INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0178/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0829
http://www.fspublishers.org

Full Length Article

Integrated Effect of Compost and Cr^{6+} Reducing Bacteria on Antioxidant System and Plant Physiology of Alfalfa

Muhammad Usman Saleem[1*], Hafiz Naeem Asghar[1], Zahir Ahmad Zahir[1] and Muhammad Shahid[2]
[1]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad*
[2]*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad*
[*]For correspondence: usmansaleem2221@gmail.com

Abstract

Toxicity of chromium is highly dependent on its oxidation state. Mainly in natural ecosystem, it persists in two stable states Cr^{3+} and Cr^{6+}. Among these states, Cr^{6+} is 100-1000 times more toxic as compared to the reduced form Cr^{3+}. Microbes (bacteria) have the ability to reduce Cr^{6+} to Cr^{3+} and this process can be further boosted with organic matter application. Present study was designated in 2016-2017 at the Wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad to investigate the reduction potential of bacteria and their role in strengthening of plant anti-oxidant system and chlorophyll contents. Three pre-isolated and well characterized microbial strains Q5, U32 and U37 alone as well as in the presence of compost were used. Strain Q5 belongs to specie *Alcaligenes,* while U32 and U37 were *Pseudomonas* specie. It was a pot experiment and tester crop was alfalfa. Soil was Spiked with potassium dichromate salt and 24 mg kg^{-1} level was maintained (This was the average concentration at the fields irrigated with tannery effluent from previous study). Chlorophyll contents (a and b), antioxidant system (ascorbate peroxidase, glutathione reductase, superoxide dismutase and catalase) and physiological attributes (photosynthetic and transpiration rate, stomatal conductance and water use efficiency) were studied by following standard analytical methods and statistical procedure. Results of the study indicated that inoculation with microbial strain U32 in the presence of compost enhanced Cr^{6+} reduction upto 53% and grain yield 44% more over the control. Overall inoculation with U32 had improved the plant growth, physiology, anti-oxidant production, alfalfa grain yield and Cr^{6+} reduction was 53% more over the control.

Keywords: Cr^{6+} reduction; Oxidative stress; Reactive oxygen species; Photosynthetic system; Plant response

Introduction

Chromium is the 2nd most important pollutant in soil and water. It's principal source of contamination are municipal and industrial waste water application to the agricultural lands (Barrera-Diaz *et al*., 2012). Among industrial source, tanneries are contributing round about 70% in contamination of chromium. In Pakistan, Karachi, Kasur, Peshawar, Muzaffargarh and Sialkot are major cities where tanneries are installed. In Punjab Sialkot and Kasur are mainly affected. Round about 650 tanning units are working in Kasur as a result of that chromium concentration has been mounted upto 2.30 mg/L in water and 2990 mg/kg in soil. Tannery effluent also contain toxic heavy metals like Pb, Ni and As (Rashid *et al*., 2012). Ultimately from where these toxic metals enter into the food chain. Among the oxidation states, Cr^{3+} is required in low quantity 50-200 $\mu g\ g^{-1}\ day^{-1}$ for the human beings. It plays role in sugar and lipid metabolism. It acts as insulin co-factor as well as lowers the level of glucose in diabetes patient and is also the part of substances that bind the excessive chromium in the body and retards the functioning of phosphotirosine phosphatase enzyme that reduce the sensitivity of insulin in body (Anonymous, 2006).

Hexavalent chromium (Cr^{6+}) is toxic at all levels of concentration, that's why it is among the 17 chemicals that are severe threat to life. It is included in Group-1 human cancer causing substances by the International Agency for Research on Cancer (IARC) (Iyer and Mastorakis, 2010; Oliveira, 2012). It mainly attacks respiratory track and causes disorders like chronic rhinitis, asthma, eye and skin irritation and chronic bronchitis. It also damages the structure of DNA, protein and lipids, causes birth defects and impairment of reproductive capacity. Cr^{3+} as well as Cr^{6+} can be taken up by the plants and this uptake is heavily dependent on oxidation state, mobility, redox potential, pH and temperature (Sharma and Adholeya, 2011).

Presence of Cr^{6+} in soil also affects the normal plant growth by interfering with number of physiological and chemical processes within the plant. It inhibits the seed germination because it interrupts the activity of amylase enzyme that inhibits the starch break-down (Shanker *et al*., 2005). Once Cr^{6+} entered into the plant, it effects below (root length, biomass and diameter) and above ground parts

To cite this paper: Saleem, M.U., H.N. Asghar, Z.A. Zahir and M. Shahid, 201x. Integrated effect of compost and Cr^{6+} reducing bacteria on antioxidant system and plant physiology of alfalfa. *Int. J. Agric. Biol*. 00: 000–000

(chlorophyll, plasma membrane and cell organelles) equally (Lie *et al*., 2008). Cr^{6+} induced oxidative stress results in lipid peroxidation (damages structure of cell membranes) (Panda and Choudhury, 2005), degradation of photosynthetic pigments resulting in decreased photosynthesis by disturbing the thylakoid arrangement (Prasad *et al*., 2001) and retard stomatal conductance (Ali *et al*., 2013). Overall physiological processes (transpiration, photosynthesis and vapour pressure deficit), chlorophyll (a and b) contents and plant defence system in the form of antioxidants reduces their efficiency (Davies *et al*., 2002; Shanker *et al*., 2003).

Remediation of chromium contaminated soils can be done by many conventional methods including physical, chemical and biological (Pugazhenti *et al*., 2005; Fu *et al*., 2017). Among the biological approaches, bioremediation is the most effective, easy and environment friendly approach than other techniques. It uses living organisms to eradicate contaminants from the system. Phytoremediation and microbial bioremediation are two broad types of the bio-remediation (Wang *et al*., 2016). Further microbial remediation is divided into number of types, reduction is the most significant type from Cr^{6+} point of view (Basha and Murthy, 2007).

During the process of reduction, chromate reducing bacteria take electrons from either electron transport chain, organic matter or any other source and transform Cr^{6+} to Cr^{3+}. Chromate reduction is further divided into aerobic and anaerobic (Debadatta and Susmita, 2012; Liu *et al*., 2017). In aerobic reduction, bacteria need oxygen to reduce Cr^{6+} to Cr^{3+} and enzyme *Chromate reductase* ChrR to carry out this process. During this process, Cr^{6+} is converted into Cr^{3+} with 3 electron shuttle. Few reactive oxygen species (ROS) are produced that cause oxidative stress to plant resulting in lipid peroxidation (Brose and James, 2010). Another enzyme "*Chromate reductase* YieF" is also used for this process and is more efficient than ChrR by producing 20-22% less ROS. Some of the known bacteria with aerobic reduction are *Psedumonaus maltophilla*, *Ps. Putida NK1*, *Bacillus megaterium* and *Alcaligens eutrophus*. In anaerobic reduction, soluble and membrane associated enzymes are involved, that are linked with electron transport for electron shuttle. Cr^{6+} receives electron from organic matter, fat and proteins as well. Enzymatic reduction of chromate is mainly carried out by cytochrome (b and c) family. Earlier scientist were unable to find that why bacteria carried out the reduction? But now few strains have been identified that got energy from reduction process. Bacteria with anaerobic reduction are *Agrobacterium radiobactor*, *Pentoea agglumerans* and *Desulfovibrio desulfuricans* (Islam, 2016; Shahid *et al*., 2017). In phytoremediation plants are used either to transform or detoxify or to accumulate the pollutants. Sunflower, Indian mustard, alfalfa, spinach and some grasses (*Poa pratensis*, *Lolium perenne* and *Festuca rubra)* are commonly used for this purpose (Tangahu *et al*., 2011; Sathish *et al*., 2015).

The process of bioremediation can be further enhanced through exogenous application of organic matter/compost in polluted soil (Yadav, 2010). Organic matter helps in burgeoning population of microbes, provide carbon for assimilation, electrons for reduction and sites for immobilization of metals as well as produces substances that modifies the rhizosphere for remediation of contaminated sites (Brown and Chaney, 2000). It contains hydroquinone (group of dissolved organic carbon) that is topmost source of electron required for Cr^{6+} reduction (Gu and Chen, 2003; Saranraj and Sujitha, 2013). These amendments also chelate heavy metals and reduce their availability resulting in better plant growth (Monte *et al*., 2009; Malaviya and Singh, 2016).

As mentioned earlier, microbial remediation, phytoremediation and organic amendments have their own individual advantages and if all these techniques are combined, it would be of massive use. In previous literature available, few studies with combined use of phytoremediation and microbial remediation are reported but data regarding these two combined with organic amendments is scares. Organic matter provides nutrients as well as improve the overall soil health that ultimately result in better plant growth (Chiu *et al*., 2009; Trebien *et al*., 2011). It also provides carbon, nutrients, energy and electrons to bacteria for better functioning. On the other hand, bacteria reduce the Cr^{6+} (Bolan *et al*., 2003) and play important role in strengthening the plant defence mechanism (Hao *et al*., 2008; Yu *et al*., 2014).

The purpose of present study was to evaluate the Cr^{6+} reduction potential of bacteria under pot conditions and to estimate the integrated effect of compost and Cr^{6+} reducing bacteria on alfalfa grain yield, chlorophyll contents, antioxidant system and its physiology under the Cr^{6+} contamination.

Material and Methods

Experiment on Chromium Contaminated Soil

Pre-isolated and pre-characterized bacterial strains Q5, U32 and U37 (isolated from three main industrial cities Sialkot, Lahore and Kasur) were used for the pot trial. Inoculum was prepared using Loria Britni media broth and standard condition for bacterial growth in shaking incubator. Required bacterial population was attained by monitoring the optical density. Trial was conducted in the wire house of "The Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad". Properly dried and grounded soil was used for trial. Cr^{6+} contents were also determined along with other physic-chemical parameters of soil (Table 1). $K_2Cr_2O_7$ salt was used to contaminate the soil and 24 mg kg^{-1} of Cr^{6+} was upheld according to the Cr^{6+} content in the soil samples from which bacteria were isolated (from previous study). Pots were filled with 10 Kg soil and compost was added in each pot @ the rate of 1.5%.

Table 1: Physio-chemical properties of the soil used for the pot trial

Characteristics	Unit	Concentration
pHs		7.92
Electrical conductivity	dS m^{-1}	1.32
Soil texture		Sandy clay loam
Organic C	%	0.245
Cation exchange capacity	$cmol_c\ kg^{-1}$	14.25
Cr^{6+} concentration	mg kg^{-1}	ND
Total chromium	mg kg^{-1}	0.2
Total Nitrogen	%	0.19
Available phosphorus	mg kg^{-1}	7.3
Available potassium	mg kg^{-1}	109.5

Table 2: Physio-chemical properties of compost used for pot trial

Characteristics	Unit	Concentration
Water holding capacity	%	58.4
Electrical conductivity	dS m^{-1}	2.7
pH		7.5
Total nitrogen	%	1.23
Total phosphorus	%	0.21
Total potassium	%	1.16
Total carbon	%	23.6
C:N ratio		19
Cr^{6+} concentration	mg kg^{-1}	ND
Total chromium	mg kg^{-1}	6

The characteristics of compost used for trial are illustrated in (Table 2). *Medicago sativa* (alfalfa) seeds were inoculated with broth and three seeds per pot were sown. Fertilizer was applied according to the recommendation. Completely randomized design with three replications was used and data was collected according to the standard methods.

Estimation of Physio-Chemical Properties of Soil Used for Pot Trial

Soil organic matter was measured by method used by Moodie *et al*. (1959) and available phosphorus through spectrophotometric method (Watanabe and Olsen, 1965). 1 N ammonium acetate was used for soil extraction for potassium determination by Flame photometer (Jenway PFP-7) and potassium concentration was calculated by using standard curve (U.S. Salinity Lab. staff, 1954).

Determination of Cr^{6+} in Soil Samples

Cr^{6+} was measured by the method used by Gheju and his co-workers, 2009. 40 mL aqua regia ($HCl:HNO_3$ = 3:1) was added in 50 mL flask having 2 g air dried soil in it. This mixture was kept for 16 h and then digested at 85°C for two hours and was allowed to cool. Then filtered and 50 mL volume was made with HNO_3. Cr^{6+} concentration was measured on spectrophotometer by using 1,5-diphenylcarbazide method. Determination was based on purple complex formation by Cr^{6+} in presence of 1,5-diphenylcabazide. After 15 min of adding colour developing reagent, the absorbance was measured at 540 nm wavelength on Evolution 300 LC spectrophotometer.

Estimation of Plant Physiological Attributes

Physiological parameters like stomatal conductance, water use efficiency, transpiration rate and photosynthetic rate were determined by using CIRUS-3 and chlorophyll contents were measured according to the method used by Arnon (1949) and SPAD value was measured through chlorophyll meter.

Estimation of Antioxidants

Ascorbate peroxidase activity was measured by method used by Nakano and Asada (1981). Superoxide dismutase (SOD) activity was assayed using a modified NBT method, catalase activity through method described by Aebi (1974), proline contents were determined according to the method described by Bates *et al*. (1973) and malondialdehyde (MDA) concentration was calculated from the difference (A532–A600) in absorbance using Beer and Lambert's equation and expressed in terms of μM MDA mg^{-1} protein (Jambunathan, 2010).

Cr^{6+} Determination in Plants

Vegetative as well as reproductive parts of the plants were digested by the method used by Humphries (1956) followed by the Cr^{6+} determination by the method used by Gheju and his co-workers, 2009.

Bacterial Identification

For proper identification at strain level, the intergenic region between 16S and 32S rRNA gene was identified through 16S rRNA technique. In this technique, the extracted region of the gene from DNA is amplified and compared with the ladder from the Gene Bank to obtain match.

Results

Results of the study revealed that inoculation in the presence of compost enhanced the Cr^{6+} reduction in soil, production of anti-oxidants, physiological attributes and grain yield of alfalfa. Chlorophyll 'a' and 'b' contents of plants inoculated with U32 in the presence of compost were 56% and 75% more over the contamination control under Cr^{6+} stress conditions. Alfalfa plants grown without inoculation had 55% less carotenoids. While inoculation with U32 increased 42% carotenoids and 12% overall chlorophyll contents as compared to the contamination control. Maximum recorded SPAD value was by the plants inoculated with strain U37 in the presence of compost (Table 4).

Chromate stress also affected negatively the physiological processes. However, inoculation with selected isolates had given some relief to the plants by reducing the chromate uptake by the plants. The process of photosynthesis (μmol CO_2 m^{-2} s^{-1}) was enhanced upto 58% and transpiration upto 1.1 folds by inoculating with U32 in combination with compost application as compared to the stress control plants (Table 5). Water use efficiency (mmol CO_2 mol^{-1} H_2O) was enhanced by 1.2 folds by inoculating plants with bacterial isolate Q5 in the presence of compost. Under stressed conditions, U37 performed better and improved the stomatal conductance by 2.7 folds as compared to respective stress control plants and Q5 performed better under non-stressed conditions and enhanced the process upto 53% compared to the control (Table 5).

Performance of plant defence system (antioxidants) is of much more importance because their enhanced concentration alleviates the stress from plant and provide normal conditions for plant growth and development. Under normal conditions, the production of antioxidants was non-significant with control plants, while under contamination was significant. The ascorbate peroxidase production was 1.42 folds higher than the stress control plants when inoculated with strain U32 along-with compost application (Fig. 1). Bacterial strain Q5 enhanced the superoxide dismutase (μmol g^{-1} fw) and catalase production upto 1.76 (Fig. 2) and 2.02 folds (Fig. 5) as compared to the contamination control. Similar strain had enhanced the concentration of stress indicator (proline) upto 1.17 (Fig. 3) folds over the contamination control plants. The production of MDA increases with increasing concentration of Cr^{6+} and contrary to this, inoculation had neutralized the metal effect by reducing Cr^{6+} into Cr^{3+}. Maximum MDA was produced in plants of control under stress (78%) and minimum in plants inoculated with U32 followed by U37 (Fig. 4).

One of the prime objective of using Cr^{6+} reducing bacteria was chromate reduction. Maximum Cr^{6+} reduction (56%) was carried out in soil where compost was applied along with Q5 inoculation. While U32 has reduced the Cr^{6+} upto 53% as compared to the contamination control and was at par with the isolate U37 (Fig. 6). When we talk about chromate uptake, maximum Cr^{6+} was found in plants of contamination control and minimum in plants inoculated with U32 (68%). In arial parts of the contamination control, maximum Cr^{6+} was translocated and minimum in U32 inoculated plants (4.4 folds) less compared to control in shoots and no Cr^{6+} was detected in grain. Farmer required 'grain yield' was reduced upto 28% from the plants of contamination control. Maximum grain yield (44% more over the stress control plants) was obtained when inoculated with U32 in the presence of compost compared to contamination control (Table 6). Pre-isolated and pre-characterized bacterial strains were further re-verified for the particular bacteria through 16S rRNA technique. Isolate U32 and U37 were from *Pseudomonas* specie and Q5 belongs to specie *Alcaligenes* (Table 3).

Table 3: Bacterial identification through 16S rRNA technique

Sr. No	Code	Identification	Similarity index (%)
1	Q5	*Alcaligenes faecalis*	100
2	U32	*Pseudomonas gessardii*	99
3	U37	*Pseudomonas fluorescens*	99

Table 4: Effect of inoculation and compost application on chlorophyll contents of alfalfa

Treatments	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	SPAD Value
	mg g^{-1}	mg g^{-1}	mg g^{-1}	
Control	1.23e-h	0.51c-f	0.51c-f	17.72fg
Cr	0.84k	0.33h	0.23h	12.40j
Q5	1.33de	0.60bc	0.61a-d	24.11bc
U32	1.28ef	0.58b-d	0.67ab	21.50bc
U37	1.45cd	0.54cd	0.62a-c	24.87c-e
Q5Cr	0.95jk	0.38gh	0.54fg	15.75g-j
U32Cr	1.01ij	0.41f-h	0.47fg	13.40f-h
U37Cr	1.1g-j	0.43e-h	0.39gh	17.21ij
Comp	1.32de	0.53c-e	0.58a-e	22.08cd
Comp+Cr	1.07h-j	0.39gh	0.32h	13.57h-j
Comp+Q5	1.56bc	0.72a	0.66b	29.70a
Comp+U32	1.63ab	0.75a	0.72a	26.61a
Comp+U37	1.75b	0.66ab	0.67ab	28.82ab
Comp+Cr+Q5	1.13f-j	0.48d-g	0.45c-f	18.16e-g
Comp+Cr+U32	1.25e-g	0.58b-d	0.48b-f	16.46d-f
Comp+Cr+U37	1.19e-h	0.55c-f	0.41e-g	19.86f-i

Values sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

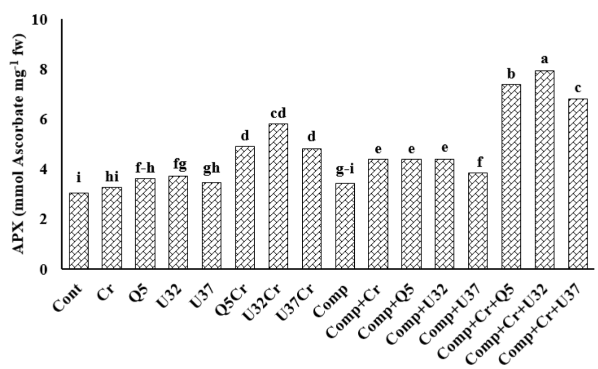

Fig. 1: Effect of combined application of organic amendment and Cr^{6+} reducing bacteria on APX concentration of leaves of alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

Discussion

Ferocious effects of heavy metals are the consequence of burgeoning population of the world and industrialization. Heavy metals have affected the human, plant and animal health equally. Broadly heavy metals are divided into two categories beneficial (Zn, Fe and Cu) and toxic (Cr, Pb and As). Heavy metals are under the category of in-organic pollutants that can only be transformed from one form to another (Mulligan *et al*., 2001; Merdy *et al*., 2009).

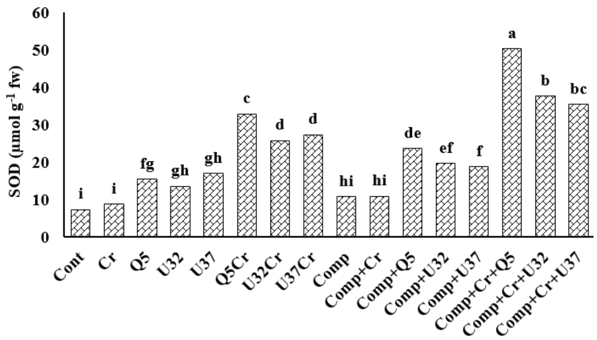

Fig. 2: Effect of combined application of organic amendment and Cr^{6+} reducing bacteria on SOD content of leaves of alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

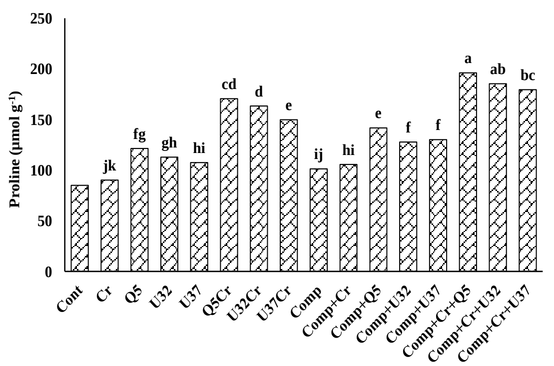

Fig. 3: Effect of combined application of organic amendment and Cr^{6+} reducing bacteria on proline production in alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

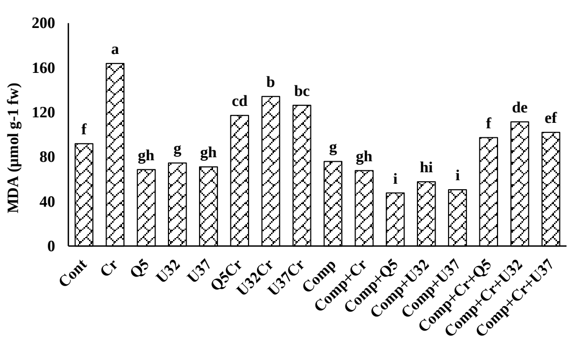

Fig. 4: Effect of combined application of organic amendment and Cr^{6+} reducing bacteria on MDA content in alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

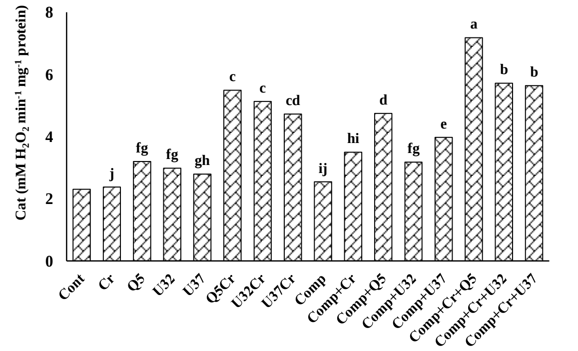

Fig. 5: Effect of combined application of organic amendment and Cr^{6+} reducing bacteria on catalase content in alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

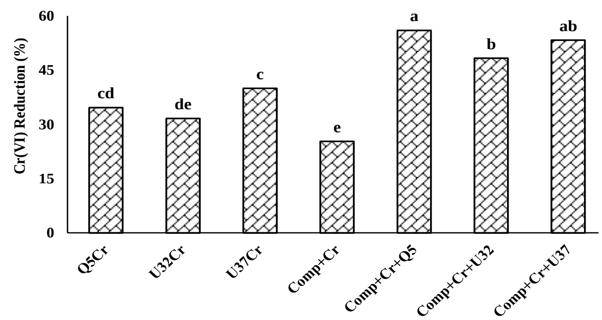

Fig. 6: Effect of Cr (VI) reducing bacteria and organic amendment on Cr^{6+} transformation under alfalfa where bars sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

Certain microbes have the ability to transform these metals through processes like oxidation reduction, methylation/de-methylation and bioaccumulation. In the present study, focus was on the reduction of Cr^{6+} to Cr^{3+}, plant physiology and anti-oxidant system. Bacterial reduction process requires an electron source, organic carbon and nutrients. For this purpose organic matter in the form of compost played significant role in providing all these things together.

Results of the study showed that the presence of Cr^{6+} has reduced the efficiency of the plant physiological processes but bacterial inoculation alone as well as along-with compost application provided relief significantly. Cr^{6+} had reduced the photosynthesis process by disturbing the ultra-structure of chloroplast (one of the primary target of the Cr^{6+} in plants) by changing the arrangement of thylakoids (site of photosynthetic reaction). The transpiration process and stomatal conductance were also reduced due to the interference of chromate with the opening and closing of stomata. This all might be due to the ability of the bacteria to alleviate the chromate stress by reducing it into the chromite (Cr^{3+}). As Cr^{3+} is less mobile so its uptake was reduced 10-100 times compared to the Cr^{6+} (Parameswari *et al*., 2009). On contaminated sites, Cr^{6+} reducing bacteria are under the dire need of electron donors that were provided by the exogenous application of compost (organic amendment) (Ahmed *et al*., 2016).

Table 5: Effect of inoculation and compost application on physiological parameters of alfalfa

Treatments	Photosynthetic rate	Transpiration rate	Stomatal Conductance	Water Use Efficiency
	μmol $CO_2 m^{-2} s^{-1}$	mmol H_2O $m^{-2} s^{-1}$	mmol H_2O $m^{-2} s^{-1}$	mmol CO_2 mol^{-1} H_2O
Control	14.26ef	2.72fg	373f	10.53d-f
Cr	8.62i	0.71i	96i	4.85i
Q5	18.96bc	4.14bc	511c	14.79c
U32	17.24cd	3.28de	474d	13.28c-e
U37	15.78de	3.76cd	495cd	13.39cd
Q5Cr	12.31f-h	2.01fg	253i	8.92f-h
U32Cr	11.27g-i	2.10fg	207j	7.17hi
U37Cr	10.38hi	2.38fg	222j	7.54g-i
Comp	15.12de	2.92e-g	412e	11.63d-f
Comp+Cr	11.10g-i	2.97ef	159k	5.72i
Comp+Q5	22.63a	4.74a	573a	20.93a
Comp+U32	20.69ab	4.47ab	540b	17.72b
Comp+U37	20.12ab	4.28ab	560ab	18.95ab
Comp+Cr+Q5	10.38hi	2.52gh	338gh	10.45fg
Comp+Cr+U32	13.60e-g	3.66f	311h	8.79gh
Comp+Cr+U37	11.97f-h	2.43g	359fg	9.22f-h

Values sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

Table 6: Effect of inoculation and compost application on Cr^{6+} distribution in plant and grain yield of alfalfa

Treatments	Cr(VI) in root	Cr(VI) in shoot	Cr(VI) in grain	Grain yield
	mg g^{-1}	mg g^{-1}	μg g^{-1}	g $plant^{-1}$
Control	0	0	0	4.33c-f
Cr	16.564a	7.1307a	0.052a	3.35f
Q5	0	0	0	5.08a-d
U32	0	0	0	5.22a-d
U37	0	0	0	4.08d-f
Q5Cr	8.7433de	3.5344d	0	4.08d-f
U32Cr	9.2685c-e	4.6321c	0.030c	4.56c-e
U37Cr	10.64b-d	3.9351cd	0.033c	4.33c-f
Comp	0	0	0	4.58c-e
Comp+Cr	11.417bc	6.9288ab	0.045b	3.83ef
Comp+Q5	0	0	0	5.58ab
Comp+U32	0	0	0	6.08a
Comp+U37	0	0	0	5.33a-c
Comp+Cr+Q5	6.9647ef	1.8912e	0.02d	4.58b-e
Comp+Cr+U32	5.28f	1.31e	0	4.82b-e
Comp+Cr+U37	7.6292ef	2.1072e	0.0123e	4.71b-e

Values sharing the same letters are statistically non-significant (Tukey's test, $p < 0.05$)

Organic amendments along with electron provision also enhanced the bacteria population by providing the energy and carbon, immobilizes metals, and rhizospheric modifications resulted in enhanced remediation process (Brown and Chaney, 2000). Root, shoot and reproductive parts Cr^{6+} analysis showed that roots contain higher concentration as compared to the arial parts. It might be due to decreased translocation of Cr^{6+} to the plant arial parts due to less accumulation (Ali *et al*., 2013; Gutierrez-Corona *et al*., 2016). This type of metal apportioning within the various plant was reported by Licina *et al*., 2007; Yen *et al*., 2017.

As a result of Cr^{6+} uptake, excessive reactive oxygen species (free radicals including OH^-, H_2O_2, O_2^-, O_3 and peroxides) were produced (Sharma *et al*., 2012) that disturbed the balance between their production and detoxification and caused oxidative damage to the plants. It resulted in scavenging of biomolecules (nucleic acids, lipids amino acids and proteins), irreparable metabolic changes that leads to the cell death (Dhir *et al*., 2009). Presence of ROS severely impaired the selectivity of permeable membrane by interfering with structure of lipids in it. To overcome the pernicious effects of Cr^{6+}, plants have efficient defence system in the form of antioxidants (Alaraidh *et al*., 2018).

Enzymatic antioxidants (catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase) (Gamalero *et al*., 2009) played pivotal role in scavenging of ROS (Rajkumar *et al*., 2012). Inoculation with Cr^{6+} reducing bacteria in the presence of the organic amendment may had enhanced the production of antioxidants significantly by alleviating the Cr^{6+} from the plants. It might also be due to the bacterial ability to produce higher concentrations of enzymatic antioxidants when exposed to the higher concentration of the heavy metals (Islam *et al*., 2014). Particularly catalase, is a heme containing enzyme that catalyses the dis-mutation of hydrogen peroxide into water and oxygen (Etesami, 2018). It is indispensable for the scavenging of ROS under chromate stress (one molecule of CAT can scavenge 6 million molecules of H_2O_2 per minute) (Azpilicueta *et al.*, 2007). So the bacteria living under chromate stress in association with plant may produce antioxidants for plant to overcome the situation. On the other hand, compost provide electrons required for the reduction process as well as carbon, nitrogen and energy to the bacteria for the assimilation (Hossain and Komatsu, 2013). Under stress, the composition of phospholipids of cell membrane is also changed due to increase the phospottidylcholine production and lower the phosphotidylamine concentration. Bacteria can also alter the situation by lowering the concentration of phospotidylcholine and increasing the phosphotidylamine (Pereyra *et al*., 2006). Stress indicator (proline) concentration was increased to alert the plant to activate its defence system timely. So increased concentration of Cr^{6+} results in higher concentration of proline too (Islam *et al*., 2014). Zhou *et al.* (2009) found similar kind of results that metal stress increased the production of proline in *Medicago sativa* L. Cr^{6+} reduction was the ultimate goal of the study to elevate the stress from the plants. Bacteria carried out the process of reduction to elevate the stress from themselves that ultimately benefitted the plants as well (Rajkumar *et al*., 2012). Recent studies have shown that bacteria also gain the energy from the process of reduction. Another possible reason can be that the bacterial genetic makeup has such abilities that it can alter itself according to the conditions (Maleki *et al*., 2017).

Conclusion

Isolate U32 *Pseudomonas fluorescens* can reduce Cr^{6+} under pot conditions. Combined use of strain U32 and compost enhanced chlorophyll contents, physiological attributes, antioxidant enzymes and grain yield under Cr contaminated soil. However, multi-sites field evaluation is required to confer the approach.

References

Aebi, H., 1974. Catalase. *In: Methods of Enzymatic Analysis*, pp: 673–684. Bergmeyer, H. (Ed.). Academic Press, New York, USA

Ahmed, E., H.M. Abdulla, A.H. Mohamed and A.D. El-Bassuony, 2016. Remediation and recycling of chromium from tannery wastewater using combined chemical–biological treatment system. *Proc. Saf. Environ. Prot.*, 104: 1–10

Alaraidh, I.A., A.A. Alsahli and E.S. Abdel-Razik, 2018. Alteration of antioxidant gene expression in response to heavy metal stress in *Trigonella foenum-graecum* L. *S. Afr. J. Bot.*, 115: 90–93

Ali, H., E. Khan and M.A. Sajad, 2013. Phytoremediation of heavy metals-concepts and applications. *Chemosphere*, 91: 869–881

Anonymous, 2006. Standards of medical care in diabetes. *Diab. Care*, 29: 4

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1

Azpilicueta, C.E., M.P. Benavides, M.L. Tomaro and S.M. Gallego, 2007. Mechanism of CATA3 induction by cadmium in sunflower leaves. *Plant Physiol. Biochem.*, 45: 589–595

Barrera-Diaz, C.E., L.L. Violeta and B. Bryan, 2012. A review of chemical, electrochemical and biological methods for aqueous Cr (VI) reduction. *J. Hazard. Mater.*, 223/224: 1–12

Basha, S. and Z.V.P. Murthy, 2007. Kinetic and equilibrium models for biosorption of Cr (VI) on chemically modified seaweed, *Cystoseira indica*. *Process Biochem.*, 42: 1521–1529

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205–207

Bolan, N.S., D.C. Adriano and R. Natesan, 2003. Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. *J. Environ. Qual.*, 32: 120–128

Brose, D.A. and B.R. James, 2010. Oxidation-reduction transformations of chromium in aerobic soils and role of electron-shuttling quinones. *Environ. Sci. Technol.*, 44: 9438–9444

Brown, S.L. and R.L. Chaney, 2000. Combining by-products to achieve specific soil amendment objectives. *In: Land Application of Agricultural, Industrial, and Municipal By-Products*, pp: 343–360. Power, J.F. and W.A. Dick (eds.). Soil Science Society of America, USA

Chiu, C.C., C.J. Cheng, T.H. Lin, K.W. Juang and D.Y. Lee, 2009. The effectiveness of four organic amendments for decreasing resin-extractable Cr(VI) in Cr(VI)-contaminated soils. *J. Hazard. Mater.*, 161: 1239–1244

Davies, F.T., J.D. Puryear, R.J. Newton, J.N. Egilla and J.A.S. Grossi, 2002. Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth, and gas exchange. *J. Plant. Nutr.*, 25: 2389–2401

Debadatta, D. and M. Susmita, 2012. Simultaneous reduction of phenol and chromium from textile industry effluent using mixed culture of microorganisms. *J. Environ. Res. Dev.*, 7: 344–349

Dhir, B., P. Sharmila, P.P. Saradhi and S.A. Nasim, 2009. Physiological and antioxidant responses of *Salvinia natans* exposed to chromium-rich wastewater. *Ecotoxicol. Environ. Saf.*, 72: 1790–1797

Etesami, H., 2018. Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: mechanisms and future prospects. *Ecotoxicol. Environ. Saf*., 47: 175–191

Fu, R., D. Wen, X. Xia, W. Zhang and Y. Gu, 2017. Electrokinetic remediation of chromium (Cr)-contaminated soil with citric acid (CA) and polyaspartic acid (PASP) as electrolytes. *Chem. Eng. J.*, 316: 601–608

Gamalero, E., G. Lingua, G. Berta and B.R. Glick, 2009. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. *Can. J. Microbiol.*, 55: 501–514

Gheju, M., I. Balcu and M. Ciopec, 2009. Analysis of hexavalent chromium uptake by plants in polluted soils. *Ovidius Uni. Ann. Chem.*, 20: 127–131

Gu, B. and J. Chen, 2003. Enhanced microbial reduction of Cr(VI) and U(VI) by different natural organic matter fractions. *Geochim. Cosmochim Ac.*, 67: 3575–3582

Gutierrez-Corona, J.F., P. Romo-Rodríguez, F. Santos-Escobar, A.E. Espino-Saldana and H. Hernandez-Escoto, 2016. Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology. *World J. Microbiol. Biotechnol.*, 32: 191

Hao, X.H., S.L. Liu, J.S. Wu, R.G. Hu, C.L. Tong and Y.Y. Su, 2008. Effect of long-term application of inorganic fertilizer and organic amendments on soil organic matter and microbial biomass in three subtropical paddy soils. *Nutr. Cycl. Agroecosyst.*,81: 17–24

Hossain, Z. and S. Komatsu, 2013. Contribution of proteomic studies towards understanding plant heavy metal stress response. *Front. Plant Sci.*, 3: 310

Humphries, E.C., 1956. *In: Modern Methods of Plant Analysis*, p: 481. Paech, K. and M.V. Tracy (eds.). Springer-Verlag, Germany

Islam, F., T. Yasmeen, Q. Ali, S. Ali, M.S. Arif, S. Hussain and H. Rizvi, 2014. Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicol. Environ. Saf.*, 104: 285–293

Islam, H.S., 2016. Bioremediation of chromium. *Res. J. Chem. Environ.*, 20: 1

Iyer, V.G. and N.E. Mastorakis, 2010. Unsafe chromium and its Environmental Health Effects of Orissa Chromate Mines. *In: Recent Advances Energy and Environmental Technologies and Equipment.* pp: 111–122. Proc. Int. Conf. Universitatea Politechnica, Bucharest, Romania

Jambunathan, N., 2010. Determination and detection of reactive oxygen species (ROS), lipid peroxidation and electrolyte leakage in plants. *In: Plant Stress Tolerance*, pp: 291–297. Humana Press, USA

Licina, V., S. Antic-Mladenovic and M. Kresovic, 2007. The accumulation of heavy metals in plants (*Lactuca sativa* L., *Fragaria vesca* L.) after the amelioration of coalmine tailing soils with different organo-mineral amendments. *Arch. Agron. Soil Sci.*, 53: 39–48

Lie, D., J. Zou, M. Wang and W. Jiang, 2008. Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defense system and photosynthesis in *Amaranthus viridis* L. *Bioresour. Technol.*, 99: 262

Liu, Y., R. Jin, G. Liu, T. Tian and J. Zhou, 2017. Effects of hexavalent chromium on performance, extracellular polymeric substances and microbial community structure of anaerobic activated sludge in a sequencing batch reactor. *J. Chem. Technol. Biotechnol.,* 92: 2719–2730

Malaviya, P. and A. Singh, 2016. Bioremediation of chromium solutions and chromium containing wastewaters. *Crit. Rev. Microbiol.*, 42: 607–633

Maleki, M., M. Ghorbanpour and K. Kariman, 2017. Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. *Plant Gene*, 11: 247–254

Merdy, P., L.T. Gharbi and Y. Lucas, 2009. Pb, Cu and Cr interactions with soil: sorption experiments and modelling. *Colloids Surf. A.,* 347: 192–199

Monte, M.C., E. Fuente, A. Blanco and C. Negro, 2009. Waste management from pulp and paper production in the European Union. *Waste Manage.*, 29: 293–308

Moodie, C.D., H.W. Smith and R.A. McCreery, 1959. Organic matter determination. *In: Laboratory Manual for Soil Fertility*, pp: 31–39. Department of Agronomy, State College of Washigton, Pullman, USA

Mulligan, C.N., R.N. Yong and B.F. Gibbs, 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Eng. Geol.,* 60: 193–207

Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22: 867–880

Oliveira, H., 2012. Chromium as an environmental pollutant: insights on induced plant toxicity. *J. Bot.*, 2012: 1–8

Panda, S.K. and S. Choudhury, 2005. Chromium stress in plants. *Braz. J. Plant Physiol.*, 17: 95–102

Parameswari, E., A. Lakshmanan and T. Thilagavathi, 2009. Effect of pre-treatment of blue green algal biomass on bio-adsorption of chromium and nickel. *J. Algal Biomass Utili.*, 1: 9–17

Pereyra, M.A., C.A. Zalazar and C.A. Barassi, 2006. Root phospholipids in Azospirillum-inoculated wheat seedlings exposed to water stress. *Plant Physiol. Biochem*., 44: 873–879

Prasad, M.N.V., M. Greger and T. Landberg, 2001. *Acacia nilotica* L. bark removes toxic elements from solution: corroboration from toxicity bioassay using *Salix viminalis* L. in hydroponic system. *Int. J. Phytorem.,* 3: 289–300

Pugazhenti, G., S. Sachan, N. Kishore and A. Kumar, 2005. Separation of chromium (VI) using modified ultra-filtration charged carbon membrane and its mathematical modelling. *J. Member. Sci.,* 254: 229–239

Rajkumar, M., S. Sandhya, M.N.V. Prasad and H. Freitas, 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnol. Adv.,* 30: 1562–1574

Rashid, H., J. Takemura and A.M. Farooqi, 2012. Investigation of subsurface contamination due to chromium from tannery effluent in Kasur District of Pakistan. *J. Environ. Sci. Eng.,* 1: 1007–1024

Saranraj, P. and D. Sujitha, 2013. Microbial bioremediation of chromium in tannery effluent: a review. *Int. J. Microbiol. Res.*, 4: 305–320

Sathish, T., N.V. Vinithkumar, G. Dharani and R. Kirubagaran, 2015. Efficacy of mangrove leaf powder for bioremediation of chromium (VI) from aqueous solutions: kinetic and thermodynamic evaluation. *Appl. Water Sci.*, 5: 153–160

Shahid, M., S. Shamshad, M. Rafiq, S. Khalid, I. Bibi, N.K. Niazi, C. Dumat and M.I. Rashid, 2017. Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. *Chemosphere,* 178: 513–533

Shanker, A.K., R. Sudhagar and G. Pathmanabhan, 2003. Growth, Phytochelatin SH and antioxidative response of sunflower as affected by chromium speciation. *In*: *2nd Int. Congr. Plant Physiol. Sus. Plant Prod. Chang. Environ*. New Delhi, India

Shanker, A.K., C. Cervantes, H. Loza-Tavera and S. Avudainayagam, 2005. Chromium toxicity in plants. *Environ. Int.*, 31: 739–753

Sharma, P., H.S. Gujral and B. Singh, 2012. Antioxidant activity of barley as affected by extrusion cooking. *Food Chem.,* 131: 1406–1413

Sharma, S. and A. Adholeya, 2011. Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi. *Int. Biodeter. Biodegrad.*, 65: 309–317

Tangahu, B.V., S.R.S. Abdullah, H. Basri, M. Idris, N. Anuar and M. Mukhlisin, 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng.*, 201: 1–31

Trebien, D.O.P., L. Bortolon, M.J. Tedesco, C.A. Bissani and F.A.O. Camargo, 2011. Environmental factors affecting chromium-manganese oxidation-reduction reactions in soil. *Pedosphere*, 21: 84–89

U.S. Salinity Lab. Staff, 1954. Diagnosis and improvement of saline and alkali soils*. In: USDA Handbook No. 60,* US Govt. Printing Office, Washington, D.C., USA

Wang, C., H. Deng and F. Zhao, 2016. The remediation of chromium (VI)-contaminated soils using microbial fuel cells. *Soil Sediment Contam. Int. J.*, 25: 1–12

Watanabe, F.S. and S.R. Olsen, 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from the soil. *Soil Sci. Soc. Am. J*., 29: 677–678

Yadav, S.K., 2010. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S. Afr. J. Bot.*, 76: 167–179

Yen, H.W., P.W. Chen, C.Y. Hsu and L. Lee, 2017. The use of autotrophic *Chlorella vulgaris* in chromium (VI) reduction under different reduction conditions. *J. Taiw. Ins. Chem. Eng*., 74: 1–6

Yu, B., H. Zhang, W. Xu, G. Li and Z. Wu, 2014. Remediation of chromium-slag leakage with electricity cogeneration via a urea-Cr (VI) cell. *Sci. Rep.*, 4: 5860

Zhou, Z.S., K. Guo, A.A. Elbaz and Z.M. Yang, 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of *Medicago sativa*. *Environ. Exp. Bot.,* 65: 27–34

(Received 02 February 2018; Accepted 24 July 2018)