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Bioremoval of heavy metals by algae with determination of their molecular responses using the COMT assay

Nuha Falih Kadhim[1] and Kareem Talib Kahashan[2]

1Department of Biology, College of Science ,University of Babylon/Iraq.
2Faculty of Science , University of Kufa ,Iraq.

Email: sci.nuha.falih@uobabylon.edu.iq , ORCID: 0000-00003-3101-2891

***Corresponding author:** sci.nuha.falih@uobabylon.edu.iq

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Abstract:

The present study included testing the ability of three types of algae, which include *Oscillatoria sp*., *Westiellopsis prolifica*, and *Stigonema sp*. to reduce the values of some heavy elements. Wastewater from the final sedimentation tanks of the Al-Ma'mara wastewater treatment plant was used as culture media for growing algae. by cultivating each algae alone in wastewater under constant laboratory conditions at 25 ± 20 ° C and in a light system 16: 8 hours Illumination: Darkness ,Leaving a wastewater sample without adding algae as a control

The results showed the efficiency of algae in reducing sodium values and the highest reduction rates recorded by *Oscillatoria sp.* and *Stigonema sp.* were 43.4% and 35.9% respectively, while *W. prolifica* recorded a reduction rate of 27.3% . Algae also recorded efficiency in reducing potassium, as *Oscillatoria sp*. recorded a higher reduction efficiency of 71.2%, while *W. prolifica* and *Stigonema sp.* recorded the highest reduction rate of 43.1% and 32.1% respectively. Algae recorded iron reduction rates throughout the treatment period, and reduction rates reached 98.3% for both *Oscillatoria sp.* and *Stigonema sp.* while *W. prolifica* recorded higher reduction rates of 100%. Algae also recorded a reduction of copper of 61.8% and 53.03% for *Oscillatoria sp.* and *W. prolifica* respectively, while *Stigonema sp.* recorded lower reduction rates of 38.3%. *Stigonema sp.* recorded a higher efficiency in reducing zinc values of 86.8%, while *Oscillatoria sp.* and *W. prolifica* recorded a reduction rate of 69.2% and 72.74% respectively..As for cadmium, *Oscillatoria sp.* recorded the highest reduction rate of 72.7%, while *W. prolifica* and *Stigonema sp.* recorded reduction rates of 66.4% and 53.3% respectively. As for the role of algae in treating lead, the results indicate that the highest rates recorded when treating with *Oscillatoria sp.*, *W. prolifica* and *Stigonema sp.* reached 99.6%.

On the other hand, bioremediation showed a clear effect on molecular properties of algae, this was demonstrated by the high values of Comet assay indicators, which include tail length, Comet length, tail moment which indicate the effect of treatment on DNA after completion of the treatment.

Key words: bioremediation, algae, heavy metals , molecular properties

Introduction

Water is the basis of life for living organisms, and therefore its preservation is essential for these organisms in order to survive. Household wastewater is considered one of the most important sources that have a negative impact on water quality (1). It releases large amounts of pollutants, including trace elements and salts. Heavy metals are not capable of biodegradation, but they accumulate in living tissues, causing long-term effects (2). Conventional treatment methods to reduce heavy metal pollution include various mechanisms such as chemical precipitation, ion exchange, activated carbon adsorption, membrane filtration, electrochemical removal, and advanced oxidation (3). However, these traditional methods remain inefficient due to several drawbacks, including incomplete removal, low selectivity, consumes a lot of energy, costs are high, and Production of highly toxic waste. Therefore, researchers have turned to using alternative, low-cost, safe, and more effective methods to reduce heavy metals, which is the use of biological processes in the treatment (4) .

biological processes used living or dead organisms such as bacteria, algae, and fungi, whose cells contain carbohydrates and peptides in their walls with hydroxyls, ketones, phosphates, aldehydes, amines, and carboxyl groups responsible for the absorption and synthesis of metals (5). Heavy metals such as Manganese, nickel, iron, zinc consider as essential element for algal growth and this known as ‘trace elements’ they are used as micronutrients, while other heavy metals such as cadmium, lead, and mercury are considered toxic to algae and have no specific biological function (6). Detailed research and studies of the structure of the cells of the physical and chemical student helped in discovering the role of algae in reducing environmental pollutants, especially in the field of removing heavy elements from polluted water. Some algae showed the ability and strength to endure and survive in water polluted with heavy elements (7).

Where Algae have the ability to tolerate heavy metals through different mechanisms. The uptake of heavy metals by algae occurs either by absorption or bioaccumulation. Bioaccumulation is a completely energy-independent process, which usually results in the binding of element ions to the algal cell wall (8) . This process actually involves the use of living or dead algal cells and involves mechanisms for absorption and cation exchange. Bioaccumulation means the increase in the concentration of any chemical substance in the body of an organism over time compared to the concentration of this substance in the environment, The accumulation of heavy metals by algae occurs by one or a combination of the following mechanisms: active transport along the algal cell membrane, entrapment by some cellular components, exchange of cations or complexation and finally adsorption (9). The complex composition of the cell walls of some algae which has affinity for some metal ions and also carries a negative charge, this is related to some groups such as carboxylic, phosphoric and sulfhydryl groups which capable of binding metals through ion exchange (8). Therefore, and considering the ability of algae to remove metals, the aim of the current research includes demonstrating the ability of some algae in the process of bioremediation of wastewater with a statement of the effect of treatment on the molecular indicators of the algae studied through the comet method

Materials and Methods

Algae (isolation, purification and cultivation)

The special solutions for BG11 medium used in growing algae were prepared by dissolving a certain amount of salts listed in Table (1) and then dissolved in sterile water and completed to (1) liter with distilled water with adjusting the pH to about (7) (10). Then 10 ml of pure algae were taken and added to a flask containing 90 of the culture medium and incubated at a temperature of 25 ± 20 degrees with a light period of eight hours darkness: sixteen hours light for 14 days. Then, the growing algae were multiplied by transferring 100 ml of the growing algae to a flask containing 900 ml of the medium and incubated for 14 days.

Table 1: Shows the components of BG11 medium

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

Trace metal mix A5	Amount
H_3BO_3	2.86 g
$MnC_{l2}.4H_2O$	1.81 g
$ZnSO_4.2H_2O$	0.222 g
$NaMoO_4.2H_2O$	0.39 g
$CuSO4.5H_2O$	0.079 g
$Co(NO3)_2.6HO$	0.049 g
Deionized water	1 L

Treatment of Municipal wastewater with algae

Treatment with algae were carried out by taking one liter flask and placing in them 850 ml of domestic wastewater. 150 ml of the required culture was added to each treatment after reaching the stationary phase with three replicates for each treatment (11) , taking the control treatment that contained only 1 liter of wastewater. The treatments were placed in the culture room and a specific volume of the culture was taken every three days for fifteen days to conduct heavy element concentration tests .

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Determination of Total Heavy Metals

The (12) method was followed which include digesting the wastewater with 5 mL of concentrated nitric acid and then measuring using the Atomic Absorption Spectrophotometer and expressing the final product in mg / l. While sodium and potassium ions were estimated according to the method (13) by using a flame photometer.

Assessment of DNA damage (Comet Assay)

The algae were crushed with Phosphate buffer solution, then 40 µl of proteinase K were added, then centrifuged and the precipitate was taken with the addition of a small amount of Phosphate buffer solution, then 50 µl of suspension was taken and brushed on the slide, with added 500 µl of Low melting agarose. The process was carried out under yellow or dim light to prevent DNA damage by ultraviolet rays. Then, Lysing solution was poured on the slides and kept for half an hour at 4°C. The solution then was removed with added of Alkaline solution and left at 10°C. It was transferred to the transfer device where the slides were arranged horizontally in Electrophoresis solution TBE at a concentration of 1X. After running the electrophoresis 24 device for 60 minutes, the TBE solution was removed and the slides were stained with Ethidium Bromide and left for hours. The slides Then photographed and the some dimensions were taken by use a fluorescence microscope (14, 15) where the amount of DNA damage estimated by estimating some indicators which include tail length ,tail moment and Comet length .

Results and Discussion

The current study showed a distinct role for the algae under study in removing some elements. Algae showed high efficiency in reducing sodium values and the highest reduction rates recorded on day 12 for each of *Oscillatoria sp.* and *Stigonema sp.* were 43.4% and 35.9%, respectively ,while *W. prolifica* recorded the highest reduction rate of 27.3% on day 3 compared to the control treatment, which recorded the highest reduction rate of 13.7% on day 6 Figure (1) .

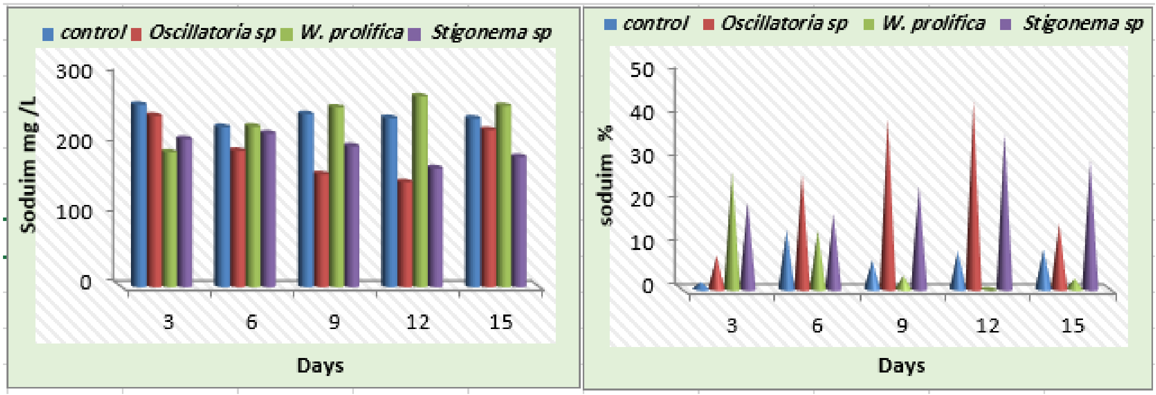

Figure (1) Concentrations and percentages of sodium removal

As for potassium, *Oscillatoria sp.* recorded high efficiency in reduction it compared to the rest of the algae throughout the treatment period, and the highest reduction rate recorded was 71.2% on day 6. As for *W. prolifica* , it recorded the highest reduction rate of 43.1%. While *Stigonema sp.* recorded the highest reduction rate of 32.1% on day 3 compared to the control treatment, which recorded the highest reduction rate of 25.3% on day 6 Figure (2).

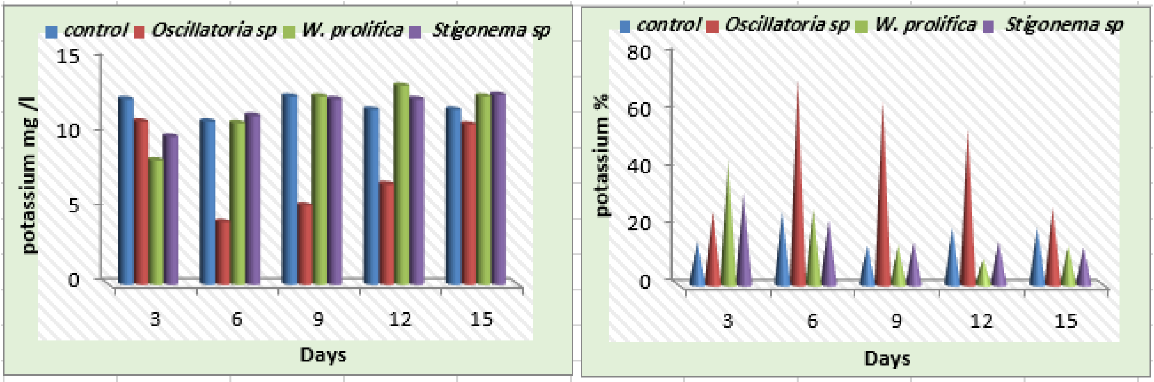

Figure (2) Concentrations and percentages of Potassium removal

The role of algae in removing both sodium and potassium may be due to the fact that Many algae have a good ability to adapt to salinity changes through a number of biological strategies such as the production and accumulation of osmotic solutions or through the operation of an effective Na+/K+ pump system (16). The operation of this pump contributes to In the development of osmotic stress and thus affecting turgor pressure and cell volume (17). On the other hand, Recently observed through research the formation of new vacuoles is observed in single or multicellular algal cells after exposure to osmotic stress (18). Recent research has shown that the formation of vacuoles during exposure To a very salty solution occurs to store sodium ions (19) . So adaptation can be summarized in two stages , the first stage involves responding to salinity changes with a rapid change in turgor (cells with walls) or volume (cells without walls) resulting from high water flow along the osmotic gradient, the second stage involves osmotic control which is The change in the concentration of osmotically active solutes in the cell until a new equilibrium is reached and both stages are considered part of the feedback signal that provides osmotic adaptation (20) .

As for the rest of the heavy elements, the algae under study have proven to be highly efficient in removing iron, *Oscillatoria sp.* and *Stigonema sp.* recorded reduction rates 98.3% on days 3 and 6 for the first alga and day 6 for the second. As for *W. prolifica*, it recorded higher reduction rates of 100% on day 15 of treatment compared to the control treatment which recorded the highest reduction rate of 13.5% on day 3 of treatment Figure (3). The ability of the studied algae to remove iron may be due to the fact that iron is considered one of the essential elements necessary for the growth of algae, as it plays a distinct role in photosynthesis, respiration, and nitrogen fixation (21) .In addition, it is considered a cofactor in enzyme systems and a very important component in cytochrome as well as in iron oxidation and reduction proteins (22).

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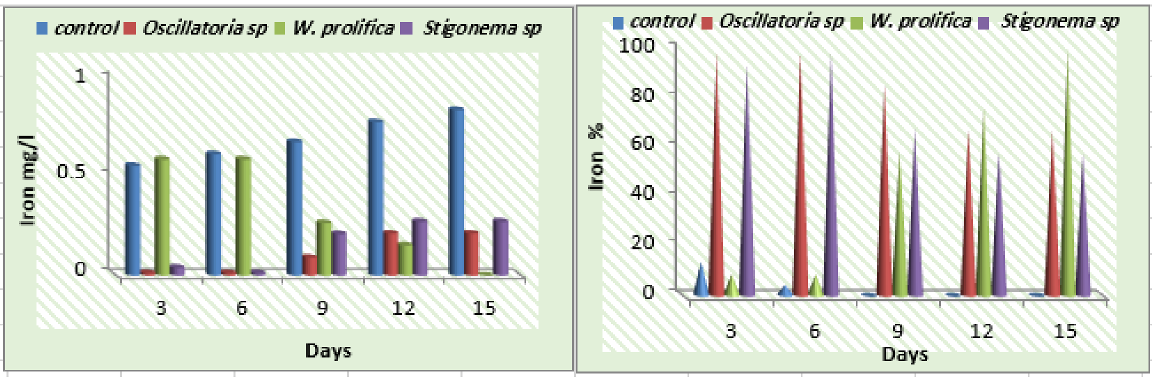

Figure (3) Concentrations and percentages of Iron removal

The role of algae in reducing copper is shown in Figure (4), as *Oscillatoria sp.* and *W. prolifica* recorded a role in reducing it throughout the treatment period, and the highest reduction rate of 61.8% and 53.03%, respectively, was recorded on day 3 of treatment. As for *Stigonema sp.*, it recorded lower reduction rates of 38.3% on day 6 compared to the control treatment, which recorded slight reduction rates of 11.78% on days 3 and 6. This study indicated the efficiency of algae in reducing copper values in domestic water. This is due to the fact that copper is one of the basic elements, as it is considered a nutrient and important element in the cellular metabolism process and is considered an important and basic component in metabolic enzymes (23) .

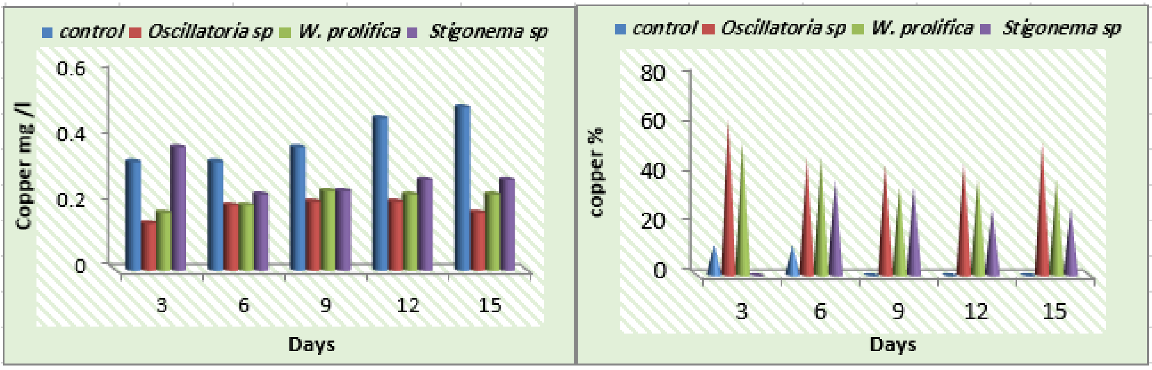

Figure (4) Concentrations and percentages of Copper removal

Algae also recorded efficiency in reducing zinc values as shown in Figure (5). Where *Stigonema sp.* recorded the highest reduction rate of 86.8% on day 3. *Oscillatoria sp.* and *W. prolifica* recorded the highest reduction rate of 69.2% on days 12 and 15 and 72.74% on day 15 respectively. The control treatment recorded reduction rates throughout the treatment period, but they were lower than the treated water, as the highest reduction rate recorded for it reached 53.4% on day 15. The efficiency of algae in removing zinc may be due to the ability of algae to absorb, which occurs in two stages (24). The first stage includes adsorption in to some materials found outside the cell such as polysaccharides, and in the cell wall such as carboxyl groups, sulfates and phosphates which are considered non-metabolizing. The second stage includes absorption in addition to the accumulation of minerals inside the cell. The both stages are affected by many factors such as pH, type of algae, and concentration of heavy metals (25).

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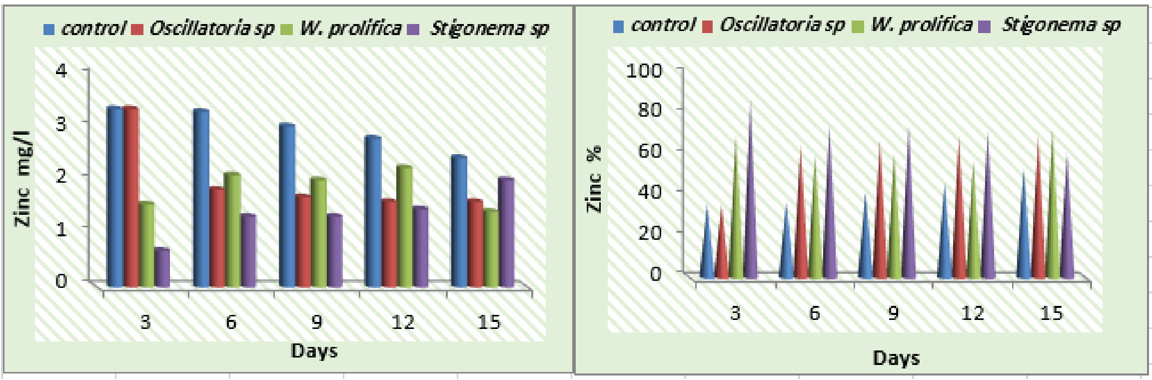

Figure (5) Concentrations and percentages of Zinc removal

As for cadmium, *Oscillatoria sp.* recorded the highest reduction rate reaching 72.7% on day 9, while *W. prolifica* and *Stigonema sp.* recorded the highest reduction rates reaching 66.4% on day 12 and 53.3% on day 15 respectively. Compared to the control treatment which did not record reduction rates during the treatment period Figure (6). Cadmium removal by algae may occur through intracellular accumulation complemented by extracellular fixation (26). In addition, the carboxyl group in the algal cell wall has been shown to be responsible for cadmium removal in both *Coelastrella sp.* and *Chlorella vulgaris* (27). Some studies have also shown that algae respond to cadmium exposure by forming cadmium complexes within chloroplasts as a removal mechanism (28).

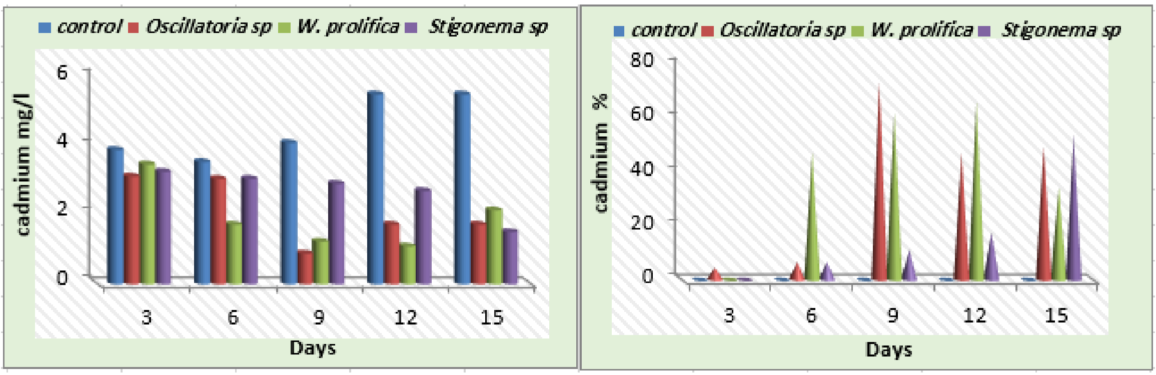

Figure (6) Concentrations and percentages of Cadmium removal

As for the role of algae in treating lead in domestic water, the results indicate that the highest rates recorded when treating with algae *Oscillatoria sp.* , *W. prolifica* and *Stigonema sp.* reached 99.6% for most of the treatment period, while the control treatment recorded a reduction rate of 45.7% on the 15th day of treatment Figure (7) .The efficiency of the studied algae in reducing lead was consistent with the study of (29) which proved the ability of algae to remove lead ions from wastewater and attributed this to the mechanism of metabolic bioaccumulation of algae, where the living cells of algae control the absorption of the metal by controlling the mineral balance system of the metal that has been absorbed. After a short period of time, the algae cells try to isolate the unwanted metal either in the vacuoles or in the organelles inside other cells for possible export from the cell using flow systems.

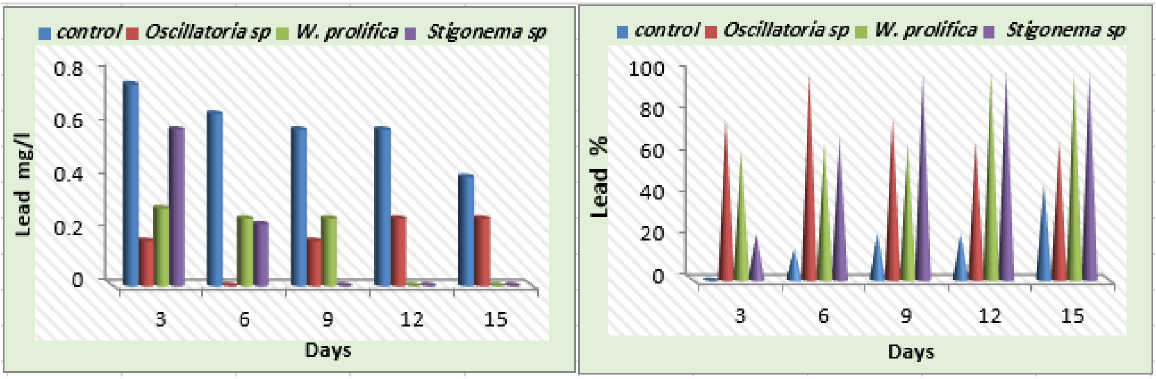

Figure (7) Concentrations and percentages of Lead removal

Estimation of DNA damage in algae under investigation

The comet assay is a sensitive and useful technique for monitor the genotoxicity, where allows to detection the DNA damage at single cell level and investigate intercellular differences (30). The DNA damage was estimated by estimating some indicators which include (tail length), (tail moment) and (comet length) . Where the damage in the DNA molecule was classified in to three categories according to the degree of damage as shown in Figure (8).

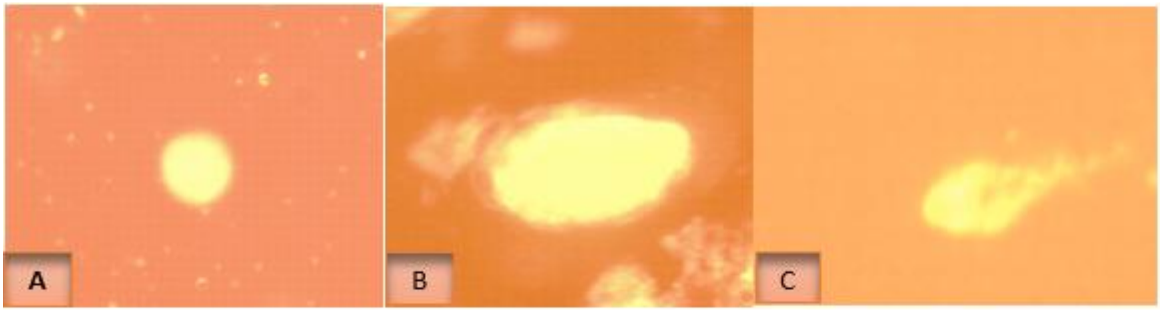

Figure (8) Types of DNA damage in algae under study according to the Comet assay technique and in the following order: **Class (A) control - Class (B) Medium damage- Class (C) High damage**

The current study recorded a clear effect of bioremediation on the molecular characteristics of the algae under study, as the values of the aforementioned indicators increased in *Oscillatoria sp.* where Comet length values increased after treatment to reach (56.2 micrometers) compared to the control algae, which recorded a value of (44 micrometers).Tail length values also increased to record the highest values, which reached (8.19 micrometers), compared to the control algae, which recorded (3.4 micrometers). As for Tail moment values, they were around (0.47 micrometers), while the lowest value was recorded for the control algae, which reached (0.11 micrometers) Figure (9).

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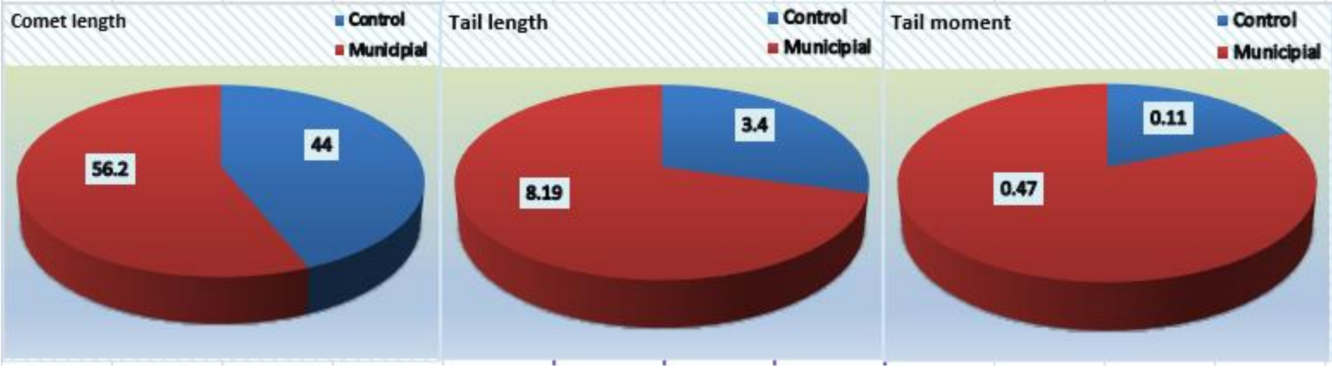

Figure (9): values of some Comet Assay Indicators in *Oscillatoria sp.*

The results indicate the presence of clear damage in the DNA molecule in *W. prolifica* algae at the end of treatment. The highest values recorded in Comet length reached (49.5 micrometers) in the algae after treating the wastewater, and the lowest values reached (26.5 micrometers) in the control algae. Tail length values in the algae increased after treatment to reach (6.5 micrometers), while the control algae recorded the lowest value, reaching (2.04 micrometers). As for the Tail moment values, they also increased in the algae after completing the wastewater treatment to be around (0.21 micrometers), and the lowest value was recorded in the control algae, reaching (0.06 micrometers) Figure (10).

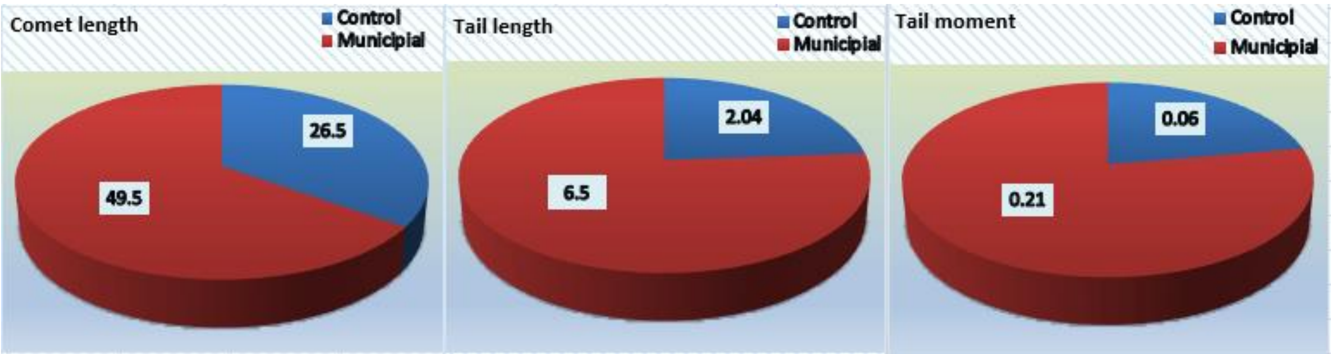

Figure (10): values of some Comet Assay Indicators in *wes sp.*

Stigonema sp. showed clear damage after completion of treatment, as Comet length values increased to 53.3 micrometers after treating wastewater compared to the control algae , which recorded the lowest values (32.5 micrometers). Tail length values increased to record the highest values (4.4 micrometers) after treating wastewater compared to the control algae , which recorded (3.5 micrometers). As for Tail moment values, their value in the control algae was around (0.10 micrometers), and the highest value was recorded in the algae after treating wastewater, which reached (0.14 micrometers) Figure (11).

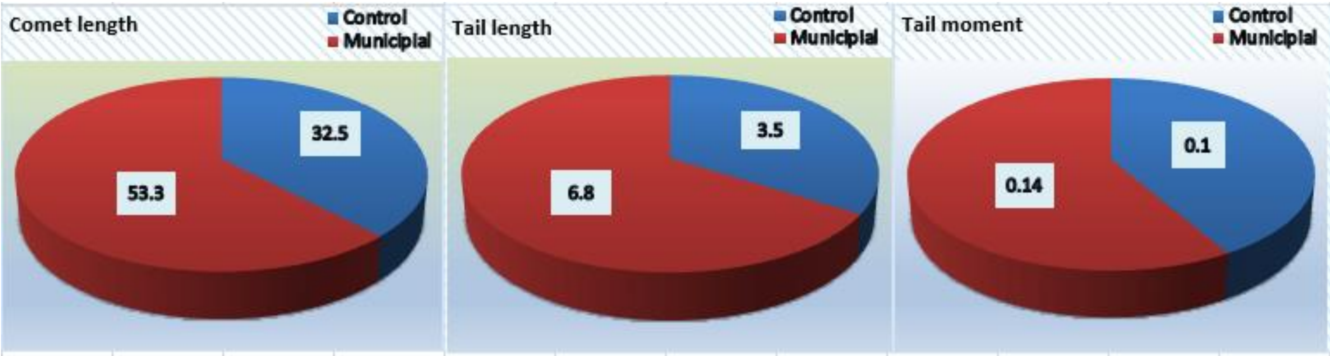

Figure (11) :values of some Comet Assay Indicators in *Stigonema sp.*

The high indicators of the comet analysis technique for the studied algae are evidence of their exposure to stress during the treatment process. where this assay detection of DNA damage such as alkali-labile sites, single-strand breaks and also other modifications of the molecules which induced in cell by physical and chemical agents (31). Under the alkaline conditions, DNA base-pairing is disrupt, also, Single-strand breaks which occur after alkaline treatment or by DNA damaging substances can cause the formation of "comets" after DNA migration in electrophoresis , where After staining the DNA with a fluorescent dye, the damage of cells can appear as comets which composed from comet head and a tail of migrated DNA fragments , this assay is able to detect DNA damage which induced by intercalating agents, alkylating agents, and also oxidative damage (32). Reactive oxygen species (ROS) consider as active genotoxic agents (33), where many studies demonstrated that the ROS are major sources for DNA damage by nucleotides removal , strand break, and many modifications of the bases in nucleotides (34).

Conclusions

The current study proved the efficiency of all algae in reducing the values of heavy elements with a clear superiority of Oscillatoria sp. in the reduction process, compared to the control sample (sewage water without algae). The bioremediation also showed a clear effect on the molecular characteristics of the algae through the increase in the values of the indicators due to the Comet assay technique, which is an indication of the effect of the DNA molecule in the current algae after completing the treatment compared to the control sample (algae grown in BG11 medium.

Author Contributions Statement

All research stage: laboratory work, collection and analysis of data, research write were done by the author.

Declaration of competing interest

The author confirms that there was no competing interest with others.

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References

1- Gad, A.; Ali, R. and El-Zeiny, A. Negative impacts of man-made activities on water quality, Egypt, Conference: Conference of integrated water resources management in the Mediterranean At: Agadir, Morocco, 2011.

2- Mosleh, Y.; Mofeed, J.; Nafea, El-Saye and Heham , S. Use of the microalga *Scenedesmus obliquus (Turpin) Kutzing* to remove some heavy metals from the industrial wastewaters, Egyptian Journal of Aquatic Biology and Fisheries, 25(5): 337 – 354. 2021. DOI: 10.21608/ ejab f. 2021.199738.

3- Ogemdi, I. K. and Gold, E. E. Physico-Chemical Parameters of Industrial Effluents from a Brewery Industry in Imo State, Nigeria, Advanced Journal of Chemistry-Section A, 1(2), 66-78. 2018. https:// doi.org / 10.29088/sami/ajca.2018.1.6678.

4- Mosleh, Y.; Mofeed, J.; Nafea , El-Saye; Heham, S. and Mofeed, J . Heavy Metals Removal from Industrial Wastewater Using Dry Green Macro Alga *Ulva lactuca Linnaeus* ,Egyptian Journal of Aquatic Biology & Fisheries , 27(2): 343 – 360 . 2023. DOI: 10.21608 / ejab f. 2023 .292265.

5- Wang, J. and Chen, C. Biosorbents for heavy metals removal and their future a review," Biotechnol. Adv., 27: 195-226. (2009). DOI: 10.1016/j.biotechadv.2008.11.002.

6- Jais, N.M.; Mohamed, R.; Al-Gheethi, A. and Hashim, M.A .The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. Clean Technol Environ Policy 19:37–52. 2017. DOI : 10.1007/s10098-016-1235-7.

7- Kotrba, P. Microbial biosorption of metals—general introduction. In: Kotrba P, Mackova M, Macek T (eds) Microbial biosorption of metals. Springer, Dordrecht, pp 1–6. 2011.

8- Karna, R. ; Rao, U. ; ma, L.; Subramanian, G. and Maruthi Mohan, P. Biosorption of toxic metal ions by alkali-extracted biomass of a marine cyanobacterium, *Phomidium valderianum* BDU 30501. World Journal of Microbiology and Biotechnology, 15, 729-732. (1999). DOI: 10.1023/a:1008992510890.

9- Al Ghais , S. M. and Varadharajulu , S. The Bio-Removal of Heavy Metals from Wastewaters a Green Approach, International Research Journal of Engineering and Technology (IRJET), 7: 1775 – 1781. 2020.

10- Andersen, R. A. Algal Culturing Techniques. Physiological Society of America, Elsevier Academic press , 589P. (2005). DOI: 10.1016/j.jembe.2006.05.002.

11- Al-Rubaie, G. H. Using algae in treating household wastewater. Master's thesis, College of Science, Al-Mustansiriya University. 2003.

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

13- APHA. (American public health Association) .Standard method for the examination of Water and Waste Water, 16th. Ed. New York.1911pp. 1985.

14- Banath , J.P.; Fushiki, M. ;Olive, P.L . Rejoining of DNA single and double-strand breaks in humane White blood cells exposed to ionizing radiation . Int J Radiat Biol,73, pp 649. 1998. DOI: 10.1080 / 095 530098141906.

15- Kumari ,S.; Rastogi ,R.P. Singh; K.L.; Shailendra, P.S. and Rajeshwar ,P.S. DNA detection strategies .EXCLI Journal ,7:44-62. 2008.

16- Slama, I.; Abdelly, C.; Bouchereau, A.; Flowers, T.; Savouré, A. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann. Bot. 115, 433–447. 2015. https://doi.org/10.1093/aob/mcu239.

17- Erdmann, N.; Hagemann, M. Salt acclimation of algae and cyanobacteria: A comparison. In Algal Adaptation to Environmental Stresses; Rai, L.C., Gaur, J.P., Eds.; Springer: Berlin, Germany, pp. 323–361. 2001. DOI:10.1007/978-3-642-59491-5_11.

18- Kirst, G.O. Salinity tolerance of eukaryotic marine algae. Annu. Rev. Plant Physiol., 41, 21–53. 1989. https://doi.org/10.1146 / annurev. pp.42.010191. 200001

19- Singh, R.; Upadhyay, A.K.; Chandra, P.; Singh, D.P. Sodium chloride incites reactive oxygen species in green algae *Chlorococcum humicola* and *Chlorella vulgaris*: Implication on lipid synthesis, mineral nutrients and antioxidant system. Bioresour. Technol., 270, 489–497. 2018. doi: 10.1016/j.biortech.2018.09.065.

20- Li, X.; Yuan, Y.; Cheng, D.; Gao, J.; Kong, L.; Zhao, Q.; Wei, W.; Sun, Y. Exploring stress tolerance mechanism of evolved freshwater strain *Chlorella sp*. S30 under 30 g/L salt. Bioresour. Technol., 250, 495–504. 2018. DOI: 10.1016/j.biortech.2017.11.072.

21- Boyd P., Law S., Wong C. The decline and fat iron-induced subarctic phytoplankton bloom. Nature. 428 (6982), 549. (2004).

22- Huang Y., Wei C., Liao Q. Biodegradable cationic starch with high C/N ratio for Chlorella vulgaris cells concentration: Regulating microalgae flocculation performance by pH. Bioresource Technology. 276, 133. 2019. doi: https://doi.org/10.1016/j.biortech.2018.12.072.

23- Ajeel, A., M ; A. Mehdi, A, L. Effect Of Eruca Sativa Seeds Powder As Feed Supplementation On Some Physiological Traits Of Male Lambs. Journal of Life Science and Applied Research., 1, 20-30. 2020. DOI:10.59807/jlsar.v1i1.13.

24- Kaplan, D.; Richmond, A. and Hu, Q. Absorption and adsorption of heavy metals by microalgae. In: Richmond, A., and Hu, Q, Ed. Handbook of Microalgal Culture: Applied Phycology and Biotechnology, second ed. Wiley-Blackwell, p. 602. 2013. https:// doi.org /10.1002 / 9781118567166.ch32.

25- Suresh, K.K, Dahms H.-U., Won, E.-J., Lee, J.-S., and Shin, K.-H. Microalgae–A promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 113, 329. 2015. DOI: 10.1016/j.ecoenv.2014.12.019

26- Yang, J.; Cao, J.; Xing, G.; Yuan, H. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae *Chlorella minutissima* UTEX2341. Bioresour. Technol., 175, 537–544. 2015. https:// doi.org/10.1016/ j. biortech.2014.10.124.

27- Plöhn, M.; Escudero-Oñate, C. and Funk, C. Biosorption of Cd (II) by Nordic microalgae: Tolerance, kinetics and equilibrium studies. Algal Res., 59, 102471. 2021. https://doi.org/10.1016/j.algal.2021.102471

28- Mendoza-Cózatl, D.G. and Moreno-Sánchez, R. Cd^{2+} transport and storage in the chloroplast of *Euglena gracilis*. Biochim. Biophys. Acta (BBA)-Bioenerg., 1706, 88–97. 2005. https:// doi.org/10.1016/ j.bbabio.2004.09.010.

29- Diaconu, L. I. ; Covaliu-Mierlă ,C. I.; Păunescu ,O.; Covaliu ,L. D. ;Iovu ,H. and Paraschiv , G. Phytoremediation of Wastewater Containing Lead and Manganese Ions Using Algae.. Biology , 12, 773. 2023. DOI:10.3390/biology12060773.

30- Desai, S. R.; Verlecar, X. N.; Nagarajappa and Goswami, U. Genotoxicity of cadmium in marine diatom *Chaetoceros tenuissimus* using the alkaline Comet assay. Ecotoxicology, 15, 359-363. 2006. DOI: 10.1007/s10646-006-0076-2

31- Prado, R.; Díaz, R.; Rioboo, C.; Abalde, J.; Herrero, C. and Cid, A. Use of the comet assay to evaluate pesticide toxicity on non-target microalgae. In Genotoxicity: Evaluation, Testing and Prediction; Kocsis, A., Molnar, H., Eds.; Nova Science Publishers: Hauppauge, NY, USA, pp. 311–319. 2009.

32- Henderson, L.; Wolfreys , A.; Fedyk, l,; Boumer, G. and Windebauk, S. The ability of the Comet assay to discriminate between genotoxins and Cytotoxins. Mutagenesis, 13, 89-94. 1998. DOI: 10.1093/mutage/13.1.89.

33- Gaivao, I.; Sierra, L. M. and Comendador, M. A. The w/w+ SMART assay of Drosophila melanogaster detects the genotoxic effects of reactive oxygen species inducing compounds. Mutat Res, 440, 139-145. 1999. DOI: 10.1016/s1383-5718(99)00020-0.

34- Cooke, M. S.; Evans, M. D.; Dizdaroglu, M. and Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J, 17, 1195-1214. 2003. DOI: 10.1096/fj.02-0752rev