Egyptian Journal of Aquatic Biology and Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27 (6): 317 – 327 (2023)
www.ejabf.journals.ekb.eg

Efficiency of Biological Removal of Mineral Elements from Wastewater Using the Blue-Alga *Oscillatoria* sp.

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ARTICLE INFO
Article History:
Received: Oct. 28, 2023
Accepted: Nov. 17, 2023
Online: Dec. 5, 2023

Keywords:
Microalgae,
Mineral,
Removal,
Accumulation,
Biomass,
Wastewater

ABSTRACT

The present study made significant contributions to the application of a green approach for removing metals from wastewater using microalgae. Bioelimination, which involoves the use of biological material to accumulate and concentrate pollutants from aqueous solutions enables ecologically an acceptable recovery and/or elimination of these pollutants. This research specifically aimed to evaluate the efficacy of the blue-green alga/cyanobacterium *Oscillatoria* sp. in the wastewater treatment procedure. The growth rate was promising, especially in low concentrations of wastewater. Additionally, the study explored the potential of using algae for wastewater treatment by reducing the proportion of mineral elements in the algal culture filtrate and examining the possibility of mineral accumulation within the algal cells. Notably, minerals such as phosphate, magnesium, manganese, sodium, iron, sulfur and calcium showed significant differences ($P \leq 0.05$) at the probability level. Additionally, it was noted that the highest percentage of mineral nutrient removal was 10% with high efficiency, although in high wastewater concentrations, it was somewhat less efficient.

INTRODUCTION

Wastewater is one of the main risks to public health since it is frequently dumped into bodies of water without being fully or even partially treated, especially in developing nations. This leads to an increase in the pollution of the water and a loss of biodiversity in the body of water, as well as the consumption of these wastes by aquatic organisms and their entry into the water. The food chain is the basis of many health problems **(Boretti & Rosa, 2019)**. Therefore, reusing wastewater has many advantages, especially when water is scarce. Biological treatment using microorganisms is one of the modern methods that are both safe for health and do not add a financial or technical burden to production costs. Furthermore, the life processing system stands out as a significant advantage, relying on microorganisms such as bacteria, algae, fungi, and protozoa that live in these waters **(Acién *et al.*, 2016)**.

This pollution stems from the disposal of waste and factory effluents into water bodies, a consequence of chemicals usage in activities such as agricultural pesticides, household insects and agricultural fertilizers. Industrial waste mixes with sewage water in the sewage of some cities in large quantities, causing an increase in the proportion of toxic pollutants such as heavy metals **(Mishra, 2012)**. The techniques employed in wastewater treatment differ, encompassing chemical and physical methods. Some methods rely on the combination of the two approaches, while others are based on biological methods **(Rout *et al*., 2020)**.

The bio-treatment system is one of the best types of systems used since it is a healthy and less expensive method. In addition, this system depends on living organisms such as bacteria, fungi, algae and plants that cross the water as their habitat **(Marcilhac *et al.*, 2014)**.

Microalgae wastewater treatment is a more environmentally friendly method of reducing nitrogen and phosphorus and removing metals from wastewater. Not only does this method not demand energy or the use of chemicals, but it also helps mitigate slime formations. In fact, when algae are employed in biological treatment, it goes hand in hand with the production of fuel and economically significant chemicals **(Ferro *et al*., 2018)**.

Therefore, attention to the remedy of this waste has become very important and the focus of many researchers. The use to assess eutrophication, organic pollutants, and inorganic pollutants using algae has recently become increasingly relevant. By utilizing the chlorophyll formation, for example, water collected from aquatic systems with a total nitrogen content can be estimated spectrophotometrically, giving us an idea of the degree of eutrophication **(Ferro *et al*., 2018)**.

The removal of geavy metals involves various mechanisms, such as flocking, adsorption, absorption of metals and anions. Specific mechanisms for heavy metal removal include complexation, precipitation, oxidation, microbiological activity, and uptake. The first is low-concentration metabolism-related uptake in their cells, while the second is biosorption, a non-active adsorption process. While, the second method is a passive biosorption process **(Chekroun & Mourad, 2013; Mohsenpour *et al*., 2021)**.

Oscillatoria is a genus of filamentous cyanobacteria that is typically found in freshwater. It has been investigated for its ability to remove heavy metals from contaminated water via adsorption and absorption mechanisms. Adsorption is the attachment of heavy metal ions to the surface of Oscillatoria cells. Oscillatoria's cell wall contains functional groups, such as carboxyl, amino, and phosphate groups that can interact with heavy metal ions. These functional groups can interact with heavy metals via complexation or ion exchange processes, resulting in their adsorption on the cell surface. On the other hand, absorption entails the incorporation of heavy metal ions into the cellular structure of Oscillatoria. Heavy metal ions that have been deposited onto the cell surface can permeate into the cells via passive or active transport pathways. Heavy metals can bind to internal components like proteins and enzymes or accumulate in vacuoles **(Katırcıoğlu *et al.*, 2008; Azizi *et al.*, 2012; Atoku *et al.*, 2021)**.

MATERIALS AND METHODS

Microalgae and aquaculture wastewater

In this study, we used Cyanophyta microalgal species *Oscillatoria* sp. and obtained pure algae isolates from the Marine Science Center at the University of Basrah/ Iraq. This microalga was pre-cultivated in BG11 medium. The cultivation was conducted under specific conditions, including a 14:10-hour light: dark cycle, and a temperature of 25°C. At the intiation of the experiment, the weight of *Oscillatoria* sp. was set at 4 grams. Wastewater was amassed from secondary sedimentation basins at Hamdan Station in the Abu Al-Khasib area of Basra Governorate/ Iraq, where it was dined before being released.

Bioremediation by using the investigated cyanobacterium

Concentrations of 10, 25, 35, and 50% were prepared from the wastewater obtained from the station. Deionized water was used to make up each concentration, and the resulting mixtures were sterilized with an autoclave. One of these concentrations served as the control. Afterward, these concentrations were used to grow the algae mentioned in the research to discover its efficiency in removing metal elements from wastewater after being injected with the same weight of *Oscillatoria* sp. **(Al-Jubouri *et al*., 2017)**.

At the end of each week throughout the three-week experiment, the sample was filtered to separate the filtrate from the algal cultures. The first sample was taken before the transplant process, and the concentrations of mineral elements contained in it were determined. This process allowed for the assessment of the efficiency of bio-removal at the end of each week.

Approximately, 5ml of the sample was extracted and placed in a clean Teflon tube. To this, 10ml of nitric acid HNO3 was added. After allowing it to sit for 24 hours, water was introduced to the sample and heated for dissolution. The solution was filtered before bringing the volume up to 15ml with distilled water, and the measurment was conducted using an ICP ms Agilent7500, manufactured in the United States. The obtained values were then compared to those from ICP using a dilution factor to obtain the final results. Subsequently, the algae removal rate was calculated using the following equation:

$$\text{Perce. of total removal} = \frac{\text{Contaminated conce. before treatment} - \text{Contaminated conce. after treatment}}{\text{Contaminated conce. before treatment}} * 100$$

$$\text{Total removal \%} = \frac{\text{Initial conce. before treatment} - \text{Final conce. after treatment}}{\text{Initial conce. before treatment}} * 100$$

Statistical analysis

Data were analyzed using the SPSS V.20 program. Differences between groups were calculated for statistical significance using one-way ANOVA. The data were investigated at a probability level of $P \leq 0.05$.

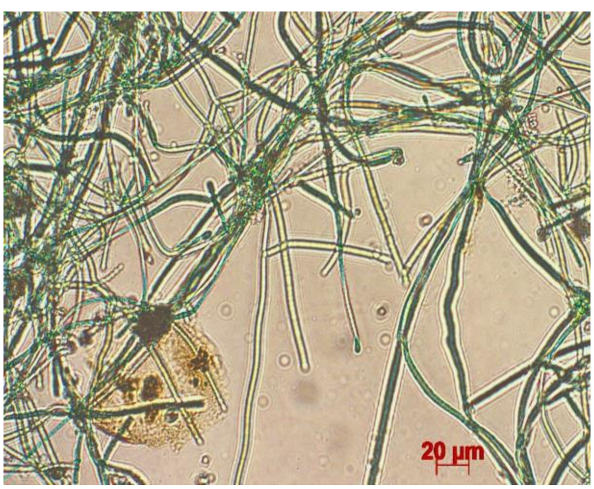
RESULTS

1. Growth rate of microalgae in wastewater

The main components responsible for algal growth are carbon, nitrogen, phosphorous, and sulfur. Metals like potassium, sodium, calcium, iron, and magnesium act as micronutrients for algae growth. The emphasis is usually on nitrogen and phosphorus uptake; however, the technological aspects of heavy metal removal by algal biomass are also important **(Cai *et al*., 2013)**.

In this study, algae were cultivated using wastewater from secondary sedimentation ponds, and growth was observed across all concentrations. There was a decrease in the weight of the biomass alga at the end of the experimentation, but an increase in the weight of the biomass alga was noted after weeks two and three. During the first three weeks of the experiment, the 25% concentration increased significantly, but it appeared to decrease during the fourth and final week of the experiment.

The results showed that the weight of the algae in the 35 per cent concentration increased slightly in the first week and decreased in the third and fourth weeks. Furthermore, the results showed that the weight of the mass increased during the experimental period with a concentration of 50%, as shown in Figs. (1, 2).



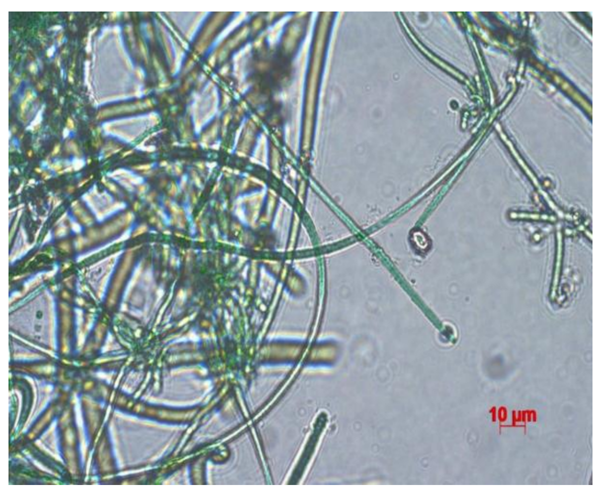


Fig. 1. Light microscopy micrographs of *Oscillatoria* sp. microalgae

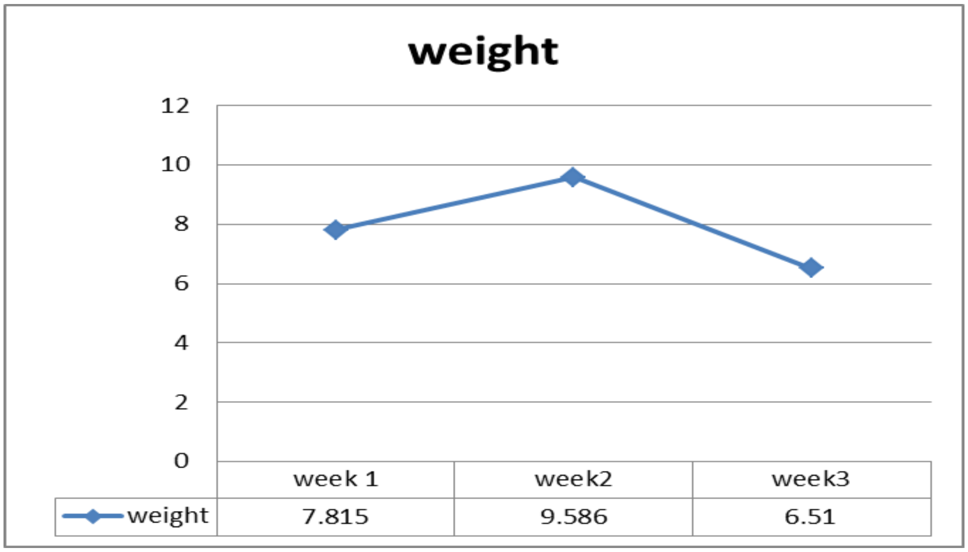


Fig. 2. Growth rate of *Oscillatoria* sp. microalgae

1.1. Mineral elements bioremoval efficiency

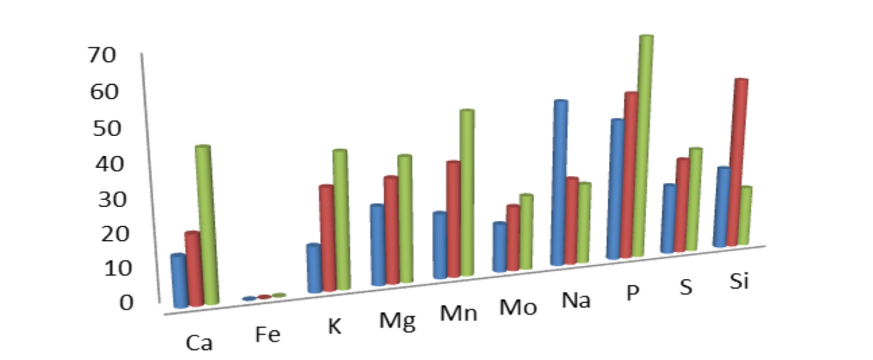
The concentrations of mineral elements in each infiltrate and biomass of the studied algae were measured during the research, and the concentrations of mineral elements and their accumulation in each infiltrate and biomass of the algae were determined. The elements phosphorus, sodium, manganese, magnesium, lithium, iron, sulfur, potassium, molybdenum, and calcium were found in high concentrations in wastewater collected from the secondary sedimentation station.

1.3. Efficiency of bioremoval of mineral elements in the filtrate

During the first week, the pollutant concentration in all tests decreased dramatically. Pollutant removal gradually levelled off until the end of treatment. Overall, the culture of *Oscillatoia* sp. in contaminated wastewater demonstrated thriving algal biomass during the removal of certain elements such as Fe, P, S, Mg and Mn.

1.3.1 Mineral elements removal efficiency in filtrate concentration 10%.

The results showed that phosphorus had the highest biological elimination rate in the first week, reaching 44 %. Conversely, potassium demonstrated the lowest percentage elimination, with a value of 14%. In the second week, phosphorus elimination increased to 52%, morbidity was 20%, and by the third week, it further rose to 69%. The removal rates for phosphorus and sodium were 15%, while iron removal was 0% in this process. Significant differences were observed for all elements except iron, at the probability level of $P \leq 0.05$ (Fig. 3).

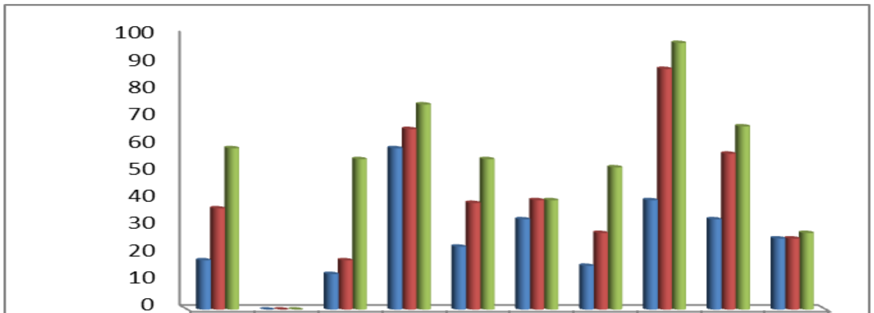


	Ca	Fe	K	Mg	Mn	Mo	Na	P	S	Si
week 1	15	0	14	24	20	15	51	44	22	26
week 2	21	0	31	32	35	20	27	52	30	54
week3	45	0	41	38	50	23	25	69	33	19

Fig. 3. Mineral elements removal efficiency in filtrate concentration 10%

1.3.2. Mineral elements removal efficiency in filtrate concentration 25%.

In the first week, the highest elimination rate was for Mg, with a 59% elimination rate, and the lowest was for K, with a 13% elimination rate. Afterward, P comes with a 40% elimination rate, and the removal percentage for sodium was 16%. Whereas, the removal percentage of S was 33%. In the second week, the phosphorus was 88% and K was 18%. The elimantion rate of Ca, Mg, Mn, Mo, Na, and S was 37, 66, 23, 40, 28, 57%, respectively. In the final week, the average reduction of pollutants was the lowest, with phosphorus registering the highest percentage at 97.5% and magnesium at 77%. Conversly, molybdenum had the lowest percentage at 40%, while potassium was recorded at 55%, calcium at 59%, and manganese at 55%. The differences were statistically significant ($P \leq 0.05$) for all elements except for iron, as illustrated in Fig. (4).



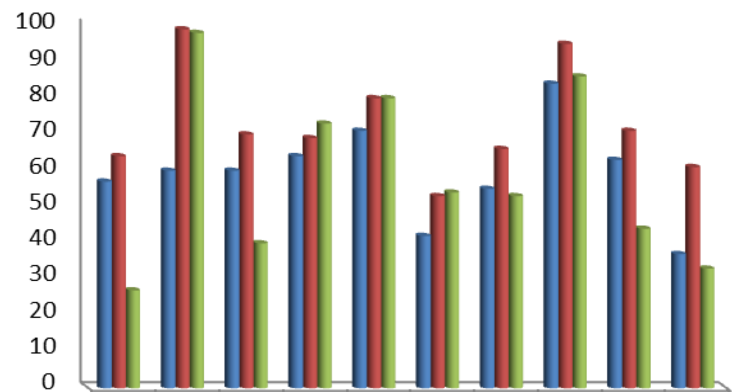
	Ca	Fe	K	Mg	Mn	Mo	Na	P	S	Si
week 1	18	0	13	59	23	33	16	40	33	26
week 2	37	0	18	66	39	40	28	88	57	26
week3	59	0	55	75	55	40	52	97.5	67	28

Fig. 4. Mineral elements removal efficiency in filtrate concentration 25%

1.3.3 Mineral elements removal efficiency in filtrate concentration 35%.

The element phosphorus had the highest percentage of elimination in the first week at 84 %, followed by 71 % for manganese, 64% for magnesium, and iron with a percentage of elimination of 60%. The total percentage of potassium removed was also 60%, and the percentage of silicon removed was 37%.

During the second week, the higest percentage of biological elimantion was observed for iron, with an elimination rate of 99%. Phosphorus followed closely with a rate of elimination of 95%, and manganese exhibited an elimination rate of 80%. Additionally, sulfur had an elimination rate of 7%, potassium at 70%, magnesium at 69%, and calcium at 64%. Upon comparing the third week to the previous two weeks, the highest percentage of elimination was observed for iron at 98%, followed by phosphorus at 86%, manganese at 80%, magnesium at 73%, sodium at 53%, and sulfur at 44%. Significant differences were noted for all elements except for iron, with a probability level of $P \leq 0.05$, as illustrated in Fig. (5).



	Ca	Fe	K	Mg	Mn	Mo	Na	P	S	Si
week 1	57	60	60	64	71	42	55	84	63	37
week 2	64	99	70	69	80	53	66	95	71	61
week3	27	98	40	73	80	54	53	86	44	33

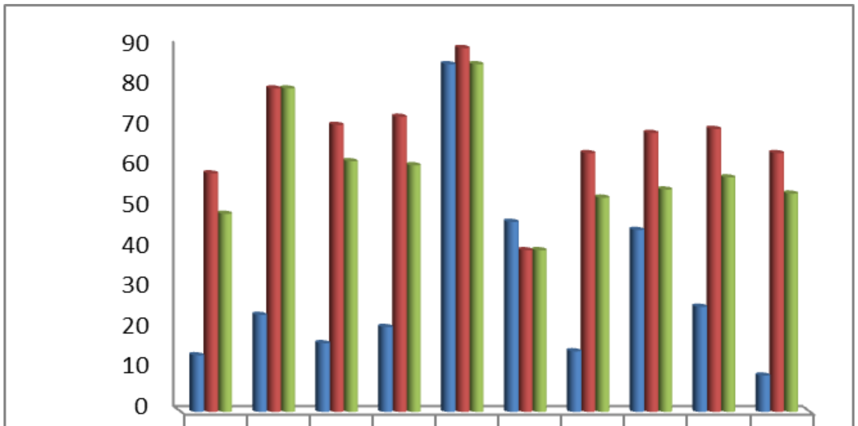
Fig. 5. Mineral elements removal efficiency in filtrate concentration 35%

1.3.4. Mineral elements removal efficiency in filtrate concentration 50%.

At the 50% concentration, the highest percentage removal was observed for manganese at 86%. Phosphorus exhibited a removal rate of 45%, sulfur was removed by 26%, magnesium by 21%, and potassium by 17%.

In the second week of the experiment, the highest percentage of removal was achieved by magnesium, reaching the highest rate at 73%. Following closely were iron removal at 69%, phosphorus removal at 64%, and sodium removal at 59%. Calcium exhibited a removal rate of 59%, while molybdenum had the lowest removal rate at 40%.

In the third week of the experiment, the highest percentage of iron removal was 80%, followed by potassium at 62%, sulfur at 58%, phosphorus at 55%, sodium at 53%, calcium at 49%, and molybdenum at 40%. The observed differences were statistically significant at the probability level of $P \leq 0.05$, as depicted in Fig. (6).



	Ca	Fe	K	Mg	Mn	Mo	Na	P	S	Si
week1	14	24	17	21	86	47	15	45	26	9
week2	59	80	71	73	90	40	64	69	70	64
week3	49	80	62	61	86	40	53	55	58	54

Fig. 6. Mineral elements removal efficiency in filtrate concentration 50%

DISCUSSION

The researchers confirmed that the presence of nutrients in wastewater provides an ideal environment for algae growth and reproduction, as well as a rise in fresh and dry weight. Dilution, on the other hand, results in a drop in the biomass relative to greater concentrations, which occurs when wastewater contents are diluted and become insufficient **(Ubando, 2021)**. Additionally, the researchers **Sekaran *et al.* (2013)** stated that treating sewage water with algae results in high-quality water that may be used for a variety of industrial and agricultural uses, as well as the utilization of the algal mass produced by algae for its high nutritional value.

The absorption of mineral elements by algae occurs in two stages: The first is short-term adsorption, involving physical adsorption on the cell's surface. This rapid process takes place within the first few hours of exposure. The second stage is long-term absorption, which includes bioaccumulation and occurs after the initial adsorption process. It is noted that Oscillatoria sp. utilizes phosphorus, as it is essential for algal growth **(Lee & Lee, 2001)**. Moreover, this contributes to the production of phosphour-containing compounds, such as Adenosine triphosphate (ATP) and Nicotinamide adenine dinucleotide Phosphate-oxidase (NADPH). Furthermore, phosphorus can be eliminated by abiotic phosphorus assimilation into biomass and abiotic precipitation **(Hvitved *al*, 2009)**.

Calcium is one of the common elements in water. It is a nutrient that is needed by plants and aquatic organisms in small amounts and can reduce the solubility of carbon dioxide in water **(Allen *et al*., 2000)**. Algae actively reduce magnesium ion values, potentially attributed to the utilization of magnesium ions by microalgae in the formation of chlorophyll pigment. As the algae's growth increases, so does the production of chlorophyll, resulting in the consumption of magnesium ions—the central atom of chlorophyll. Consequently, the magnesium ion concentration in the medium decreases **(White & Broadley, 2003)**.

It was noted through research that growth in dilute concentrations of wastewater, which itself contains dilute concentrations of iron, was an encouraging medium for further growth, but at higher concentrations. On the other hand, a decrease in growth was observed, evidenced by a reduction in the weight of soft algae. This decline is attributed to the accumulation of iron in the algae cells, thereby inhibiting biological processes within the cells. This inhibition manifests through the reduction of photosynthetic pigments, such as chlorophyll and carotene, as well as a decrease in the unsaturated fatty acids of the algae membranes. Moreover, this is what the researchers confirmed that the microalgae required both macro and micronutrients and nutrient optimization for maximum biomass production. Micronutrients are necessary in the regulation of nitrogen assimilation and have an impact on the photosynthesis pathway. Furthermore, concentrations of three micronutrients vary, such as iron chloride, sodium molybdenum oxide, and manganese chloride **(Singh *et al*., 2011)**.

CONCLUSION

It has been found that microalgae are among the organisms that can be used in wastewater treatment, as well as using wastewater as a medium for algae growth to benefit from it in various fields. Microalgae also contribute to reducing the levels of pollutants by significant proportions. The study recommends expanding the research in the field of the wastewater treatment. Regarding the use of algae for other species and genera, it is a less expensive method than the methods that are currently being used.

REFERENCES

Acién, F. G.; Gómez-Serrano, C.; Morales-Amaral, M. D. M.; Fernández-Sevilla, J. M. and Molina-Grima, E. (2016). Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment?. *Applied microbiology and biotechnology*, *100*: 9013-9022.

Al-Azzawi , A.J.M.. An ecological study of phytoplankton in some sewers in the northern part of the general estuary . Master Thesis. College of Sciences . Baghdad University ; 2004 (Iraq). 137 pp.

Al-Jubori, M. H.; Al-Janabi, J. D. and AI-Shahrii, Y. J. (2017). Study of using some microalgae in treatment of domestic wastewater. *Tikrit Journal of Pure Science, 22*(10): 84-94.

Atoku, D. I., Ojekunle, O. Z., Taiwo, A. M., and Shittu, O. B. (2021). Evaluating the efficiency of Nostoc commune, Oscillatoria limosa and Chlorella vulgaris in a phycoremediation of heavy metals contaminated industrial wastewater. *Scientific African*, *12*: e00817.

Azizi, S. N.; Hosseinzadeh Colagar, A. and Hafeziyan, S. M. (2012). Removal of Cd (II) from aquatic system using Oscillatoria sp. biosorbent. *The Scientific World Journal*, *2012*.

Boretti, A. and Rosa, L. (2019). Reassessing the projections of the world water development report. *NPJ Clean Water*, *2*(1): 15.

Cai, T.; Park, S. Y. and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renewable and Sustainable Energy Reviews*, *19*: 360-369.

Chekroun, K. B. and Baghour, M. (2013). The role of algae in phytoremediation of heavy metals: a review. *J Mater Environ Sci*, *4*(6): 873-880.

De Godos, I.; Blanco, S.; García-Encina, P. A.; Becares, E. and Muñoz, R. (2009). Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. *Bioresource Technology*, *100*(19): 4332-4339.

Ferro, L.; Gentili, F. G. and Funk, C. (2018). Isolation and characterization of microalgal strains for biomass production and wastewater reclamation in Northern Sweden. *Algal research*, *32*: 44-53.

Ferro, L., Gorzsás, A.; Gentili, F. G. and Funk, C. (2018). Subarctic microalgal strains treat wastewater and produce biomass at low temperature and short photoperiod. *Algal research*, *35*: 160-167.

Katırcıoğlu, H., Aslım, B., Türker, A. R., Atıcı, T., and Beyatlı, Y. (2008). Removal of cadmium (II) ion from aqueous system by dry biomass, immobilized live and heat-inactivated Oscillatoria sp. H1 isolated from freshwater (Mogan Lake). *Bioresource technology*, *99*(10): 4185-4191.

Lee, K. and Lee, C. G. (2001). Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering*, *6*: 194-199.

Marcilhac, C.; Sialve, B.; Pourcher, A. M.; Ziebal, C.; Bernet, N. and Béline, F. (2014). Digestate color and light intensity affect nutrient removal and competition phenomena in a microalgal-bacterial ecosystem. *Water research*, *64*: 278-287.

Mishra, F. K. P. and Mahanty, N. B. (2012). *Characterization of sewage and design of sewage treatment plant* (Doctoral dissertation).

Mohsenpour, S. F.; Hennige, S.; Willoughby, N., Adeloye, A. and Gutierrez, T. (2021). Integrating micro-algae into wastewater treatment: A review. *Science of the Total Environment*, *752*: 142168.

Rout, P. R.; Zhang, T. C.; Bhunia, P. and Surampalli, R. Y. (2021). Treatment technologies for emerging contaminants in wastewater treatment plants: A review. *Science of the Total Environment*, *753*: 141990.

Sekaran, G.; Karthikeyan, S.; Nagalakshmi, C. and Mandal, A. B. (2013). Integrated Bacillus sp. immobilized cell reactor and Synechocystis sp. algal reactor for the treatment of tannery wastewater. *Environmental Science and Pollution Research*, *20*: 281-291.

Singh, A.; Nigam, P. S. and Murphy, J. D. (2011). Renewable fuels from algae: an answer to debatable land based fuels. *Bioresource technology*, *102*(1): 10-16.

Ubando, A. T.; Africa, A. D. M.; Maniquiz-Redillas, M. C., Culaba, A. B., Chen, W. H.; and Chang, J. S. (2021). Microalgal biosorption of heavy metals: a comprehensive bibliometric review. *Journal of Hazardous Materials*, *402*:123431.

White, P. J. and Broadley, M. R. (2003). Calcium in plants. *Annals of botany*, *92*(4), 487-511.