Algal Bioremediation: Heavy Metals Removal And Evaluation Of Biological Activities In Sewage Plant

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Abstract

This study aimed to grow algae to remove heavy metals and nutrients from wastewater. The biomass that was produced can be used to create bioactive compounds. *Oscillato riarubscens and Ankistrodesmus monoraphids* were grown in Tobruk wastewater, which removed about 92% of the nitrogen from the medium. The cumulative effects of *Scenedesmus acutus*, *Ankistrodesmus monoraphids* and *Euglena gracilas* also resulted in a 91% reduction in phosphorus from wastewater from Tobruk and Derna in eastern Libya that is discharged into the Mediterranean Sea.Following reductions in Cd, Zn, and Pb levels of 88%, 83%, and 58%, *Ankistrodesmus monoraphids, Euglena gracilas*, *Lyngbya muscular and Scenedesmus acutus* were grown in Tobruk wastewater. While *Scenedesmus acutus* and *Nitzschia palea* saw Pb reduction of 43% and *Ankistrodesmus monoraphids, Scenedesmus acutus, Euglena gracilas, Nitzschia palea* and *Oscillato riarubscens* reduced Zn by 75%., *Euglena gracilas* saw reductions in Cd of 97% using the same algae in the Derna wastewater. Most algae showed notable activity against *Bacillus cereus* and *Escherichia coli* in both aqueous and ethanolic extracts, but only a weak zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The following is a list of the bacterial strains inhibited in an order calculated by inhibition zone, in order of importance: *O. rubscens* > *Mixture* > *L. muscula* > *N. palea* > *A. monoraphids* > *E. gracils* > *S. acutus.*

Keywords: Wastewater, Algae, Heavy Metals, Bacterial Strains

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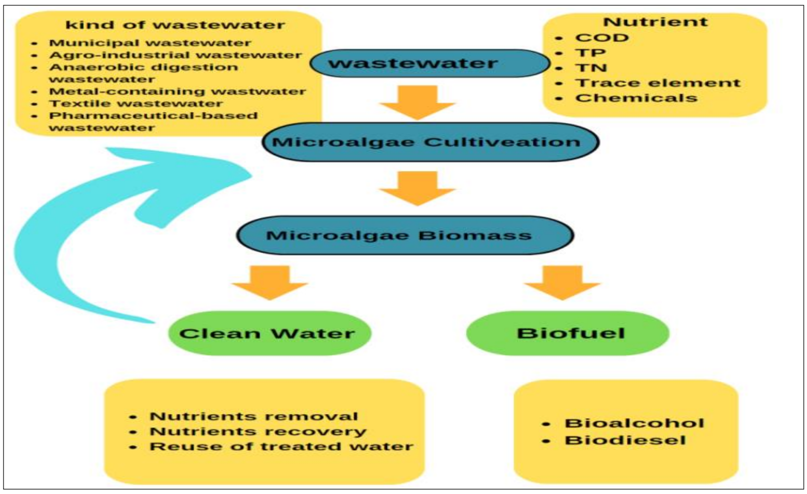
Introduction

A notable part of the treatment of urban wastewater is played by cloacal algae. This is global and might be considerably more effective in tropical areas when the climate is warm and the sun is at its best. With net sequestration of CO_2, algae play a crucial role in the recycling of oxygen and carbon dioxide. Moreover, algae can ingest nitrogen and phosphorus into the cells, acting as fertilizers by recycling minerals. Also, algae clean domestic wastewater of germs. [1, 2, 3, 4, 5] As a result, algae have a variety of cleaning effects on sewage effluents. Oxidative algal ponds don't need an outside source of oxygen because they create their own while also consuming CO_2 created by bacteria, which lowers the environmental carbon load. Algae and bacteria coexist in symbiotic ways in oxidation ponds. They give aerobic bacteria the oxygen they need to biodegrade the organic pollutants in wastewater, and in exchange, they use the bacteria's carbon dioxide and the sun's energy to synthesize carbohydrates. Yet, algae go beyond just exchanging oxygen and carbon dioxide; they also increase the concentration of dissolved oxygen, alter pH, and even secrete inhibitory compounds that harm bacterial activity. The latter effect is advantageous in that it eliminates anaerobic bacteria, the majority of which are harmful, as well as organic and inorganic pollutants that were released into the environment as a result of home, agricultural, and industrial water activities. The normal primary and secondary treatment processes of this wastewater have been introduced in a growing number of places, to eliminate the easily settled materials and to oxidize the organic material present in wastewater. The final result is a clear, apparently clean effluent that is discharged into natural water bodies [6] However, due to the release of refractory organics and heavy metals, this secondary effluent is highly nitrogen and phosphorus-rich, leading to eutrophication and other long-term issues. [7] Compared to traditional wastewater treatment, using algae for wastewater treatment has several intriguing benefits. Cost-effective treatment, minimal energy requirements, a decrease in sludge formation, and the generation of algal biomass are all benefits of algae-based treatment. [8] Algae can occasionally aid in the tertiary treatment stage's elimination of infections. Numerous researchers have developed methods for taking advantage of the algae's capacity for rapid growth and nutrient removal, which primarily results from the assimilation of nutrients as the algae grow. However, other nutrient-stripping phenomena also happen, such as ammonia volatilization and phosphorus precipitation because of the high pH the algae cause. [9] The involvement of six microalgae in the laboratory for the removal of nutrients and heavy metals from two wastewater treatment plants at Tobruk and Derna, eastern Libya, will be highlighted in the current study. The resulting biomass can be used for the extraction of bioactive compounds. [Fig1]



Simulations of wastewater treatment using microalgae biomass production for befoul generation and trace metal and nutrient removal are shown schematically.

Results and Discussions

Pollutants, microbes, and other solids suspended or dissolved in wastewater include nutrients. The wastewater that is discharged into water bodies is hazardous to the environment and causes various health issues in people. One significant environmental issue brought on by the release of nutrient-rich wastewater into water bodies is eutrophication. Several methods are used to remove nutrients (phosphorus and nitrogen) from wastewater, including chemical treatments and conventional biological methods. However, its application is restricted by its high cost and increased sludge production, so researchers are currently concentrating on microalgae for nutrient removal from wastewater because it is less expensive and produces less sludge. They display the physicochemical composition of the collected wastewater employed as a culture medium in Table 1. We examined the influent and effluents for total organic carbon (TOC), total nitrogen (TN), total phosphate (TP), and heavy metals. We discovered them to be present in high amounts in the influent than in the effluents.

Table (1). List of physico-chemical compositions of Tobruk and Derna Plants, Libya.throughout the study period,(data are means of 3 replicates).

Items	Tobruk		Derna	
	Influent	Secondary effluent	Influent	Secondary effluent
pH	8.0	7.8	7.9	7.6
Color	Brownish	Yellow	Brownish	Yellow
Total nitrogen (TN, mg/L)	18	15	19	23
Total phosphate (TP, mg/L)	3.6	2.4	3.7	3.8
Total organic carbon (TOC, mg/L)	5.1	3.4	4.7	5.2
BOD (mg/L)	583	410	633	582
Ca (mg/L)	29.58	30.03	26.59	22.47
Cd (mg/L)	0.085	0.073	0.581	0.5221
Pb (mg/L)	0.962	0.948	1.064	0.974
Zn (mg/L)	0.063	0.057	0.084	0.081
Cu (mg/L)	0.045	0.038	0.017	0.025
Fe (mg/L)	0.17	0.06	0.14	0.12
K (mg/L)	8.67	10.81	8.59	10.08
Mg (mg/L)	4.9	5.1	4.6	5.2
Na (mg/L)	27.3	24.6	23.3	21.9

Over the summer of 2022, we identified 13 microalgae species (Table 2), comprising 5 *Chlorophyta taxa*, *4 Cyanophyta taxa* and two *Bacillariophyceae taxa*, *Euglenophyta taxa* in Tobruk and Derna plants, Libya. The frequency of algae presence varied between sites. The dominant algae which high frequency of occurrence were *Ankistrodesmus, Nitzschia, Scenedesmus,* and *Euglena* (100%) followed by *Ulothrix* (80%), and (57%), *Oscillatoria*. The algal *taxa Chlamydomonas, Chlorella, Lyngbya* and *Navicula* were encountered with moderate frequency (50%), while the others were low and rare occurrences. Algae isolated from wastewater treatment plant locations often adapt to and grow better under culture conditions. Many species are isolated or employed in microalgae-based wastewater treatment, including members of the genera *Scenedesmus, Chlamydomonas, Chlorella, Micractinium,* and *Actinastrum*. [10] When cultivated under mixotrophic conditions, they knew these microalgae to boost biomass and lipid production. [11], [12]

Table (2). List of algal taxa identified throughout the study period in Tobruk and Derna Plants, Libya.

Algal taxa	Tobruk			Derna			Frequency %
	1	2	3	4	5	6	
spirogyra	+	+		+			30
Ankistrodesmus	+	+	+	+	+	+	100
Chlamydomonas				+	+	+	50
Chlorella	+	+	+				50
Scenedesmus	+	+	+	+	+	+	100
Ulothrix	+	+	+	+	+		80
Osillatoria	+	+	+				57
Spirullina	+			+			30
Navicula	+	+		+			50
Nitzschia	+	+	+	+	+	+	100
Euglena	+	+	+	+	+	+	100
Phacus	+						20
Lyngbya				+	+	+	50

Microalgae are a diverse yet highly specialized group of microorganisms that have adapted to various ecological habitats. *Scenedesmus acutus* was the best alga growing on wastewater medium for 10 days based on either O,D, or chlorophyll-a or dry weight, followed by *Ankistrodesmus monoraphids, Scenedesmus acutus, Mixture, Euglena gracilas*, *Nitzschia palea*, *Oscillato riarubscens*, and *Lyngbya muscular* irrespective of the wastewater used. *Scene-desmus acutus, Ankistrodesmus monor aphids, and Nitzschia palea* grew at the Tobruk plant after 10 days, with algal biomass of 30.4, 25.7, and 23.7 mg dry weight / L, respectively. In Derna wastewater, the highest levels of algal biomass were found in *Nitzschia palea* (42.2mg/L), *Ankistrodesmus monoraphids* (38.7mg/L), and *Scenedesmus acutus* (36.4 mg/L) (Table 3). Nitrogen is a significant macro element in microalgae as a component of biomass, accounting for 1% to over 10% of total mass. It is also an important role in regulating the lipid content of algal cells [13]. The results showed microalgae cultures may grow in both types of wastewater effluents. *Oscillato riarubscens and Ankistrodesmus monoraphids* cultivated in Tobruk wastewater reduced nitrogen to about 92% of the medium. On the other hand, *Scenedesmus acutus*, *Euglena gracilas*, *Oscillato riarubscens* and *Lyngbya muscular* grown in Tobruk and highly reduced Derna wastewater nitrogen than the other algae tested after 10 days (Table 4,5).

Table (3). Algal growth in wastewater of Tobruk and Derna plants for 10 days under controlled conditions,(data are means of 3 replicates).

Items	Tobruk plant						Derna plant					
	Optical Density		Chlorophyll-a (mg/L)		Dry weight (mg/L)		Optical Density		Chlorophyll-a (mg/L)		Dry weight (mg/L)	
	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days
A.monoraphids	0.04	0.37	0.59	4.46	0.43	25.7	0.04	0.42	0.55	7.05	0.47	38.7
S. acutus	0.04	0.41	0.55	4.07	0.56	30.4	0.04	0.39	0.47	6.13	0.52	36.4
E. gracils	0.04	0.26	0.62	3.91	0.54	15.8	0.04	0.42	0.60	3.85	0.61	35.8
N. palea	0.04	0.51	0.53	3.58	0.49	23.7	0.04	0.51	0.53	3.92	0.59	42.2
O. rubscens	0.04	0.58	0.28	2.65	0.36	13.6	0.04	0.52	0.31	2.39	0.32	32.8
L muscula	0.04	0.43	0.32	1.84	0.38	15.9	0.04	0.55	0.28	2.31	0.36	25.3
Mixture	0.04	0.71	0.57	3.95	0.5	23.2	0.04	0.71	0.57	4.11	0.54	35.2

Table (4). Effect of algae addition to post treated wastewater after 10 days , on the contents of , phosphorus and nitrogen ,BOD at Tobruk station , Libya. ,(data are means of 3 replicates).

Items	Phosphorus (mg/L)			Nitrogen (mg/L)			BOD (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction
A.monoraphids	0.11	0.01	91	1.2	0.1	92	583	213	63
S. acutus	0.11	0.01	91	1.2	0.2	83	583	184	68
E.gracils	0.11	0.01	91	1.2	0.2	83	583	136	77
N. palea	0.11	0.02	82	1.2	0.2	83	583	189	68
O. rubscens	0.11	0.03	73	1.2	0.1	92	583	176	70
L muscula	0.11	0.02	82	1.2	0.3	75	583	127	78
Mixture	0.11	0.02	82	1.2	0.1	92	583	142	76

Table (5). Effect of algae addition to post treated wastewater after 10 days , on the contents of , phosphorus and nitrogen ,BOD at Derna station , Libya. ,(data are means of 3 replicates).

Items	Phosphorus (mg/L)			Nitrogen (mg/L)			BOD (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction
A.monoraphids	0.11	0.01	91	1.1	0.3	73	633	226	64
S. acutus	0.11	0.01	91	1.1	0.2	82	633	211	67
E.gracils	0.11	0.01	91	1.1	0.2	82	633	186	71
N. palea	0.11	0.02	82	1.1	0.4	64	633	183	71
O. rubscens	0.11	0.02	82	1.1	0.2	82	633	175	72
L muscula	0.11	0.04	64	1.1	0.2	82	633	261	59
Mixture	0.11	0.02	82	1.1	0.3	73	633	169	73

Phosphorus was decreased to 91% from Tobruk and Derna wastewater types by *Scenedesmus acutus*, *Ankistrodesmus monoraphids* and *Euglena gracilas* at the same time (Table 4,5). Phosphorus is an essential element for growth and other mechanisms, such as energy transmission and DNA production [14], Its precipitation and assimilation into biomass, as well as intracellular polyphosphate dynamics, have been proposed as P removal mechanisms, especially at high pH values [15]. It is assumed that biomass uptake is the primary mechanism for TN and TP removal. As a result, the influent with a higher growth rate has a high ratio of the removed TN and TP (Table 4 and 5). Since nitrogen (N) is an essential constituent of all structural and functional proteins in algal cells and phosphorus (P) is a major macronutrient that is central to cellular metabolic processes by forming many structural and functional components required for normal growth and development of microalgae, an increase in algal biomass (BBM) was found under N and P-rich conditions (BBM) when the N concentration was 40.87 mg/ L and the P concentration was 51.5 mg/L. Due to the low concentrations of N and P compared to BBM, algal biomass was shown to be decreasing in wastewater conditions. For 13 of the microalgae isolates under study, secondary municipal wastewater supported biomass productivity rates between 20 and 31mg/L/day, with Scenedesmus sp. AMDD is the most productive. [15],[10] A high BOD level indicates an excess of organic carbon. The addition of wastes with high BOD values to aquatic ecosystems causes oxygen depletion. The higher the BOD of a waste source, the higher its polluting power. The results of this study are presented in Tabs. 4 and 5, which showed that *Lyngbya muscular* and *Euglena gracilas* cultivation in these wastes for 10 days resulted in a relatively high removal of BOD in the Tobruk plant, 78% and 77%, respectively. Nevertheless, the algae that reduced BOD (approximately 72%) Derna plants were *Oscillato riarubscens* followd (71%) to *Euglena gracilas* and *Nitzschia palea*. Algal population growth allows for the further decomposition of organic materials by producing oxygen. Its oxygen production replenishes the oxygen required by heterotrophic bacteria. Oxidation ponds tend to fill because of the settling of bacterial and algal cells formed during sewage decomposition. Overall, oxidation ponds are inefficient, and effluents containing oxidized products must be removed from the ponds regularly [17]. Elevated pH can enhance N removal from pond liquid via ammonia volatilization and P removal via phosphate precipitation [19]. As a result, the pH can influence both algal growth and the effectiveness of N and P removal in wastewater treatment. The role of algae in wastewater treatment was also studied, as well as their affinity for heavy metals based on high negative surface change. In laboratory cultures, heavy metal uptake by microbial cells depends on free ion activity [20]. The data in tables 6 and 7 show that after 10 days, *Ankistrodesmus monoraphids, Euglena gracilas* and *Lyngbya muscular* grown in Tobruk wastewater reduced Cd to 88%, Zn to 83% *Scenedesmus acutus*, and Pb to 58% *Euglena gracilas*. Using the same algae in Derna wastewater *Euglena gracilas* reduced Cd by the most (97%), while *Scenedesmus acutus* and *Nitzschia palea* reduced Pb by 43%, and *Ankistrodesmus monoraphids, Scenedesmus acutus, Euglena gracilas, Nitzschia palea* and *Oscillato riarubscens* reduced Zn by 75%. It is worth noting that the mixture of six algae was the best reducer in terms of either nutrients or heavy metals. In this respect, the cyanobacterial cell wall includes metal-binding functional groups such as amine, carboxylic, thiol, sulfhydryl, and phosphoric. Nevertheless, the efficacy of adsorption is highly dependent on the type of metal.ion, the number of charges, and the affinity of the binding site for each metal [21]. According to [22], histidine present on the cell wall can bind Copper Cu^{+2} because it provides a bidentate site. Amine and carboxylic groups can also combine with copper bidentate. The presence of methionine as one of the amino acids is significant, which may result in metal binding to sulfhydryl groups. Moreover, polysaccharides may function as chelators [23], [24] suggested two possible mechanisms for heavy metal effect on algae: first, displacement of an essential metal ion from the central and functional part of the enzyme protein, and second, interference with sulfhydryl (-SH) groups, which often determine the secondary and tertiary structure of the proteins. Nevertheless, [25] discovered that heavy metals (Ni, Cu, Zn, Co, Fe, and Hg) at concentrations ranging from 1 to 200 ppm affected the survival and motility of algae.

Table (6). Effect of algae addition to post treated wastewater after 10 days , on the contents of heavy metals (cadmium, lead and zink)at Tobruk station , Libya.,(data are means of 3 replicates).

	Cadmium (mg/L)			Lead (mg/L)			Zinc (mg/L)		
Items	**0 day**	**10 days**	**% reduction**	**0 day**	**10 days**	**% reduction**	**0 day**	**10 days**	**% reduction**
A.monoraphids	0.08	0.01	88	0.96	0.5	48	0.06	0.02	67
S. acutus	0.08	0.02	75	0.96	0.7	27	0.06	0.01	83
E.gracils	0.08	0.01	88	0.96	0.4	58	0.06	0.03	50
N. palea	0.08	0.02	75	0.96	0.5	48	0.06	0.02	67
O. rubscens	0.08	0.02	75	0.96	0.7	27	0.06	0.02	67
L muscula	0.08	0.01	88	0.96	0.5	48	0.06	0.03	50
Mixture	0.08	0.01	88	0.96	0.4	58	0.06	0.01	83

Table (7). Effect of algae addition to post treated wastewater after 10 days , on the contents of heavy metals (cadmium, lead and zink)at Derna station , Libya.,(data are means of 3 replicates).

	Cadmium (mg/L)			Lead (mg/L)			Zinc (mg/L)		
Items	**0 day**	**10 days**	**% reduction**	**0 day**	**10 days**	**% reduction**	**0 day**	**10 days**	**% reduction**
A.monoraphids	0.58	0.06	90	1.06	0.7	34	0.08	0.02	75
S. acutus	0.58	0.04	93	1.06	0.6	43	0.08	0.02	75
E.gracils	0.58	0.02	97	1.06	0.8	25	0.08	0.02	75
N. palea	0.58	0.05	91	1.06	0.6	43	0.08	0.02	75
O. rubscens	0.58	0.05	91	1.06	0.8	25	0.08	0.02	75
L muscula	0.58	0.04	93	1.06	0.7	34	0.08	0.03	63
Mixture	0.58	0.02	97	1.06	0.5	53	0.08	0.02	75

Aqueous and ethanol extracts of *A. monoraphids*, *S. acutus*, *E. gracils*, *N. palea*, *O. rubscens*, *L muscular*, and a mixture enriched with wastewater from Tobruk and Derna Plants were evaluated in vitro against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacteria (*Staphylococcus aureus* and *Bac* (*Escherichia coli*, and *Pseudomonas aeruginosa*). Most algal extracts exhibited significant activity against *Bacillus cereus* and *Escherichia coli* but had a low zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (Figure 1&2). In both the Tobruk and Derna plants, ethanolic extracts of all algae were more effective against bacteria than aqueous extracts. The inhibition zone determined the inhibitory effect of algae on bacterial strains in the following order*: O. rubscens, > L muscular >Mixture > N. palea> E.gracils> S. acutus> A.monoraphids* indicating that antibacterial agent secretion may be related to algal species and algal nutrition.

Materials and Methods:

Wastewater Sampling for Microalgae Isolates

Water samples were collected during the summer from municipal wastewater mixed with agricultural drainage for the Tobruk and Derna plants, Libya, which was supporting an obvious algal bloom. Following collection, samples were stored in 10 L plastic bottles and kept on ice in the dark. When the wastewater was returned to the laboratory, the pH was measured using a pH meter (Orion 290A), and it was between 7.8and 7.6, with a temperature between 18 °C and 20 °C. All samples were microscopically examined for the presence of new species. [26], [27], [28], [29] were used to identify the algae. The second step toward successful isolation is the removal of contaminants, particularly those that can outcompete the target species, such as zooplankton that feed on algae. Dilution, single-cell isolation by micropipette, and agar streaking techniques were used.

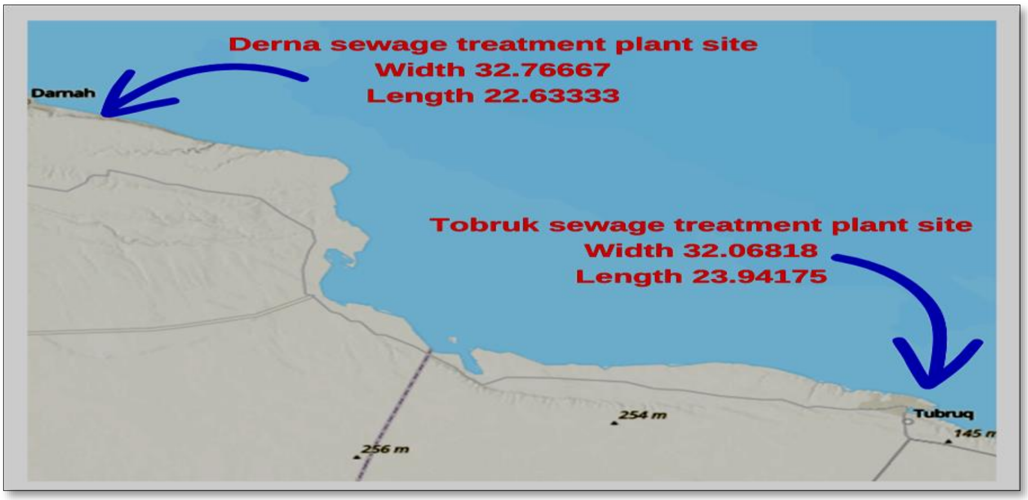


Fig 2: Libya's eastern coastline overlooks the Mediterranean Sea, and there are two sewage treatment plants: Tobruk and Derna

Wastewater Sampling for Cultivation of Microalgae Strains

They collected sequentially domestic wastewater influent and effluent samples from the Tobruk and Derna plants, in Libya. Sterilized sampling bottles were used to collect the samples. The influent was pedimented automatically, the secondary effluent was treated with aeration, and the tertiary effluent was treated with UV radiation. The wastewater's physicochemical characterization was evaluated and analyzed using different methods described in the following paragraphs. To remove microorganisms and suspended solids, water samples were immediately filtered through a 0.2 0.2 µm nylon membrane filter. In the interest of examining the potential of the selected microalgae species for integrated wastewater treatment and antibacterial production, the algal suspension was centrifuged and washed three times with sterilized distilled water. The algal suspension's initial optical density (OD) was set to 1.5, and 500 mL of the algal suspension was used as the initial inoculums. The selected microalgae species were grown at 27 ^oC in 10-liter conical flask photobioreactors containing wastewater and illuminated (50 mol photons m^{-2} s^{-1}) by cool fluorescent lamps with a 12:12 L:D cycle. 0.3% CO_2-enriched air was bubbled into the culture medium. The optical density at 680 nm was measured on different days with a spectrophotometer (Thermo, 300, USA) to determine algal growth.

Evaluation of Nutrient Removal

Centrifugation was used to harvest microalgae cells, and the supernatant was filtered through a micro-syringe filter (0.2 m). Total nitrogen (TN) and total phosphorus (TP) were measured in filtrated solution (TIC). The TN and TP levels in wastewater samples were determined using the Persulfate Digestion and Ascorbate methods, respectively [30]. To measure TN, 20 gm NaOH was dissolved in 500 mL DW, then 15 gm Potassium Persulfate ($K_2S_2O_8$) was added and thoroughly mixed (Persulfate solution). 1 mL of persulfate solution was added to 5 mL of the sample and autoclaved at 120 °C for 30 minutes in a kit tube.

Room temperature was used to cool the kit tubes. 1 mL HCl was added to 5 mL of autoclaved kit tube solution (1:16 DW). Using a spectrophotometer (Thermo, 300, UV-VIS), the absorbance of the final solution was measured at 220 nm. After an extensive data analysis of the standard curve, TN concentration was calculated using the following equation: OD = 0.215 TN concentration. 6 gm Ammonium molybdate for measuring TP. 4 H_2O was dissolved in 300 mL DW, 0.24 g of potassium antimonyl tartrate was added, 120 mL of H_2SO_4 2: 1 (80 mL of H_2SO_4 + 40 mL DW) was added, 5 g of Ammonium Sulfamate ($NH_4OSO_2NH_2$) was added, and the solution was finally completed to 500 mL by DW. The solution was combined with another containing 7.2 gm Ascorbic Acid dissolved in 100 mL DW. 5 mL of sample was mixed with 0.5 mL of the mixed solution. At 880 nm, a Spectrophotometer UV/Vis (Thermo, 300) was used to measure the sample. An ELAN DRC II Inductively Coupled Atomic Absorption Spectrophotometer (AAS) was used to analyze metals (Thermo Scientific, FS96, USA). A pH meter was used to measure the pH. (Orion 290A). The nutrient removal rate percentage was calculated by dividing the difference between the first and final-day concentrations by the first-day concentration and multiplying the result by 100. The experiments were carried out in triplicate, and the results are presented as means. The cultures were placed in the same conditions as the blank experiments (wastewater without inoculums).

Algal Biomass Assessment

The biomass of microalgae was measured as dry weight at 0 and 10 days after growth.

Estimation of Chlorophyll-a Content

The expression of pigments over time can determine the growth of an algal cell. Acetone extraction is a method of extracting pigments from algae cells. Chlorophyll-a concentration is defined as the absorbance (A) reading of the pigment extract at a specific wavelength relative to a solvent blank in a spectrophotometer. [31]

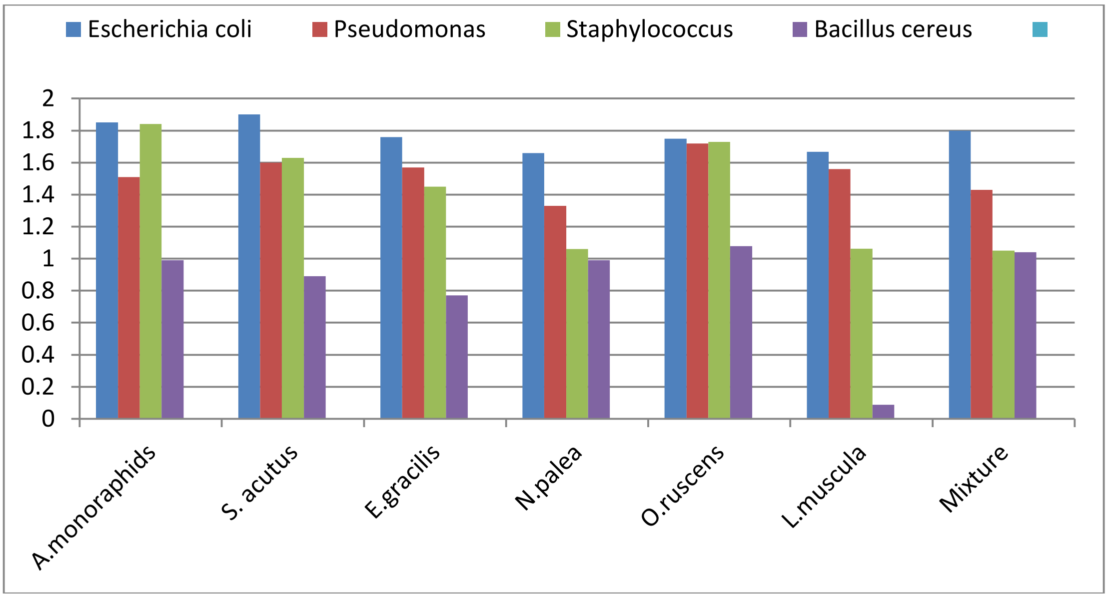
Antibacterial Activity

One gram of dried algae biomass was extracted in a mortar and pestle with water (aqueous) and/or ethanol and stored overnight at 4 °C for complete extraction. After 10 minutes of centrifugation at 10,000 g, the supernatant was collected. At 40 °C, they concentrated the solvent extracts under reduced pressure. The dry residue was redissolved in dimethyl sulfoxide (DMSO) and stored at 4 °C until it was used in the bioassay. The antibacterial activity of microalgae extracts was determined using an agar plate diffusion test (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*).

Filter paper disks (5 mm) were saturated with 20 liters of a 1 mg ml^{-1} test solution, dried, and placed on nutrient agar plates with the test microorganisms. Plates were incubated at 37 °C for 30 minutes, and they measured inhibition zones [32] and [27].

Conclusions

Cloacal algae can remove nutrients and heavy metals, as animal feed, and as an antibacterial agent. This dual process has several advantages, including lower costs, lower energy input, and lower greenhouse gas emissions. Microalgae have a wide range of applications in biotechnology. In terms of medical biotechnology applications, microalgae are potential sources of high-value products such as nutraceutical and bioactive molecules that could lead to the discovery of new drugs. Another application that has piqued the interest of researchers is the use of microalgae as a biological tool for monitoring and assessing environmental toxicants. According to the findings of this study, microalgae can clean wastewater for nutrients and heavy metals. Except for cadmium, the levels of investigated metals were lower in sewage sludge than in pig slurry solids. They may increase the risk of Cd contamination of soil from the application of sludge and pig slurry solids in industrially polluted areas where plants and animals may become ill, contaminating the entire food chain. The antibacterial activity of the six algal species against selected strains of human pathogenic bacteria varies depending on the extraction solvent. We need more phytochemical research to identify the components responsible for these extracts' antibacterial activity against bacteria.



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