Sustainable water management for cultivating *Triticum aestivum L*. using treated wastewater with *Chlorella vulgaris*

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ABSTRACT: This study attempts to address some of the sustainable development goals of the United Nations, specifically, climate action, clean water and sanitation. With the advance of climate change, water shortage is becoming a challenge all over the world. There is a growing global trend for wastewater reuse. Therefore, the present study aimed to investigate the efficiency of the microalgae *Chlorella vulgaris* in removing nutrients and some heavy metals from EL-Tabia drainpipe at the El-Tabia region in Alexandria, Egypt, and the potential use of algal treated wastewater in irrigating wheat plants (*Triticum aestivum* L., cv. Masri 3). *Chlorella vulgaris* successfully eliminated 2.85% aluminium, 7.14% iron, 48.33% manganese, 50% cadmium, 96.30% copper and 98.63% zinc. Wheat plants were irrigated with untreated wastewater (WW), treated wastewater (TWW) or tap water (TW). Plant irrigated with TWW showed an improvement in shoot biomass (39%), protein (40%) and carbohydrate (32%) content, chloroplast ultrastructure, whereas the activity of a catalase (18.1), guaiacol peroxidase (2.13%) and superoxide dismutase (11.7%), malondialdehyde (57%) and H_2O_2 (64%) contents decreased compared to plants irrigated with WW. Overall, this study suggests the potential of using algal-treated wastewater for crop irrigation which could be a sustainable strategy for disposing of wastewater. However, risk assessment of using treated wastewater on public health is a major concern.

Keywords: Phycoremediation, Sustainability, Wastewater, Heavy metals, Water scarcity

INTRODUCTION

The impact of climate change is increasing worldwide, which in turn leads to water scarcity (Mainardis *et al*., 2022), as the increase in the rate of evaporation and the change in rainfall patterns leads to severe water shortage (Hristov *et al*., 2021). In addition, the increasing population growth and human activities resulted in more intense competition for water use. The arid and semi-arid regions are among the areas that will be most negatively affected by water scarcity, as it is expected that about 1.5 billion people will suffer from water shortage in these regions (Abou-Shady *et al*., 2023). The agriculture sector is the largest consumer of about 70% of freshwater (Garcia-Garcia and Jagtap, 2021). Globally, 380 billion cubic meters of wastewater are produced every day. Expectations indicate this rate to increase by 24% by 2030 and 51% by 2050 (Qadir *et al*., 2020). In this respect, sustainable wastewater management strategies are needed (Alvarez-Holguin *et al*., 2022).

There is a growing trend around the world to use treated wastewater for crop irrigation. This includes treating domestic, industrial, and agricultural wastewater (Jesse *et al*., 2019; Zhang *et al*., 2020; Chen *et al*., 2023). The use of treated wastewater in agriculture has many benefits, such as decreased eutrophication (Qadir *et al*., 2020; Penserini *et al*., 2024; Rápalo-Cruz *et al*., 2024), decreased chemical fertilization needs and supply crops with essential nutrients (Lahlou *et al*., 2021), alleviate the harmful effects associated with disposing of wastewater (Demir and Sahin, 2020) and promoting a circular economy approach (Khan *et al*., 2022). Several studies have reported that the use of treated wastewater for crop irrigation improves soil physiochemical properties and increases the productivity of crops (Inyinbor *et al*., 2019; Alvarez-Holguin, *et al*., 2022). Libutti *et al*. (2018) reported that the use of treated wastewater for irrigation improved the soil quality and productivity of tomatoes and cabbage under Mediterranean conditions. Likewise, the use of treated wastewater had a positive effect on growth parameters and active constituents of the flat-topped yet (Maaloul *et al*., 2019). The effect of treated wastewater on broad beans as well as their effect on plant-aphid interaction was investigated by Shannag *et al*. (2021). They concluded that treated wastewater was as effective as fresh water in boosting plant growth.

Phycoremediation is an environmentally valuable and low-cost approach. It supports green technology for high nutrient recovery, biofuel production and wastewater treatment with microalgae (Kumar *et al*., 2024). *Chlorella vulgaris* is used for wastewater treatment because it can successfully remove phosphorus nitrogen, chemical oxygen demand and biochemical oxygen demand across a wide variety of retention times, which typically range from 10 hours to 42 days (Azizi *et al*., 2020).

Common wheat (*Triticum aestivum*), is one of the most important cereal grains in the world (Moshawih *et al*., 2022). It is considered a good source of energy and nutrients, as it contains carbohydrates, protein, fibres in addition to minerals and vitamins (Biel *et al*., 2020). Moreover, it has medicinal properties such as anti-Inflammatory (Lim *et al*., 2021), antimicrobial (Rajoria *et al*., 2016), anticancer activity (Zhang *et al*., 2018), Cholesterol-Lowering Effect (Tong *et al*., 2014) and protection against chronic superficial gastritis (Kan *et al*., 2020). Abdelazez *et al*. (2024) stated that climate change led to a significant increase in water requirements for wheat cultivation in Egypt.

The wastewater from the Amia drain in the El-Tabia area of Alexandria, Egypt, includes both irrigation runoff and industrial effluents from various local industries (Akl *et al*., 2023). This study aimed to investigate the effectiveness of *C. vulgaris* in treating wastewater and examine the reuse of this treated wastewater for irrigating *Triticum aestivum* L., cv. Masri 3 plants, which copes with the sustainable development goals declared by the United Nations (2, 6, 12, 13). To the best of our knowledge, this research is the first to assess the feasibility of reusing treated wastewater for irrigation in the El-Tabia region.

MATERIALS AND METHODS

Wastewater gathering and characterization

Wastewater sample was amassed from El-Tabia drainpipe at the El-Tabia region in Alexandria, Egypt (Figure 1). Parameters (pH, Carbonate, Bicarbonate, Electrical Conductivity, Total Dissolved Solids, Total Phosphorous, Chemical oxygen demand, Calcium, Potassium, Magnesium, Sodium, Fluoride, Chloride, Nitrate, Nitrite, Sulfate, and Phosphate) were determined using standard methods for water and waste examination (APHA, 2012). Also, Leveraging Inductively Coupled Plasma-Emission Spectrometry (ICP-ES) with Ultra Sonic Nebulizer (USN) Perkin Elmer optima 3000, USA., Major trace metals were detected, notably aluminium, cadmium, cobalt, chromium, iron, copper, manganese, and zinc.

Microalgal Culture

The microalga exploited in this study was *Chlorella vulgaris* Beyerinck [Beijerinck]. It was obtained from the Institute of the Oceanography and Fisheries at Alexandria, Egypt. *Chlorella vulgaris* was cultured for 14 days (temperature at 25±3ºC, 43.7 µmol/m^2/s and 12:12 hour light/dark regime) in 5000 ml Erlenmeyer flasks utilizing Bold's Basal Medium (Bold, 1949; Bischoff and Bold, 1963) as a control medium or 100% wastewater.

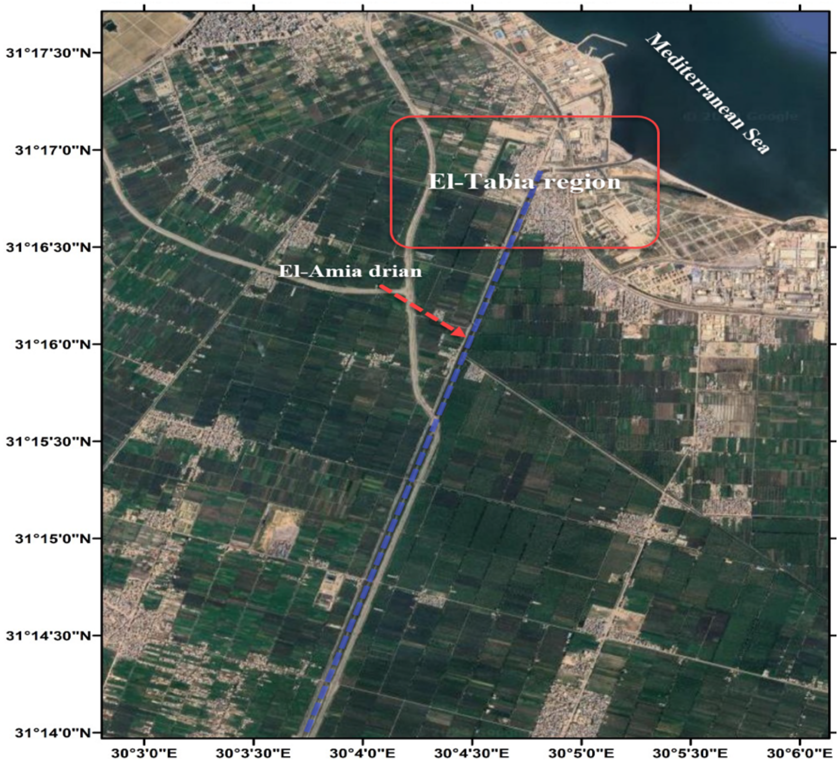

Figure 1. El-Amia drain at El-Tabia region, Alexandria, Egypt.

Growth measurements

Cell counting: Cell counting of *C. vulgaris* was examined under an optical microscope every two days, by using modified Neubauer hemocytometer.

Biomass estimation: The biomass concentration was evaluated spectrophotometrically via measurement of the culture absorbance at 625 nm (OD_{625}) Converti *et al*., (2009). A calibration curve revealed a relationship between OD_{625} and dry biomass concentration using the equation; y=4.203x (R^2=0.990) Converti *et al*., (2009). Where y is the concentration expressed in milligrams of dried biomass per litre of medium (mg/l) and x is the optical density at 625 nm.

Experimental design and treatments

Wheat grains (*Triticum aestivum* L., cv. Masri 3) were obtained from the Agricultural Research Center in Giza, Egypt. Before germination, after 3 minutes of immersion in a 0.1% sodium hypochlorite solution, the grains were carefully rinsed with distilled water. After being sterilized, the grains were submerged in distilled water at room temperature for 24 hours. The grains were sprouted in containers with sandy clay soil and incubated under natural conditions (16: 8 hours light/dark regime, temperatures of 28/23 ± 2°C, and a light intensity (23 µmol $m^{-2}s^{-1}$)). The pots were irrigated every two days with tap water (T), untreated wastewater (WT), and wastewater treated with *C. vulgaris* (TW) to 80% field capacity for 21 days. On the 21st day, the seedlings were harvested, carefully rinsed with water, gently blotted to remove excess water, and then separated into shoots and roots for subsequent growth parameter estimation and chemical analysis.

Determination of growth parameters and chlorophyll fluorescence

Shoot and root dry weights were measured for each treatment. Chlorophyll fluorescence was detected using an OS-30P pulse-modulated chlorophyll fluorometer (Opti-sciences, Hudson, USA), as described by Van Kooten and Snel, (1990). Before every measurement, leaves were dark-adapted for 30 minutes via leaf-staples. The minimum fluorescence (Fo) has been established by flipping on the weak detecting light and measuring Fo. To maximize fluorescence yield (Fm), the leaves were subjected to a 0.1s saturating flash at 6000 µmol m^2 s^{-1}. The variable to maximal fluorescence ratio (Fv/Fm) was automatically determined based on Fo and Fm measurements [Fv/Fm = (Fm-Fo)/Fm].

Determination of total protein, total carbohydrates and total lipids

Total proteins and total carbohydrates for both *T. aestivum* leaves and *C. vulgaris* microalga were estimated as reported by Lowry *et al*. (1951) and Dubois *et al*. (1959), respectively. The lipid content of *C. vulgaris* has been determined by Bligh and Dyer method, (1959).

Determination of lipid peroxidation and hydrogen peroxide content

The level of lipid peroxidation in *T. aestivum* was determined using the thiobarbituric acid (TBA) assay, which measures the malondialdehyde (MDA) content as a byproduct of the lipid peroxidation reaction. MDA concentration had been evaluated using a spectrophotometer (T80+, PG Instruments Limited, Leics, United Kingdom) and premeditated exhausting its extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968). The hydrogen peroxide content in *T. aestivum* was determined using the Velikova *et al*. (2000) method.

Antioxidant enzyme activity

Fresh leaves samples were extracted for antioxidant enzymes according to Azevedo Neto *et al*. (2006).

Catalase activity: Catalase action was assessed in accordance with the method of Beers and Sizer (1952), with some adjustments as explained by Azevedo Neto *et al*. (2006).

Guaiacol peroxidase activity: Guaiacol peroxidase activity was determined as explained by Urbanek *et al*. (1991). Enzyme activity was quantified by the quantity of tetraguaiacol produced employing its molar extinction coefficient (26.6 mM^{-1} cm^{-1}).

Superoxide dismutase activity: Superoxide dismutase activity was assessed as depicted by Giannopolitis and Ries, (1977) by assessing its capacity to block the photochemical reduction of nitroblue tetrazolium chloride (NBT).

TEM and EDX

The second leaf fragments from three treatments (T, W, and TW) were repaired as stated by the method depicted by Spurr, (1969). A diamond knife was used to cut ultra-thin pieces of leaves on an ultramicrotome (Leica EM UC6, Germany), which were then put on copper grids with 300 square mesh. The cell ultrastructure was seen and photographed using a transmission electron microscope at various magnifications. Also, A JEOL JSM-IT200 Scanning

Electron Microscope (SEM) (Tokyo, Japan) was used to conduct an energy-dispersive X-ray (EDX) study of the elemental distribution in the dried leaves at 0 to 12 keV.

Statistical analysis

Results are conferred as the mean of three replications in each treatment. All data has been analyzed for variance via IBM SPSS v.27.0 software. The differences between the means were compared with the least significant difference test at $p \leq 0.05$ (Steel and Torrie, 1980).

RESULTS

Wastewater characteristics

The characterization of the wastewater collected from the El-Tabia drain, the treated wastewater by *Chlorella vulgaris* after 14th days of culturing and the standards water limit for irrigation according to FAO/2016 are shown in Table 1. It was clear that, most of the values of wastewater and treated wastewater parameters (bicarbonate, total dissolved solids, electrical conductivity, calcium, potassium, sodium, magnesium, fluoride, nitrate, phosphate, sulfate, aluminium, chromium, cobalt, Iron, and manganese) were within the normal range for irrigation as set by FAO guidelines. Also, wastewater had the highest values of potassium (44 mg/l), cadmium (0.022 mg/l), copper (0.325 mg/l) and zinc (0.825 mg/l) in comparison with treated wastewater and FAO limit.

It was noticed that *C. vulgaris* decreased the concentrations of total dissolved solids, total phosphorus, chemical oxygen demand, calcium, potassium, magnesium, sodium, fluoride, chloride, nitrite, nitrate, phosphate and sulphate from 1684, 1.270, 101, 77.79, 44, 50.54, 400.0, 0.29, 667, 7.9, 1.4, 1.6 and 124 mg/l in wastewater sample to 2.370, 0.322, 88, 44.59, 27, 43.64, 385.0, 0.28, 0.28, 587, 0.2, 0.1, 0.2 and 109.4 mg/l, respectively. Also, As shown in Figure 2, *C. vulgaris* decreased the concentrations of aluminium, iron, manganese, cadmium, copper and zinc by 2.85, 7.14, 48.33, 50, 96.30 and 98.63%, respectively.

Growth measurements, Total carbohydrates, proteins and lipids of *Chlorella vulgaris*

C. vulgaris cell number and biomass concentrations are presented in Figures. 3,4. The measured maximal cell number and biomass of *C. vulgaris* cultured with 100% wastewater on the 12th day of culturing were 495×10^4 cells/ml and 1.207 mg/l, which are higher than those at Bold basal medium (163×10^4 cells/ml and 0.858 mg/l).

Data representing total carbohydrates, total lipids and total proteins of *C. vulgaris* cultured for 14 days in Bold's Basal medium and 100% wastewater under controlled batch culture conditions (43.7 µmol/m^2/s, 12:12 hrs. light/dark regime), were illustrated in Table 2. The maximum value of total carbohydrates (357.15 ± 0.06 mg/g dry weight), total proteins (212.57 ± 0.01 mg/g dry weight) and total lipids (322.98 ± 0.01 mg/g dry weight) was recorded at 100% wastewater.

Effect of different water sources on growth parameters and chlorophyll fluorescence of *Triticum aestivum*

As presented in Figure 5, Regarding the fresh weight and dry weight of both roots and shoots, there was a significant difference between plants that were irrigated with WW and those that were irrigated with TWW. Plant irrigated with TWW showed higher root fresh weight (by 31.8%), root dry weight (by 35%), shoot fresh weight (by 21%), shoot dry weight (by 39%) compared to plants irrigated with WW. However, there is no significant difference in the above-mentioned parameters was noticed among plants irrigated with TWW and TW. It was observed that chlorophyll fluorescence was greater in *T. aestivum* plants irrigated with treated wastewater compared to wastewater (Figure 6).

Effect of different water sources on protein content and total carbohydrates of *Triticum aestivum*

Algal treatment of wastewater induced a significant increase in the total protein and carbohydrate content of wheat plants by 40% and 32% respectively (Figure 7).

Effect of different water sources on lipid peroxidation and H_2O_2 contents of *Triticum aestivum*.

As shown in Figure 8, malondialdehyde and H_2O_2 contents decreased significantly in wheat plant irrigated with TWW by 57% and 64% respectively compared to plants irrigated with WW.

Effect of different water sources on catalase, guaiacol peroxidase and superoxide dismutase activities of *Triticum aestivum*

As presented in Figure 9, algal treatment of wastewater markedly affected the activity of antioxidant enzymes. Maximum values of catalase, guaiacol peroxidase and superoxide dismutase activities were recorded in wheat plants irrigated with

Table 1. Characterization of the wastewater and treated wastewater by *C. vulgaris*.

Parameters	Unit	Untreated Wastewater	Treated wastewater	FAO limit/ 2016
pH	-	9.12	9.80	6.5–8.4
Carbonate	(mg/l)	72	0.00	*
Bicarbonate	(mg/l)	53	204	600
Electrical Conductivity	(dS/cm)	2.630	2.370	3
Total Dissolved Solids	(mg/l)	1684	1514	2000
Total phosphorus	(mg/l)	1.270	0.322	*
Chemical Oxygen Demand	(mg/l)	101	88	*
Calcium	(mg/l)	77.79	44.59	400
Potassium	(mg/l)	44	27	2
Magnesium	(mg/l)	50.54	43.64	60
Sodium	(mg/l)	400.0	385.0	900
Fluoride	(mg/l)	0.29	0.28	1.0
Chloride	(mg/l)	667	587	0–1100
Nitrite	(mg/l)	7.9	0.2	*
Nitrate	(mg/l)	1.4	0.1	10
Phosphate	(mg/l)	1.6	0.2	2
Sulfate	(mg/l)	124	109.4	1000
Aluminum	(mg/l)	0.035	0.034	5
Cadmium	(mg/l)	0.022	0.011	0.01
Chromium	(mg/l)	ND	ND	0.10
Cobalt	(mg/l)	ND	ND	0.05
Copper	(mg/l)	0.325	0.012	0.20
Iron	(mg/l)	0.014	0.013	5
Manganese	(mg/l)	0.060	0.031	0.20
Zinc	(mg/l)	0.825	0.014	2.0

ND: Not detected; *: indicates data unattainability

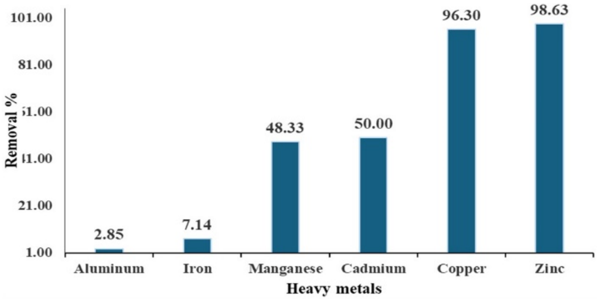

Figure 2. Removal percentage of heavy metals (aluminium, iron, manganese, cadmium, zinc and copper) from 100% wastewater using *C. vulgaris*.

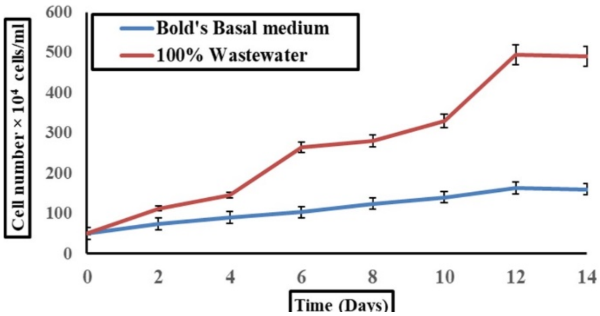

Figure 3. Cell number of *C. vulgaris* cultured with Bold's Basal medium and 100% wastewater under controlled batch cultured conditions.

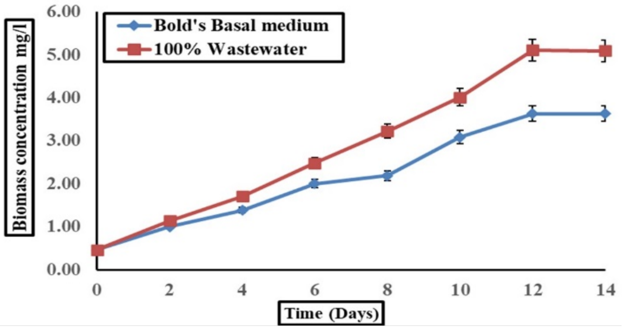

Figure 4. Biomass concentration (mg/l) of *C. vulgaris* cultured with Bold's Basal medium and 100% wastewater under controlled batch cultured conditions.

WW, followed by TWW irrigated plants, whereas the minimum values were recorded in plants irrigated with TW.

Effect of different water sources on ultrastructure of *Triticum aestivum* leaves

TEM micrograph of wheat leaves is presented in Figure 10. Leaf mesophyll cells of wheat plants irrigated with TW or TWW have defined cell wall, continuous cell membrane and well -developed nuclear envelope (Figure 10 A,E,D). In contrast, plants irrigated with WW exhibited observed changes in

Table 2. Total carbohydrates, proteins and lipids content (mg/g dry weight) of *C. vulgaris* cultured in 100% wastewater and Bold's Basal medium under controlled batch culture conditions.

	Total carbohydrates	**Total proteins**	**Total lipids**
Bold's Basal medium	122.16 ± 0.05	63.58 ± 0.01	99.01 ± 0.01
100% wastewater	357.15 ± 0.06	212.57 ± 0.01	322.98 ± 0.01
F-values	2748*	8283*	7523*

Values represent means ± SD, *= significant difference at P< 0.05.

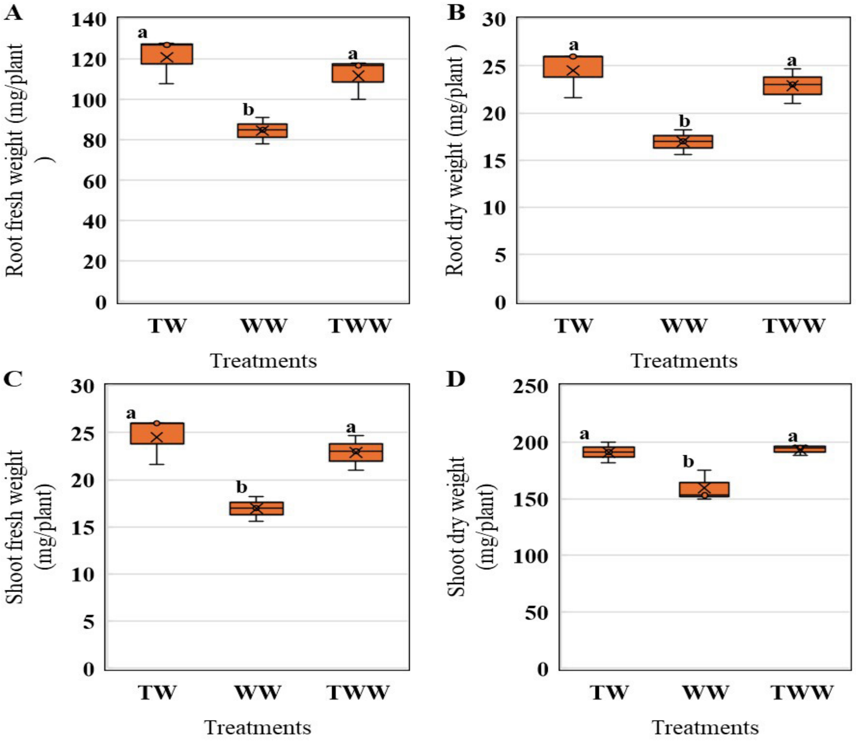

Figure 5. Effect of different water sources on root fresh weight (A), root dry weight (B), shoot fresh weight (C) and shoot dry weight (D) of *Triticum aestivum*. TW: tap water; WW: untreated wastewater; TWW: treated wastewater. A significant difference at P<0.05 was denoted by different letters on the bars.

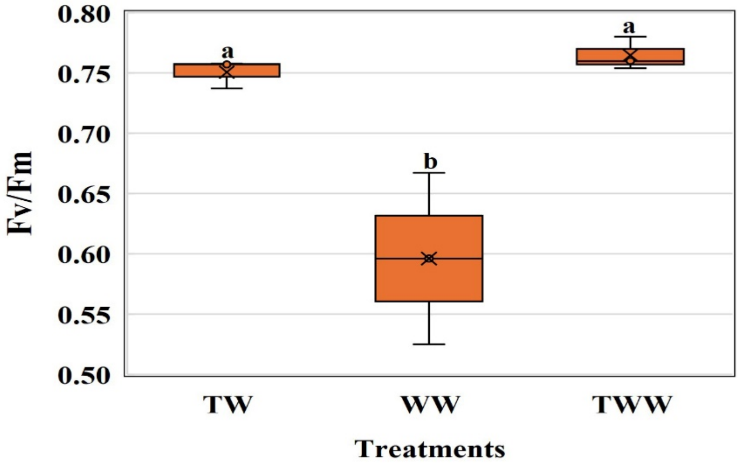

Figure 6. Effect of treated wastewater on chlorophyll fluorescence of *Triticum aestivum*. TW: tap water; WW: untreated wastewater; TWW: treated wastewater. A significant difference at P<0.05 was indicated by different letters on the bars.

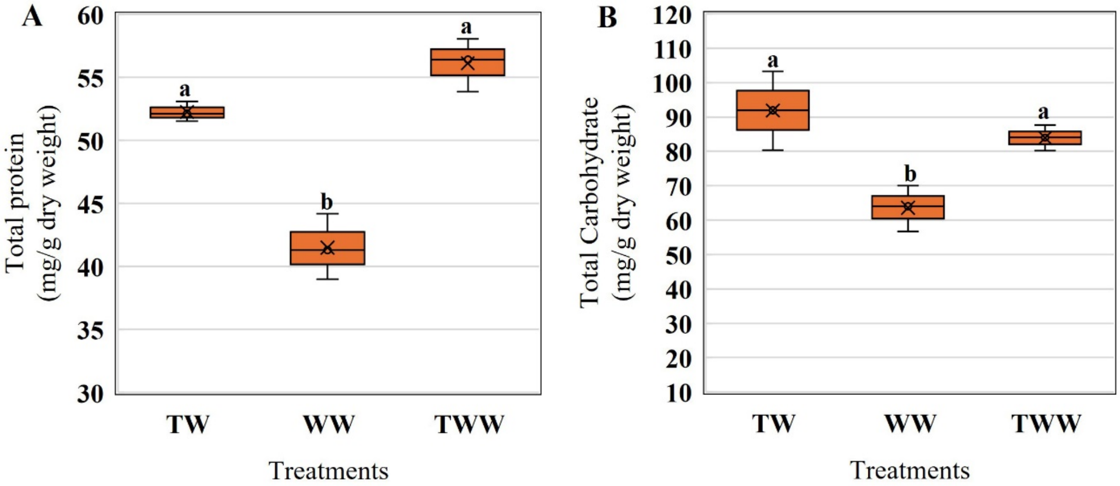

Figure 7. Effect of different water sources on protein content (A), total carbohydrates (B) of *Triticum aestivum*. TW: tap water; WW: untreated wastewater; TWW: treated wastewater. A significant difference at P<0.05 was indicated by different letters on the bars.

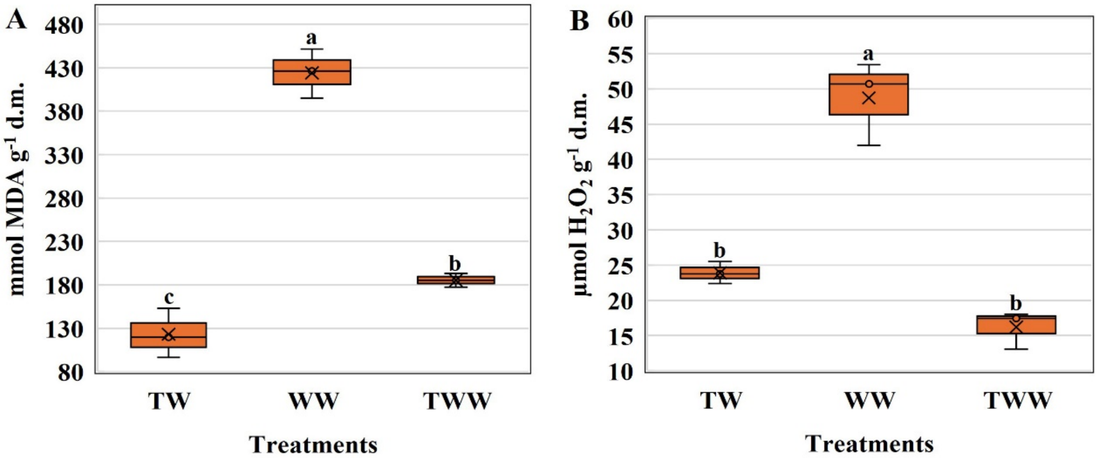

Figure 8. Effect of different water sources on lipid peroxidation (A), H_2O_2 content (B) of *Triticum aestivum*. TW: tap water; WW: untreated wastewater; TWW: treated wastewater. A significant difference at P<0.05 was indicated by different letters on the bars.

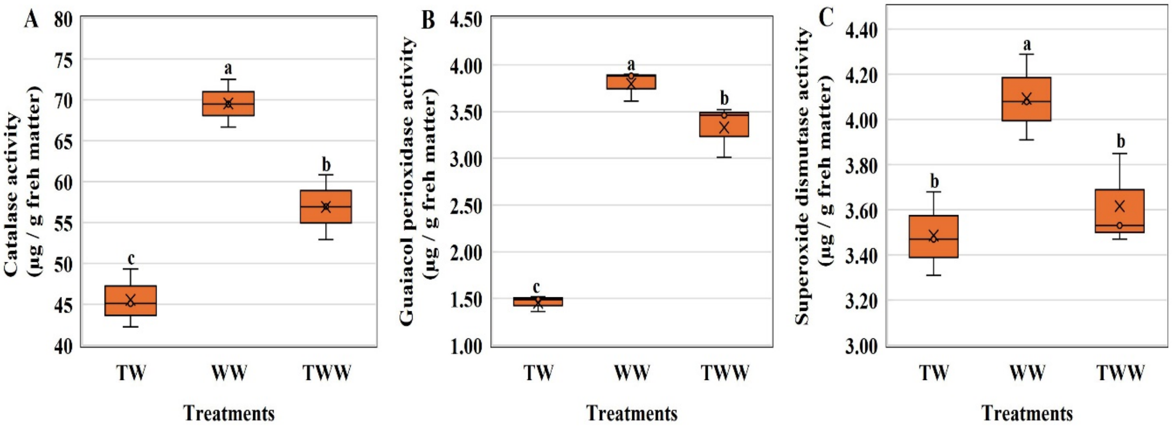

Figure 9. Effect of different water sources on catalase (A), guaiacol peroxidase (B) and superoxide dismutase (C) activities of *Triticum aestivum*. TW: tap water; WW: untreated wastewater; TWW: treated wastewater. A significant difference at P<0.05 was indicated by different letters on the bars.

their ultrastructure (Figure 10I). leaves of plant irrigated with WW have abnormal spherical chloroplast with disrupted thylakoid membranes (Figure 10 I,J,K), whereas plants irrigated with TW or TWW have typical elliptical chloroplast and well-organized thylakoid membranes (Figure 10 B,F).

EDX

Figure 11 depicts the normal EDX pattern for leaves of *Triticum aestivum* plant irrigated with tap water (A), treated wastewater (B) and wastewater (C). This EDX pattern shows the presence of C, O, and N signals. In addition, a signal of Si ion, which increased obviously in plant irrigated with wastewater (C). Furthermore, the EDX pattern for the leaf that irrigated with tap water and treated wastewater did not display the characteristic signal of Cu, however the plant that was irrigated with wastewater showed three signals of the Cu ion.

DISCUSSION

The farmers in Egypt have been forced to reuse untraditional water sources for irrigation purposes due to limited freshwater and the growing population. However, a precise assessment of such water quality is necessary to avoid potential hazards as the contaminants in the wastewater may reach the food chain and cause serious health complications if not treated safely. (Cherfi *et al*., 2015; Abbas *et al*., 2020). Many researchers investigated the efficiency of treated wastewater for crop irrigation (Alkhamisi *et al*., 2011; Bozdogan, 2015; Elfanssi *et al*., 2018; Reda *et al*. 2020; Rápalo-Cruz *et al*., 2024). One of the most promising technologies for wastewater treatment is using microalgae (Luo *et al*., 2016; Putri and Huang, 2020). Currently, 80% of vegetable crops are irrigated with treated wastewater (Funmilola *et al*., 2019).

Several investigations have shown that *Chlorella* sp. can effectively eliminate nutrients (e.g., N and P) and organic contaminants from assorted resources of wastewaters, particularly when processed via a settling process, an activated sludge method, or just dilution with water or culture media (Wang *et al*., 2010; Kadir *et al*., 2018; Kumar *et al*., 2019; Plohn *et al*., 2021; Kong, *et al*., 2021). In this work, *C. vulgaris* removed total phosphorus, calcium, potassium, magnesium, sodium, fluoride, chloride, nitrite, nitrate, phosphate and sulphate by 74.6, 42.6, 38.6, 3.7, 3.4, 11.9, 97.4. 98.2, 87.5 and 11.7%, respectively.

Chlorella has been evaluated for the removal of heavy metals and a variety of other metals from wastewater, such as Al, Cd, Cu, Fe, Mg, Mn, Ni, Ur, and Zn (Mehta and Gaur, 2005; Leong and Chang, 2020; Spain *et al*. 2021; La Bella *et al*., 2022). The biosorption of metals with *Chlorella* encompass chiefly electrochemical adsorption of metal ions onto the cell surface through definite intracellular molecules such as phytochelatins, biochemical ligands, and metallothioneins accompanying cell walls and cytoplasmic membranes (Mehta and Gaur, 2005). According to our results, *C. vulgaris* removed the concentrations of aluminum, iron, manganese, cadmium, copper and zinc by 2.85, 7.14, 48.33, 50, 96.30 and 98.63%, respectively. The values of these elements were within the permissible range accepted by FAO (FAO, 2016).

In this work, the improvement in growth parameters (cell number and biomass) in *C. vulgaris* cultured with 100% wastewater might be attributable to the presence of bicarbonate, nitrate, nitrite and phosphorus in the wastewater. It was shown that *C. vulgaris* utilizes dissolved CO_2 and bicarbonate ions metabolized by carbonic anhydrase into CO_2 as carbon sources for photosynthesis and utilizes phosphorus and nitrogen for metabolic activity. Also, the high rate of microalgal biosynthesis directs to increase biomass (Molazadeh *et al*., 2019; Singh *et al*., 2019; Ñañez *et al*., 2024).

Since carbon is utilized by microalgae for the biosynthesis of lipids and carbohydrates, its assimilation plays a crucial role for energy storage compounds (Chang *et al*., 2018; Hernández-García *et al*., 2019). Also, when microalgal cells undergo nitrogen limitation, they undergo a metabolic shift from protein synthesis to carbohydrate synthesis (Kusmayadi *et al*., 2024). Accordingly, the current investigation demonstrated that a higher removal of carbonate, nitrite, and nitrate from the wastewater may be responsible for a boost in total carbohydrates and lipids more than protein levels in *C. vulgaris* grown in 100% wastewater.

The result of this study revealed that there is a significant improvement in the biomass, total protein and carbohydrate contents of wheat plants when irrigated with TWW compared to WW and at a level approaching the biomass of plants irrigated with TW. Previous research has demonstrated that treated wastewater improved plant growth and yield of several plants, including sunflower (Moazzam-Khan *et al*., 2009), olive (Bedbabis *et al*., 2010; Tekaya *et al*., 2016), tomatoes (Cirelli *et al*. ,2012; Jahan *et al*., 2019), rice (Alghobar and Suresha, 2016), oat (Moradi *et al*., 2016), broad bean (Shannag *et al*., 2021) and

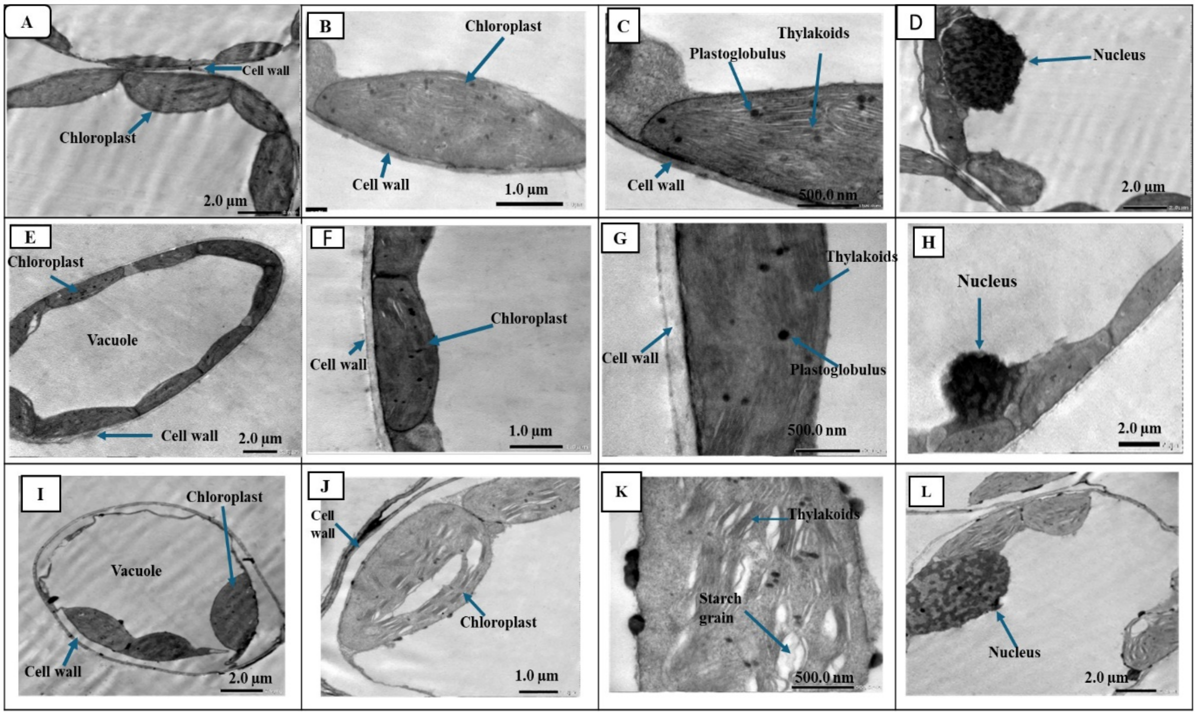

Figure 10. Transmission Electron Microscope (TEM) micrograph of *Triticum aestivum* leaves irrigated with tap water (A, B, C & D), treated wastewater (E, F, G &H) and untreated wastewater (I, J, K & L).

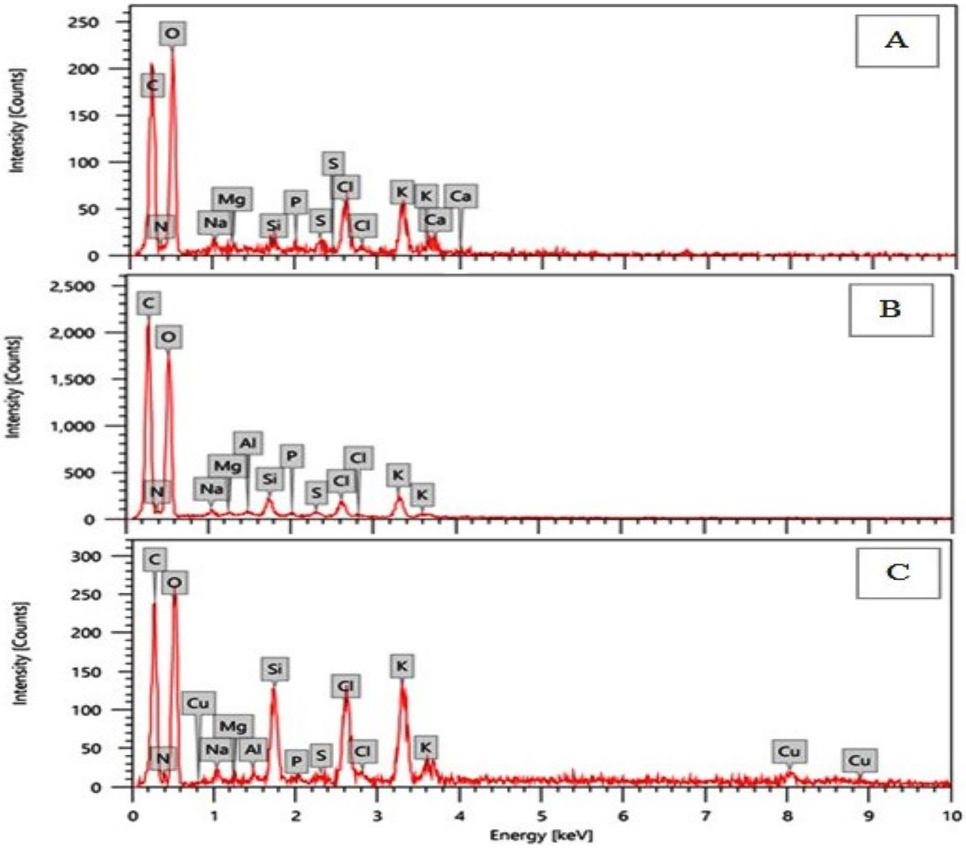

Figure 11. Energy dispersive X-ray (EDX) analysis of *Triticum aestivum* leaves irrigated with tap water (A), treated wastewater (B) and untreated wastewater (C).

barely (Alvarez-Holguin, *et al*., 2022). These can be explained by the presence of valuable nutrients in the treated wastewater, which are reflected in crop yield (Aghtape *et al*., 2011; Becerra-Castro *et al*., 2015; Reda *et al*., 2020). These results run in contrary to those of Mkhinini *et al*. (2018) who found that plants grown under freshwater irrigation had a higher biomass than those grown under treated wastewater irrigation.

In the recent study, the plants that were irrigated with TWW had the highest value of chlorophyll fluorescent, followed by those that were irrigated with TW and finally, WW. Parallel results are noted by Tekaya *et al*. (2016) who found that the use of TWW in the irrigation of olive trees resulted in a significant increase in stomatal conductance, chlorophyll fluorescence and the photosynthetic rate. In addition, Oyiga *et al*. (2016) and Hajihashemi *et al*. (2020) observed that irrigation of wheat plants with wastewater leads to a significant reduction in chlorophyll fluorescence. The low value of chlorophyll fluorescence observed for plants irrigated with WW in the present study could be related to stress. Previously Shu *et al*. (2012) and Song *et al*. (2013) found that plants under stress showed lower values of chlorophyll fluorescence. These findings were consistent with the results of leaf ultrastructure as the plants irrigated with WW revealed alternation in the shape of chloroplast and drastic changes in thylakoid membranes.

This study showed that the activities of catalase, guaiacol peroxidase and superoxide dismutase as well as malondialdehyde and H_2O_2 contents were significantly higher in plants irrigated with WW compared to those irrigated with TW or TWW. This could be related to higher level of heavy metals in wastewater (Kalavrouziotis *et al*., 2012). Comparable results were notified by Hashem *et al*. (2013) who recorded a significant increase in the activity of antioxidant enzymes in turnip, lettuce, and tomato plants when irrigated with wastewater. It is well known that the presence of high levels of heavy metals in irrigation water leads to oxidative damage (Shi *et al*., 2010) and altered metabolisms (Liang *et al.* 2007). As reported in several research, heavy metal stress causes drastic changes in the action of antioxidant enzymes including guaiacol peroxidase, catalase and superoxide dismutase (Cho *et al*., 2000; MacFarlane *et al*., 2001; Sai Kachout *et al*., 2009). In this work, the lower activities of antioxidant enzymes in the algal treated wastewater could be attributed to the efficiency of *C. vulgaris* in the removal of heavy metals from WW.

CONCLUSION

This study shows that using the microalgae *Chlorella vulgaris* for wastewater treatment is an eco-friendly method for removing nutrients and some heavy metals from the wastewater in the study area. Consequently, the treated wastewater after the removal of microalgal biomass may be a good choice for using in irrigation of *Triticum aestivum* L., cv. Masri 3 plant, according to FAO/2016 guidelines for irrigation. This is confirmed by enhancing the growth parameters and chlorophyll fluorescence, proteins and carbohydrates of *T. aestivum* plant when irrigated with treated wastewater by *C. vulgaris*. This study may contribute to the resilience of water resources and agricultural practices in Egypt by using *C. vulgaris* -treated wastewater in crop irrigation. Nevertheless, it is crucial to investigate the impact of crops irrigated with wastewater on human health.

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الإدارة المستدامة للمياه لزراعة نبات القمح باستخدام مياه الصرف الصحي المعالجة باستخدام طحلب الكلوريلا فولجارس

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الملخص العربي

تسعى هذه الدراسة إلى تحقيق بعض أهداف الأمم المتحدة في مجال التنمية المستدامة، ولا سيما الإجراءات المتعلقة بالمناخ والمياه النقية والصرف الصحي. ومع زيادة اثار التغير المناخي، أصبح نقص المياه يشكل تحديا في جميع أنحاء العالم .وتماشياً مع ذلك، هناك اتجاه عالمي متزايد نحو إعادة استخدام المياه المستعملة. ولذلك كان الهدف من هذه الدراسة هو التحقق من كفاءة طحلب كلوريلا فولجاريس في إزالة المغذيات وبعض المعادن الثقيلة من مصرف العامية في منطقة الطابية بالإسكندرية، مصر، والاستخدام المحتمل لمياه الصرف الصحي المعالجة بالطحلب في ري نبات القمح صنف مصري 3 . وقد أظهرت النتائج قدرة الطحلب المستخدم من تنقية مياه الصرف الصحي من الألمونيوم بنسبة 2,85 % و 7,14 من الحديد و48,33 % من المنغنيز، و50 % من الكادميوم، و96,30 % من النحاس، و98,63 % من الزنك. تم ري نباتات القمح بمياه صرف غير معالجة، أو مياه صرف معالجة باستخدام الطحلب أو مياه حنفية .وأظهرت النباتات المروية باستخدام الطحلب تحسناً في الكتلة الحيوية للمجموع الخضري (39%)، والبروتين (40 %)، والكربوهيدرات (32 %)، وتحسن في التركيب التشريحي الدقيق للبلاستيدات الخضراء في ورقة نبات القمح، في حين انخفض نشاط انزيم الكتاليز(18%)، والبيروكسيديز(213 %)، سوبر أكسيد ديسميوتاز (11.7 %)، والمالونديالدهيد (57 %)، ومحتويات فوق أكسيد الهيدروجين (64 %) مقارنة بالنباتات المروية بمياه الصرف الصحي . إجمالاً، تشير هذه الدراسة إلى إمكانية استخدام مياه الصرف المعالجة بالطحالب في ري المحاصيل التي يمكن أن تكون استراتيجية مستدامة للاستفادة من تلك المياه. ومع ذلك، يعتبر تقييم مخاطر استخدام مياه الصرف الصحي المعالجة على الصحة العامة أمرًا مقلقًا للغاية.