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Differential antioxidative enzyme responses of *Jatropha curcas* L. to chromium stress

Santosh Kumar Yadav [a], Monika Dhote [a], Phani Kumar [b], Jitendra Sharma [a], Tapan Chakrabarti [a], Asha A. Juwarkar [a,*]

[a] *Environmental Biotechnology Division, National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur, Maharashtra 440020, India*
[b] *Defence Institute of High Altitude Research, Ministry of Defence, Govt. of India, India*

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ABSTRACT

Chromium (Cr) tolerant and accumulation capability of *Jatropha curcas* L. was tested in Cr spiked soil amended with biosludge and biofertilizer. Plants were cultivated in soils containing 0, 25, 50, 100 and 250 mg kg^{-1} of Cr for one year with and without amendment. Plant tissue analysis showed that combined application of biosludge and biofertilizer could significantly reduce Cr uptake and boost the plant biomass, whereas biofertilizer alone did not affect the uptake and plant growth. Antioxidative responses of catalase (CAT), ascorbate peroxidase (APX) and glutathione S-transferase (GST) were increased with increasing Cr concentration in plant. Hyperactivity of the CAT and GST indicated that antioxidant enzymes played an important role in protecting the plant from Cr toxicity. However, APX took a little part in detoxification of H_2O_2 due to its sensitivity to Cr. Therefore, reduced APX activity was recorded. Reduced glutathione (GSH) activity was recorded in plant grown on/above 100 mg kg^{-1} of Cr in soil. The study concludes that *J. curcas* could grow under chromium stress. Furthermore, the results encouraged that *J. curcas* is a suitable candidate for the restoration of Cr contaminated soils with the concomitant application of biosludge and biofertilizer.

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1. Introduction

Nowadays, development has become synonymous with deforestation and progress has become synonymous with pollution. Because of the increased mining and industrial activities in the late 19th and early 20th century, the pollution due to heavy metals has increased considerably worldwide [1]. Chromium and its compounds have multifarious industrial uses and are extensively employed in leather processing and finishing industry, production of refractory steel and electroplating cleaning agents. In India, 2000–3200 tones of elemental Cr escapes into the environment annually [2], and very high levels of Cr (VI) contamination, 14,800 mg kg^{-1} in ground water and 25,900 mg kg^{-1} in soil have been reported in United Chrome Products Site in Corvallis Oregon [3].

Contamination of soil and ground water due to the use of Cr in various anthropogenic activities has become a serious source of concern to plant and animal scientists over the past decade. Chromium is a toxic element to higher vascular plants and is detrimental to plants growth, development and reproduction [4]. Chromium toxicity in plants is observed at multiple levels from reduced yield to inhibition on enzymatic activities and mutagenesis [5]. The physiological impact of Cr contamination in soil and water is dependant on the speciation viz., Cr (III) and Cr (VI). Speciation of Cr in the soil differentially affects the mobilization of the metal, subsequent uptake and resultant toxicity in the plant system. In view of the seriousness of Cr pollution, considerable efforts have been made to develop suitable methods for the reclamation of Cr contaminated soil [6].

The use of plants as a vegetation covers for the phytoremediation of land contaminated by heavy metals does seem to have considerable potential. There is plenty of evidence from the natural establishment of trees on contaminated sites that some types of trees can survive under such adverse conditions [7]. Plant species in association with mycorrhizae have shown promising results for rehabilitation of Cr contaminated lands in and around tannery industrial areas [8]. The growth and metabolisms of plants are adversely affected by increasing levels of these metals in the soil environment [9]. Soil amendment is a major requirement for the successful establishment of vegetation in metal-contaminated soils. The addition of amendments such as sewage sludge, fly ash, pig manure is effective in lowering the metal toxicity of soil and provides a slow release of nutrient sources such as N, P, K to support plant growth [10–13].

Some authors reported that many abiotic stresses, including exposures to heavy metals, induces free radicals that may dam-

* Corresponding author. Tel.: +91 712 2249764; fax: +91 712 2249900.
E-mail address: aa_juwarkar@neeri.res.in (A.A. Juwarkar).

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age plants major cell macromolecules [14,15]. Plant cells are able to respond to elevated levels of reactive oxygen species (ROS) by activating their antioxidative defence systems [16]. The external activities include exudation of chelators to bind metal. The intracellular activities include alteration of cell membrane or to generate reactive oxygen species (ROS) and create oxidative stress situations [17]. The antioxidative system includes, metabolites GSH, ascorbate (AsA) and the enzymes ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), superoxide dismutases (SOD), catalases (CAT) and peroxidases (PODs) are involved in detoxification of O_2^-, and H_2O_2 respectively, thereby preventing the formation of OH radicals [18,19].

Reclamation of Cr contaminated soils through utilization of industrial waste and economically important plant species is a valuable option. For long-term remediation, metal tolerant species are commonly used for revegetation of metal spoil lands. Oil seed plants can be used as pioneer species to solve the problem of oil crisis, which can grow on Cr contaminated lands. Considering economic value of *Jatropha curcas* plant, it was selected in the present study, due to its draught resistance and tolerance to abiotic stress environment [20,21]. Thus, the present work aims to evaluate the effects of chromium stress and the soil amendment with biosludge and biofertilizer in growth, activities of some antioxidative enzymes such as CAT, APX, GST and reduced glutathione (GSH) contents in *J. curcas*.

2. Materials and methods

2.1. Soil, biosludge, biofertilizer and preparation of Cr spiked soil

Experimental soil was collected from 0 to 20 cm depth from the premises of National Environmental Engineering Research Institute (NEERI), Nagpur, India. It was air-dried and sieved through a 2 mm mesh sieve. Standard soil characterizations were performed. Chromium was added to give nominal concentrations of 0 (without Cr), 25, 50, 100 and 250 mg Cr kg^{-1} by mixing with potassium dichromate ($K_2Cr_2O_7$) in to soil. The Cr (VI) salt ($K_2Cr_2O_7$) was dissolved in distilled water, sprayed on the soil and mixed thoroughly. Then, Cr treated soil samples were incubated in a pot and kept for 60 days. Soil without addition of chromium served as the control. Biosludge was collected from M/s Gujarat Refinery, Vadodara, India. Biosludge, which was generated from the biologically effluent treatment plant (ETP) of refinery and was fully matured. Biosludge was air-dried for four weeks and sieved through a 2 mm mesh. *Azotobacter chroococcum* strain AAJ85 was used as biofertilizer, which was earlier isolated at NEERI from chromium contaminated soil.

The soil and biosludge were characterized following the standard methods. pH (solid:deionised water = 1:2, w/v) and electrical conductivity (EC; solid:deionised water = 1:2, w/v) of the samples were measured using a glass electrode pH meter and EC meter, respectively. Percent organic carbon, total N, P, K were analyzed as per standard methods [22,23]. Texture was assessed using the International pipette method. Total concentration of chromium and other metals in soil was determined using 0.5 g soil sub-samples digested with acidic mixture of HNO_3:$HClO_4$. After digestion, metal concentration was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 4100 DV, PerkinElmer, USA) (Tables 1 and 2). Physico-chemical properties of soil and biosludge used in the experiment are given in Table 1.

Table 1
Physico-chemical properties of soil and biosludge used for the pot experiment.

Parameters	Soil	Biosludge
Soil texture	Clayey	–
Electrical conductivity (mS cm^{-1})	0.47 ± 0.05	6.46 ± 1.2
Organic carbon (%)	0.48 ± 0.05	17.2 ± 4.0
pH	8.5 ± 0.1	6.38 ± 0.5
Total element concentrations (mg kg^{-1})		
Nitrogen	179 ± 25	1680 ± 122
Phosphorous	188 ± 14	552 ± 96
Potassium	186 ± 29	270 ± 22
Iron	3512 ± 722	1668 ± 471
Copper	93.6 ± 5.9	372 ± 15
Zinc	30.6 ± 3.2	34.6 ± 2.0
Chromium	31.2 ± 2.2	–
Lead	6.2 ± 0.08	17 ± 2.1

2.2. Experimental design

A pot experiment was conducted to assess growth, accumulation and enzymatic activities of *J. curcas* in different concentration of Cr spiked soil amended with biosludge and/or biofertilizer under different treatments. The treatments selected included: Treatment 1 (T1): soil + chromium; Treatment 2 (T2): soil + chromium + biosludge; Treatment 3 (T3): soil + chromium + biofertilizer; Treatment 4 (T4): soil + chromium + biosludge + biofertilizer. Further, each treatment was subjected to 5 sub-treatment viz. 0, 25, 50, 100 and 250 mg kg^{-1} of Cr in soil. Finally, the experiments were followed by completely randomized block design with a 5 × 4 factorial arrangement with total 20 treatments. Four replicates of each treatment were kept and in each pot, one plant was maintained.

The data of plant biomass, metal accumulation and enzymes activity in *J. curcas*, under different treatments were analyzed using the SigmaPlot 11 software program (Systat Software Inc., San Jose, USA) by two-way analysis of variance (ANOVA) to compare treatments with control and between means of the different treatments. All values are given the mean of four replicates ± standard deviation.

2.3. Plant

Seeds of *J. curcas* were obtained from M/s Shrishail Nursery, Nagpur and identified comparing with authentic specimens at the herbarium of Department of Botany, R.T.M. Nagpur University, Nagpur. Seeds were grown in earthen pots in nursery, and placed in field

Table 2
Total chromium concentration (mg kg^{-1}) in the different treatments before plant growth experiment.

Chromium concentration in soil (mg kg^{-1})	Treatments			
	T1	T2	T3	T4
0	30.5 ± 3.4	30.2 ± 2.4	31.1 ± 0.8	30.8 ± 1.3
25	60 ± 6.2	60 ± 6.1	61 ± 2.4	63 ± 3.8
50	80 ± 11.4	95 ± 2.5	73 ± 16.5	73 ± 14.1
100	123 ± 25.2	154 ± 13.7	132 ± 14.2	128 ± 12.9
250	345 ± 39.5	356 ± 32.7	323 ± 25.6	358 ± 49.5

Mean ± standard deviation; *n* = 4.

Table 3
Biomass (g dry weight/pot) of *J. curcas* grown on different concentrations of chromium spiked soil amended with biosludge and biofertilizer for a period of one year.

Chromium concentration in soil (mg kg^{-1})	Treatments			
	T1	T2	T3	T4
0	84 ± 10.3	151 ± 18.1	108 ± 11.8	165 ± 24.2
25	70 ± 9.4	130 ± 15.3	82 ± 11.4	148 ± 16.1
50	50 ± 9.2	127 ± 7.1	60 ± 7.3	145 ± 11.3
100	35 ± 5.4	66 ± 5.5	44 ± 4.6	74 ± 4.4
250	ns	31 ± 3.1	ns	34 ± 4.1

'ns'– not survived; mean ± standard deviation; $n = 4$.

receiving normal day light, temperature and humidity. Plants having approximately same height and weight were carefully uprooted after 15 days of sowing and transferred into experimental pots.

2.4. Plant growth experiment

Experimental pots were filled with 14 kg of Cr contaminated soil thoroughly mixed with biosludge at the rate of 50 tons per hectare to improve soil conditions and plant growth. Broth culture of *A. chroococcum* having titre value of 16×10^8 CFU ml^{-1} was used as biofertilizer for inoculation of seedlings at the time of plantation. Soil moisture was maintained at 40–50% of the maximum field water holding capacity by adding distilled water during the experimental period. Plants were grown under the net/glass house with natural environmental conditions. The plants were harvested after 12 months to analyze growth performance, heavy metal accumulation and enzymes activities. Whole plant was separated into roots and shoots (stems and leaves) for Cr uptake and enzymes analysis. Fresh plant tissues were used for antioxidant/enzymatic activity. All parts were washed very carefully with deionized water for four times and blotted dried. Plant tissues were oven dried at 70 °C, and constant dry weights were recorded. The dried plant materials were ground to less than 1 mm and sieved through nylon mesh for metal analysis. The concentrations of Cr in leaf, stems and root of plants were determined using 0.5 g plant sub-samples digested with acidic mixture of HNO_3:$HClO_4$, and analysis was done in triplicate by ICP-OES (Optima 4100 DV, PerkinElmer, USA).

2.5. Antioxidative enzyme extraction and measurements

Plant material (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone (w/v) at 4 °C with mortar and pestles (0.1 g FW/ml buffer), filtered through four layers of cheese cloth and centrifuged at 15,000 × *g* at 4 °C for 20 min. The supernatant obtained was designated as crude enzyme extract and was used for various antioxidant enzyme assays. The protein content was determined in plant samples following the procedure given by Bradford [24].

2.5.1. Catalase (CAT), EC 1.11.16

CAT activity was assayed by a UV–vis spectrophotometer. The enzyme extract (40 μl) was added to 9.96 ml of H_2O_2 phosphate buffer pH 7.0 (0.46 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer), CAT activity was determined by measuring the rate change of H_2O_2 absorbance in 60 s with a UV–vis spectrophotometer at 250 nm [25].

2.5.2. Ascorbate peroxidase (APX), EC 1.11.1.11

APX assay was performed using the method of Koricheva et al. [26]. The enzyme extract was further centrifuged at 16,000 × *g* for 15 min at 4 °C after addition of 0.2 mM ascorbic acid. The 3 ml reaction mixture consisted of 50 mM phosphate buffer (pH 7), 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA and 0.1 ml enzyme (sample). The reaction was started with the addition of 0.1 mM H_2O_2. Decrease in absorbance for a period of 30 s was measured at 290 nm in UV spectrophotometer.

2.5.3. Glutathione S-transferase (GST) (EC 2.5.1.18)

One gram plant samples were extracted in 5 ml medium containing 50 mM phosphate buffer, pH 7.5, 1 mM EDTA and 1 mM DTT. The enzyme activity was assayed in a reaction mixture containing 50 mM phosphate buffer, pH 7.5, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was initiated by the addition of 1 mM GSH and formation of S-(2,4-dinitrophenyl) glutathione (DNP-GS) was monitored an increase in absorbance at 340 nm to calculate the GST specific activity [27].

2.5.4. Glutathione (GSH) measurement

The assay was based on sequential reduction by NADPH in the presence of glutathione reductase (GR). About 0.2 g of fresh tissue was extracted in 100 mM phosphate buffer, pH 7.5, containing 0.5 mM EDTA. The assay mixture in 1 ml contained 150 μl buffer containing 125 mM phosphate and 6.3 mM EDTA, pH 6.5, 700 μl of 0.3 mM NADPH, 100 μl of 3 mM DTNB, and 50 μl processed sample. The reaction was initiated by addition of 10 μl of GR and the change in absorbance at 412 nm was recorded [28].

3. Results and discussion

3.1. Plant biomass and chromium accumulation

Pot trial experiments were conducted over one year, and growth performance of *J. curcas* was observed in Cr contaminated soils amended with biosludge and biofertilizer. Growth of *J. curcas* was significantly affected by Cr and varied with soil Cr levels. Chromium reduced the biomass of the plant in dose and treatment dependent manner (Table 3). Biosludge amended treatment (T2) improved biomass (65–110% respectively) than T1 treatment. Combined application of biosludge and biofertilizer (T4) further improved the growth performance of the plant as compared with T2 treatment. While biofertilizer alone (T3) showed less improvement than the remaining two treatments i.e. T2 and T4 (Table 3). Our recent studies showed a similar observation with amendments of dairy sludge and biofertilizer [9,12,13]. Similar observations were also observed by other investigators where they emphasized that organic amendment resulted in successful revegetation of metal-contaminated soil [29,30]. The results suggested that biosludge application was the primary factor influencing growth performance. The quality and productivity of soils can be improved by the addition of organic amendments to soil [30]. Therefore, application of biofertilizer and organic materials are necessary for decreasing the toxicity of metals in the soil.

Chromium uptake by *J. curcas* significantly (ANOVA, $P < 0.05$) affects the growth of the plant which was reflected by the decrease in biomass (Table 3). *J. curcas* accumulated Cr in concentration dependent manner (Fig. 1). The amount of Cr accumulated by different plant tissues (roots > shoots) varied significantly (ANOVA, $P < 0.05$), Cr was not found in leaves of plant. Roots accumulated

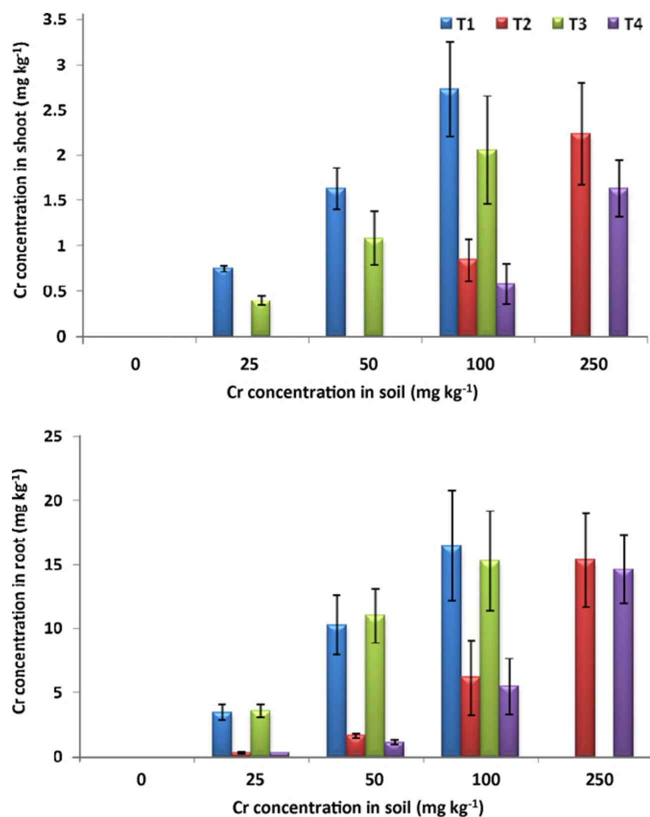

Fig. 1. Chromium accumulation by *J. curcas* grown on different concentrations of Cr contaminated soil amended with biosludge and biofertilizer after one year. Mean ± standard deviation; *n* = 4.

maximum amount (16.46 mg kg^{-1}) of Cr when plant exposed to 100 mg kg^{-1} Cr in treatment T1. The maximum Cr (2.74 mg kg^{-1}) content in shoot was also recorded at same concentration and treatment (T1). While, biosludge application reduced Cr uptake to 6.18 and 5.52 mg kg^{-1} in T2 and T4 treatments respectively. Present study also confirmed that organic amendment in heavy metal-contaminated soil reduced the bioavailability and uptake of metals in *J. curcas* [13,30]. Furthermore, Cr distribution in the shoots varied with its root concentration levels. Plants grown in lower concentrations of Cr spiked soils i.e. 25 and 50 mg kg^{-1} were observed without translocation of metal into their shoots by the application of biosludge (T2 and T4) treatments. It was also observed that in higher concentrations of Cr spiked soils, there was a great variation in metal accumulation capability of roots and shoots. The reason for the high accumulation in roots could be because Cr (VI) is immobilized in the vacuoles of the root cells to render it non-toxic, which may be a natural toxicity response of the plant. Since Cr (VI) must cross the endodermis via symplast, the Cr (VI) in cells is probably readily reduced to Cr (III) and retained in the root cortex cells [31]. Another important reason for the lack of transport of Cr from roots to shoots could be because the plants lack any specific mechanism of transport of Cr, as it is a toxic and nonessential element to plant growth. The results are in confirmation with the report of Khan [8]. Application of biosludge to metal-contaminated soil increased the plant nutrients because of its high organic matter which also acted as metal chelator thereby reducing the uptake and toxicity of Cr to the plants and enhanced in growth.

3.2. Antioxidant enzyme activities

Comparing the activities of the antioxidant enzymes such as CAT, APX and GST, it is evident that uptake of Cr induced a strong antioxidative response in *J. curcas* (Figs. 2 and 3). The activities of the antioxidant enzymes increased with increased Cr concentration and followed similar pattern as metal uptake by the plant (Fig. 1). This indicated that metal uptake was associated with

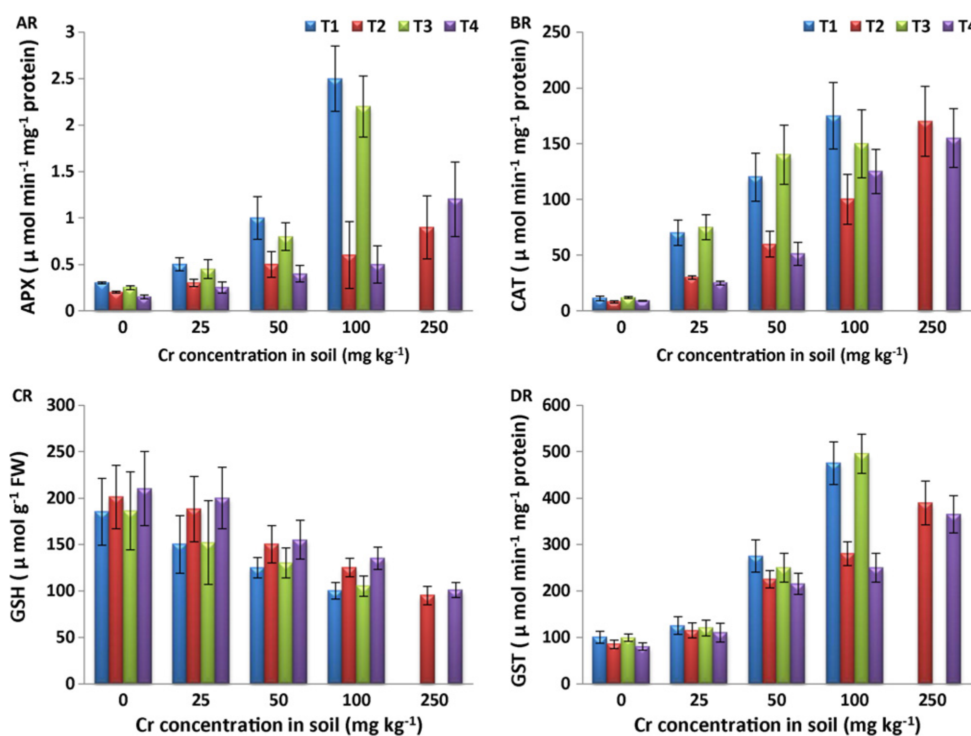

Fig. 2. Changes in the specific activities of (AR) APX (BR) CAT (CR) GSH and (DR) GST in the roots of *J. curcas* in chromium contaminated soil amended with biosludge and biofertilizer after one year. Mean ± standard deviation; *n* = 4.

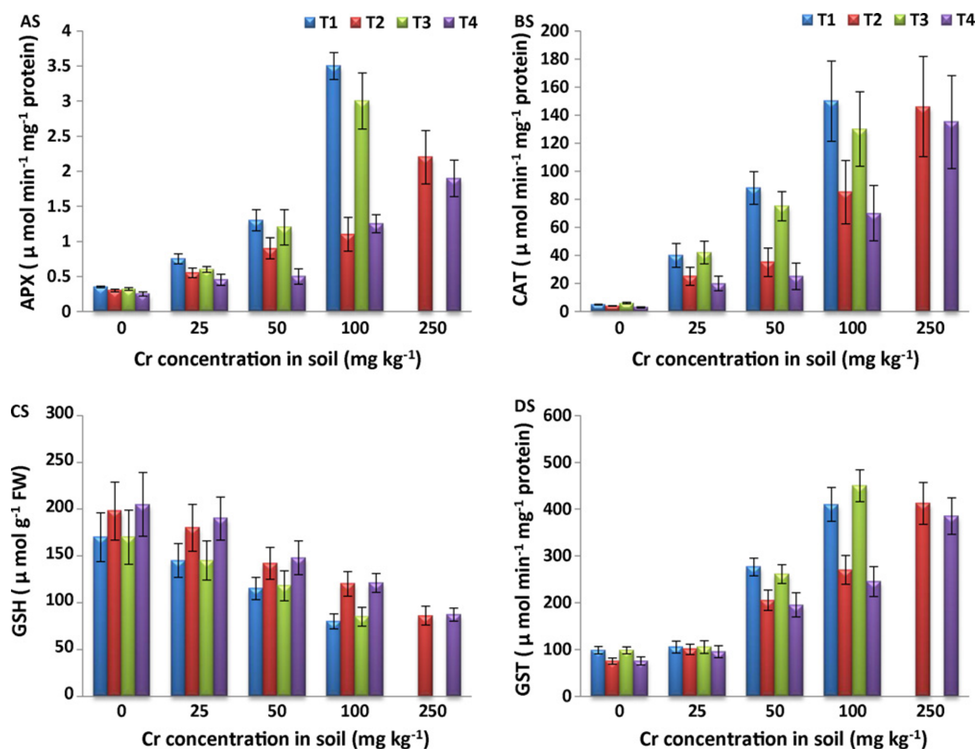

Fig. 3. Changes in the specific activities of (AS) APX (BS) CAT (CS) GSH and (DS) GST in the shoots of *J. curcas* in chromium contaminated soil amended with biosludge and biofertilizer after one year. Mean ± standard deviation; $n = 4$.

high activities of antioxidative enzymes. Excessive levels of H_2O_2 could be minimized through the activities of CAT and APX. In control plants (0 mg kg^{-1} of Cr), the CAT activity was in between 8 and 12 μ mol min^{-1} mg^{-1} protein. While with the increased concentrations of Cr in soil, the enzyme activity was also increased from 25 to 175 μmol min^{-1} mg^{-1} protein. Similar response was observed in APX activity. Plant grown in biosludge alone (T2) and biosludge along with biofertilizer (T4) amended soil accumulate lesser amount of Cr (Fig. 1) resulted in both the enzyme activities were significantly (ANOVA, $P < 0.05$) suppressed in treatments T2 and T4 (Figs. 2 and 3). Biosludge application to metal-contaminated soil reduced the bioavailability of metals resulted in low accumulation of Cr were recorded in plant grown on biosludge (T2) and biosludge along with biofertilizer (T4) amended treatments, which was also reflected in activity of antioxidant enzymes. Biofertilizer alone (T3) treatment did not affect the metal availability in soil, therefore metal uptake in plants were similar to control treatment (T1), resulted in similar activity of antioxidant enzymes were observed in T3 treatment as compared with control (T1) plants. Low accumulation of Cr in roots and shoots may be a natural process or phenomenon of plants to protect from oxidative stress. The increase in the slower rate of CAT and APX at lower metal concentrations (25 mg kg^{-1} of Cr) may be indicative of a lower stress. The higher APX activities were observed in shoots (Fig. 3) than roots (Fig. 2), whereas in case of the CAT activities, the opposite was true. This showed that both the enzymes were functioning concurrently to remove H_2O_2 in different parts of the plant [32]. Jain et al. [33] observed that the decline in the specific activity of CAT with increase level of Cr concentration. But in the case of *J. curcas*, the activity of CAT was increased with increasing concentration of Cr in soil, whereas in amended treatment the activity of CAT was reduced in the plant indicating that organic amendments to metal spiked soil reduces the toxicity of Cr to the plant. The glutathione S transferase (GST) is known to be responsive to biotic and abiotic stresses and it has not been characterized with respect to their antioxidative roles in plants. The activity of GST was similar as compared with CAT activity in plant (Figs. 2 and 3). Chromium treatment caused a significant increase (ANOVA, $P < 0.05$) in GST activity in both roots and shoots (Figs. 2 and 3), which was also significantly (ANOVA, $P < 0.05$) suppressed in plant grown in treatments T2 and T4. The GST activity was increased drastically on exposure of plant to high Cr contaminated soil (100–250 mg kg^{-1}) and recorded highest at 100 mg kg^{-1} of Cr concentration in control treatment (unamended soil, T1) and slightly less in treatment T3 (biofertilizer alone). While in treatments T2 and T4, no significant changes were observed at low level of Cr contaminated soil, but at high level of Cr contamination (250 mg kg^{-1}) there were elevated activity of GST was recorded. The control plant (T1) showed elevated level of GST due to metal toxicity, whereas plant grown on amended treatment (T2 and T4) showed less activity of GST proved that amendments of biosludge and biofertilizer reduced the Cr toxicity to the plant. The GSH acts as conjugate substrate for GST activity and simultaneously it also contribute to formation of metal chelator. The increased level of Cr toxicity, compelled plant cells to overproduce the activity of GST to maintain equilibrium in cell system. As reported GST activity is induced in pumpkin by oxidant like $K_2Cr_2O_7$ [34]. The H_2O_2 formed by the superoxidation of active oxygen species was quenched by catalase. However, APX took a little part in quenching of H_2O_2 due to its sensitivity to the Cr. Therefore, reduced APX activity was recorded in Cr treated *J. curcas*. Chromium stress induced the production of the GST, which involved in Cr detoxification in plant.

In addition to the effect of Cr on antioxidative enzymes, it may also affect non-enzyme antioxidants like GSH, which was also involved in a plant's oxidative defense. It also maintains the cellular redox status and serves as substrate for phytochelatin synthesis, showed a concentration and time-dependent decrease in its level with increased concentrations of metal [35]. Both roots and shoots of *J. curcas* were performed significant (ANOVA, $P < 0.05$) decreasing activity of GSH with increased concentrations of Cr in plant. Roots of *J. curcas* grown in all concentration i.e. 0, 25, 50 and 100 mg kg^{-1} of Cr in soil, showed decreased activity of GSH in treatment T1 (con-

trol) with increase Cr in plant. Whereas plant grown in biosludge amended treatment (T2) GSH activity in *J. curcas* was increased as 8.0%, 20.2%, 16.7% and 20.0% respectively and combination with biofertilizer (T4), it was raised up to 11.9%, 25.0%, 19.54% and 25.9% respectively as compared with T1 treatment, while single biofertilizer (T3) could not much improved GSH activity than T1 treatment (Fig. 2). Shoots of *J. curcas* also indicated similar sort of GSH activity (Fig. 3). A crucial role for GSH in combating oxidative stress in plant has been suggested previously [18]. Various levels of metal induced depletion of GSH have been reported in different plant species [36–38]. GSH is known for non-enzymatic defense system, which acts as a radical scavenger producing oxidized glutathione (GSSG) [39]. The depletion of GSH under metal stress condition contribute in the utilization of GSH by GSH peroxidases during enzymatic repair of lipid peroxides [40] and also in the synthesis of metal binding proteins named phytochelatins [37]. As GSH activity in amended treatments (T2 and T4) was slightly increased, helped in synthesis of phytochelatins and increase plants metal tolerance [41]. Noctor et al. [19] has reported that stimulation of reduced glutathione (GSH) biosynthesis was observed under stress conditions in poplar trees. The interconversion of reduced and oxidized forms of glutathione to maintain redox status of the cell as well as to scavenge free radicals could have caused a decrease in GSH. Based on how GSH responded to Cr concentrations in *J. curcas*, it revealed that GSH is involved in Cr detoxification through its activity in plant grown on higher concentrations of Cr spiked soils. The differential responses of antioxidative enzymes to different amended treatments may be attributed to stimulated varied level of ROS generation in plant. Metal ions may stimulate the generation of ROS, either by direct transfer of electrons in single-electron reactions involving metal cations, or as a consequence to metal-inactivated metabolic reactions [42].

The induction of APX, CAT and GSH provides additional defense against Cr toxicity in *J. curcas*. However, some detailed research is further needed to understand the defense mechanisms in plants under heavy metal stress condition. Chromium accumulation in shoots is relatively low due to transport barriers and, moreover, the oxidative damage imposed by Cr is avoided with an altogether increase in the activities of antioxidative enzymes. *J. curcas* was able to tolerate 100 mg kg^{-1} chromium with some physiological and biochemical changes. Hence the study suggests that the *J. curcas* has a high adaptability to cope-up with Cr stress. The roots accumulated more Cr than the shoots in all treatments. Comparatively lower accumulation of Cr in shoots than roots was probably due to reduction of Cr (VI) to Cr (III), which reduces its mobility from roots to shoots. It has been reported that Cr (III) readily forms complexes with –COOH groups which inhibits the translocation of metal from roots to shoots. Biosludge application to Cr spiked soil has improved the tolerance of *J. curcas* up to 250 mg kg^{-1} of Cr and its antioxidant activity were also similar to plant grown in 100 mg kg^{-1} of Cr in soil. Finally the findings of the present study revealed that the *J. curcas* can be cultivated up to 250 mg kg^{-1} of Cr contaminated soil with amendments of biosludge combined with the suitable biofertilizer and most of Cr, taken up by the plant is accumulated in the roots parts only.

4. Conclusion

The present study concluded that the *J. curcas* could grow in Cr contaminated soil and accumulate Cr in roots followed by shoot. Chromium accumulation by *J. curcas* affects various physiological processes. Chromium-induced oxidative stress was tolerated by this plant through the hyperactivity of antioxidant defense system. The application of biosludge alone (T2) and combination with biofertilizer (T4) had the expected effect, allowed an enhancement of plant growth and consequently reducing concentration of Cr in aerial parts. Despite of higher accumulation in roots, the level of free Cr ions in roots may remain low since most of the Cr ions are either immobilized or compartmentalized in vacuoles or form Cr–phytochelatin complexes. Induction of CAT, APX, GST and GSH provide defense against Cr toxicity and keeps the metabolic activities in plants functional. Further research is needed in order to elucidate the mechanisms involved in Cr detoxification in *J. curcas*. Moreover, studying other antioxidants like POD (non-specific peroxidase), SOD and GR activities in *J. curcas* due to heavy metal stress could be useful to understand the differences observed at the physiological level. Furthermore, seeds are required to be investigated for its chromium concentrations to confirm that the *Jatropha* oil could fulfill the diesel requirement without polluting the environment.

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