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RESEARCH ARTICLE

Ameliorative role of foliar Zn-lysine application on wheat (*Triticum aestivum* L.) stressed by Tannery Wastewater

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Abstract Tannery industries discharge a high concentration of chromium (Cr) along with other heavy metals, which are hazardous for all life forms. With increasing shortage of freshwater, tannery effluent is frequently used for crop an irrigation, causing damage to plants' health. In order to address this challenge, amino acid chelate fertilizer was used to investigate the impact on wheat crops against tannery waste water. Tannery wastewater (TW) was used at different levels such as 0%, 25%, 50%, and 100% with an amendment of foliar Zn-lysine (Zn-lys) at30 mg/L. This research highlighted the positive correlation of Zn-lysine on the morpho-physiological, biochemical, and gas exchange traits under different levels of tannery wastewater. The findings of this study showed that the application of Cr-rich tannery wastewater at different treatment levels resulted in a significant reduction in plant height (23%, 31%, and 36%), the number of tillers (21%, 30%, and 43%), spike (19%, 36%, and 55%) and dry weight (DW) of grains (10%, 25%, and 49%) roots DW (17%, 41%, 56%), and shoots DW (22%, 32%, and 47%) as compared to control. Foliar-applied Zn-lys positively enhanced photosynthetic attributes, antioxidant enzymes activities and gas exchange traits by reducing the oxidative stress alone and under Cr stress. The concentration of Cr in roots (21%, 37%, 38%) and shoots (11%, 36%, 37%) was reduced by the foliar application of Zn-lys at different treatment levels. These findings conclude that Zn-lys served as a protector for the growth and development of wheat and has an incredible potential to inhibit the phytotoxicity induced by excess Cr.

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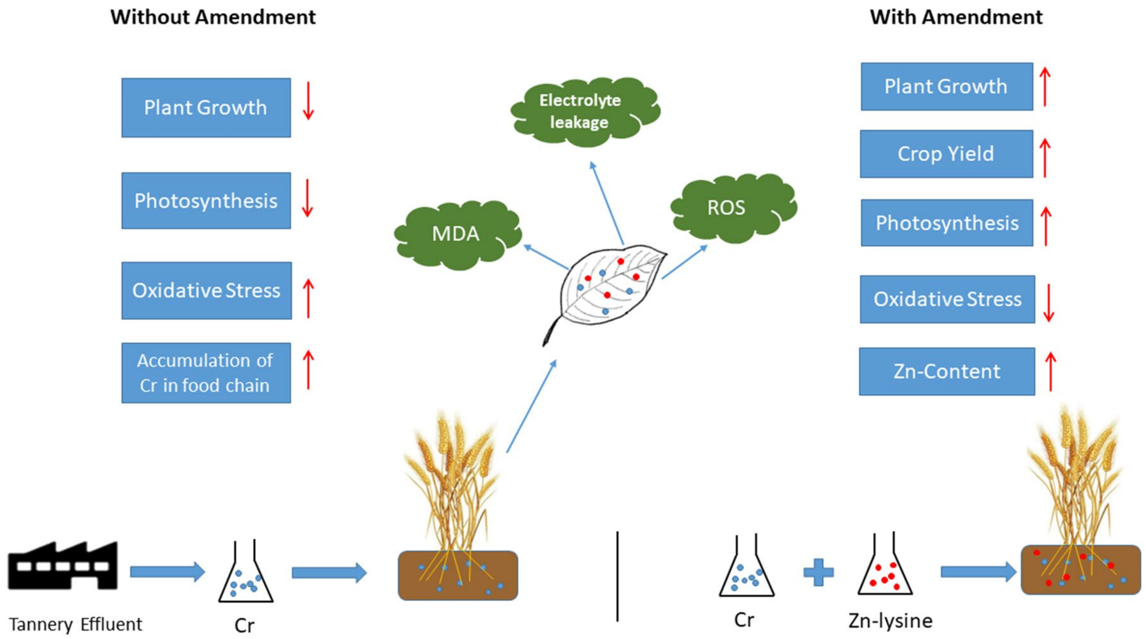
Graphical abstract



Keywords Chromium · Zn-lysine · Photosynthetic · Oxidative stress · Phytotoxicity · Wheat

Introduction

For the last few years, the over-growing population and increased industrialization and urbanization have generated unprecedented levels of heavy metals (HMs). Heavy metals have posed a major challenge to living beings as well as a threat to plant survival (Ashraf et al. 2017). Tanneries have contributed a lot to soil and water contamination in different areas of Pakistan, especially central Punjab including the Sialkot district and have widely released persistent toxins including the HMs (Khalid et al. 2017). Agricultural land of Sialkot city is being used for the cultivation of edible crops and due to the extreme scarcity of freshwater; agricultural practices use 70% of produced tannery wastewater for irrigation purposes direct after the primary treatment (Kamran et al. 2019). Generally, arable farmland doesn't always contain high levels of metals, but when irrigated with industrial wastewater, it raises the level of HMs. Furthermore, irrigated land with tannery wastewater is a menace to plant health as well as human health due to its transfer and accumulation into the food chain from lower to higher trophic levels (Li et al. 2018; Sarfraz et al. 2022). Amongst these HMs, tannery wastewater has discharged a high influx of Cr, considered as a hazardous and toxic element for the environment. It has also been reported that HMs pose a danger to the entire ecosystem and could contribute to catastrophic health consequences (Zaheer et al. 2020a). Although wastewater carries micronutrients that make the plants growth easier, it also usually helps to minimize various fertilizers containing N, P, and K but the prominent factor discharged from the leather processing industries is the presence of excessive Cr (Hussain et al. 2018).

Wheat (*Triticum aestivum* L.) is known as bread wheat that is generally consumed worldwide especially in Pakistan as a staple food; hence it is a key-player in traditional human healthcare (Rizvi et al. 2020). Sialkot is the land of several tannery factories and half of its land is being used for agricultural purposes. Wastewater irrigation is a common practice due to inadequate and low freshwater supply. When wheat accumulates HMs, stress triggers different responses and results in detrimental consequences such as a loss of yield (StanišićStojićet al. 2016). The excess Cr in the aerial parts of the plant show inhabitation of plant growth (Maqbool et al. 2018). Plants uptake Cr from contaminated soils along with several other essential components (Farid et al. 2018). Moreover, excess Cr accumulation induces oxidative stress and morpho-physiological reduction (Zaheer et al. 2019), enhanced electrolyte leakage, increased activities of antioxidant enzymes (Imran et al. 2019) and distortion of the plant cell. Moreover, the intake of Cr through Cr-contaminated food may cause severe illness and health issues like ulcer, itching, liver disease and lung cancer (Farid et al. 2018).

Various approaches can help to reduce Cr accumulation in plant species and alleviate HM stress. One of the remedial

Table 1 Physicochemical properties of soil used in the experiment

Attributes	Values
pH	7.01
EC (dS/ cm)	0.86
NaCl (%)	5.16
Bicarbonates (mg L^{-1})	113
Chlorides (mg L^{-1})	65.9
Mn (mg kg^{-1})	0.008
Mg (mg kg^{-1})	0.96
K (mg kg^{-1})	11.2
Na (mg kg^{-1})	111.4
Ca (mg kg^{-1})	16.4

approaches, amino acid (lysine) as a chelate, has been introduced in recent years to impede heavy metals absorption and enhances plant growth and production (Saleem et al. 2020). Zinc (Zn) is an essential element, and its deficiency has shown a negative response to plant growth, yield, and nutritional content. Lysine is also an essential component that makes proteins. Lysine increases the nutritional quality of plants. Thus, scientists have introduced the Zn-lys method that has the potential to work efficiently under plant stress conditions. Zn chelated fertilizers with lysine are environmentally sustainable, boost crop growth and quality by fortifying Zn and reduce HMs levels (Rafie et al. 2017). It might be the competition between metals and essential nutrients as Zn makes the complex with lysine and translocate more efficiently than the Cr (Zaheer et al. 2022). Numerous studies have been performed in the past on foliar Zn-lys fertilization for various crops such as rice and spinach under excess Cr (Bashir et al. 2018; Zaheer et al. 2020a, b) and for wheat under excess Cd (Rizwan et al. 2017).

However, there is no research on the effect of Zn-lys on wheat plant under tannery wastewater. Therefore, this experiment investigated (1) the impact of excess Cr in the wheat crop irrigated with tannery wastewater in soil. (2) The ameliorative role of amino acid-chelated nutrients (Zn-lys) was accessed. (3) Various considerations, such as morpho-physiological and biochemical characteristics, root and shoot Cr and Zn concentrations of the wheat plants were investigated to evaluate the role of Zn-lys chelate alone and under tannery wastewater. (4) To observe the impact of Zn-lys on wheat plant against oxidative stress.

Materials and methods

Sample collection

The soil was collected (0–20 cm depth) from an agricultural field, far from the industrial or contaminated area. The soil was sieved at 2 mm to remove undesirable particles. Table 1 includes complete detail of the physicochemical characteristics of the soil used in this study. Seeds were taken from Punjab Seed Corporation, Gujranwala Center, Punjab, and tannery wastewater was collected from a leather field company located on Wazirabad Road, Sialkot. Solutions for treatments were prepared by adding tannery wastewater (TW) to distilled water. Various physic-chemical characteristics of tannery wastewater were analyzed by following the method of APHA (2005) and results are given in Table 2.

Table 2 Physic-chemical parameters of tannery wastewater measured before utilization in the experiment

Attributes	Tannery water constituents	Permissible values
Color	Black grey	–
pH	7.7	6.5–8.4
EC(μS/cm)	2.4	< 1.5
Total dissolved solids (TDS)	24,377.5	< 450
Sodium Adsorption Ratio (SAR)	28.9	< 7.5
Zn mg/L	2.25	2.00
Cr mg/L	4.21	0.10
Ni mg/L	0.25	0.20
Pb mg/L	1.25	5.00
Co mg/L	Nil	0.05

Permissible values: Ayers and Westcot (1985); Hassan et al. 2013

Experimental setup and treatments

A pot experiment was conducted for this study. The experiment was carried out with wheat as a test plant under atmospheric conditions with 24/12 °C day/night temperature at the time of seed sowing and 36/20 °C at the harvesting time period. The study design was a completely randomized design (CRD) since three replicates were used for each treatment. Every porcelain pot was loaded with 6 kg of soil and was sown with seven seeds and only five seedlings remained in each pot after thinning. Different levels of tannery wastewater (0, 25, 50, and 100%) were used along with the foliar application of Zn-lys (30 mg/L). During the three months of the growth period, a 1-L volume of tannery wastewater was administered to the plants every week. Plants were thoroughly sprayed with deionized water in the control treatment, and a 2-L of Zn-lysine was applied via six foliar applications per treatment. Moreover, in the entire experimental phase, the Zn-lys was applied at specific intervals, i.e., 2nd, 4th, 6th, 8th, 10th, and 12th week. A specific volume of nutrients in the form of urea (N), diammonium phosphate (P), and potassium sulfate (K) were supplied in the pots to provide adequate plant nutrients as described in Rizwan et al (2017). Pots were rotated frequently to minimize the spatial impact on the plants; weeds were removed manually and crushed into the same pot. After 90 days of treatments, plants were then separated between roots and shoots. Roots were washed vigorously with distilled water to eliminate soil particles.

Different levels of tannery wastewater (TW) (0, 25, 50, 100%) and one level of Zn-lys (30 mg/L) were applied in the following combinations with three replicates for each treatment, T_1: Control, T_2: 0% TW + 30 mg/L Zn-lys, T_3: 25% TW, T_4: 25% TW + 30 mg/L Zn-lys, T_5: 50% TW, T_6: 50% TW + 30 mg/L Zn-lys, T_7: 100% TW, T_8: 100% TW + 30 mg/L Zn-lys.

Morpho-physiological characteristics

Plant height data was recorded right after harvesting and then plant parts were dried in the oven at 70 °C for 72 h for dry weight measurement by using the weighing balance in grams (g). Tillers were measured using a measuring tape. Collected tillers were dried and weighed on a digital balance to determine the dry weight of spikes in g. These spikes were threshed separately, and the grains were dried and counted using a top-loading weighing balance in g. Roots were washed with 1.0% hydrochloric acid (HCl) to eliminate Cr adhered to the root surface. Moreover, fresh leaves of a certain weight (0.5 mg) were crushed into powder form and stored in bottles containing 80% acetone to extract leaf pigments. The sample bottles were placed in the cool dark for 24 h and thereafter placed in a centrifuge machine at 10,000 rpm for 5 min. The supernatant solution was used to analyze pigments at a particular wavelength (480, 645, and 663 nm) by using a spectrophotometer (Halo DB20/DB-20S, Dynamica Labs, London, UK). Chlorophyll content was estimated by using the equation of Lichtenthaler (1987). After 90 days of sowing, the gas exchange parameters, i.e., photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency were determined by using Infra-Red Gas Analyzer (IRGA, CI-340, Analytical Development Company, Hoddesdon, England) on a bright sunny day from 10 to 11 am, when plants were photosynthetic ally active.

Measurement of electrolyte leakage (EL) and malondialdehyde (MDA)

Fresh leaves (50 mg) were taken after 90 days, put in 10 mL of distilled water in glass test tubes, and then EL (EC_1) was measured by autoclaving the sample for 2 h at 32 °C. Afterward, the same procedure was carried out again by placing the samples at 121 °C for 20 min and EC_2 was measured. Finally, leaf EL was calculated by the following equation (Dionisio-Sese and Tobita 1998).

$$\mathrm{EL} = \left(\mathrm{EC}_1/\mathrm{EC}_2\right) \times 100$$

MDA concentration was measured according to Heath and Packer (1968) with modifications suggested by Dhindsa et al. (1981) and Zhang and Kirkham (1992). Fresh leaves (0.25 mg) were homogenized with 0.1% thiobarbituric acid 5 mL and centrifuged (10 min, 12,000 g, 4 °C). After this, 4 mL 20% trichloroacetic acid in 0.5% thiobarbituric acid was added to 1 mL supernatant. The solution was kept in boiling water for 30 min then quickly cooled in the ice bath and centrifuged (10,000 g, 10 min). The absorbance of the mixture was measured at 532 nm, 600 nm and the value for nonspecific absorbance at 600 nm was subtracted from 532 nm. Then the concentration of MDA was calculated through an extinction coefficient of 155 $\mathrm{mM}^{-1}\,\mathrm{cm}^{-1}$.

Measurement of antioxidant enzymes activities

The activities of antioxidant enzymes in the leaves of the wheat plant were measured after 90 days when the variation between treatments was noticeable. The concentration of ascorbate oxidase (APX) and catalase (CAT) was measured by following the methodology suggested by Nakano and Asada (1981) and Aebi (1984) respectively. Similarly, the activities of superoxide dismutase (SOD) and peroxidases (POD) were measured by following the protocol suggested by Zhang (1992).

Heavy metals (Zn and Cr) concentration in plant parts

After completion of the experiment (90 days), 1.0 g of dry samples of roots and shoots were taken in the flask having 20 mL of HNO_3. The mixture was heated for 20 min by slowly raising the temperature to 250 °C on the hot plate. After that, the solution was cooled; 10 mL of $HClO_4$ was added, and then the mixture was heated until it was colorless by adding hydrogen peroxide (H_2O_2) drop by drop. After cooling, the mixture was filtered using a filter paper (Whatman no 1) and the solution was made up to 100 mL by adding distilled water (Greenberg et al. 1998). The concentration of Zn and Cr was determined by the Atomic Absorption Spectrophotometer (AAS) (Model: Savant AA, Australia).

Statistical analysis

Data obtained from all treatments were presented as an average of three replicates ± S.D. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize considerable variance by using Statistix 10.0 version software.

Table 3 Effect of different concentrations of tannery wastewater alone and/or in combination with Zn-lys on agronomic traits of wheat

Treatments	Tannery effluent Concentration			
	T.E 0	T.E 25%	T.E 50%	T.E 100%
	Plant Height (cm)			
0 mg/L Zn-lys	87.33 ± 1.67b	67.56 ± 1.80e	59.90 ± 1.56f	55.80 ± 0.50 g
30 mg/L Zn-lys	93.65 ± 0.91a	83.26 ± 1.33c	77.63 ± 0.66d	71.53 ± 1.20e
	Shoot Dry Weight (g)			
0 mg/L Zn-lys	29.30 ± 0.67b	22.87 ± 1.11d	19.99 ± 0.27e	15.40 ± 0.93f
30 mg/L Zn-lys	34.66 ± 1.19a	27.80 ± 0.24bc	25.53 ± 0.94c	19.48 ± 0.81e
	Root Dry Weight (g)			
0 mg/L Zn-lys	19.62 ± 0.55b	16.19 ± 0.74 cd	11.61 ± 0.72e	8.59 ± 0.57f
30 mg/L Zn-lys	23.51 ± 0.60a	18.29 ± 0.41bc	17.69 ± 1.18bc	14.53 ± 1.09d
	Spike Dry Weight (g)			
0 mg/L Zn-lys	3.20 ± 0.09b	2.60 ± 0.10d	2.04 ± 0.05f	1.42 ± 0.06 g
30 mg/L Zn-lys	3.93 ± 0.15a	2.92 ± 0.04c	2.33 ± 0.06e	1.95 ± 0.06f
	Grain Dry Weight (g)			
0 mg/L Zn-lys	16.35 ± 0.21b	14.70 ± 0.18c	12.25 ± 0.13d	8.33 ± 0.11f
30 mg/L Zn-lys	17.93 ± 0.15a	15.86 ± 0.06b	14.60 ± 0.34c	10.33 ± 0.57e

Data was recorded after the 90 days of seed sowing to the completion of treatments. Values are demonstrated as means of three replicates along with standard deviation. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize the significant difference at $P < 0.05$. Different small letters indicate that values are significantly different at $P < 0.05$

Results

Morphological traits

Alterations in various growth parameters like plant height, root, and shoot DW was estimated at different tannery wastewater treatment levels with and without foliar application of Zn-lys. The data on growth and biomass characters of *T. aestivum* is given in Table 3. According to this study, chlorosis was observed in T_3, T_5 and T_7 (25%, 50%, and 100% TW only) and compared to the controls and Zn-lys treated plants. However, the toxic content of Cr had a significant effect on plant height, root, and shoot DW relative to the control plant T_1 (irrigated without wastewater). The plants treated with Zn-lys without tannery wastewater in T_2 enhanced the morphological parameters compared with T_1. A highly significant reduction was observed in the tannery wastewater only irrigated plants (T_3, T_5 and T_7) however, the application of Zn-lys enhanced growth and biomass parameter in $T_{4,}$ T_6 and T_8 under tannery wastewater. Maximum plant height (87%) was observed in $T_{4,}$ and the lowest (69%) in T_5 compared with the combined application of tannery wastewater and Zn-lys. Consequently, the foliar-applied Zn-lys is advantageous to the plant's health and has often strengthened the morphological characteristics. Although the significant feature was obvious in this research when plant was treated with T_4 (25% TW + 30 mg/L Zn-lys), it showed a slight reduction in both growth and biomass parameter relative to the same dosage of Zn-lys in T_6 and T_8. It could be attributed to an increasing level of tannery wastewater. Overall, the lowest plant height, root, and shoot DW, spike and grain DW decreased significantly as compared to controls by 36%, 43%, 55%, 49% and 56% in T_7 (100% TW + 0 mg/L Zn-lys) respectively, in contrast, under the same level of tannery wastewater with Zn-lys, the growth and biomass attribute dramatically improved. The number of tillers reduced significantly under tannery effluents with a tendency to increase when Zn-lys was applied in combination with all tannery wastewater treatments (Table 3).

Photosynthetic pigments and gas exchange parameters

The increased concentration of Cr processed water decreased drastically the chlorophyll content of all treatments relative to T_1 and T_2 (Fig. 1). The deterioration of biochemical characters in the leaves of the stressed plant was noted. Consequently, the maximum chlorophyll a and b content was found in T_2, and the minimum in T_7. And also the same findings were associated with gas exchange parameters (photosynthesis rate, transpiration rate, stomatal conductance, and water use efficiency). In addition, when plants were processed without Zn lysine in $T_{3,}$ T_5 and T_7, the chlorophyll content and gas exchange characteristics decreased significantly as a result of increased TW levels. Moreover, the same treated wastewater concentration (100% TW) in both T_7 and T_8 but, in T_8, the biochemical characteristics quite increased considerably compared to T_7. Though, it is linked to the presence of Zn-lys in T_8 (100% TW + 30 mg/L Zn-lys). Although this is also the same in the

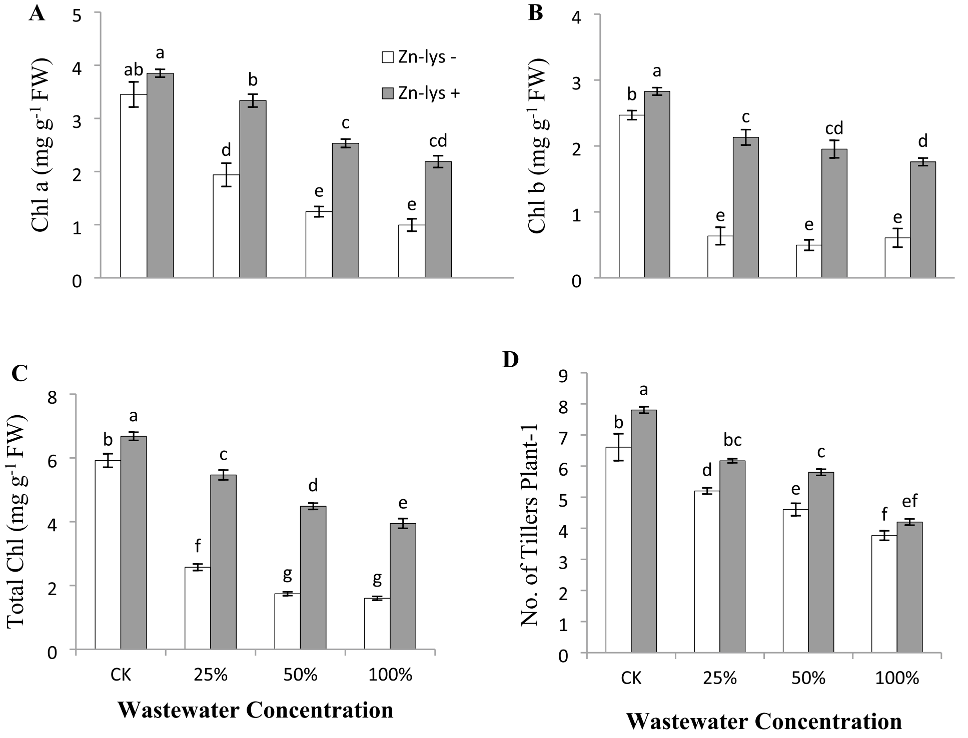


Fig. 1 Effect of tannery wastewater and Zn-lys on chlorophyll a (**A**), chlorophyll b (**B**), total chlorophylls (**C**), and number of tillers (**D**) in wheat with increasing wastewater concentrations (0, 25, 50, and 100%) treated or not with 30 mg/L Zn-lys. Data was recorded after the 90 days of seed sowing to the completion of treatments. Values are demonstrated as means of three replicates along with standard deviation. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize the significant difference at $P < 0.05$. Different small letters indicate that values are significantly different at $P < 0.05$

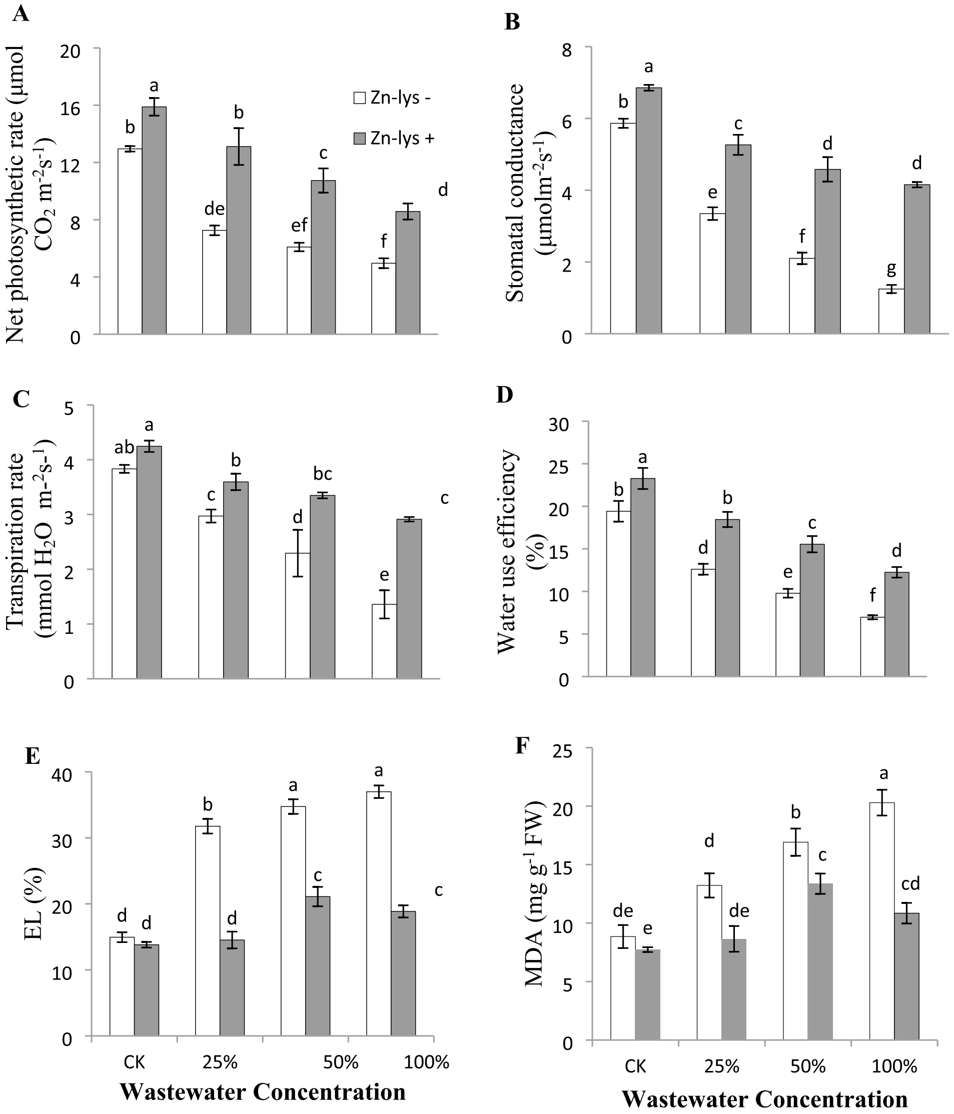
case of T_3 and T_5 which are treated alone with tannery wastewater shows drastic changes in biochemical and gas exchange attributes but when treated with the same level of TW with the combination of Zn-lys in T_4 and T_6 it improved the photosynthetic pigments and gas exchange parameters. Compared to the solo application of wastewater with the combined application of TW and Zn-lys, the impressive fact was that the amino acid chelated nutrient mitigated the dramatic impact of Cr in wheat plants, as seen in Fig. 2A–D. The highest photosynthetic and gas exchange attributes observed in the T_2 were treated with distilled water and Zn-lys except that a minor reduction was found in T_4 (25% TW + 30 mg/L Zn-lys) compared to the control. Application of Zn-lys was the same in $T_{4,}$ $T_{6,}$ and T_8 and, thus the chlorophyll content and gas exchange characteristics decreased massively in T_8 leading to enhanced Cr toxicity in plants. Moreover the percentage of chlorophyll a, chl b, photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency under 25% TW with 30 mg/L Zn-lys was as follows: 96%, 86.5%, 98%, 93%, 89% and 95% respectively. At 50% wastewater treatment with 30 mg/L Zn-lys was as follows 73%, 79%, 81%, 87%, 78% and 80% and at 100% TW with 30 mg/L Zn-lys was as: 63%, 71%, 65%, 78%, 72% and 63% respectively.

Effect of Zn-lysine on EL and MDA and antioxidant enzyme activities

Under the Zn-lysine application, the present results showed lower electrolyte leakage (EL) and malondialdehyde (MDA) content (Fig. 2E–F). The high content of MDA and EL was seen in T_7 at 100% wastewater. The addition of Zn-lys under 100% TW significantly lowered the MDA and EL production. Amino acid chelated nutrient (Zn-lys) has demonstrated a favorable response towards oxidative stress in the wheat plant. The maximum EL and MDA were in T_7 whereas; the minimum was in T_4 compared to T_1 and T_2. Therefore, the data in Fig. 2E–F accurately described the oxidative damage in the leaves of the wheat plant under TW. Interestingly, the same level of Zn-lys treatment was observed in T_2; which marginally decreased the level of EL and MDA in T_2 because of the absence of TW. Under 0%, 25%, 50%, and 100% TW treatments with Zn-lys at 30 mg/L, MDA (13%, 35%, 21%, and 47%) and EL (8%, 54%, 39%, 49%) decreased compared to those treatments treated without Zn-lys. However, this oxidative stress was mitigated by the application of Zn-lys. Higher content of EL and MDA in T_5 and T_7 might be due to the toxicity of Cr stress in plants.

Different antioxidant enzyme activities in leaves were also investigated to assess the alleviation of oxidative stress in wheat Fig. 3. The results demonstrated a significant increase in antioxidant activities (SOD, POD, CAT, and APX) in the combined application of tannery wastewater and Zn-lys which is T_4, T_6, and T_8. T_2 has the highest level of all antioxidant enzyme activities when compared to other treatments. In comparison between T_1 and T_2, the enzyme activities increased more in the presence of Zn-lys. Zn-lys showed an ameliorative role by increasing antioxidant enzyme

Fig. 2 Effect of tannery wastewater and Zn-lys on photosynthetic rate (**A**), stomatal conductance (**B**), transpiration rate (**C**), water use efficiency (**D**), electrolyte leakage (**E**) and malondialdehyde (**F**) in the leaves of wheat with increasing wastewater concentrations (0, 25, 50, and 100%) treated or not with 30 mg/L Zn-lys. Data was recorded after the 90 days of seed sowing to the completion of treatments. Values are demonstrated as means of three replicates along with standard deviation. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize the significant difference at $P < 0.05$. Different small letters indicate that values are significantly different at $P < 0.05$



activities and decreasing the oxidative stress in leaves of the wheat plant. This result indicated that the enzyme activities in stressed plants are improved by the application of Zn-lys.

Impact of Zn-lysine on Cr and Zn concentration in plants

The exogenous application of Zn-lys significantly reduced the concentration of Cr in wheat plants as shown in Table 4. In both roots and shoots of the wheat plants, the concentration of Cr decreased as well as the highest tendency of Zn was seen in these treatments (T_4, T_6 and T_8) under the foliar application of Zn-lys. The higher Cr concentration pattern was distinctly measured in T_3, T_5, and T_7, which were treated only with tannery wastewater. Because of the ameliorative effect of amino-acid chelated fertilizer; the wheat plant easily tolerated the TW stress conditions. In comparison between T_7 (100% TW) to T_8 (100% TW + 30 mg/L Zn-lys), the T_7 showed a marginally higher content of Cr, which is attributed to the absence of Zn-lys in T_7. Results showed a higher concentration of both Zn and Cr in roots than in shoots. Furthermore, the presence of Zn was seen at the T_1 plant, which was a bit higher than T_3, T_5 and T_7. It might be due to the Cr toxic level under these treatment conditions. The reduction content of Cr in the plant was as follows: $T_4 > T_6 > T_8$ compared to untreated treatments with Zn-lys. Zn content in roots (32%, 52%, 63%) and shoots (40%, 57%, 64%) was reduced by the application of tannery wastewater at different levels of treatments (25%, 50%, and 100%) as compared to control. Notably, the mixed application of tannery wastewater with Zn-lys demonstrated an improvement in the concentration of Zn in the various organs of T_4, T_6, and T_8 when compared to the TW only treatments T_3, T_5, and T_7.

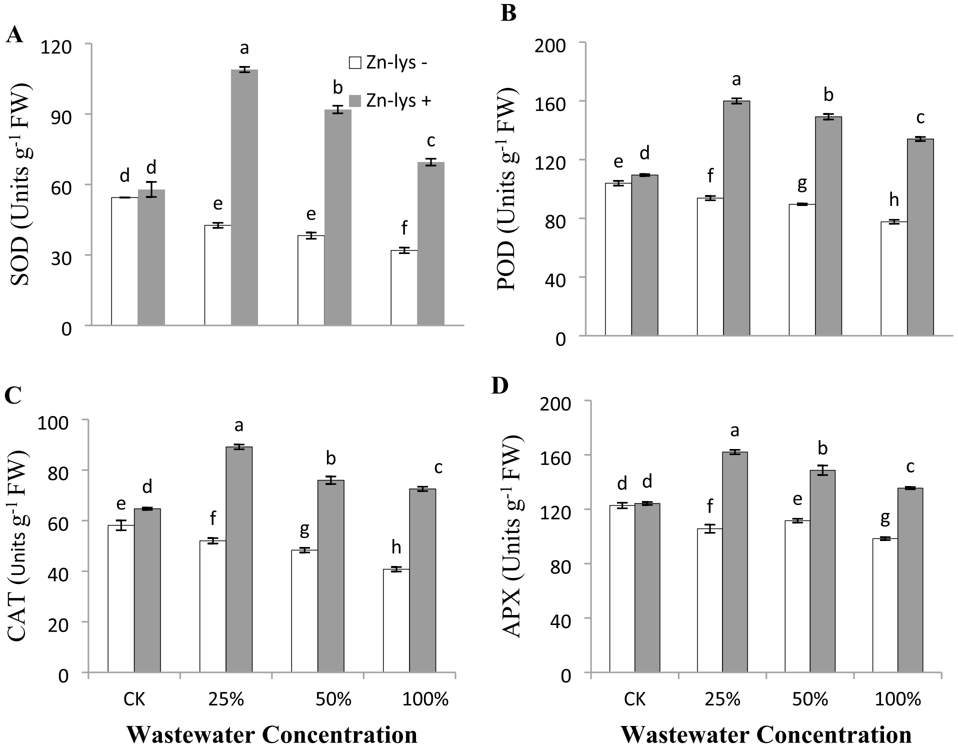


Fig. 3 Effect of tannery wastewater and Zn-lys on SOD (**A**), POD (**B**), CAT (**C**) andAPX (**D**) in the leaves of wheat with increasing wastewater concentrations (0, 25, 50, and 100%) treated or not with 30 mg/L Zn-lys. Data was recorded after the 90 days of seed sowing to the completion of treatments. Values are demonstrated as means of three replicates along with standard deviation. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize the significant difference at $P<0.05$. Different small letters indicate that values are significantly different at $P<0.05$

Table 4 Effect of different concentrations of metals present in tannery wastewater alone and/or in combination with Zn-lys on heavy metals uptake and accumulation in wheat

Treatments	Cr concentration in root (mg kg^{-1})	Cr concentration in shoot (mg kg^{-1})	Cr accumulation in root (µg plant^{-1})	Cr accumulation in shoot (µg plant^{-1})
CK	0.00 ± 0.00 g	0.00 ± 0.00f	0.006 ± 0.01d	0.00 ± 0.00e
Zn-lys	0.03 ± 0.05 g	0.001 ± 0.001f	0.82 ± 1.37d	0.03 ± 0.03e
T.E 25%	60.37 ± 0.65c	16.26 ± 0.88de	978.02 ± 53.14a	371.77 ± 22.64d
T.E 25% + Zn-lys	47.62 ± 1.34f	14.50 ± 0.62e	871.29 ± 43.07abc	403.43 ± 20.94 cd
T.E 50%	82.86 ± 1.70b	27.59 ± 1.23b	962.71 ± 76.89a	551.90 ± 32.33a
T.E50% + Zn-lys	52.01 ± 0.81e	17.62 ± 1.38d	919.78 ± 47.55ab	450.92 ± 51.43bc
T.E 100%	89.17 ± 0.98a	32.36 ± 0.96a	766.63 ± 55.50c	498.41 ± 43.10ab
T.E 50% + Zn-lys	54.84 ± 0.84d	20.40 ± 1.36c	797.35 ± 69.63bc	396.92 ± 18.69 cd
Treatments	Zn concentration in root (mg kg^{-1})	Zn concentration in shoot (mg kg^{-1})	Zn accumulation in root (µg plant^{-1})	Zn accumulation in shoot (µg plant^{-1})
CK	59.18 ± 0.95e	31.25 ± 1.76e	1161.14 ± 36.59e	916.65 ± 71.20d
Zn-lys	134.43 ± 1.40a	71.28 ± 1.61a	3160.78 ± 103.78a	2471.93 ± 136.17a
T.E 25%	40.52 ± 0.70f	18.61 ± 0.90f	656.28 ± 30.66f	426.45 ± 40.74e
T.E 25% + Zn-lys	129.99 ± 1.84b	65.25 ± 1.20b	2377.23 ± 33.34b	1814.23 ± 41.75b
T.E 50%	28.24 ± 1.20 g	13.53 ± 0.69 g	327.37 ± 7.13 g	270.51 ± 14.29ef
T.E50% + Zn-lys	112.32 ± 1.68c	49.37 ± 1.00c	1987.47 ± 130.86c	1260.51 ± 50.70c
T.E 100%	21.77 ± 1.02 h	11.14 ± 0.38 g	187.07 ± 13.54 g	171.46 ± 6.91f
T.E 100% + Zn-lys	100.04 ± 1.17d	44.67 ± 1.16d	1454.37 ± 124.39d	869.59 ± 19.13d

Data was recorded after the 90 days of seed sowing to the completion of treatments. Values are demonstrated as means of three replicates along with standard deviation. Analysis of variance (ANOVA) was applied, and data was compiled by Tukey's post hoc test followed by all para-wise comparisons among the mean values of treatments to recognize the significant difference at $P<0.05$. Different small letters indicate that values are significantly different at $P<0.05$

Discussion

Outcomes of the present study revealed that the Cr toxicity decreased the phenotypic parameter but, the Zn-lysine enhanced such parameters (Table 2). These results are in line with the findings of Maqbool et al (2018) who reported that Cr stress conditions markedly reduced growth parameters of spinach plants. More Cr accumulation in plants reduces nutrient migration which eventually inhibits plant growth (Tauqeer et al. 2016). Rafie et al (2017) reported that Zn-amino chelate enhanced the growth parameters in onion. In addition, another study documented that foliar-applied Zn-lys have been beneficial to the growth of wheat plants grown on Cd-contaminated soil (Rizwan et al. 2017). This study supports our present research that Zn-lysine promotes the growth of wheat plants under wastewater stress. Similar positive response of amino-chelate nutrients has already been present in previous research by Ghasemi et al (2014). The increase might be due to the accompanying effect of Zn and lysine. Amino acids may also involve many plants structural components and various physiological responses (Nasholm et al. 2009; Rizwan et al 2017). Amino-chelate fertilizer has optimized the reduction of wastewater stress in the morphological attributes of wheat. Because of its capability to boost nutrient uptake and protect against environmental stresses, Zn-lysine is a generally recognized amino-chelate fertilizer. Through complexes with toxic heavy metals, amino acids play a vital role in reducing the mobility of metals in active plant parts (Souri 2016). Therefore, the outcome of this research work indicated a massive reduction in the growth and biomass of wheat plants due to Cr toxicity but augmented those parameters significantly under Zn-lys application.

The TW at different levels (25%, 50%, and 100%) decreased the photosynthetic and gas exchange attributes, as shown in Figs. 1 and 2A–D. The presence of Cr in the plant destroys the electron transport chain reaction or degeneration of chloroplasts. This further reduces the photosynthetic activity as well (Singh et al. 2013). This study revealed that the supplementation of Zn-lysine improves the level of photosynthetic and gas exchange attributes (Figs. 1 and 2A–D). Zinc is actively engaged in the stabilization of membrane structural integrity and protection of chloroplasts in some plants (Zaman et al. 2017; Zaheer et al. 2019). It is also apparent that the foliar-applied Zn-lysine enhanced the photosynthetic and other gas exchange parameters in the presence of toxic Cr content in the tested plant. Zaheer et al (2019) concluded that Zn-lysine boosts photosynthetic activity under abiotic stress conditions. Enhancement in photosynthetic pigments might be due to the presence of amino acids complex with micronutrients (Rizwan et al. 2017). The lower chlorophyll content was seen in different varieties of plants i.e., wheat, spinach, mung bean, and sunflower under Cr toxicity (Ali et al. 2015; Farid et al. 2017; Zaheer et al. 2020a, b). Previous research work also supported these findings that improvement of photosynthetic performance and gas exchange attributes in spinach plant as a consequence of substantial uptake of Zn (Zaheer et al. 2019).

In accordance with Ahmad et al. (2017) the production of ROS and EL induces severe oxidative damage under wastewater stress. Oxidative stress was also noted in wheat plant under metals accumulation (Abbas et al. 2017; Rizwan et al. 2017). Cr and Cd-induced oxidative stress was reported in number of plant varieties such as spinach, wheat, *Brassica napus*, sunflower, and maize plant (Abbas et al. 2017). Foliar applied Fe-lys and Zn-lys reduced oxidative stress in different plant varieties (Farid et al. 2017; Zaheer et al. 2020a, b). It is well documented that the toxicity of metal stress induces oxidative stress in plants (He et al. 2017). Different amino acids like glutamine, glycine, lysine, and arginine with Zn play a crucial role to overcome the concentration of MDA and EL in several plants (Mohammadi and Khoshgoftarmanesh 2014). The present study also concluded that the amino-chelate fertilizer reduced oxidative stress and enhanced the antioxidant activities when irrigated with tannery wastewater (Figs. 2E–F and 3). Another study supported the findings that foliar application of Zn-lys improved antioxidant enzymes activities in wheat plants under Cd stress (Rizwan et al. 2017).

This research shows that when the level of TW increases, it increases the concentration of Cr in both roots and shoots of the plants (Table 3). Cr has a bit more specificity accumulation capability in plants (Shahid et al. 2017). Cr toxicity makes it conceivable to show severe damage to plants resulting in the subsequent deterioration of crop yields (Farid et al. 2019). Plant roots accumulate HMs from the soil and then distribute to the various organs of the plant body (Lajayer et al. 2019). When plants extract Cr or other HMs from the soil, the plant is, therefore, unable to absorb other essential minerals which eventually lead to structural destruction and further reduce the growth and development of the plant (Yu et al. 2018). The increased concentration of TW in different levels reduced the Zn content in both organs of the plants (Table 3). Reduction in the essential nutrient (Zn) may lead to the reluctance of the roots to accumulate in the presence of higher Cr stress in the soil (Tauqeer et al. 2016). Micronutrient-amino chelator has been well documented in various studies that raise Zn and decrease Cr levels in different plant species such as wheat, spinach, cabbage, tomatoes, and onions (Ghasemi et al. 2013a; Ghasemi et al. 2014; Rafie et al. 2017; Rizwan et al. 2017; Zaheer et al. 2020a, b). Supplementary Table 1 shows the role of lysine with essential nutrients extracted from studies reported in recent past. Amino acids form complexes with HMs, lowering metal mobility in different plant parts (Sharma et al. 2016). In addition, amino-chelate fertilizers (Zn-lys) boost morpho-physiological traits and improve the ability of wheat plants to persist under Cr stress conditions. In the present study, the dramatic impact of Cr decreased, and wheat plant growth was considerably boosted. Furthermore, the application of Zn-lysine improved plants' capacity to compete with Cr stress.

Conclusions

This study indicates a significant outcome on wheat plants against tannery wastewater treatment. Foliar Zn-lysine application had a beneficial influence on the morpho-physiological and gas exchange traits of the studied plants. The number of parameters reduced dramatically in tannery wastewater treated plants. Eventually, the level of micronutrients within these treatments was also lowered due to the toxicity of chromium stress. Furthermore, Cr toxicity enhances oxidative stress in the leaves of wheat plants in T_3, $T_{5,}$ and $T_{7,}$ but at the same time, Zn-lysine induces positive effect to overcome stress in plant leaves of T_4, $T_{6,}$ and T_8. The Cr concentration was reduced in roots and shoots at different levels (T_4, $T_{6,}$ and T_8) when compared to the treatments irrigated only with tannery wastewater (T_3, $T_{5,}$ and T_7). Finally, our findings highlighted that Zn-lysine is advantageous against metal toxicity in wheat plants. However, field experiments and further analyses are required to verify these results.

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Authors' contributions WS, MF and NK designed the main idea and content of the article including writeup of different sections. ZFR, AN, SA collected the data and wrote different portion of the article. NN and SA, SF designed the graphical representation of data abstracted from different articles. WS, MF, ZA and SA critically reviewed the article. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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