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Research Article

Assessing the Scope of Growing Brassica Campestris L. in Soil Spiked with Arsenic, Chromium and Copper: Effect on Growth, Antioxidants and Oil Yield

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Keywords

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- Oil yield

Abstract

Study was undertaken to assess the response of *Brassica campestris* L. exposed to different metals {Cu, Cr(VI), As(III), As(V)} on accumulation, antioxidants, nutrient status, toxicity and oil yield. Results showed translocation of metals to the upper part and its sequestration in the leaves without significantly affecting seed weight and oil yield. In seeds, accumulation of as was below detection limit, however, the accumulation of 3.15 mg/kg of Cr was recorded. Seeds collected from Cu treated plants have shown no difference in essential metal content as compared to control. Amongst the four metal treatments, Cr was the most toxic as evident from the decrease in growth parameters and chlorophyll content along with increase in malondialdehyde content. The activities of antioxidant enzymes varied for different metals. In view of these findings, *Brassica campestris* L. can be recommended for cultivation on as and Cu contaminated soil for oil production only.

INTRODUCTION

The global need for food production is increasing with the rise of population. To meet the demand, food crops are being cultivated in farms contaminated with toxic metal(s) which raises question on the quality of agricultural products grown there. The problem manifests to a wider scale in developing countries due to various constraints. One common practice amongst all developing countries is the use of treated industrial waste water for irrigation. Such waste water is often a cocktail of various heavy metals and metalloids, both essential and toxic. Numerous reports are available on the effect of toxic metal(s)/loid(s) to plants grown in such areas [1-3].

Brassica campestris (yellow mustard) is a tolerant plant towards heavy metals and is cultivated widely across Indian subcontinent for oil production. The oil cake left out after extraction of oil is also fed to milch cattle; if the crop is contaminated it would pass-on through the food chain affecting the consumer. Owing to the importance of this crop and its reported tolerance towards heavy metals, many studies have been devoted to study the translocation of toxic metals to the various parts of *Brassica* sp. in different varieties and its tolerance mechanism [1,4-7]. Most of these studies were carried out in hydroponics conditions and the conclusion drawn from these studies are quite different from natural field conditions. It is important to study the effect of plants to toxic metal(s)/loid(s) in simulated soil conditions to understand the metal translocation between various parts of the plant and its inflicted toxicity. A comprehensive and comparative study has not yet been reported on the effect of different metals/metalloids on fully grown *Brassica campestris* under field condition.

Several studies have been undertaken to study the effect of single metal on a particular crop [6-8], however, studies investigating the comparative effect of different metal (loids) on a particular plant/crop are very few. Copper is an essential micronutrient required as a cofactor in some enzymes, however, it is toxic at higher concentrations. Arsenic exists mostly in the environment as As (III) or As (V), with former being abundant in reduced conditions than the later. There are many reports on

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ground water As contamination from the state of West Bengal and Uttar Pradesh, India, which lies in the upper and middle Gangetic plains, which has been chiefly attributed to the contamination of the agricultural products growing in these regions [9]. Arsenic can induce toxicity to plants through its interaction with sulfhydryl groups of proteins and enzymes [10] and through an increase of reactive oxygen species in cells, consequently causing cell damage [11]. In industries, Cr (VI) compounds are used for tanning of hides and metal plating leading to wide spread contamination in the environment [2]. Excessive accumulation of Cr, which is not an essential metal for plant growth [12], has been shown to interact with essential nutrients [13]. Toxic metal (loids) are also reported to affect the nutrient uptake and overall nutrient homeostasis in the plant [14].

Hence, this study attempts to comparatively assess the effect of Cr, As (III), As (IV) and Cu on a tolerant plant *Brassica campestris*, on its antioxidant defense property, alteration in nutrient uptake and on oil content.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of *Brassica campestris* L cv. T-42 were purchased from commercial seed supplier. Seed were disinfected by 30% H_2O_2 treatment for 10 min, washed thrice with MilliQ water and left in a beaker for soaking water for 24h in dark. The imbibed seeds (10 nos.) were sown in earthen pot (23 cm in diameter) to a depth of 0.5 cm, containing garden soil (9 kg) for germination. The plants were allowed to grow in the institute's experimental garden under natural sunlight and temperature (15 – 25^{o}C) in a randomized block design with four replicates (in separate pots). The pots were watered daily till germination of seeds and thinned out to retain five uniform seedlings after 10d. The plants were allowed to grow for 20d and treated with different concentrations of metals/metalloids. Metals and metalloids have been used in the study, however, for convenience, the word metals have been used commonly for all the elements. Plants without treatment served as control (C). Two treatments (30 and 80 mg/kg dw) of the different metals i.e. Cu, As (V) and Cr were made using $CuSO_4$, $Na_2HAsO_4.7H_2O$ and $K_2Cr_2O_7$, respectively. In view of toxicity of As (III) to the plants, three treatments (30, 50, 80 mg/kg dw) were made using $NaAsO_2$. For convenience, the different concentrations of metals were abbreviated as Cu-(30) and Cu-(80), As(V)-30, As(V)-80, Cr-(30) and As(III)-30, As(III)-50. However, the plants treated with 80 mg/kg dw of As (III) and Cr did not survive. The soil in the pots were tilled for aeration and weeded for proper growth of the plants. Care was taken to avoid leaching from the pots and watered with about 500 ml of water. The plants were harvested after 15d (first harvest) and 30d (second harvest) of treatment. Further, three plants were grown till maturity of the seeds for estimation of metal levels, seed weight and oil content.

Growth, biochemical parameters and oil content

Fresh weight (FW), root and shoot lengths were taken immediately after harvesting. The following methods were used for the estimation of chlorophyll [15], protein [16], malondialdehyde (MDA) [17], glutathione (GSH) [18], superoxide dismutase (SOD) [19], ascorbate peroxidase (APX) [20], guaiacol peroxidase (GPX) [21,22] (Smith et al. 1988), catalase (CAT) [23].

Crushed seeds were extracted with hexane and solvent was evaporated after filtration. The weight of the oil was determined by weighing till constant weight.

Metal accumulation and soil extractable metal concentration

Oven-dried tissue plant samples were ground and digested using HNO_3 on a hot plate and the volume was made up using MilliQ water. The digested solution was filtered using whatman No. 42 filter paper before analyzing on AAS (GBC ∑ Avanta). DTPA extractable fraction was obtained using method of Lindsay and Norvell [24] and EDTA fraction by Quevauviller et al. [25]. For bioavailable As, the arsenic fractionation procedure of Onken and Adriano [26] was followed. The different extractable fraction of metals and arsenic concentration present is shown in Table-1.

Quality control and statistical analysis

Analytical data quality of metals was ensured through analysis of sewage sludge samples of Resource Technology Corporation (EPA Certified Reference material) (Catalog No. CRM029-050; Lot No. JC029a). To determine significant differences between treatments, Duncan Multiple Range test (DMRT) was determined. In Tables and Figures, the values are marked by letters for the significance level as compared to control.

RESULTS

Metal accumulation in plant biomass

The metal accumulation in different part of the plant after 30d of treatment is presented in Table 2. The accumulation of Cu in the roots increased with the increase of metal treatment; however, there was less translocation to the upper parts as compared to C. The toxic metal (As (III)-30, As(V)-30, Cr-30) treated plants have also shown lower translocation of Cu to the upper part, as compared to the C. Such trend was not observed at higher concentrations of As (As (III)-50 and As (V)-80) treated plants. In Cu, Cr and As(V) treated plants, the accumulation (mg/kg dw) of Mn in roots ranged between 27.45 and 34.26 as compared to 18.51 in C, hence, showing more accumulation in these treatments except in As(III)-30. The translocation of Mn in leaves of all these sets was found more. As compared to C, Fe accumulation increased in the roots in all the treatments. The accumulation of Zn in Cr-30 treated roots (104.31 mg/kg dw) and its translocation to leaves (71.16 mg/kg dw) were the highest among all the treatments. In rest of the treatments, Zn translocation to leaves was less. In Cu treatments, the accumulation of Fe in leaves increased with increase in concentration (Cu-30 to Cu-80) and were also observed to be higher than C. Whereas, the accumulation of Fe in leaves of As(III) and As(V) treated plants decreased with increase in As concentration as compared to C. Similar to the Fe accumulation in Cu treated plants, Fe accumulation in leaves increased in Cr treated plants, however the translocation to leaves of As(III) and As(V) treated plants decreased, as compared to C. In Cr treated plants, the accumulation of Cr was recorded higher in roots (134.10) than leaves (17.49) showing least translocation to upper part. The levels of phosphate have not shown any significant change in all the treatments as compared to C, however, the level of phosphate was lowest in As treated plant.

In seeds (Table 3), the accumulation of Fe, Mn and Zn increased with increase in concentration of Cu, As(III) and As(V). However, in the seeds of As(III)-50 treated plants, higher accumulation of Fe (164.74 mg/kg) was recorded. In rest of the treatments, Fe accumulation ranged 120.52-160.73 mg/kg. Interestingly, the level of Cu was found below detection limit in As and Cr treated plants. Similarly, the level of As was also found BDL in As treated plants. In Cu treated plants, non-significant difference in the accumulation of Cu in the seeds was observed as compared to C. In case of Cr treated plants, an accumulation of 1.84 Cr mg/kg dw was found in the seeds.

Seed weight and oil content

No significant difference was observed in the oil content (Table 4) between the different metal treatments both in comparison to C and also between the plants exposed to increased metal concentration. Cu treated plants have shown non-significant increase in seed weight, as compared to C, and also against all other treatments. Overall, comparison between all the metals studied at 30 mg/kg dw indicated that Cr imparted highest toxicity against C, leading to significant decline in seed production. However, no significant difference in seed yield was observed in As(III) and As(V) treated plants.

Growth parameters and lipid peroxidation

The exposure of the metals to the plants, in general, exhibited non-significant changes in growth parameters (Figure 1a, b). There was no significant difference in fresh weight, root and shoot lengths between the treatments and also when compared with C, except for Cr-30 where significant decrease in shoot length was observed after 30d. Decrease of 43.8 (fresh weight) and 32.2% (shoot length) was observed in Cr treated plants. These results revealed that the highest toxicity among the metals was induced by Cr.

There was no significant difference in the chlorophyll contents (Figure 2A) between different treatments when compared with C, after 15d, however, the level increased in As(V)-80 with respect to As(III) and Cr treated plants after 15d. With increase in individual metal concentrations, there was no significant difference in chlorophyll content in Cu and As (V) treated plants at both the growth periods, however, it decreased significantly in As (III) treated plants at 30d. The plants could not survive in Cr concentration higher than Cr-30. As compared to C, significant decrease in chlorophyll content was observed in Cr and higher concentration of As treated leaves of the plants at 30d. Interestingly, no significant difference was observed in carotenoid content (Figure 2B) at both the growth periods in all the treatments. Similarly, the protein content (Figure 2C), at 15d showed no significant difference between the treatments except for decrease ($p<0.05$) in the leaves of Cr treated plants. At 30d, significant increase was observed in Cu-30 treated plants as compared to all other treatments and non-significant increase with respect to C. As compared to C, non-significant decrease was observed in Cu-80, As(V)-30, As(III)-30 and Cr-30 and significant decrease ($p<0.05$) in As (V)80 and As(III)50 treated plants. With increase in individual metal concentration, protein content decreased in all set of treatments at 30d.

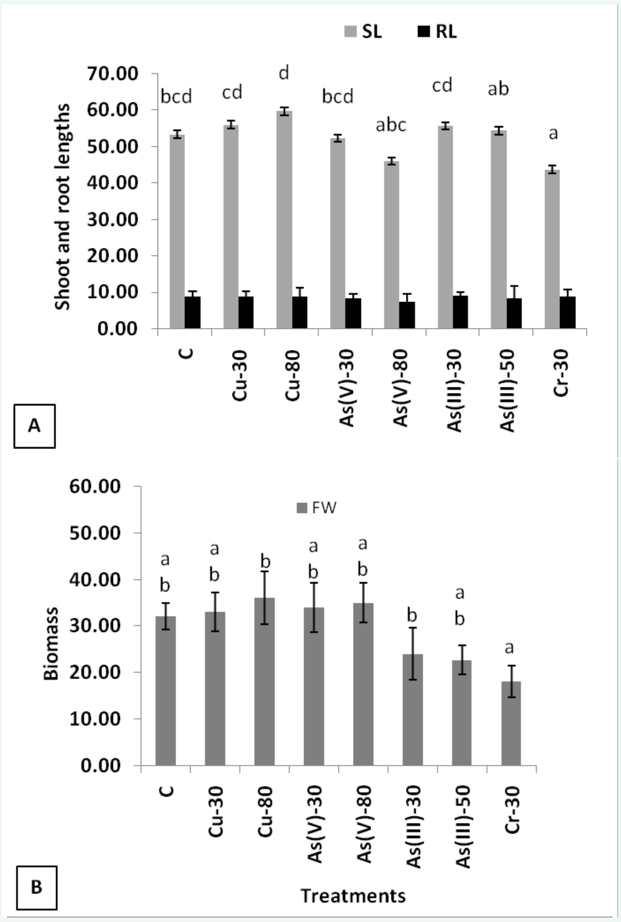

Figure 1 The effect of metal contaminated soil in (A) shoot and root lengths (cm) and (B) fresh weight (g) in the plants grown on metals spiked soil after 30d of metal treatments. All the values are means of four replicates ± SD. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$). SL= Shoot length, RL= Root length.

The level of MDA (Figure 2D), a by-product of lipid peroxidation, was observed to be high (non-significantly) in all the treated plants at both the growth periods against C, except in Cr-30 where significant increase of 42% was observed at 15d. With increase in individual metal concentrations within each metal treatment, an increase in MDA content was observed at both the growth periods. At 15d, an increase of 13.98, 5.88 and 14.7% was recorded in Cu-80, As(V)80 and As(III)30, respectively as compared to lower metal concentration.

Antioxidant enzymes

As compared to C, no significant difference was observed in SOD (Figure 3A) activity of the leaves in any of the treatments at both the growth periods, except for a significant increase in Cu-30 after 15d, and decrease in As(III) after 30d. Maximum increase of 34% was observed in Cu-30 ($p < 0.05$) after 15d and decrease of 29% in As(III)-30, after 30d, as compared to their respective C. At 30d, there was not much difference in SOD activity (U/min/g fw) of Cu, As(V), and Cr treated leaves ranging between 388.5 to 436.0 against C (433.08). Although, it showed an increasing trend with increase of individual metal concentrations (Cu, As(V) and As(III)) within each treatment.

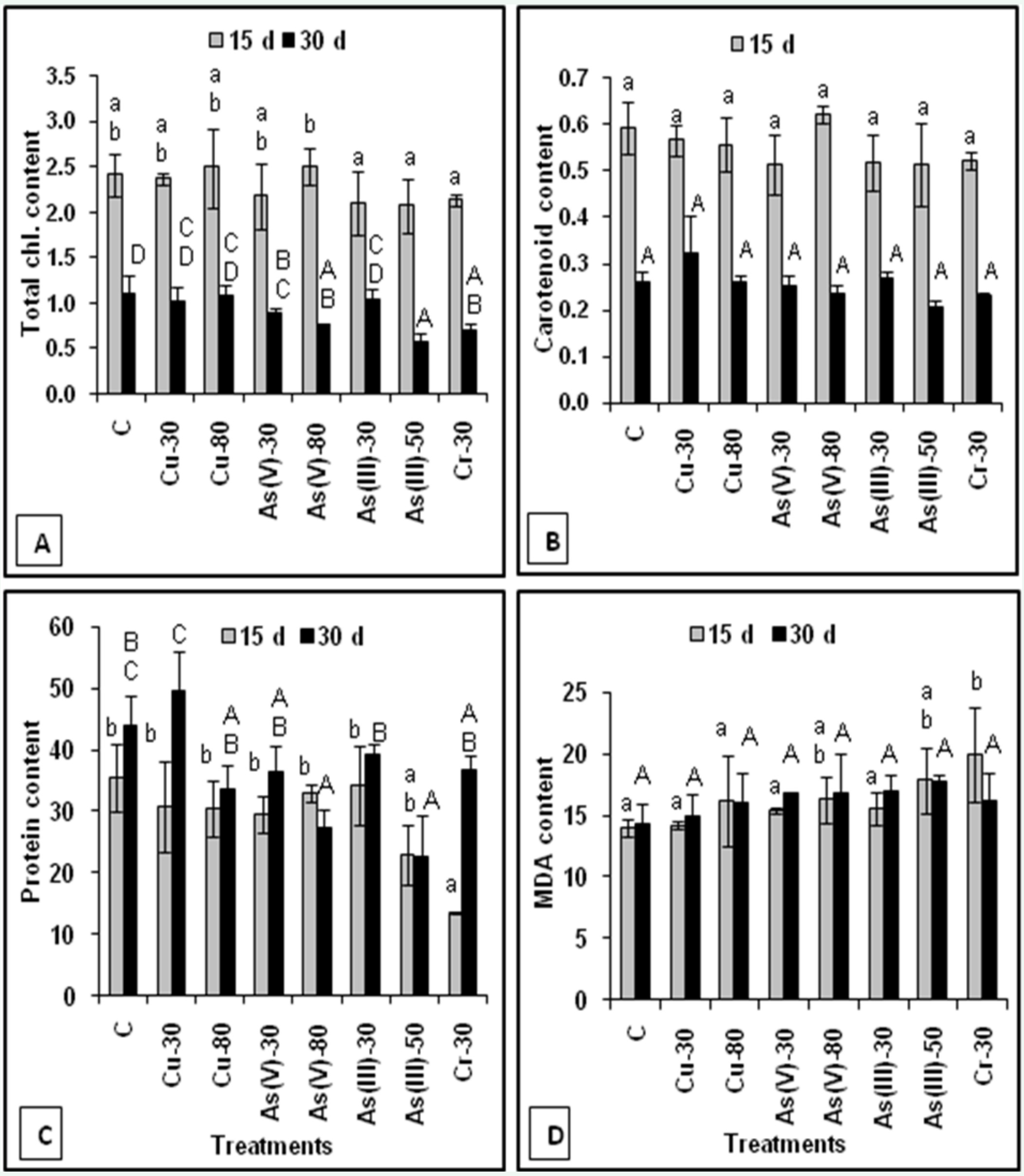

Figure 2 The effect of metal contaminated soil in (A) total chlorophyll, (B) carotenoid and (C) protein contents (mg/g fw) and (D) MDA content (mmol/g fw) in the plants grown on metals spiked soil after 15 and 30d of metal treatments. All the values are means of four replicates ±SD. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$). SL= Shoot length, RL= Root length.

CAT, APX and GPX are H_2O_2 capturing enzymes. There was no significant difference in the CAT activity (µmol/min/g fw) in the leaves after 15d. Its activities ranged between 13.31 - 21.80 against 17.58 in C, except for a significant increase of 58% and 82% observed in As(III)-30 and Cr-30 treated plants, respectively, as compared to C (Figure 3B). After 30d, CAT activity increased in all the treatments as compared to C, except in As(V) treated plants. An increase ($p < 0.05$) of 39% in Cu-80 and 31% in Cr-30 treated leaves was observed in CAT activity as compared to C, after 30d.

GPX activity (mmol/min/g fw) decrease in all the treatments (Figure 3C) with respect to C after 15d, except for Cu-30, As(III)-30 and Cr-30 where an increase was observed. Maximum decrease of 29% was observed in As(V)-80, however, no significant change was observed in all other treatments which ranged between 3.09 and 3.85 against C (3.59). At 30d, there was no significant difference in GPX activities in the leaves of the treated plants as compared to C except for significant decrease of 32% in As(III)-50. With reference to As treatments, GPX activity has been observed to decrease in all the treatments with increase in concentration at both the growth periods.

The activity of leaf APX (Figure 3D) increased in Cu and As(V) treated plants as compared to C, after 30d, and also with increase of the concentrations of individual metal (Cu, As(V)). In case of As(III) and Cr treated plants, the APX activity in the leaves has shown decrease as compared to C after 30d and it was almost the

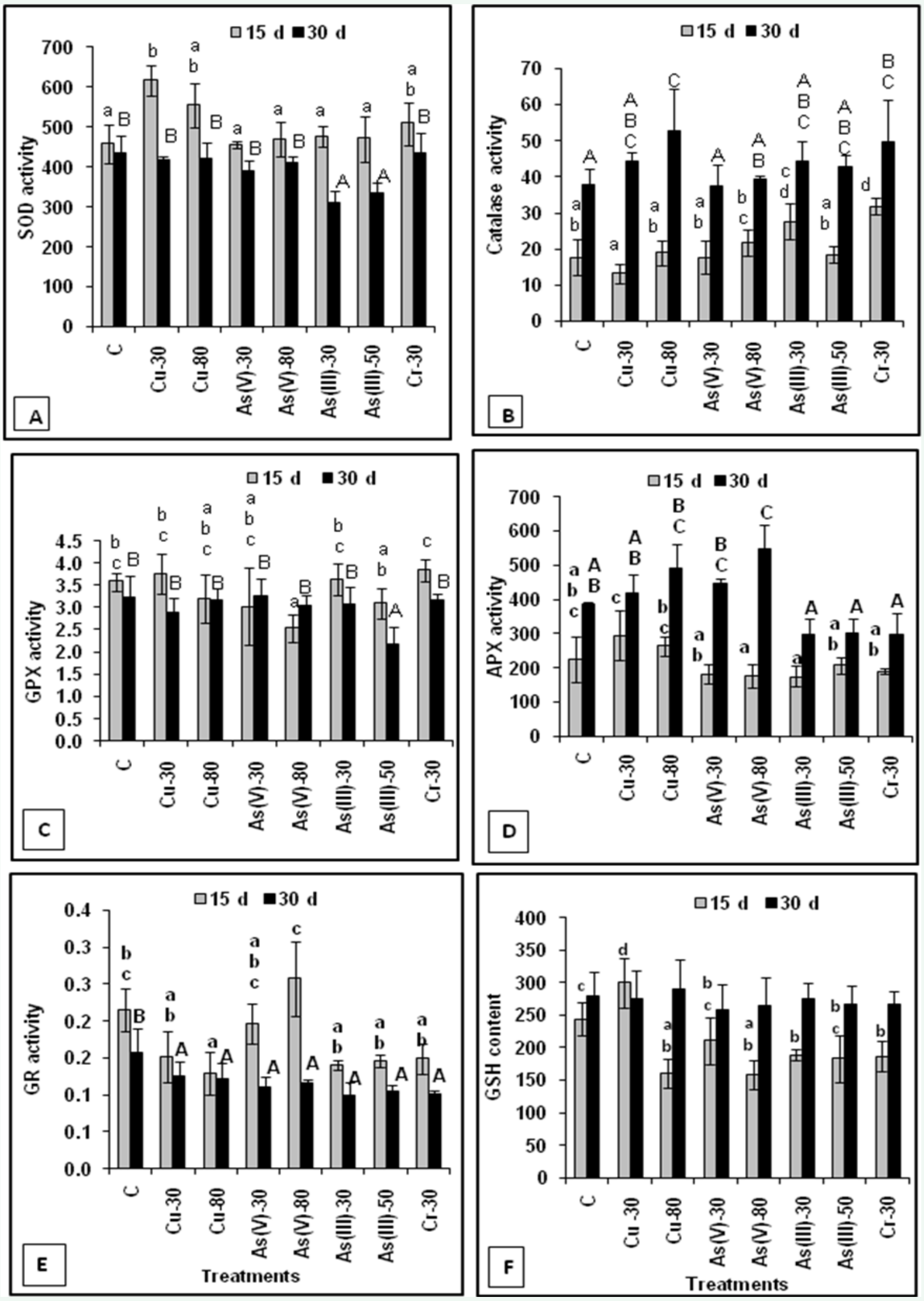

Figure 3 The effect of metal contaminated soil in (A) SOD (U/min/g fw), (B) catalase (μmol/min/g fw), (C) GPX (mmol/min/g/ fw), (D) APX (μmol/min/g fw), (E) GR (mmol/min/g fw) and (F) GSH content (μmol/g fw) in the plants grown on metals spiked soil after 15 and 30d of metal treatments. All the values are means of four replicates ±SD. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$). SL= Shoot length, RL= Root length.

same in these two treatments. After 15d, no significant difference was observed in APX activities, as compared to C among all the treatment. However, it was found to increase (31%) non-significantly in Cu-30 treated plants, followed by decrease (17%) at Cu-80, compared to C.

Glutathione reductase activities in leaves (Figure 3E) decreased in all sets of treatment with respect to C at both the exposure periods, except in As(V)-80 where an increase of 24% was observed after 15d. Similar levels of decrease (38%) in GR activity were observed in As(III) and Cr treated at 30d against C.

Glutathione, which plays an important role in cellular detoxification of metal ions, decreased in all set of treatments,

Table 1: Level (mg/Kg dw) of extracted metals (As with NH_4Cl) and other metals (with EDTA and DTPA) from metal soil spiked after harvesting of the plants. All the values are means of four replicates ±SD. BDL = Below detection limits.

Treatments (mg/Kg)	**Cu**	**Zn**	**M n**	**Fe**	**Cr**	**As**
	EDTA					**NH_4Cl**
C	27.16±1.82	6.74±0.14	60.43±1.34	130.41±1.96	BDL	BDL
Cu-30	36.12±1.12	8.70±0.18	61.67±0.85	136.42±2.17	BDL	BDL
Cu-80	64.65±14.53	8.70±0.18	62.21±1.95	137.88±4.75	BDL	BDL
As(V)-30	27.20±0.97	7.44±0.17	61.00±0.17	137.67±2.17	BDL	11.57±0.35
As(V)-80	29.04±0.87	7.13±0.43	60.51±1.07	138.66±0.68	BDL	12.82±0.52
As(III)-30	32.22±0.77	10.15±0.21	61.35±0.67	138.76±0.37	BDL	15.62±3.10
As(III)-50	23.47±0.07	8.02±0.85	61.66± 0.39	137.26±1.36	BDL	21.41±0.74
Cr-30	27.26±1.06	7.30±0.19	60.25±1.18	135.18±0.57	17.11±2.19	
	DTPA					
C	4.10±2.15	3.03±0.22	8.81±0.26	15.20±2.94	BDL	BDL
Cu-30	27.38±0.61	2.85±0.06	7.97±0.36	13.99±1.28	BDL	BDL
Cu-80	43.22±4.57	3.17±0.08	10.92±0.59	21.23±5.36	BDL	BDL
As(V)-30	2.37±0.21	2.46±0.13	8.91±1.30	14.04±0.82	BDL	BDL
As(V)-80	2.72±0.76	2.37±0.02	11.00±0.40	14.54±8.85	BDL	BDL
As(III)-30	2.31±0.17	3.56±0.10	12.34±0.82	15.17±0.69	BDL	BDL
As(III)-50	1.54±0.29	2.85±0.08	10.17±0.11	16.15±0.57	BDL	BDL
Cr-30	2.08±0.26	2.67±0.15	8.18±0.48	7.58±8.69	BDL	BDL

Table 2: Accumulation of metals in different parts of the plants after 30d of treatment. All the values are mean of four replicates±SD. R = roots, S = stems, L = leaves. BDL = Below detection limits.

Treatments (mg/ Kg)/ Plant's parts	**Metals accumulation (mg/kg dw)**						**P (mg/g dw)**
	Cu	**Mn**	**Zn**	**Fe**	**Cr**	**As**	
CR	9.98±4.4	18.51±0.9	59.06±6.5	359.2±35.5	BDL	BDL	1.86±0.31
CS	7.69±3.8	18.77±1.7	35.10±0.7	182.9±22.1	BDL	BDL	0.62±0.27
C L	8.10±0.5	65.63±11.0	54.77±5.4	514.07±65.1	BDL	BDL	0.89±0.07
Cu-30R	17.06±1.9	27.45±6.0	56.28±7.0	560.31±57.9	BDL	BDL	1.53±0.11
Cu-30S	5.85±1.2	11.83±0.1	42.39±7.2	237.98±18.0	BDL	BDL	0.96±0.14
Cu-30L	6.66±0.6	61.16±5.4	48.60±9.2	491.8±72.0	BDL	BDL	1.17±0.04
Cu-80R	32.99±6.7	27.68±2.5	82.85±15.4	577.9±36.4	BDL	BDL	1.48±0.12
Cu-80S	11.80±0.5	15.82±0.2	32.33±2.7	180.9±41.8	BDL	BDL	0.60±0.07
Cu-80L	13.63±1.6	75.53±12.2	60.10±6.2	687.6±51.4	BDL	BDL	0.95±0.18
As(V)-30R	51.52±7.0	32.66±6.2	66.80±7.6	526.1±115.2	BDL	374.5±75.4	1.31±0.22
As(V)-30S	5.37±1.5	16.90±1.8	28.28±2.3	165.46±37.5	BDL	245.1±54.3	0.55±0.08
As(V)-30L	6.65±0.6	54.04±2.2	62.65±10.3	483.8±30.0	BDL	162.6±20.3	0.85±0.04
As(V)-80R	3.06±1.2	28.90±9.7	65.33±10.0	611.0±41.0	BDL	663.1±7.6	1.10±0.02
As(V)-80S	5.55±0.7	26.17±8.5	51.43±8.8	315.7±31.1	BDL	143.2±34.3	0.43±0.07
As(V)-80L	5.20±1.4	48.95±11.5	55.85±3.8	451.9±3.3	BDL	177.6±17.2	0.83±0.12
As(III)-30R	23.34±5.3	17.40±2.1	69.08±2.0	381.5±31.5	BDL	442.8±12.9	1.17±0.06
As(III)-30S	8.57±2.8	17.54±3.7	42.85±2.0	220.8±34.2	BDL	211.0±2.9	0.68±0.05
As(III)-30L	4.63±0.4	47.07±4.6	68.78±8.0	416.3±54.5	BDL	131.0±1.7	0.87±0.08
As(III)-50R	4.68±0.6	34.26±7.7	70.42±0.4	401.0±25.5	BDL	413.0±32.0	1.01±0.12
As(III)-50S	10.08±2.4	16.53±2.9	34.78±8.0	183.7±14.0	BDL	281.6±14.3	0.62±0.08
As(III)-50L	4.14±0.3	53.08±9.0	48.48±9.0	350.6±26.4	BDL	159.1±27.1	0.88±0.08
Cr-30R	13.68±0.4	33.05±4.9	165.09±18.7	595.5±86.7	134.1±17.0	BDL	1.00±0.07
Cr-30S	6.42±1.5	16.28±2.3	47.52±7.6	155.6±20.5	15.1±3.6	BDL	0.61±0.04
Cr-30L	7.01±0.4	80.54±1.7	71.16±1.9	608.9±59.4	17.5±1.7	BDL	1.10±0.18

except Cu-30, where an increase of 23% was observed as compared to C after 15d (Figure 3F). Within each set of metal treatments, the level of GSH was observed to be higher at lower metal treatment than its respective higher levels, after 15d. There was no significant difference in GSH contents (μmol/g fw) among all set of treatments ranging between 258.2 and 289.2 against C (279.6) after 30d.

DISCUSSION

In this investigation, *B. campestris* L. were exposed to different metal (loids) to understand the pattern of uptake under the test condition. The study recorded higher accumulation of As in the root than in the shoot of *B. campestris*, showing poor translocation to upper parts of the plant. The phenomenon may be a defense strategy of the plant to restrict the translocation of toxic metal to the upper parts [1,27] and such strategy has also been observed in *Brassica juncea* [4] and sunflower [28,4] reported unbound As(V) and As(III) species in xylem sap of *Brassica juncea*. The majority of As remains as an As(III)-tris-thiolate complex. The binding of these non-essential metals to the thiol biomolecules in the roots and its restricted translocation to the upper parts is indicative of adaptive mechanism of the plant. In Cr treated plants, high accumulation of Cr in the roots of *B. campestris* could be due to its immobilization to vacuoles of the root cells. The uptake of nutrient elements by *B. campestris* was also influenced by the presence of toxic metal in the soil. The accumulation of Mn was observed to be high in Cr and higher doses of Cu treated plants. Similar observation of Mn accumulation pattern was also reported in *Brassica juncea* [3,29]. In contrast to Cr and Cu treatments, Mn accumulation decreased in As treated leaves of the plant which has also been reported by Mokgalaka-matlala et al. and Sinha et al. [29,30]. There are several reports [3,29] which conform to the findings of the present study showing high accumulation of Fe, Mn and Zn in the plants grown on Cr contaminated soil. Based on these results, it can be inferred that the presence of toxic metals can greatly influence the uptake of essential metals and it can vary from one plant to another. Such data are very important for those plants/crops which are being consumed by human and animals; there is a need for investigation [2,6,29]. The chemical properties of As(V) and PO_4^{-3} are very similar, hence, there is strong evidence that AsO_4^{-3} and phosphate (PO_4^{-3}) are taken up by the same transporters in plant roots [30]. As observed in this study, the level of PO_4 in the shoots and leaves of As (V) treated plants were lower than that in the plants treated with As(III) and other metals.

The study also shows that there was not much variation in the level of essential metals in the seeds of the treated plants, except in Cu, where the level was below detection limit (0.1 ppm). In contrast to this, *Brassica campestris* L.(cv. Pusa Jaikisan) grown on industrially contaminated substrate has been reported to accumulate essential and toxic metals including Cu in the seeds [31]. Similarly, another oil bearing plant, sesame (*Linum usitatissimum*) have also been reported to accumulate Cu along with other essential metals in its seeds [6]. The level of As and Cr in the seeds was below detection limit (BDL) in the plants receiving lower metal concentration. This may be due to the saturation in the formation of thiol-metal complex by thiol containing metabolites resulting in the reduction of translocation of unbound metals to the seed.

Table 3: Accumulation of metals in seeds of *B. campestris* All the values are means of four replicates±SD. Different letters indicates significantly different values (DMRT, $p < 0.05$). BDL = Below detection limits.

Treatments (mg Kg^{-1})	Metals accumulation (mg kg^{-1} dw)					
	As	Cu	Mn	Zn	Fe	Cr
C	BDL	3.94±1.73	23.06±2.80	67.30±10.92	137.77±30.69	BDL
Cu-30	BDL	4.06±0.54	20.65±0.98	67.83±9.18	120.52±13.72	BDL
Cu-80	BDL	5.51±0.55	24.28±3.47	75.10±13.79	141.77±26.33	BDL
As(V)-30	BDL	BDL	20.27±5.33	69.59±5.10	141.91±28.41	BDL
As(V)-80	9.12±0.86	BDL	26.20±3.97	85.64±11.78	152.97±18.68	BDL
As(III)-30	BDL	BDL	21.68±5.20	66.47±11.33	160.73±47.51	BDL
As(III)-50	BDL	BDL	27.18±6.94	74.35±0.92	164.74±24.23	BDL
Cr30	-	BDL	24.01±2.57	79.68±4.57	121.62±32.88	BDL

Table 4: Seeds weight (g/100 seed) and oil content (g/100 g seed) of *B. campestris*. All the values are means of four replicates±SD. Different letters indicates significantly different values (DMRT, $p < 0.05$).

Treatments (mg/Kg)	Weight (g) of 100 seeds	Oil (g)/100 g seeds
C	351.40±34.63^{bc}	38.82±1.4
Cu-30	396.97±38.57^{c}	41.39±7.80
Cu-80	356.00±13.06^{abc}	37.19±1.4
As(V)-30	332.85±22.60^{a}	36.86±1.42
As(V)-80	293.57±28.59^{ab}	36.96±0.3
As(III)-30	348.56±13.60^{ab}	39.94±1.32
As(III)-50	345.60±6.24^{a}	38.30±2.8
Cr30	310.87±2.48^{a}	37.71±2.2

No significant difference was observed in the oil contents among the metal treated plants. In contrast, decrease in oil content was reported in *Brassica juncea* at higher concentration of As(V) [29]. High Zn content in the seeds of metal treated plants was observed. Cakmak [32] explained that high level of Zn in the seeds to play an important physiological role during seed germination and also protect them from oxidative damage through the detoxification of reactive oxygen species. The oil content in the seed of metal treated *B. campestris* (T-42) did not vary significantly from the control in all the treatments. However, Ahuja et al. [8] reported variation in the oil content in the seeds of sixty four genotypes of *Brassica campestris* L.

Decrease in growth parameters due to the accumulation of toxic metals/(loids) in the plants is well reported [5,29,33]. The growth parameters and photosynthetic pigments were not much affected in Cu and As(V) treated *B. campestris,* this may be due to its tolerant nature which is widely reported [31]. In contrast, Sinha et al. [29] reported a decrease in growth parameters in *Brassica juncea* treated with Cu and also in As(V) treated Indian mustard [7]. However, in this study it was observed that As(V) showed no effect on growth parameters. Shanker et al. [34] reported that Cr toxicity in the plants is observed at multiple levels, from reduced yield, due to effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. The negative effect of As(III) on growth parameters may be attributed to the higher affinity of As(III) with thiol groups, where energy are diverted for production of more such metabolites [35].

The destabilization of plasma-membrane is generally measured in terms of lipid peroxidation, which is the index for damage due to production of toxic oxygen free radicals under metal (loid) stress. In this study, maximum increase in MDA content of Cr treated plants after 15d, suggests higher toxicity than the other metals. Cr ions is reported to block the electron flow in PS II, leading to production of free oxyradicals [34] resulted in lipid peroxidation. The increase in MDA levels was comparatively less in Cr treated *B. campestris* as compared to *Brassica juncea* [29] which point towards the best tolerance mechanism of *B. campestris*. The MDA content of *B. campestris* in As(III) and As(V) treated were similar. This may be possibly due to reduction of As(V) to As(III) in plants by arsenate reductase, after which the same toxicity mechanism occurs.

The enzymatic antioxidants (SOD, APX, GPX, CAT, GR) exhibits a well concerted mechanism of oxidative defense, showing redundancy in different plants and against different elicitors. SOD functions as a reactive oxygen species scavenger by converting O_2^- to H_2O_2. Further, H_2O_2 is converted to O_2 and H_2O by APX, CAT and GPX. Higher levels of SOD activity in all the metal treated plants after 15d with respect to control, suggests that initial shock from the toxic metals resulting into the production of O_2^-, however after acclimatization, the production of O_2^- decreased resulting in decrease of SOD activity after 30d [37]. On the contrary, the activities of APX and CAT in later stages of growth were higher, exhibiting a possible redundancy between the antioxidative enzymes and different stages of growth. In Cu and As(V) treated *B. campestris*, APX and CAT activities, in general, have increased with concentration, however, the decline in the level of APX in As(III) and Cr(VI) treated plants, suggests that APX is more vulnerable to As(III) and Cr(VI) toxicity than CAT. The activity of SOD in Cu treated plants were the highest after 15d and decreased with concentration, which could be due to Cu being a cofactor of CuSOD which may have enhanced the synthesis of CuSOD.

It has been reported that the plants detoxify As by reducing As(V) to As(III), which is subsequently detoxified via forming complexes with thiol-reactive peptides such as ɣ -glutamylcysteine, GSH and PCs [4,33]. GR reduces GSSG to GSH, however, high activity of GR in As(V) in conjuncture with no rise in the level of GSH was observed in this study. Raab et al**.** [28] studied the mechanistic details of As detoxification in the plants and reported time-dependent formation of various arsenite–PC complexes in the roots, stems and leaves of sunflower **(***Helianthus annuus*) in response to As exposure. This may be the reason for the decrease in GSH content in *B. campestris* in As treated plants.

CONCLUSION

Among all the metals studied, Cr was more toxic as evident from the results obtained for fresh weight, chlorophyll content, seed weight, seed oil and concomitant increase in MDA content. Among the two species of As, As(III) was more toxic than As(V) as the plants could not survive in higher doses and the effect on growth parameters was greater in As(III) treated plants. The level of As and Cu in the seed was below detectable limit and no significant difference in the other essential metals. The plant can be grown on sites contaminated with these metals for oil production as the translocation of As to seeds was below detectable limits. The activities of antioxidant enzymes varied for different metals, where the activity of SOD and APX increased in the plants treated with Cu. CAT activity increased in the plants treated with lower doses of As(III) and Cr. This demonstrated the varied defense mechanisms induced in *B. campestris* towards different metals.

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