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Treated Wastewater by Selected Microalgae for Irrigation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Water is a major natural resource that supplies our demands in a precise manner. We must conserve and utilise every drop of water. Irrigation is considered the primary user of freshwater. Irrigation of land accounts for about 80% of overall freshwater usage. Reusing treated waste water could be an alternative method for increasing water resources. Many countries are using wastewater as an irrigation resource to meet urban demand and manage water scarcity. Microalgae-based water treatment is a viable bio refinery approach that promotes environmental and economic sustainability. Phycoremediation is a cost-effective, environmentally friendly, secure, and substitute method for wastewater treatment. In a polite lab experiment lasting 30 days, two microalgal strains *Chlorella vulgaris* and *Trichormus variabilis* were used as bioremediation agents for sewage water collected from Bahr El-Baqar, El- El-Sharkya Governorate, Egypt. The goal of the current study was to assess how inoculated microalgae (*Chlorella vulgaris* and *Trichormus variabilis*) contributed to the phycoremediation of sewage effluent, and then a pot experiment was

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conducted to test the water's potential for use in ornamental plant irrigation, due to decrease water consumption of fresh water. The study's findings revealed that sewage wastewater had a decrease in pH, electrical conductivity, total dissolved solids, phosphorous, potassium, ammonium, nitrate, biological oxygen demand, and chemical oxygen demand after 30 days of experimentation, some heavy metals were absorbed by both algal strains, while others were practically completely removed. Moreover, the treated waste water could be used for irrigation of ornamental plant.

Keywords: Phycoremediation; sewage water; Chlorella vulgaris; Trichormus variabilis.

1. INTRODUCTION

Water is a source of life and energy for all living organisms on earth, but millions of people worldwide are concerned about a lack of fresh and clean drinking water. Rapid industrialization, population growth, and unplanned urbanization have all contributed significantly to severe water pollution. Worldwide wastewater releases approximately 0.4 trillion m^3 per year, contaminating approximately 5.5 trillion m^3 of water per year. As a result, all countries should be worried about treating and reusing this massive amount of wastewater. The increased need for residential water due to population expansion, improved living conditions, and the expanding industrial sector will increase the amount of wastewater produced, supporting treated water reuse globally (Younas and Younas 2022). Untreated domestic and industrial wastewater discharge contaminates both surface and ground water with erratic pollutants. With groundwater scarcity and increasing pollution, it is critical to make the water reusable by removing pollutants; thus, wastewater treatment has become both an ecological and an unavoidable demand. The current wastewater treatment processes are extremely costly and energy-intensive. Microalgae have a high potential for producing purified high-value products (e.g., pigments and polyunsaturated fatty acids) and serve as a source of bioavailable nutrients in aquaculture feeds; however, harvesting costs limit production (Beltrán-Rocha et al., 2022). Microalgae-based remediation, also known as phycoremediation, could be used as an alternative to current treatment methods (Sharma et al., 2014). Microalgae b) ioremediation is particularly effective due to their ability to convert solar energy into useful biomasses and assimilate nutrients such as phosphorus and nitrogen that cause eutrophication during photosynthesis (El-Sheekh et al., 2016). The process of using algae for remediation is known as phycoremediation removing a surplus of nutrients from wastewater and as a result, the pollutants in wastewater are reduced. This technology can be used in conjunction with conventional treatment. process in a cost-effective and long-term manner. The role of algae an efficient role in the removal of nitrate - nitrogen (NO_3 -N), nitrogen (NH4 -N), phosphorus (P), and potassium (K), biological oxygen demand (BOD_5), and chemical oxygen demand (COD_5) COD is an abbreviation for CO_2 demand (Khan et al., 2019).

Chlorella vulgaris (*C. vulgaris*) is a unicellular green alga whose photoautotrophic development is often limited by nutrient depletion (particularly nitrogen), light attenuation, pH shift, carbon limitation, and photosynthetic oxygen formation (Yuvraj and Singh, 2016).

Trichormus variabilis's (*T. variabilis*) (syn *Anabaena variabilis* ATCC 29413) (Abedi et al., 2019) is a Nostocoidae subfamily filamentous cyanobacterium found in freshwater and soil environments. *T. variabilis* has high auto-flocculation capacity, temperature tolerance, and biofertilizer potential1 strongly suggest that research on this species should be expanded in the future and its primary enzymatic functions, which make it suitable for applications in bioremediation, biofertilization and biohydrogen production (Salleh et al., 2017). Cyanobacteria are excellent biosorbents that are often found in water and soil habitats (Kulal et al., 2020 and Touliabah et al., 2022).

Agricultural irrigation is currently the greatest treated water user globally; thus, this provides huge potential opportunities for water reuse in both industrialized and developing countries (Ungureanu et al., 2020). *Tagetes erecta* is a genus of annual or perennial herbaceous plants belonging to the sunflower family (Asteraceae). *T. erecta*, also known as American marigold, has larger flowers than *T. patula*, popularly known as French marigold, which has smaller flowers (Zhang et al., 2022). Yellow, orange, golden, or bicolored flowers are held above or tucked into the finely textured dark green foliage. The plant grows to a height of 1 to 3 feet and a spread of

0.5 feet. Leaves are placed in an opposite/subopposite pattern, and the leaf kinds are odd pinnately complex. The edge is dentate, and the form is oblong. The leaf blade length is less than 2 inches, and the leaf colour is green. The flower's colors are orange, yellow, gold, and bicolored. The blossom is spectacular (Lalit et al., 2020).

Therefore, the objective of this study is to utilize both microalgal strains *C.vulgaris* and *T. variabis* to clean up swage water, and then reuse this water to cultivate ornamental plants in a pot experiment to determine whether it is able to be used in agriculture.

2. MATERIALS AND METHODS

2.1 Materials

- Sewage water sample was collected from Bahr El-Baqar effluent station, El-Sharkya Governorate, Egypt.
- Microalgal strain *C. vulgarisa* and *T. variabilis* were obtained and cultured at Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

2.2 Experimental Design

Sample of agriculture sewage water were collected from Bahr El -Baqar agricultural swage station, El-Sharkya Governorate, Egypt. sample was analyzed at laboratory of Microbiology Department, Soils waters and Environmental Institute, Agriculture Research Center (ARC), Giza, Egypt. These samples were exposed to chemical characterization (Table 1). Treatments were as follows:

T1: Sewage water treated with *Chlorella vulgaris*

T2: Sewage water treated with (*Trichormus variabilis*).

Cells of *Chlorella vulgaris* (*C. vulgaris*). and *Trichormus variabilis* (*T. variabilis*) were inoculated in 10-liter glass tanks containing sewage water samples: sewage (100% sewage water) with algae cell cultures at a concentration of 5%. Water samples were treated with algae for 30 days while being illuminated and aerated. The treated water samples were centrifuged to precipitate the algal mats. The growth of microalgae on waste was analyzed, and biomass was extracted and tested for nutritional potential in water samples. On 10th, 20th and 30th day of incubation algal biomass was harvested by muslin cloth 2-3 times followed by centrifugation and processed for manure production. The dry weight of different algal biomass is given in, while total chlorophyll was analyzed according to Macking (1941).

Table 1. Sewage water analysis before Phycoremediation treatments

Parameter	Initial value in sewage water
pH	7.40
EC (dS/m)	1.60
TDS (mg/ml)	1025
COD (mg O_2/ml)	94.30
BOD (mg O_2/ml)	65.60
CO_3^{--}(mg/L)	-
HCO_3^-(mg/L)	5.88
Cl^-(mg/L)	9.86
Po_4^-(mg/L)	2.40
No^{3-}(mg/L)	1.4 0
NH_4^-(mg/L)	2.10
SO_4^- (mg/L)	0.27
Ca^{2+} (mleq/L)	4.29
Mg^{2+}(mleq/L)	1.92
Na^+(mleq/L)	9.04
K^+ (mleq/L)	0.76
Cu (mg/L)	32.60
Fe (mg/L)	318.50
Mn (mg/L)	1.70
Zn (mg/L)	2.00

2.2.1 Chemical analysis for parameters and elements

To determine whether a water is suitable for irrigation, factors such as pH, electrical conductivity (EC), total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD), anions like carbonate (CO_3^{-2}), bicarbonate (HCO_3^{-}), chloride (Cl^{-}), sulphate (SO_4^{-2}), cations such as calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^{+}), potassium (K^{+}), salts like nitrate-nitrogen (NO_3^{-}), ammonia-nitrogen (NH_4^{+}), and some elements as copper (Cu), ferric (Fe) ,manganese (Mn) and zinc (Zn). An estimate of microalgae phycoremediation was obtained from the difference between the initial and end readings.

2.3 Agriculture Experiment

Tagetes erecta plants were cultivated in a pot experiment in the Agriculture Research Center greenhouse in Giza, Egypt. To investigate the potential of treated sewage water after phycoremediation for use in agriculture. The experimental treatments were Plants received

1. Domestic water,
2. untreated sewage water,
3. Domestic water *C. vulgaris*
4. Domestic water+ *T. variabilis*
5. treated sewage water with (*C. vulgaris*) strain
6. treated sewage water with (*T. variabilis*) strain.

After 45 days of planting plant samples were collected from each treatment to determine, plant height, dry weight, pigments content by method of Moran(1961), proline according to Irigoyen et al. (1992) and some anions (CO_3^{-2}), (HCO_3^{-}), (Cl^{-}), ($SO4^{-2}$), cations such as (Ca^{+2}), (Mg^{+2}), (Na^{+}), (K^{+}), salts like (NO_3^{-}), (NH_4^{+}), and some elements as (Cu), (Fe), (Mn) and (Zn), in plant leaves and soil samples to investigate dehydrogenase activity (Casida etal., 1964), CO_2 (Gaur et al., 1971)and the same anions, cations and salts analyzed in plant. Table (2) shows the physical and chemical parameters of the soil used in the agriculture experiment.

Table 2. Physical and chemical properties of experimental soil

Particle size distribution*			Chemical properties**						Textural class
Clay	Silt	Sand	O.M. (%)	EC dSm-1 (1:5)	Available mg/g			pH (1:2.5)	
	%				N	P	K		Clay loam
40.8	35.4	23.8	1.84	0.99	45.00	12.5	191.90	7.75	

Table 3. Chemical analysis of domestic water

Parameter	Domestic water
pH	7.4
EC(dS/m)	0.88
TDS(mg/ml)	560
COD (mg $O_{2/}$ml)	4
BOD (mg O_2/ml)	<100-300
CO_3^{--}(mg/L)	-
HCO_3^{-}(mg/L)	5.3
Cl^{-}(mg/L)	2.11
No^{3-}(mg/L)	1.4
NH_4^{-}(mg/L)	2.1
SO_4^{-} (mg/L)	1.34
Ca^{2+} (mleq/L)	3.82
Mg^{2+}(mleq/L)	2.88
Na^{+}(mleq/L)	1.84
K^{+} (mleq/L)	0.21
Cu (μg/L)	0/1
Fe (μg/L)	0.01
Mn (μg/L)	0.01
Zn (μg/L)	0.01

3. RESULTS

3.1 Cyanobacteria Growth Analysis

Bioremediation and biodegradation are potential methods for decontaminating contaminated areas by leveraging microbial catabolic capabilities. Results of this study demonstrated that *C. vulgaris* and *T. variabilis* were found to be very effective in reducing sewage water contaminations. *C. vulgaris* and *T. variabilis* cells adapted to wastewater, resulting in high growth rates, biomass and chlorophyll content throughout the experiment. Biomass dry weight and chlorophyll content were deduced from the data in Tables (4 and 5) after 10, 20 and 30 days. The highest dry weight after 20 days of experiment was 2.94 gL^{-1} recorded by *C. vulgaris* treatment, whereas *T. variabilis* recorded the highest dry weight of 1.97 gL^{-1}, A biomass of 2.35 g L^{-1} was recorded for *C. vulgaris*, while *T. variabilis* treatment while the lowest biomass was 1.54 g L^{-1} at the end of the experiment, then at 20 days' dry weight decreased in both treatments due to the accumulation of some contaminants. The chlorophyll content increased with treatment time, with both algal strains reaching their peak after 30 days. After 30 days, the chlorophyll concentration of both algal strains rose, with *C. vulgaris* having the higher chlorophyll content than *T. variabilis*. Abedi, et al., 2019 b) reported that exposing *T. variabilis* cells to increasing quantities of wastewater would allow them to gradually adapt and tolerate the "stress factors" found in the wastewater.

3.2 Sewage Water Analysis

3.2.1 pH value, EC and total dissolved salts (TDS)

The primary parameters for determining irrigation water quality are pH, electrical conductivity (EC) and TDS. Total dissolved solids (TDS) is the total amount of metals, cations, anions, minerals, and salts that have been dissolved in water. Irrigation water with high EC and TDS causes salt accumulation and increases soil water osmotic potential, reducing crop water availability and yield (Tessema et al., 2022). To avoid secondary soil salinization, irrigation water quality parameters such as electrical conductivity and TDS should be in the range of 0.7-3 dS/m and 450-2000 mg/L, respectively, according to FAO. In this study, the initial pH, EC and TDS of sewage wastewater were 7.4, 1.6 dS/m and 1025 mg/L, respectively. After 25 days of phycoremediation, in both microalgal treatments, pH decreased in 10 days of inoculation to but increased to 7.2 and 7.1 in *T. variabilis* and *C.vulgaris* respectively but after 30 days of culture pH increased to 7.8 in both treatments. EC had decreased significantly to 1.06 and 1.12 dS/m *T. variabilis* and *C. vulgaris* consequently after 30 days. TDS in the initial sample of sewage water was 1025 (mg/ml) after 10 days, the reduction in TDS was approximately 97% *T. variabilis* and 96.4% *C. vulgaris* after 30 days (Table 6).

3.2.2 Biological oxygen demand (BOD) and chemicaloxygen demand (COD)

BOD and COD are indicators of the amount of organic pollutants in wastewater. Wastewater with high BOD and COD levels reduces the availability of dissolve oxygen to aquatic organisms. As a result, before discharging wastewater into fresh water bodies, the BOD and COD loads should be reduced. To estimate the decomposable organic compounds in water bodies, a standard COD and BOD 5 test over a 30d ay period is typically used. *C. vulgaris* and *T. variabilis* were found to be very effective in reducing BOD and COD and thus increasing the DO content of sewage wastewater in the current study. COD and BOD were measured in the initial sample at 94.3 and 65.6 mg O_2ml^{-1}, respectively. After 30 days of experimentation, both microalgal strains reduced COD by 93.2% *for T. variabilis* and 92.5% for *C. vulgaris*, while BOD was reduced by 91.7% by *T. variabilis* and 91.5% by *C. vulgaris* (Table 7).

3.2.3 Changes in NPK content in sewage water

Plants' primary nutrients are nitrogen, phosphorus, and potassium. Algae absorb nitrogen as nitrate and ammonia, phosphorus as phosphate, and potassium as K^+ ions. Algae inoculation in sewage wastewater considerably lowered nitrate, ammonia, phosphate, and potassium levels. The initial concentrations of nitrate, ammonium, phosphate and potassium in sewage wastewater were 1.4 mg/L, 2.1 mg/L, 2.4 mg/L, and 0.67 mg/L, respectively (Table 1). Data in Table (8) demonstrate significant decreases in nutrients content NO_3^-, NH_4^-, Po_4^{-3}, and K^+ due to the presence of microalgal strains in sewage water. After 30 days of phycoremediation by *T. variabilis* nutrients were reduced to 0.54mg/ L (61%), 0.56mg/ L (73%), 1.37 mg/ L (59%), and 0.18 mg/ L (73%), respectively and *C. vulgaris* the nutrients were lowered to 0.47 mg/ L (66 %), 0.55 mg/ L (74 %), 0.81 mg/ L (66.2%), and 0. 2 mg/ L (70%), respectively.

3.2.4 Removal of some minerals from sewage water

By analyzing the sewage water used for this study, four heavy metals-copper, iron, and lead were found in significant concentrations. Cu, Fe, Mn and Zn values were recorded 32.6, 318.5, 17 and 20 (µg/L), respectively. *C. vulgaris* and *T. variabilis* are promising species for the remediation of metal-contaminated water. Thus, their ability to extract Cu, Fe, Mn and Zn ions from contaminated sewage water was studied. The presence of each microalgal strain *T. variabilis* and *C. vulgaris* in sewage water led to decrease all heavy metals Cu decreased by 73.9 and 71.6%, Fe by 73.9 and 71.6%, Mn by 73.9 and 71.6% while Zn by 73.9 and 71.6% , our results were matched with Ahammed (Ahammed et al. 2023) (Table 9).

Table 4. Biomass dry weight in 100 ml culture algae after harvesting

Parameter	*C. vulgaris*			*T. variabilis*		
	10 days	**20 days**	**30 days**	**10 days**	**20 days**	**30 days**
Dry wt. (g L^{-1})	1.89	2.94	2.35	1.78	1.97	1.54

Table 5. Total Chlorophyll (µg/ml)

Treatment	Initial	10th day	20th day	30th day
C. vulgaris	6.19	7.8	8.6	9.51
T. variabilis	5.47	6.9	7.8	8.63

Table 6. pH, Ec and TDS after Phycoremediation of sewage water by *T. variabilis and C. vulgaris* after 10, 20 and 30 days

Parameter	Final value					
	T. variabilis			*C. vulgaris*		
	10 d	**20 d**	**30d**	**10 d**	**20 d**	**30 d**
pH	7.2	7.5	7.8	7.1	7.6	7.8
EC (dS ml^{-1})	1.3	1.14	1.06	1.45	1.28	1.12
TDS (mg ml^{-1})	325	284	218.2	286.5	242.6	221.4

Table 7. COD and BOD Phycoremediation of sewage water by *T. variabilis and C.vulgaris* after 10, 20 and 30 days

Parameter	Final value					
	T. variabilis			*C. vulgaris*		
	10 d	**20 d**	**30d**	**10 d**	**20 d**	**30 d**
COD (mg/ml)	7.33	7.11	6.42	7.25	7.52	7.04
BOD (mg/ml)	6.24	6.18	5.46	6.02	5.82	5.57

Table 8. Phycoremediation of some nutrients in sewage water by *T. variabilis and C.vulgaris* after 10, 20 and 30 days

Parameter	Final value					
	T. variabilis			*C. vulgaris*		
	10 d	**20 d**	**30d**	**10 d**	**20 d**	**30 d**
NO_3 (mg/L)	0.7	0.62	0.54	0.7	0.58	0.47
NH_4 (mg/L)	0.58	0.54	0.56	0.54	0.57	0.55
PO_4 (mg/L)	1.32	1.03	0.73	1.37	1.12	0.81
K (mleq/L)	0.47	0.30	0.18	0.49	0.32	0.20

Table 9. Phycoremediation of some minerals in sewage water by *T. variabilis and C. vulgaris* after 10, 20 and 30 days

Elements	Initial value	Final value					
		T. variabilis			*C. vulgaris*		
	0d	10 d	20 d	30d	10 d	20 d	30 d
Cu (µg/L)	32.6	27.2	13.4	8.5	12.3	14.7	9.3
Fe(µg/L)	318.5	211.2	202.6	191.5	210.3	209.8	196.5
Mn (µg/L)	17	0.02	<0.02	<0.02	0.02	<0.02	<0.01
Zn(µg/L)	20	12.3	9.2	4.2	13.2	9.8	5.4

3.3 Agriculture Experiment

Treated wastewater is reused in a variety of settings, including agricultural, urban, industrial, environmental, and recreational areas, as well as to support groundwater supplies. In metropolitan areas, treated wastewater is used as an alternative water resource to irrigate parks and recreation areas, sports fields, school gardens, roadways, and refuges EPA, 2004. Worldwide, treated wastewater is mostly used in agricultural irrigation and reuse in other areas based on the demands of the country (Kesari et al., 2022). Adequate study is needed to determine the potential of using treated wastewater, as well as the effects on crops. Therefore, the study was planned to examine the effect of irrigating treated wastewater on a non-food crop *Tagetes erecta*. Results of the agriculture experiment demonstrated non-significant difference between using domestic water and sewage water treated with cyanobacteria.

3.3.1 Plant analysis

Plant growth features rose normally in all treatments, with the exception of plants irrigated with waste water, which showed the lowest growth characteristics. Table (10) shows plant growth characteristics such as plant height, dry weight, pigment content, and proline accumulation on plant leaves after 25 days of experiment. Plants watered with domestic water treated with *C. vulgaris* and *T. variabilis* reached their maximum plant heights of 56 and 55 cm, respectively, whereas plants irrigated with treated sewage water treated with each microalgal strain showed no significant difference. Dry weight and pigments followed the same pattern, with dry weight increasing in plants irrigated with domestic water treated with *C. vulgaris* and *T. variabilis* to 6.77 and 7.44 g respectively. There was a minor difference between plants watered with treated sewage water and those irrigated with domestic water with cyanobacteria, with the latter having the lowest dry weight. Total chlorophyll and carotenoids, plants watered with domestic water treated with *C. vulgaris* had the highest concentration (2.69 and 1.67 mg/g), whereas plants irrigated with sewage water had the lowest content (1.61 and 0.77 mg/g). Proline accumulation under stress has been linked to stress tolerance in several plant species, therefore maximum proline content (3.05 mg/g) reported in leaves of plants irrigated by untreated sewage water than other plants in our experiment.

Data in Table (11) showed the effect of treated wastewater and domestic domestic water on N, P, K% and some minerals content in of *Tagetes erecta* plant after 25 days of experiment. Plants watered with untreated sewage water showed the highest N, P, and K% due to their high organic matter concentrations of 1.39, 5.11, and 6.47%, respectively. Plants irrigated with domestic water treated with *C. vulgaris* and *T. variabilis* had 1.17, 1.14 N%, 3.35, 3.02 P%, 4.55, 4.29 K%, respectively, while plants watered with treated sewage water with each microalgal strain showed no significant changes. Fe, Mn, Zn, and Cu were analyzed in plant tissue to determine how untreated and treated sewage water affected *T. erecta* plants compared to domestic water. Results demonstrated that all elements recorded the highest concentrations in plants irrigated with untreated sewage water: Fe (3669), Mn (83.2), Zn (92.9), and Cu (329.40) mg/kg plant tissue. While all elements decreased in plants irrigated with treated wastewater with strain *T. variabilis* Fe (330.50), Mn (26.7), Zn (32.10) and Cu (10.10) mg/kg and plants irrigated with domestic water recorded the lowest element concentration in plant tissue, Fe (362.59), Mn (39.33), Zn (35.32), and Cu (6.19) mg/kg.

3.3.2 Soil analysis

In the same trend as plant study, soil samples were exposed to determine the effect of irrigating plants with treated wastewater vs domestic water on soil biological activity as measured by dehydrogenase enzyme activities (DHA) and CO_2 levels after 25 days (Table 12). Results demonstrated that, the presence of cyanobacteria increased the microbial activities in soil, the highest dehydrogenase activity and CO_2 were (1.16 mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{-1}) and (880 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1}) respectively recorded by treatment of domestic water + *T. variabilis* followed by (1.04 mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{-1}) and (780 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1}) which recorded by treatment of domestic water + *C. vulgaris,* then treatment of untreated sewage water, while, domestic water treatment (control) resulted in the lowest microbial activity in both dehydrogenase enzyme and CO_2.

Table (13) shows the effects of treated sewage water and domestic water on N, P, K, and certain mineral content in *T. erecta* plant rhizosphere soil after 25 days of experimentation. Plants watered with untreated sewage water had the highest N, P, and K content in their rhizosphere soil, due to their high organic matter concentrations of 164.40, 10.39, and 654.5 $mg.kg^{-1}$, respectively. Plants irrigated with domestic water treated with *C. vulgaris* and *T. variabilis* had 156.10, 157.50 N, 9.13, 9.04P, 152.06, 138.06 K $mg.kg^{-1}$, respectively, while plants watered with treated sewage water with each microalgal strain showed no significant changes. Fe, Mn, Zn, and Cu concentrations were measured in rhizosphere soil of *T. erecta* plants to investigate how untreated and treated sewage water affected *T. erecta* rhizosphere compared to domestic water. Results demonstrated that all elements recorded the highest concentrations in rhizosphere soil of plants irrigated with untreated sewage water: Fe (4.00), Mn (9.31), Zn (15.04), and Cu (6.05) mg/g soil. Plants irrigated with treated wastewater with strain *T. variabilis* had lower element concentrations (2.10, 6.11, 9.72, and 3.29 mg/g soil), while plants irrigated with domestic water had slight lower concentrations (2.12, 6.43, 10.03, and 3.01 mg/g soil). However, the soil of plants watered with treated sewage water by each of the microalgal strains showed an insignificant rise in element concentration compared to domestic water.

4. DISCUSSION

Microalgae use a defensive mechanism against harmful metals to sustain themselves. Metals create compounds with proteins in the cell membrane, allowing them to accumulate without hindering development. Apart from that, build-up of the toxic metal can inhibit cell growth if concentrations are so high that the organism's defense mechanism is unable to overcome its effects. Algal cell size is also correlated with the levels of hazardous metals (Manuela etal., 2010 and Dewi etal., 2018). Blue green algae, or cyanobacteria, are perfectly adapted to carry out these tasks due to their great degree of environmental adaptability. There have been attempts to achieve this goal with a few freshwater and marine blue-greens. Through their metabolic processes, these characteristics, which are promoted by the abundance of algae forms in eutrophic waters, are a typical phenomenon that can be controlled to remove different types of inorganic and associated substances (Lebkuecher et al., 2015).

Arbib et al. (2014) and Zhao et al. (2016) reported that pH increases during microalgae growth due to a shift in the chemical balance between carbon dioxide, carbonic acid, carbonate, and hydrogen. CO_2 initially mixes with H_2O to generate H_2CO_3, which then separates into HCO_3 and H^+. The carbonic acid is then dissociated in carbonate. The TDS levels in sewage wastewater after phycoremediation were within the irrigation maximum limit. *Chlorella* sp. reduced TDS in sewage wastewater by 96% (Khan et al., 2019). TDS is a significant chemical characteristic of water that indicates the presence of numerous minerals such as nitrate, nitrite, phosphate, sulphates, metallic ions, alkalis, and acids in both colloidal and dissolved forms (Nayar, 2020). TDS was reduced due to the consumption of dissolved solid from wastewater, which is nutrient rich for the growth of microalgae. *Cholrella* sp. reduced EC and TDS by 90-98% from common effluent treatment plant) tertiary treated wastewater (Malla et al., 2015). Algae may reduce TDS through the mechanism of bioabsorption/adsorption for several types of dissolved solids in wastewater are responsible for reducing TDS to the lowest level (Azarpira et al., 2014).

The ability of microalgae species to tolerate and survive in high COD levels is indicated by the high rate of COD removal (Wang etal., 2018). Abdel-Raouf et al. (2012) found that high BOD levels in wastewater can decrease dissolved oxygen due to the respiratory demands of bacteria and algae metabolizing organic materials (Holmes et al., 2020). Very large reductions in BOD utilizing various algal species,

Table 10. Effect of treated wastewater and domestic water on growth features of *T. erecta* plant after 25 days

Treatments	Plant height (Cm)	Dry weight (gm)	Pigments		Proline (mg/g)
			Total chlorophyll (mg/g)	Carotenoids (mg/g)	
Domestic water (control)	48(b)	5.88	2.60	1.02	2.05
Domestic water + *T. variabilis*	55(a)	6.77	2.69	1.38	1.38
Domestic water + *C. vulgaris*	56(a)	7.44	2.83	1.67	1.25
Sewage water	34(c)	4.91	1.61	0.77	3.05
Sewage water treated with *T. variabilis*	53(a)	6.67	1.97	1.17	1.81
Sewage water Treated with *C. vulgaris*	52 (a)	6.35	2.21	1.06	1.63

Table 11. Effect of treated wastewater and domestic water on NPK% and some elements content in of *T. erecta* plant tissue after 25 days

Treatments	%			mg/kg			
	N	P	K	Fe	Mn	Zn	Cu
Domestic water (control)	1.13	3.44	4.43	362.59	39.33	35.32	6.19
Domestic water + *T. variabilis*	1.17	3.02	4.29	330.50	26.7	32.10	10.10
Domestic water + *C. vulgaris*	1.14	3.35	4.55	375.80	70.8	83.50	18.30
Sewage water	1.39	5.11	6.47	3669	83.2	92.90	329.40
Sewage water treated with *T. variabilis*	1.04	3.13	4.62	335.20	32	34.60	7.12
Sewage water treated with *C. vulgaris*	1.08	3.20	4.81	347.40	39	35.80	6.70

Table 12. Effect of treated wastewater and domestic water on dehydrogenase activity and CO2 in rhizosphere soil of *T. erecta* plant after 25 days

Treatments	Dehydrogenase (mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})	CO_2 (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})
Domestic water (control)	0.85	750
Domestic water + *T. variabilis*	1.16	880
Domestic water + *C. vulgaris*	1.04	870
Sewage water	0.99	780
Sewage water treated with *T. variabilis*	0.97	754.4
Sewage water treated with *C. vulgaris*	0.94	780.5

Table 13. Effect of treated wastewater and domestic domestic water on N, P, K% and some elements content in rhizosphere soil of *T. erecta* plant after 25 days

Treatments	mg kg^{-1} soil			mg g^{-1} soil			
	N	P	K	Fe	Mn	Zn	Cu
Domestic water (control)	148.70	8.86	171.32	2.12	6.43	10.03	3.01
Domestic water + *T. variabilis*	156.10	9.04	138.06	2.10	6.11	9.72	3.29
Domestic water + *C. vulgaris*	157.50	9.13	152.06	2.24	6.28	10.14	3.24
Sewage water	164.40	10.39	654.5	4.00	9.31	15.04	6.05
Sewage water treated with *T. variabilis*	141.40	10.21	731.5	3.04	7.10	11.35	3.21
Sewage water Treated with *C. vulgaris*	130.20	8.60	644.8	3.10	7.22	12.18	3.65

confirming that microalgae are the best options for wastewater purification and improvement in its physicochemical properties (Kshirsagar, 2014 and Azarpira et al., 2014) demonstrated. The lowering of COD value can be attributed to the chemical oxidation of carbon in organic pollutants, which releases carbon dioxide. Similar results obtained by El-Sheekh (2016) who reported that Algal treatment of water samples reduced BOD and COD levels gradually. Additionally, faster biodegradation and bioconversion of organic matter by algae may also contribute. The chemical oxidation of carbon present in organic pollutants, which releases carbon dioxide, is responsible for the drop in COD value; similarly, faster biodegradation and bioconversion of organic matter due to algae (Tufail et al., 2020).

According to Kube et al. (2020), *C. vulgaris* is capable of significantly reducing all forms of nitrogen, particularly ammonia and nitrate levels. They also discovered that *C. vulgaris* had a nearly 70 to100% phosphate removal efficiency in wastewater. A significant reduction in both nitrate and phosphate concentrations (up to 92%) remediated by *C. vulgaris* grown in wastewater Sharma *et al. (2020)* demonstrated that, the gradual decrease in phosphorus levels in culture media is due to this nutrient being absorbed from wastewater by microalgae such as *T. variabilis*, *Nostoc* sp and *chlorella* sp., which is required for its growth (Salgueiro *et al., 2016)*. As previously proven by Yaakob et al. (2021), the content of phosphorus in the medium is directly related to the growth of the microalga. Furthermore, phosphorus concentration is frequently a limiting factor in microalgae growth (Chowdury et al., 2020) and cells can ingest and store this mineral, reducing the amount of phosphate in the effluent. *Chlorella* sp. grown in urban wastewater reduced ammonia nitrogen by 96.8-98.4% and nitrate nitrogen by 94.2-97.8% (Kiran et al., 2014). Microalgae can remove nitrates and phosphates from wastewater with over 90% effectiveness. Furthermore, it can use nitrate and phosphate to regulate metabolic pathways. This paragraph elaborates on the microalgae-based absorption process (Chai et al., 2021).

Wastewater contains many inorganic and organic contaminants, as well as heavy metals like chromium (Cr), manganese (Mn), lead (Pb), zinc (Zn), ferric (Fe) and copper (Cu) (Priya et al., 2021). Algae have a high surface area to volume ratio, a high heavy metal tolerance, the ability to grow autotrophically or heterotrophically, the ability to manipulate genetics, phytochelatin expression, and phototaxy (Kumar & Gunasundari 2018).Blue-green algae (cyanobacteria) biosorption is high in vitamins and proteins. Even when the cells are dead, the biomass can absorb and adsorb heavy metals from aquatic solutions. Unlike traditional methods, cyanobacteria procedures do not generate toxic sludge and are very effective, simple to run, and cost effective for treating vast amounts of wastewater with low pollutant concentrations (Prakash &Awasthi, 2013). Cyanobacteria are excellent biosorbents that are often found in water and soil habitats (Cepoi et al., 2016). *T. variabilis* exposure to contaminants can elicit slow changes in gene expression, causing the organism to adapt to growth in previously harmful substances. For example, persistent cultivation of this species in a medium containing a high concentration of Cu $(NO_3)_2$ resulted in the development of a Cu-resistant strain with broad resistance to Cd, Zn, and Ni (Abedi etal.,2019). Sewage effluent including heavy metals as Fe^{3+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cd, Ni, Co, and Cr was found to promote algae and cyanobacteria growth, algae can be effective biosorbents due to their low cost, large surface area, and strong binding affinity (El Bestawy 2019). *C. vulgaris* effectively reduces pollutants from wastewater, this algae species offers a cost-effective alternative for purifying effluent before it is released into natural systems. These solutions appear to be both cost-effective and environmentally friendly. Future research should focus on using algae species to promote functional phycoremediation techniques, given these encouraging outcomes (Das, et al., 2017).

Treated wastewater, as a substitute for freshwater, makes a significant contribution to agricultural irrigation, helping to overcome the worldwide water deficit. Treated wastewater irrigation has significantly relieved water shortage issues in many parts of the world. However, it has the potential to drastically diminish environmental carrying capacities while also providing major economic, sociological, and ecological benefits in the future (Ungureanu etal., 2020). Plants utilize various metal tolerance mechanisms to adapt to their environment and survive (Gratão etal., 2019). Heavy metals have restricted plant life cycles, altering plant primers and secondary metabolites via biochemical and physiological processes (Kisa,2019). In recent decades, there has been a revived interest in studying abiotic variables that regulate secondary metabolism during plant growth *in vitro* and *in vivo* (Ahmad et al., 2018). Algal cells' biosorption ability was significantly affected by operational conditions such as pH, initial metal ion concentration, doses, contact duration, and temperature. This alga's metal biosorption is due to the presence of metal binding functional groups (C-N, -OH, COO-, -CH, C=C, C=S, and -C-) and its porous shape, which aids in heavy metal bio-sorption (Ahmad et al., 2018). Results of the study were matched with El Bestawy et al. (2019) who reported that, different microalgal species, whether free or fixed, may effectively remove metals from water or wastewater, even at high concentrations, and have selective preferences. The soil microbial population plays a vital role in managing the ecosystem's material cycle (Raj et al., 2021). Soil microbes play an important role in decomposing organic matter, cycling nutrients, fertilizing the soil, and forming soil structure, because soil microorganisms produce soil organic carbon, which improves soil fertility and water-retaining ability (Basu etal., 2021). According to Barone et al. (2019), the presence of microalgae increases microbial activities in soil, therefore the rhizosphere soil of treatments of treated sewage water recorded the lowest DHA activity and CO_2 contents than other treatments., heavy metals have an impact on soil enzymatic activity as well as the structure of the microbial community. The findings of this investigation were consistent with those of Priya et al., (2022) who revealed that heavy metals have been reduced in sewage water treated by each microalgal strain to levels suitable for irrigation (Naz et al., 2022).

5. CONCLUSION

There are already wastewater purification systems that use microalgae to recover nutrients from wastewater more effectively than prior methods. Microalgae are effective at treating sewage discharge. Microalgae (*Chlorella vulgaris*) and (*Thricuromus viriabilis*) effectively cleanse sewage effluent. They absorbed the greatest contaminants from collected sewage effluent after 10-15 days. The algal biomass obtained after 30 days of phycoremediation typically comprised 6% N, 1% P, and approximately 0.48% K, which is higher than the bulk of accessible organic manure sources. Selective algae help to reduce pollution load in sewage effluent and allow the same water to be reused for agriculture. The physicochemical parameters range of the phycoremediated wastewater is within the recommendations for irrigation water.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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