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EVALUATION OF REMEDIATION ABILITY OF *PONGAMIA PINNATA* (L.) PIERRE UNDER HEXAVALENT CHROMIUM STRESS SOIL CONDITIONS

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ABSTRACT

The increase in demands for industrial and mining products alters the conventional ecosystem approach and attainment of sustainable development. Production of industrial products, processing of metals and protection of environment are intimately connected with one another and make a challenge for sustainable growth and development of human-beings. In the industrial and mining environment, the release of heavy metals like hexavalent chromium [Cr(VI)] in solid, liquid or gaseous states influence the soil health of the region. Its concentration exceeding the threshold limits is highly toxic and expresses in the form of manifold health problems. The health of crop plants and animal husbandry are not spared from its toxic effects. The Cr (VI) is a highly toxic, mobile, inter-convertible form of chemical element mostly used in industrial applications for its tensile strength and anti-corrosion ability. The soil pollution due to high Cr(VI) load is a negative attribute of mining and industrial developmental activities. Restoration of soil quality in these mining and industrial areas is highly essential for sustainable development and healthy living. Confinement of this toxic element in the closed biological system is a move towards reducing its load in the soil profile. In the present approach *Pongamia pinnata* (L.) Pierre is experimented as a renewable closed biological system for improving the soil health of Cr(VI) rich mining and industrial sites. In this experimental set up the assessment of Cr(VI) content in selected parts of this experimental plant species and the rhizospheric soil of their growth was performed using the standard methodologies of APHA (1998). The positive aspect of this approach is the survival of this species under high soil Cr(VI) concentration and differential accumulation of this toxic element in this biotic system. The targeted plant species was able to accumulate approximately 50 % of the soil Cr(VI) within a period of 135 days from the initiation of treatment. The order of accumulation of Cr(VI) was found to be root > leaf > stem. Compared to the limitations of physical, chemical and microbiological techniques, this process is sustainable in the long run, cheaper and has least negative interference with other components of the environment. Further work in this area has the possibility to improve the efficiency of Cr(VI) intake by this living system.

KEY WORDS: Biotic, Heavy metals, Health, Hexavalent chromium stress, Industrial and mining activities, Toxic

INTRODUCTION

The rise in global population coupled with technological advancement in the last few decades have led to large scale industrial growth. The industries heavily relied upon heavy metals and their alloys for several applications. The improper disposal of wastes rich in heavy metals like hexavalent chromium, Cr(VI) from industrial and mining sites impart toxic effects on soil health of the region. The Cr(VI), due to its high mobility percolates down the earth and can contaminate the highly precious resources like groundwater. The runoff water solubilise the Cr(VI) loosely held by soil particles and with the loading of Cr(VI), surface water of the adjoining area can get contaminated. Heavy metal pollution in the environment is a major problem which may be attributed to the toxicity expressed by the metals even at extremely low concentration (Kalidhasan *et al*., 2016). The Cr(VI) is widely acclaimed as a genotoxin, mutagen, and carcinogen (Das *et al.*, 2017; Saikia *et al.*, 2014).

The Cr(VI) is released as a soil contaminant from natural as well as anthropogenic sources (Das *et al.*, 2018). However, the chances of Cr(VI) led soil contamination is more from anthropogenic sources as compared to natural sources. The anthropogenic release of Cr(VI) as a soil contaminant mostly takes place from industrial and mining activities (Das *et al.*, 2021). The release of Cr(VI) into the environment through industrial effluents is widely acknowledged. After release, it is directly or indirectly incorporated into the lithosphere and present in the soil in varieties of oxidation states with different levels of solubility and stability.

Out of all the forms of this heavy metal chromium (Cr), the moderately soluble Cr(VI) is highly reactive (Das *et al.*, 2021). It has the ability to form stable complexes with divalent cations present in the soil even at low concentration. The presence of this heavy metal in toxic concentrations, affects the soil and the exposed organisms. The selectively permeable bio-membranes and the carrier proteins present in these membranes are the possible gateways for the entry of this Cr(VI) in living cells from the contaminated soil. Exceeding the threshold concentration, the Cr(VI) expresses its lethal effects on human health. The formation of reactive oxygen species (ROS) during its chemical reactivity inside the living cells induces the alterations at the levels of structural integrity and metabolism of the contaminated cells.

Selected plants having the ability to tolerate and grow on Cr(VI) contaminated soils can be utilized for phyto-extraction of Cr(VI) from contaminated soils, as they skip the adverse secondary effects of other physical, chemical and biological methods. The hyper-accumulation of Cr(VI) in plant biomass can lead to reduce the concentration of Cr(VI) in the contaminated soil. The present study is designed with an attempt to evaluate the tolerance, growth and accumulation ability of *Pongamia pinnata* (L.) Pierre under Cr(VI) contaminated soil conditions.

MATERIALS AND METHODS

Selection of plant species

The selection of *Pongamia pinnata* (L.) Pierre as the plant species for the present experiment was done on the basis of its cosmopolitan distribution and its ability to grow on soil regimes of chromite mining and industrial area at Sukinda of Odisha in India. It had attained high importance value index (IVI) in a study done at Kalinga Nagar Industrial sites, situated at Duburi of Odisha in India. One of the supporting factors behind its selection is its easy natural reproduction. It is capable of natural reproduction using seeds and suckers on the soils of industrial and mining belts without any pre-treatment.

Selection and germination of seeds for the experiment

The healthy seeds were selected randomly from the collected certified seeds of *Pongamia pinnata* (L.) Pierre plants. The intact seeds selected for the purpose were surface sterilized using 0.1% mercuric chloride. The sterilized seeds were sown in rows on moist soils in trays maintained at 28 ± 2°C temperatures with 16 hours of light and 8 hours of dark cycle.

Experimental set up

The experiment was done on the basis of randomized block design with control plant labelled as T_0 and plants on Cr(VI) treated soils labelled as T_1 [50 µg/g Cr(VI)], T_2 (100 µg/g), T_3 (150 µg/g), T_4 (200 µg/g) maintained in triplicates. The 45 day plants labelled as T_0 to T_4 were allowed to grow on individual pots filled with 3 kg of homogenized soil of specified Cr(VI) concentration under greenhouse condition maintained with mean temperature 28 ± 2°C and humidity 80 ± 2%. The water content of the soil was maintained at 75% of its water-holding capacity with monitoring at an interval of 24 hour.

Estimation of Cr(VI) tolerance index of targeted plant species

The tolerance index of the selected plant species under variable soil Cr(VI) concentrations was calculated on the basis of accumulation in fresh weight of biomass following standard methodology

(Sinha *et al.*, 2014).

Cr(VI) Tolerance index = (MBT / MBC) ×100

where,

MBT = Mean biomass of Cr(VI) treated plants

MBC = Mean biomass of control plants

Evaluation of variation in selected phenotypic parameters

The variations in selected phenotypic parameters of *Pongamia pinnata* (L.) Pierre on soils with variation in Cr(VI) concentrations were evaluated following standard methodologies (Yoshida *et al.*, 1976).

Determination of Cr(VI) accumulation in selected plant parts

The accumulation of Cr(VI) in selected parts of targeted plant species like shoots and roots under variation in soil Cr(VI) was determined using atomic absorption spectroscopy following the standard methodology (APHA, 1998). The dried plant samples in powdered form were acid digested using 0.5 M HNO_3. The analysis of [Cr(VI)] in acidic solution was also done using UV-Vis spectrophotometer as a confirmatory test. The coloured complex formed due to the interaction of Cr(VI) and 1,5-diphenylcarbazide was analyzed at 540 nm for the determination of Cr(VI) in selected plant parts (Das *et al.,* 2022). The [Cr(VI)] in plant parts were determined after a gap of 90, 180 and 270 days of soil treatment respectively. The index values like bioconcentration factor (BCF) and translocation factor (TF) were calculated using the methods of Sajad *et al.* (2020) to estimate the remediation potential of the targeted plant species.

$BCF = C_{Plant}/C_{Soil}$

where

C_{Plant}= [Cr(VI)] in plant and

C_{Soil}= [Cr(VI)] in soil

$TF = C_{Shoots}/C_{Roots}$

where

C_{Shoots}= [Cr(VI)] in plant shoot and

C_{Roots}= [Cr(VI)] in plant root.

Statistical analysis

The experimental data were analyzed at p = 0.05 and 0.01using IBM SPSS statistics 21.0 software.

RESULTS AND DISCUSSION

Tolerance of targeted plant species to Cr(VI) contaminated soil conditions

The tolerance of targeted plant species, *Pongamia pinnata* (L.) Pierre to Cr(VI) contaminated soil conditions is evaluated on the basis of computed index values. It shows, the decrease in tolerance index with an increase in Cr(VI) input to the soil during the study. The minimum tolerance index (51.08%) is revealed during the growth of the targeted plants on soil treated with 200 µg Cr(VI)/g soil (Table 1). Similarly, the maximum tolerance index (80.00%) is observed in plants grown on soil with 50µg Cr(VI)/g soil. The plants show insignificant tolerance index, exceeding the soil Cr(VI) concentration of 200 µg/g soil. The plants showing minimum 50% tolerance index are able to tolerate and grow on Cr(VI) contaminated soil conditions (Baker *et al.*, 1994). It indicates that the targeted plant species growing on soils with Cr(VI) concentration up to 200 µg/g soil is meeting the required minimum tolerance index value of 50%. The increase in soil Cr(VI) toxicity, possibly leads to metabolic alterations responsible for impaired plant growth, considerable reduction in plant biomass and reduction in tolerance capacity.

Table 1. Estimation of tolerance index of *Pongamia pinnata* (L.) Pierre. on Cr(VI) treated soil

Soil treatment code	Cr(VI) concentration in treated soil (in µg/g soil)	Tolerance Index of *Pongamia pinnata* (L.) Pierre. (in %)
T_1	50	80.00
T_2	100	74.20
T_3	150	63.55
T_4	200	51.08

The earlier studies on tolerance of many plants to soil Cr(VI) concentration of 100 µg/g soil revealed the reduction in tolerance. The soil Cr(VI) toxicity symptoms in plants is expressed as reduced plant growth on Cr(VI) treated soil conditions. The high tolerance index in plants like ragweed (70%), coastal bermuda grass (72%), switch grass (75%), and vetiver grass (68%) shows tolerance to such toxic soil (Shahandeh and Hossner, 2000). However, under hydroponics, during exposure to an initial Cr(VI) concentration of 10 mg/l for a period of 30 days, three plant species like *T. pallida*, *G. luteoalbum* and *A. philoxeroides* showed 87%, 58% and 35% of tolerance index, respectively. The plants with 35% and 58% of tolerance index showed acute symptoms of toxicity under treatment condition as compared to being maintained as control. The plant, *T. pallida* with high tolerance index (87%) demonstrated no toxicity symptoms. However, under hydroponics, the plants failed to tolerate a higher concentration of Cr(VI) solution (20 mg/l), for a prolonged exposure period of 60 days (Sinha *et al.*, 2014). The *Glycine max* plants expressed high tolerance percentage on soil contaminated with chromium concentration of 0.5 mg/Kg soil and the value is subsequently low with increase in chromium concentration to 25 mg/Kg soil (Amin *et al.*, 2014). It supports the experimental findings of the current study that *Pongamia pinnata* (L.) Pierre. is a suitable tolerant species for growth on Cr(VI) contaminated soil conditions as it can sustained on high level of contamination of soil up to 200 µg Cr(VI)/g soil.

Effect of soil Cr(VI) level on selected phenotypic parameters of *Pongamia pinnata* (L.) Pierre

The effect of variation in the soil Cr(VI) level on selected phenotypic parameters of plant is recorded and it shows significant differences in its influence. The variation in growth parameters of plant like length of shoot and root is correlated with the fluctuation in soil Cr(VI) concentration. After 90, 180 and 270 days of soil treatment, a significant reduction in mean shoot length of plant is observed with increase in soil Cr(VI) concentrations, at $p = 0.05$ and 0.01. It is observed that progressive reduction in mean shoot length is visible from T_0 (control) to T_1 (50 µg/g soil), T_2 (100 µg/g soil), T_3 (150 µg/g soil), and T_4 (200 µg/g soil).

After 90 days of treatment, the mean shoot length of control plant is 54.767±0.205 Cms. and reduced to 25.633±0.124 Cms. in T_4 (Table 2). After 270 days of treatment, the mean shoot length of control plant is 99.533±0.339 Cms. and reduced to 56.400±0.294 Cms. in T_4. After 180 days of treatment, the mean shoot length of control plant and plant on treated soil is intermediate to that of 90 and 270 days of post-treatment period.

The reduction in mean shoot length expressed linear relationship with variation in soil Cr(VI) concentrations with a coefficient of 0.914 (Figure 1a). It may be due to the toxic impact of soil Cr(VI) on genetic constituents of the cell and its altered expressions. The cytotoxicity in plants exposed to soil treated with Cr(VI) is a possible factor for reduction in shoot length. It is supported by the findings of the study reported earlier (Sinha *et al.*, 2014).

Similarly, after 90, 180 and 270 days of soil treatment, the mean root length of plant reduced significantly with increase in soil Cr(VI) concentrations, at $p = 0.05$ and 0.01. The mean root length of plant maintained on untreated soil (T_0) and treated soils (T_1 - T_4) increases with the increase in days of post-treatment from 90 – 270 (Table 3).

After 90 days of treatment, the mean root length of control plant is 46.720±0.163 Cms. and reduced to 20.089±0.202 Cms. in T_4. After 270 days of treatment, the mean root length of control plant is 81.74±0.204

Table 2. Variation in shoot length of *Pongamia pinnata* (L.) Pierre during growth on Cr(VI) treated soil

Soil treatment code	Cr(VI) concentration of treated soil (in µg/g soil)	Mean shoot length of plant during growth on treated soil (in Cms.)		
		90 Days	180 Days	270 Days
T_0(control)	0	54.767±0.205	77.233±0.205	99.533±0.339
T_1	50	48.133±0.124	54.267±0.249	62.200±0.216
T_2	100	38.067±0.094	44.033±0.124	60.033±0.205
T_3	150	37.733±0.205	42.167±0.169	59.300±0.244
T_4	200	25.633±0.124	35.033±0.047	56.400±0.294

Table 3. Variation in root length of *Pongamia pinnata* (L.) Pierre during growth on Cr(VI) treated soils

Soil treatment code	Cr(VI) concentration of treated soil (in µg/g soil)	Mean root length of plant during growth on treated soil (in Cms.)		
		90 Days	180 Days	270 Days
T_0(Control)	0	46.720±0.163	68.225±0.163	81.740±0.204
T_1	50	38.125±0.115	43.222±0.166	51.200±0.141
T_2	100	30.025±0.052	35.273±0.052	44.233±0.124
T_3	150	28.100±0.081	34.244±0.102	42.555±0.124
T_4	200	20.089±0.202	27.333±0.047	36.010±0.082

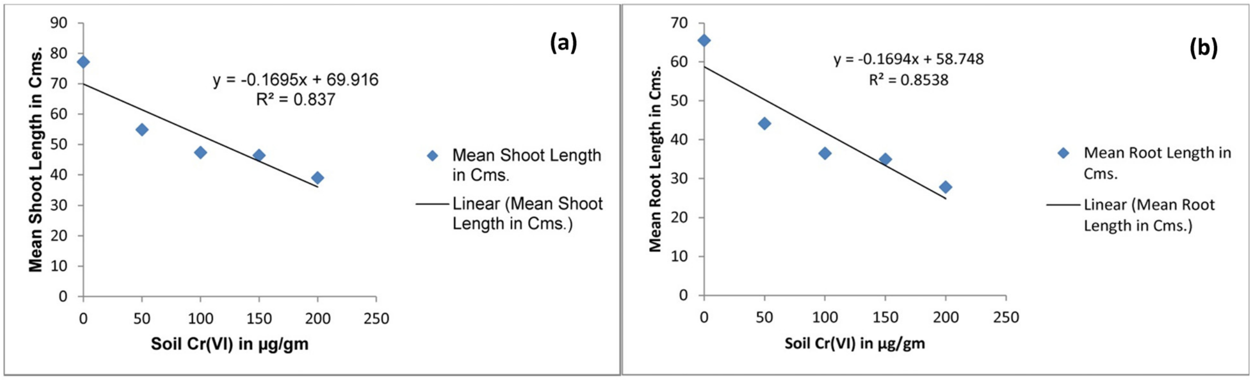

Fig. 1. Influence of variation in soil Cr(VI) concentrations on **(a)** mean shoot length and **(b)** mean root length of *Pongamia pinnata* (L.) Pierre over a period of 270 days

Cms. and reduced to 36.01±0.082 Cms. in T_4. After 180 days of treatment, the mean root length of control plant and plant on treated soil is intermediate to that of 90 and 270 days of post-treatment period.

The reduction in mean root length shows linear correlation with variation in soil Cr(VI) concentrations with a coefficient of 0.924 (Figure 1b). It may be due to the toxicity of Cr(VI) present in the soil and its adverse effects on division and expansion of root cells. The structural or functional alterations of root cells due to chemical stress is a possible factor for reduction in mean root length of plant on soil treated with Cr(VI). It is getting support from the observations made earlier (Sinha *et al.*, 2014; Vajpayee *et al.*, 2000).

The mean shoot length is more than the mean root length of plant on Cr(VI) treated soil. It may be due to the direct exposure of plant roots to toxic surroundings like Cr(VI) treated soil (Sinha *et al.*, 2014). The increase in soil Cr(VI) has the possibility of disturbing the physico-chemical equilibrium of the soil in a direction to disturb the growth of plant roots on Cr(VI) treated soil (Bolan *et al.*, 2003; Li *et al.*, 2018).

Assessment of Cr(VI) accumulation in selected plant parts

Pongamia pinnata (L.) Pierre. grown on untreated (T_0) and Cr(VI) treated soil (T_1- T_4) is found tolerant to soil Cr(VI) concentrations from 50 µg/g soil to 200 µg/g soil. The assessment of Cr(VI) shows that significant amount of it is accumulated in selected plant parts like shoot, root and leaf, during its growth on soils labelled as T_0 to T_4 (Figure 2).

Under each treatment conditions the soil Cr(VI) concentration was found to decrease with gradual increase in time, while the accumulation of Cr(VI) in various plant parts increased. Soil treated with 50 µg/g of Cr(VI) was found to exhibit a decline in the concentration of the toxic heavy metal and reduced to 33.03 µg/g after a duration of 270 days. The Cr(VI) concentration of soil initially treated with 100 µg/g of the heavy metal finally reduced to 65.23 µg/g. Similarly soils with initial treatment of 150 and 200 µg/g of Cr(VI), reduced to 86.53 µg/g and 125.6 µg/g respectively after the 270 days study period. The percentage reduction in Cr(VI) concentration from soils under different treatment conditions (50 µg/g, 100 µg/g, 150 µg/g, and 200 µg/g) after the entire study period was found to be 33.94, 34.77, 42.31 and 37.2 respectively. Increased Cr(VI) toxicity of the soil leads to increased accumulation in plant parts and a corresponding increase in the reduction of the metal from soil with respect to the exposure time. Similar results were obtained in a study wherein *Tagetes erecta* was exploited for its ability to accumulate Cr from electroplating effluents contaminated soils. The study revealed an increase in Cr concentration in the biomass of the plant with an increase in Cr concentration of the soil and the time of exposure (Chitraprabha and Sathyavathi, 2018). In the current study, a decrease in percentage reduction of soil Cr(VI) at 200 µg/g of treatment conditions may be due to higher toxicity levels that may adversely impact plant accumulation to certain extent. *P. pinnata* subjected to 50 µg/g of Cr(VI) showed maximum accumulation in the leaf of the plant followed by the roots and stems. In rest of the soil Cr(VI) treatment conditions (100, 150, and 200 µg/g) the root showed maximum accumulation followed by the stems and leaves after a 90 days period. However, analysis at 180 days and 270 days post treatment, revealed a change in the

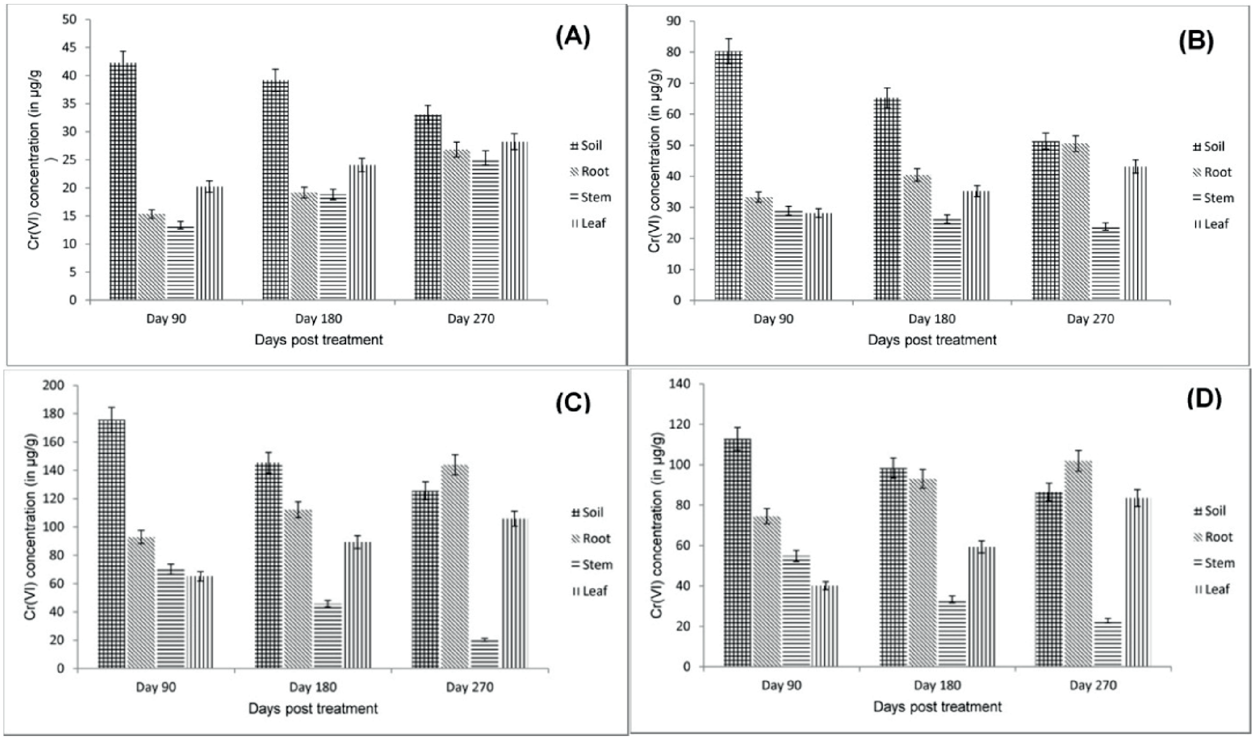

Fig. 2. Accumulation of Cr(VI) in different plant parts and treated soil analysed at regular intervals of 90, 180 and 270 days under different Cr(VI) soil concentrations (**A** = 50 µg/g, **B** = 100 µg/g, **C** = 150 µg/g and **D** = 200 µg/g).

accumulation pattern showing higher accumulation in roots, followed by leaves and then the stems (Roots>Leaves>Stems). This may be possibly due to an increased translocation of Cr(VI) to the aerial parts with an increase in soil concentration of the toxic heavy metal. Similar results were also obtained in case of *T. pallida* when subjected to a Cr(VI) concentration of 10 mg/l of nutrient solution (Sinha *et al.*, 2014). Bashri *et al.*, (2016) assessed the accumulation capacity of *Amaranthus viridis* and *Amaranthus cruentus* subjected to Cr(VI) concentrations of 10 µM and 50 µM in a hydroponic set up. *A. viridis* was found to accumulate higher amount of Cr(VI) in its biomass as compared to *A. cruentus*. In both the plants the accumulation was higher in roots followed by the shoots.

Estimation of bioaccumulation factor and translocation factor

The plant exhibited a bioaccumulation and translocation factor of more than 1 in almost all the treatment conditions, thereby signifying its utility as a hyper accumulator of Cr(VI) (Figure 3). This is supported by the fact that plant species having a BCF and TF values of more than 1 can be regarded as potential candidates for remediation purposes (Nazir *et al.*, 2011). The maximum BAF and TF was found to be 2.43 and 2.24 respectively and was observed in plants (T_1) subjected to a soil Cr(VI) concentration of 50 µg/g. A recent study by Saravanan *et al.* (2019) showed similar results wherein *Vigna mungo* showed highest BAF and TF values at 50 µg/g of soil Cr(VI) concentrations. The BAF and TF values decreased with an increase in soil Cr(VI) content. Lowest BCF and TF values of 1.3 and 0.88 respectively was observed in case of the plants (T_4) exposed to Cr(VI) soil concentration of 200 µg/g. A rise in BCF value under each treatment conditions and with the time supports more accumulation of Cr(VI) in the roots as compared to other plant parts. The plants exhibited a decrease in the BCF and TF values with a corresponding increase in the Cr(VI) soil concentration. This may be attributed to the fact that high concentration of Cr(VI) may prove to be toxic to the plants. Such a condition may have adverse effect on plant metabolism leading to reduced uptake of the heavy metal by the plant. Another probable reason is that Cr(VI) may have inhibited processes like cellular division and would have caused damage to the root system, thereby decreasing the uptake efficiency (Sinha *et al.*, 2014). Cr(VI) is also known to decrease

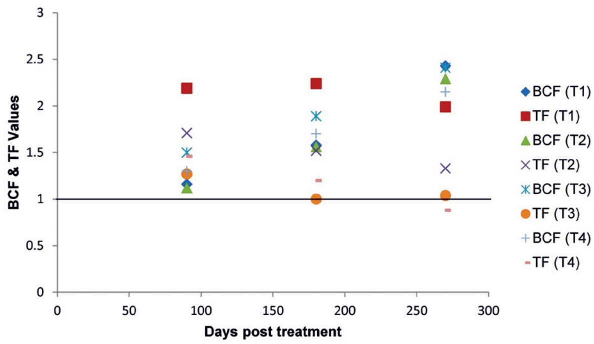

Fig. 3. Bioconcentration factor (BCF) and Translocation factor (TF) demonstrated by *Pongamia pinnata* (L.) Pierre under different soil Cr(VI) conditions over a period of 270 days of study.

photosynthesis rate in plants and may hinder the translocation of the toxic metal (Vajpayee *et al.*, 2000).

CONCLUSION

The tolerance of *Pongamia pinnata* (L.) Pierre to soil Cr(VI) concentrations up to 200 µg/g makes it a promising tool for the effective remediation of the contaminated environment. Despite toxicological symptoms, the targeted plant species manages well to survive and accumulate high concentration of Cr(VI) in its biomass. The high bioaccumulation, and translocation factor of more than 1 in almost all the cases of toxic conditions and over a period of 270 days of study demonstrates the plant's ability as a phytoremedial tool to remediate Cr(VI) contaminated mining and industrial soils. Further research in this regard could be carried out to improve the efficacy of Cr(VI) uptake by the targeted plant thereby rendering the environment harmless.

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