Article

Ameliorating the Cr toxicity for rapeseed (*Brassica napus L.*) growth and biomass through the foliar application of micronutrients chelation with amino acids grown in tannery wastewater

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Received; Accepted; Published: date

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Abstract: Contamination of soil with toxic heavy metals due to wastewater irrigation is grave concern globally, leather industry is one of the main sectors in Pakistan, releasing toxic heavy metals like chromium via discharge of untreated wastewater. Use of untreated tannery wastewater for irrigation may significantly affects the growth, yield and fertility of the soil. Present study was conducted to examine the harmful impacts of tannery wastewater on *Brassica napus L* under the exogenous application of micronutrient chelated with amino acid Zn-lysine and Fe-lysine under varying level of tannery wastewater (0. 33, 66, 100%). Results revealed a considerable decline in the growth of *Brassica napus L* with the increasing concentration of tannery wastewater. Maximum decline in plant height, number of leaves, root length, fresh and dry biomass of root and leaves of the *Brassica napus L* were recorded at the maximum level of tannery wastewater application I.e 100% compared to the plants in control treatment. Similarly, contents of carotenoid and chlorophyll, gas exchange attributes and activities antioxidant enzymes were also reduced significantly with the increasing concentration of tannery wastewater. However, the interactive combine exogenous application of Zn-lys and Fe- lys effectively mitigated Cr induced noxious effects on *Brassica napus L* and improve growth traits, photosynthesis, biomass and antioxidant defense mechanism of the plants though limiting the generation of reactive oxygen species. Promotive application of Zn-lys and Fe-lys reduced the accumulation and uptake of toxic Cr, while boost up the uptake of essential micronutrients like Zn and Fe in different tissues of the plants. Results concluded that exogenous application of micronutrients chelated with amino acid like lysine successfully mitigate Cr stress in *Brassica napus L* by improving the morpho-physiological characteristic and nutrients uptake in plants. Field level, supplementation of theses amino acids with micronutrients could be used an effective strategy for the alleviation of metal stress for other important crops.

Keywords: Amino acid + micronutrients, tannery wastewater, Cr stress, antioxidant defense system, *Brassica napus L*

1. Introduction

Contamination of soil with toxic heavy metals is a pressing and complex problem intensely felt worldwide, particularly in densely industrialized and populated areas [1]. Rapid growth of industries and manufacturing operations like smelting, melting, electroplating and mechanical set-ups leads to the extensive contamination of soil with contaminates like heavy metals [2]. Excessive application of fertilizers, pesticides, herbicides and bio solids to various crops results in buildup of these contaminates in soil [3, 4]. In terrestrial ecosystem soil act as both source and sink of heavy metal contaminants. Extreme accumulation of heavy metals in arable soil not only causes pollution of aquatic and terrestrial environment, but also escalate the likelihoods of human exposure with heavy metals [5, 6]. Copious public health distress rises if food products grown on such polluted soil came into national markets, which ultimately expose a huge number of populations to health threats of heavy metals contamination [7].

Prompt industrial and economic developments throughout the world has led to range of environmental concerns including the contamination of soil with toxic heavy metals [8]. Production of industrial sector is considered main cause of soil pollution, water pollution and air pollution [9]. Tanning industry is categorized as major source of environmental contamination [10, 11]. Tanning industries produces many multifarious and exceedingly loaded effluents that requires the prior treatment before final disposal [12]. Tanning industries are principal source of chromium pollution in Pakistan and different parts of the world as reported in different studies [13, 14]. Chromium is most important tanning agent in tannery industries. Chromium is main contaminant as it poses enormous toxic effect on various crops grown on Cr contaminated soil [15, 16].

Chromium is potentially noxious metal and it does not have any vital function in metabolic activities of plants [17]. Industrial process mainly (tanning effluents) along with natural and anthropogenic activities resulted in amplified accumulation of chromium in surrounding environment [4, 8]. Accumulation of Cr in water and soil directly affects the plant physiology, animal and human health, and it may easily accumulate in food chain, that can be grave concern for the health of both secondary and tertiary consumer as well [18]. Chromium stress negatively affects the physic-chemical processes in plants that eventually leads to the plant death [19]. Unnecessary accumulation of Cr in plant body resulting into severe toxicity as shown by decline in growth and biomass, degradation of chlorophyll, excessive production of reactive oxygen species (ROS), hindrance in nutrients uptake, demolition of cellular ultrastructure and disarray in antioxidant system [11, 16]. Therefore, it is essential to discover management option to reduce the Cr accumulation in plants grown on contaminated soil and lessen risk to human health.

Globally, Rapeseed (*Brassica napus* L) is considered as essential oilseed crop and regarded as second major source of vegetable oil after soybean in the world [20]. Oil

Rapeseed (*Brassica napus* L) has a tremendous capacity to combat the stress induced by the heavy metal toxicity [21]. Among different heavy metals, contamination of Cr has shown noticeable influence on growth, ultra-structural, physicochemical and molecular profiling of the *Brassica napus* [22]. *Brassica napus* has the ability to tolerate various kind of heavy metals stresses, owing to its physiological and biological processes, it also has the capacity to withstand the chromium stress [11, 22].

To date, many scientific approaches have been employed to remediate the heavy metal stress in plants, particularly by using exogenous applied amino acids. Amino acids play a very critical role in metal compartmentation, transport and tolerance in plants. Zinc and iron chelated fertilizers complexed with lysine as amino acid reported to improve the growth and yield of the crops [23, 24]. Fruitful application of micronutrients expressively mitigates the toxic effects of heavy metals stress in plants [25, 26]. Both iron and Zinc are important micronutrients as they play very critical role in various metabolic process in plants including photosynthesis, DNA synthesis and respiration [14]. Zinc chelated with amino acid lysine effectively mitigate the Cr stress in rice plant and improved the growth of plant through Zn fortification [13, 27]. Similarly, previous study also reported that Fe-lysine can be effectively used as operative amendment in the reduction of Cd stress in rice plant [28]. However very little is known about the role of both Zn-lysine and Fe-lysine about the mitigation of heavy metals stress in pants.

There are various studies about the application of Zn and Fe-lys on different crops under range of heavy metal stresses [13, 27-29], where there is no significant literature present on the combine application of Zn and Fe-lys on crops. Therefore, present study aims to estimate the effects of various concentration of Zn and Fe-lysine on Brassica napus L grown under various level of tannery wastewater application. At the end, we will able to understand (1) the impacts of different levels of tannery wastewater and combine application of Zn and Fe–lys on the growth and biomass of the plant, (2) gas exchange attributes and pigments of photosynthesis, (3) Cr induced oxidative stress and response of antioxidant machinery, (4) accumulation and uptake of micronutrients (Zn, Fe) and Cr in various portions of Brassica napus L plant under tannery wastewater application. This experimental study will provide foundation for evolving strategies by using micronutrients chelated with amino acid in order to reduce the risk related with Cr stress for the purposes of sustainable production of crops.

2. Material and Methods

2.1 Description of site and soil sampling

Soil surface samples (0-22cm) were carefully taken from the botanical garden, Department of Botany, University of the Punjab Lahore. Soil samples were crushed softly with hands, all visible portions of dead and flourishing vegetation along with other dirt particles were watchfully detached at the site. Samples of soil were air dried cautiously. Debris and mud particles were removed from the dried soil with the help of 2-mm sieve. All samples of soil were kept in plastic bag at room temperature until used for further analysis.

2.2 Determination of soil and wastewater physico-chemical characteristics

Total organic content of the soil was estimated via subsequent titration and dichromate oxidation with ferrous ammonium sulfate according to the method describe [30]. Soil water holding capacity was determined by following the procedure defined by [31]. Similarly, soil ions, sodium adsorption ratio (SAR) and electrical conductivity (EC) were also carefully estimated with the method given by [31]. Soil sampling was performed with the assistance of ammonium bicarbonate diethylenetriamine penta acetic acid (AB-DTPA) solution for the appropriate measurement of extractable trace elements [32]. Physiochemical characteristics of the soil under study are given in Table 1S. Tannery wastewater used in this present experimental study was carefully collected from tannery industries located in Kasur, Punjab Pakistan. Physiochemical features of tannery wastewater used in the study were carefully estimated according to the set protocols given for the estimation of water and wastewater (APHA, 1995). Comprehensive details of major characteristics of tannery wastewater used in this experiment are presented in Table 2S.

2.3 Experimental design

Soil based pot experimental study was performed in botanical garden under natural condition with 25 °C and 10 °C average temperature of day and night. During experimental study average humidity recorded was 65%. Health seeds of rapeseed *(Brassica napus* L.) were obtained from Ayub Agriculture Research Institute Faisalabad, Pakistan. Seeds of *Brassica napus L* were sterilized with sodium hypochlorite (4%), washed carefully three times, and then soaked watchfully in ultrapure water for nearly 4 hours. *Brassica napus L* seeds were uniformly sowed with hand in plastic experimental pots filled with 5kg of soil approximately. Experiments was conducted accordingly with completely randomized design (CRD) with three replicates of each treatment. After germination time of two weeks *Brassica napus L* seedling were treated with the foliar application of Zn and Fe-lys (Zn–lys 0, 10 mg/L and Fe–lys 0, 5 mg/L) along with treatment of tannery wastewater with different levels (0, 33, 66 and 100%). Hand sprayer was used for the foliar application of both Zn and Fe-lys to *Brassica napus L* plants. Plants under control treatment were sprayed with distilled water. In order to mitigate the crucial deficiency of macronutrients, potassium sulphate (SOP) and phosphate were carefully applied to plants as fertilizer by following the methodology described by Bashir et al (2018).

2.4 Treatments

In total there were total eight treatments as follows, T1(control): Cr 0, Zn lys 0 and Fe lys 0 mg/ L, T2: Cr 0, Zn lys 10 and Fe lys 5 mg/ L, T3:Cr 33, Zn lys 0 and Fe lys 0 mg/ L, T4: Cr 33, Zn lys 10 and Fe lys 5mg/ L, T5: Cr 66, Zn lys 0 and Fe lys 0 mg/ L, T6: Cr 66, Zn lys 10 and Fe lys 5mg/ L, T7: Cr 100, Zn lys 0 and Fe lys 0 mg/ L, T8: Cr 100, Zn lys 10 and Fe lys 5mg/L.

2.5 Plant Harvesting

After 60 days of sowing, seedling of *Brassica napus L* plants were carefully harvested to measures the important agronomic characteristics of the plant including plant height, shoot length, root length, fresh and dry biomass of roots and shoots, number of leaves and leaf area.

2.6 Estimation of Chlorophyll Contents and Gas Exchange Parameters

Extended upper most leaves of the plant were used for the estimation of carotenoid and chlorophyll used for the measurement of transpiration rate, photosynthesis, water use efficiency and stomatal conductance by following the methodology described by [33].

2.7 Estimation of MDA, EL, H_2O_2 Antioxidants Enzymes

Contents of MDA were carefully measured by using the method described by [34]. Electrolyte leakage (EL) in roots and shoots was measured by the estimation of initial EL (EL_1) and Finial EL (EL_2). EC_1 was estimated by retaining the samples of roots and shoots in a tube having known volume of distilled water, heated for 2 hours at approximately 32 °C. Whereas EC_2 was measured by heating the sample again for about 20 minutes at temperature of 120 °C. Final EL was estimated by following the protocol described by [35]. Method laid by [36] was used to measure the contents of H_2O_2 in leaves and roots of *Brassica napus L* plant.

2.8 Estimation of Antioxidants Enzymes

Activities of SOD, POD, APX and CAT were calculated with the help of (Halo DB-20S, Dynamica, UK) spectrophotometer. 0.05 M buffer solution of Phosphate (PO_4^{3-}) was used to standardize all the samples at pH of 7.8. The activities of Antioxidants such as SOD, POD, APX and CAT measured by following the method of [37], [38], [39] and [40] respectively.

2.9 Estimation of Cr, Zn and Fe Contents

Samples were carefully rinsed with hydrochloric acid (diluted), then dried at 70 °C and grinded to very fine powdered form. Around 1 g sample of *Brassica napus L* plant tissues (roots, stem, leaves) was cautiously placed in muffle furnace for 6 hours at temperature of 600 °C till burned to ashes and then digested with hydrochloric acid (HCL) and nitric acid (HNO3) in ratio of 3:1. Resultant specimen was kept overnight and further digested with the addition of same solution of 5mL. Final volume of 50 ml was made with the addition of deionized water. Concentration of Cr, Zn and Fe were estimated by using atomic absorption spectrometer as recommended by [41].

2.10 Statistical analysis

Present experiment was developed in completely randomized design. All data presented are mean of three replicates. Experimental data was statistically estimated by one-way analysis of variance (ANOVA) through use of software (SPSS, version 16.0) subsequently, Tukey's HSD post hoc test was effectively used for the accurate estimation of multiple comparison among different means.

3. Results

3.1 Growth-related attributes

Growth attributes of *Brassica napus L* including morphological traits showed significant reduction due to the subsequent application of tannery wastewater in different concentration (0,33,66,100%). Maximum decline in the growth-related attributes of the plant were noticed at maximum level of tannery wastewater application i.e. (100%). It was witnessed greatly that increasing concentration of wastewater significantly reduced when compared to the plants grown without

addition of wastewater (Fig 1). Maximum decline in all agronomic attributes was detected by 67, 67, 86, 36, 90, 83, 89 and 82% respectively with the mixing of wastewater at 100% concentration in contrast the plants grown without the wastewater irrigation. We also elucidated from this study, successful application of both Zn-lys and Fe-lys improved the growth attributes of the plant even grown under various levels of wastewater treatments. Collaborative application of Zn-lys (10 mg/L) and Fe-lys (5 mg/L) considerably boost up the all the growth characteristic of rapeseed (*Brassica napus L*) at every level of tannery waste water treatment (0, 33, 66, 100%) as presented in Fig 1 and 2. Zn and Fe–lys effectively improved the all mentioned agronomic traits by 33, 25, 40, 25, 32, 23, 40 and 29% under the supplementation of 100% wastewater, as compared to the treatment with zero application of Zn and Fe–lys.

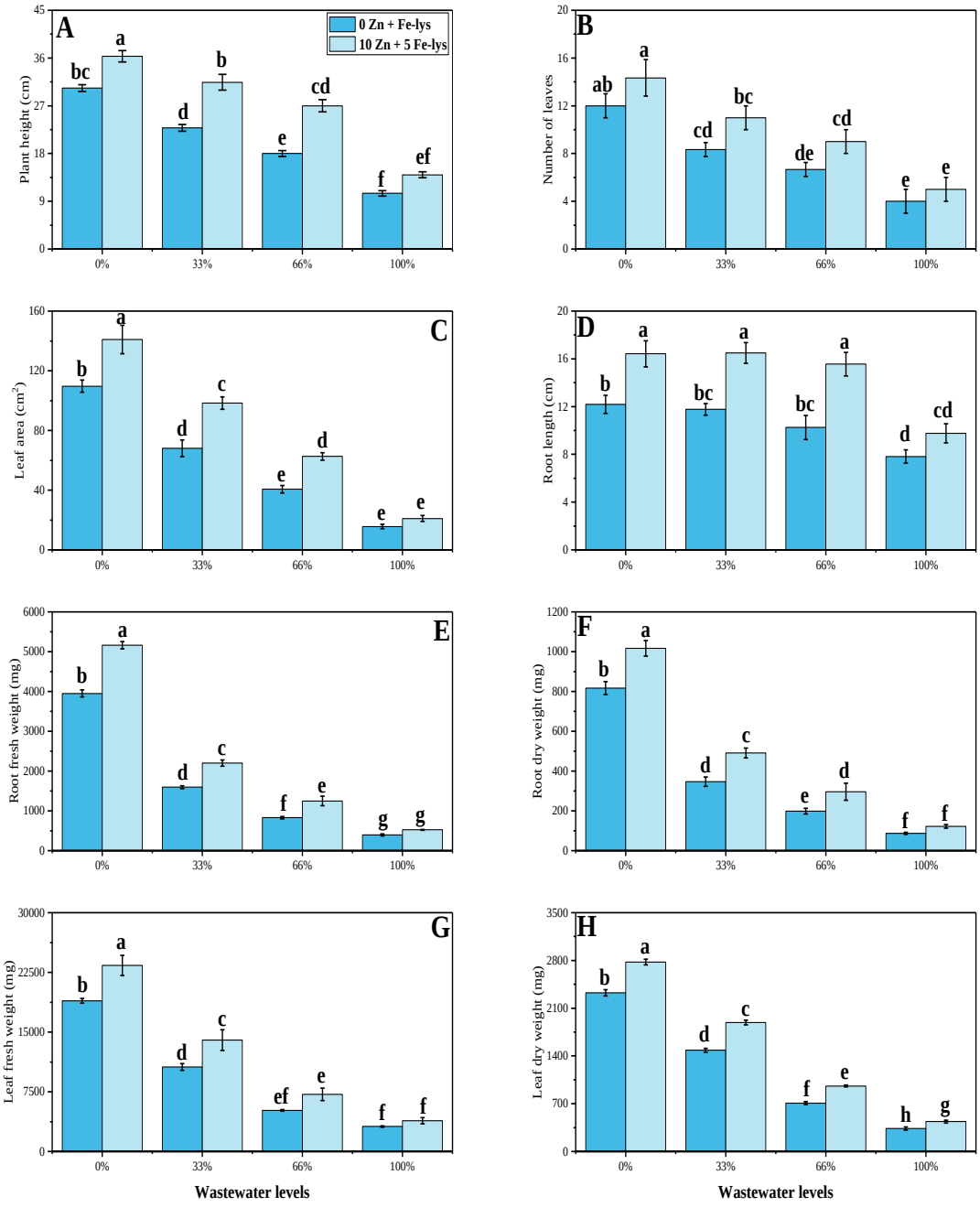

Figure 1 Influence of various concentrations of wastewater on morphological traits in the supplementations of Zn and Fe-lys on *B. napus*.

3.2 Gaseous exchange attributes and chlorophyll contents

Gaseous exchange characteristics, carotenoids and total chlorophyll contents of *B. napus* along with various concentrations of wastewater treatments and with and without application of Zn-lys (10 mg/L) and Fe-lys (5 mg/L) are given in Fig 2. Mixing of wastewater significantly reduced photosynthetic measurements of *Brassica napus L*, in comparison to the plants under control treatment, 100% application of tannery wastewater reduces the photosynthetic measurements by 68, 57, 62, 57, 76 and 60 % respectively. Whereas, exogenous application of both Zn-lys (10 mg/L) and Fe-lys (5 mg/L) significantly ($P < 0.05$) improves gas exchanges attributes, contents of carotenoids and total chlorophyll in *Brassica napus L* even at all levels of tannery wastewater applications. As presented in Fig. 2, all above mentioned characteristics of the plants were significantly improved by 46, 30, 30, 42, 34 and 31% respectively under the combine application of both Zn-lys and Fe-lys in plants irrigated with 100% tannery wastewater treatment.

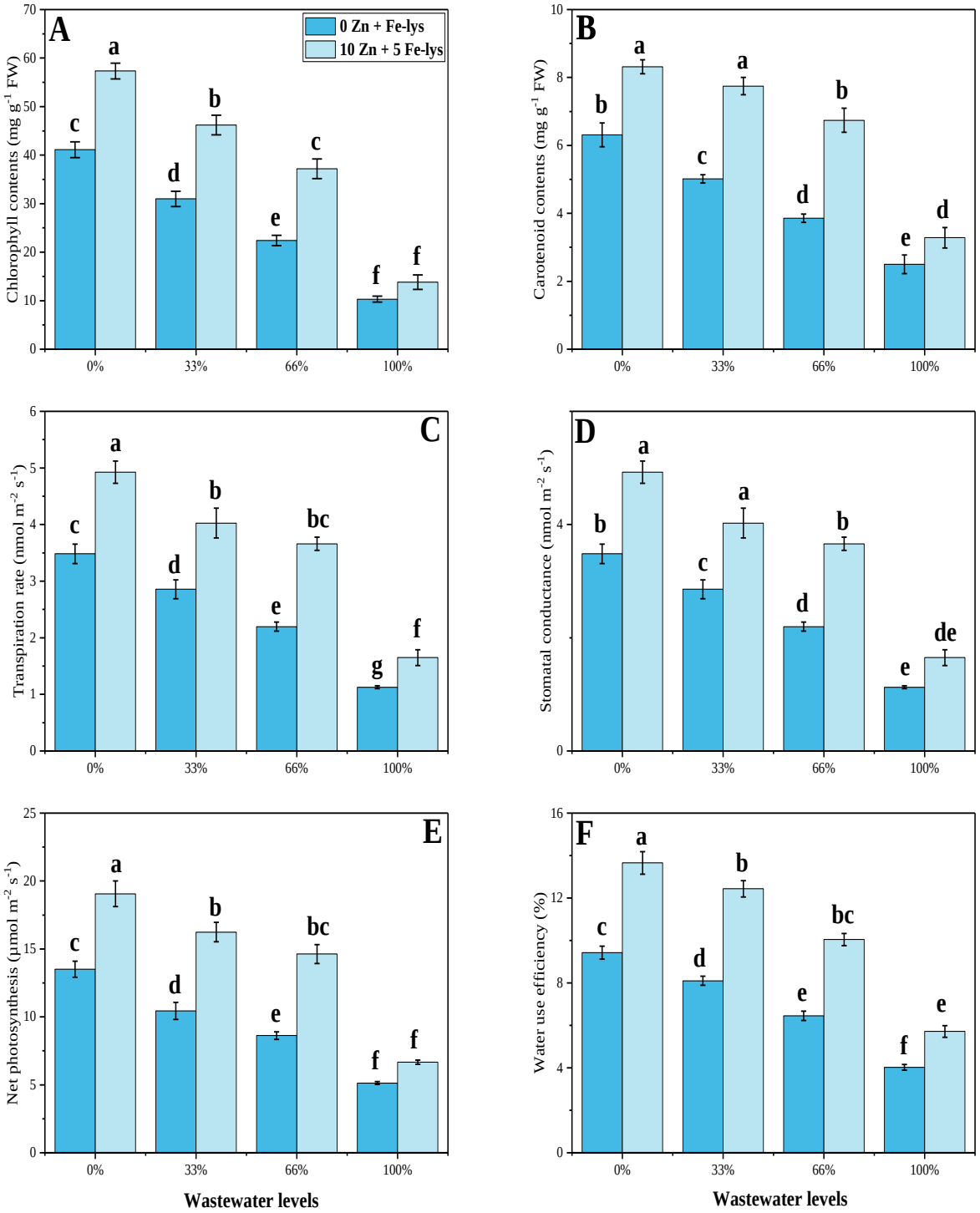

Figure 2 Influence of various concentrations of wastewater on photosynthetic measurements in the supplementations of Zn and Fe-lys on *B. napus*.

3.3 Oxidative stress biomarkers

Increase in the concentration of tannery wastewater application imposed severe oxidative stress on *Brassica napus L* via increasing the contents of oxidative stress biomarkers in all organs of the plants. Results showed that increasing concentration of wastewater application increased the contents of Electrolyte leakage (EL), MDA and H_2O_2 and maximum increase in these parameters was reported at 100% tannery

wastewater treatment as contrast with the plants grown without wastewater. Whereas, exogenous application of both Zn-lys and Fe-lys mitigate the oxidative stress by lowering the contents of MDA, H2O2, EL in all organs of the plants as contrast with the plants with no application of Zn and Fe-lysine. At maximum concentration of tannery wastewater treatment i.e. 100%, combine application of Zn-lys and Fe-lys reduced the concentration of Electrolyte leakage (EL) by 27 and 32% in roots and leaves of the *Brassica napus L*, similarly contents of both MDA and H2O2 were declined by 20, 26, 32 and 39% in boot roots and leaves of the plant as presented in Fig. 3.

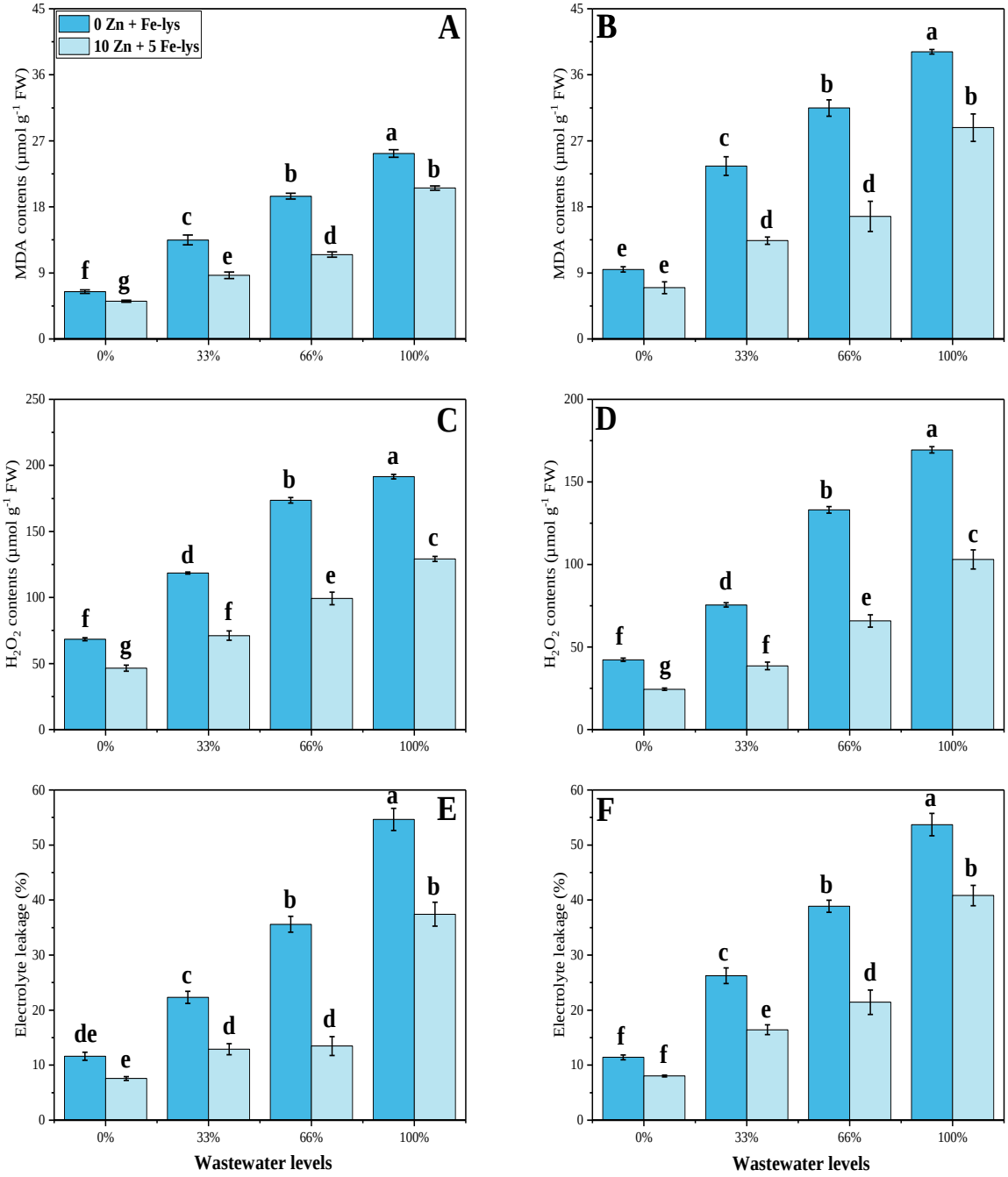

Figure 3 Influence of various concentrations of wastewater on oxidative stress indicators in the supplementations of Zn and Fe-lys on *B. napus*.

3.4 Antioxidant enzymes activities

Activities of different enzymatic antioxidant compounds in all organs of the plant were estimated in order to determine the influence on *B. napus*. In this experimental study, results showed that a significant declined was noticed in the activities of all-important antioxidants including CAT, POD, APX and SOD in both roots and leaves of the *Brassica napus L* plants as given in Fig. 4. At highest level of tannery wastewater (i.e. 100%), the actions of antioxidants start declined. Although, combine supplementation of Zn, Fe-lys improved the activities of all-important antioxidant CAT, POD APX and SOD in all organs of the *Brassica napus L* even at maximum concentration of tannery wastewater (100%). Combine supplementation of Zn, Fe-lys enhanced the actions of important antioxidant enzymes in plant under all range of (0, 33, 66 and 100%) tannery wastewater application. Actions of antioxidants were enhanced to 74, 57, 56, 63 in leaves and 50, 47, 60 and 83 in roots of *Brassica napus L* through combine application of Zn, Fe-lys under Cr stress generated by tannery wastewater application as presented in Fig (4).

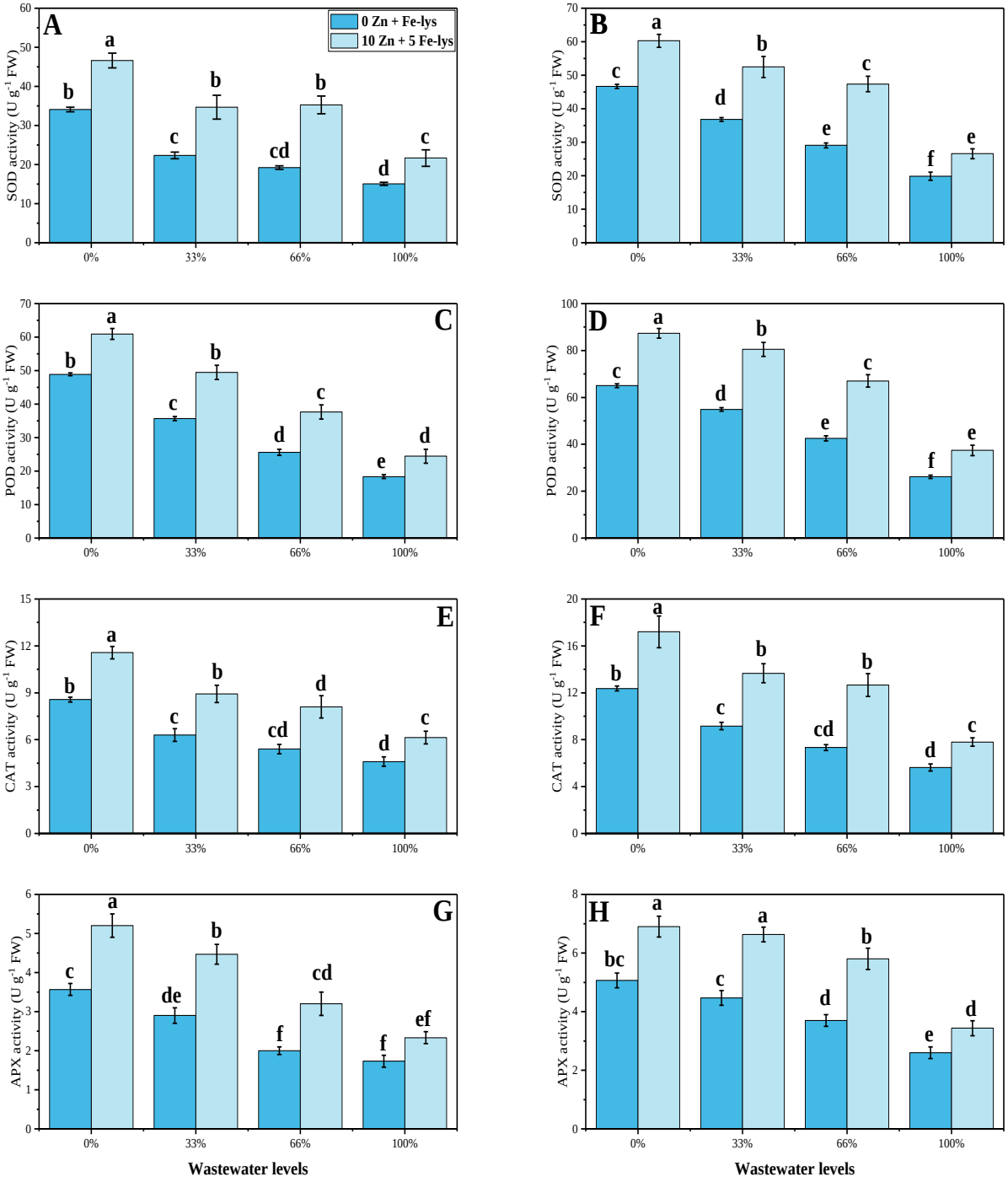

Figure 4 Influence of various concentrations of wastewater on antioxidant activities in the supplementations of Zn and Fe-lys on *B. napus*.

3.5 Uptake of Cr, Zn and Fe

Accumulation and uptake of Cr, Zn and Fe in different parts (i.e. roots, shoots) of the *Brassica napus L* were investigated under varying levels of Cr stress generated due to successive application of tannery wastewater. Results depicting the contents of Cr, Zn and Fe in *B. napus* grown in different treatments of wastewater with and without supplementation of Zn-lys and Fe-lys are given in Fig. 5. Results revealed that increasing concentration of Cr in various portions of the plants were recorded with increase in the level of tannery wastewater treatment, while at the same time increasing

level of tannery wastewater treatment continuously reduces the contents of Zn and Fe in different organs of the plants. Maximum contents of Cr (154 mg kg^{-1} DW and 45 mg kg^{-1} DW) were observed in roots and leaves of the *B. napus* plant under the application of 100% tannery wastewater application. As contrast to the control, increases in the level of tannery wastewater application contents of both Zn and Fe were continuously declined and maximum decease reported in roots was 55 and 60% while 50 and 75% reduction in leaves of the plant under 100% irrigation of tannery wastewater. However, combine application of both Zn and Fe lysine declined the overall contents of Cr in various parts of the plant. A significant reduction of Cr by 30 and 33% was observed in different organs of the *B. napus* while maximum improvement in contents of Zn and Fe were recorded by 28 and 32 in roots and 31% and 33 % in leaves of the plant under maximum concentration of tannery wastewater treatment (100%) as given in Fig. 5.

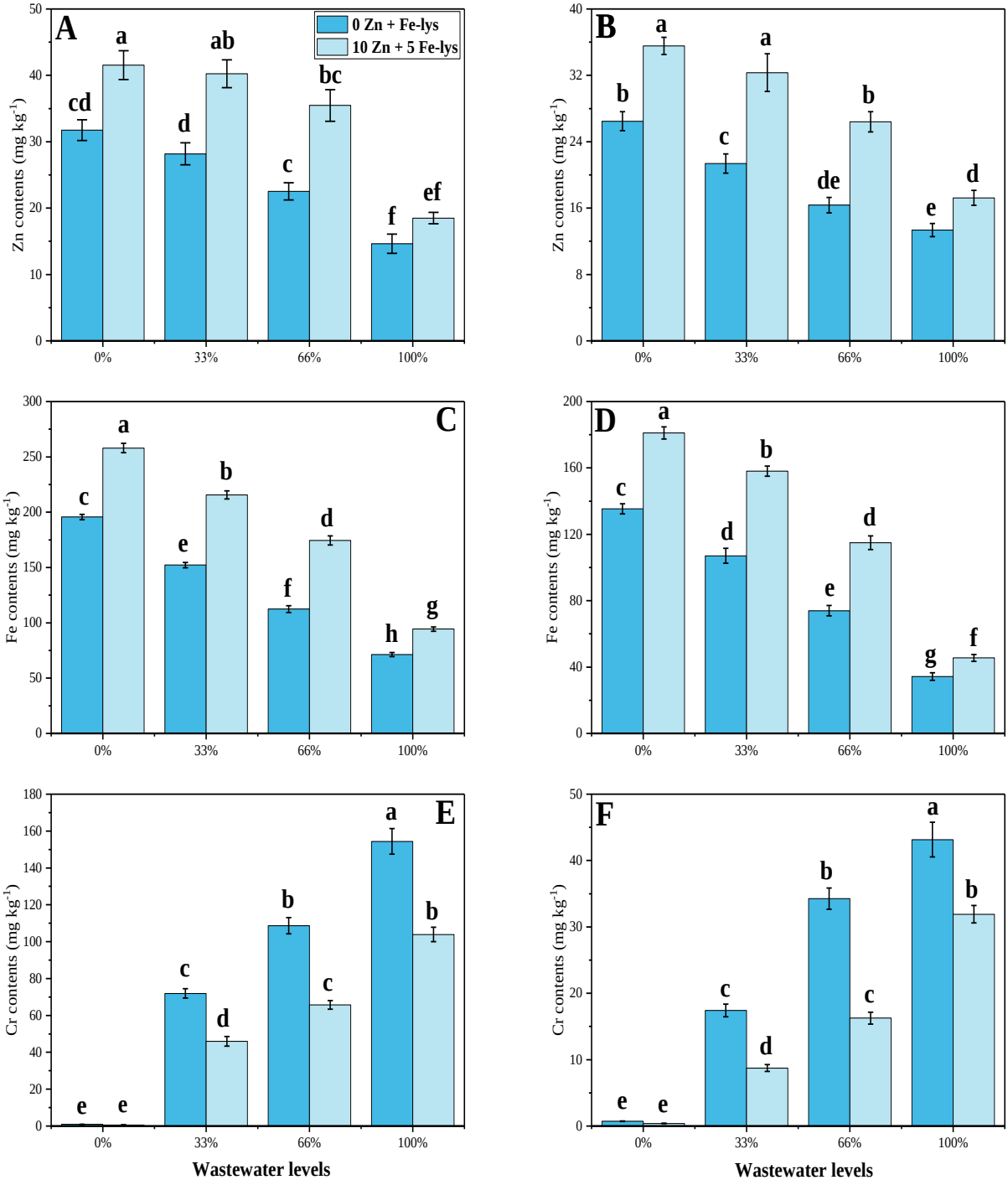

Figure 5 Influence of various concentrations of wastewater on uptake of Zn, Fe and Cr in the supplementations of Zn and Fe-lys on *B. napus*.

3.6 Relationship

We also constructed a correlation analysis to signifying the differences between various attributes studied in our experiment (Fig. 6). We have noticed that Cr concentration in the roots were positively associated with Cr concertation in the shoots and MDA contents in the roots while negatively associated with all other morpho-physiological traits studied in this experiment. Similarly, MDA contents in the roots was positively associated with Cr contents in the roots of the plants while negative correlated Zn and Fe contents and other morpho-physiological traits

studied in this experiment. These relationships showed a close connection between different morpho-physiological attributes of *B. napus* studied in this experiment.

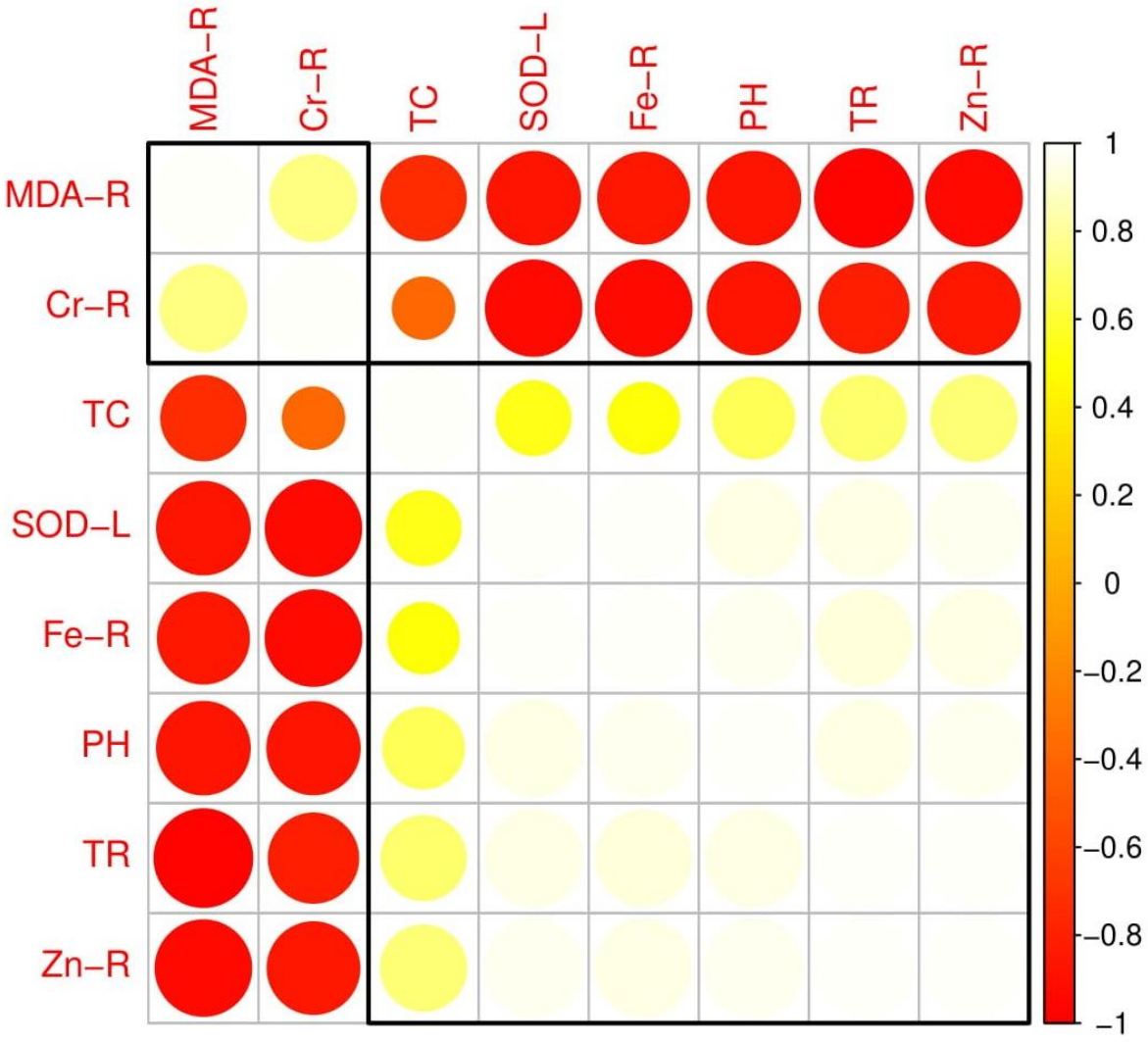

Figure 6 Pearson's correlation between various morpho-physiological parameters.

4. Discussion

Economic, social development, population growth and water shortage are becoming severe issues globally [42]. Freshwater scarcity is gradually observed as a worldwide systemic risk [9, 10, 13]. Extraordinary demand of water for the production of food crops led to growing scarcity of freshwater resources in different parts of the world [43]. Limited supply of freshwater put so much pressure on the farmers in developing countries to utilized wastewater as alternative source of water supply. Wastewater irrigation is a common practice in developing nations to recompense the water shortage [44]. Irrigation of soil with wastewater provide sufficient quantity of micronutrients to the plants [45]. Besides this, wastewater also contains some very toxic potential toxic heavy metals (PTMs), which have the ability to seriously affect the human health and whole ecosystem [46]. Toxicity of heavy metals is a burning issue worldwide. Heavy metals are usually required in trace amount, however excessive concentration of toxic metals could be detrimental for both growth and productivity of improvement crops plants [47].

Chromium toxicity due to tannery wastewater irrigation is widely reported in various studies in different part of the world [16]. Among different heavy metals chromium is considered as highly toxic metal [15]. Excessive use of tannery wastewater for irrigation would eventually results in the subsequent releases of Cr in the surrounding environment. Excessive discharge of Cr could be extremely toxic for normal plant growth, morphological and physio-biochemical processes and membrane integrity of plants [48]. Result of present study revealed that morphological traits of the plant including growth-related attributes significantly reduced through the supplementation of tannery wastewater in soil (Fig. 1). Chromium has the ability to decline the growth attributes as previously reported in different plants including *Brassicca napus* [7], *Zea mays* [49] *Helianthus annuus* [50] and *Triticum aestivum* [51]. Cr stress leads to significant reduction in plant growth, as Cr obstructs the nutrients availability for the aerial parts which eventually leads to the hindered growth of the plant. Similarly finding were reported in *B. juncea* [52]and *B. campestris* [53] where Cr considerably restricts the nutrients availability and finally restrict the growth of the plants. In this study, supplementation with Zn-lys and Fe-lys considerably reduced the Cr induced decrease in the growth-related attributes of the *Brassicca napus L* under varying concentration of tannery wastewater. Both zinc-lysine and iron-lysine are widely recognized as (amino chelated) fertilizers and they have the profound capacity to boost up the uptake of necessary nutrients and safeguard the plants against external stresses. Amino acids have shown tremendous potential of making complexes with different toxic metals and eventually reducing their mobility [29]. In our study, combine exogenous supplementation of Zn-lys and Fe-lys improve the plant height, biomass (fresh + dry) compared to the treatment with zero Zn and Fe-lysine application. Findings from this experiment are similar with the outcome of previous studies specifying promotive supplementation of Zn-lys and Fe- lys in improving the growth and biomass of different plants in metal stress environment [13, 27, 28]. This enhancement in the growth-related attributes is attributed to the active role of amino acids different ultra-structural elements [54]. [54] reported that amino acids also possess stimulation effect for the proper growth and yield.

Results revealed that gas exchange parameters and chlorophyll contents of the *Brassicca napus L* were reduced under the application of wastewater. Considerable reduction in the growth and biomass (fresh weight + dry weight) of the plant is also attributed to the decline in light harvesting components including carotenoids, chlorophyll a, chlorophyll b and photochemistry of the plant. Cr induced reduction in photosynthesis and contents of chlorophyll and carotenoids were previously recorded in different plants including maize, rice spinach and sunflower [22, 49]. In present study, a gradual increase in the gas exchange parameters and chlorophyll contents of the *Brassicca napus L* were recorded with the combine application of Zn-lysine and Fe lysine as given in Fig (1). Amino acids also help to improve the contents of chlorophyll in plants leading to developed degree of photosynthesis. Similarly, [55] also stated that amino acids improves the plant growth by improving the availability of nutrients and photosynthetic assimilation in plants. Significant improvement in photosynthetic

machinery, gas attributes in *Brassicca napus L* with the combine application of Zn-lys and Fe-lys under Cr stress generated due to tannery wastewater treatment is attributed to the higher uptake of both Zn and Fe with the subsequent decline in Cr accumulation. Results indicates that combine application of both these micronutrients along with lysine effectively improve the gas exchange attributes and chlorophyll contents of the plants as presented in Fig (3). Similar finding were witnessed by [29] and [28] in wheat and rice under Cd stress.

Metal stress is grave risk for the plant growth, toxic contents of metals results in the over formation of ROS as compared to no stressed plants [11, 13]. Enhancing contents of Cr with increases in the concentration of wastewater boost up the production of ROS, EL and MDA and H_2O_2 in all organs of the plant (Fig. 3). Cr stress has extensively studied for the unwanted increases in oxidative stress and lipid peroxidation in various plants including *Brassica juncea L*, *Brassica oleracea botrytis L* and *Cymbopogon flexuosus* [22, 49, 56]. In our investigation, combine supplementation with both Zn-lys and Fe lys reduce the overall generation of ROS, MDA, EL and H_2O_2 in *Brassicca napus* exposed to tannery wastewater irrigation via boosting up the activities of all-important antioxidant enzymes like APX, SOD, CAT and POD (Fig 3 and 4). Amino acids have the ability to restrict the generation of ROS in various plants. Supply for various amino acid increase the actions of antioxidant enzymes as previously reported in soya bean plant [57]. Similarly supply of amino acid chelated with micronutrients like Zn and Fe improves the activities and efficiency of various antioxidant enzymes in rice and spinach [13, 58]. Combine Exogenous application of both Zn-lys and Fe-lys played a very crucial role in the scavenging of ROS and safeguarding the *Brassicca napus L* from Cr toxicity by restricting its accumulation in plant.

Our results stated that, increases in the level of tannery wastewater application in soil resulted in significant accumulation of Cr contents in different parts of the plant by restricting the uptake of essential nutrients like Zn and Fe as given in Fig (5). High concentration of Cr concentration in various tissues of plants were also detected previously by different experimental studies [7, 12, 13]. It is well documented that plants which already absorbed a considerable amount of heavy metals are unable to accumulate the vital micronutrients as they possess the failing photosynthesis machinery and disturbed ultra-structure of chloroplast [20, 59, 60]. It is also observed that, restriction in the uptake of necessary micronutrients is attributed to the inability of plants roots to successfully absorb these vital nutrients under the uncompromising stress of Cr toxicity [16]. Much higher accumulation of Cr in plants significantly effects the yield, morpho-physiological and physio-biochemical attributes of the plants [4, 15]. Although, our finding recommended that combine external supplementation with Zn-lys and Fe-lys effectively restricts the accumulation and uptake of Cr and improved the accumulation of both Zn and Fe in various tissues of the plant (Fig 6). Our findings depicting that, application of Zn-lys improve the concentration of Zn in various plants like *Triticum aestivum L* [29], *Oryza sativa* [27] and *Spinacia oleracea* [13]. Similarly Fe-lys enhanced the contents of Fe in *Oryza sativa* [28] and *Zea mays* [49].

5. Conclusions

Our results confirmed that plant growth, photosynthesis and antioxidant system were adversely affected by Cr stress generated as a result of tannery wastewater application. Although, the oxidative stress and uptake/accumulation of Cr were enhanced with increasing levels of wastewater applications. Combine exogenous application of both Zn-lysine and Fe-lysine significantly improved the morpho-physiological traits and defense system to mitigate the oxidative stress and Cr toxicity via activation of vital antioxidant enzymes. Nevertheless, imminent research is required to study the mechanism of both combine and individual application of amino acid chelated with different micronutrients like Zn and Fe on different plants grown on polluted soil contaminated with other heavy metals under field conditions.

Authors contribution: Conceptualization, Ihsan Elahi Zaheer; Data curation, Hafiza Sana Yousaf and Sajid Fiaz; Formal analysis, Ihsan Elahi Zaheer, Muhammad Hamzah Saleem, Afifa Malik, Zohaib Abbas and Sajid Fiaz; Funding acquisition, Xiukang Wang, Hafiza Sana Yousaf, Afifa Malik, Muhammad Rizwan and Sajid Fiaz; Investigation, Ihsan Elahi Zaheer, Xiukang Wang, Zohaib Abbas, Muhammad Rizwan and Sajid Fiaz; Methodology, Hafiza Sana Yousaf, Afifa Malik, Zohaib Abbas and Muhammad Rizwan; Project administration, Shafaqat Ali; Resources, Xiukang Wang; Software, Afifa Malik; Visualization, Shafaqat Ali and Muhammad Hamzah Saleem; Writing – original draft, Shafaqat Ali and Muhammad Hamzah Saleem; Writing – review & editing, Shafaqat Ali and Muhammad Hamzah Saleem

Acknowledgments

Thanks to Higher Education Commission of Pakistan for providing financial assistance. The findings of this study are a part of Doctorate studies of Ihsan Elahi Zaheer.

Funding: The publication of the present work is supported by the National Key Research and Development Program of China (grant no. 2017YFC0504704) and the National Natural Science Foundation of China (51669034, 41761068, 51809224).

Conflicts of Interest: The authors declare that there is no conflicts of interest.

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