Comparative Study of Metal Translocation from Tannery Sludge Amended Soil to *Capsicum annuum* L. under the Influence of Chelants: Effect on Growth Parameters

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Abstract

The study comparatively assess the enhancement of metal translocation to shoot edible part and overall growth effect in *Capsicum annuum* L. grown on tannery sludge ammended with three different chelants. A comparison between the effects of application of three chelants on the growth and metal accumulation in *C. annuum* L. grown on tannery sludge amended soil was conducted to assess the enhancement of the metal translocation to the shoots, edible part and the overall effect on the growth. The analysis of the results revealed that all the morphological parameters (except number of leaves), chlorophyll and protein contents increased in the plants by the addition of chelants in 25% tannery sludge (TS) after 120 d as compared to the plants grown on T (garden soil). The plants treated with nitriloacetic acid (NTA) have shown better growth than ethylediamine tetraacetic acid (EDTA) and citric acid (CA) as compared with no addition of chelants. The accumulation of Cr in the leaves increased (72.03±6.03) by the addition of CA in 25% TS, in contrast to Ni and Cd after 120 d. Similarly, translocation of Cr and Zn increased in the edible part by the addition of EDTA, compared plants chelants. Comparison of Cr accumulation level in the leaves among three chelants, showed higher level in CA treated plants, whereas, Ni proved otherwise. However, in view of the higher translocation of Cr and Zn, it is advisable to grow plants on NTA alongwith 25% TS amended soil.

Key words: Chromium, Tannery sludge, *Capsicum annuum*, Chelants, Accumulation.

1. Introduction

Use of synthetic chelants has been widely reported to enhance the solubility of metals in soils and their subsequent uptake and translocation in the shoots of the plant (Kos and Lestan, 2003). Chen and Cutright (2001) reported the use of chelants as a safer alternative over soil acidification to increase the mobility of the metal within the soil as well as its uptake. It was reported that toxic heavy metals also reach the edible parts beyond permissible limits (Sinha *et al*., 2006). Alternatively, the plants have an exclusion strategy which involves avoidance of metal uptake and its translocation from roots to the shoots when exposed to contaminated substrates. However, the bioavailability of heavy metals thus increased by the addition of chelants which results into the breakdown of the exclusion mechanism, eventually exhibiting higher uptake (Dahmani - Muller *etal*.,2000). Nat-urally, root exudates, especially low molecular weight organic acids (acetic, oxalic, fumaric, citric and tartaric acid) are able to form soluble complexes and chelates with metal ions (Chen *et al*., 2003) resulting into the uptake of the metal. Experimentally, citric acid has been found to assist in the accumulation of Ni in shoots of Indian mustard (do Nascimento *et al*., 2006).

The dried and pulverized tannery sludge cakes are amended into the agricultural fields as a soil conditioner (Gupta and Sinha, 2007). The practice is well reported and advocated as a cheap waste management technique in many developing countries (Wong *et al*., 2001). However, the practice increases the risk of contamination of the edible produce from such farms. Sinha *et al*. (2006) have reported about the accumulation of metals in the leaves of *Capsicum annuum* grown on tannery waste contaminated sites and subsequently to the fruit pods.

C. annuum also known as sweet pepper is a herbaceous, annual plant, belonging to Solanaceae family, which are adapted to warm climates and are cultivated for its fruit pods, used as a spice and for medicinal purpose (Surh and Lee, 1996). India is the world’s largest producer of sweet pepper approximately over 25% of the world’s production, followed by China 24%, Spain 17% and Mexico

8%. The production of sweet pepper in India is increasing annually (1544 kg per hectare in 2005 to 1550 kg per hectare in 2009). In 2008, the production was 12,44,000 tonnes (http://faostat.fao.org/site) and 7,50,000 tonnes in 2009 (http://www.commodityonline.com). The plant is relatively tolerant towards toxic metals, however, the translocation to the edible parts is a matter of concern and needs detailed investigations. Since, *C. annuum* is a tolerant plant which is widely grown across India and can serve as a good candidate for phytoremediation. Unfortunately, the studies related to the phytoremediation potential of the plant are scanty.

Although, the use of chelant for enhanced uptake of toxic metals from contaminated soil, has been widely advocated by several workers in the past (Chen and Cutright, 2001; Chen *et al*., 2003; do Naschimento *et al*., 2006) but due to the virtue of enhanced bioavailability of metals, the edible produce from such treatment may contain higher levels of metals. Among chelants, ethylediamine tetraacetic acid (EDTA) is one of the most widely used and can produce the highest metal extraction efficiency. However, EDTA and EDTA-heavy metal complexes can be toxic to plants and soil microorganisms and may also persist in the environment due to its low biodegradability (Bucheli-Witschel and Egli 2001; Grčman *et al*., 2003) and eventually contaminate the groundwater. Whereas, compounds like citric acid (CA) and nitriloacetic acid (NTA) are costly but easily decomposable. Kulli *et al*. (1999) and Kayser *et al*. (2000) have reported the enhancement of the uptake of heavy metals from degraded soil by the application of NTA. The prospect of using chelants for enhanced phytoremediation lies in optimization of dose of application and selection of cheaper and ecofriendly chelants.

It has also been experienced through several earlier studies (Singh *et al*., 2004; Singh and Sinha., 2004) that tannary sludge (TS) amendment upto 25% to 35% in soil, favors the growth of crop plants. Thus, it was hypothesized that TS amendment of 25% would favor maximum growth of the plants and application of chelants would further increase the metal translocation. In this study, an attempt has been made to comparatively assess the performance of three different chelants in terms of their enhancement of metal bioavailibility in tannery sludge amended soil and subsequent uptake and translocation of the metals *C. annuum* L. and its cumulative effect on the growth of the plant.

2. Materials and Methods

Seeds of *C. annuum* obtained from Shriram Bioseed Genetics India Limited, Hyderabad, India, were germinated in a nursery. Uniform sized plantlet was transplanted to terracotta earthen pots (30 cm) in diameter, each in three replicates, containing different TS amendments and chelants. The TS amemdments (10 and 25%) were prepared with pulverized and sieved TS cakes collected from Jajmau, Kanpur, India and uncontaminated garden soil (T) from National Botanical Research Institute, Lucknow. The amendments (10% and 25% TS) was prepared by mixing appropriate proportions of both TS and T (10 parts of TS and 90 parts of T for 10% and 25 parts of TS and 75 parts of T for 25%). The chelant treatments with the substrates were prepared by spiking different synthetic chelants separately at the rate of 10.745 mM, 10.465 mM and 10.041 mM of EDTA, CA and NTA, respectively. For convenience, the abbreviations of the amendments are presented in Table 1. Pots were placed in the open under natural sunlight, in a complete randomized design (n=3) at an average diurnal temperature of 17-27°C. Optimized amount of tap water (500 ml day^{-1}) was used for irrigation, avoiding any leakage from the pots. Plants were harvested at 90 and 120 d, and washed repeatedly under running tap water to remove the adhered soil particles and foliar depositions. Fresh weight (FW), root length (RL) and shoot length (SL) were measured, number of leaves of the plant were counted immediately after harvesting. Chlorophyll content was estimated from fresh leaves of the plant (100 mg) after extraction in 80% acetone (Arnon, 1949). Protein content in the leaves and roots of the plants were determined using bovine serum albumin (BSA) as standard (Peterson, 1977). Thoroughly washed leaves, fruit and root samples of freshly collected plants were oven dried at 80^{o}C till constant weight. Dried plant samples were grinded and digested in HNO_3 and $HClO_4$ (3:1) and analysed for Cr, Cd, Ni, Mn, Cu and Zn on GBC Σ Avanta, Atomic Absorption Spectrophotometer (Sinha *et al*., 2006). DTPA extractable

Table 1: Abbreviations used for different substrates in the experiment

S. No.	Substrates	Symbols
1	Garden soil + 10.745 mM EDTA	E
2	Garden soil + 10.745 mM + 10% Tannery Sludge	E10
3	Garden soil + 10.745 mM + 25% Tannery Sludge	E25
4	Garden soil + 10.465 mM CA	C
5	Garden soil + 10.465 mM + 10% Tannery Sludge	C10
6	Garden soil + 10.465 mM + 25% Tannery Sludge	C25
7	Garden soil + 10.041 mM NTA	N
8	Garden soil + 10.041 mM NTA +10% Tannery Sludge	N10
9	Garden soil + 10.041 mM NTA + 25% Tannery Sludge	N25
10	Garden soil	T
11	Garden soil + 10% Tannery Sludge	T10
12	Garden soil + 25% Tannery Sludge	T25

metals were estimated by the method of Lindsay and Norvell (1978). The data of 90 and 120 d were subjected to Duncan's Multiple Range Test (DMRT) separately, at significant level ($p<0.05$). The treatments (T, T10 and T25) were considered as controls and comparison was made with their respective chelant treated plants i.e. E10, C10 and N10 were compared with T10. Results are presented as mean of at least three replicates ± standard deviation.

3. Results and Discussion

The results of physico-chemical properties and total metals analysis in the contaminated soil and TS are presented in Table 2.

3.1. Effect on growth parameters

The morphological parameters of *C. annuum* grown in various treatments are presented in Fig. 1. An overall increase in fresh weight (Fig. 1A) was observed in all the chelant with respective TS treatments after 120 d, as compared to T10 and T25, except for C10. However, the FW of the plants grown on E and C were not significantly different as compared to T, except for N, where significant increase was observed after 120 d. The number of leaves per plant (Fig. 1B) in all the chelant amendments (E, C, N) was observed to increase significantly as compared to T at both the growth periods. As compared with TS amendments, number of leaves increased non-significantly in all the chelant treatments (E25, C25, N25) at 120 d of growth, compared to T25. Shoot lengths (SL) of the plants (Fig. 1C) grown on different chelant amended substrates (E25, C25, N25) were observed to exhibit significant increase after 120 d as compared to T25, and maximum was observed in NTA. As compared to T, after 90 d the SL was reduced in all the chelant treatments (E, C, N), however, no change was observed after 120 d. Plants grown on N10 were found to be tallest (55 cm) after 120 d. The root length (RL) (Fig. 1D) of all the plants treated with chelants (E, C, N) were higher than T after 90 d, which was contrary to the SL. At 120 d, the RLs were observed to decrease significantly in all chelant treatments (E, C, N), as compared to T. In contrast, it increased significantly in all the chelant treatments (E25, C25, N25) as compared to T25 after 120 d. Similar to these findings, several authors have also reported that plants of *Helianthus annuus* receiving EDTA and HEDTA at the rate of 1 and 2 g kg^{-1} have shown reduction of biomass, however, when grown on soil amended with 0.5 g kg^{-1} of EDTA, exhibited better growth and higher in biomass, compared to the other chelants (HEDTA) (Chen and Cutright, 2001). Overall, morphological parameters of the plants grown on 25% TS with addition of chelants have shown better growth than without chelants after 120 d. The increase in the morphological parameters could be due to availability of essential nutrients by the application of chelants. Similar observations have been encountered during several earlier studies (Singh *et al*., 2004), where the increase in the photosynthetic pigments in the plants grown of TS amended soil have been attributed to enhanced bioavailability of essential metals. The plants of *C. annuum* has also been found to be tolerant towards metals (observed phenomenon), whereas, the growth of other plants (*Chrysanthemum coronarium* and *Phaseolus vulgaris*) have been reported to decline with application of EDTA and EDDS (Luo *et al*., 2006). Similarly, Jean *et al*. (2007) also observed that plants of *Datura innoxia* treated with 5 and 10 mmol EDTA kg^{-1} of soil, showed signs of wilting as after one day of the treatment, and they eventually died during their experiment. Hsiao *et al*. (2007) also observed that *Brassica juncea* receiving chelants i.e. EDTA, DTPA, CA and oxalic acid resulted into significant reduction in dry matter of the shoots as compared to the control shoots. Quite similar to these reports, in this study, the reduction of FW, number of leaves and SL of E, C and N as compared to T can be attributed to the adverse effect of the chelants on the plant's growth.

The overall trend of the total chlorophyll and carotenoid contents (Fig. 2A, B), demonstrated that the plants grown on chelant amended substrates had higher chlorophyll content than grown only on TS amended soils after 120 d. Significant increase ($p \leq 0.05$) in total chlorophyll content was observed after 120 d in plants receiving C25, as compared to T25. On the contrary, a decline in the chlorophyll content was observed in all the chelant treatments after 90 d as compared to T25. A similar trend was also observed in chlorophyll a and b contents (Fig. 2C, D). The addition of chelants in TS amended soil has shown increase in the carotenoid content. After 120 d, the highest increase in carotenoid content was observed in the plants grown on T25 (331.8%), E25 (142.8%), N25 (62.7%) and C25 (41.6%), as compared to T, E, N and C, respectively. Carotenoid is known to have antioxidative property (Halliwell, 1987) and exhibited higher levels could be for countering the reactive oxygen species generated due to excess heavy metals. With TS amendments, significant increase in leaf and root total soluble protein was observed after 120 d for E25 and C25 as compared to T25 (Fig. 3A, B). Significant decrease in root protein content was observed in the plants treated with chelants i.e. E, C and N as compared to T after 90 d, which could be due to the combined effect of the chelants and the increased mobility of the toxic metals. Similarly, significant decrease was also observed in E10, C10 and N10 as compared to T10, after 120 d. The use of lower amendments of TS in agricultural fields has been recommended by earlier studies as a soil conditioner and no deleterious effect on the plants was observed (Singh *et al*., 2004). The increase in chlorophyll and protein contents observed in the plants grown on TS amendments are in congruence to findings by Singh *et al*. (2004), which may be due to the increase in bio-availability of essential metals (Cu and Zn). The bioavailability of these metals (Table 3) have been found to increase with increase in TS amendment from 10% to 25%, whereas, Mn was found to decrease with increase in TS, which have shown a sharp decline in all the chelant treatments receiving 10% TS amendments. The order of mobilization for Mn after 120 d was, E25 (242.07%) > N25 (94.4%) > C25 (86.3%), as compared to T25.

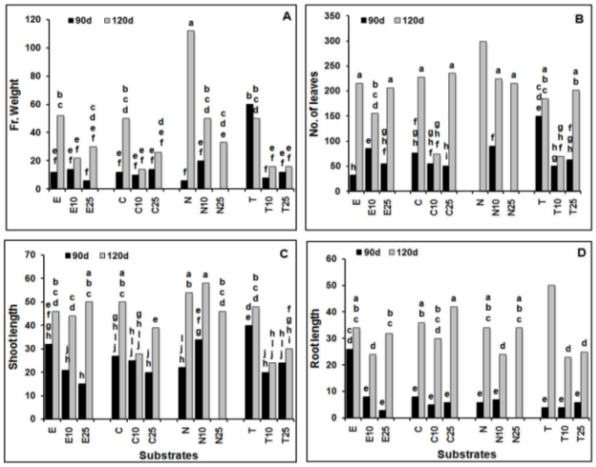

Fig. 1: Effect in morphological parameters of *C. annuum* grown on different substrates after 90 and 120 d. A: Fresh weight (g plant^{-1}), B: shoot length (cm), C: root length (cm) and D: No. of leaves (plant^{-1}). Values marked with similar alphabets are not significantly (Duncans test, p<0.05) different

Table 2: Physico-chemical analysis and total metals (µg g^{-1} dw) in tannery sludge (TS) and control soil (T)

Parameters	Amendments (%)	
	T	25% TS
BD (Mg g^{-3})	0.4	1.3
Clay (%)	10	18
pH (1:2.5)*	6.7±0.0	7.1±0.2
EC (µS cm^{-1})*	461.0±0.0	662.0±63.6
CEC (cmol kg^{-1})*	32.2±2.60	48.4±2.7
OC (%)*	0.42±0.08	0.78±0.01
Water soluble Na (µg g^{-1})	36.2±2.6	52.3±4.51
Water soluble K (µg g^{-1})	16.9±1.65	11.6±2.21
OM (%)*	0.7±0.02	1.3±0.1
N-NO$_3$ (µg g^{-1})	8.2±0.12	53.09±3.21
N-NH$_4$ (µg g^{-1})	2.1±0.71	23.25±2.18
Available P (µg g^{-1})	12.0±1.01	14.63±1.02
	Metals (µg g^{-1} dw)	
Total Na	48.36±2.35	14692±939.5
Total K	21.2±1.48	42178.6±6579.75
Fe	16664±522	15534.5±29.775
Zn	82.5±1.3	147.7±3.48
Mn	246.1±23.3	215.7±22.85
Cu	10.8±0.5	45.4±0.5
Cr	16.4±2.7	18738.7±62.15
Pb	12.8±1.3	10.2±0.9
Ni	138.2±16.7	208.5±17.95
Cd	0.5±0.09	13.2±0.4

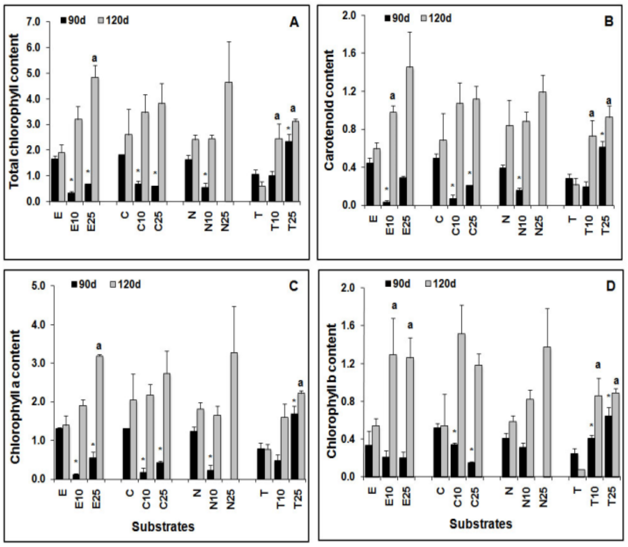

Fig. 2: Effect on plant pigment contents (mg g^{-1} fw) of *C. annuum* grown on different substrates at 90 and 120 d. A: Total chlorophyll; B: Carotenoid; C: Chlorophyll a and D: Chlorophyll b. Values marked with different alphabets (lower case for 90 d and upper case for 120 d) are significantly (Duncans test, p<0.05) different

3.2. Accumulation of toxic metals

As a general trend reported in several other studies (Gupta and Sinha, 2006, 2009; Sinha *et al*., 2006), the roots (Fig. 4) accumulated more metals than the leaves. CA and EDTA have been reported to have a high affinity for Cr and Ni, respectively (Jean *et al*., 2007). The critical leaf Cr concentration in most plants seems to fall between 1 and 10 mg kg^{-1} DW.

A significant increase in accumulation of Cr in the leaves was observed with C10 and C25 as compared to T10 and T25, respectively after 120 d, while reverse was observed in the roots. The higher uptake of the Cr in the plants receiving CA as chelants has been explained by Jean *et al*. (2007) that the competition of CA with Cr(VI) happens on the surface of the soil. This mechanism allows CA to be more efficient in desorbing Cr than EDTA. In their study the accumulation of Cr by *Datura innoxa* was reported to be in the range of 1 - 2 µg g^{-1} in leaves which declined after two applications of 5 mmol kg^{-1} and also in single or double application of 10 mmol kg^{-1} of CA, and have been proclaimed that the plant behaves as a Cr and Ni tolerant species without being a hyperaccumulator. However, the plants of *C. annuum* have shown an accumulation (µg g^{-1} DW) of Cr (72.03±6.03) in C25 after 120 d, as compared to Cr accumulation (6.69±2.69) when grown on T after 120 d. According to Kabata-Pendias and Pendias (2001) who reported that Cr levels in plants growing in normal soils are usually less than 1 and rarely exceed 5 mg kg^{-1}. Therefore, the level of Cr in control, observed in this study is just above the limit (6.7±2.7), but in view of the high standard deviation, it can be assumed within range. Therefore comparing with these studies, it implies that the plants of *C. annuum* is quite tolerant towards Cr and can serve as a good tool to phytoremediate TS contaminated sites along with CA. In roots, the highest accumulation (611.6±60.9 µg g^{-1} DW) was observed in T25 followed by E25 (319.0±10.7 µg g^{-1} DW) after 120 d. As compared with Cr accumulation (25.9±2.2) in *Helianthus annuus* grown on 100% TS reported by Singh *et al*. (2004), the observed Cr accumulation (µg g^{-1} DW) by *C. annuum* in T25 is 29.9±4.6 after 120 d is higher and the highest accumulation was observed in C25 (72.0±6.75). Thus, it illustrates that plants of *C. annuum* has higher potential for Cr remediation from TS.

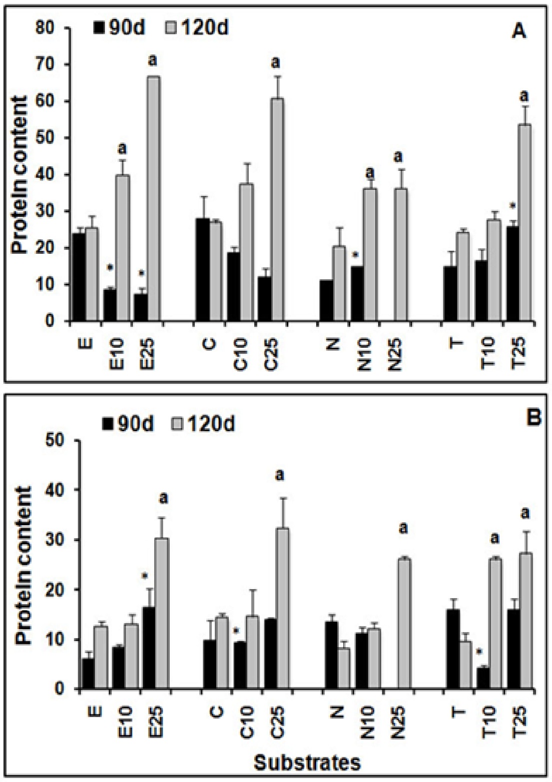

Fig. 3: Effect on protein content (mg g^{-1} fw) in the leaves (A) and roots (B) of *C. annuum* grown on different substrates after 90 and 120 d. Values marked with different alphabets (lower case for 90 d and upper case for 120 d) are significantly (Duncans test, $p<0.05$) different. All the values are means of three replicates ±SD

Cd accumulation (µg g^{-1} DW) in the roots of E25 (14.0±3.2) and C25 (13.0±3.3) was found to be higher than T25 (10.4±2.0) after 120 d, whereas, significant decrease in the leaves (Fig. 4C) was observed in E25 (3.5±1.3), C25 (4.4±1.5) and N25 (3.5±0.6) as compared to T25 (10.4±2.0) after 120 d. Similarly, as compared with T10 after 120 d, significant decrease in Cd levels in all the 10% TS chelants treatments was observed in the roots (Fig. 4D). Accumulation of Cd in *Helianthus annuus* treated with 1g kg^{-1} (~2.5 mM) and 2 g kg^{-1} (~5.3 mM) of EDTA was reported to be ~100 to 200 mg kg^{-1} (Chen and Cutright, 2001). Lai and Chen, (2006) reported an accumulation of ~25 to 27 mg kg^{-1} in *Dianthus chinensis* treated with 2 and 5 mmol kg^{-1} of EDTA, respectively. Meers *et al*. (2005) reported accumulation of Cd (mg kg^{-1}) in the range of ~ 2.3 - 3.0 in *Helianthus annuus* receiving 0.8 to 2.0 mmol of EDTA, ~1.8 to 2.0 in plants receiving 25 to 100 mmol of CA and ~ 1.7 - 1.8 in plants receiving 0.8 mmol of NTA. Cd concentrations of uncontaminated soils are usually below 0.5 mg kg^{-1} but can reach up to 3 mg kg^{-1} (Wang *et al*., 2007). Based on the data presented from the study and comparing it with other studies, it can be concluded that the plants of *C. annuum* is not a good candidate for Cd phytoremediation. Similarly, no enhancement of Ni uptake in the plants was observed as there was no difference between E, C and N as compared to T after 90 d, which declined further after 120 d (Fig. 4E). As compared with T25, there was a significant decrease in Ni accumulation in the roots of all the chelant treatments (Fig. 4F) after 120 d, whereas in leaves, significant decline in uptake was observed in E10 and C10 after 90 d as compared to T10. Meers *et al*. (2005) reported accumulation of Ni (mg kg^{-1}) in the range of ~ 4.2 - 4.5 in *Helianthus annuus* receiving 0.8 to 2.0 mmol of EDTA, ~3 to 4 in plants receiving 25 to 100 mmol of CA and ~6.0 to 6.3 in plants receiving 0.8 mmol of NTA.

3.3. Accumulation of essential metals

Mn is an essential micronutrient required throughout all stages of plant development which needs at least 30 mg kg^{-1} DW in tissues regardless of plant species for optimal growth (Marschner, 1995). The level of Mn accumulation (µg g^{-1} DW) in the plants receiving only chelants did not show any significant increase except for C (75.5±4.3) whereas, the maximum uptake of Mn was recorded in N10 (98.5±9.6) and T10 (102.3± 5.5) without any significant difference between them (Fig. 4G). In roots, level of Mn in all the plants receiving chelants i.e. E (179.8±6.4), C (179.5±19.0) and N (170.7±32.3) were high than T (133.3±23.7) after 120 d, however, the highest level was observed in N10 (224.6±35.4) (Fig. 4H). Cu is essential for plants and the natural concentration of Cu found in plants usually ranges from 5 to 25 mg kg^{-1} DW (Epstein, 1997). The level of Cu in the leaves of all the chelant treatment plants i.e. E (4.4±0.3 and 6.5±0.6), C (6.8± and 7.2±0.6) and N (7.0±2.10 and 13.0±1.6) after 90 and 120 d were higher than that of T (1.0 and 3.07±1.0) (Fig. 4J, L), respectively. Similarly, significant increase in Cu contents were observed in E10 (14.9±1.2) and N10 (15.0±2.5) after 120 d, as compared to their respective values of T10 (9.7±1.2) (Fig. 4I) whereas, as compared to T25, significant increase was observed only in E25 (24.3±5.5). Zinc is essential for plants and animals. Zn tissue concentration of less than 15 mg kg^{-1} DW renders a plant deficient, whereas a concentration over 400 mg kg^{-1} DW becomes phytotoxic. Zn levels in the leaves of the chelant treated plants were generally higher than without chelant treated plants (Fig. 4K) where E10 had significantly higher Zn level (46.51±2.5) as compared to T10 (35.7±7.8) after 120 d. Whereas, in the roots, the Zn levels in the chelant treated plants were lower than that of T10 and T25 after 120 d. The levels in E25 (39.3±3.3) and C25 (21.8±1.3) have shown a significant decrease. The observed increase in level of metals in the plants receiving chelants is in congruence to several earlier studies. Luo *et al*. (2006) observed that the application of EDTA at a rate of 3 mmol kg^{-1} significantly increased the concentrations of Cu, Pb, and Zn in the shoots of the plants. The enhancement is reported to be more pronounced in the dicotyledonous than the monocotyledon plants. Although, enhancement in metal accumulation has been observed with chelant application but large differences have been reported (Huang *et al*., 1997) in stimulating effects of chelants on the metal accumulation in the shoots of different species of the plants. Jean *et al*. (2007) also observed that the application of CA did not have any significant effect on Cr accumulation in the roots and leaves of *Datura innoxia*. Meers *et al*. (2005) reported accumulation of Zn and Cu (mg Kg^{-1}) in *Helianthus annuus* in the range of ~ 140 to 160 and ~12 to 15, respectively in plants receiving 0.8 to 2.0 mmol of EDTA, ~80.0 to 100.0 and 7.0 to 8.0, respectively in plants receiving 25 to 100 mmol of CA and ~ 120.0 to 125.0, respectively in plants receiving 0.8 mmol of NTA. Hence, the plants of *C. annuum* have shown less accumulation of Zn but the overall accumulation of Cu was higher.

Shoot to root ratio of metals expresses the translocation factor, higher values denote lower translocation and *vice-versa*. Higher translocation was observed for Zn, Cr, Cu and Ni, in most of the chelant amendments as compared to T, T10 and T25 after 120 d.

Table 3: Levels of DTPA extractable metals from different substrates after 90 and 120 d

Substrates	**Metals (μg g^{-1} dw) after 90 d**						
	Cr	**Cu**	**Zn**	**Mn**	**Ni**	**Pb**	**Cd**
E	0.17±0.10$^{\mathbf{a}}$	BDL	BDL	28.4±0.9^{f}	0.4±0.0^{a}	0.3±0.2^{a}	0.2±0.1^{a}
E10	0.91±0.17^{b}	3.1±0.3$^{\mathbf{b}}$	BDL	15.9±1.2$^{\mathbf{b}}$	0.3±0.1$^{\mathbf{a}}$	1.9±0.3$^{\mathbf{b}}$	5.4±0.3^{a}
E25	4.31±0.28$^{\mathbf{c}}$	8.7±0.5$^{\mathbf{c}}$	1.8±0.4$^{\mathbf{bc}}$	18.7±0.7$^{\mathbf{c}}$	1.5±0.2^{d}	6.2±0.2^{d}	16.2±1.1^{de}
C	0.2±0.02$^{\mathbf{a}}$	BDL	BDL	19.4±0.9$^{\mathbf{c}}$	0.5±0.2$^{\mathbf{ab}}$	BDL	0.01±0.0$^{\mathbf{a}}$
C10	1.53±0.48$^{\mathbf{b}}$	3.3±0.2$^{\mathbf{b}}$	BDL	17.9±0.8$^{\mathbf{c}}$	0.5±0.2$^{\mathbf{a}}$	1.7±0.4$^{\mathbf{b}}$	7.2±0.1$^{\mathbf{bc}}$
C25	3.5±0.5$^{\mathbf{c}}$	9.8±0.2$^{\mathbf{d}}$	2.5±0.3$^{\mathbf{cd}}$	17.9±0.8$^{\mathbf{b}}$	1.4±0.6^{d}	7.6±0.2$^{\mathbf{e}}$	16.2±1.1$^{\mathbf{e}}$
N	BDL	BDL	BDL	25.6±2.0$^{\mathbf{e}}$	0.3±0.1$^{\mathbf{a}}$	BDL	BDL
N10	1.503±0.39$^{\mathbf{b}}$	3.7±0.2$^{\mathbf{b}}$	BDL	12.3±1.1$^{\mathbf{a}}$	0.4±0.1$^{\mathbf{abc}}$	2.2±0.9^{b}	7.4±0.4$^{\mathbf{bc}}$
N25	3.96±1.0$^{\mathbf{c}}$	9.7±0.7$^{\mathbf{d}}$	3.0±0.3$^{\mathbf{d}}$	19.0 ±0.6^{c}	1.1±0.2^{cd}	7.8±0.8$^{\mathbf{e}}$	17.2±0.2$^{\mathbf{e}}$
T	1.16±0.68^{b}	3.5±0.2$^{\mathbf{b}}$	BDL	14.5±1.0$^{\mathbf{b}}$	0.5±0.2$^{\mathbf{ab}}$	1.8±0.03$^{\mathbf{b}}$	8.1±0.1$^{\mathbf{bc}}$
T10	2.231±0.25$^{\mathbf{b}}$	3.8±0.4$^{\mathbf{b}}$	BDL	21.4±0.9$^{\mathbf{d}}$	1.0±0.3$^{\mathbf{bcd}}$	3.3±0.4$^{\mathbf{c}}$	7.2±0.2$^{\mathbf{bc}}$
T25	3.395±0.69^{c}	9.2±0.5$^{\mathbf{cd}}$	3.0 ±0.2$^{\mathbf{d}}$	19.7±1.3^{cd}	1.4±0.0$^{\mathbf{d}}$	8.0±0.1$^{\mathbf{e}}$	17.6±0.6^{cd}
	Metals (μg g^{-1} dw) after 120 d						
E	BDL	BDL	BDL	22.6±0.3^{AB}	0.2±0.13$^{\mathbf{A}}$	BDL	BDL
E10	2.61±0.82$^{\mathbf{BC}}$	2.6±0.3$^{\mathbf{B}}$	BDL	14.4±1.0^{A}	0.6±0.3$^{\mathbf{B}}$	1.0±0.0^{A}	5.4±0.4^{B}
E25	3.54±0.75$^{\mathbf{D}}$	8.8±0.6^{D}	0.9±0.7^{A}	47.2±8.5$^{\mathbf{BC}}$	1.5±0.4^{D}	7.8±0.3^{D}	10.2±0.5$^{\mathbf{D}}$
C	0.86±0.11$^{\mathbf{AB}}$	BDL	BDL	26.8±1.3$^{\mathbf{AB}}$	0.7±0.6$^{\mathbf{B}}$	BDL	BDL
C10	1.65±0.41$^{\mathbf{BC}}$	3.8±0.8$^{\mathbf{BC}}$	BDL	16.8±1.8$^{\mathbf{AB}}$	0.8±0.3$^{\mathbf{B}}$	1.5±0.4^{AB}	7.0±0.3$^{\mathbf{BC}}$
C25	3.53±0.6$^{\mathbf{D}}$	11.4±1.9$^{\mathbf{E}}$	3.7±1.2^{B}	25.7±0.9$^{\mathbf{BC}}$	1.4±0.4$^{\mathbf{CD}}$	9.7±2.4^{D}	18.8±3.4$^{\mathbf{F}}$
N	BDL	BDL	BDL	50.3±1.0^{C}	0.9±0.04^{D}	BDL	BDL
N10	3.05±0.59$^{\mathbf{D}}$	5.5±0.4^{C}	BDL	21.2±1.8$^{\mathbf{AB}}$	1.1±0.1$^{\mathbf{BCD}}$	4.4±0.4^{C}	10.3±0.4$^{\mathbf{D}}$
N25	2.89±0.34$^{\mathbf{D}}$	10.6±1.2$^{\mathbf{E}}$	3.0±1.0$^{\mathbf{B}}$	26.8±4.2$^{\mathbf{AB}}$	1.6±0.2$^{\mathbf{D}}$	8.0±1.0^{D}	17.3±3.7^{EF}
T	0.21±0.0$^{\mathbf{A}}$	BDL	BDL	19.1±1.2$^{\mathbf{AB}}$	0.7± 0.2$^{\mathbf{B}}$	BDL	BDL
T10	2.58±0.223$^{\mathbf{CD}}$	4.1±1.2$^{\mathbf{BC}}$	BDL	28.5±9.8$^{\mathbf{AB}}$	0.7±0.02$^{\mathbf{BC}}$	3.2±0.1^{BD}	8.2±0.2^{CD}
T25	3.15±0.88$^{\mathbf{D}}$	10.4±2.2$^{\mathbf{DE}}$	2.5±2.2^{B}	24.5±1.4$^{\mathbf{AB}}$	1.0±0.1$^{\mathbf{BC}}$	7.4±3.5^{D}	15.1±1.5^{D}

Values marked with similar alphabets are not significantly (Duncan's test, $p<0.05$) different, (lower case after 90 d and upper case after 120 d) .

All the values are mean of three replicates ±SD. BDL = Below detection limit

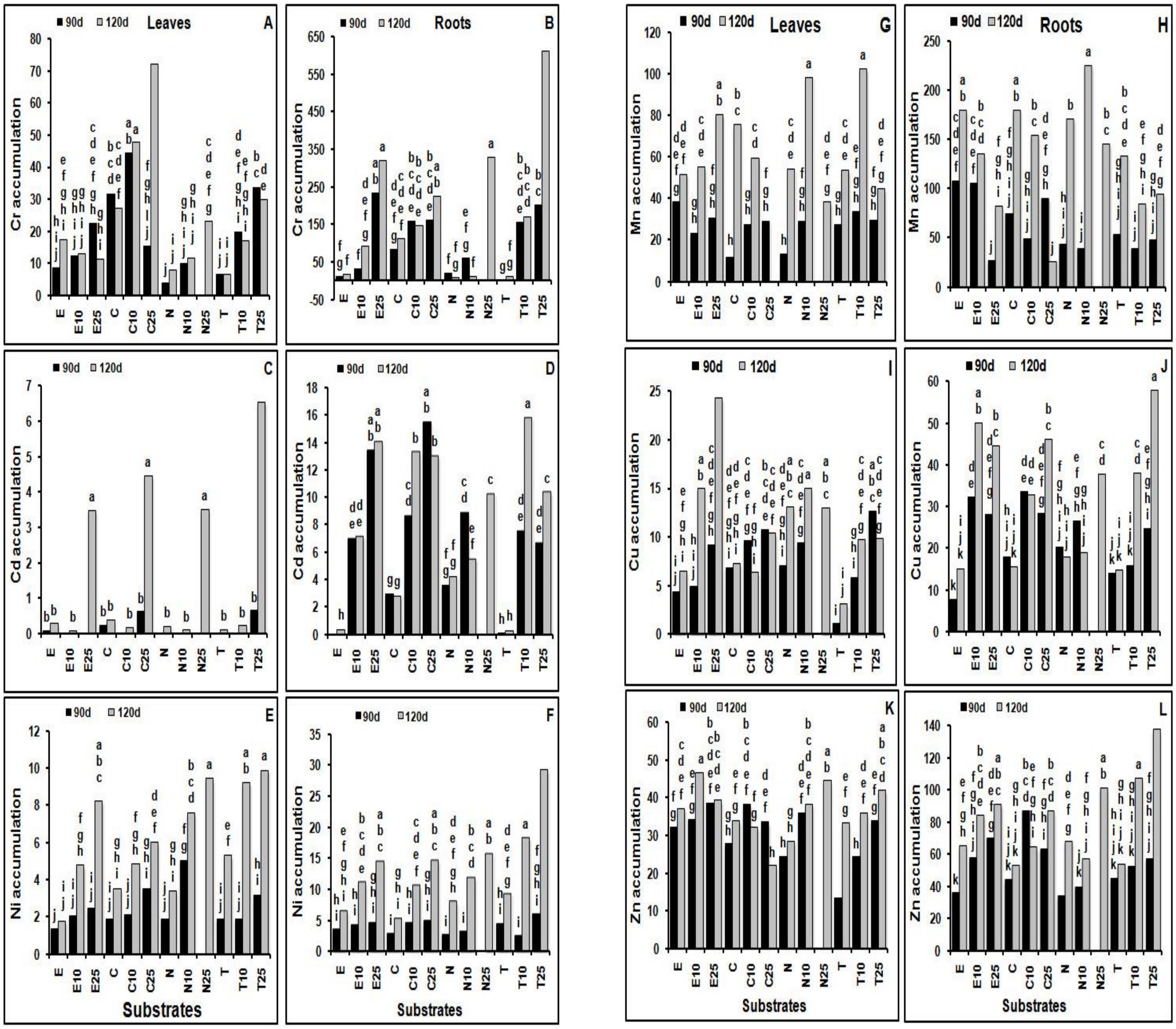

Fig. 4: Accumulation (mg g^{-1} dw) of metals in the leaves and roots of *C. annuum* grown on different substrates after 90 and 120 d. Cr in A: leaves; B: Roots; Cd in C: leaves; D in roots; Ni in E: leaves; F: in roots; Mn in G: leaves; H: roots; Cu in I: leaves; J: roots, Zn in K: leaves; L: roots. Values marked with different alphabets (lower case for 90 d and upper case for 120 d) are significantly (Duncans test, p<0.05) different. All the values are means of three replicates ±SD

Table 4: Level of toxic metals in the fruit pod of *C. annuum* grown on different substrates after 120 d

Substrates	**Metals (µg g^{-1} dw)**					
	Cr	**Cu**	**Mn**	**Zn**	**Ni**	**Cd**
E	BDL	12.8±1.7$^{(b)}$	17.6±2.1$^{(c)}$	32.1±9.2$^{(c,d)}$	1.0±0.03$^{(a)}$	BDL
E25	72.03±6.8$^{(c)}$	9.8±0.23$^{(a)}$	BDL	22.1±3.2$^{(a,b)}$	6.5±0.1$^{(c,d)}$	0.2±0.0$^{(a)}$
C	BDL	9.4±0.5$^{(a)}$	18.7±3.2$^{(c)}$	21.3±0.8$^{(a)}$	1.7±0.1$^{(a)}$	0.2±0.0$^{(a)}$
C10	5.12$^{(a)}$	10.8$^{(a)}$	63.4 $^{(e)}$	34.8$^{(d)}$	4.2$^{(a,b)}$	0.9$^{(a)}$
N	3.20±1.9$^{(a)}$	9.0±1.6$^{(a)}$	17.7±3.9$^{(c)}$	22.3±2.3$^{(a)}$	2.6±1.1$^{(a)}$	0.2±0.0$^{(a)}$
N25	20.1±6.5$^{(b)}$	11.1±1.0$^{(a)}$	12.7±2.1$^{(b)}$	30.2±5.1$^{(c,d)}$	8.9±1.8$^{(c)}$	1.3±0.4$^{(a)}$
T	2.6±0.1$^{(a)}$	7.1±0.6$^{(a)}$	22.1±1.1$^{(d)}$	25.9±3.4$^{(a,b,c)}$	4.3±2.2$^{(a)}$	0.1±0.0$^{(a)}$
T25	15.2±0.4$^{(b)}$	9.6±2.4$^{(a)}$	10.2±1.4$^{(b)}$	27.7±0.3$^{(b,c,d)}$	7.7±0.8$^{(b,c)}$	4.1±0.5$^{(b)}$

Values marked with similar alphabets are not significantly (Duncans test, p<0.05) different. All the values are mean of three replicates ±SD. BDL = Below detection limit

3.4. Metal translocation to the fruit pods

The metal content (µg g^{-1} dw) in the fruit pods of the plant have been presented in the Table 4. Most of the plants grown over 10% TS, with various chelant amendments did not bear fruit pods. Among the toxic metals, pods grown on E25 have shown to accumulate higher levels of Cr (72.03±6.8) followed by N25 (20.1±6.5) as compared to those without chelants T (2.56±0.09). It is noteworthy that the level of Zn was higher in the fruits against the low bioavailability of the metal (Table 3). The highest accumulation was observed in E (32.1±9.2) followed by N25 (30.2±5.09). The ability of the plant to translocate higher levels of Zn to its aerial parts are also corroborated from the fact that the plants have shown progressively increasing trend of Zn accumulation in its leaves with increase in TS amendment (Fig. 4K). Among the roots, highest level was observed in T25 (Fig. 4L). The presence of high level of Zn has been reported in the shoots (~310) and seeds (156.38±6.63) of fenugreek (*Trigonella foenum-graecum* L.). Similarly, Sinha *et al*, (2006) have reported about the level of Zn accumulation in leaves (94.64±0.59) and seeds (81.72±6.78) of *C. annuum*, grown on tannery wastewater contaminated site of Kanpur. In the same study they reported the level of Zn in wheat seeds range between 52.69 to 198.99 and in yellow mustard range between 95.79 and 148.62. In all these reports and the data presented in this study indicates that the Zn translocation to the seeds are high irrespective to the bioavailoability of the metal in the soil. In view of the prevalent Zn deficiency among populations of developing world, consumption of *C. annuum* grown on 25% TS amended soil can be beneficial. Cr(III) has been reported to be an essential trace element in mammals in regard to maintenance of normal carbohydrate metabolism. The Cr present in the plant tissues are reported to be in the reduced form [Cr(III)]. The minimum recommended dietary intake of Cr(III) is 50 µg d^{-1} (Anderson and Kozlovsky, 1985). It has been advocated by Zayed and Terry (2003) that spices may have higher Cr concentrations, however, in view of their very small components of the human diet, the overall human exposure towards the metal may be less. Also, the lowest Cr concentration in above ground plant tissues has always been observed in the fruits and seeds. Therefore, in view of these facts and the data obtained through study, it can be concluded that cultivation of *C. annuum* on 25% TS amended soil would result into phytoremediation and the sweet pepper produced from such cultivation can serve as spices and supplement dietary mineral uptake.

4. Conclusion

This study demonstrates that the growth of the plant treated with NTA has shown better growth than EDTA and CA. The increase in growth parameters i.e. fresh weight, shoot length, root length, total chlorophyll and carotenoid contents were observed in the plants receiving chelants as compared to T after 120 d. There was no significant difference in the bioavailabilty of toxic metals after addition of chelants in the tannery waste contaminated soil, however, significant increase in translocation of Cr in the fruits of *C. annuum* was observed in E25. The plant exhibited poor translocation of Cd to its shoots. Highest level of accumulation of Cr in the leaves was observed in plants receiving CA with 25% TS whereas, in case of roots the plants grown on 25% TS without any chelants showed highest accumulation. Translocation of Zn was higher in leaves receiving chelants after 90 d and to the fruits and were greatly influenced by presence of chelants, particularly EDTA and NTA. In view of less amount of spices used in cooking, the fruits of *C. annuum* grown on 25% TS treated with chelants (EDTA and NTA) can serve the plant for phytoremediation of toxic metals and mineral supplement in foods.

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