements such as calcium and magnesium as well as iron [38]. The results showed that the values of chlorophyll was increased slightly in *Oscillatoriasp* and*W. prolifica* and decreased in *Stigonemasp* after treatment of watertable (2). The high chlorophyll values may be due to the availability of the necessary elements for the formation of chlorophyll.Either the low values in the *Stigonemasp* may return to The high concentrations of pollutants that inhibit the production of pigments as chlorophyll [39]. The SOD is the first defensive line against ROS, which stimulates the transformation of superoxide radicals (O_2) into $H_2O_2$and O_2, which has a toxic effect on the cell, so catalase removes its toxicity by converting it to O_2 [40]. The current study indicates that the SOD values recorded in the algae under study were close to controltable (2). In fact, in the blue-green algae such as *Oscillatoriasp*, two important types of SOD [Mn, SOD, Fe, SOD] These forms protect the algae from oxidative stresses resulting from the identification or restriction of nitrogen in the medium or the addition of dyes to the feeder medium [41].

Catalase is an enzyme in almost all living organisms whose function is to break down hydrogen peroxide into water and oxygen [42]. The study showed that the enzyme content were increased in *Oscillatoriasp* and*W. prolifica* after treatment and decrease in *Stigonema sp*.table (2). The increased efficacy of Catalase under oxidative stress such as increased salinity indicates the high efficiency of algae in the removal of H_2O_2 [43]. Lipid peroxidation (LPO) is one of the vital indicators of oxidative damage that gets to aquatic organisms exposed to stress. Malondialdehyde (MDA) is a cytotoxic product and is an important indicator of the production of free radicals that cause lipid peroxidation and thus damage cellular membranes [45]. The current study showed that the MDA values increased In all Algae studied. The high content of MDA in the algae found in waste water may be related to damage by free radicals and reflects the low algal susceptibility to get rid of these roots **table (2)**.. The process of thylakoid

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membrane lesion in algae under the influence of heavy elements means increased free radical production and high lipid peroxidation [46].

Table (2) Total chlorophyll values and antioxidants in algae under study (mean ± standard deviation)

Biochemical markers Algae		Chl (mg/g)	SOD(U/mg)	Cat(U/mg)	MDAnmol/mg.
Oscillatoriasp	control	0.25±0.01 a	2.57±1.22 a	4.74±1.13 a	0.27±0.03 a
	wastewater	0.26±0.01 a	2.80±1.21 a	6.06±0.00 a	0.29±0.02 a
W. prolifica	control	0.24±0.01 a	3.23±1.70 a	9.09±3.03 a	0.17±0.02 a
	wastewater	0.40±0.10 c	2.80±1.38 a	14.14±8.08 b	0.26±0.02 c
Stigonemasp	Control	0.33±0.01 a	2.67±1.34 a	10.77±2.33 a	0.12±0.02 a
	wastewater	0.05±0.01 c	2.49±1.65 a	7.40±1.16 a	0.22±0.02 b

The different letters mean a significant difference

Conclusions

The current study showed the distinctive and effective role of blue-green algae in the reduction and treatment of the pollutants Especially the nutrients that released from the final settling ponds of central wastewater treatment plant (Almamierh).As for the biochemical changes The current study showed a clear variation in the values of chlorophyll, Catalase enzyme activity, and MDA content between increase and decrease in all the algae under study. While the activity of the SOD enzyme did not change its value in all the algae under study

References.

1-Olguin, E.J. (2003) .Phycoremediation: key issues for cost-effective nutrient removal processes. Biotechnol. , 22: 81-91.

2-**Favero**,N.; Cattalini ,F.; Bertaggia ,D. and Albergoni, V. (1996) . Metal accumulation in a biological indicator (*Ulvavigida*) from the Lagoon of Venice (Italy). Arch. Environ. Contam. Toxicol.31,9-18.

3-Hu ,S. ;Lau, K.W.K. and Wu, M.(2004). Cadmium sequestration in *Chlamydomonasbreinhardtii*. Plant Sci., 161: 987-996.

4-Rijstenhil, J.W. and Wijnholds, J.A.(1998).HPLG analysis of nonprotinethiods in planktonic diatoms. Poolsize, redox state andresponse to copper and cadmium exposure. Marin Biol.,127:45 – 54.

5-Perales-vela, H.V.; Pena-castro, J.M. and Cannizares-villanueva, R.O.(2006). Heavy metal detoxification in eukaryotic microalgae. Chemosphere, 64: 1-10 .

6-Anderson, R. A. (2005). Algal Culturing Techniques. PhysiologicalSociety of America, Elsevier Academic press,. 589P.

7-APHA.(American public health Association)(2003). Standard Methods for examination of water and waste water, 20th, Ed. Washington DC, USA.

8-APHA(American public health association).(2005).Standard methods for examination of water and wastewater.21the Ed. Washington, D.C., U.S.A.

9-Parsons, T.R. ;Mait, Y. and Laulli, C.M . (1984) . A manual of chemical and bilogical methods for seawater analysis pergamone press oxford/ U.K.

10-Murphy, J. & Riley, J.R. (1962). A modificational single solution method for determination of phosphate in natural water.Chem. Acta.*27*:31-36,mussels. Environmental Science and Technology 34, 91–99.

11-Smith, R. (2004). Current methods in aquatic science. University of Waterloo, Canada.

12-Aminot, A. and Rey , F. (2000). Standard procedure for determination of chlorophyll by spectroscopic methods . ICES Techniques in Marine Environ .Sci . , pp:1 – 17 .

13-Marklund S. and Marklund G. (1974). Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and aConvenient Assay for Superoxide Dismutase .*Eur. J. Biochem*. 47:469-474.

14-Aeibi, H. (1984). Catalase *in vitro.* Methods Enzymol.,105: 121-126.

15-Zacheo, G.;Cappello, M.S. ;Gallo, A; Santino ,A. and Cappelo, A.R.
(2000). changes associated with post-harvested ageing in Almond seeds.Lebensm-Wiss.U.Technol.,33;415-423.

16-Al-Sarraf, M. A .A. (2006). A taxonomic environmental study of phytoplankton in the Diyala and their impact on the Tigris River.PhD thesis - University of Baghdad.

17-Wetzel, R.G. (1983). Limnology.(Saunders colleges publishing, Sydney).

18-Dominic,V.J.;Soumya, M. and Nisha ,M.C.(2009). Phytoremediation efficiency of three microalgae *Chlorella vulgaris*, *Synechocystissalina* and *Gloeocapsagelatinosa* . SB Academic Review 1(1): 138-146.

19-WHO (World Health Organization) .(1996). Guideline for Drinking Water Quality Health Criteria and Other Supporting Information 2nd. Ed. Vol. 20.Geneva .

20-Abed Alasady ,R. K.(2014). Use of some types of algae and aquatic plants in the biological treatment of water treatment plants in the city of Diwaniya / Iraq. PhD thesis - University of Diwaniyah, 106 pages.

21-APHA (American public health association) (1976). Standard method for the examination of Water and Waste Water, 13th. Ed. New York.

22-Stirling, H. P. (1985).Chemical & Biological methods of water analysis for aquaculture lists.Sterling university, Scotland, 119 pp.

23-Best, G.A. and Ross, S.L. (1977). Clyde river purification board.Eastkibride, Glasgow, 87 pp.

24-Kotteswari M., Murugesan S. and Ranjith K. R.(2012).Phycoremediation of Dairy Effluent by using the Microalgae *Nostoc sp*. International Journal of Environmental Research and Development. 2(1): 35-43.

25-Lind, G.T. (1979). Handbook of common methods in Limnology, 2nded., London.

26-Sivasubramanian ,V; Subramanian, V.V.; Muthukumaran ,M. and Murali ,R.(2012).Algal technology for effective reduction of total hardness in wastewater and industrial effluents.Phykos, 42 (1): 51– 58.

27-Ashraf ,M.(2004).Some important physiological selection criteria for salt tolerance in plants"flora,vol.199.pp:361-377.

28-Kupper , H. ; Kupper , F. and Spiller , M. (1998) . In situ detection of heavy metals subtituted chlorophylls in water plants . Photosynthesis Research , 58 (2) : 123-133.

29-Munawar ,M.(1970).Limnlogical studies on fresh water ponds of Hyderabad-India .I. the biotope hydrobiology,35(1):127-162.

30-Al-Saadi ,H. A.(1994). Iraq's water environment and sources of pollution, Proceedings of the Scientific Research Conference and its role in protecting the environment against the dangers of pollution. R: 59-88. Edited by Dr. Hussein Ali Al-Saadi.Union of Arab Scientific Research Councils, General Secretariat, Baghdad, Iraq.

31-Vijayakumar, S and Manoharan, C.(2012).Treatment of Dye Industry Effluent Using Free and Immobilized Cyanobacteria.J BioremedBiodeg,Volume 3 • Issue 10.

32-Hockenbury, M. R. and Grady Jr, C. P. L.(1977). “Inhibition of nitrification— effects of selected organic compounds”. Journal Water Pollution Control Federation,49 (5): 768–777.

33-Evonne, P.Y.T.; Warwick, F.V.; Danie ,P.; Paul ,L. and De la Noüe ,J. (1997).Polar cyanobacteria versus green algae for tertiary wast-water treatment in cool climates .J. Appl .Phycol .9.:371-381.

34-Lavoie, A. andNoue, J.de.la (1985).Hyperconcentrated cultures of *Scenedesmusobliquus* .A new approach for wastewater biological tertiary treatment Wat.Res,19(11):1427-1442.

ISSN 2410-2598

35-Yaakob, Z.; Kamarudin, K.F. and Rajkumar, R.(2014).The Current Methods for the Biomass Production of the Microalgae from Wastewaters: An Overview.World Applied Sciences Journal, 31 (10): 1744-1758.

36-Goldman, C.R. & Horne, A.J. (1983). Limnology-MegrawHillint.B. co., U.S.A.

37-Lefsrud , M.G and Kopsell , D.A. (2005). Air Temperature affects biomass and carotenoid pigment accumulation in Kale and Spinach growm in a controlled Environmental .HortSci , 40 (7) : 2026 – 2030 .

38-Sarvari , E. ; Cseh , E. ; Blczer , T. ; Szigeti ,Z. ; Zaray , G. and Fodor , F. (2008). Effects of Cd on the iron re-supply induced formation of chlorophyll – protein complexes in cucumber. ActaBiologicaSzegediensis , 52 (1) : 183 – 186.

39-Saikia,M.K.;Kalita, S. and Sarma, G.C.(2011). Anexperimental investigationon growth stimulation (+) and inhibition (-) of algae (*Oscillatoria.chlorinaand Scenedesmus.quadricauda*) treated with pulpeand papermilleffluents. International Journal of Applied Biology and Pharmaceutical Technology,2(4):87-94.

40-Kachout, S.S.; Mansoura, A.B.; Leclerc ,J.C.; Jaffel,K.;Rejeb ,M.N. and Ouerghi ,Z.(2009).Effects of heavy metals on antioxidant activities of *Atriplexhortensis* and *Atriplexrosea*. journal of applied botany and food quality ,83:37-43.

41-Saha, S.K.; Swaminathan,P.;Raghavan ,C. and Uma ,L.(2010) .Ligninolytic and antioxidative enzymes of amarinecyanobacterium*Oscillatoriawillei* BDU130511 during poly R-478 decolourization.Bioresource Technology ,101:3076-3084.

42-Chelikani ,P.; Fita ,I. andLoewen, P.C. (2004).Diversity of structures and properties among catalases .cell. Mol. life Sci.,61(2) :192-208.

43-Chakraborty, S.; Santra, S. C. and Bhattacharya, T.(2010).Seasonal variation of enzyme activity and stress metabolites in eight benthic macro algae with fluctuation in salinity of sunder ban estuary ,india. indian Journal of marine science ,39(3):429-433.

44-Ohkawa, H.;Ohishi, N.andYagi, K.(1979).Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal.Biochem., 95: 351.

45-El-Sheekh, M.M.; Farghl ,A.A.M., Hani and H.S.(2016).Cultivation of *Chlorella* species in sewage and different water samples mixtures.Egypt. J. Exp. Biol. (Bot.), 12(1): 97 – 106 . .

46-Foyer C.H., Lelandais M., Kunert K.J. (1994). Photooxidative stress in plants. Physiol. Plantarum., 92: 696-717.