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Biochemical Changes on Jute Mallow Plant Irrigated with Wastewater and Its Remediation

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Abstract

Owing to fresh water paucity, farmers are using wastewater for irrigation. This study was performed to assess whether application of *Spirulina platensis*, *Chlorella vulgaris*, leaves powder of salix and Ethylenediamine tetraacetic acid could ameliorate the harmful effects of wastewater on Jute mallow. In addition to estimating heavy metals accumulation in Jute mallow leaves (edible part). The results revealed that the contents of total soluble protein in shoot and root throughout the two growth stages, yield parameters and yield components were significantly reduced in wastewater-irrigated plant. The contents of malondialdehyde, free proline, total phenols, superoxide dismutase, catalase, peroxidase and polyphenoloxidase significantly increased in wastewater-irrigated plant than control. Wastewater-irrigated plant has higher levels of Ni, Cd, Pb and Co in leaves, which were beyond recommended limits set by international regulations and the higher rates of plant enrichment was Pb. In contrast, our treatments attenuated the adverse effects of wastewater on metabolic contents, malondialdehyde, non-enzymatic and enzymatic antioxidants, yield parameters and yield components of Jute mallow. Moreover, the concentration of Ni, Cd, Pb and Co in leaves were significantly decreased. Finally, these findings suggested that *Spirulina platensis* and *Chlorella vulgaris* treatments could be used for mitigating the harmful effects of wastewater on plants.
Keywords: Wastewater irrigation; Jute mallow; Heavy metals; Biochemical changes.

Introduction

Leafy vegetables come from very broad kinds of plants and they are commonly used for food, being riches origin of antioxidants, minerals and dietary fibers **(Duma et al., 2014).** Jute mallow (*Corchorus olitorius* L.) belonging to the family Tiliaceae is one of the most critical leafy vegetable in tropical areas like Egypt, Egypt farmed around 887 ha of Jute mallow and yielded around 2173 tons with a productivity of 2.45 ton/ha **(Haridy et al., 2019).** *Corchorus olitorius* are very rich in proteins, β-carotene, vitamins (A, B, C, E) and essential minerals. In addition, are also used in herbal pharmacopoeia **(Adebo et al., 2018).**

Irrigation with wastewater (WW) may affect either the biotic and abiotic components of the environment **(Qadir et al., 2010).** Farmers in developing zones where technologies for WW treatments are limited and in arid and semi-arid regions where fresh water source is rare who require water for irrigation frequently have no other options than using WW **(Keraita et al., 2008).** The repeated applications of untreated WW resulted in significant accumulation of heavy metals (HMs) in soil **(Ghosh et al., 2012)** and in plants and their following relocate to food chain, result possible health risk to consumers **(Singh et al., 2010).** Accordingly, there is an urgency need to develop novel eco-friendly, low cost and easy to use methods to decrease the accumulation of these metals to crops for safe and healthy food production.

In the recent years, several authors focused on the uses of algae to remove the different HMs from wastes. Algae are considered especially suited for HMs chelation due to their large surface/volume ratios and the presence of metal-binding groups on their cell surfaces **(Ahmad et al., 2020).** A number of algal species such as *Chlorella* sp, *Spirulina* sp

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has been used during HMs-chelation studies and showed varying removal efficiencies **(Çelekli and Bozkurt 2011; Carfagna et al., 2013).**

Naturally available plant biomass can be also used as a biosorbent material for removal of toxic HMs from contaminated wastes **(Sekhar et al., 2003).** Salix tree (*Salix alba*) extracts are rich in many bioactive and bio-stimulant compounds, including salicylic acid. Salicylic acid was investigated by authors and proved to be effective chelating agents for HMs **(Popova et al., 2012).**

Synthetic chelating agent such as Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for HMs removal because it has a strong chelating ability for different metals **(Oh & Yoon, 2014).**

The present study was conducted to assess whether application of dry algae powder (*Spirulina platensis* and *Chlorella vulgaris*), leaves powder of salix and EDTA could ameliorate the harmful effects of WW on Jute mallow. Investigating the physiological, biochemical and yield parameters. In addition to estimating HMs uptake and accumulation in Jute mallow leaves (edible part).

MATERIALS AND METHODS

Materials:

The seeds of Jute mallow (*Corchorus olitorius* L.) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Natural *Spirulina platensis* and *Chlorella vulgaris* in the form of algae powder were obtained from National Research Center, Giza, Egypt. Fresh leaves of salix plant (*Salix alba*) were obtained from Botanical Garden, Agriculture Research Center (ARC), Giza, Egypt. The leaves were air dried for few days till a constant dry weight, then crushed into a fine powder.

Water sampling:

Water samples were collected from each irrigation source (fresh tap water and wastewater effluent). Wastewater samples were collected from El-Rahawy drain (30°11`12.3”N latitude and 31°02′53.3”E longitude), Giza, Egypt (figure1A&B) which receives all sewage of El-Giza governorate in addition to agricultural and domestic wastes of El-Rahway village without treatment. The wastewater samples were collected in plastic bottles and used in irrigation.

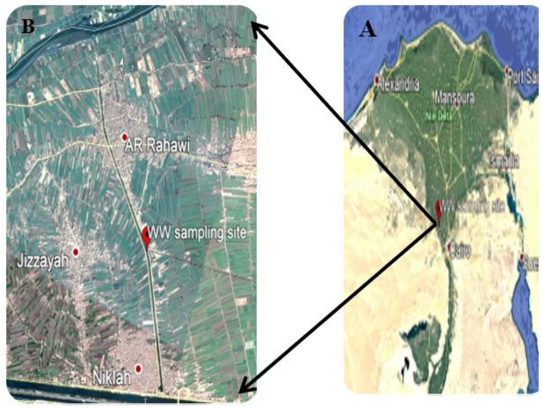

Figure (1A&B):Wastewater sampling site in El-Rahawy drain, Giza, Egypt.

Methods of planting, treatments and collection of plant samples:

A field experiment was conducted under the field conditions of the Botanical Garden, Botany and Microbiology Dept., Fac. of Sci., Al-Azhar Univ., Nasr City, Cairo, Egypt on the date 1.9.2020. The Jute mallow seeds were planted in pots (30 cm. width and 30 cm. length) containing 6.0 Kg. of clay soil. The pots were divided into 6 groups representing the following treatments:

I. Fresh tap water (control)

II. Wastewater

III; IV; V & VI. Each contains wastewater+ (*Spirulina platensis; Chlorella vulgaris*; salix and EDTA 3g/kg soil individually).

Plants of each group were treated with the above treatments (as chelate into soil) from the beginning of the experiment. The developed plants were irrigated whenever required until the complete germination. The plant samples were collected for analysis when the plants were 30 (Stage I) and 45 (Stage II) days old from planting. At the end of the growing season (100 days) analysis of the yield from the different treatments as well as the control were done.

Analysis Technique

Water Analyses

Heavy metals contents in fresh tap water and wastewater were estimated according to **(APHA, 1999),** Salinity (EC), total dissolved solids (TDs), and pH were measured (Table 1). At the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain shams university, Cairo, Egypt.

Soil Analyses

The collected soil for planting (Table 2) was air dried and digested using the acid digestion method adopted by **(Wade et al., 1992).** Soil reaction (pH), electrical conductivity (EC), total dissolved solids (TDs), Soluble cations and anions and HMs content were determined according to **(Page, 1982).** At the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain shams university, Cairo, Egypt.

Plant Analyses

Determination of metabolic contents

Contents of total soluble proteins were assayed according to the methods of **(Lowery et al., 1951).** Contents of free proline were estimated according to the method described by **(Bates et al., 1973).** Total phenolic compounds (mg/100 g of dry wt.) were carried out according to that method described by **(Daniel & George, 1972).** Total soluble carbohydrates in yield were estimated according to the method of **(Umbriet et al., 1959).**

Assays of Enzymes Activities and lipid peroxidation

Extraction of enzymes was according to **(Mukherjee & Choudhuri, 1983).** Superoxide dismutase (SOD) activities were estimated using the method of **(Marklund & Marklund, 1974).** Catalase (CAT) activities were assayed according to the methods of **(Aebi, 1983).** Peroxidase (POX) activities: were determined according to the method of **(Bergmeyer et al., 1974),** Polyphenoloxidase (PPO) activities were measured according to the methods of **(Kar & Mishra, 1976).** Lipid peroxidation was assayed as malondialdehyde (MDA) content in fresh leaves of Jute mallow according to that method described by **(Zhang et al., 2015).**

Determination of Plant Heavy Metals Contents

Heavy metals contents in the different samples of studied plants (edible plant part) were determined according to **(Parkinson & Allen, 1975).** At the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain shams university, Cairo, Egypt.

Enrichment Factor

Enrichment factor **(Barman et al., 2000)** has been calculated to determine the degree of heavy metal accumulation in the plants with respect to the control according to equation:

$$EF = \frac{\text{Concentration of metals in contaminated plants}}{\text{Concentration of metals in uncontaminated plants (control)}}$$

Statistical Analysis

Statistical calculations were done using computer programs Microsoft excel version 365 and SPSS version 25 and Minitab version 19 statistical programs. at 0.05 level of probability **(Snedecor & Cochran, 1982).** Quantitative data with parametric distribution were done using analysis of variance the One-way ANOVA and Post hoc-Tukey's test. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

Results

1.Metabolic Responses.

1.1.Total soluble proteins:

The present results (Figure 2) showed that compared to control, the total soluble protein contents were significantly decreased at stage I and stage II due to the irrigation with untreated wastewater by about 29% and 23% in shoot and by about 42% and 30% in root, respectively. However, treatments with *Spirulina platensis*, *Chlorella vulgaris*, salix and EDTA alleviated the wastewater toxicity and the highest value of total soluble protein in shoot and root at stage I and stage II was observed when treatment with WW+SA by about 9.8% and 41% in shoot at stage I and stage II, respectively. while in root was observed by about 131% at stage I and 108% at stage II as compared to control.

1.2.Free proline:

Our data in figure (3) showed that free proline contents were significantly stimulated in stage I and stage II due to the irrigation with untreated wastewater compared to control by about 92% and 63%, respectively. However, treatments with *Spirulina platensis*, *Chlorella vulgaris*, salix and EDTA alleviated wastewater-generated toxicity and the maximum reduction in free proline (except control) in stage I was observed by about 36% at WW+*Spirulina platensis* treatment, while in stage II was observed by about 38% at WW+SA treatment as compared to untreated wastewater irrigated plants.

Table (1): Physicochemical analyses of the water used for irrigation.

Parameters / Water samples	pH	EC (dS/m)	TDS (ppm)	Cations (meq L^{-1})				anions (meq L^{-1})				Macro and micro-nutrients (mg/L)						Heavy metals (mg/L)			
				Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Cl^{-}	CO_3^{2-}	HCO_3	SO_4^{2-}	P	S	Fe	Zn	Cu	Mn	Ni	Cd	Pb	Co
F.W	6.70	3.37	2156.80	6.00	4.80	0.16	22.75	24.25	0	0.60	8.85	4.81	6.68	0.12	0.01	0.04	0.09	0.05	0.03	0.02	0.05
W.W	6.50	10.11	6467.20	4.32	3.66	0.14	92.88	97.12	0	0.78	3.10	5.08	32.98	0.13	0.02	0.33	0.28	0.57	0.34	0.31	0.49

Table (2): Physicochemical analyses of the soil used for planting.

Parameters / sample	Soil texture	pH	EC (dS/m)	TDS (ppm)	Cations (meq kg^{-1})				Anions (meq Kg^{-1})				Macro and micro-nutrients (mg/kg)						Heavy metals (mg/kg)			
					Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Cl^{-}	CO_3^{2-}	HCO_3	SO_4^{2-}	P	S	Fe	Zn	Cu	Mn	Ni	Cd	Pb	Co
Soil	Clay	7.40	10.38	6640.00	4.78	1.28	0.28	97.41	98.02	0	1.66	4.07	19.15	35.46	0.43	0.13	1.37	0.39	0.20	0.01	0.93	0.04

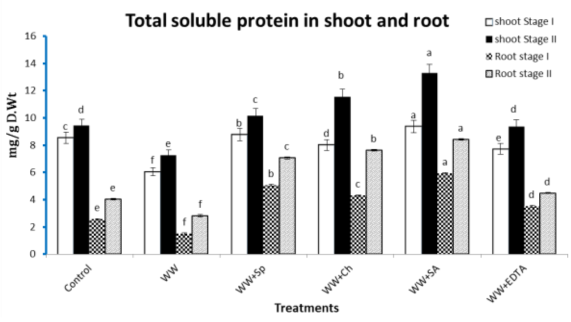

Figure (2): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on total soluble protein (mg/g. D.Wt) of Jute mallow plant. Each value is a mean of 3 replicates ± standard error of means. Different lower-case-letters are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05. Values of bars with the same letter are not significantly different.

1.3. Total phenols:

The present results (Figure 4) showed that the total phenol contents increased in Jute mallow plants that irrigated with untreated wastewater throughout the two growth stages, values were about 122% at stage I and 78% at stage II greater than the control. Conversely, the total phenol contents were considerably reduced by about 49% at stage I and about 42% at stage II when treatment with WW+ *Spirulina platensis* (except control) compared to plants irrigated with untreated wastewater.

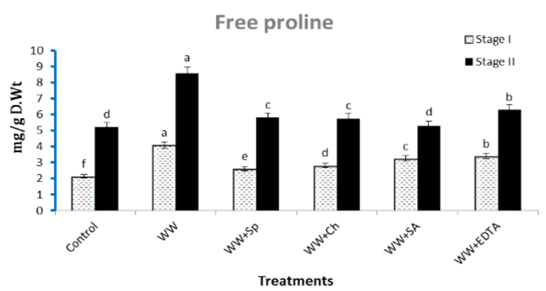

Figure (3): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on free proline (mg/g. D.Wt) of Jute mallow plant. Each value is a mean of 3 replicates ± standard error of means. Different lower-case-letters are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05. Values of bars with the same letter are not significantly different.

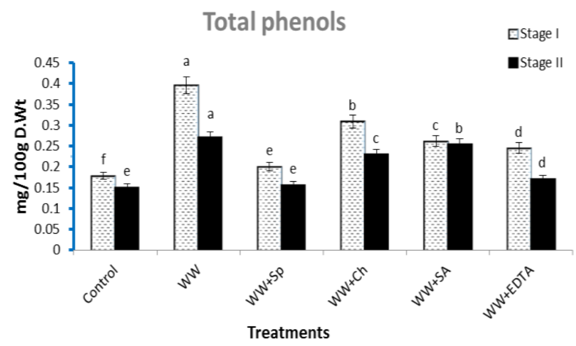

Figure (4): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on total phenols (mg/100g D.Wt) of Jute mallow plant. Each value is a mean of 3 replicates ± standard error of means. Different lower-case-letters are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05. Values of bars with the same letter are not significantly different.

2.Enzymes Activities

The activity of antioxidant enzymes increased in response to wastewater irrigation throughout the two growth stages (Figure 5 A,B,C&D). In case of stage I, the rise in activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) enzymes were about 133%, 83%, 147% and 408%, respectively under untreated wastewater irrigation as compared to control. likewise, in case of stage II the rise in activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) enzymes were 59%, 267%, 156% and 149%, respectively as compared to control.

. In contrast, treatment of Jute mallow plants with *Spirulina platensis*, *Chlorella vulgaris*, salix plant powder and EDTA reduced the highest activities of SOD, CAT, POD and PPO enzymes generated by wastewater toxic stress (Figure 5 A,B,C&D). In cases of SOD, CAT and POD enzymes except for the control, the maximum decreases in activities were recorded in *Chlorella vulgaris* treatments by about 78%, 50% and 40%, respectively at stage I and by about 58%, 64% and 35%, respectively at stage II as compared to untreated wastewater irrigated plants. likewise, in cases of PPO enzyme, the maximum decrease in the activity was recorded in WW+SA treatment (except control) by about 72% at stage I and about 54% at stage II as compared to untreated wastewater irrigated plants.

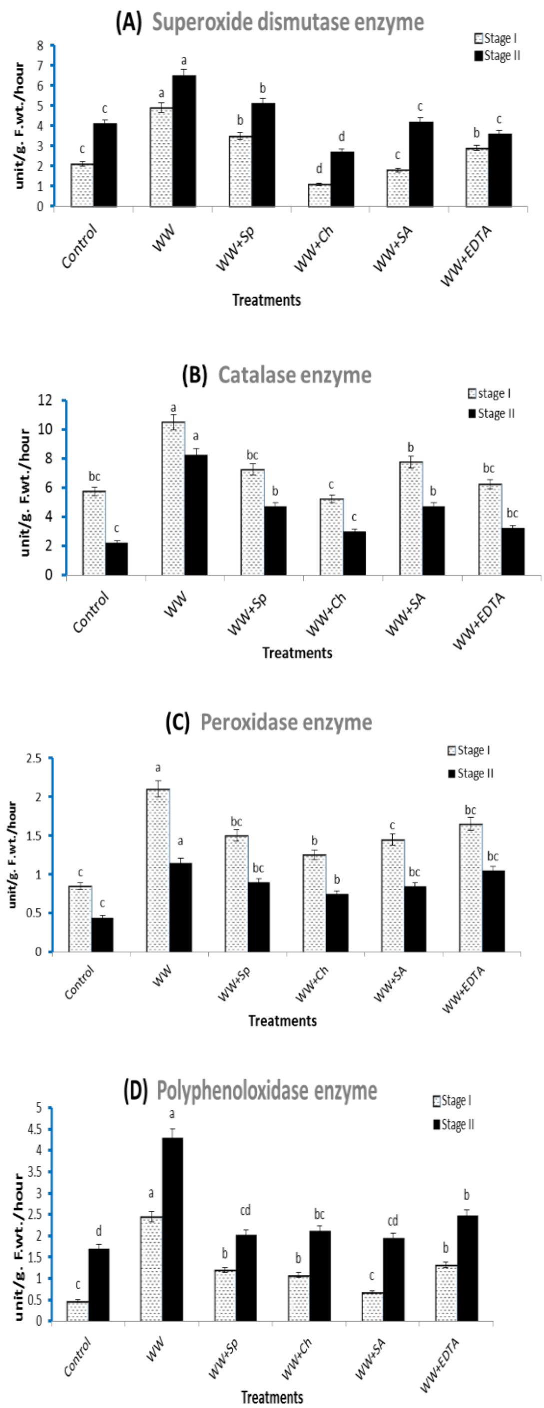

Figure (5): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on superoxide dismutase (A), catalase (B), peroxidase (C) and polyphenoloxidase (D) enzymes (unit/g F.wt./hour) of Jute mallow plant. Each value is a mean of 3 replicates ± standard error of means. Different lower-case-letters are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05. Values of bars with the same letter are not significantly different.

3.Lipid Peroxidation contents

The level of lipid peroxidation in leaves of Jute mallow was determined by malondialdehyde (MDA) contents at stage II (Figure 6). The total MDA content was significantly increased in case of WW irrigated plants by about 109% more than the control. Conversely, the lowest value of MDA content was observed when treatment with WW+Sp (except control) by about 45% compared to untreated WW irrigated plant.

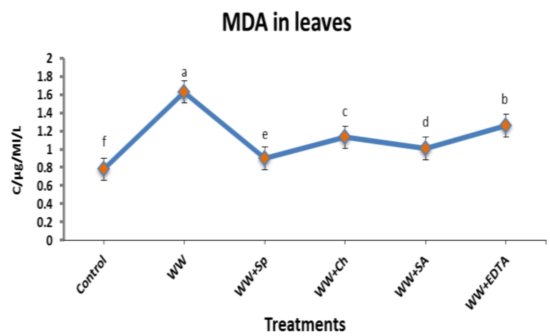

Figure (6): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on MDA contents (C/µg/Ml/L) in leaves of Jute mallow plant at stage II. Each value is the mean of 3 replicates ± standard error of means. Different lower-case-letters are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05. Values of line with the same letter are not significantly different.

4.Yield characters

Data in Table (3) showed compared to control samples that all tested yield parameters were decreased significantly under effect of WW irrigation. Where, the lowest values in the number of pods/plant, number of seeds/plant, weight of seeds (gm)/plant and weight of 1000 seeds (gm) were recorded when irrigated with wastewater which decreased by about 13%, 11%, 12%, 16% of control, respectively.

On contrary, a positive effects on all yield parameters were observed when treated with *Spirulina platensis*, *Chlorella vulgaris*, salix and EDTA compared to control (table 3). The highest value in the number of seeds/plant was recorded when the plants were treated with WW+Ch which increased by about 99% more than the control. The same trend was observed at number of pods/plant, weight of seeds (gm)/plant and weight of 1000 seeds (table 3) where, the highest values were recorded at WW+Sp treated plants which increased by about 53%, 43% and 32% more than the control, respectively.

Table (3): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on yield characters of Jute mallow plants. Each value is a mean of 10 replicates ± standard error of means.

Treatments	No. of pods / plant	No. of seeds / plant	weight of seeds (gm) / plant	weight of 1000 seeds (gm)
Control	1.50±0.38bc	196.00±3.92e	0.51±0.04cd	1.54±0.09c
WW	1.30±0.27c	174.00±3.74f	0.45±0.05d	1.30±0.11d
WW + Sp	2.30±0.29a	366.00±4.97b	0.73±0.02a	2.04±0.09a
WW + Ch	2.10±0.55ab	390.00±4.55a	0.65±0.07ab	1.81±0.10ab
WW + SA	2.00±0.31abc	305.00±4.32c	0.64±0.06ab	1.66±0.12bc
WW + EDTA	1.80±0.35abc	215.00±4.90d	0.58±0.04bc	1.77±0.11bc
HSD	0.75	9.94	0.10	0.23

Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05, values of the same column with the same letter are not significantly different.

5.Metabolic Constituents of Yield

Results in Figure (7) showed that the total soluble carbohydrates and total soluble proteins in seeds of untreated WW irrigated Jute mallow plant were reduced relative to control plants by about 24% and 5%, respectively. On contrary, the highest value of carbohydrate contents in seeds was recorded at WW+Sp treatment which increased by about 53% more than the control. Similarly, the highest value of protein contents in seeds was recorded at WW+SA treatments which increased by about 21% of control.

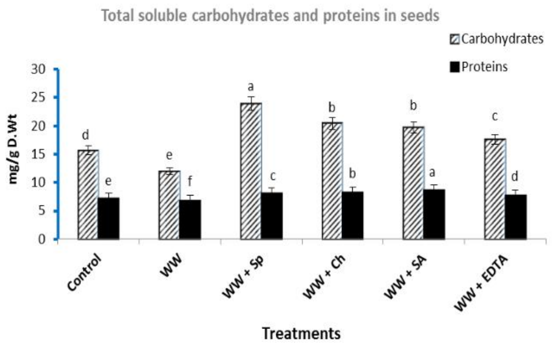

Figure (7): Effect of Fresh water, Wastewater, WW+*Spirulina platensis*, WW+*Chlorella vulgaris*, WW+salix plant powder and WW+EDTA on total soluble carbohydrates and proteins (mg/g. D.Wt) of Jute mallow seeds. Each value is a mean of 3 replicates ± standard error of means.

6.Heavy metals accumulation in leaves

The results of the heavy metal contents in Jute mallow leaves (edible part) are presented in Table (4). The accumulation of Ni, Cd, Pb and Co is greater in case of wastewater irrigated plants than control. Whereas, the lowest accumulation of Ni was detected under WW+*Chlorella vulgaris* treatment. The same trend, the lowest accumulation of Cd, Pb and Co were detected in case of treated plants with WW+Sp.

7.The Enrichment Factor

The enrichment of HMs in leaves of plant irrigated with untreated WW is as follows: Pb (12.63) > Co (11.38) > Cd (10.79) > Ni (7.64). Whereas, in case of WW+Sp treatment the enrichment of HMs in leaves of plant is as follows: Pb (24) > Ni (2.64) > Co (1.63) > Cd (1.43). In case of WW+Ch treatment the enrichment of HMs in leaves of plant is as follows: Pb (6.00) > Co (2.75) > Cd (2.29) > Ni (1.73).

In case of WW+SA treatment the enrichment of HMs in leaves of plant is as follows: Pb (5.89) > Cd (3.43) > Co (3.38) > Ni (3.00), and in case of WW+EDTA treatment the enrichment of HMs in leaves of plant is as follows: Pb (7.05) > Cd (6.86) > Co (4.88) > Ni (4.27).

Discussion

The shoot and root parts of jute mallow show lower protein content in wastewater-irrigated plant throughout the two growth stages as compared to control. These finding are supported by the studies of **Gupta et al. (2008)** and **Guo et al. (2007).** The decrease in total protein of the wastewater-irrigated plant probably corresponded with several effects due to the HMs stress **(Rizvi et al., 2020),** oxidative stress of ROS **(Gupta et al. 2010),** increased in ribonuclease activity **(Gopal & Rizvi, 2008)** or modification in gene expression **(Kovalchuk et al., 2005).**

Table (4): Heavy metals accumulation in leaves (edible part) of Jute mallow plants. Each value is a mean of 3 replicates ± standard error of means.

Treatments	Ni	Cd	Pb	Co
	mg/kg			
Control	0.011±0.001f	0.014±0.003e	0.019±0.005e	0.008±0.002d
WW	0.084±0.008a	0.151±0.009a	0.240±0.011a	0.091±0.007a
WW + Sp	0.029±0.005d	0.020±0.007e	0.097±0.002d	0.013±0.002d
WW + Ch	0.019±0.002e	0.032±0.003d	0.114±0.006c	0.022±0.004c
WW + SA	0.033±0.006c	0.048±0.003c	0.112±0.003cd	0.027±0.004c
WW + EDTA	0.047±0.005b	0.096±0.004b	0.134±0.006b	0.039±0.003b
HSD	0.003	0.008	0.016	0.006

Different lower-case-letters in the same column are significantly different by post hoc-Tukey's Honestly Significant Difference test (HSD) at P≤0.05, values of the same column with the same letter are not significantly different.

In contrast, total soluble protein contents in jute mallow were significantly increased in shoot and root by application of WW+Sp, WW+Ch, WW+SA and WW+EDTA throughout stages I and II compared to untreated WW and control plants. These results are supported by the study of **Akhtar et al., (2013).**

Free proline and total phenols were markedly stimulated due to the irrigation with untreated WW throughout the two growth stages compared to control. The higher promoting free proline and total phenol contents in response to WW irrigation may be due to the fact that free proline and polyphenols as a non-enzymatic antioxidants, plays an important role in protection against environmental stress like HMs **(Khan & Khan, 2017).** These results also are in agreement with the recent studies of **Đogić et al. (2017) and Khalilzadeh et al. (2020).**

On the other hand, the contents of free proline and total phenols were significantly decrease in response to WW+Sp, WW+Ch, WW+SA and WW+EDTA treatments compared to untreated wastewater-irrigated plant throughout stages I and II. These results indicate that the role of these treatments in alleviating the toxic effects of wastewater on plant. These results agreed with the recent studies of **Hemada et al. (2020)** and **Spain et al. (2021).**

Regarding the activities of SOD, CAT, POD and PPO enzymes, the higher activities of antioxidant enzymes SOD, CAT, POD and PPO in untreated WW irrigated plant at stages I and II are indication of the oxidative stress encountered by the plants as compared to control. The results are in agreement with **Cardoso et al. (2005)** who reported that plants protect themselves from metal toxicity by producing antioxidant enzymes that can act as scavenger for the toxicity of reactive oxygen species (ROS) produced by plants under HMs stress.

In contrast, the current results also demonstrated that the application of WW+Sp, WW+Ch, WW+SA and WW+EDTA reduced the activities of antioxidant enzymes throughout the two growth stages as compared to untreated wastewater irrigated plant. The decrease in the activities of SOD, CAT, POD and PPO enzymes may be attributed to the fact that the oxidative stress was confronted by our treatments and further increasing in the antioxidant enzymes was not required. These results in harmony with recent studies of **Lu et al. (2018)** and **Kumar et al. (2020).**

Lipid peroxidation can produce MDA when plants are under stress, and it is considered reliable indicators to the extent of oxidative injury **(Hu et al., 2012).** In the present study, the MDA increased and oxidative stress occurred in response to WW irrigation as compared to control. This is may be due to the inability of oxidative enzymes to reduce ROS levels and therefore not reducing the damage to the cell membrane. Our results are in agreement with previous study of **Yilmaz et al. (2017).** Recently, **Yildirim et al. (2019)** reported that exogenous application of Pb and Cd generally increased the contents of MDA in rocket plant.

Conversely, the MDA contents were significantly decreased after application of WW+Sp, WW+Ch, WW+SA and WW+EDTA as compared to WW irrigated plant. This may be due to the reduction of ROS production, and the extent of the antioxidant system and restoring. These results are supported by the recent study of **(Mohamed & Hassan, 2019).**

All yield parameters of Jute mallow plants significantly decreased in response to irrigation with untreated wastewater, which is in agreement with the recent finding of **(Kanwal et al., 2020).** Scientists are focused on developing and improved crops to produce more yields. So**,** the current research also investigate severe positive impacts of *Spirulina platensis, Chlorella vulgaris*, leaves powder of salix and EDTA treatments on Jute mallow yield. Significant variation in the treatments means were revealed. Maximum increased in yield attributes were noticed in those pots which treated with *Spirulina platensis* (in most cases) and *Chlorella vulgaris* (in some cases). Similar observation were enhanced by **Faheed & Fattah (2008)** showed that application of soil with 2 and 3 g dry algae/kg soil increased the yield parameters and mitigate the environmental stress. Few years ago, **Nawar & Ibraheim (2014)** reported that treatments of pea plant with algae improved the yield quality and productivity.

Data regarding metabolic constituents of yield revealed that irrigation with WW significantly affected the total soluble carbohydrate and protein contents in Jute mallow seeds compared to the control. Irrigation with untreated WW results in negative impacts in yield components. This result also was illustrated by **Hajihashemi et al. (2020).** However, the total soluble carbohydrates and proteins in seeds were significantly increased in response to the application of our tested treatments. The highest increased of total soluble carbohydrate and protein in Jute mallow seeds was recorded at S. *platensis* and SA treatments, respectively. Similar results also were obtained by **Enan et al. (2016). Huda et al. (2016)** stated that treating the rice with SA increased the protein contents. Recently, **Osman et al. (2020)** reported that treatments of broad bean seeds with algal extraction significantly increased the contents of carbohydrates and proteins.

In case of heavy metal accumulation, results from present study demonstrate that the higher rates of plant uptake and enrichment was Pb. Wastewater-irrigated plant has higher levels of Ni, Cd, Pb and Co in Jute mallow leaves than the maximum permissible value in food proposed by **FAO/WHO, (2001).** Similar to the present study, excessive accumulation of HMs under WW irrigation were reported by **Gupta et al. (2010)** in mustard, radish and taro plants; **Bamniya et al. (2010)** in *Brassica olerace* and *Spinacia oleracea*.

On the other hand, the accumulation of Ni, Cd, Pb and Co were significantly decreased by application of *Spirulina platensis*, *Chlorella vulgaris*, leaves powder of salix and EDTA on Jute mallow leaves. Similar results were recorded by **Ewais et al., (2015)** in sugar beet and **Lu et al., (2018)** in *Lemna minor*. Likewise, Significant variation in the treatments means were revealed. The maximum decreased in Ni, Cd, Pb and Co contents were noticed in those plants which treated with *Spirulina platensis* and *Chlorella vulgaris*. Similar observation were enhanced by the recent study of **Abdel-Razek et al., (2019)** reported that *C. vulgaris* and *S. platensis* can chelate some heavy metals from wastes at up to 88% with efficacy.

Conclusion

In conclusion, this study showed irrigating Jute mallow with untreated wastewater resulted in significant accumulation of heavy metals (Ni, Cd, Pb and Co) at leaves (edible part) of the Jute mallow plant. The higher rates of plant uptake and enrichment was Pb. Results of the physiological and biochemical parameters showed that the total soluble protein in shoot and root markedly reduced in untreated WW irrigated plant less than control plant at stage I and stage II. Furthermore, irrigating Jute mallow with untreated WW markedly increased the levels of lipid peroxidation and MDA contents which was accompanied by the significant increasing of non-enzymatic (free proline and total phenols) and enzymatic (SOD, CAT, POX and PPO) antioxidants activities throughout the two growth stages. All yield parameters, total soluble carbohydrate and protein contents in seeds markedly decreased. On the other hand, it is inferred from the results of present study that the application of *Spirulina platensis, Chlorella vulgaris,* salix plant powder and EDTA alleviated the toxic effects of WW via significantly decreased the accumulation of Ni, Cd, Pb and Co in edible plant part. In the meantime, the total soluble protein in shoot and root markedly increased throughout the two growth stages. The MDA, free proline and total phenols contents and SOD, CAT, POX and PPO activities significantly decreased throughout the two growth stages. All yield parameters, total soluble carbohydrate and protein contents in seeds markedly increased.

Finally, the results entail that *Spirulina platensis* and *Chlorella vulgaris* treatments were more significantly great in reducing the accumulation of HMs in leaves and improving the biochemical contents of Jute mallow. So, from the obtained results it is recommend that *Spirulina platensis* and *Chlorella vulgaris* treatments could be used as an eco-friendly applications, for mitigating the toxic effects of wastewater irrigation on plants.

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