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AN EVALUATION PHYTOREMEDIATION EFFICIENCY OF CARROT PLANTS FOR HEAVY METAL CONTAMINATED SOILS

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ABSTRACT

The present study intended to evaluate the remediation ability of carrot plants to remove Cu and Zn from the contaminated soil using growth and biochemical attributes. Furthermore, the possible mechanisms such as accumulation or exclusion regarding metal tolerance in carrot plants were also assessed. Our data showed that metal toxicity affected the plants. However, the growth of root was decreased substantially as compared to shoot under heavy metal stress. The oxidative stress indicators such as H_2O_2 and Malondialdehyde (MDA) were increased as the metal concentration increased. Physiological mechanism enlightened that the level of soluble sugars and chlorophyll content index was reduced as the metal concentration increased in the soil due to damages in the reactive centers of PSII at higher metal concentrations, Cu in particular. Furthermore, higher metal content was observed in roots as compared to shoots. Greater bioaccumulation factor and lower translocation factors were recorded for the carrot plants. The zinc metal showed relatively better physiological activities due to less metal accumulation in shoot. It is concluded that carrot plants tolerate Cu and Zn by accumulation of these ions in their roots so that this plant can be better choice for metal contaminated soil.

Key words: Phyto-remediation, carrot, heavy metal, oxidative stress, copper and zinc.

INTRODUCTION

The term heavy metal is applied to the trace metals up to 1000 ppm or less in earth's crust having a density greater than 5000 mg cm^{-3} (Wijayawardena *et al*., 2016). Among these metals copper (Cu), zinc (Zn), cadmium (Cd), nickel (Ni), chromium (Cr), cobalt (Co), mercury (Hg) and lead (Pb), are well reported (Zacarias *et al*., 2012). It was suggested that heavy metals are biologically essential at low concentrations for normal metabolic processes of plants (Diacono and Montemurro, 2010). However, non-essential elements like Pb, Hg, Cd and Sn are not required for normal metabolism. In nature, both essential and non-essential elements enter the plants but the tolerance capacities of the plants vary regarding concentration of metal and plant cultivars (Gupta *et al*., 2010).

Contamination of soil air and water by heavy metals is a serious threat to the environment. Metal toxicity is of great concern particularly for developing countries such as Pakistan where pressure on agriculture output and industrial production causes the heavy metals accumulation in the environment (Savvas *et al*., 2010; Pehluvan *et al*., 2012; Wijayawardena *et al*., 2016). The sources of heavy metals in the land are inorganic and organic fertilizers, sewage sludge, effluents from various industries, and pesticides; particularly fungicides (Gupta *et al*., 2010). Consequently, the high concentrations of the heavy metals in soil become toxic to natural flora and fauna affecting physiological and biochemical processes (Kovacik *et al.*, 2009; Hediji *et al*., 2015). The visible and non-specific symptoms of metal toxicity include rapid inhibition of seed germination, seedling and root growth stunted plants, chlorophyll degradation and enzyme inhibition (Clemens, 2006; Ali *et al*., 2013; Gangwar *et al*., 2014). In cell, most of the metals such as Cd, Ni, Pb may participate in most of the biochemical pathways which results in the formation of reactive oxygen species (ROS), which causes oxidative damage in plants (Sharma and Dietz, 2009; Gill and Tteja, 2010).

Nevertheless, the heavy metals tolerance in plants is manifested by an interaction of a genotype to its environment (Gallego *et al*., 2012). Thus, the visible injury symptoms in the form of morphological disorders regarding germination and growth can be used as predictors to assess the degree of metal tolerance. Some plant species have evolved adapting means involved in general homeostasis that may enable them to thrive on metaliferrous soils (Peralta-Videa *et al*., 2009). Plants also possess a range of cellular mechanisms starting from detoxification to tolerance against heavy metal stress. In this regards enzymatic i.e. peroxidases (POD), catalases (CAT), superoxide dismutases (SOD,) and non-enzymatic (proline, carotenoids and sugar accumulation etc.) defense systems to cope with oxidative damage are well known (Szabados *et al*., 2011; Shavalli Khan *et al*., 2014; Szollosi, 2014).

Strategically, plants can be grouped as either accumulators or excluders for their metal tolerance mechanism (Chung *et al*., 2011). In this way a technology is evolved in which plants can eliminate, sequester or destroy contaminants from water, soil and air called phyto-remediation (Nakbanpote *et al*., 2016). This technique is getting great attention in recent years because of significant variability in plant species for heavy metal tolerance based on various tolerance strategies (Sytar *et al*., 2016; Jan *et al*., 2016).

Daucus carota L. (carrot) is a root vegetable, usually orange in color and are domesticated form native to southwestern Asia and Europe. Carrot is grown in vast areas of Pakistan. Though, in modern research phyto-remediation ability of dissimilar plant species becoming major focus but some of the important crop plants still need to be closely monitored (Mahmood *et al.,* 2005). Further PSII photosynthetic efficiency in metal tolerance plants is rather scarce (Dixit *et al*., 2015). Therefore, essential workability of PS II about crop plant species is lacking in relation to their growth on metal contaminated soils. Therefore, it was imperative that the impacts of heavy metal toxicity on the plant growth and physiological responses be studied and localizes the metals within the plant. While considering the impetus for the identification of metal tolerant crops, the present study was being carried out to explore the cultivation potential of carrot plants for tolerance to Cu and Zn contamination and physiological information regarding metal tolerance is also provided.

MATERIALS AND METHODS

Collection of plant materials

The plant material/germplasm was obtained from the "Agricultural department University of Karachi" Experiment was carried out using soil culture (after texture determination) in plastic pots under natural environmental conditions. Two metals (Cu and Zn) and their combination (Cu+Zn) were used to treat the plants. Salts of Zn ($ZnSO_4$) and Cu ($CuSO_4$) were used as a source of these metals. Three levels of heavy metal treatments i.e. 20, 40, and 60 mg kg^{-1} of soil were applied apart from control. The experiment was carried out in a completely randomized block design for eight weeks and replicated three times.

Metal uptake by plant modules

The diverse biochemical attributes included metal uptake by various plant modules were calculated by using Atomic Absorption Spectrometry. In our study, a simple and quick method of digestion of plant modules was used (Huang *et al*., 2004). The samples were taken in 50 mL polypropylene tubes with 3.2 mm ventilation holes in their caps. A microwave digestion system was used for the purpose of digestion. The process comprised of two phases. In the first step, digestion of plant material with HNO_3 is carried out followed by heating the mixture at 75 ˚C (10 minutes) and 109 ˚C (15 minutes). The second phase comprised of cooling the mixture for ten minutes and after that 100 µL of H_2O_2 was added. Later the sample was heated again at 109 ˚C for 15 more minutes.

Metal tolerance index (TI), Bio-concentration factor (BCF) and Translocation factor (TF)

Metal tolerance index **(**Wang *et al*., 2014) used to predict the sensitivity of tissues to stress induced by heavy metals. Calculations were also carried out for bio-concentration factor (BCF) to evaluate the phytoremediation potential of target plant. BCF is the ratio of soil and plant concentration of Cu and Zn (Wang *et al*., 2014). To find out metal concentration in shoots/roots the method of Majid *et al*. (2012) was followed.

Chlorophyll fluorescence and Chlorophyll contents index

The chlorophyll fluorescence emissions were quantified using 8 weeks old dark adapted (30min) leaves. The pulse amplitude modulation mode of the fluorescence monitoring system (Handy PEA) was used for this purpose. Initial measurements were recorded under modulated beam of far-red light (LED typical peak at 735 nm wavelength). The maximum (Fm), original (F0) and fluorescence yields were observed at weak (0.5 µmol m^{-2} s^{-1} modulated red light with 6.8 µmol m^{-2} s^{-1} PAR. The variable fluorescence traits regarding quantum yield of PSII photochemistry were measured following Maxwell and Johnson (2000). Chlorophyll contents index was measured on fully expanded young leaves using chlorophyll contents meter (Hansatech CL-01, USA).

Estimation of Soluble sugars

The soluble sugars (mg g^{-1} FW^{-1}) were estimated following (Dey, 1990). Fresh plant material with 10 mL alcohol was heated for one hour at 600 ^{o}C in an incubator. Mixture was then extracted and final volume of 25 mL was made by adding alcohol. To 1 mL of this extract, 100 µL of diluted Phenol and 500 µL of sulphuric acid were added. This mixture was then thoroughly mixed and cooled in air. Beckman DU 640 spectrophotometer was used to quantify the absorbance at 485 nm against a standard curve of glucose solution.

H_2O_2 and MDA contents

Hydrogen peroxide (H_2O_2) and Melondialdehide (MDA) contents were measured according to the procedure of (Velikova *et al*., 2000) and (Dhindsa *et al*., 1981), respectively.

Extraction and Estimation of Antioxidant Enzymes

Antioxidant enzymes were extracted using 500 mg leaf samples were homogenized in 10 mL protein extraction buffer (Tris-HCl pH 6.810 mL DDT, 0, 1 mM EDTA 50 mg PVP).. The whole contents were centrifuged at 12,000 RPM for 10 min and later protein content was examined (Bradford 1976). Catalase (CAT) activity was measured by the method of Patterson *et al*. (1984). In a reaction tubes the H_2O_2 decomposition was observed at 240 nm and the decrease in 240 nm absorbance was used to illustrate the activity. The superoxide dismutase **(**SOD) activity was assayed by the method of Beyer and Fridovich (1987).

Statistical analysis

Statistical packages such as MINITAB and MS EXCEL were used for statistical analysis of the data. Graphic and tabulated presentation of the data was carried out using the afore-mentioned computer software. Least significant differences (LSD) were calculated by DMRT (Duncan, 1955).

RESULTS

Plant biomass

The results for root and shoot length, dry weight and fresh weight (plant biomass) of *Daucus carota* plants grown under varying levels of Cu, Zn and Cu + Zn are presented in Fig. 1. The plants exhibited a significant decline in biomass attributes under metal stresses as compared to control. Consequently, the combined metal treatment (Cu + Zn) appeared to be more drastic for plant biomass. The lowest root and shoot lengths were recorded under 60 mg kg^{-1} concentration of metal. A substantial decline in shoot length was observed at the 60 mg kg^{-1} of combined metal treatment which was 31% compared to control. Moreover, the highest increments in the shoot length were observed in 20 and 40 mg kg^{-1} Zn treatments. The results indicated a marked decline in fresh and dry mass of plants with increasing metal concentration in the growth medium. However, Carrot plants showed greater biomass under Zn treatments as compared to other treatments (Fig 1). Moreover, the highest increments in the shoot fresh mass was observed in 20 and 40 mg kg^{-1} Zn treatments. The fresh mass of shoot decreased by 50% as compared with control at 60 mg kg^{-1} of Zn followed by 36% reduction under Cu. The lowest decline shoot fresh mass i.e. 16% was recorded for Cu + Zn as compared to control. The results for the dry weight of shoot of *Daucus carota* plants grown under varying levels of Cu and Zn have been recorded. The maximum decrease in shoot dry mass was observed at 60 mg kg^{-1} of Cu + Zn treatment, where a drop of 91% in dry mass was recorded as compared with control. The same was recorded as 75% for Cu and 44% for Zn in case of individual treatments. A significantly consistent decline in root fresh mass was recorded when treated with heavy metals. The fresh biomass of root showed a concentration dependent reduction. Root fresh mass were decreased in combined metal stress (Zn + Cu). Root fresh biomass decreased by 54% as compared with control when the plants were grown under both Cu and Zn at 60 mg kg^{-1}. The same was recorded to be 48% and 46% for Cu and Zn respectively. The greatest extent of decrease in root dry mass was observed under Zn where 90, 92 and 93% drop in dry mass was observed at 20, 40 and 60 mg/kg as compared with control. Cu treatment (60 mg kg^{-1}) exhibited greater decrease in biomass as compared to control. The lowest decline in root dry mass was recorded for combined treatment of Cu and Zn. The data indicated that carrot plants exhibited an overall better shoot growth as compared to roots under metal stress. Among the metal treatments, Zn showed greater biomass under 20 and 40 mg kg^{-1} concentrations as compared to individual Cu and combined Cu treatments.

Chlorophyll fluorescence

Chlorophyll ‘a’ fluorescence parameters did not show consistent results under metal stress even in higher concentrations (Fig. 2). The non-photochemical quenching (NPQ) and non-photochemical quenching co-efficient (qN) were higher in 60 mg kg^{-1} of Cu and Cu + Zn treatments. However, the maximum quantum yield was decreased at higher metal concentrations, especially in the Cu and Zn + Cu treatments. This decline in Fv/Fm ratio was not significantly affected in plants treated with 20 and 40 mg kg^{-1} of Zn. Compared to Cu and Cu + Zn treated plants the Zn treated plants expressed better photosynthetic performance in terms of Fv/Fm ratio. However, considerable decline was found for Cu (23%) and Zn + Cu (28%) compared with the control (Fig. 2). The OJIP fluorescence curve showed non-significant differences at 20 mg kg^{-1} of Cu, Zn and Cu + Zn treatments (Fig. 3). Individual Zn treatments also showed non-significant variations except fewer than 40 mg kg^{-1} Zn treatment that expressed higher I-P curve. In 60 mg kg^{-1} metal treated plants showed some non-significant differences compared to control.

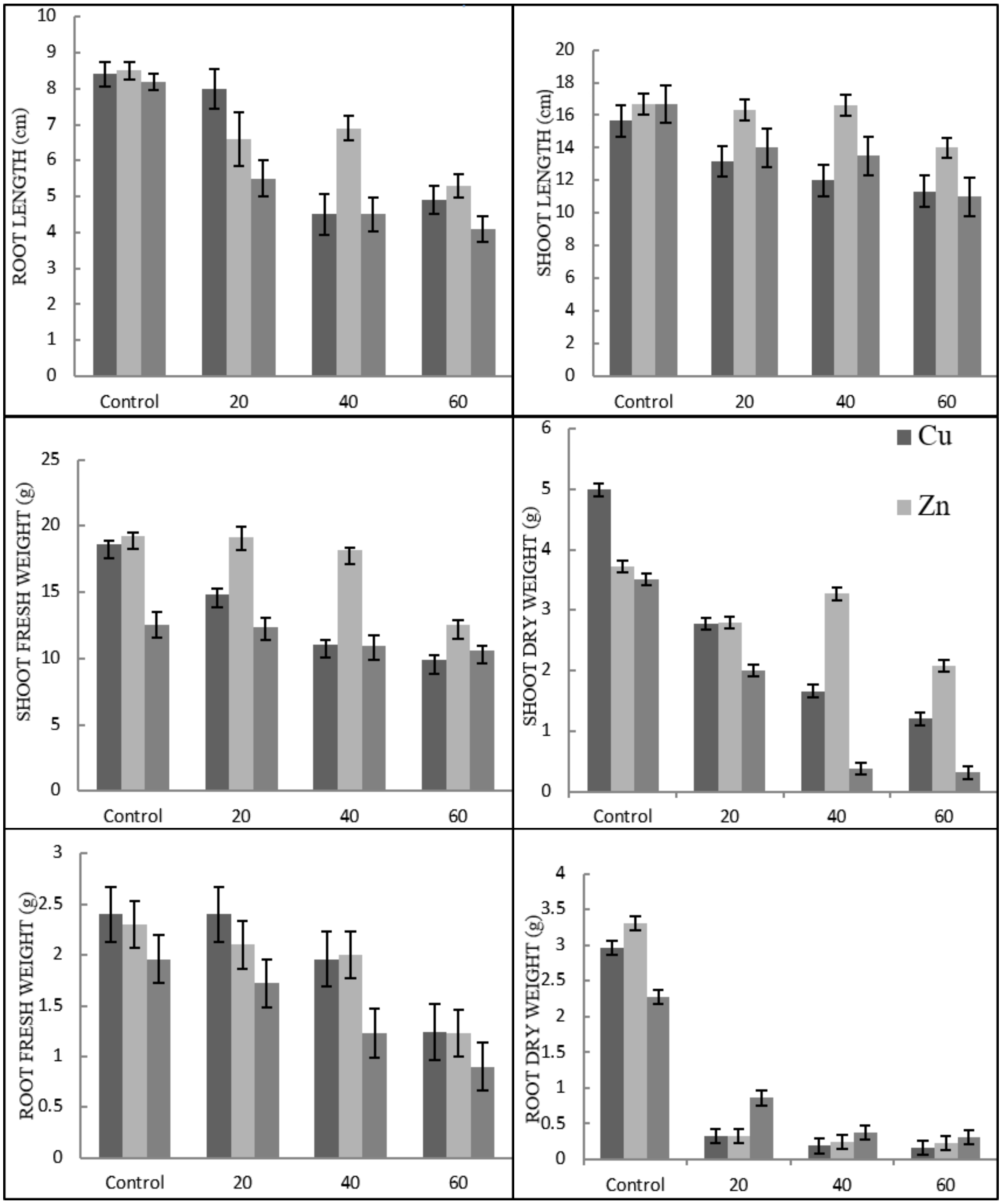

Fig. 1. Changes in shoot length, root length, shoot fresh weight, shoot dry weight, and root fresh weight and root dry weight of *Daucus carota* plants grown for eight weeks under varying levels of Cu and Zn. Vertical lines on the bar expressed the mean standard error (±).

Chlorophyll, soluble sugars and metal contents:

In Fig. 4 the results for the chlorophyll, soluble sugars and metal contents in the carrot plants when grown under heavy metal stress were displayed. The amount of chlorophyll reduced as the metal concentration increased in the soil. In 40 mg kg^{-1} the Zn treated plants expressed relatively better performance. A 23% decline was recorded for both Cu and Zn in comparison with control at 60mg/kg level. The maximum reduction was observed to be 27%

under Cu + Zn treatment as compared to control. A consistent decline in the sugar content of plants was observed at 40 and 60mg kg^{-1} concentrations. It was recorded to be 63% and 55% under 60 and 40 mg kg^{-1} respectively as compared to control. However, all the treatments showed same retarding effect on soluble sugar content. Results expressed that metal in root accumulated were more as the concentration increased in the soil (Fig. 4). Metal accumulation in roots was highest at the highest concentration of Cu + Zn (60mg kg^{-1}). Hence the metal content in the roots increased with increasing levels irrespective of the type of the treatment. All three metals were found invariable for this attribute. Zn has lower metal accumulation in roots as compared to Cu. The data for shoot metal content showed a distinct augment in the metal concentration of shoot when plants were grown under varying heavy metal levels for eight weeks (Fig. 4). It became evident from this comparison that the roots accumulated more metal than shoot. Zn treated plants has lowest metal in their shoot compared to Cu treated pants. The highest metal accumulation was observed under combined metal treatment Cu + Zn in shoot. It was observed that the Cu accumulation was not increased under 60 mg kg^{-1} as compared to lower concentrations.

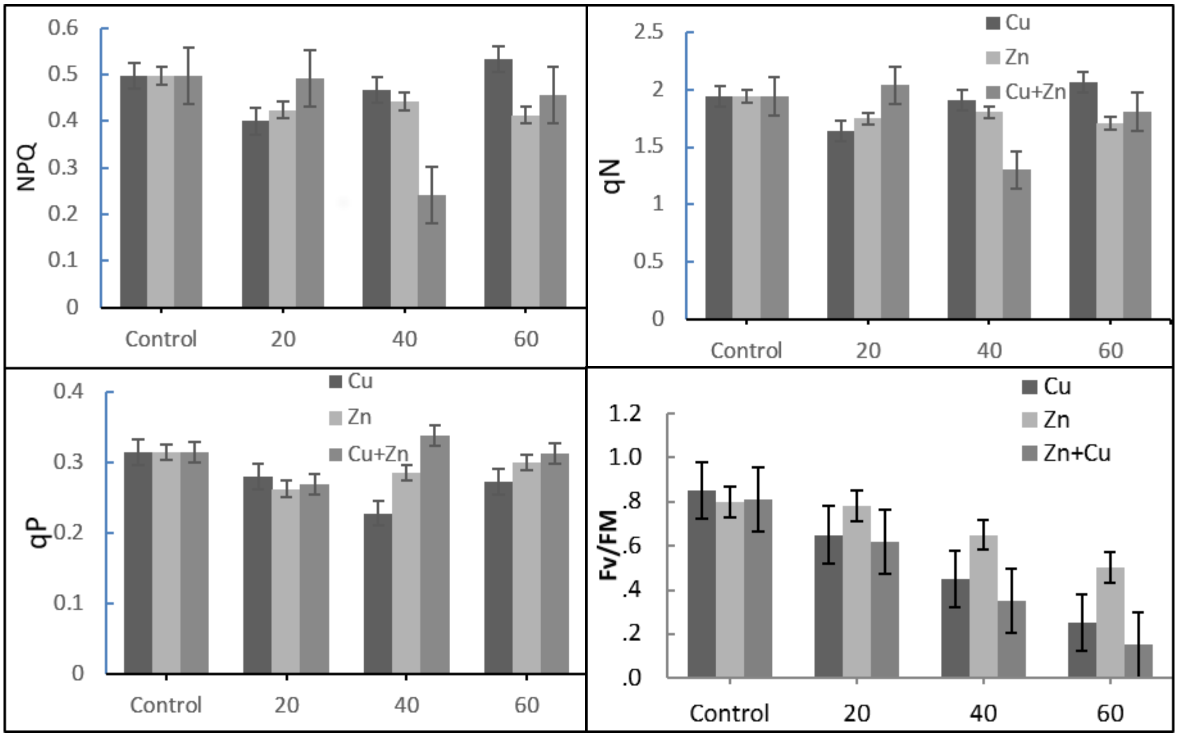

Fig. 2. Changes in non-photochemical quenching (NPQ), non-photochemical quenching coefficient (qN), photochemical quenching (qP) and maximum quantum yield (Fv/Fm) of *Daucus carota* grown for eight weeks under varying levels of Cu and Zn.
Vertical lines on the bar expressed the mean standard error (±).

MDA and H_2O_2 contents and antioxidant enzymes activities

The responses of carrot plants regarding MDA, H_2O_2 contents and antioxidant enzymes activities under varying levels of Cu and Zn concentrations was illustrated in Fig. 5. It is evident from these results that there was a steady rise in amount of MDA with the increasing levels of the heavy metals. The maximum MDA content (0.79 µmol mg^{-1} FW) was recorded in combined Cu + Zn treatment at the highest concentration (60mg kg^{-1}). The results indicated an increase in the amount of H_2O_2 in plant leaf was observed in plants when it was treated with different metals. There was a steady increase in H_2O_2 level in presence of Cu in the growth medium with the increasing levels. Whereas, in case of Zn and Cu + Zn treatments, the H_2O_2 amount increased in 40mg kg^{-1} level, While, lower values were recorded at the highest metal concentration i.e. 60mg kg^{-1}. However, under higher metal treatment, Zn showed lowest H_2O_2 contents as compared to Cu and combined Cu + Zn treatments. A steady rise in the CAT activity was observed as the concentrations of stress treatments were raised. Consequently, the maximum enzyme activity was seen at highest metal concentration (Fig. 5). CAT activity was increased under 60mg kg^{-1} Zn and Cu treatments as compared to control. There was a considerable rise in the SOD activity with increasing metal concentrations (Fig. 5). The highest increment was observed in combined metal stress treatment whereas lowest increment was observed in Zn treatments. The increment was up to 80% and 55% increase for 60 mg kg^{-1} Cu + Zn and Cu respectively.

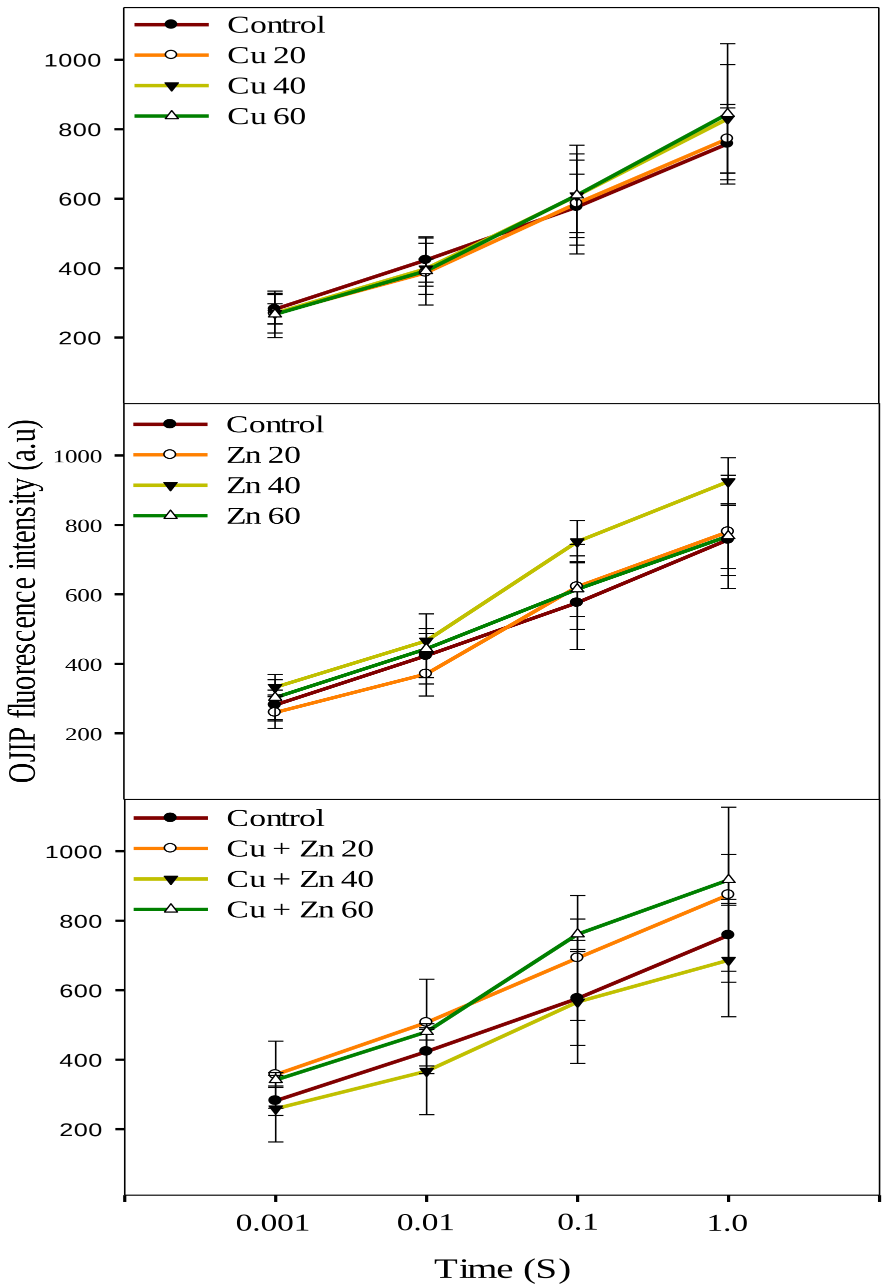

Fig. 3. Changes in OJIP curve of *Daucus carota* grown under varying levels of Cu and Zn stress. Vertical lines on the line expressed the mean standard error (±).

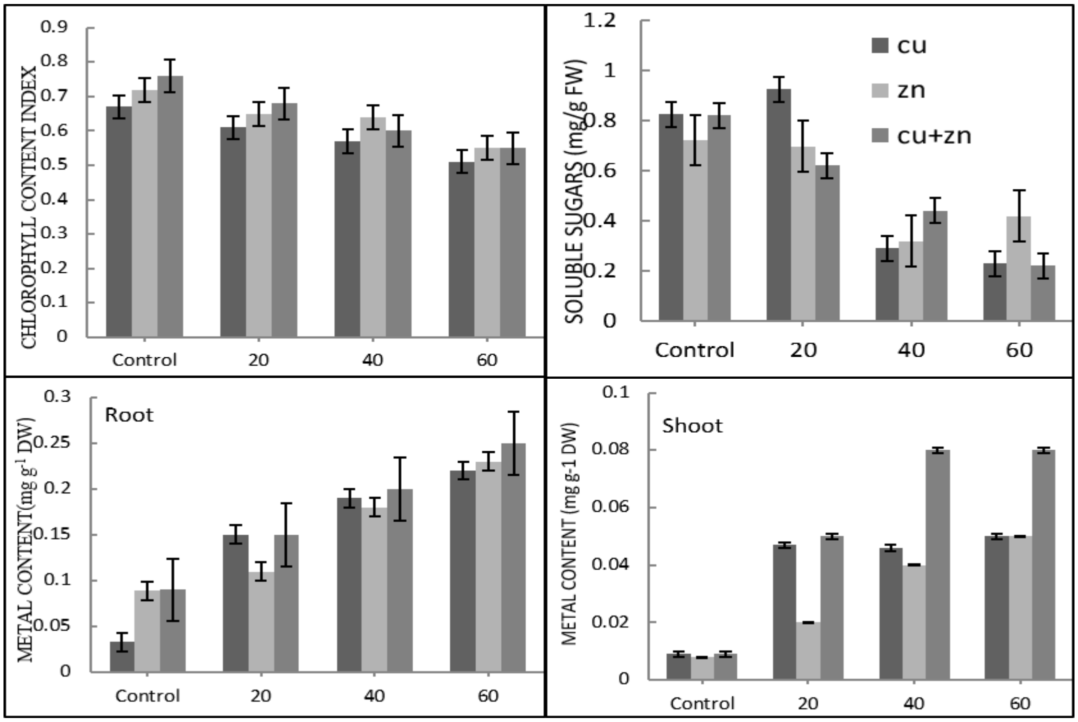

Fig. 4.Changes in chlorophyll content index, soluble sugar, shoot metal contents and root metal contents of *Daucus carota* grown under varying levels of Cu and Zn stress. Verticle lines on the bar expressed the mean standard error (±).

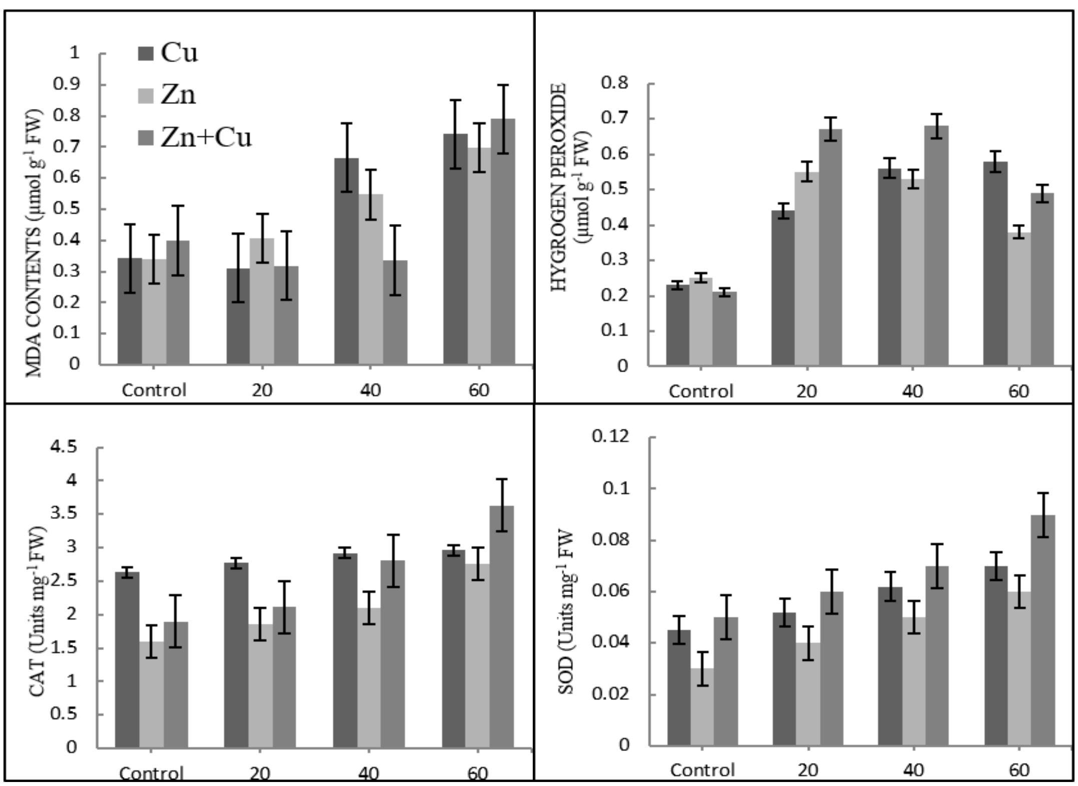

Fig. 5. Changes in MDA, H_2O_2 contents, CAT and SOD activities of *Daucus carota* grown under varying levels of Cu and Zn stress. Vertical lines on the bar expressed the mean standard error (±).

Table 1. Tolerance Indices (TI) % ± S.E. of *Daucus carota* grown for eight weeks under varying levels of Cu and Zn. (n= 3)

	Root Tolerance Index			Shoot Tolerance Index		
	Metal Levels (mg/kg)					
Metal Treatments	20	40	60	20	40	60
Cu	5.88 ± 0.05	6.7 ± 0.06	5.41 ± 0.04	3.19 ± 0.02	3.63 ± 0.03	2.93 ± 0.02
Zn	5.71 ± 0.04	6.78 ± 0.06	5.47 ± 0.04	3.06 ± 0.02	2.23 ± 0.005	1.72 ± 0.01
Cu+Zn	3.04 ± 0.02	3.53 ± 0.03	4.51 ± 0.03	1.59 ± 0.001	2.36 ± 0.005	1.85 ± 0.02

Values are the mean of three replicates ± standard error (SE)

Table 2. Bioaccumulation Factor (BF) and Translocation Factor (TF) of *Daucus carota* grown for eight weeks under varying levels of Cu and Zn. (n= 3).

	BIOACCUMULATION FACTOR (BF)			TRANSLOCATION FACTOR (TF)		
	Metal Levels (mg/kg)					
Metal Treatments	20	40	60	20	40	60
Cu	0.59 ± 0.05	0.85 ± 0.033	1 ± 0.04.	0.066 ± 0.001	0.24 ± 0.022	1.5 ± 0.03
Zn	0.59 ± 0.04	1 ± 0.043	1.13 ± 0.032	0.18 ± 0.03	1.2 ± 0.06	0.086 ± 0.005
Cu + Zn	0.69 ± 0.044	0.75 ± 0.036	1 ± 0.056	0.01 ± 0.001	0.053 ± 0.002	1.1 ± 0.045

BF= Metal concentration of plant/ Metal concentration of soil,
TF= Metal content in shoot/ Metal content in root
Hyper accumulating plants have BF <1 and TF<1 (Majid *et al*., 2012).

Tolerance Index (TI), Bioaccumulation (BF) and Translocation Factor (TF):

The tolerance indices of plant modules reflect the tissue sensitivity of plants in response to the heavy metal stress in the growth medium. The statistics for the TI of roots and shoots of *D. carota* plants have been presented in Table 1. These results indicated greater TI of root as compared with shoots of the plants under study. The highest TI (5.88 ± 0.05 for Cu and 5.71 ± 0.04 for Zn) were observed at lesser metal level (20mg/kg) for Cu and Zn in root tissues. Moreover, lowest tolerance indices (Cu = 5.41 ± 0.04 & Zn = 5.47 ± 0.04) were recorded at the highest metal concentration (60mg/kg) for these heavy metals. For combined metal treatment, the maximum tolerance index was observed at the highest concentration. Bioaccumulation factor corresponds to the proportion of metal concentration in the soil to that of plant. Whereas, the Translocation factor is the ratio of metal content of shoot and root. Plants which have BF<1 and TF<1 are referred to as hyper accumulators. Plants under study exhibited BF greater than one for Cu and Cu + Zn treatments (Table 2) at the highest metal concentration (60mg/kg). It was observed that BF was greater than 1 at 40 and 60 mg/kg for Zn. These results indicated that plants showed higher BF in the maximum metal level. On the other hand, less than 1.0 value was recorded for translocation factor at all levels of all the three metal treatments (Table 2).

DISCUSSION

The plant growth and development are greatly affected by the solutes uptake from the soil. The development of the root system of *D. carota* plants was observed both in the presence or lack of toxic metals in the soil. Significant differences were recorded for the root growth when plants were grown under copper and zinc individually and in combination as compared to control. The results clearly represented an adverse effect of metal toxicity in plant growth. The plants exhibited a significant decline in their biomass accumulation with increasing metal concentration. The reduction of root length appeared to be more drastic under Cu treatments. Similar results were recorded for combined treatments (Cu + Zn). Moreover, the effect of combined treatment of both metal proved to be more retarding to the root growth as compared with the individual treatment applications. The greater negative effect of metals used in this investigation were observed in root growth with increasing metal concentration as compared to shoot growth. These findings are in agreement with several other studies which described the affects of heavy metal toxicity in different plant species and for diverse metals such as Ni, Mn, Cd, Cu, Cr, Pb, Zn (Tiecher *et al*., 2016; Ali *et al*., 2013; Dixit *et al*., 2015; Sumiahadi and Acar, 2018).

In this investigation, the carrot plants exhibited an overall better shoot growth under Zn treatments as compared Cu stress. (Meldau *et al*., 2012) proposed that under metal stress, the biosynthetic capacity of the plants is redirected from growth to defense. For this reason, carrot showed growth reduction under stresses environments. However, Zn treated carrot plants did not showed substantial decline in biomass. Our results suggested that carrot plats accumulated Zn metals in their roots and reduced the translocation of Zn towards the shoot. Reduction in shoot metal accumulation leads to the tolerance in carrot plants under Zn treatments.

The heavy metals alter the chloroplast ultra-stucture and reduce the net photosynthetic rate, stomatal conductance and leaf transpiration (Souza *et al*., 2011). In this study, the amount of chlorophyll was reduced as the metal concentration increased in the soil. The influence of Cu was observed to be more profound in contrast with Zn treatment. The photochemical efficiency of PSII in dark-adapted plants with the PSII fully open is expressed Fv/Fm (maximum quantum yield). Fv/Fm is a good indicator of change in the photosynthetic quantum conversion rate (Vernay *et al*., 2007) ours results showed that it was more sensitive to the Cu and combined Zn+Cu treatments compared to other treatment.

The decline in the chlorophyll content index in carrot plants after exposure to elevated metal treatments under study may imply that these heavy metals are able to influence the chlorophyll synthesis (Tiecher *et al*., 2016). These results are also in agreement with other workers who observed a considerable decline in chlorophyll content and quantum yield (Fv/Fm) of grasses treated with heavy metals (Vernay *et al*., 2007; Jiang *et al*., 2010; González *et al*., 2012). The conversion of light energy into chemical energy is reduced under environmental stresses (Umar and Siddiqui, 2018). The proportion of inactive reaction centre can be determined through the measurement of photochemical quenching (qP) (Moradi and Ismail, 2007). Decline in qP showed the disintegration of light harvesting complex from PSII under abiotic stress (Wu *et al*., 2010; Umar and Siddiqui, 2018). Our results indicated that qP was not significantly declined under higher concentrations of Zn and Cu. This might be due to the lower metal uptake in carrot plants. To improve photosynthetic efficiency, plant tends to absorb more sunlight and unfortunately excessive absorption accelerates the quantity of inactive reaction centre. In this scenario, plant activates its regulatory mechanism, the non-photochemical quenching (NPQ). Our results indicated that the NPQ was non-significantly changed under Zn and Cu treatments.

It was revealed from the literature that heavy metal toxicity affects the growth and development of plants to a greater proportion than any other stress (Gill and Tutaja, 2010). The heavy metal stress disrupts the physiological and biochemical processes by triggering the processes for generation of reactive oxygen species (ROS), which results in oxidative stress. The ROS are present to the partially reduced form or activated derivatives of oxygen radical. Therefore they are comprised of both free radicals (O^{-2}, $OH^{-\cdot}$, $OH^{-2\cdot}$) and non-radical forms (H_2O_2) in the plant system. The compound effects of these species cause metabolic disorders, damaging cellular infrastructure and enhancing senescence processes. The accumulation of excessive amount of ROS in plant system cause cytotoxic effects (Shahid *et al*., 2014) deteriorating DNA, lipids, proteins and reduction in organic osmolytes (Yang *et al*., 2003; Siddiqui *et al*., 2008; Gill and Tutaja, 2010; Siddiqui and Khan, 2011; Siddiqui *et al*., 2013). The results of the study showed that different levels of Cu and Zn either alone or in combine form caused increase in oxidative stress, inhibited root proliferation and stunted growth in carrot (*Daucus carota* L.). Accumulation of excessive amounts of Cu and Zn produce negative effects on normal cellular functioning of plants generate more free radicals, hydrogen peroxide content cause lipid peroxidation at cellular level (Dixit *et al*., 2001; Srivastara *et al*., 2005; Cao *et al*., 2009; Gill and Tuteja, 2010). It is inferred from this study that quantity of MDA content and H_2O_2 increased with increasing level of Cu and Zn metal indicating higher oxidative stress in carrot plants. This effect resulted in deteriorating plasma membrane structures concurrent reduction in quality of plant regulators (Chakrabarty *et al*., 2009; Meng *et al*., 2009). To reduce the concentration of ROS, plants have enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) for improving healthiness of plants under metal stress (Sandalio *et al*., 2001; Cetinkaya *et al*., 2014; Tiecher *et al*., 2016). So, the synthesis of proteins and mainstreaming of enzymatic activities are influenced directly under Cu and Zn stress environment. The plants vary greatly to assimilation of Cu and Zn in terms of quantum of uptake oxidative damage and up regulation of antioxidant enzyme system. The enhanced amount of CAT could eliminate the toxicity of H_2O_2 and other peroxidases in realm of heavy metal stress. The up-regulation of SOD and POX would mitigate the adverse effects of oxidative stress (Cetinkaya *et al*., 2014; Chamsddin *et al*., 2009; Singh *et al*., 2007; Moravcova *et al*., 2018). The greater CAT and SOD activities explained that the carrot plants bear Zn and Cu tolerance by reducing ROS production inside the leaf cells.

The increase in metal contents was observed to be concentration dependent. It was observed that roots appeared to have more metal content than shoot. Several other studies also suggested that the uptake and accumulation of various heavy metals by certain plants are varied when they were grown under polluted soil conditions **(**Luo *et al*., 2012; Poşta *et al*., 2015). It is suggested that due to less accumulation of metals in shoot, carrot plants tolerated the metal contents and expressed non-significant decline in photosynthetic efficiencies. Furthermore, Zn accumulation was lowest in shoots hence Zn treated plants showed better tolerance compared to Cu treated plants. The tolerance indices (TI) of plant modules reflect the tissue sensitivity of plants in response to the heavy metal stress in the growth medium. It was observed that roots of *D. carota* appeared to be less sensitive to metal stress than shoots.

Bioaccumulation (BF) and Translocation Factor (TF)

Bioaccumulation corresponds to the proportion of metal concentration in the soil to that of plant. Whereas, the Translocation factor (TF) is the ratio of metal content of shoot and root. These results strongly suggest that plants were accumulating heavy metals in their roots. It was reported that some species, are exemplified by their capacity to mount up greater magnitude of metals in various plant parts (Zakka *et al*., 2014). These plants are regarded as Hyper-accumulators which achieved a plant-to-soil metal-concentration ratio greater than one (>1).

Conclusion

It was concluded that carrot plant is a good phytoremediator which not only tolerates the metal stress accumulating the metals in their roots and reduces the uptake of heavy metals by the shoot but also keeps the substantial photosynthetic efficiencies with greater biomass production and better antioxidant enzymes activities even under heavy metal stress.

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