Environmental Science and Pollution Research (2024) 31:14043–14058
https://doi.org/10.1007/s11356-024-31869-2

RESEARCH ARTICLE

Eco-friendly approach to decrease the harmful effects of untreated wastewater on growth, yield, biochemical constituents, and heavy metal contents of carrot (*Daucus carota* L.)

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Received: 6 June 2023 / Accepted: 2 January 2024 / Published online: 25 January 2024

Abstract

Here, the impact of irrigation using untreated wastewater (WW) on carrots (*Daucus carota* L.) was examined. We hypothesized that the addition of ethylenediaminetetraacetic acid (EDTA), dry algal powder (*Spirulina platensis* or *Chlorella vulgaris*), and *Salix alba* leaves powder would function as chelators for harmful contaminants in wastewater. The findings showed that irrigation of carrot plants with the sampled untreated wastewater led to significant decreases in the shoot lengths, fresh, dry weights of shoots and roots at stage I, the diameter of roots, pigment content, carotenoids, total soluble carbohydrate content, and soluble protein content. Furthermore, a significantly increased level of proline, total phenols, and the activities of polyphenol oxidase (PPO), peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT) was identified in stage I samples. In contrast to the stage I, the length of the roots, the number of leaves on each plant, wet and dry weights of the stage II roots were all greatly enhanced. In spite of the increased yield due to the wastewater irrigation, carrot roots irrigated with wastewater had significantly more cadmium (Cd), nickel (Ni), cobalt (Co), and lead (Pb) than is considered safe. Our data clearly show that the application of *Spirulina platensis*, *Chlorella vulgaris*, EDTA, and leaves powder of salix was able to alleviate the toxicity of wastewater on carrot plants. For example, we recorded a significant decrease in the accumulation of carrot's Cd, Ni, Co, and Pb contents. We conclude that the treatments with *Spirulina platensis* and *Chlorella vulgaris* can be utilized as eco-friendly tools to lessen the damaging effects of wastewater irrigation on carrot plants.

Keywords Wastewater irrigation · Heavy metals · Carrot · Algae · Salix · EDTA

Responsible Editor: Gangrong Shi

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Introduction

Carrot (*Daucus carota*) is the most common root vegetable in the Apiaceae family, and it is one of the top ten vegetable crops worldwide. Carrot roots are commonly used in cooking owing to their high content of carotenoid pigments, dietary fiber, vitamin A, vitamin C, and a variety of other vitamins and minerals (El-Batal et al. 2023; Que et al. 2019). Egyptian farmers usually encounter a shortage of irrigation water. Moreover, many sewage stations are located very close to agricultural lands, which forces farmers to use that water that is not treated at all for irrigation whenever they have a deficiency in water supply (Al-Zou'by et al. 2017; Fouda et al. 2021b; Selim et al. 2021). Africa has experienced long-term variations in rainfall of various lengths and intensities (Amarteifio et al. 2006). Non-traditional supplies are frequently employed to meet the rising demand for irrigation water (El-Sersawy et al. 2021; Mapanda et al. 2005). In addition, because of urbanization and increased population, there is a greater water demand, which makes these resources exceedingly limited. On the other hand, large amounts of wastewater provide an issue along with the tremendous water use (Amarteifio et al. 2006; Fouda et al. 2022). Wastewater has a wide range of elements that are affected by various factors including the municipal water supply's composition, the type of added water, and the level of received treatment. In Egypt, many farmers were forced to use wastewater to irrigate their crops, due to a lack of freshwater (Nowwar et al. 2023). Some authors reported that at least 240,500 feddans in the North Delta are irrigated using effluent from the Al-Gharbiyyah Al-Raisi drain (Antar et al. 2012). Also, wastewater from the Bahr El-Baqar drain, which is situated in the eastern portion of Egypt's Nile Delta, is used to irrigate roughly 800,000 feddans (Abdelrazek 2019).

Salts, algae, pathogenic bacteria, viruses, oxygen-demanding items, sediments, grease, oil, scum, pesticides, and heavy metals are common components of domestic wastewater (Hamza et al. 2021). Turbidity, suspended particles, total dissolved solids, acidity, and dissolved oxygen are some of the properties used to describe wastewater (Farid et al. 1993). Uncontrolled wastewater irrigation can lead to the buildup of heavy metals in the soil and may negatively impact plant growth (Malik et al. 2004). As a result, through plants' absorption, contamination of the food chain may occur. Also, the plants have the potential to accumulate these metals in hazardous amounts in a variety of edible plant components, which is dangerous for human health (Nzediegwu et al. 2019; Saied et al. 2021).

Therefore, a lot of work should be directed toward developing new environment-friendly, inexpensive, and user-friendly techniques to minimize the accumulation of these toxic pollutants in crops. Hence, we can assure safe crops and the environment. In this regard, both living and dead algal biomass can be employed as heavy metal adsorbents (Alharbi et al. 2022). Nevertheless, many studies have indicated that chelating metals using dead algae may be more successful and more cost-efficient than employing live cells (Pham et al. 2021). Moreover, the chelation of heavy metals during wastewater treatment was frequently accomplished using dried plant materials (Albdaiwi et al. 2022). Many bioactive and biostimulant substances can be found in the dried salix (*Salix alba*) materials. The major and most important compound of salix is the plant hormone salicylic acid (SA). Plants that live in challenging environments are thought to benefit physiologically from SA (Gupta et al. 2017). EDTA as a synthetic chemical is the most efficient for heavy metal removal (Nowwar et al. 2022, Oh and Yoon 2014).

This study was carried out to evaluate whether applying dry algae powder (*Spirulina platensis* or *Chlorella vulgaris*), *Salix alba* leaf powder, and ethylenediaminetetraacetic acid (EDTA) might lessen the harmful effect of wastewater on carrot plants. Following wastewater irrigation and the application of different treatments, morphological, physiological, biochemical, and yield parameters were measured, as well as the estimated heavy metal uptake and accumulation in carrot root (edible part).

Materials and methods

Materials

Daucus carota L. seeds (Chantenay cultivar) and *Salix alba* leaves were supplied by the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. We bought algae from the National Research Centre in Giza, Egypt. The leaves of *S. alba* were air-dried (32 °C) till a constant dry weight and were crushed to a fine powder.

Sampling of wastewater

For irrigation of carrot plants with contaminated water, samples from the El-Rahawy drainage station in Giza, Egypt (30°11′12.3″ N, 31°02′53.3″ E) were used. This station takes the sewage from the governorate of El-Giza as well as household and agricultural waste from the community of El-Rahway. Without any post-collection treatment, wastewater samples were utilized for irrigation. The positive control was fresh tap water.

Planting, treatments, and plant sampling

The pot experiment was conducted in the botanical garden of Al-Azhar University, Faculty of Science, Botany and Microbiology Department. This study was crumbled block a completely randomized design replicates. Carrot seeds were sown in pots that contained clay soil. While we were investigating the stress-relieving potential of various soil additions, we decided the optimal dose would be 3 g/kg of additive soil. Pots were then divided into six different groups (each group has five replicates) as follows: In the first group, seeds were grown using fresh tap water (control) and the soil has no supplements. In the second one, seeds were grown using wastewater (WW) with no soil supplement. Third, seeds were grown using wastewater while the soil was supplemented with dried *Spirulina platensis* powder (WW + Sp). Fourth, seeds were treated with wastewater while the dried *Chlorella vulgaris* powder was added to the soil (WW + Ch). Fifth, wastewater was used while the *Salix alba* leaves powder was added to the soil (WW + SA). In the sixth group, a combination of wastewater and ethylenediaminetetraacetic acid (WW + EDTA) was applied. For a period of 6 weeks, the developed plants were irrigated with fresh tap water (control) and wastewater collected at a rate of 1000 mL per pot every 5 days. Until the experiment's conclusion, the watering intervals were then raised to occur every 8 days. During the trial, three fertilizations were carried out 14, 28, and 35 days following planting. Each pot received 240 mg dm^{-3} of $MgNO_3$ and 250 mg dm^{-3} of KNO_3 during the first two fertilizations. Per pot, 100 mg dm^{-3} of urea was sprayed at the most recent fertilization. The equal amount of fertilizer in the form of nutrient solution was applied to each pot. When the plants were 90 (stage I) and 110 (stage II) days old, samples were taken. The roots that were harvested) yielded roots) from each group at the end of the growing season (181 days) were evaluated for their quality.

Chemical analysis

Wastewater analysis

Total dissolved solids (TDs), electrical conductivity (EC), and pH of wastewater and fresh tap water were all measured using a pH/electric conductivity meter (914 pH/Conductometer Metrohm AG). After that, water samples were immediately acidified with diluted HNO_3, preparing them for different element analyses with an atomic absorption spectrophotometer (PerkinElmer 3100) (Rice et al. 2012). The concentration of potassium (K) was measured using a flame photometer, CORNING M410, while the concentration of phosphorus (P) was calculated using the molybdenum blue method and measured at 700 nm using a spectrophotometer (UNICO Vis Model 1200, USA) (Allen et al. 1974). On the other hand, ammonia–nitrogen (NH_3-N) concentrations were estimated calorimetrically by using the nesslerization method (Golterman 1991). In the following step, 20 mL of the test solution was well combined with 1 mL of sodium salicylate solution; then, 1 mL of Nessler's reagent was added, and the mixture was stirred. After 15–30 min, the generated color was detected at 420 nm after the reaction had taken place. Titrimetric analysis was used to quantify the chemical oxygen demand (COD), whereas the 5-day biochemical oxygen demand approach was utilized to estimate

Table 1 Chemical and biological properties of irrigation waters

Water characters	Water sample type	
	Fresh water	Wastewater
pH	7.11 ± 0.17	7.33 ± 0.21
EC (dS/m)	3.71 ± 0.09	11.12 ± 1.02
TDS (ppm)	2372.5 ± 31.1	7113.9 ± 33.1
COD (mg/L)	5.35 ± 1.22	416.90 ± 2.21
BOD (mg/L)	2.10 ± 0.09	182.60 ± 2.11

Data represented by the means of three replicates ± standard error (n = 3, ± SE). WHO Standard Limits: pH (6.5–8.5), TDS (≤ 2000), COD (≤ 10), BOD (≤ 6) (Organization 2022)

Table 2 Chemical analysis of irrigation waters

Water characters		Water sample type	
		Fresh water	Wastewater
NH_3-N (mg/L)		7.11 ± 0.17	7.33 ± 0.21
Cations (meq/L)	Ca^{2+}	7.15 ± 1.04	4.75 ± 0.08
	Mg^{2+}	5.28 ± 3.01	4.03 ± 0.10
	K^+	0.18 ± 0.03	0.15 ± 0.04
	Na^+	25.03 ± 4.01	102.17 ± 0.31
Anions (meq/L)	Cl^-	26.68 ± 6.04	106.83 ± 1.21
	CO_3^{2-}	0	0
	HCO_3^-	0.66 ± 0.22	0.86 ± 1.02
	SO_4^{2-}	9.74 ± 1.11	3.41 ± 0.11
Macro and micro-nutrients (mg/L)	P	5.29 ± 0.09	5.59 ± 0.13
	S	7.35 ± 0.06	36.28 ± 1.05
	Fe	0.13 ± 0.08	0.14 ± 0.03
	Zn	0.01 ± 0.01	0.02 ± 0.01
	Cu	0.04 ± 0.01	0.36 ± 0.11
	Mn	0.10 ± 0.03	0.31 ± 0.08
Heavy metals (mg/L)	Ni	0.06 ± 0.01	0.63 ± 0.12
	Cd	0.03 ± 0.01	0.37 ± 0.07
	Pb	0.02 ± 0.01	0.34 ± 0.10
	Co	0.06 ± 0.02	0.54 ± 0.16

Data represented by the means of three replicates ± standard error (n = 3, ± SE). WHO Standard Limits: NH_3-N (6.5–8.5), Fe (0.3), Zn (5.0), Cu (2.0), Mn (0.08), Ni (0.07), Cd (0.003), Pb (0.01), and Co (0.05) (Organization 2022) the biochemical oxygen demand (BOD) (Delzer and McKenzie 2003) (Table 1 and 2).

Soil chemical analysis

The soil samples were then exposed to air drying before being digested by acid mixtures at a temperature of 80 °C for 8 h at a ratio of 5:1:1 v/v/v $HNO_3/H_2SO_4/HClO_4$ (Wade et al. 1984). To filter the translucent digests, Millipore cellulose nitrate membrane filter paper with a 0.45-µm pore size was used after mixing the digests with dis. H_2O for up to 50 mL. The quantity of solubilized cations, anions, and heavy metals was determined using the absorption spectrophotometer (PerkinElmer 3100 Atomic). Using a spectrophotometer, nitrogen was measured at 660 nm and phosphorus at 700 nm. Using the molybdenum blue method and the Kjeldahl technique, the concentrations of total soluble nitrogen (N) and phosphorus (P) were measured, respectively (Allen et al. 1974). To measure potassium (K), the flame photometer was used (CORNING M410) (Allen et al. 1974). In order the purpose of measuring the pH of the soil (1 g soil/5 mL water), TDs, and EC, a pH/electric conductivity meter (914 pH/Conductometer Metrohm AG) was used (Page et al. 1982). According to the WHO Guidelines, the maximum permissible levels for various metal ions in soils were tested (Organization) 2022) as presented in Table 3 and 4.

Plant material analysis

Morphological analysis

The length of shoots and roots for eight different individual plants were investigated in centimeter; leaf numbers were also counted for the same group of plants (Amin et al. 2021). Following clipping, plants in each treatment were weighed for fresh weight determination of both shoots and roots (estimated as g/plant). Plant materials were dried at 70 °C till they reached the constant dry weight. After that, the dry weight was recorded as the final weight (Abdel-Hamid et al. 2021).

Table 3 Chemical and biological properties of soil sample

Soil characters	Soil sample
Texture	Clay
pH	7.14 ± 1.01
EC (dS/m)	11.42 ± 1.20
TDS (ppm)	7304.00 ± 3.19

Data represented by the means of three replicates ± standard error (*n* = 3, ± SE)

Table 4 Chemical analysis of soil sample

Soil characters		Soil sample
Cations (meq/L)	Ca^{2+}	5.26 ± 0.12
	Mg^{2+}	1.41 ± 1.21
	K^{+}	0.31 ± 0.11
	Na^{+}	107.15 ± 2.09
Anions (meq/L)	Cl^{-}	107.82 ± 3.41
	CO_3^{2-}	0
	HCO_3^{-}	1.83 ± 1.22
	SO_4^{2-}	4.48 ± 0.43
Macro and micro-nutrients (mg/kg)	**N**	24.40 ± 1.41
	P	21.07 ± 1.33
	S	39.01 ± 2.20
	Fe	0.47 ± 0.19
	Zn	0.14 ± 0.09
	Cu	1.51 ± 0.21
	Mn	0.43 ± 0.11
Heavy metals (mg/kg)	**Ni**	0.22 ± 0.14
	Cd	0.01 ± 0.01
	Pb	1.02 ± 0.13
	Co	0.04 ± 0.01

Data represented by the means of three replicates ± standard error (*n* = 3, ± SE). WHO Standard Limits: Fe (7.0), Zn (50), Cu (100), Mn (20), Ni (5.0), Cd (0.7), Pb (50), and Co (65) (Organization 2022)

Physiological and biochemical analyses

Chlorophyll and carotenoid contents The pigment content of fresh carrot leaves was determined using a method described before (Ismail et al. 2021; Lightenthaler 1987). Briefly, 1 g of fresh leaves was crushed in a mortar with 80 mL (80% v/v) acetone with 0.5 g calcium carbonate, then centrifuged for 5 min at 8000 rpm and filtered. The filtrate was transferred to a volumetric flask and diluted to 100 mL with acetone (80%). The optical density of the extracted compounds was evaluated using a spectrophotometer (Jasco model V-530, Tokyo, Japan) at 645, 663, and 470 nm for chlorophyll a, chlorophyll b, and total chlorophyll, and 440 nm for carotenoids. The pigment content was calculated in mg/g FW.

Carbohydrate and protein contents Using anthrone-sulfuric acid, total soluble carbohydrates were calculated using UV spectrophotometry (UNICO Vis Model 1200, USA) in accordance with the methodology of (Umbreit et al. 1957). Total soluble protein contents were measured with a UV spectrophotometer (UNICO Vis Model 1200, USA) and determined using the Bio-Rad protein assay, following (Lowry 1951) instructions.

β-Carotene contents The concentrations of β-carotene contents were calculated using the method that was given by Nagata and Yamashita (1992)**.** For that, 1 g of fresh carrot root was homogenized (IKA-WERKE T10 Basic) for 2 min to a homogeneous mass using 10 mL of an acetone-hexane mixture (2:3) followed by centrifugation at 5000 rpm for 10 min at 20 °C. The absorbance of the clear supernatant was determined at 453, 505, 645, and 663 nm using the UV/VIS spectrophotometer (Cary 50 Scan). The following equation was used to calculate the amount of β-carotene (Nagata and Yamashita 1992):

$$\beta - carotene(mg/100mL) = (0.216 \times A_{663}) - (1.22 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453}) \quad (1)$$

where A = absorbance.

Free proline

The concentrations of free proline were calculated using the method that was given by Bates et al. (1973). Using this method, 10 mL (3%) sulfosalicylic acid was used to homogenize 0.5 gm dry plant material. Whatman No. 2 filter paper was used to filter the homogenate. Two milliliters of the filtrate was subjected to a 1-h reaction with 2 mL of glacial acetic acid in a test tube submerged in boiling water. The ninhydrin was prepared by heating 1.25 g in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid, stirring until it dissolved and then allowed to cool. An cold bath marked the end of the reaction. Four milliliters of toluene was added to the reaction mixture, and it was rapidly stirred for 15 to 20 s using a test tube stirrer. Using a UV spectrophotometer (UNICO Vis Model 1200, USA), the toluene-containing chromophore was removed from the aqueous phase and heated to room temperature, and the absorbance was measured to 520 nm. Proline content was given in milligrams per gram of dry mass.

Total phenols The total phenolic compounds (mg 100 g^{-1} of dry wieght) were determined using the method described by Diaz and Martin (1972) as follows: After being extracted for at least 24 h at 0 °C using 1 g of dried defeated ground leaves in 5–10 mL of 80% ethanol, the alcohol was purified. Three times, 5–10 mL of 80% ethanol was used to remove the leftover residue. Using 80% ethanol, the cleared extract was eventually finished to a volume of 50 mL. In a dry test tube, an aliquot of 0.5 mL of the prior extract and 0.5 mL of Folin-Denis reagent were well combined, and the tube was vigorously shaken for 3 min. After thoroughly mixing 1.0 mL of the 35% saturated Na_2CO_3 solution, 3 mL of distilled water was added. Using 0.5 mL of 80% ethanol and reagents alone as a blank, the development color was measured at 725 nm by a UV spectrophotometer (UNICO Vis Model 1200, USA) after an hour.

Enzyme activities Enzyme extracts were prepared according to the method described by Mukherjee and Choudhuri (1983) Briefly, leaf tissues were homogenized in 10 mL of phosphate buffer (0.1 M, pH 6.8) at ice-cold temperatures. This was followed by centrifugation at 12,000 rpm and 4 °C for 20 min. The activity of the examined enzymes was immediately assessed using the supernatant.

Superoxide dismutase (SOD) activity was evaluated by measuring the suppression of pyrogallol autoxidation, as described by Alharbi et al. (2022). Pure water (3.6 mL), 0.1 mL of enzyme, 5.5 mL of 50 mM phosphate buffer (pH 7.8), and 0.8 mL of 3 mM pyrogallol (dissolved in 10 mM HCl) made up the solution (10 mL). Using a UV spectrophotometer, the rate of pyrogallol reduction was determined at 325 nm. The amount of enzyme that caused a 50% inhibition of pyrogallol's auto-oxidation rate at 25 °C was considered one unit of enzyme activity.

Catalase (CAT) activity was analyzed according to the method of Chen et al. (2000). In a 3 mL volume, the assay mixture included 50 mL of sample extract, 15 mM H_2O_2, and 100 mM potassium phosphate buffer (pH 7.0). The amount of enzyme that decreased 50% of the H_2O_2 in 60 s at 25 °C was defined as one unit of enzyme activity.

Peroxidase activity (POX) was measured according to the method of Pütter (1974), the reaction mixture was as follows: after adding 2 mL of 20 mm of pyrogallol, 5.8 mL of 50 m of phosphate buffer at pH 7.0, 0.2 mL of enzyme extract, and 2 mL of 20 mm of H_2O_2. The rate of increase in absorbance as pyrogallol was measured spectrophotometrically using a UV spectrophotometer within 60 s at 470 nm and 25 °C.

The polyphenol oxidase (PPO) activity was determined as described previously (Kar and Mishra 1976)**.** The sodium acetate buffer (pH 5.0) containing 0.1 M catechol was utilized as the substrate. The reaction took place for 60 min at 30 °C, and measurements were taken at 395 nm (Ultrospec 2000). The expression for polyphenoloxidase activity was shifts in optical density/min/g fresh mass.

Plant heavy metal content determination

The concentrations of heavy metal concentrations in the collected samples (edible plant parts) were quantified using an atomic absorption spectrophotometer (PerkinElmer 3100) (Parkinson and Allen 1975).

Statistical analysis

The statistical calculations were carried out in Microsoft Excel 365 and SPSS 25 (Statistical Software for the Social

Sciences). Quantitative data with parametric distributions were compared using analysis of variance for one-way ANOVA and post hoc test, Tukey's, both with a 0.05 probability threshold. The artwork and figures were performed using computer programs Microsoft Excel version 365 and GraphPad Prism program (version8).

Results and discussion

Growth traits

Approximately, 70% of all freshwater withdrawals are used in the agricultural sector, although in some underdeveloped nations, this percentage might exceed 95% (Bruinsma 2017). Egypt uses the Nile River's water for agricultural irrigation to the tune of almost 80% of its total volume. As a result, and without any prior treatments, farmers would use wastewater for crop irrigation in locations that have no access to fresh water (Antar et al. 2012). Based on the the results shown in Table 5 and 6, wastewater irrigation had no effect on the length, fresh or dry weight of *Daucus carota* shoots at stages I and II, or the fresh and dry weights of roots at stage I. Similarly, the use of wastewater had no effect on the number of leaves, fresh and dry weights, or root lengths in stage II. In comparison to the control and wastewater treatments, the application of *S. platensis*, *C. vulgaris*, *S. alba* plant powder, and EDTA considerably enhanced the lengths, fresh and dry weights, and number of leaves at stages I and II.

In case of shoot lengths, the maximum increases were observed by about 31 and 12% when the plants were treated with *S. platensis* at stages I and II, respectively, as compared to the wastewater-irrigated plant. In case of root lengths, the maximum increases were observed by about 37 and 27% when the plants were treated with *C. vulgaris* at stages I and II, respectively. The maximum increases in the number of leaves were showed by about 32% at stage I after the plants were treated with *S. platensis*, while the maximum

Table 5 Effect of different treatments on the shoot length (cm), root length (cm), and numbers of leaves /plant of *Daucus carota* plant

Treatments	Shoot length (cm)		Root length (cm)		Number of leaves/plant	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	17.0 ± 0.82ab	18.5 ± 1.3ab	9.7 ± 1.4c	16.0 ± 1.4b	4.7 ± 0.8c	7.3 ± 0.5c
WW	14.5 ± 0.9b	16.5 ± 1.0b	11.3 ± 2.4bc	18.5 ± 1.3b	5.5 ± 0.6bc	8.0 ± 0.8c
WW + Sp	19.0 ± 2.2a	20.8 ± 0.5a	13.2 ± 2.4b	20.5 ± 0.6ab	7.3 ± 0.5a	11.3 ± 1.0b
WW + Ch	18.0 ± 0.8a	20.5 ± 2.7a	15.5 ± 1.6a	23.5 ± 1.0a	6.2 ± 0.4ab	11.5 ± 0.6b
WW + SA	18.5 ± 1.0a	19.8 ± 0.6a	13.3 ± 1.9b	19.3 ± 1.5ab	6.8 ± 0.8a	13.5 ± 1.3a
WW + EDTA	17.0 ± 1.2ab	18.3 ± 1.0ab	11.8 ± 2.2b	20.8 ± 1.3b	6.5 ± 0.6ab	11.0 ± 0.8b
HSD	2.79	3.04	4.63	2.73	1.35	1.95

Different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference test (HSD) at $p \leq 0.05$; values of the same column with the same letter are not significantly different. The data are represented as the mean of value ± SE ($n = 3$). Control is fresh water, WW is wastewater, WW + Sp is wastewater + *Spirulina platensis,* WW + Ch is wastewater + *Chlorella vulgaris*, WW + SA is wastewater + salix plant powder, and WW + EDTA is wastewater + EDTA

Table 6 Effect of various treatments on the fresh and dry weights (g) of shoots and roots of *Daucus carota* plant. Each value is a mean of 8 replicates ± standard error of means

Treatments	F.Wt. shoot (gm)		D.Wt. shoot (gm)		F.Wt. root (gm)		D.Wt. root (gm)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	3.6 ± 0.7d	5.6 ± 0.3c	0.3 ± 0.1b	0.6 ± 0.2b	9.9 ± 0.9bc	12.8 ± 1.7e	0.9 ± 0.14ab	1.4 ± 0.5c
WW	3.6 ± 0.2d	5.3 ± 0.4c	0.3 ± 0.1b	0.4 ± 0.1b	8.6 ± 0.8c	14.1 ± 1.8de	0.8 ± 0.1ab	1.6 ± 0.4bc
WW + Sp	4.7 ± 0.3bc	6.7 ± 0.6b	0.6 ± 0.1ab	1.0 ± 0.2a	11.4 ± 0.8ab	17.9 ± 1.4 cd	1.0 ± 0.2ab	2.4 ± 0.4ab
WW + Ch	5.7 ± 0.8ab	7.6 ± 0.5b	0.6 ± 0.2a	1.1 ± 0.1a	10.6 ± 0.6b	22.2 ± 1.5bc	0.8 ± 0.2b	1.8 ± 0.5abc
WW + SA	6.4 ± 0.8a	10.7 ± 0.4a	0.6 ± 0.2ab	1.2 ± 0.1a	12.3 ± 0.8a	26.3 ± 1.6a	1.1 ± 0.1a	2.8 ± 0.5a
WW + EDTA	4.8 ± 0.3 cd	7.4 ± 0.6b	0.5 ± 0.1ab	1.1 ± 0.2a	11.2 ± 0.6ab	23.3 ± 1.3ab	0.9 ± 0.2ab	2.2 ± 0.5abc
HSD	1.08	0.91	0.32	0.31	1.51	4.26	0.33	1.03

Different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$; values of the same column with the same letter are not significantly different. The data are represented as the mean of value ± SE ($n = 3$). F.Wt. is denoting the fresh weight; D.Wt. is referring to the dry weight; control is fresh water; WW is wastewater; WW + Sp is wastewater + *Spirulina platensis;* WW + Ch Is wastewater + *Chlorella vulgaris*; WW + SA is wastewater + Salix plant powder; and WW + EDTA is wastewater + EDTA increases in the number of leaves were showed by about 69% at stage II after the plants were treated with salix powder. On the other hand, the maximum increases in the shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight were observed by about 78, 102, 100, 200, 43.87, 37, and 75% at stages I and II, respectively, when the plants were treated with salix powder as compared to the wastewater-irrigated plant.

Several researchers have noted the promoting effects of microalgae and macroalgae with regard to the growth of plants. For instance, the morphological properties of tomato (*Solanum lycopersicum*), such as the length and dry weight of the shoots, roots, and nodes, were enhanced when irrigated with polysaccharide extracted from three microalgae of *Arthrospira platensis*, *Porphyridium* sp., and *Dunaleilla salina*, as compared to the control (Rachidi et al. 2020). The presence of different stimulants such as sulfates, carbs, uronic acids, and high protein levels may cause these growth benefits following treatment with microalgae. These varied components may raise the concentration of phytohormones such as IAA and gibberellic acids, which promote cell division, cell growth, and tissue differentiation (Fouda et al. 2021a; Saucedo et al. 2015). Moreover, Swiss chard's growth and pigment content was enhanced when it was sprayed with varied quantities of *Chlorella vulgaris* extracts (Hajnal-Jafari et al. 2020).

Simulative effects of salicylic acid (the major component of *Salix* leaves) have been reported on the wheat plant under cobalt pollution of soils by Mohamed and Hassan (2019); they showed that the cobalt element causes a remarkable reduction of fresh and dry biomass, and shoot height. In addition, maize plants were treated to promote development in the presence of high NaCl concentrations by either soaking the seeds or spraying them with various amounts of salicylic acid (El-Khallal et al. 2009). The effect of EDTA in protecting the plant from the potentially damaging effects of exposure to certain environmental stresses was investigated (Saleem et al. 2020). Also, the application of EDTA to *Brassica napus* L. reduced the inhibitory effects of heavy metal stress, which led to an increase in root length, leaf area, plant height, fresh and dried leaf weights, and root weights (Habiba et al. 2015).

Photosynthetic pigments

Data analysis demonstrated that the concentrations of photosynthetic pigments in the carrot plants decreased in comparison to the control after wastewater irrigation throughout both growth stages (Table 7). The chlorophyll (a) was significantly declined by about 4% and 27%, while the content of chlorophyll (b) was reduced by about 15% and 23%, total chlorophyll (a + b) by about 7% and 27%. Also, the contents of carotenoids were significantly decreased by about 25% and 37%, at the stages I and II less than those of the controls, respectively. The result might be explained by an accumulation of heavy metals, which could prevent certain enzymes from functioning and, as a consequence, prevent further metabolic processes from occurring (Shakya et al. 2008)**.** Moreover, the presence of heavy metals in wastewater can have an impact on photosynthetic processes directly or indirectly. This might result in the Calvin cycle's enzymatic activities being inhibited and a CO_2 deficit brought on by the closed stomata (Bertrand and Poirier 2005). The reduction in the chlorophyll content associated with heavy metal stress may be caused by the inhibition of enzymes involved in the formation of chlorophyll (Żurek et al. 2014). Also, Bharwana et al. (2013) revealed that cotton plants that are exposed to heavy metal stress, such as that caused by lead, have considerably reduced chlorophyll (a, b, and total chlorophyll) and carotenoid concentrations in comparison to

Table 7 Effect of irrigation of carrot plant with freshwater (control), wastewater (WW), wastewater + *Spirulina platensis* (WW + Sp), wastewater + *Chlorella vulgaris* (WW + Ch), wastewater + salix plant powder (WW + salix), and wastewater + EDTA (WW + EDTA) on the photosynthetic pigment contents (mg/g fresh weight)

Treatments	Chlorophyll (a) mg/g fresh weight		Chlorophyll (b) mg/g fresh weight		Chlorophyll (a + b) mg/g fresh weight		Carotenoids (mg/g fresh weight)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	7.0 ± 0.1d	7.8 ± 0.1e	3.4 ± 0.2 cd	3.0 ± 0.2b	10.4 ± 0.3c	10.8 ± 0.3c	0.8 ± 0.1c	0.8 ± 0.1c
WW	6.7 ± 0.1e	5.7 ± 0.1f	2.9 ± 0.1d	2.3 ± 0.1c	9.7 ± 0.2d	7.9 ± 0.3d	0.6 ± 0.1d	0.5 ± 0.02d
WW + Sp	7.7 ± 0.1b	9.2 ± 0.1c	3.8 ± 0.2bc	3.7 ± 0.2a	11.6 ± 0.3b	12.9 ± 0.2a	0.9 ± 0.1bc	0.8 ± 0.03c
WW + Ch	8.4 ± 0.1a	10.7 ± 0.1a	4.2 ± 0.1ab	2.6 ± 0.1bc	12.6 ± 0.2a	13.2 ± 0.2a	1.0 ± 0.01ab	1.0 ± 0.01b
WW + SA	7.4 ± 0.1c	9.5 ± 0.1b	3.3 ± 0.2d	4.1 ± 0.2a	10.7 ± 0.3c	13.6 ± 0.3a	1.01 ± 0.1a	1.2 ± 0.03a
WW + EDTA	8.5 ± 0.1a	8.3 ± 0.1d	4.4 ± 0.2a	3.7 ± 0.2a	12.9 ± 0.3a	12.0 ± 0.2b	0.8 ± 0.02c	0.8 ± 0.02c
HSD	0.22	0.23	0.48	0.47	0.69	0.70	0.11	0.09

Different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$; values of the same column with the same letter are not significantly different. Data are represented by means of three replicates ± SE the control. The increase of reactive oxygen species (ROS) might be the source of heavy metal-induced suppression of normal pigment accumulation (Kasim 2005).

On the other hand, the analysis of variance revealed that all of the treatments (*C. vulgaris, S. platensis*, salix powder, and EDTA) considerably enhanced the amounts of chlorophyll and carotenoids in carrot plants. In case of stage I, the maximum increases in the chlorophyll a, b, and a + b were observed by about 27, 52, and 33% when the plants were treated with EDTA, respectively, as compared to the wastewater-irrigated plant. While the highest value of the carotenoid content was showed by about 68% after the plants were treated with salix powder. In case of stage II, the maximum increases in the chlorophyll b, a + b, and carotenoid were observed by about 78, 72, and 140%, respectively, when the plants were treated with salix powder, while the highest value of the chlorophyll a was observed by about 88% after the plants were treated with *Chlorella vulgaris* as compared to the wastewater-irrigated plant.

Probably, this phenomenon could be due to the chelating effect of EDTA and the other extracts which may have chelating factors that alleviate the toxic effects of wastewater. This increase in the photosynthetic pigments could be attributed to our finding that these treatments were able to lower the heavy metal accumulation as shown in Table 10. Our findings are in agreement with several previous studies that reached the same conclusion on the efficacy of algal extracts in increasing the amounts of various plant pigments (Hajnal-Jafari et al. 2020; Rachidi et al. 2020). Also, it was shown that salix plant extract stimulated the production of photosynthetic pigments in barley and wheat plants (Arfan et al. 2007; El-Tayeb 2005). In accordance with these results, the treatment of plants with EDTA under heavy metal stress was able to increase the chlorophyll contents (Saleem et al. 2020).

Metabolic responses

Carbohydrate, protein, proline, and phenol contents

In the present studies, irrigation with wastewater significantly reduced the total soluble carbohydrate and protein contents of carrot shoots and roots at stages I and II compared to the control (Fig. 1). Data analysis revealed that irrigation with wastewater caused the total soluble carbohydrates in carrot shoots to decrease from 15.4 (control) to 11.3 mg/g dry weight at stage I with a percentage of 26.5% and from 16.5 (control) to 12.5 mg/g dry weight at stage II with a percentage of 24.2% (Fig. 1A). Similarly, the root's carbohydrate content decreased from 35.6 and 46.5 (control) to 28.8 and 37.7 mg/g dry weight with percentages of 19.1% and 18.9% at stages I and II, respectively (Fig. 1B). However, all treatments alleviated the wastewater toxicity on carbohydrates and protein contents. The maximum carbohydrate content of the shoot was 22.11 mg/g dry weight attained in the presence of *S. platensis* at stage II, compared to wastewater treatment (16.49 mg/g dry weight), while in the root system was 62.42 mg/g dry weight compared to 46.50 mg/g dry weight of wastewater treatments at the same stage. On the other hand, the highest values for shoot protein content were achieved in the presence of EDTA (9.3 mg/g dry weight) at stage 1 as compared to wastewater and freshwater (5.62 and 7.94 mg/g dry weight), respectively (Fig. 1C), but in the root system, *S. platensis* appeared the highest values 10.62 mg/g dry weight as compared to wastewater and freshwater (4.92 and 6.82 mg/g dry weight), respectively, during the first stage (Fig. 1D). These findings are in agreement with those reported by Akhtar et al. (2013); they claimed that protein levels of shoot and roots of *Vigna radiata* L. growing under salt stress were improved by the application of salicylic acids as compared to the control. Wastewater contains a huge amount of heavy metals that contribute to decreasing the total carbohydrate and protein in the wastewater-irrigated plants (Rizvi et al. 2020)**;** this could be attributed to a number of reasons including the increased oxidative stress of ROS (Gupta et al. 2010), the increased ribonuclease activity (Gopal and Rizvi 2008), and/or the altered gene expression of key metabolic genes (Kovalchuk et al. 2005)**.**

On the other hand, irrigation with untreated wastewater led to a significant increase in the concentrations of free proline and total phenol in both stage I and stage II, as shown in Fig. 2. The addition of EDTA resulted in the greatest reduction in the free proline (6.76 mg/g dry weight and 5.78 mg/g dry weight at stages I and II), with decreasing percentages of 42.7% and 33.5% in stages I and II, respectively as compared to wastewater-irrigated plant. Different treatments had different abilities in reducing the free proline as follows: EDTA, salix, *S. platensis*, and *C. vulgaris* for stage I and EDTA, *S. platensis*, *C. vulgaris*, and salix for stage II (Fig. 2A). It is interesting to note that all of the treatments showed a considerable reduction in the total phenolic compounds in comparison to the wastewater treatment. Analysis of variance revealed that total phenols can be decreased in the presence of *S. platensis* (038 mg/100 g dry weight)*, C. vulgaris* (34 mg/100 g dry weight), salix (39 mg/100 g dry weight), and EDTA (33 mg/100 g dry weight), with percentages of 24%, 32%, 22%, and 34%, respectively, at stage I, and 28%, 34.6%, 15.4%, and 21.2% during stage II in comparison to the wastewater treatment (Fig. 2B). Non-enzymatic antioxidants like as proline and polyphenols play an important function in protecting against environmental stress such as wastewater irrigation (Mehla et al. 2017). Irrigation with wastewater that is containing different heavy metals enhanced proline production in *Salicornia europaea* compared to irrigation with fresh water (Khalilzadeh et al. 2020). After irrigation with wastewater containing aluminum-heavy metal, *Lupinus termis* L. also

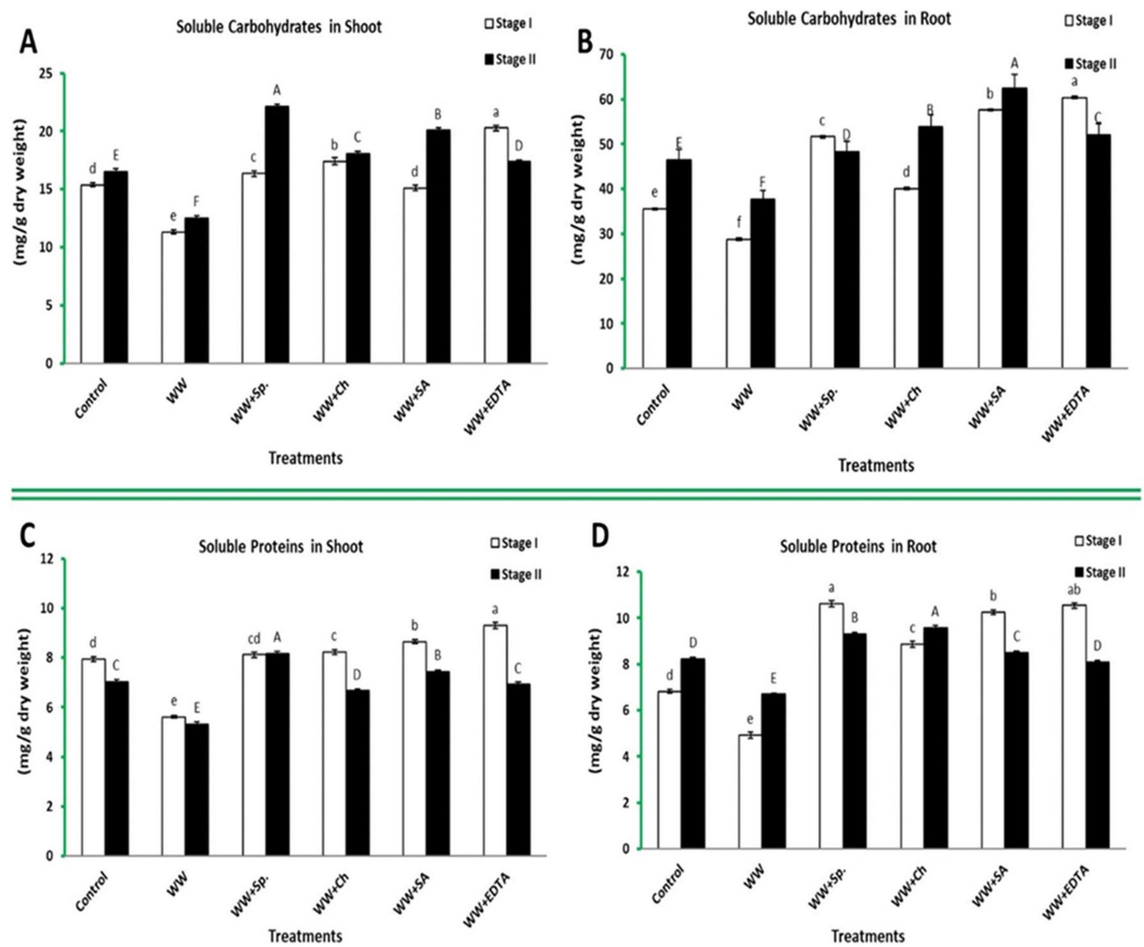

Fig. 1 Effect of different treatments on the total soluble carbohydrates and proteins (mg/g dry weight) in both shoots and roots of *Daucus carota* plants. **A**, **B** Soluble carbohydrates in the shoot and root, respectively; **C**, **D** Soluble protein content in the shoot and root of the carrot, respectively. Control is referring to irrigation with fresh water, WW is irrigation with wastewater, WW + Sp is irrigation with wastewater in the presence of *Spirulina platensis*, WW + Ch is irrigation with wastewater in the presence of *Chlorella vulgaris*, WW + SA is irrigation with wastewater in the presence of salix plant powder, and WW + EDTA is irrigation with wastewater in the presence of EDTA. Each value is the mean of 3 replicates ± standard error ($n = 3, \pm$SE). The different letters (small and capital letters) are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. Values of bars with the same letter are not significantly different

had a higher amount of some non-enzymatic antioxidants such as proline and phenols. The heavy metal contents declined in Lupine beans after receiving treatment with salicylic acid (the major component of salix) (Moustafa et al. 2020). Furthermore, Spain and coauthors found that microalgae are a viable method for relieving the hazardous effects of heavy metal-containing wastewater by biosorption. The intricate and distinctive structure of algal cell walls may in part explain this occurrence (Spain et al. 2021).

Enzyme activity

Antioxidant enzyme activities such as polyphenol oxidase (PPO), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) were increased in consequence of wastewater irrigation throughout the two growth stages (Fig. 3). The activity of the stage I antioxidant enzymes SOD, CAT, POX, and PPO increased by 181%, 91%, 170%, and 212%, respectively, in comparison to the control. The activity of the SOD, CAT, POX, and PPO enzymes increased by 169%, 150%, 322%, and 376%, respectively, in stage II. On the other hand, carrot plants treated with *S. platensis*, *C. vulgaris*, *Salix alba* leaf powder, and EDTA demonstrated reduced of SOD, CAT, POX, and PPO activities (Fig. 3). The greatest drop-in activity was observed for SOD, POX, and PPO enzymes at stage I with the EDTA treatment, with percentages of 59%, 62%, and 60%, respectively. *Chlorella vulgaris* powder caused a decrease in CAT activity by 46% compared to its activity when the wastewater was used. In contrast,

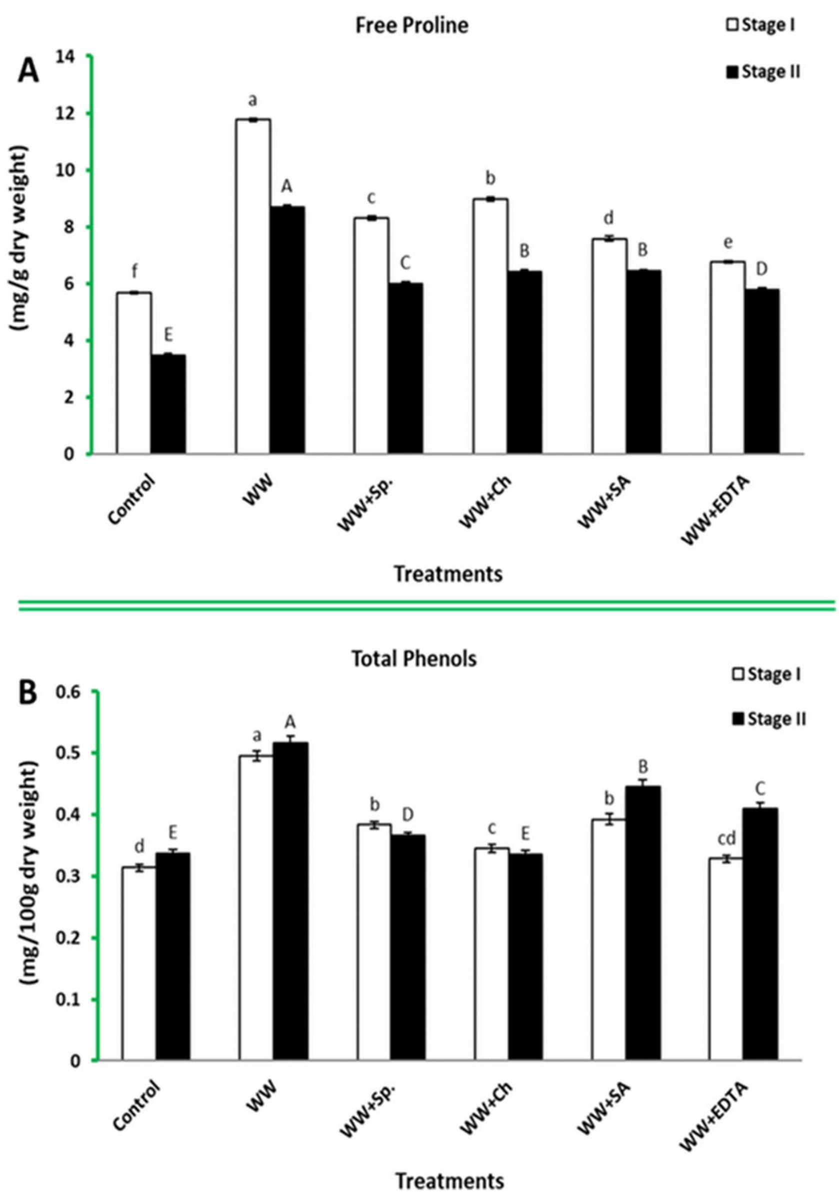

Fig. 2 Effect of different treatments on free proline (mg/g dry weight) and total phenol contents (mg/100 g dry weight) of *Daucus carota* plants. **A** Free proline. **B** Total phenols; the abbreviation of different treatments was discussed as shown in Fig. 1 legend. Each value is a mean of 3 replicates ± SE. The different letters (small and capital letters) are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. Values of bars with the same letter are not significantly different

the effects of the EDTA treatment were to reduce the activities of CAT, POX, and PPO by about 54%, 60%, and 69% respectively at stage II. However, the treatment with *Spirulina platensis* resulted in the greatest reduction in the activity of SOD, which was 42% lower than the control. Heavy metal buildup is increasing in crops as a result of continuous wastewater irrigation, which raises the activity of antioxidant enzymes to counteract the damaging effects (Singh and Agrawal 2010). Due to their high toxicity and in ability of the plant to biodegrade them, heavy metals can cause oxidative damage and death of plants (Cho and Park 2000, Hamza et al. 2022). The antioxidant system of plants, particularly enzyme antioxidants such as POX, PPO, CAT, and SOD, functions as the first defined line of defence in the plant's ability to survive these unfavorable circumstances. This is because if the ROS are not promptly destroyed, they would create lethal effects to the plant (Caverzan et al. 2016; Khalil et al. 2021). In the current study, untreated wastewater irrigation of carrot plants boosted the antioxidant enzyme activities at stages I and II in comparison to controls. By accelerating the conversion of hazardous ROS to non-toxic molecules, the increase in POX, PPO, CAT, and SOD activities and higher concentrations of non-enzymatic antioxidant plants would reduce the dangers of oxidative stress (Kang et al. 2013). In this regard, the SOD and CAT levels in the leaves and roots of the *Eichhornia crassipes* plant steadily increased as the Pb concentration was increased (Malar et al. 2016). Moreover, the effects of various Cd concentrations (10, 50, 100, and 500 M) on cucumber (*Cucumis sativus* L.) plant enzymes were investigated. Their findings showed that the level of SOD,

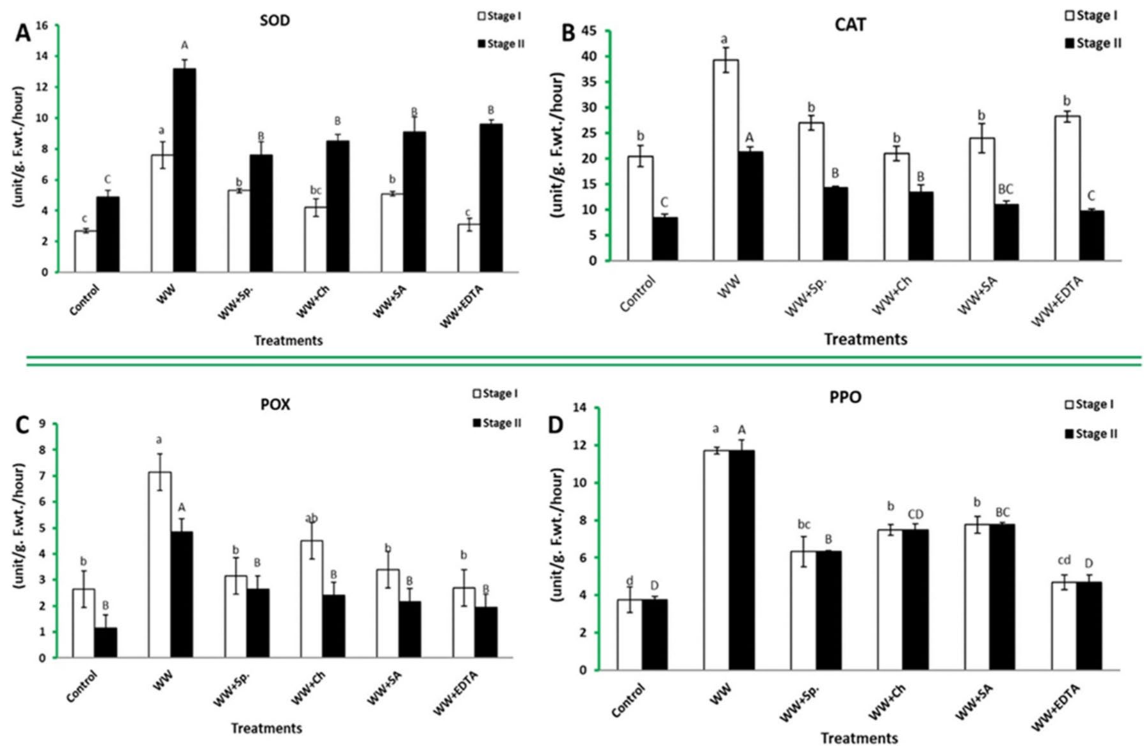

Fig. 3 Effect of different treatments on superoxide dismutase (**A**), catalase (**B**), peroxidase (**C**), and polyphenol oxidase (**D**) enzymes (unit/g fresh weight/hour) of *Daucus carota* plants. The abbreviation of different treatments was discussed as shown in Fig. 1 legend. Each value is a mean of 3 replicates ± SE. The different letters (small and capital letters) are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. Values of bars with the same letter are not significantly different

CAT, and POX activities in root and shoot was elevated with the rising Cd concentrations (Sun et al. 2015). In the same trend, the POX, SOD, and CAT activities in the Glycine max plant increased with Zn contamination (Mishra and Prakash 2010)**.** Additionally, it was shown that Vicia faba plants that were watered with wastewater had an increased PPO enzyme activity compared to plants that were irrigated with water from the Nile (Ismaiel et al. 2014). Also, the activity of PPO increased significantly by Pb exposures (Kaur et al. 2010).

However, the use of *C. vulgaris*, *S. platensis*, *Salix alba* leaf powder, and EDTA treatments decreased the detrimental effects that wastewater had on the environment, as shown by the activities of antioxidant enzymes throughout the two stages that were investigated. These findings could be supported by Noriega et al. (2012), who found that salicylic acid reduces the effects of oxidative stress caused on by heavy metals in soybean plants. Similar to this, the use of salicylic acid reduced the negative effects of a few heavy metals in *Lemna minor* plants as shown by a drop in the antioxidative enzymes (Lu et al. 2018)**.** In this context, the use of *S. platensis* has the potential to chelate several kinds of heavy metals from wastewater and reduce the harmful impacts of these metals (Dinesh Kumar et al. 2020).

Yield characteristics

Morphological traits

The results of the data analysis in Table 8 showed that the yield statistics of carrot plants irrigated with wastewater, including root length, diameter, and fresh and dry weight of root, were not significantly distinct from each other when compared to the control. All treatments caused a significant improvement when compared to the control and/or the wastewater treatments. Carrot plants that were treated with the wastewater in the presence of salix leaf extract had the highest values of yield traits, which increased with percentages of 34.52, 33.12, 97.89, and 72.46% of root length, diameter, fresh weight, and dried weight of root, respectively, in comparison to the plants that were irrigated with the wastewater alone. Salix's tolerance to heavy metals may result from the production of phytochelatin (Ali et al. 2003). Similarly, salix species have exhibited significant potential for the phytoextraction of heavy metals (Wani et al. 2011). The role that algae may play in improving the yield characteristics of crops was recorded by Faheed and Fattah (2008); they revealed that the enhancement of the

Table 8 Effect of different treatments on yield characters of *Daucus carota* plants

Treatments	Root length (cm)	Root diameter (cm)	F.Wt. root (gm)	D.Wt. root (gm)
Control	18.3 ± 1.3d	7.5 ± 0.9 cd	16.02 ± 1.5d	1.8 ± 0.7c
WW	19.0 ± 0.8d	7.7 ± 0.6d	18.04 ± 1.5d	2.1 ± 0.6bc
WW + Sp	26.3 ± 1.0ab	8.8 ± 0.5abc	30.8 ± 1.6bc	3.2 ± 0.5ab
WW + Ch	27.5 ± 1.0ab	9.6 ± 0.5ab	33.5 ± 1.3ab	2.4 ± 0.6abc
WW + SA	28.3 ± 1.0a	10.3 ± 0.9a	35.7 ± 1.1a	3.6 ± 0.6a
WW + EDTA	25.5 ± 0.6b	8.6 ± 0.6bc	28.6 ± 1.3c	2.9 ± 0.6abc
HSD	2.14	0.72	3.83	1.31

Freshwater (control), wastewater (WW), wastewater + *Spirulina platensis* (WW + Sp), wastewater + *Chlorella vulgaris* (WW + Ch), wastewater + salix plant powder (WW + SA), and wastewater + EDTA (WW + EDTA). Different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$; values of the same column with the same letter are not significantly different. Each value is a mean of 10 replicates ± SE

yield characteristics and reduction of the environmental stresses could be accomplished by adding 2 and 3 g of dry algae/kg soil. Also, Nawar and Ibraheim (2014) revealed that because algae contain a variety of nutrients, treating pea plants with them can increase both productivity and yield quality.

Metabolic constituents of the yield

The results shown in Table 9 indicated that, at the yield stage, the concentrations of total soluble proteins, total soluble carbohydrates, and carotene in the roots of carrot plants irrigated with untreated wastewater were significantly less than those in control plants (irrigated with fresh water), with percentages of 11.8%, 26.4%, and 18.2%, respectively. On other hand, all of the investigated additions significantly increased the level of total soluble carbohydrates and β-carotene when compared to the control and wastewater treatments isolated. As a response to the *Salix alba* addition, the greatest value of β-carotene was obtained. While the usage of *Chlorella vulgaris* was accompanied by the highest soluble carbohydrate content. At the yield stage, all the used biostimulants significantly increased the total soluble protein of carrots; the highest value was seen with the WW + Sp treatment. Salix's role in bringing the protein levels up was stated previously by Huda et al. (2016); they claimed that SA (the major compound in salix) treatment improved the protein content of rice. In recent years, broad bean seeds had been treated with *Spirulina* extract to considerably boost the contents of protein and carbohydrates (Lerer et al. 2021). Wastewater caused an increase in the free proline and total phenolic contents by 69.6 and 50%, respectively, in comparison to the control. *Chlorella vulgaris* can lessen the harmful effects of wastewater by reducing free proline and total phenols by 46.7% and 50%, respectively, when compared to the wastewater alone (Table 7). Moreover, the following various treatment processes were able to lessen the toxic effects of wastewater irrigation in the following order: salix > *Spirulina platensis* > EDTA.

Heavy metal accumulation in root at the yield stage

The results of the study on the levels of heavy metals present in edible part of carrot roots are shown in Table 10. According to the standard, the cadmium, nickel, cobalt, and lead concentrations in the carrot roots of a plant that is irrigated with wastewater have been found to be higher than the maximum permissible

Table 9 Effect of different treatments on the β-carotene (mg/100 g F.Wt.), total soluble carbohydrates and proteins (mg/g. D.Wt.), free proline (mg/g. D.Wt.), and total phenols (mg/100 g D.Wt.) in the roots of *Daucus carota* roots

Treatments	β-Carotene	Soluble carbohydrates	Soluble proteins	Free proline	Total phenols
Control	0.5 ± 0.1de	53.01 ± 0.3e	8.1 ± 0.1a	2.8 ± 0.1f	0.2 ± 0.01f
WW	0.5 ± 0.01e	39.0 ± 0.2f	6.6 ± 0.1d	9.2 ± 0.1a	0.4 ± 0.03a
WW + Sp	0.6 ± 0.01bc	68.7 ± 0.2b	7.4 ± 0.1b	6.9 ± 0.03d	0.3 ± 0.03c
WW + Ch	0.7 ± 0.02ab	71.6 ± 0.2a	6.9 ± 0.1c	4.9 ± 0.1e	0.2 ± 0.04e
WW + SA	0.8 ± 0.1a	57.9 ± 0.2d	7.3 ± 0.1b	7.5 ± 0.04b	0.3 ± 0.1d
WW + EDTA	0.6 ± 0.03 cd	60.4 ± 0.2c	6.2 ± 0.1e	7.2 ± 0.04c	0.3 ± 0.03b
HSD	0.05	0.28	0.10	0.05	0.054

Freshwater (control), wastewater (WW), wastewater + *Spirulina platensis* (WW + Sp), wastewater + *Chlorella vulgaris* (WW + Ch), wastewater + salix plant powder (WW + SA), and wastewater + EDTA (WW + EDTA). Different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$; values of the same column with the same letter are not significantly different. Each value is a mean of 3 replicates ± SE

Table 10 Heavy metal accumulation in roots (edible part) of *Daucus carota* plant irrigated with wastewater in the presence and absence of various treatments

Treatments	Ni mg/kg D.Wt	Cd	Pb	Co
Control	0.04 ± 0.001e	0.08 ± 0.003e	0.020 ± 0.006e	0.007 ± 0.002d
WW	0.305 ± 0.003a	0.360 ± 0.007a	0.401 ± 0.008a	0.288 ± 0.006a
WW + Sp	0.027 ± 0.003d	0.044 ± 0.004d	0.035 ± 0.005e	0.063 ± 0.004b
WW + Ch	0.040 ± 0.002c	0.065 ± 0.005c	0.114 ± 0.003c	0.032 ± 0.005c
WW + SA	0.018 ± 0.004de	0.049 ± 0.003 cd	0.077 ± 0.004d	0.015 ± 0.004d
WW + EDTA	0.086 ± 0.004b	0.098 ± 0.005b	0.136 ± 0.006b	0.068 ± 0.003b
HSD	0.005	0.008	0.009	0.007
Permissible limit*	0.10	0.10	0.20	0.10

The different lowercase letters in the same column are significantly different by post hoc Tukey's honestly significant difference (HSD) test at $p \leq 0.05$; values of the same column with the same letter are not significantly different. Each value is a mean of 3 replicates ± SE

*Permissible limits are according to FAO/WHO (FAO 2019; Organization 2020)

level in the food (FAO 2019; Organization 2020). On the other hand, all the tested treatments showed a significant reduction in the accumulation of these heavy metals. For example, in the presence of salix, Ni and Co in the wastewater-irrigated plants were 0.018 and 0.015 mg/kg D.Wt. declined from 0.305 and 0.288 mg/kg D.Wt, respectively, when the wastewater was used alone. On the other hand, in the presence of *spirulina*, Cd and Pb in wastewater treatment plants were 0.044 and 0.015 mg/kg D.Wt. compared to the wastewater treatment only (0.360 and 0.401 mg/kg D.Wt., respectively). Similar to the current study, the high buildup of heavy metals in plants including mustard, radish, and taro plants that were irrigated with wastewater has been documented in numerous literature**,** *Brassica olerace* and *Spinacia oleracea* (Bamniya et al. 2010), sugar beet (Ewais et al. 2015), and *Lemna minor* (Lu et al. 2018). In this concern, *C. vulgaris* and *S. platensis* present in soils can effectively chelate heavy metals from waste with a percentage that may reach up to 88% (Abdel-Razek et al. 2019).

Conclusions

It seems possible that when carrot plants were watered with wastewater, the levels of free proline, total phenols, SOD, CAT, POX, and PPO tended to rise. The heavy metals Ni and Co were decreased from 0.305 and 0.288 mg/kg D.Wt. (for plant irrigated with wastewater) to 0.018 and 0.015 mg/kg D.Wt., respectively, due to using of salix. Also, the highest decrease of Cd and Pb was achieved in presence of *Spirulina* from 0.360 and 0.401 mg/kg D.Wt. to 0.044 and 0.015 mg/kg D.Wt., respectively. On the other hand, it led to a big drop in the created roots' photosynthesis pigments, soluble carbohydrates, proteins, and β-carotene. Data showed that the highest reduction of total phenols was achieved in presence of *S. platensis, C. vulgaris,* salix, and EDTA with percentages of 24%, 32%, 22%, and 34% at stage I and 28%, 34.6%, 15.4%, and 21.2% during stage II, respectively. In our study, all of the treatments that were tried were able to reduce the amount of heavy metals in the roots. It seems that *Spirulina platensis* and salix leaves were the best additions. So, both of them could be used in the future as eco-friendly chelators to lessen the bad effects of wastewater on carrot plants and maybe other important food plants as well.

Acknowledgements We want to acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R182), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Author contribution A.A, A.Ab, M.A, and A.N: conceptulation. A.A, A.Ab, M.A, A.N, A.F, M.I, A.M, M.A, A.E, A.S, and H.S: data analysis. A.F, M.I, A.M, M.A, A.E, A.S, H.S: visualization; A.A, A.Ab, M.A, A.N, A.F, M.I, A.M, M.A, and A.E: project manager. A.A, A.Ab, M.A, A.N, A.F, M.A, and A.E: funding. A.A, A.Ab, M.A, A.N, A.F, M.I, and A.M: writing the draft. A.A, A.Ab, M.A, A.N, A.F, M.I, A.M, M.A, A.E, A.S, and H.S: revision. A.A, A.Ab, A.E, A.S, and H.S: supervisor.

Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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