

Effects of EDTA and aqueous plants extract on the developmental and stress tolerance attributes of *Spinacia oleracea* and *Brassica rapa* under sewage water regime

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Abstract

Sewage water is causing a potential threat to agriculture sector due to industrial effluents having heavy metals. Present investigation was carried to study the role of ethylenediaminetetraacetic acid (EDTA) or aqueous extracts of *Hyacinth* and *Hedychium* on soil quality and growth of spinach and turnip plants irrigated with sewage water (SW). Treatment of plants with SW resulted in an increment of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) activities. However, EDTA or plant extracts further enhanced their activities. At both stages of development of the tested crops, a substantial increase was found in the content of proline and total phenols, indicating the strengthening of the antioxidant protection mechanism to boost the oxidative effects of SW stress. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) studies revealed considerable variation in the protein profile among the different treatments, with an expression of some unique proteins obvious with other treatments. SW treatments increased heavy metals (HM) content in soil and plants; however, EDTA or plant extracts greatly decreased the levels of HMs in both shoots and

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roots and soils. The present study results suggest that the application of EDTA or aqueous plant extracts can be a useful strategy for phytoextraction in areas irrigated with sewage water.

Keywords: antioxidants; biochemical constituents; heavy metals; proline; SDS-PAGE

Introduction

Farmers worldwide are forced to use unconventional methods to increase food supply due to a lack of quality irrigation water and inefficient management in the use of existing water resources (Guo and Sims, 2000; Soliman *et al.*, 2020). In this regard, sewage water (SW) for agricultural purposes has been over-used to boost crop growth and production (Zeid and Abou El Ghate, 2007). Which severely influences the soil's physical and chemical properties, causing a reduction in the fertility index (Nahar and Shahadat, 2021). The use of municipal sewage effluent for crop management and production is worked out by several researchers (Ali *et al.*, 2011; Asgharipour and Azizmoghaddam, 2012). However, it is important to note here that proper management practices and crop selection can minimize the detrimental impacts on soil properties and, consequently, on the yield of crops while using these polluted waters (Ali *et al.*, 2011). The use of low-quality water or wastewater for irrigation has received significant attention due to the increasing crisis in the availability of freshwater (Khan *et al.*, 2017; Valipour and Singh, 2016). Sewage usually contains a variety of essential inorganic and organic ingredients that are considered valuable for the life cycle of plants (Nahar and Hossen, 2021). However, contaminated water, including sewage, sludge, etc., is rich in toxic ions that negatively impact flora and fauna around the globe by influencing soil characteristics (Singh *et al.*, 2009; Zeid and Abou El Ghate, 2007). In addition, the accumulation of toxic ions in food crops results in significant health risks in humans (Singh *et al.*, 2010). Heavy metal (HM) accumulation in plants grown on soil mixed with sludge has been reported previously (Elloumi *et al.*, 2016; Singh and Agrawal, 2007). Therefore, prolonged irrigation with SW causes the build-up of micronutrients in plants and exaggerated accretion of HM in soil which eventually impairs growth and crop quality (García *et al.*, 1991). Availability of HMs and higher concentrations of other ions in SW result in a considerable decline in the development and productivity of major crop plants through alterations of the physiological and biochemical attributes (Singh and Agrawal, 2012). As a result, cleaning up the environmental system for maintaining the growth, quality and quantity of plant production and ensuring human health risk assessment under SW irrigation is much needed with enormous attention globally (Dar *et al.*, 2016). In this connection, various plants have been recognized to potentially remove toxic HMs from wastewater through their uptake or chemical precipitation (Singh and Agrawal, 2007).

Many chelating compounds are used by different researchers to facilitate the phytoextraction process. However, ethylenediaminetetraacetic acid (EDTA) is considered as the cheapest and most suitable complexing compound for preventing HM toxicity in environment (Xie *et al.*, 2008). There is growing attention to the environmental risk and health hazards resulting from the HM contaminants in the soil and groundwater (Masindi and Muedi, 2018). The establishment of exposure is the most variable aspect of the risk assessment concerning metal ion and contaminant pathways for the target organism. To mitigate the damaging effects of these toxic metal ions, EDTA assisted phytoextraction has been suggested as an alternative remediation approach for the reclamation of polluted soils. The chelating amino polycarboxylic acid and EDTA have been recorded to enhance tissue accumulation of HMs. Phyto-extraction was suggested as a promising approach and an alternative remediation method for treating polluted soils to mitigate these toxic HM ions (Ebrahimi, 2013; Hasan *et al.*, 2019).

Against this backdrop, the current research investigates the effects of EDTA and *Hyacinth* and *Hedychium* aqueous plant extracts on the soil properties and spinach and turnip grown on soils irrigated with SW. Therefore, we hypothesized that the application of EDTA and plant extract could enhance the chelation

capacity for enhanced phytoextraction purposes and could increase the physio-biochemical and oxidative stress parameters in spinach and turnip against the toxic metals.

Materials and Methods

Physico-chemical characteristics of sewage water and soil

Soil sample representing the entire field was collected from a depth of 1-15 cm before transplanting and after the completion of the growing period of crops. The samples collected were air-dried and grounded through a pestle and mortar, and then it was sifted through a 2 mm sieve. The sewage water was collected from the sewage station of Six October City Giza, Egypt. Both soils and SW samples were analysed for the content of nutrients (micro and macro) and HMs (in micro ranges). The properties of collected soil and SW are presented in Table 1. Depending on the variables, the following parameters were analysed.

Table 1. Characteristics of sewage water used for irrigation

Parameter	pH	TSS	COD	Sulfides	Nitrogen	Phenol	Oil	Phosphate	Zn	Ni	Cd	Fe	Cr	Cu	Pb	Mn
		mg/L								ppm						
Sewage water	8.1	862	436	0.07	15	0.01	3.2	2.5	1.42	0.26	0.29	0.49	0.53	2.50	3.17	0.30

pH and electrical conductivity

The pH was measured using a glass electrode pH meter in a water suspension of 1:2.5 as described by Jackson (Jackson, 1973). The EC was analysed with the help of a traditional EC meter following the tested procedure outlined in Sasaki *et al.* (2003).

Soil organic carbon

The soil sample 20000 mg was mixed with 60 ml of 6% H₂O₂ and heated in a water bath for 30 min to oxidize the organic matter present. The carbonates present were dissolved by adding 200 ml of HCl by keeping the mixture overnight. The filtrate was made Cl free by filtering through Whatman filter paper and washed with double distilled water (DDW). Further, the contents were mixed with 400 ml DDW in a beaker. To separate the finer particles in the sample, 8 ml of 1N NaOH was added. The soil separates were dispersed by stirring for 10 min with a mechanical stirrer. A 1L volume was made using DDW. The clay, silt, and sand fractions were finally analysed following the pipetting protocol mentioned by Rattan *et al.* (2015).

Micronutrient analysis

The concentrations of selected HMs in soil, SW, soil and tested plant samples were determined following the method of Lindsay and Norwell (Lindsay and Norvell, 1978). The samples were extracted in DTPA, and the content of Cd, Zn, Pb, Ni, Cr, Cu, Fe and Mn was determined by atomic absorption spectrophotometer (AAS-4141).

Preparation of aqueous extracts and EDTA

The aqueous plant extracts of *Hyacinth* or *Hedychium* were prepared by homogenizing fresh plant tissues 5.0 gm in 1 L of water. Aqueous extracts of both *Hyacinth* and *Hedychium* and 50 mM EDTA were applied to normal and sewage-treated seedlings.

Plant material, growing conditions and experimental treatments

The present study selected two test plants, spinach (*Spinacea oleracea* var. *local*) and turnip (*Brassica rapa* var. *rapa*). Seeds of spinach and turnip were taken from the Agricultural Research Center (ARC), Giza, Egypt. Vigorous and uniform-sized seeds were sown in well-prepared plots. After ten days of germination, healthy seedlings were transplanted into pots (25 cm diameter) filled with 5 kg garden soil. After successful seedling establishment (ten days after transplantation) pots were irrigated with: (1) tap water (control), (T1) sewage water, (T2) SW + EDTA (50 mM), (T3) SW + Hyacinth aqueous plant extracts, and (T4) SW + Hedychium aqueous plant extracts. Pots were maintained at the experimental farm station of Botany and Microbiology Department, Faculty of Science, Al-Azhar University. After 32 (stage I; 32 days after treatment, DAT) and 47 (stage II; 47, DAT) days of growth, plants were analysed for further growth and biochemical parameters as discussed below. At the end of the growth season, 75 days, data on biomass parameters [total above-ground biomass; TAGB] and total protein and carbohydrates were analysed as visual indicators for the response to the applied treatments during the experiment.

Table 2. Different treatment design

Treatment	Sewage water
Control	×
T1	SW
T2	SW+EDTA
T3	SW + <i>Hyacinth</i> extract
T4	SW + <i>Hedychium</i>

Determination of total soluble protein, proline, and total phenol

Lindsay and Norvell (1978) protocol were used to estimate complete soluble protein by extracting fresh tissue in phosphate buffer and precipitating the protein in the supernatant by adding TCA. After centrifuging at 5000 rpm, the pellet was dissolved in NaOH and protein content was estimated in alkaline medium by reacting with Folin–Ciocalteu (FC) reagent (1N).

The Bates *et al.* (1973) protocol was employed for measuring the content of proline. A 500 mg dry powdered tissue was extracted in 3% sulphosalicylic acid. First, the slurry was centrifuged for 20 min at 3000 rpm; after that 2 mL supernatant was mixed with 2 mL glacial acetic acid and 2 mL ninhydrin reagent in a water bath 100 °C for 1 h. The tubes having samples were then kept on an ice bath, and proline was separated by using toluene. Finally, the optical density was read at 520 nm.

The method described by (Diaz Martin, 1972) was followed for determining total phenols by reacting plant extract with FC reagent and measuring the optical density at 765 nm.

Assay of catalase, superoxide dismutase, peroxidase, and polyphenol oxidase

For activities of peroxidase (POD), polyphenol oxidase (PPO), Superoxide dismutase (SOD) and catalase (CAT) methods described by (Matta and Dimond, 1963; Aebi, 1984; Chen *et al.*, 2000; Khan *et al.*, 2021), respectively. The fresh leaf tissues were used for assaying the activities of SOD, POD, PPO, and CAT. The activity of POD was determined by monitoring the formation of tetra guaiacol and an absorbance change was recorded for 2 min at 470 nm. The CAT activity was noted by examining absorbance change for 2 min at 240 nm in a 1 mL reaction mixture with EDTA, 100 mM phosphate buffer (pH 7.0), H₂O₂ and 100 µL enzyme extract.

Analysis of elements in shoots of the two tested crop plants at the final stage of growth

The micronutrients and HMs viz Zn, Ni, Cd and Pb in plant samples were determined according to the method of (Lindsay and Norvell, 1978) as described above by atomic absorption spectrophotometer (AAS-4141).

Determination of total soluble protein, total carbohydrate, and biomass content of the two crop plants evaluated at the final stage of growth

The complete soluble protein was measured following the (Laemmli, 1970) method as described above. Anthrone-based method (Irigoyen *et al.*, 1992) was used to measure total carbohydrate and absorbance measured at 625 nm. Firstly, dry leaves (0.5 g) in 5 ml of 95% ethyl alcohol were homogenized. After that, 1ml chilled extract was treated with 3 ml anthrone reagent (150 mg anthrone, 100 ml of 72% H₂SO₄, W/W). The samples were left undisturbed for 10 min in a boiling water bath. Finally, the absorption was taken at 625nm using a PD-303 model spectrophotometer by using glucose as standard and expressed as mg g⁻¹ DW of leaves. Above-ground portions of the tested crop plants were collected using clippers at 75 DAT for biomass content. From each treatment, five plants with intact roots were detached and transferred to the laboratory to measure biomass content.

Protein fractions in the leaf of SW irrigated spinach and turnip plants

The methods of (Laemmli, 1970) and (Studier, 1973) were followed for protein fingerprinting using SDS-PAGE. The gels were photographed, scanned, analysed using Gel Doc VILBER LOURMAT system.

Statistical analysis

Data presented was subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using LSD multiple range test and at 5% level of probability using Statistix 8.1 software. R plot software was used to carry out PCA (principal component analysis) and Pearson's correlation of all the data.

Results

Characteristics of sewage water

The chemical properties, nutrient profile and HM content in sewage used are presented in Table 1. The results showed that SW showed slightly alkaline pH; however, it showed high EC, organic C, and available P, medium in available high in total N, phosphate and oil content.

Effect of sewage water and its interaction with EDTA or aqueous plant extracts of Hyacinth and Hedychium on soil quality

Soil samples after harvesting were collected from a depth of 10 cm from all pots. Results showed that HMs accumulated in soils irrigation with SW. The results in Table 3 showed that soil irrigated with SW exhibited a significant increase in the accumulation of HMs like Pb, Zn, Cd and Ni. Effect of EDTA and the water extracts of plants (*Hyacinth* or *Hedychium*) were found obvious as decreased accumulation of HMs in soil irrigated with SW. Application of EDTA and SW proved much effective in reducing the collection of HMs than the *Hyacinth* or *Hedychium* extracts (Table 3).

Effect of EDTA and extracts of Hyacinth and Hedychium on the shoot and root protein content

Shoot and root protein content decreased at both crop plants' growth stages due to SW treatment. Relative to control, decline in protein was 43.61% (shoot) and 24.57% (root) in spinach and 11.05% (shoot) and 10.67% (root) in turnip after 30 days of SW treatment. In both root and shoot tissues, the decline in protein content was mitigated considerably by EDTA and extracts of *Hyacinth* and *Hedychium*. In shoot (30 days after treatment), protein content increased by 43.92% and 18.76% in EDTA, 57.98% and 17.42% in SW + *Hyacinth* and 32.87% 11.23% in SW + *Hedychium* treated spinach and turnip seedlings, respectively, over the SW treated ones. A similar trend was observed in root tissues (Table 4).

Table 3. Effect of sewage water (SW) with and without applied EDTA or plant extracts of *Hyacinth* and *Hedychium* on the soil quality.

Parameters	Soil before experimental treatments	Sewage water	Soil after treatments			
			T1	T2	T3	T4
pH	7.3	8.1	5.4	7.0	6.3	6.8
EC	3.8	3.2	3.9	1.9	2.2	2.3
Zinc	0.967	1.420	1.200	1.00	1.00	1.1
Nickel	N-D	0.262	0.090	N-D	N-D	N-D
Cadmium (Cd)	N-D	0.290	0.160	N-D	N-D	N-D
Iron	114.62	0.490	126.32	42.23	68.18	61.25
Chromium (Cr)	0.342	0.534	0.744	0.353	0.443	0.411
Copper	0.040	2.500	2.177	0.74	0.92	1.01
Lead	0.092	3.172	2.67	0.099	0.116	0.120
Manganese	N-D	0.300	0.486	N-D	N-D	N-D

All values are means of three replicates ± standard deviation at level of p 0.05. Where T1=sewage water, T2= SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*.

Table 4. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on shoot and root protein content in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages

Treatments	Shoot Protein (mg/g-1 Dw)				Root Protein (mg/g-1 DW)			
	<i>S. oleraceae</i>		<i>B. rapa</i>		<i>S. oleraceae</i>		<i>B. rapa</i>	
	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	12.33±0.50 ^a	13±0.33 ^a	42.35±0.8 ^a	38.53±1.02 ^{ab}	6.46±0.5 ^a	7.57±1.15 ^a	55.15±1.39 ^{ab}	69.21±1 ^c
T1	7.63±0.55 ^d	7.33±0.77 ^c	33.20±0.86 ^d	34.27±0.92 ^c	5.22±0.79 ^c	5.81±0.23 ^b	47.69±1.29 ^{ab}	61.82±1.21 ^d
T2	11.57±0.52 ^b	10.55±0.62 ^c	41.04±0.83 ^{ab}	40.7±1.07 ^a	5.63±0.5b ^c	5.05±0.51 ^b	73.17±1.78 ^a	80.22±1.36 ^a
T3	11.04±0.26 ^b	11.58±0.61 ^b	35.68±1.03 ^c	40.24±1.42 ^{ab}	6.32±0.49 ^{ab}	5.6±0.62 ^b	41.75±5.58 ^b	71.25±0.87 ^{bc}
T4	10.37±0.37 ^c	9.74±0.61 ^c	40.77±0.95 ^b	38.12±1.01 ^b	5.63±0.62 ^{bc}	4.64±0.62 ^b	62.42±1.33 ^{ab}	73.27±1.05 ^b

All values are means of three replicates ± standard deviation at level of p 0.05. Where T1=sewage water, T2= SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test (p≤0.05)

Effect of SW with and without EDTA or aqueous extracts of Hyacinth and Hedychium on proline and phenols

Application of SW to spinach and turnip resulted in significant enhancement in the content of proline in both root as well as shoot tissue (Figure 2). Relative to control, proline content in shoot increased by 260.72% in spinach and 90.76% in turnip after 15 days of treatment while it increased by 75.50 and 73.32% in root respectively due to SW treatment. However, increase was 655.67% and 190.63% in SW + EDTA, 352.07% and 138.86% in SW + *Hyacinth* and 312.71% and 187.10% in SW + *Hedychium* in shoot tissues of spinach and turnip respectively over the control. Older seedlings i.e., 30 days after treatment, showed more proline content as compared to younger seedlings. During both experimental stages content of proline was more significantly increased due to EDTA application in both the root and shoot tissues (Figure 5).

Total phenol content increased significantly in both crop species (*Spinacia oleracea* and *Brassica rapa*) after 15 and 30 days of treatment; however, the content was more in seedlings after 30 days of treatment (Figure 3). Relative to control, 30 days after treatment of SW content of phenols increased by 47.88% and 27.70% in shoot and root of spinach and by 14.55% and 33.16% in shoot and root of turnip. However, SW + EDTA increased content of phenols by 61.95% (shoot) and 105.86% (root) in spinach and by 77.14% (shoot) and 94.88% (root) in turnip over control (Table. 6). Application of *Hyacinth* and *Hedychium* to SW treated seedlings also resulted in a further significant increase in phenols over SW treated seedlings in both tested crops at both stages (Table 6).

Table 5. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on shoot and root proline content in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages

Treatments	Shoot Proline (mg/g-1 Fw)				Root Proline (mg/g-1 FW)			
	<i>S. oleraceae</i>		<i>B. rapa</i>		<i>S. oleraceae</i>		<i>B. rapa</i>	
	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	0.21±0.1 ^e	0.23±0.14 ^c	0.21±0.07 ^d	0.24±0.12 ^d	0.36±0.1 ^d	0.40±0.08 ^c	0.28±0.1 ^e	0.30±0.12 ^d
T1	0.76±0.12 ^d	0.83±0.13 ^d	0.41±0.04 ^c	0.38±0.1 ^c	0.64±0.18 ^c	0.66±0.02 ^d	0.48±0.07 ^d	0.53±0.10 ^c
T2	1.59±0.15 ^a	1.9±0.15 ^a	0.62±0.11 ^a	0.62±0.1 ^a	0.86±0.14 ^a	0.90±0.09 ^a	0.79±0.1 ^a	0.88±0.10 ^a
T3	0.95±0.20 ^b	1.4±0.18 ^b	0.51±0.07 ^b	0.58±0.1 ^b	0.80±0.13 ^b	0.82±0.09 ^b	0.62±0.1 ^c	0.52±0.10 ^c
T4	0.87±0.12 ^c	0.94±0.18 ^c	0.61±0.04 ^a	0.61±0.07 ^a	0.68±0.19 ^c	0.72±0.1 ^c	0.69±0.12 ^b	0.67±0.09 ^b

All values are means of three replicates ± standard deviation at level of p 0.05. Where T1=sewage water, T2= SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test (p≤0.05)

Table 6. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on shoot and root phenol content in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages

Treatments	Shoot Phenol (mg/g ¹ Fw)				Root Phenol (mg/g ¹ FW)			
	<i>S. oleraceae</i>		<i>B. rapa</i>		<i>S. oleraceae</i>		<i>B. rapa</i>	
	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	1.14±0.27 ^d	1.29±0.2 ^d	0.66±0.02 ^d	0.73±0.14 ^c	0.76±0.15 ^c	0.87±0.09 ^d	0.82±0.1 ^c	0.81±0.15 ^c
T1	1.59±0.22 ^c	1.91±0.13 ^c	0.86±0.15 ^c	0.84±0.16 ^d	0.83±0.15 ^d	1.11±0.21 ^b	0.91±0.1 ^b	
T2	1.84±0.3 ^b	2.09±0.23 ^b	1.21±0.30 ^b	1.29±0.15 ^b	1.11±0.25 ^a	1.79±0.07 ^a	1.40±0.12 ^a	1.58±0.19 ^a
T3	2.03±0.19 ^a	2.65±0.24 ^a	1.14±0.32 ^b	1.21±0.14 ^c	0.90±0.12 ^c	0.93±0.23 ^c	1.411±0.25 ^a	0.86±0.20 ^c
T4	1.88±0.28 ^b	1.93±0.24 ^c	1.52±0.33 ^a	1.63±0.22 ^a	0.97±0.1 ^b	1.05±0.23 ^b	0.96±0.13 ^b	0.85±0.16 ^c

All values are means of three replicates ± standard deviation at level of p 0.05. Where T1=sewage water, T2= SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test (p≤0.05)

Effect of EDTA or aqueous plant extracts on antioxidant enzyme activities treated with SW

Results depicting the effect of EDTA and aqueous extracts of *Hyacinth* and *Hedychium* on CAT, SOD, POD and PPO activity in spinach and turnip are shown in Figure 4. It was observed that significant enhancement in CAT, SOD, POD and PPO activities in both spinach and turnip plants irrigated with S.W over the control at both growth stages. However, further enhancement was observed due to the application of EDTA or extracts of *Hyacinth* and *Hedychium*. Relative to control, after 15 days of treatment activity of CAT, POD and PPO increased by 4.71, 118.42, and 54.95% in spinach and by 36.66, 176.66 and 14.46% in turnip, respectively due to SW application (Figure 1). In spinach, further enhancement of 12.61%, 16.66% and 0.135% in CAT, 211.44%, 96.98% and 56.02% in POD and 9.01%, 10.75% and 8.13% in PPO due to SW + EDTA, SW + *Hyacinth* and SW + *Hedychium* over SW treated plants after 15 days (stage I) of treatment. While as turnip registered an enhancement of 26.82%, 5.69% and 3.25% in CAT, 74.69%, 12.04% and 40.36% in POD and 0.11%, 3.92% and 16.35% in PPO due to SW + EDTA, SW + *Hyacinth* and SW + *Hedychium*. After 30 days of treatment, a similar trend was observed with SW + EDTA treated seedlings exhibiting much obvious increase (Figure 1).

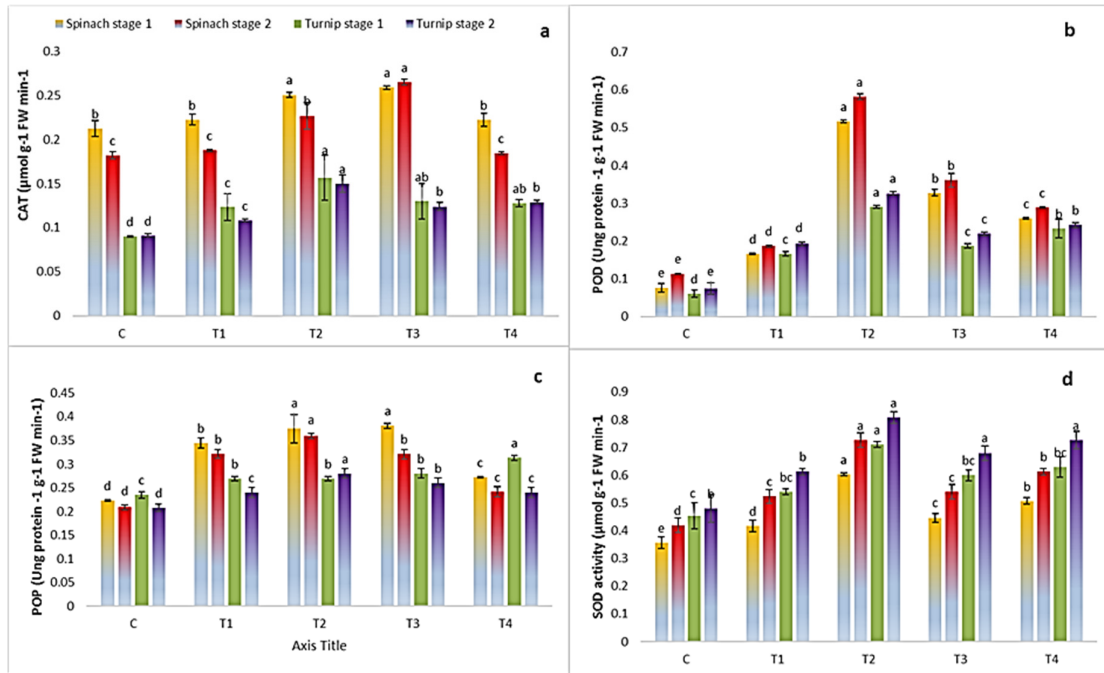


Figure 1. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on antioxidants enzymes (a) CAT activity, (b) POD, (c) POP and, (d) SOD content in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages

All values are means of three replicates \pm standard deviation at level of p 0.05. Where T1=sewage water, T2=SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test (p \leq 0.05)

Metal ion content

SW treatment resulted in the accumulation of toxic metal ions like Ni, Cd and Zn and Pb in both spinach and turnip; however, application EDTA, and extracts of *Hyacinth* and *Hedychium* resulted in declined accumulation of these metal ions analyzed (Table. 7).

Table 7. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on Ni, Cd, Zn and Pb uptake in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages.

	Ni		Cd		Zn		Pb	
	<i>S. oleraceae</i>	<i>B. rapa</i>	<i>S. oleraceae</i>	<i>B. rapa</i>	<i>S. oleraceae</i>	<i>B. rapa</i>	<i>S. oleraceae</i>	<i>B. rapa</i>
C	0.001 \pm 0.006 ^c	0.014 \pm 0.001 ^d	0.012 \pm 0.005 ^c	0.001 \pm 0 ^b	0.025 \pm 0.008 ^c	0.032 \pm 0.008 ^c	0.012 \pm 0.00 ^b	0.051 \pm 0.003 ^d
T1	0.12 \pm 0.01 ^b	0.073 \pm 0.001 ^b	0.34 \pm 0.03 ^a	0.37 \pm 0.006 ^a	0.057 \pm 0.01 ^b	0.58 \pm 0.07 ^a	0.19 \pm 0.14 ^a	0.25 \pm 0.03 ^b
T2	0.18 \pm 0.01 ^a	0.26 \pm 0.02 ^a	0.33 \pm 0.04 ^a	0.23 \pm 0.19 ^a	0.24 \pm 0.032 ^a	0.27 \pm 0.03 ^b	0.21 \pm 0.12 ^a	0.32 \pm 0.02 ^a
T3	0.026 \pm 0.005 ^d	0.022 \pm 0.001 ^{cd}	0.046 \pm 0.005 ^{bc}	0.06 \pm 0.004 ^b	0.037 \pm 0.001 ^{bc}	0.037 \pm 0.001 ^b	0.116 \pm 0.006 ^{ab}	0.067 \pm 0.001 ^{ab}
T4	0.053 \pm 0.002 ^c	0.032 \pm 0.001 ^c	0.061 \pm 0.01 ^b	0.052 \pm 0.004 ^b	0.054 \pm 0.004 ^b	0.14 \pm 0.013 ^c	0.115 \pm 0.003 ^{ab}	0.14 \pm 0.06 ^{ab}

All values are means of three replicates \pm standard deviation at level of p 0.05. Where T1=sewage water, T2=SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test (p \leq 0.05)

Effect on total protein, carbohydrate content and biomass accumulation

Treatment of spinach and turnip with SW resulted in increased accumulation of carbohydrates by 74.30% and 43.11%, respectively over the control. Increase in carbohydrates in SW + EDTA was 64.00% and 92.11%, in SW + *Hyacinth* 10.76% and 47.05%, and in SW + *Hedychium* 25.53% and 31.35% in spinach and turnip respectively over control. Biomass productivity increased by 7.43% and 5.97% in spinach and turnip

over control due to SW treatment. Percent increase in SW + EDTA, SW + Hyacinth and SW + Hedychium was 19.51%, 24.48% and 7.43% in spinach and 15.97%, 2.40% and 4.87% respectively in turnip (Figure 2).

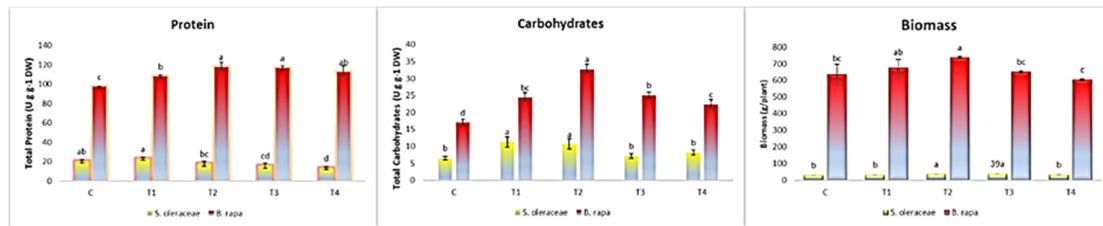


Figure 2. Effect of sewage water (SW), EDTA and extract of *Hyacinth* and *Hedychium* on total protein, carbohydrate content and biomass accumulation in spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) at two different stages

All values are means of three replicates \pm standard deviation at level of $p < 0.05$. Where T1=sewage water, T2=SW+EDTA, T3=SW+ *Hyacinth* extract and T4=SW+ *Hedychium*. Averages with the same matching lower-case letters in the same column are not significantly different according to LSD grouping test ($p \leq 0.05$)

3Influence of EDTA and Hyacinth or Hedychium extract application on the expression of proteins (SDS- PAGE protein banding patterns)

Irrigation of SW to spinach and turnip resulted in a significant alteration in the expression of proteins (Figure 8 and 9). Certain unique proteins were expressed in both seedlings due to EDTA and extracts of *Hyacinth* or *Hedychium*. A variation of the effective manner in the number, molecular weight and density of protein bands was observed. In spinach, the variability analysis among three treatments appeared 39 protein bands. *Hyacinth* extract treatment resulted in nine protein bands, EDTA in eight, and *Hedychium* extract in seven protein bands.

In contrast, as SW resulted in eight protein bands while as control gave only seven protein bands. Related to the markers, the molecular weight of expressed polypeptides was determined, ranging from 87.225 to 10.626 KDa. The polypeptide bands in all treatments (monomorphic or common polypeptide) went from a molecular weight of 87.225 to 13.356 KDa with 89.7 % polymorphism (Table 3) which may be related to spinach plants. Unique polypeptide markers in spinach plants irrigated with SW and treated with tested treatments ranged from 73.95 to 10.62 KDa with 10.3% polymorphism. These appeared bands could be polypeptide markers related (Tables 3 and 4). However, in turnip, the variability analysis among different treatments appeared a total of 34 protein bands. It was observed that *Hedychium* extract gave thirteen protein bands, EDTA showed only seven protein bands, and *Hyacinth* extract showed three protein bands, respectively.

In comparison, SW showed four 4 protein bands while control plants showed seven protein bands. The molecular weight of polypeptides determined related to the protein marker ranged from 105.978 to 17.158 KDa. The molecular weight of prominent polypeptide bands in all treatments (monomorphic or common polypeptide) ranged from 42.00 to 22.97 KDa with a percentage of 44.2%. These bands may be related to turnip; however, the most prominent specific polypeptide alterations (polymorphic bands) ranged in molecular weight from 53.67 to 17.15KDa with the percentage of 35.3%, and these bands may be specific to the tested treatments. The unique polypeptide markers that appeared in turnip seedlings irrigated with SW and treated with tested treatments ranged from 105.97 to 21.09 KDa with a percentage of 20.5%. These bands may be related to polypeptide markers (Tables 3 and 4).

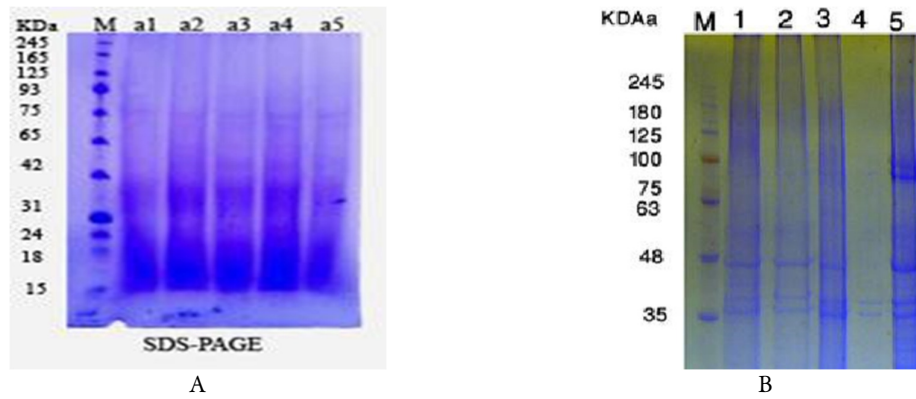


Figure 3. Protein fractions of (A) spinach and (B) turnip plant irrigated with SW with and without EDTA and extracts of *Hyacinth* or *Hedychium* using SDS-PAGE

M: Marker. 1: irrigated with tap water (control). 2: irrigated with SW. 3: SW + EDTA 4: SW + *Hyacinth*. 5: SW + *Hedychium*. Monomorphic (Common polypeptide). Polymorphic (Specific polypeptide). Unique (Polypeptide marker) or (genetic marker). - =Absence of band and + = presence of band.

Table 8. Protein fractions in leaf of SW irrigated spinach and turnip plants treated with EDTA and extracts of *Hyacinth* and *Hedychium* using SDS-PAGE

<i>Spinach oleracea</i>								<i>Brassica rapa</i>							
MW	Lane1	Lane2	Lane3	Lane4	Lane5	Frequency	Polymorphism	MW	Lane1	Lane2	Lane3	Lane4	Lane5	Frequency	Polymorphism
87.225	+	+	+	+	+	1.000	Monomorphic	105.978	-	-	-	-	+	0.200	Unique
73.950	-	-	-	+	-	0.200	Unique	98.111	-	-	-	-	+	0.200	Unique
52.315	-	-	+	-	-	0.200	Unique	53.670	+	+	+	-	+	0.800	Polymorphic
51.819	-	-	-	+	-	0.200	Unique	42.002	+	+	+	+	+	1.000	Monomorphic
38.693	+	+	+	+	+	1.000	Monomorphic	38.247	+	+	+	+	+	1.000	Monomorphic
32.185	+	+	+	+	+	1.000	Monomorphic	30.014	-	-	-	-	+	0.200	Unique
21.300	+	+	+	+	+	1.000	Monomorphic	26.883	-	-	-	-	+	0.200	Unique
18.058	+	+	+	+	+	1.000	Monomorphic	25.653	-	-	-	-	+	0.200	Unique
15.604	+	+	+	+	+	1.000	Monomorphic	24.547	+	-	-	-	+	0.400	Polymorphic
13.356	+	+	+	+	+	1.000	Monomorphic	22.976	+	+	+	+	+	1.000	Monomorphic
10.626	-	+	-	-	-	0.200	Unique	22.476	+	-	+	-	+	0.600	Polymorphic
								21.212	-	-	+	-	-	0.200	Unique
								21.096	-	-	-	-	+	0.200	Unique
								17.158	+	-	+	-	+	0.600	Polymorphic

Table 9. Polymorphism and genetic markers in leaf of SW irrigated spinach and turnip plants treated with EDTA and extracts of *Hyacinth* and *Hedychium* using SDS-PAGE

Bands	Lane1	Lane2	Lane3	Lane4	Lane5	Percent polymorphism	Lane1	Lane2	Lane3	Lane4	Lane5	Percent polymorphism
Mono	7	7	7	7	7	89.7	3	3	3	3	3	44.2
Poly	0	0	0	0	0	0	4	1	3	0	4	35.3
Unique	0	1	1	2	0	10.3	0	0	1	0	6	20.5
Total bands	7	8	8	9	7	100	7	4	7	3	13	100

PCA and Pearson’s correlation analysis

Principal component analysis clusters together input and response variables into different groups on the base of correlation. PCA in the present research work separated both crop plants into two different groups. PCA help us to compare treatment of sewage water and the total response of plants on biochemical level. In both crops PCA divided all four treatments into different classifications, which shows different effects of treatments on both plants (Figures 4 and 5). PCA was also performed to compare treatments of pot experiment for their accumulative effect on plant response traits. In both varieties the PCA divided all the five treatments into distinct divisions, indicating dissimilar effects of these treatments from each other (Figure 4). Root

protein, shoot protein, fresh weight and carbohydrates, biomass, phenol was grouped together that showing the enhanced response of these traits under normal and exposure to sewage water (Figure 5). Antioxidants and phenol were clustered together, inferring their same trend of increasing response under EDTA assisted phytoextraction. The various biochemical attributes were positively or negatively attributed (Figure 5). Furthermore, the relative impact of each treatment on the positive and negative regulation of numerous plant attributes is depicted in this diagram. The Pearson correlation coefficient discloses a substantial negative correlation among plant parameters and sewage water.

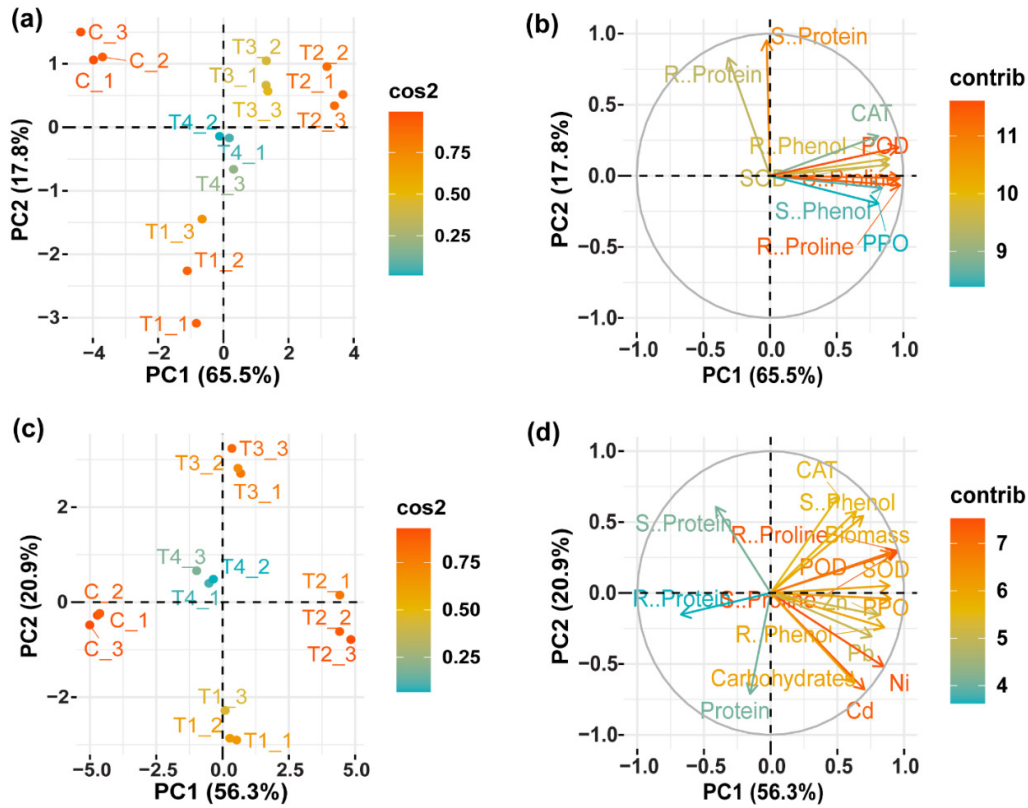
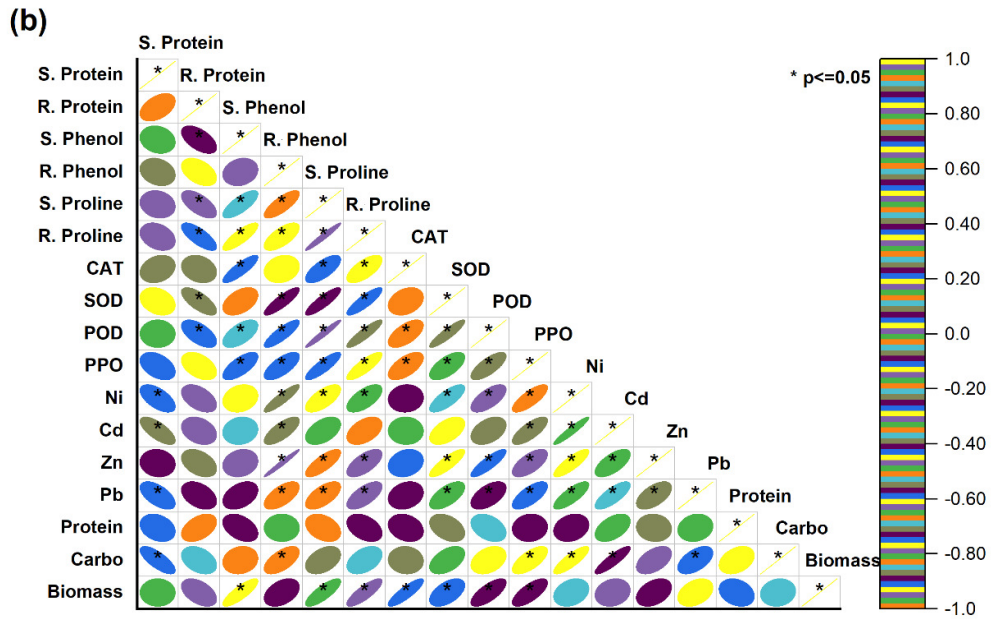
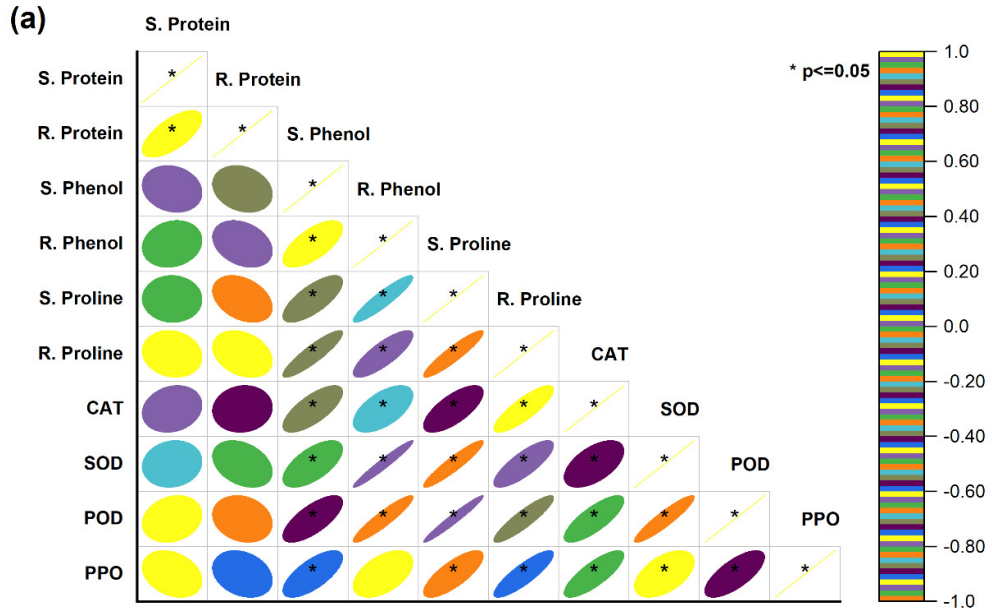


Figure 4. The PCA biplot shows the effect of sewage water, EDTA and plants extract on correlation among different growth attributes and treatments of spinach and turnip plants

Figure 4.1 (a,b) shows the correlation among treatments of spinach at stage 1 and 2 respectively while (c,d) shows the effect on different treatments on turnip at stage 1 & 2.



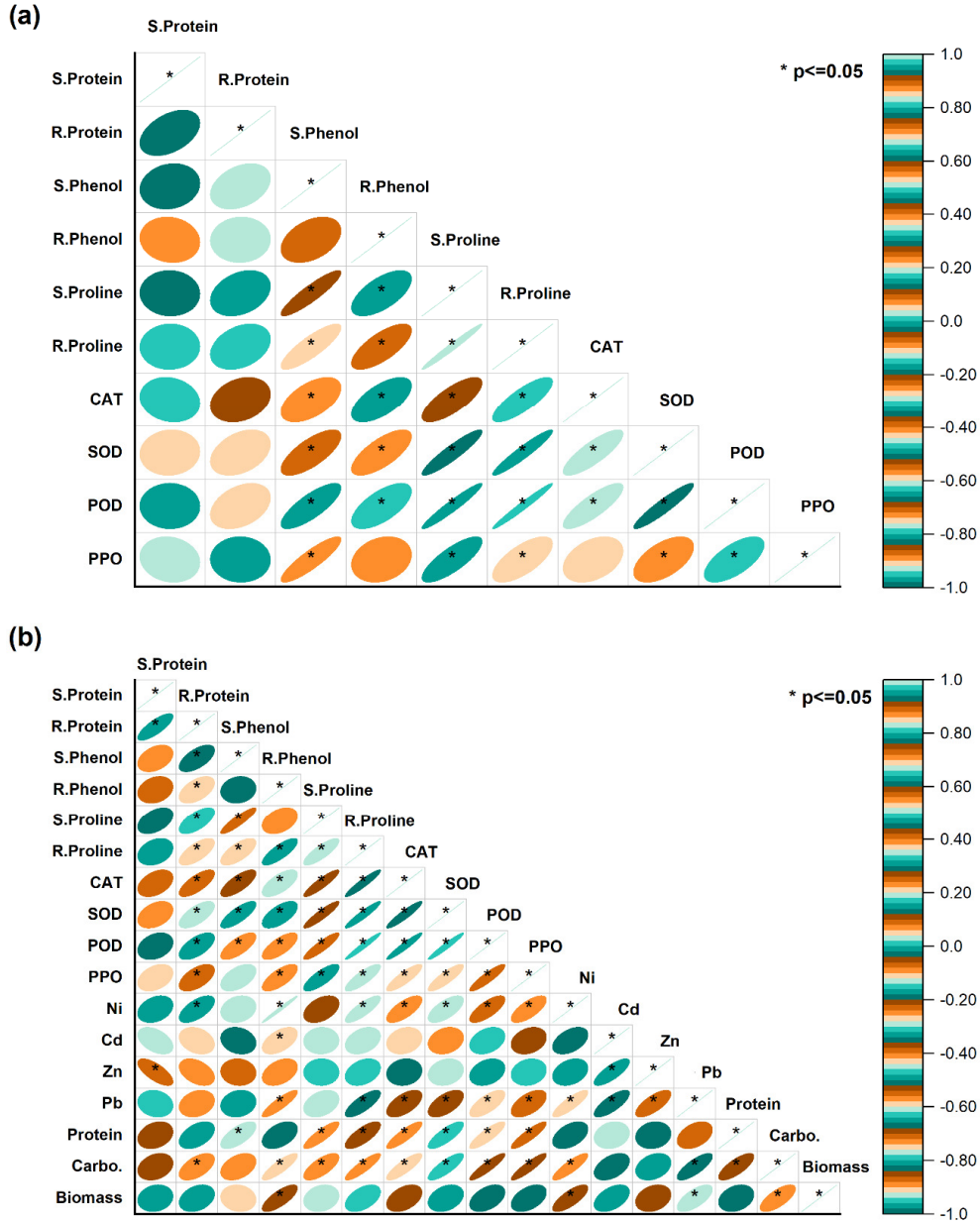


Figure 5. The Pearson's correlation shows the effect of Sewage water, EDTA and plant extracts on correlation among different growth attributes of spinach and turnip plants. 5.1 on left side shows (a,b) The correlation of spinach at stage 1 while (c,d) shows correlation at stage 2. Hence 5.2 (a,b) shows the correlation in turnip plants growth attributes at stage 1 while (c,d) shows correlation at stage 2.

Discussion

There has been continuous usage of sewage as a fertilizer and a source of irrigation in most areas of the world; in addition, it is considered a rich source for supplying the nutritional needs of the plants (Balkhair and Ashraf, 2016). However, the harmful constituents present in the sewage are least known to the farmers, thereby

rendering the crops less productive and degrading the quality of the yield product. Accumulation of toxic metal ions within the edible part of crops can severely damage humans. Therefore, it is important to devise management strategies to lessen the adverse effects of sewage irrigation. In this connection, developing novel management strategies like using chelating agents or aqueous plant extracts to remove toxic ions from sewage irrigated soils can mitigate the damaging effects. Therefore, the present research investigated the impact of EDTA and *Hyacinth* and *Hedychium* on the growth and alleviation of adverse effects of SW in vegetable crops i.e., spinach and turnip. The characteristics of SW showed pH tending towards alkalinity with high EC, organic carbon and available P, however available K, Ca, and Mg was in the medium range.

In contrast, as available N was considerably high. Sewage waters often contain potentially toxic elements viz- Pb, Cr, As, Cu, Zn, B, Co, Mo, Mn etc., most of which are non-essential and induce toxic effects to plants, animals and humans (Ahanger *et al.*, 2017). One of the damaging consequences of toxic metal ions is the excessive generation of ROS. To counteract the ROS-triggered deleterious effects, plants have built-in adaptive mechanisms like antioxidant system, osmolyte, and secondary metabolite accumulation. In the present study, SW treatment boosted the antioxidant enzyme activity and proliferation of phenolic compounds and proline. CAT, POD and PPO activity increased due to SW treatment; however, application of EDTA and extracts of *Hyacinth* and *Hedychium* further enhanced their activity, imparting quick elimination of ROS. ROS must be neutralized to maintain their optimal levels (Elkelish *et al.*, 2019; Zaid and Wani, 2019) and up-regulation of the antioxidant system reflects increased membrane functioning and cellular integrity (Cirilli *et al.*, 2017). CAT and POX have a significant role in neutralizing hydrogen peroxide in the cytosol and plasma membranes. The activity of POX is regulated by phenolic compounds (Soliman *et al.*, 2020). Increased activity of antioxidants results in significant protection of major plant functions like photosynthesis, electron transport, photo-assimilate production, enzyme functioning and mineral assimilation (Cirilli *et al.*, 2017; Foyer, 2018). Maintenance of ROS at optimal levels mediate the signalling events, maintain redox homeostasis (Ahanger *et al.*, 2018) in present study up-regulation of antioxidant following the treatments with EDTA or water extracts of *Hyacinth* or *Hedychium* may have contributed to growth improvements under SW treatment by the maintenance of major cellular structures, and their functioning like membranes permeability and, photosynthetic apparatus. Very least has been known about the efficiency of water extracts of plants against the damaging effects of toxic metal ions in vegetable crops. In addition, an increasing number of non-enzymatic antioxidant compounds are being defined, such as proline, and phenol compounds, with roles in scavenging ROS.

The optimal synthesis of phenols further strengthens the antioxidant system's functioning. It is believed that secondary metabolites contribute immensely to stress tolerance whenever the enzymatic antioxidant system fails to assuage the damage (Zaynab *et al.*, 2018). Phenolic acids constitute the principle polyphenols biosynthesized in plants and exert diverse functions during plant interactions with biotic and abiotic environments and are thought to be required for plants' survival in the environment (Isah, 2019; Shahid *et al.*, 2020). They stabilize membrane functioning by eliminating excess ROS, especially H₂O₂ by regulating the functioning of peroxidases like guaiacol peroxidase. In the current study, phenol contents were incremented significantly in both tested crops irrigated with SW as compared with control plants at both developmental stages. SW contains many non-essential and toxic trace elements, which induce negative effects in plants (Ahanger *et al.*, 2020). Soil pollution with the HMs triggers the generation of toxic ROS such as superoxide radicals (O₂^{•-}) and hydrogen peroxide (H₂O₂) in plants resulting in photoinhibition, enzyme inhibition and retarded growth (Gill and Tuteja, 2010; Wani *et al.*, 2012; Sharma *et al.*, 2020). Oxidative stress caused by HMs induces damage to lipids, proteins and nucleic acids of cells. The present effect of SW + *Hyacinth* was obvious in shoot phenols of leafy vegetable (spinach) and root phenols of root vegetable (turnip), imparting 85.39% and 84.15% enhancement in phenols contents in spinach shoots and turnip roots, respectively. In plants, phenolics play a critical role in developmental processes like biosynthesis of lignin and pigments' biosynthesis and imparting structural safety and solidity support (Akram *et al.*, 2017; Kubalt, 2016). Uptake, translocation, and the sub-cellular localization of toxic metal ions have damaging consequences on major plant

functioning (Wani *et al.*, 2012). EDTA chelates toxic metal ions and enhances the synthesis of secondary metabolites, thereby assisting the crop plants in withstanding the SW-induced toxicity better. These phenolics mediate the defence responses of crop plants against the biotic and abiotic agents that cause various devastating diseases. Plant flavonoids constitute a structurally divergent class of secondary products involved in plant defence against biotic or abiotic stresses like phytoalexins, phytoanticipins, structural barriers and plant defence genes activators (Soshinkova *et al.*, 2013). The present investigation provides evidence favouring SW irrigation mediated induction of phenolic metabolism as a response to abiotic stress.

Abiotic stresses induce changes in the water relations, and in connection to that, compatible osmolytes have big roles. Among the compatible osmolytes- proline equilibrates the proteins and lipid membranes, scavenges free radicals, brings cellular and redox homeostasis (Dar *et al.*, 2016). In the current work, it was noted that all treatments significantly enhanced proline contents in both plants (spinach and turnip) during both stages of plant growth. In spinach, plants effect was much pronounced in SW + EDTA followed by SW + *Hyacinth*. On the other hand, SW + *Hyacinth* was the most effective treatment, followed by SW in turnip plants. Proline functions as a protein-compatible hydrotrope (Wani *et al.*, 2012; Abdallah *et al.*, 2020), thereby alleviating the acidosis in the cytoplasm and maintaining an appropriate ration of NADP/NADPH compatible with metabolism during stress (Chi *et al.*, 2019). Enhanced proline accumulation efficiently mitigates the damaging consequences of abiotic stresses providing the ability to withstand the damage (Akhkha *et al.*, 2019).

Besides this, the content of total protein and carbohydrates gets increased. The results also predicted the significant accumulation of the complete soluble proteins and carbohydrates in two crops. The increase of total soluble proteins and carbohydrates due to SW has been worked out by others (Guangqiu *et al.*, 2007; Aldoobie and Beltagi, 2013). Increased accumulation of carbohydrates, sugars, and the expression of specific proteins due to Zn, Pb, Cd, Ni and Cr application has been reported in *Phaseolus vulgaris* (Guangqiu *et al.*, 2007). In addition, increased phenol and carbohydrate content has been reported due to Zn, Cu and Cd stress in *Aegiceras corniculatum* (Udawat *et al.*, 2016). As observed in the present study, SW is also rich in certain toxic metal ions, leading to increased carbohydrate and protein accumulation in crops. Accumulation of specific proteins during stress exposure controls growth and development by integrating specific tolerance mechanisms, mediating response elicitation (Akhkha *et al.*, 2019). Proteins regulate key processes like scavenging of ions, responses to hypoxia, cellular mobility and cellular growth and development regulation during a range of stresses (Wang *et al.*, 2017; Vollmer and Bark, 2018; Akhkha *et al.*, 2019). In the present study, it was observed that the application of SW resulted in the expression of specific proteins. Application of SW alone and with EDTA or plant extracts to both spinach and turnip induced a significant increase in biomass of the two crops. EDTA has been extensively used for chelating agents, thereby enhancing the phytoextraction of various HMs. This obvious effect of EDTA was due to the improved bioavailability of metal ions in polluted soil. In the current research study, total proteins including biosynthetic proteins, are expressed in response to various treatments. Proteins have a prominent role in the resistance to biotic or abiotic stress, either healthy or challenged plants. The quantitative, qualitative and activity of proteins were determined using SDS-PAGE in response to plants with SW with EDTA or plant extract. It was observed that by using SDS-PAGE, new protein patterns were obtained, having a different density of bands under other treatments. Nevertheless, the ions of HM present in wastewater could exert toxic impacts by causing profound disturbances in vital processes like photosynthesis, biosynthesis of pigments, metabolism of proteins and membranes.

In the present work, it was observed that two crops (spinach and turnip) plants irrigated with SW exhibited significant accumulation of HMs ions like Pb, Zn, Cd and Ni in shoots at both growth stages over the control. On the other hand, EDTA and water plant extracts (*Hyacinth* or *Hedychium*) application considerably decreased the accumulation of HMs in plants irrigated with SW. In an experiment conducted at Calcutta, Mitra and Gupta (Brar *et al.*, 2000) reported that in radish, gourd, spinach, and cauliflower plants, the contents of HMs were comparatively higher in sewage irrigated plants than their respective controls. In leaves and tubers of potato plants cultivated under sewage irrigated soils, the contents of metals were more

elevated than groundwater irrigated soils (Abdallah, 2020). Root crops and leafy vegetables in comparison to grain crops accumulate higher amounts of HMs.

The total contents of Pb, Ni, Cd Cr and Fe were higher in soils irrigated with SW than soils irrigated with tap water. Mahata (2004) reported that the content of Pb was almost 11.5 times more in soils irrigated with lead battery effluent as compared to canal or tube well-irrigated soils. However, Antil (2004) found that the EC, organic C, and contents of Pb, Ni and Cd of soil increased on cycle industrial effluent compared to tube well-irrigated soils. The excess presence of effluents rendered productive land unproductive, and thus soil turned rusty and fluffy. The presence of toxic trace elements was also found high in this soil.

On the other hand, the effect of EDTA and water plant (*Hyacinth* or *Hedychium*) found that considerable decrease of accumulation of HMs contents in soil irrigated with SW. Concerning the effect EDTA and water plant (*Hyacinth* or *Hedychium*) on the irrigated plants with SW, it was found that EDTA shows considerable significant decreases in HMs (Pb, Zn, Cd and Ni) contents followed by *Hyacinth* and *Hedychium*, respectively. Aquatic plants like *Hyacinth* or *Hedychium* are known to uptake and accumulate contaminants in their tissues from the challenged habitats and are thus known as bio-accumulators [66]. They have high tolerance capacity against HMs as they absorb large them to a considerable extent. Previously it is reported that *Hyacinth* has a high potential to remove potentially toxic trace metals and other pollutants from aquatic habitats (Mahamadi, 2010). In addition, sewage purification by *Hyacinth* has not yet been executed to a larger extent in some parts of the world. The chelating aminopolycarboxylic acid, EDTA has been recorded to enhance tissue accumulation of HMs (Ebrahimi, 2013).

Conclusions

The present investigation demonstrated that using SW amended with EDTA or extracts of *Hyacinth* or *Hedychium* considerably improves crop and soil quality by reducing the accumulation of toxic metal ions (Pb, Ni and Cd). Results of the present study imply that using biological agents and eco-friendly approaches to lessen the sewage wastewater treatment mediated damage to crop growth and yield. Reduced accumulation of metals in edible plant parts due to EDTA and plant extract treatment can significantly enhance the quality of crops and protect human health.

Authors' Contributions

Data curation, G.S.H.A.; Formal Analysis, A.A.K., S.M., M.H.S. and A.M.A.; Funding Acquisition, M.H.S.; Investigation, A.A.K.; Methodology, A.A.K., A.A.A, M.S.A; Project Administration, H.S.M. and M.S.A; Resources, E.A.E, M.M.H; Supervision, A.A.K.; Writing-original draft, M.H.S. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abdallah MM-S, El Sebai TN, Ramadan AAE-M, El-Bassiouny HMS (2020). Physiological and biochemical role of proline, trehalose, and compost on enhancing salinity tolerance of quinoa plant. *Bulletin of the National Research Centre* 44(1):1-13. <https://doi.org/10.1186/s42269-020-00354-4>
- Aebi H (1984). Catalase *in vitro*. *Methods in Enzymology* 105:121-126. [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3)
- Ahanger MA, Aziz U, Alsahli A, Alyemeni MN, Ahmad P (2020). Combined kinetin and spermidine treatments ameliorate growth and photosynthetic inhibition in *Vigna angularis* by up-regulating antioxidant and nitrogen metabolism under cadmium stress. *Biomolecules* 10(1):147. <https://doi.org/10.3390/biom10010147>
- Ahanger MA, Gul F, Ahmad P, Akram NA (2018). Environmental stresses and metabolomics—Deciphering the role of stress responsive metabolites. *Plant Metabolites and Regulation Under Environmental Stress* 53-67. <https://doi.org/10.1016/b978-0-12-812689-9.00003-0>
- Ahanger MA, Tomar NS, Tittal M, Argal S, Agarwal R (2017). Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiology and Molecular Biology of Plants*, 23(4):731-744. <https://doi.org/10.1007/s12298-017-0462-7>
- Akhkha A, Al-Radaddi ES, Al-Shoaibi AK (2019). The impact of treated and untreated municipal sewage water on growth and physiology of the desert plant *Calotropis procera*. *Journal of Taibah University for Science* 13(1):746-754. <https://doi.org/10.1080/16583655.2019.1605650>
- Akram NA, Shafiq F, Ashraf M (2017). Ascorbic acid—a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in Plant Science* 8:613. <https://doi.org/10.3389/fpls.2017.00613>
- Aldoobie N, Beltagi M (2013). Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. *African Journal of Biotechnology* 12(29):4614-4622. <https://doi.org/10.5897/ajb2013.12387>
- Ali HM, El-Mahrouk E-SM, Hassan FA, El-Tarawy MA (2011). Usage of sewage effluent in irrigation of some woody tree seedlings. Part 3: *Swietenia mahagoni* (L.) Jacq. *Saudi Journal of Biological Sciences* 18(2):201-207. <https://doi.org/10.1016/j.sjbs.2010.08.001>
- Asgharipour MR, Azizmoghaddam HR (2012). Effects of raw and diluted municipal sewage effluent with micronutrient foliar sprays on the growth and nutrient concentration of foxtail millet in southeast Iran. *Saudi Journal of Biological Sciences* 19(4):441-449. <https://doi.org/10.1016/j.sjbs.2012.06.001>
- Balkhair KS, Ashraf MA (2016). Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi Journal of Biological Sciences* 23(1):S32-S44. <https://doi.org/10.1016/j.sjbs.2015.09.023>
- Bates LS, Waldren RP, Teare I (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* 39(1):205-207. <https://doi.org/10.1007/bf00018060>
- Brar M, Malhi S, Singh A, Arora C, Gill K (2000). Sewage water irrigation effects on some potentially toxic trace elements in soil and potato plants in northwestern India. *Canadian Journal of Soil Science* 80(3):465-471. <https://doi.org/10.4141/s99-106>
- Chen Y, Cao X, Lu Y, Wang X (2000). Effects of rare earth metal ions and their EDTA complexes on antioxidant enzymes of fish liver. *Bulletin of Environmental Contamination and Toxicology* 65(3):357-365. <https://doi.org/10.1007/s001280000136>
- Chi YH, Koo SS, Oh HT, Lee ES, Park JH, Phan KAT, . . . Chae HB (2019). The physiological functions of universal stress proteins and their molecular mechanism to protect plants from environmental stresses. *Frontiers in Plant Science* 10:750. <https://doi.org/10.3389/fpls.2019.00750>
- Cirilli M, Caruso G, Gennai C, Urbani S, Frioni E, Ruzzi M, . . . Muleo R (2017). The role of polyphenoloxidase, peroxidase, and β -glucosidase in phenolics accumulation in *Olea europaea* L. fruits under different water regimes. *Frontiers in Plant Science* 8:717. <https://doi.org/10.3389/fpls.2017.00717>
- Dar MI, Naikoo MI, Rehman F, Naushin F, Khan FA (2016). Proline accumulation in plants: roles in stress tolerance and plant development. *Osmolytes and plants acclimation to changing environment: emerging omics technologies*. Springer, pp 155-166. https://doi.org/10.1007/978-81-322-2616-1_9
- Diaz DH, Martin GC (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *American Society for Horticultural Sciences Journal* 97:651-654.

- Ebrahimi M (2013). Effect of EDTA application on heavy metals uptake and germination of *Echinochloa crus galii* (L.) Beave in contaminated soil. International Journal of Agriculture and Crop Sciences 6(4):197-202. <https://doi.org/10.1007/bf00386988>
- Elkelish AA, Soliman MH, Alhaithloul HA, El-Esawi MA (2019). Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism. Plant Physiology and Biochemistry 137:144-153. <https://doi.org/10.1016/j.plaphy.2019.02.004>
- Elloumi N, Belhaj D, Jerbi B, Zouari M, Kallel M (2016). Effects of sewage sludge on bio-accumulation of heavy metals in tomato seedlings. Spanish Journal of Agricultural Research 14(4):17. <https://doi.org/10.5424/sjar/2016144-9210>
- Foyer CH (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and Experimental Botany 154:134-142. <https://doi.org/10.1016/j.envexpbot.2018.05.003>
- García C, Hernández T, Costa F (1991). Study on water extract of sewage sludge composts. Soil Science and Plant Nutrition 37(3):399-408. <https://doi.org/10.1080/00380768.1991.10415052>
- Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48(12):909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Guangqiu Q, Chongling Y, Haoliang L (2007). Influence of heavy metals on the carbohydrate and phenolics in mangrove, *Aegiceras corniculatum* L., seedlings. Bulletin of Environmental Contamination and Toxicology 78(6):440-444. <https://doi.org/10.1007/s00128-007-9204-9>
- Guo L, Sims R (2000). Effect of meatworks effluent irrigation on soil, tree biomass production and nutrient uptake in Eucalyptus globulus seedlings in growth cabinets. Bioresource Technology 72(3):243-251. [https://doi.org/10.1016/S0960-8524\(99\)00115-7](https://doi.org/10.1016/S0960-8524(99)00115-7)
- Hasan M, Uddin M, Ara-Sharmeen I, Alharby F, Alzahrani H, Hakeem Y, Zhang L (2019). Assisting phytoremediation of heavy metals using chemical amendments. Plants 8(9):295. <https://doi.org/10.3390/plants8090295>
- Irigoyen J, Einerich D, Sánchez-Díaz M (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiologia Plantarum 84(1):55-60. <https://doi.org/10.1111/j.1399-3054.1992.tb08764.x>
- Isah T (2019). Stress and defense responses in plant secondary metabolites production. Biological Research 52. <https://doi.org/10.1186/s40659-019-0246-3>
- Jackson M (1973). Soil chemical analysis prentice hall of India. Pvt. Ltd. New Delhi, pp 498. <https://doi.org/10.1002/jpln.19590850311>
- Khan A, Anwar Y, Hasan M, Iqbal A, Ali M, Alharby HF, ... Hasanuzzaman M (2017). Attenuation of drought stress in Brassica seedlings with exogenous application of Ca²⁺ and H₂O₂. Plants 6(2):20. <https://doi.org/10.3390/plants6020020>
- Khan AA, Wang T, Hussain T, Ali F, Shi F, Latef AAHA, ... Zainab N (2021). Halotolerant-*Koccuria rhizophila* (14asp)-induced amendment of salt stress in pea plants by limiting Na⁺ uptake and elevating production of antioxidants. Agronomy 11(10):1907. <https://doi.org/10.3390/agronomy11101907>
- Kubalt K (2016). The role of phenolic compounds in plant resistance. Biotechnology and Food Sciences 80(2):97-108.
- Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680-685. <https://doi.org/10.1038/227680a0>
- Lindsay WL, Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal 42(3):421-428. <https://doi.org/10.2136/sssaj1978.03615995004200030009x>
- Masindi V, Muedi K (2018). Environmental contamination by heavy metals, heavy metals. In: Hosam El-Din MS, Aglan RF (Eds). IntechOpen. <https://doi.org/10.5772/intechopen.76082>
- Matta A, Dimond A (1963). Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology 53(5):574. <https://doi.org/10.1111/j.1439-0434.1988.tb00989.x>
- Nahar N, Shahadat HM (2021). Influence of sewage sludge application on soil properties, carrot growth and heavy metal uptake. Communications in Soil Science and Plant Analysis 52(1):1-10. <https://doi.org/10.1080/00103624.2020.1836201>
- Rattan R, Datta S, Chhonkar P, Suribabu K, Singh A (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. Agriculture, Ecosystems & Environment 109(3-4):310-322. <https://doi.org/10.1016/j.agee.2005.02.025>

- Sasaki N, Suehara K-I, Kohda J, Nakano Y, Yang T (2003). Effects of CN ratio and pH of raw materials on oil degradation efficiency in a compost fermentation process. *Journal of Bioscience and Bioengineering* 96(1):47-52. [https://doi.org/10.1016/s1389-1723\(03\)90095-8](https://doi.org/10.1016/s1389-1723(03)90095-8)
- Shahid A, Malik S, Zhu H, Xu J, Nawaz MZ, Nawaz S, . . . Mehmood MA (2020). Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment* 704:135303. <https://doi.org/10.1016/j.scitotenv.2019.135303>
- Sharma A, Kapoor D, Wang J, Shahzad B, Kumar V, Bali AS, . . . Yan D (2020). Chromium bioaccumulation and its impacts on plants: an overview. *Plants* 9(1):100. <https://doi.org/10.3390/plants9010100>
- Singh A, Agrawal M (2012). Effects of waste water irrigation on physical and biochemical characteristics of soil and metal partitioning in *Beta vulgaris* L. *Agricultural Research* 1(4):379-391. <https://doi.org/10.1007/s40003-012-0044-4>
- Singh A, Sharma RK, Agrawal M, Marshall F (2009). Effects of wastewater irrigation on physicochemical properties of soil and availability of heavy metals in soil and vegetables. *Communications in Soil Science and Plant Analysis* 40(21-22):3469-3490. <https://doi.org/10.1080/00103620903327543>
- Singh A, Sharma RK, Agrawal M, Marshall FM (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* 48(2):611-619. <https://doi.org/10.1016/j.fct.2009.11.041>
- Singh R, Agrawal M (2007). Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. *Chemosphere* 67(11):2229-2240. <https://doi.org/10.1016/j.chemosphere.2006.12.019>
- Soliman MH, Abdulmajeed AM, Alhathloul H, Alharbi BM, El-Esawi MA, Hasanuzzaman M, Elkelish A (2020). Saponin bioprimer positively stimulates antioxidants defense, osmolytes metabolism and ionic status to confer salt stress tolerance in soybean. *Acta Physiologiae Plantarum* 42(7):1-13. <https://doi.org/10.1007/s11738-020-03098-w>
- Soshinkova T, Radyukina N, Korolkova D, Nosov A (2013). Proline and functioning of the antioxidant system in *Thellungiella salsuginea* plants and cultured cells subjected to oxidative stress. *Russian Journal of Plant Physiology* 60(1):41-54. <https://doi.org/10.1134/s1021443713010093>
- Studier FW (1973). Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *Journal of Molecular Biology* 79(2):237-248. [https://doi.org/10.1016/0022-2836\(73\)90003-x](https://doi.org/10.1016/0022-2836(73)90003-x)
- Udawat P, Jha RK, Sinha D, Mishra A, Jha B (2016). Overexpression of a cytosolic abiotic stress responsive universal stress protein (SbUSP) mitigates salt and osmotic stress in transgenic tobacco plants. *Frontiers in Plant Science* 7:518. <https://doi.org/10.3389/fpls.2016.00518>
- Valipour M, Singh VP (2016). Global experiences on wastewater irrigation: challenges and prospects. *Balanced urban development: options and strategies for livable cities*. Springer, Cham, pp 289-327. https://doi.org/10.1007/978-3-319-28112-4_18
- Vollmer AC, Bark SJ (2018). Twenty-five years of investigating the universal stress protein: function, structure, and applications. *Advances in Applied Microbiology* 102:1-36. <https://doi.org/10.1016/bs.aambs.2017.10.001>
- Wang X-F, Su J, Yang N, Zhang H, Cao X-Y, Kang J-F (2017). Functional characterization of selected universal stress protein from *Salvia miltiorrhiza* (SmUSP) in *Escherichia coli*. *Genes* 8(9):224. <https://doi.org/10.1016/bs.aambs.2017.10.001>
- Wani AS, Irfan M, Hayat S, Ahmad A (2012). Response of two mustard (*Brassica juncea* L.) cultivars differing in photosynthetic capacity subjected to proline. *Protoplasma* 249(1):75-87. <https://doi.org/10.1016/bs.aambs.2017.10.001>
- Xie CZ, Healy T, Robinson P, Stewart K (2008). Determination of EDTA in dairy wastewater and adjacent surface water. Paper presented at the Proceedings of World Academy of Science, Engineering and Technology. <https://doi.org/10.1504/ijesd.2012.049189>
- Zaid A, Wani SH (2019). Reactive oxygen species generation, scavenging and signaling in plant defense responses. In: *Bioactive Molecules in Plant Defense*. Springer, pp 111-132. https://doi.org/10.1007/978-3-030-27165-7_7
- Zaynab M, Fatima M, Abbas S, Sharif Y, Umair M, Zafar MH, Bahadar K (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis* 124:198-202. <https://doi.org/10.1016/j.micpath.2018.08.034>
- Zeid I, Abou El Ghathe H (2007). Effect of sewage water on growth, metabolism and yield of bean. *Journal of Biological Sciences* 7(1):34-40. <https://doi.org/10.3923/jbs.2007.34.40>



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