International Journal of Nanotechnology and Application (IJNA)
ISSN 2277-4777
Vol. 3, Issue 3, Aug 2013, 1-10

PLANT GROWTH PROMOTING POTENTIAL OF NANO-BIOREMEDIATION UNDER CR (VI) STRESS

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ABSTRACT

The present investigation reports on the tolerance efficiency of *Brassica Juncea (Indian mustard)* against hexavalent Chromium (Cr (VI)) in the presence of zero valent nanoiron (ZVNI). The effect of farmyard manure (FYM), a natural organic matter used as an amendment on Cr (VI) detoxification was also studied. The seeds of *Brassica Juncea* are planted in the pot containing Cr (VI) contaminated soil with various concentrations of ZVNI and FYM. There was a positive linear relationship between amount of FYM added and Cr (VI) reduction in soil. The concentration of Cr (VI) in soil was monitored using UV-Vis Spectroscopy for every 5 days during the experimental period of 30 days. The results indicated that the combined impact of FYM and ZVNI demonstrated the highest removal efficiency compared to ZVNI alone in the reduction of Cr (VI). The tolerance efficiency of *Brassica Juncea* was found to be 100% and 95% in transplantation and germination methods respectively.

KEYWORDS: Tolerance Efficiency, Chromium, *Brassica Juncea*, Farm Yard Manure, UV-Vis Spectroscopy

INTRODUCTION

Industrial effluents loaded with Cr (VI) particularly at sites associated with metal plating, wood processing, leather tanning, metal corrosion inhibition, and pigment production cause hazard to humans and other forms of life. Cr (VI) is soluble in water and hence much more mobile, toxic and carcinogenic. Moreover, the USEPA has set a maximum contamination level (MCL) of 0.1mg/L for chromium in drinking water. Conventional methods for removal of Cr (VI) are often cost prohibitive and complicated. Some of these methods release toxic sludge and the disposal of which is again creating secondary environmental quality impact. On the other hand bioremediation technologies are being sought as less expensive and efficient alternatives in Cr (VI) remediation. However, bioremediation often takes longer than other treatments, such as excavation and it is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.

Indian mustard (*B.Juncea)*, a hyperaccumulated plant, has been used to phytoextract several heavy metals, including Cr (VI) from contaminated soils and water (Bolan et al., 2003). The phytotoxicity threshold concentration of Cr(VI) in the plant tissue, corresponding to 50% growth retardation PT_{50} was found to be 4.4-8.2 mg/kg soil(Mohan et al.,2006). The Cr(VI) is weakly adsorbed and is readily available for plant uptake, while Cr(III) is strongly retained onto soil particles (Lemke et al.,2010). Thus reduction of Cr (VI) to Cr(III) can enhance the immobilization of Cr, thereby rendering it less bioavailable. Bioremediation of metal contaminated soils includes technologies that involve biological agents such as higher plants, microorganisms and organic amendments. The soil amendment with organic matter like FYM improves the soil properties such as porosity, specific gravity, microbial activity, supply of carbon and nutrient sources like total N, P, K and Ca etc(Andrew et al.,2012). Organic matter plays a pivotal role in reduction of Cr (VI) providing a source of electron-donor and increase the amount of dissolved organic carbon (DOC) or by enhancing solubilization of the soil organic matter. The easily oxidisable organic carbon and DOC fractions provide the energy source for the soil microbes (Chang et al.,1992) involved in the reduction of metals.

Iron plays an important role in contaminant mobility, sorption and breakdown due to its role as an electron donor (i.e. during the oxidation of Fe^{2+} to Fe^{3+}), and, in its various mineral forms, as a precipitant substrate. The recent rapid development of the field of nanotechnology during the last two decades has been given the great interest in using zero valent iron nanoparticles (ZVNI) as soil and groundwater remediation tool. As a strong reductant, ZVNI can degrade a wide range of pollutants by adsorption and chemical reduction. ZVNI has been successfully used for the treatment of Cr(VI) contaminated soil and groundwater that makes use of the enhanced reactivity surface area, and/or enhanced mobility of ZVNI, to produce more rapid or cost-effective clean-up of wastes(Li et al.,2006). The ZVNI is inexpensive, non-toxic and serves as a strong reductant and hence alters targeted chemicals to non-toxic compounds.

This study aimed to investigate the capability of the reduction of Cr (VI) by bio-nanoremediation involving ZVNI assisted with FYM in decreasing the phytotoxicity of Cr (VI) .The removal efficiencies of these combinations were calculated in relation to various concentrations of ZVNI and FYM in the soil during the period of 30 days. This study also compares the tolerance efficiencies of germination and transplantation of *B.Juncea* in Cr(VI) contaminated soils.

MATERIALS AND METHODS

Seeds of Indian mustard (*Brassica Juncea)* were procured from Acharya NG Ranga Agricultural University, Tirupati, India. Two chromium metal polluted soil samples i.e., surface soil (S_1=0-15 cms) and subsurface soil (S_2=15-30 cms) were collected form Ranipet tanneries, TamilNadu, India using standard USEPA operating procedures. The samples were air-dried until totally dry, passed through a 2 mm sieve, packed in plastic bags and then stored in dark at 4 °C. The major chemical stock used in this experiment was 1, 5-diphenylcarbazide for estimation of Cr(VI) concentration.

The synthesis of the ZVNI particles involved the reductive precipitation process using stoichiometric amount of sodium borohydride ($NaBH_4$) dissolved in 0.1M solution of NaOH and 30% ethanol solution of $FeSO_4.7H_2O$. The procedure for ZVNI synthesis followed the method of He and Zhao (2005), Sun et al., (2007). According to this method, $NaBH_4$ (0.75 M) solution was added drop wise to $FeSO_4.7H_2O$ (0.1 M) solution at 1:1 volume ratio. The synthesized ZVNI particles were separated with a magnet and rinsed with de-oxygenated milli-Q water three times and methanol three times before drying with nitrogen gas. Then, the solution was homogenized for 20 min in a nitrogen gas. The ZVNI particles were formed according to the reaction:

$$4Fe^{+2}(aq) + 3BH_4^- + 9H_2O \rightarrow 4Fe^0(S) \downarrow + 3H_2BO_3 + 12H^+(aq) + 6H_2(g) \uparrow \quad (1)$$

Nanoscale Zero Valent Iron Characterization

Morphological studies of ZVNI were carried out by using Transmission electron microscope (TEM) (HITACHI, H-7500). The Localized Surface Plasmon Resonance (LSPR) of ZVNI was recorded using Schimazdu UV 2450 UV–Vis spectrophotometer. The stability and particle size distribution studies of ZVNI were carried out using zeta potential fitted with dynamic light scattering (SZ-100 nanopartica, HORIBA).

Germination and Transplantation Experiments

The germination experiment involved seeds of *Brassica Juncea* which were sown in commercial soil (CS) and contaminated soil. The seeds were soaked in water for 24 hours and germinated in soil. Duration of the experiment was a period of 30 days. Six treatments on contaminated surface (S_1=0-15cms) and subsurface soils (S_2=15-30cms) were used in this experiment. They were

T_{11}= S_1+ ZVNI (0.1ppm)+FYM (50g/Kg soil) + *Brassica Juncea*

T_{12}= S_1+ ZVNI (0.1ppm)+FYM (100g/Kg soil) + *Brassica Juncea*

T_{13}= S_1+ ZVN I(0.2ppm)+FYM (50g/Kg soil) + *Brassica Juncea*

T_{14}= S_1+ ZVNI (0.2ppm)+FYM (100g/Kg soil) + *Brassica Juncea*

T_{15}= S_1+ ZVNI (0.3ppm)+FYM (50g/Kg soil) + *Brassica Juncea*

T_{16}= S_1+ ZVNI (0.3ppm)+FYM (100g/Kg soil) + *Brassica Juncea*

The same treatments were repeated for subsurface soil (T_{21}-T_{26}). Germination and growth parameters were characterized by observing number of sprouts, % of height of the seedling and % of plant survival. The transplantation experiments were carried out using one week of age after seedling in commercial soil. They were transplanted into pots containing contaminated soil. This observation compares the tolerance efficiencies such as % of height of the seedling and % of plant survival in all treatments with germination experiment. These parameters in both germination and transplantation were compared with those of control units (Commercial Soil). The soils in both the experiments were maintained with 80% of moisture content.

Analytical Study

The aim of this test was to compare the tolerance of *B.Juncea* in the germination and transplantation experiments in Cr(VI) contaminated soils. The germination, tolerance efficiency of plants in all the treatments were compared with control treatment (CS). The efficiency of Cr (VI) removal in soil was conveniently monitored using UV-Vis Spectrophotometry with 1,5 diphenylcarbazide as a complexing agent.

RESULTS AND DISCUSSIONS

The UV–Vis spectrum of ZVNI showed its absorption maxima at 300 nm (Figure1) which is a characteristic of monodispersed iron nanoparticles.

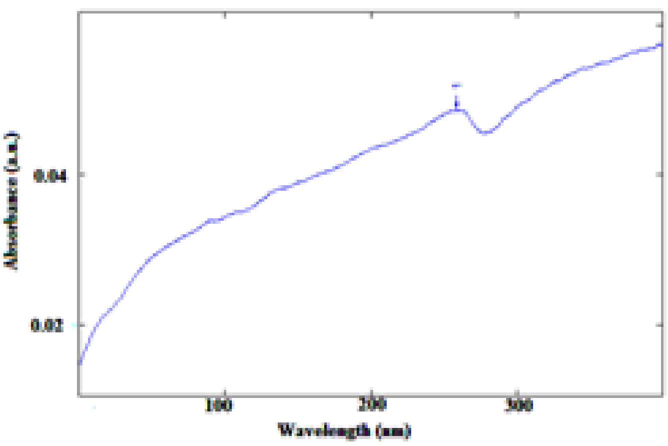


Figure 1: UV-Vis Spectroscopy of ZVNI

The TEM image of formed ZVNI was shown in figure 2. After the examination of more than 200 nanoparticles, a particle size distribution was calculated, which indicates that 90% of the particles were within the range of 70 nm .The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system.

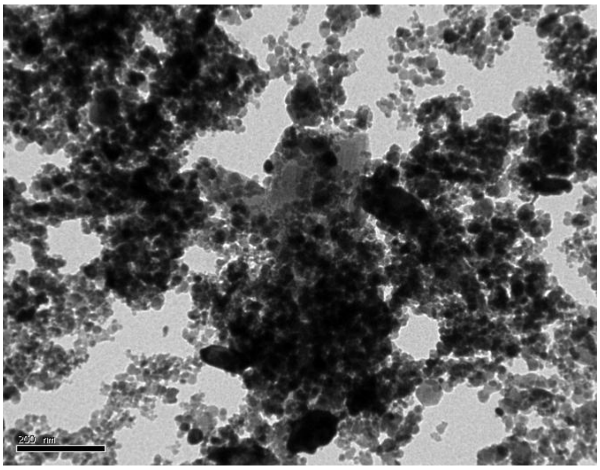


Figure 2: TEM Image of ZVNI

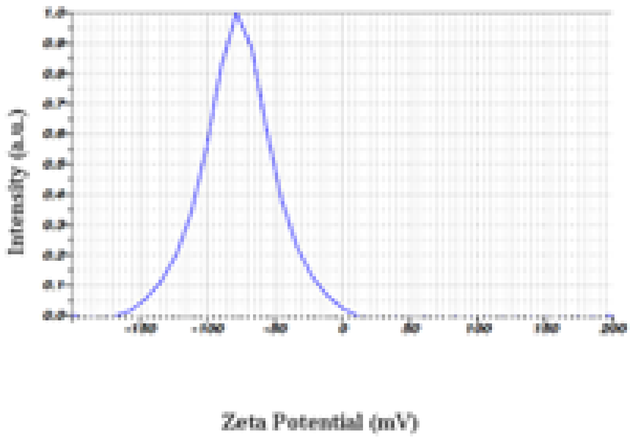


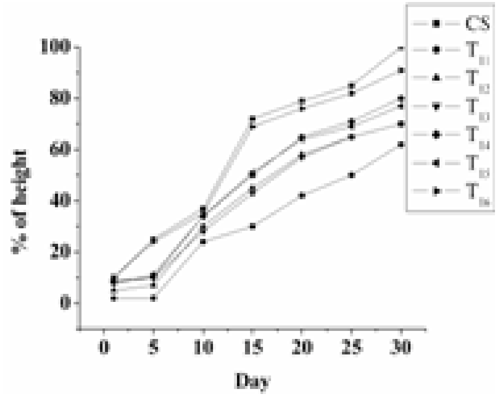
Figure 3: Zeta Potential of ZVNI

The zeta potential of ZVNI synthesized is -70.8mV (Figure 3). Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable.

Tolerance of germination and Transplantation of Brassica Juncea

The experiment was designed to investigate the germination and transplantation tolerance of Cr (VI) in all the treatments. The germination of *Brassica Juncea* was sprout at 80%, 61%, 67%, 62% and 69% in CS, T_{15}, $T_{16,}$ T_{25} and T_{26} treatments respectively. Transplantation was selected inorder to compare the survival rates of the plant with germination. The results indicated that tolerance efficiencies in transplantation and germination were 100% and 95% respectively at higher combined treatment of ZVNI and FYM when compared to those of control experiment.

It was observed that there were low percentages of germination, height of seedling and plant survival in T_{11} and T_{21} in germination experiments where the concentrations of ZVNI and FYM were low (Figure 4,5).



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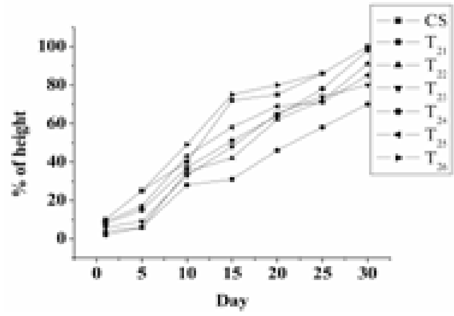

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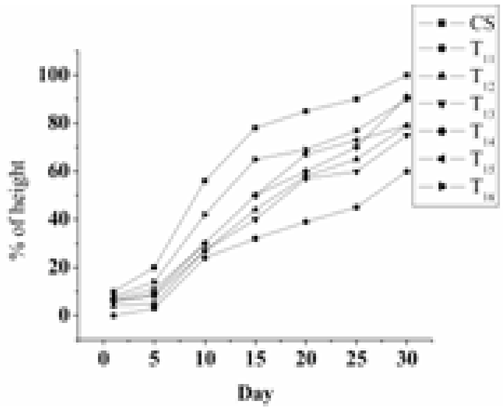

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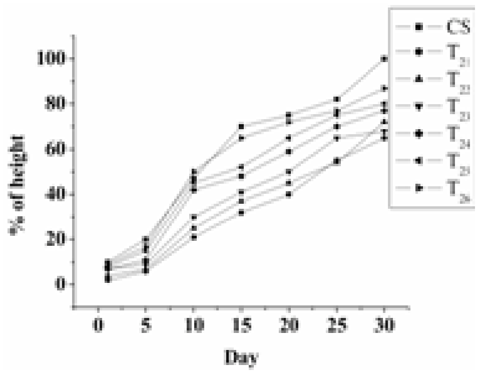
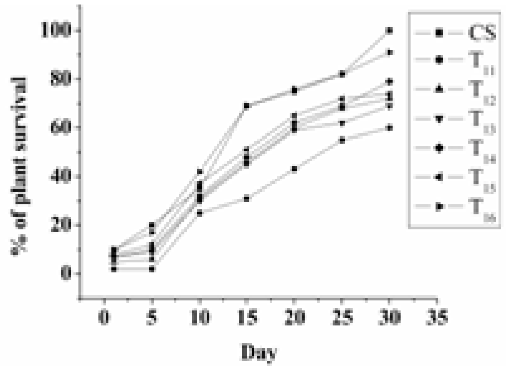


(d)

Figure 4: Tolerance Efficiency of *B.Juncea* (a) % of Height in Surface Soil by Transplantation (b) % of Height in Sub-Surface Soil by Transplantation (c) % of Height in Surface Soil by Germination (d) % of Height in Sub-Surface Soil by Germination



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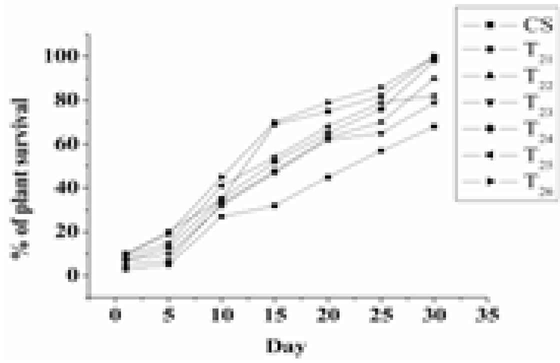

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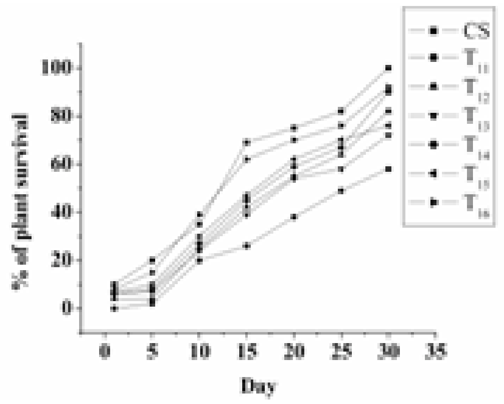

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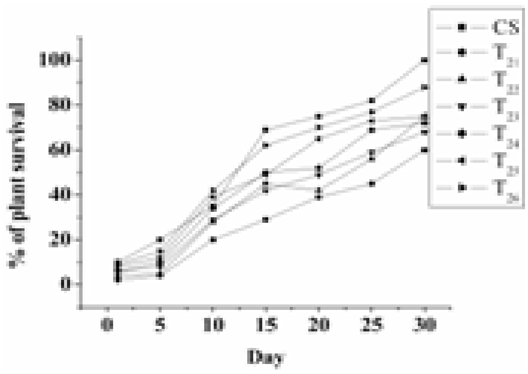


(d)

Figure 5: Tolerance Efficiency of *B.Juncea* (a) % of Plant Survival in Surface Soil by Transplantation (b) % of Plant Survival in Sub-Surface Soil by Transplantation (c) % of Plant Survival in Surface Soil by Germination (d) % of Plant Survival in Sub-Surface Soil by Germination

The results of germination, transplantation tests of tolerance study of plant with increased dose of ZVNI and FYM demonstrated that *B.juncea* presented the good rates of germination, % of height of seedlings and % of plant survival in Cr (VI) contaminated soils.

Physicochemical Properties of Amended Soils

The physicochemical properties of soils amended with FYM were presented in Table 1

Table 1: Physicochemical Properties of Contaminated Soils after Amended with FYM

Property	Surface Soil	Sub-Surface Soil
Sand (%)	66.68 ±0.6	56.72±0.2
Silt (%)	7.25±0.3	5.37±0.5
Clay (%)	25.07±0.5	37.91±0.85
pH	4.87±0.05	4.62±0.04
Organic matter (%)	14.02±0.3	14.85±0.54
Electrical Conductivity(mS)	0.976	1.041
Cr(VI)(ppm)	108.5147±0.23	106.7544±0.1
Fe(ppm)	20.0274±0.02	17.5789±0.3
Zn(ppm)	1.4932±0.1	0.8907±0.2

Effect of FYM on Tolerance Efficiency of *B.juncea*

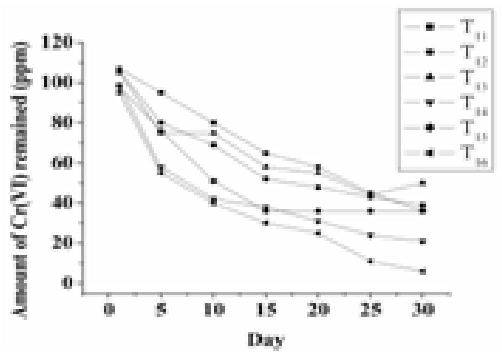
The effect of organic matter attachment on mineral–metal interactions depends on the nature of mineral surfaces (Kahle et al.,2004) of organic matter and of their environmental dependent interaction (Warren et al.,2001). The organic matter which is also rich in ammonical nitrogen undergoes nitrification (oxidation) resulting in the release of protons. This may be one of the reasons for the decrease in soil pH.

As a general rule, at low pH the adsorption of metal ions to mineral surfaces is increased by the organic matter because of the pKs of some functional groups, especially carboxylic groups. Deep soil organic matter has a very long residence time, and implicitly a large potential in metal retention (Cornelia et al.,2011). The increase in Cr (VI) reduction in the presence of OM may also result from the increase in microbial activity.

The increase in Cr (VI) reduction may be due to manure addition resulted from the enhancement of specific microbial population which reduces Cr (VI) indicating the larger contribution of microbial activity (Losi et al.,1994)This resulted from both increased supply of organic carbon and nutrient sources such as nitrogen and phosphorous to the micro-organisms.

The earlier research has proved that ZVNI can be used to remediate Cr(VI). The recent work has considered the role of organic amendment in order to mobilize colloidal ZVNI. The organic matter plays a beneficial role in decreasing the aggregation of ZVNI and attachment of ZVNI to soil surfaces by a combination of electrostatic and steric stabilization effects (Richard et al.,2009)

The sorption of organic matter onto the surfaces of ZVNI particles can serve several functions such as reducing aggregation, modifying surface charge of the aggregates to decrease electrostatic interactions between aggregates and the soil particles, help in target component removal (Hydutsky et al.,2007). Hence the organic matter assisted for facilitating the enhanced reactivity of ZVNI for the reduction of Cr(VI). The amount of Cr (VI) remained after the reduction by means of the combined effect of ZVNI and FYM in the soil treatments is shown in figure 6.



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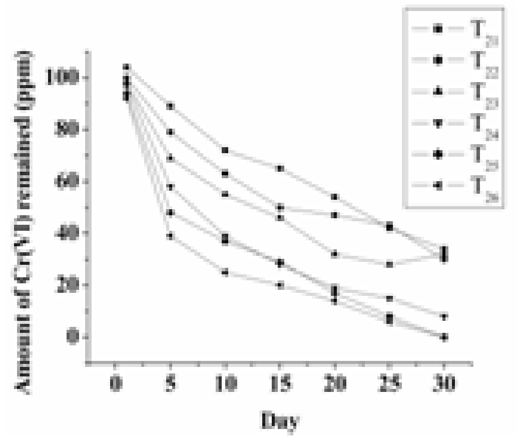

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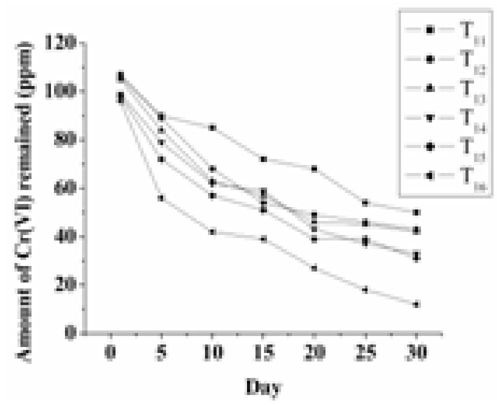

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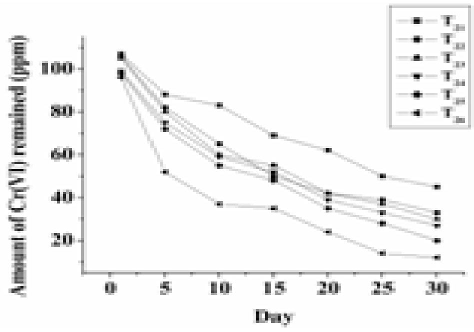

(d)

Figure 6: Amount of Cr (VI) Remained in Soil of *B.juncea* by (a) Transplantation in Surface Soil (b) Transplantation in Sub-Surface Soil (c) Germination in Surface Soil (d) Germination in Sub-Surface Soil

The distribution of Cr (VI) in soil components may be fractionized into different forms such as organic-bound, oxide-bound, residual fraction, soluble and exchangeable fractions. It has often been observed that the addition of inorganic compounds such as lime and zeolite increase the oxide-bound and residual metal fractions. But the addition of organic matter such as farm yard manure increases the organic-bound fraction (Knox et al.,2000) Generally the exchangeable and soluble fractions of Cr (VI) are considered to be plant available. Hence, the addition of FYM decreases the subsequent availability of Cr (VI) for plant uptake. Organic amendment has been shown to affect the chemistry (i.e., pH, organic acids, soil solution composition) and biology (e.g., microbial community) of soil. It is being recognized that the soil immediately surrounding plant roots (rhizosphere) is a modified microbiological and chemical environment due to plant-soil-microbe interactions. The addition of soil amendment changes the soil chemistry and therefore influences the transformation, mobility and bioavailability of Cr(VI) to the plant (Park et al.,2011). In this study, our speculation for Cr (VI) reduction to Cr (III) and decrease of phytotoxicity in *B.juncea* in the presence of FYM can be explained by three mechanisms (i) The organic matter reduces the soil pH and thereby reduces the Cr (VI) by adsorption. (ii) stimulates the microbial activity that enhance the reduction of Cr (VI) to Cr (III) (iii) modifying the electrostatic interactions between ZVNI and soil particles by increasing the surface reactivity of ZVNI. Hence the addition in FYM masks the bioavailability of Cr (VI) for plant uptake and thus reduces the phytotoxicity. Thus by considering all the factors above, the extent of Cr (VI) reduction is increased by increase in the FYM concentration, and there was significant relationship between the Cr (VI) reduction and FYM concentration.

CONCLUSIONS

Results from this study indicate that the addition of ZVNI and FYM in Cr(VI) contaminated soil enhanced the reduction of Cr(VI) to Cr(III) by increasing the microbial activity, electron-donor groups and stabilization ZVNI, thereby decreasing the bioavailability of Cr(VI) for plant uptake. The results of germination and transplantation experiments showed that the tolerance efficiencies of *B.Juncea* like germination, % of height of seedling and plant survival increased higher rates in transplantating than that of germination. It is concluded that the combined effect of FYM and ZVNI is significant compared to anyone of the individuals in remediating Cr (VI) contaminated soils.

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