Chapter VI:

SIMULTANEOUS BIOREMEDIATION OF ARSENIC AND CHROMIUM(VI) FROM CONTAMINATED SOIL

6.1 General

Removal of heavy metal(loid)s such as arsenic and chromium from soil is a challenging task as they are bound to soil matrix and hence, are difficult to mineralize. Several methods, such as physical, chemical and biological, have been employed for their removal from soil (Gholizadeh and Hu, 2021). However, most of these methods are highly cost-intensive (Satapute et al., 2019). Heavy metal(loid)s, due to their non-biodegradability, persist in soil for a long time. They are permanently fixed by soil and interact with soil through ion exchange, precipitation, adsorption and complexion (Dhaliwal et al., 2020). In addition, toxic heavy metals present in compost are deterrent to agricultural applications and their accumulation in dietary plants is a potential threat to human and environmental health (Arora et al., 2008; Singh and Kalamdhad, 2012). Several researchers have reported the use of microorganisms in combination with plants to remove contaminants at a higher rate and in greater amounts (Mushtaq et al., 2022; Yang et al., 2020). Studies have reported the effect of arsenic or chromium individually on plant growth and physiology. In spite of arsenic and chromium cooccurring in soil, their combined effect on plants is very little, and studies on crop plants are even scarce (Islam et al., 2015).

The objective of this study was to remove arsenic and chromium simultaneously from soil with the help of *Pseudomonas aeruginosa* strain K7Pb and *Proteus alimentorum* strain TY6. This work also involved (i) a comparison of different extraction mediums to assess the efficiency of bacteria for removal of arsenic and chromium from soil (ii) evaluation of plant growth-promoting traits of the bacteria through root and shoot length and chlorophyll assessment.

6.2 Effect of initial concentration on removal efficiency

The removal efficiency increased with time for all samples. For arsenic, removal was higher when it was the sole contaminant in soil as compared to when mixed with chromium. The removal from control was minimal for all three pollutants [As(III), As(V) and Cr(VI)]. After 15 days, maximum removal efficiency of 61.82±2.47% was obtained for As(III) using TY6 (Figure 6.1) and 64.18±3.21% for As(V) using K7Pb (Figure 6.2). For Cr(VI), the maximum removal obtained was 54.54±2.72% for TY6 (Figure 6.3) and 56.06±2.80% for K7Pb (Figure 6.4) after 15 days. The removal efficiency also varied with the extraction medium used. In general, the maximum removal efficiency obtained was highest for $CaCl_2$ in case of arsenic and NaOH in case of chromium. It was observed that pollutant removal decreased with an increase in concentration, which can be a result of lower bacteria growth and enzyme production rate.

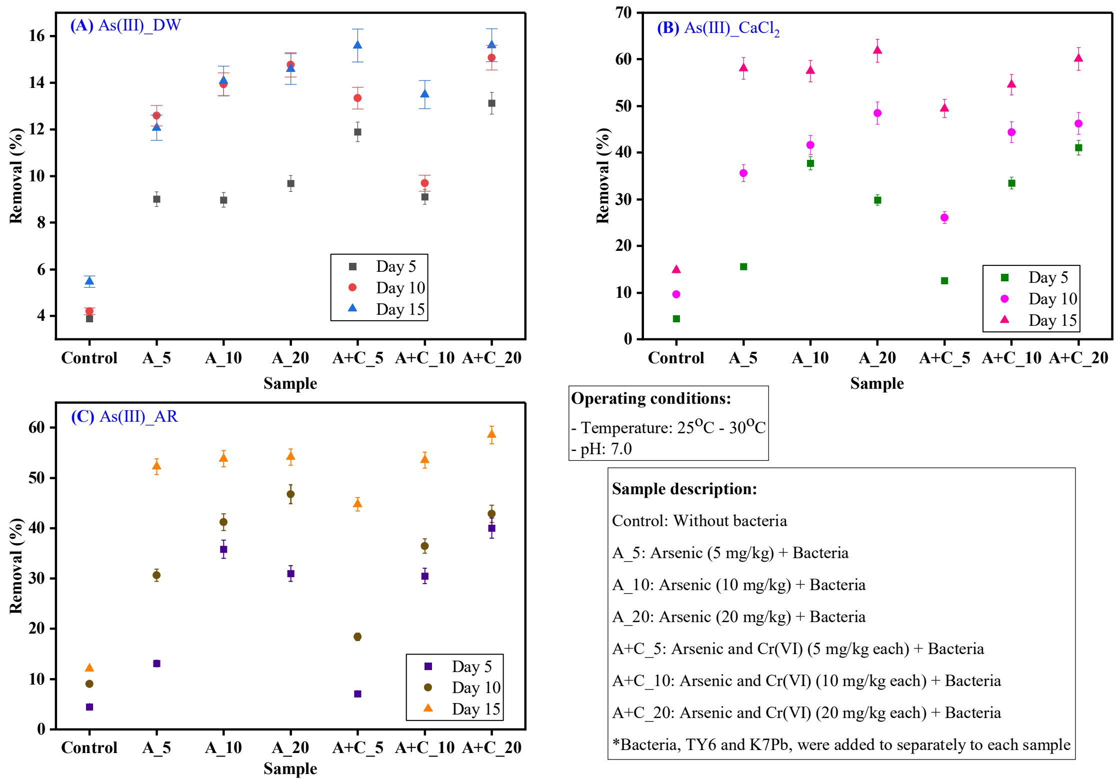

Figure 6.1: Removal of As(III) from soil using TY6 using different extraction agents

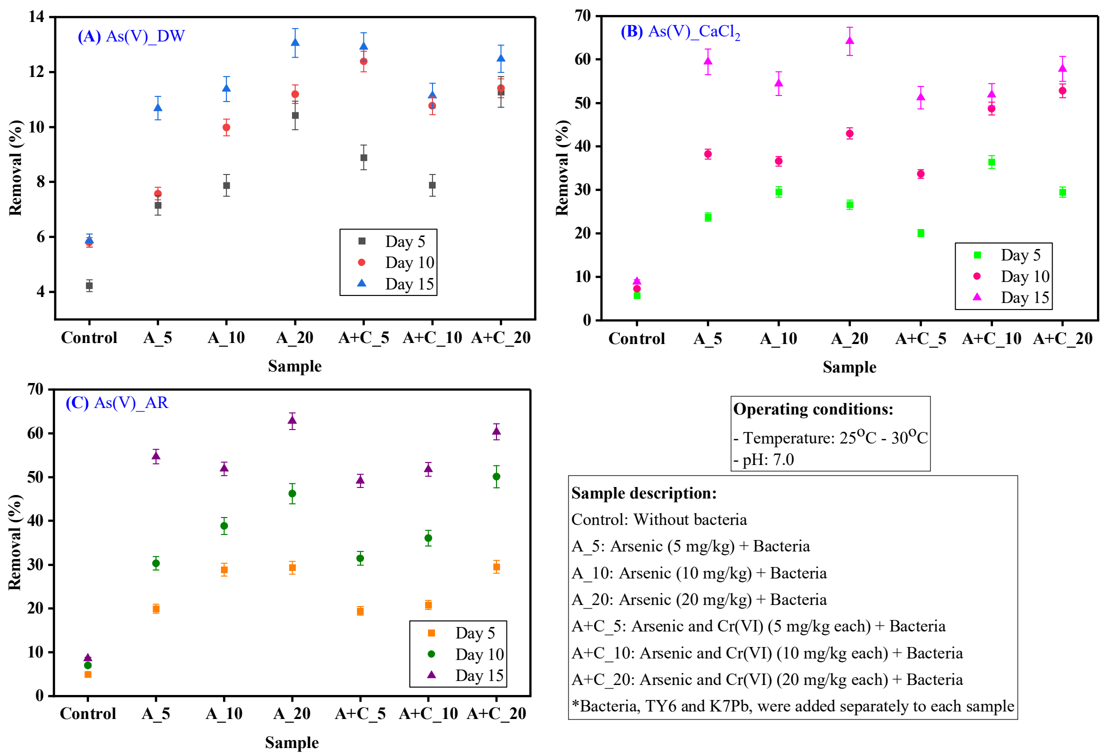

Figure 6.2: Removal of As(V) from soil using K7Pb using different extraction agents

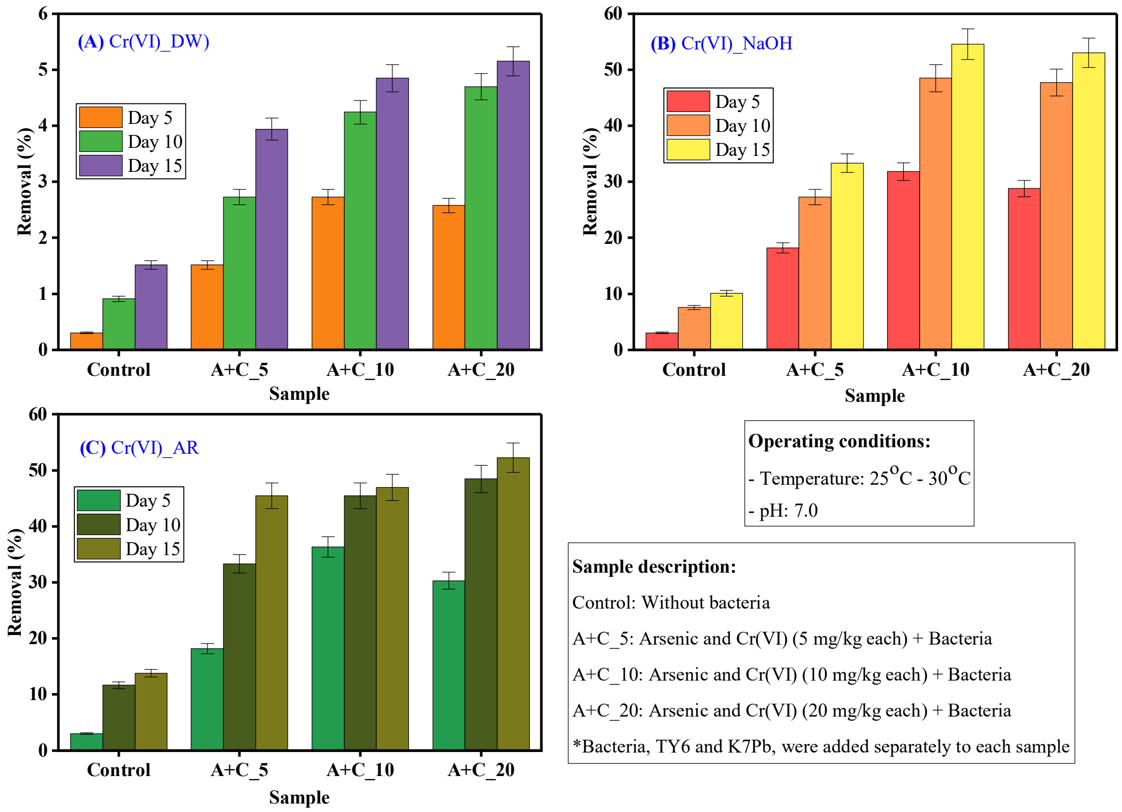

Figure 6.3: Removal of Cr(VI) from soil using TY6 using different extraction agents

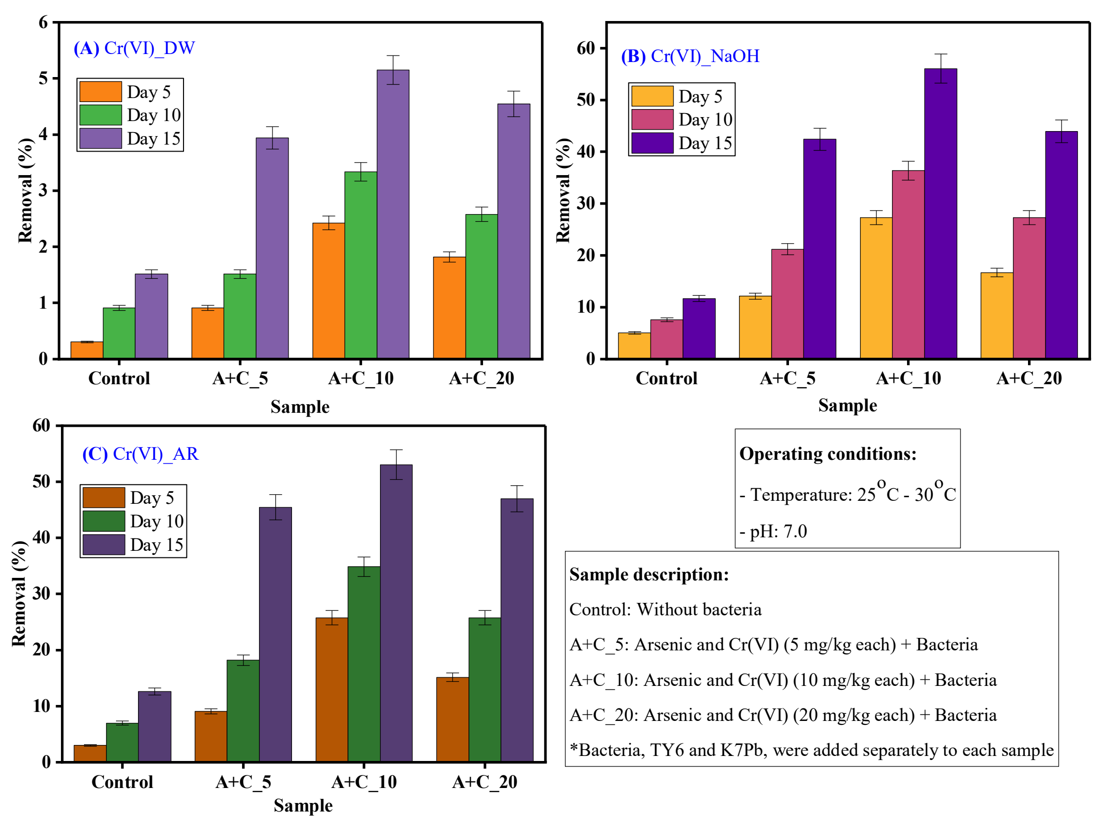

Figure 6.4: Removal of Cr(VI) from soil using K7Pb using different extraction agents

Chatterjee et al. (2009) reported that owing to its toxicity, a higher concentration of Cr(VI) poses selection pressure and thus lengthens the growth phase of the bacteria. Also, it was observed that the removal rate decreased with an increase in time. For arsenic, the rate of removal was almost the same in the first five and last ten days. A similar trend was also observed in the case of chromium. In bacteria, bioaccumulation is known to take place in two stages: the first stage involves rapid removal through physical adsorption, and the second stage is slower and depends on the metabolism of bacteria (Alam and Ahmad, 2011). Jeyasingh and Philip (2005) found that bacteria isolated from the contaminated sites of Tamil Nadu Chromates and Chemicals Limited (TCCL), Ranipet, Tamil Nadu, India could reduce 5.6 mg/g Cr(VI) completely in 20 days. *Pannonibacter phragmitetus* sp. could remove 97.8% (from 462.8 to 10 mg/kg) of Cr(VI) from soil after 10 days (Chai et al., 2009). Karn et al. (2017) reported that *Pseudomonas* sp. XS4 decreased arsenic content from 50 mg/kg to 0.07 mg/kg after 7 days of treatment. It was also found that the use of $FeCl_3$ greatly influenced the adsorption of As(V).

Studies have also reported the use of microorganisms in association with plants to increase the removal efficiency and decrease the time taken for removal of heavy metal(loid)s. Babu et al. (2015) reported that *Pseudomonas koreensis* AGB-1 promoted the growth of *Miscanthus sinensis*, which in turn enhanced phytostabilization and subsequent remediation of arsenic, cadmium, copper, lead and zinc in mine areas. *Ensifer* sp. M14, an As(III) oxidizing bacteria, has been known to promote the growth of *Medicago sativa* (alfalfa) and enhance arsenic uptake in plants (Debiec-Andrzejewska et al., 2020). Liu et al. (2011) reported that 2.2% - 4.5% of arsenic could be removed from soil through biovolatilization after 30 days. In sterilized soil samples without bacteria, no removal of arsenic and chromium was observed (not shown in the graph), which shows that biotic factors are essential for the detoxification of heavy metal(loid)s in soil.

6.3 Effect of chromium on arsenic removal efficiency

The presence of chromium was found to affect the removal efficiency of bacteria for arsenic (Figures 6.1 and 6.2). Removal of arsenic by both TY6 and K7Pb, was relatively lower in the presence of chromium as compared to arsenic alone. The presence of multiple metals is known to cause a competitive effect, and bacteria have a selective affinity towards certain elements. In contaminated sites, single toxic metals rarely exist, they almost always exist in combination with other elements. Cr(VI) is highly soluble in water and thus more mobile (Wolińska et al., 2013), also this solubility may result in its greater availability to the bacteria. *Pseudomonas aeruginosa* has been known to be effective chromium reducers

(Malaviya and Singh, 2014) which may cause selective metal interaction and hence, reduced arsenic removal.

Also, Cr(VI) is more toxic as compared to Cr(III) (Chen et al., 2021; Deepa and Mishra, 2020; Naz et al., 2016) and thus, it further increases the stress on the bacterial population. Since biosorption involves adsorption, Cr(VI) with lower ionic radii compared to As(III) and As(V) gets preferentially adsorbed on the binding sites available on the bacteria and consequently, the arsenic removal efficiency decreases. The influence of various metals on bacterial remediation capacity can be site-specific and may be affected by several factors such as type of metal(loid)s, concentrations, and bacterial community (Priyadarshanee and Das, 2021). Thorough laboratory and field experiments are required to assess and understand the complex relationship that exists between soil, metal, environment and microbial community.

6.4 Effect of extraction medium on removal of arsenic and chromium

The use of an extraction agent plays an important role in the recovery of elements from the soil media, as the amount of recovered element may depend on the extraction medium used. Either a single chemical or a mixture of chemicals can be used for the extraction of metal(loid)s from soil media. In this study, only a single chemical was used per method as it minimizes environmental pollution and is also cost-effective. For arsenic, the removal efficiency was lowest when DW was used as the extraction medium and highest with $CaCl_2$. In general, the removal efficiency varied with different extraction agents in the order DW < AR < $CaCl_2$. A comparison has been shown in Figures 6.1 and 6.2. For As(III)_TY6, after 15 days, the maximum removal efficiency obtained was 14.58±0.73% in case of A_20 with DW, 61.82±2.47% for A_20 with $CaCl_2$, and 58.53±1.75% for A+C_20 with AR. With As(V)_K7Pb, after 15 days, the maximum removal efficiency obtained was 13.05% in case of A_20 with DW, 64.18±3.21% for A_20 with $CaCl_2$, and 62.79±1.88% in case of A_20 with AR.

For Cr(VI)_TY6, maximum removal efficiency of 5.15±0.26% with A+C_20 with DW, 54.54±2.73% for A+C_10 with NaOH and 52.27±2.09% for A+C_20 with AR was obtained after 15 days. For Cr(VI)_K7Pb, 5.15±0.18% in case of A+C_10 with DW, 56.06±2.80% for A+C_10 with NaOH, and 53.03±2.12% in case of A+C_10 with AR was obtained after 15 days. The removal efficiency varied with different extraction agents in the order DW < AR < NaOH, in general. A comparison has been shown in Figures 6.3 and 6.4. Several studies have reported the use of various agents for the extraction of arsenic and chromium from soil medium (Alam and Tokunaga, 2006; Kim et al., 2016). Deionized or distilled water has been found to be the least effective agent for arsenic extraction from soil by several researchers (Alam et al., 2001; Anawar et al., 2008).

Alam et al. (2001) have used several extraction agents, namely potassium chloride, potassium nitrate, potassium sulfate, and sodium perchlorate. It was found that these solutions were ineffective as only 0.3% of arsenic could be extracted from the soil and the process was found to be heavily dependent on pH. Other components present in soil can also dissolve with a decrease in pH. Hence, neutral pH may serve as the optimal condition for arsenic extraction and damage to soil can also be avoided. Use of mixed acid reagents such as AR is one of the most common methods employed for the extraction of arsenic from soil and is used to extract arsenic bound to relatively insoluble phases and minerals. Recovery with this method, in some cases, has been reported to be in the range of 74% - 110% (Hudson-Edwards et al., 2004). The use of strong acids or alkalis as an extraction agent may result in severe damage to soil properties. The extraction was highest for $CaCl_2$ but, in some cases, the extraction ability of AR was comparable to $CaCl_2$. The high extraction using $CaCl_2$ can be attributed to the complexation effect of chloride (Pueyo et al., 2004).

Generally, cation exchange solutions only extract the weakly adsorbed exchangeable metals in the soil and thus may result in lower extraction efficiency. Complexing agents are able to extract organic and carbonate-bound as well as exchangeable fractions and hence, are reported to have higher extraction efficiency (Ramtahal et al., 2015). NaOH used for extraction of Cr(VI) produces alkaline conditions that prevent its reduction from Cr(VI) to Cr(III) state, leading to increased extraction efficiency. Sonication breaks down the soil matrix and NaOH chemically alters the soil matrix; both facilitate the solubilization of Cr(VI) and thus provide a synergistic effect in the extraction of Cr(VI) from the soil.

6.5 Accumulation of arsenic and chromium in plants

Arsenic and chromium uptake by root and shoot of chickpea plants have been shown in Figure 6.5. The uptake of metal(loid)s in plants increased with an increase in arsenic and chromium concentration. Accumulation of both arsenic and chromium was higher in the shoot than in the root. However, a decrease in arsenic uptake was observed in the presence of chromium. The maximum accumulation of arsenic was 52.31±2.61 mg/kg in roots and 17.46±0.87 mg/kg in shoots in the case of As(V)_K7Pb. For As(III)_TY6 it was 42.70±2.13 mg/kg in roots and 15.50±0.77 mg/kg in shoots. In presence of Cr(VI), the uptake decreased to 41.34±2.06 mg/kg and 12.67±0.63 mg/kg in roots and shoots, respectively, for

As(V)_K7Pb. On the other hand, it decreased to 31.75±1.58 mg/kg and 12.05±0.70 mg/kg respectively, for As(III)_TY6. For Cr(VI), the maximum uptake observed was 27.46±1.37 mg/kg and 19.35±0.96 mg/kg in roots and shoots, respectively for K7Pb_Cr(VI), whereas it was 23.24±1.16 mg/kg and 17.35±0.86 mg/kg, respectively for TY6_Cr(VI). The results showed that accumulation was higher in roots than in shoots.

Tripathi et al. (2015) reported a maximum uptake of 12 mg/kg in roots of chickpea plant. Bacterial species have also been known to reduce arsenic stress in plants. Tripathi et al. (2017) reported that *Trichoderma* sp. helps reduce arsenic stress on chickpea and also restore anatomical deformation of the plants. Gulz et al. (2005) reported that maximum arsenic uptake was 566±27.3 mg/kg in silty loam and 601±10.0 mg/kg in sandy loam in roots of *Zea mays* and *Helianthus annuus* plant, respectively. Arsenic accumulation in plants have been reported to follow the order roots>shoots>leaves (Chou et al., 2014). However, the order of uptake in shoots and leaves can change with time. Khan et al. (2012) reported that *Paenibacillus lentimorbus* enhanced plant growth and could accumulate 4.10±0.27 mg/kg and 1.89±0.005 mg/kg Cr(VI) in root and shoot, respectively. *Mesorhizobium* strain RC3 has been known to promote chromium uptake and increase seed yield (Wani et al., 2008). Chromium accumulation in roots was over 40 mg/kg and around 30 mg/kg in shoots. Low uptake of arsenic can be due to methylation of arsenic in the rhizosphere by the bacteria as recently dimethyl arsonic acid has been found to be more phytotoxic as compared to As(V) (Tripathi et al., 2017). The uptake of metal(loid)s may also depend on the type of soil and the availability of nutrients to plants. The similarity of arsenic to phosphorous can result in competition between elements and may result in higher uptake of arsenic in plants.

6.6 Plant growth and chlorophyll assessment

Shoot lengths of plants were found to be greater in the presence of bacteria as compared to those without bacteria (Figure 6.6). Some of the samples used have been shown in Figures C1 and C2 (refer to Appendix C). In case of TY6, maximum growth was 19.05±5.38 cm after 15 days in presence of As(III), whereas it was 18.90±6.02 cm in presence of As(III) and Cr(VI), but for control (without bacteria) the maximum shoot length obtained was 9.83±3.46 cm for As(III) and 9.28±4.14 cm for Cr(VI). For K7Pb, 21.26±2.40 cm was the maximum shoot length after 15 days with As(V) and 17.12±9.43 cm with As(V) and Cr(VI), whereas for control, the maximum growth observed was 10.37±6.01 cm for As(V). A blank sample was also used for comparison without any bacteria or metal(loid)s contaminant, and the maximum growth observed was 27.52±7.40 cm after 15 days.

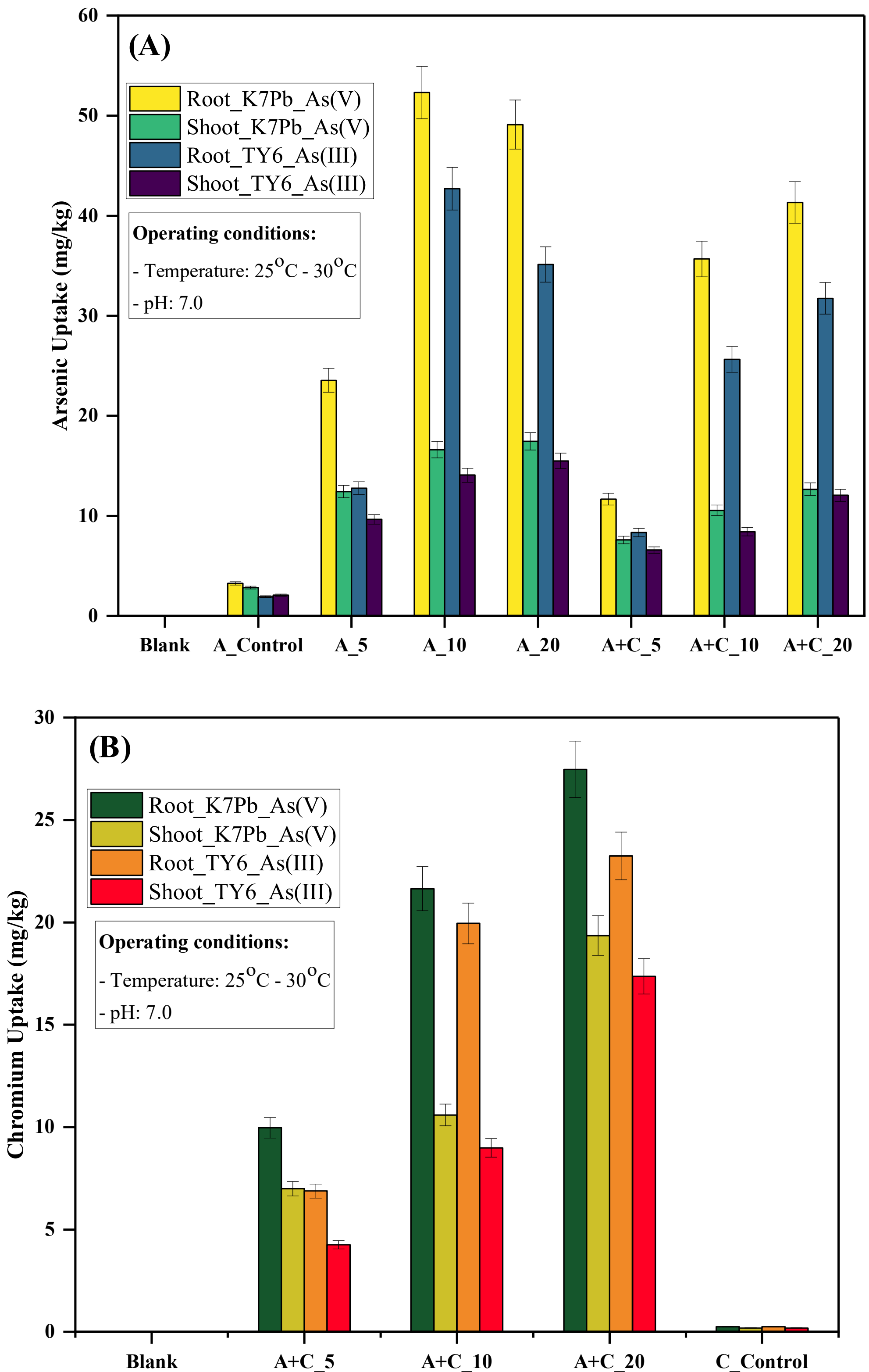

Figure 6.5: (A) Arsenic uptake **(B)** Chromium uptake in roots and shoots of plants mediated by TY6 and K7Pb

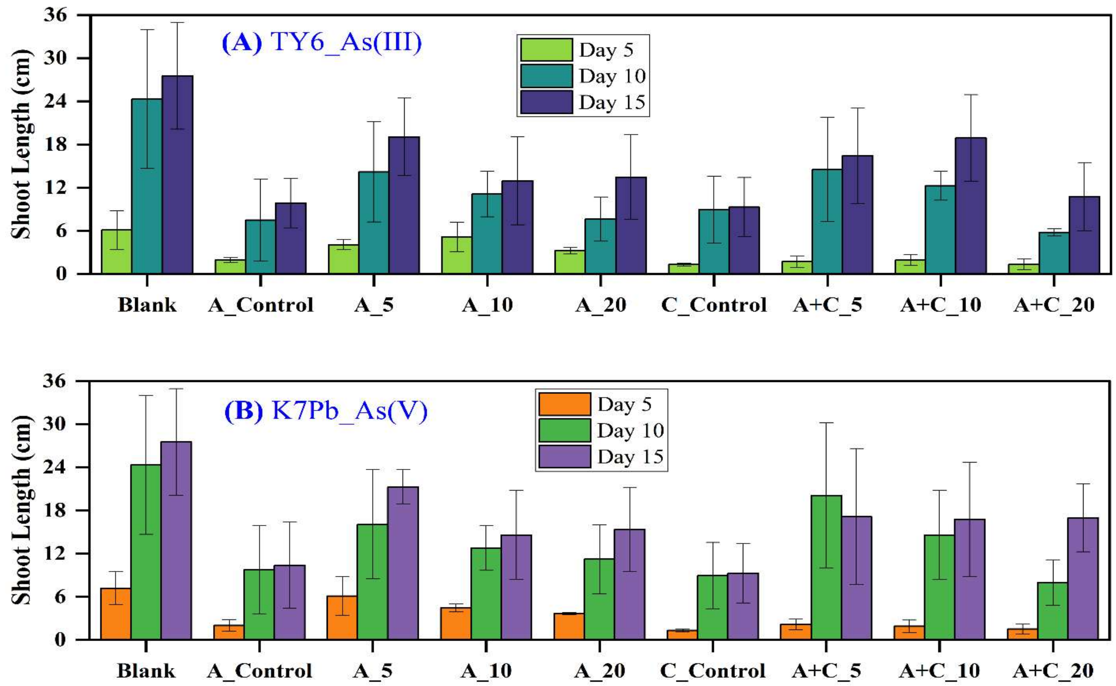

Figure 6.6: Effect of bacteria **(A)** TY6 **(B)** K7Pb on shoot growth of plants

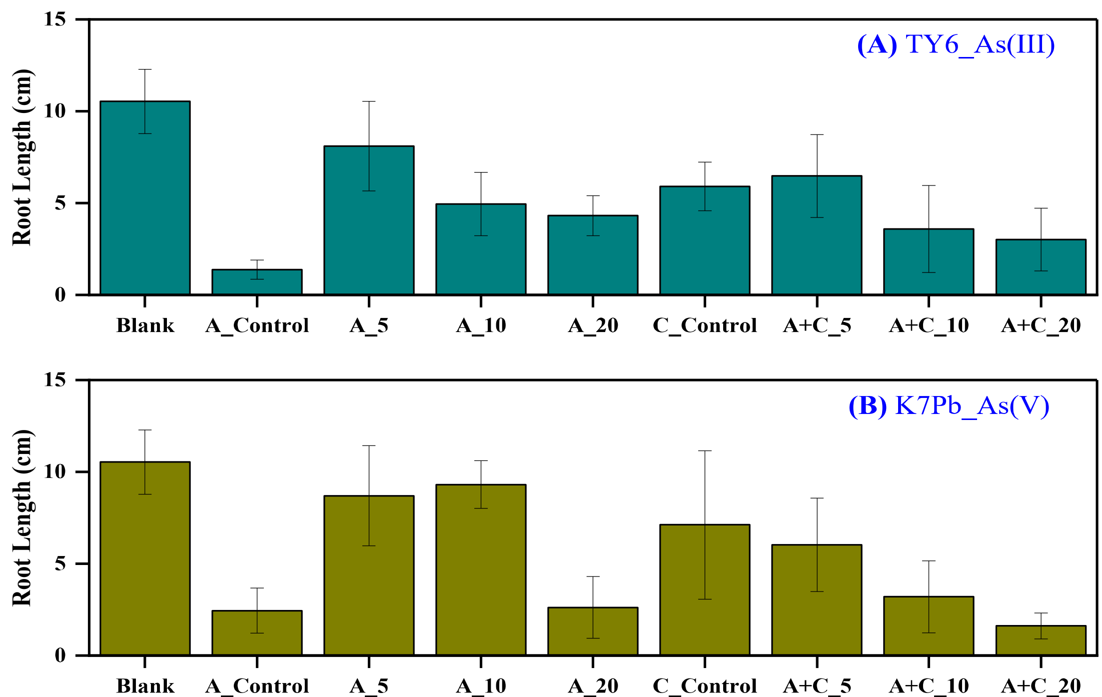

Figure 6.7: Effect of bacteria **(A)** TY6 **(B)** K7Pb on root growth of plant after 15 days

The results indicated that the presence of bacteria and metal(loid)s greatly affect plant growth. The shoot length decreased in presence of arsenic and Cr(VI), whereas bacteria helped increase the growth. Root growth for blank was 10.53±1.74 cm after 15 days.

Maximum root growth in case of K7Pb obtained was 9.31±1.29 cm, whereas for TY6, it was 7.32±3.52 cm. It was observed that, in general, the root length for K7Pb_As(V) was greater as compared to TY6_As(III) (Figure 6.7).

When both arsenic and chromium were present, root length was found to be lower for all cases, suggesting that arsenic and Cr(VI) had synergistic effects on the inhibition of root growth. Islam et al. (2015) have reported that when arsenic and chromium were present simultaneously in soil, chromium accumulation was relatively higher in roots of jute (*Corchorus olitorius* L.) due to their immobilization in vacuoles which resulted in lower growth of roots. Lower growth of control samples indicated that inhibition may be a result of phytotoxicity due to arsenic and chromium. The generation of reactive oxygen species results in the inhibition of energy-linked functions of mitochondria and affects chlorophyll synthesis and plant growth (Tripathi et al., 2017). Chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations were analyzed in leaves of chickpea plants after 15 days and have been reported in Table 6.2. It was observed that the leaves of plants from soil samples with bacterial isolates had higher chlorophyll content as compared to control samples, which had no bacteria.

Plants grown in soils contaminated with heavy metal(loid)s suffer from stunted growth, low biomass accumulation, low yield, etc. Heavy metal(loid)s are also known to trigger osmotic stress imbalance in plants (Mashabela et al., 2023). Better growth and increased chlorophyll content in soils with bacteria indicated that resistance and detoxification of arsenic and Cr(VI) by TY6 and K7Pb help regulate the osmotic pressure in chickpea plants and improve their growth. Stress due to heavy metal(loid)s inhibits the uptake of minerals. Reduced concentrations of minerals such as Mn^{2+} and Fe^{3+} due to the toxic effects of arsenic and chromium can result in decreased photosynthetic pigments (Islam et al., 2014) as can be seen in control samples. Arsenic and chromium stress have been known to decrease K^{+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} uptake in plants and result in nutritional imbalance. For sterilized soil samples, no growth or germination of seeds was observed (not shown), which employed that only abiotic factors are not capable of supporting plant growth and it requires the interaction of both biotic and abiotic components.

One of the most common ways by which bacteria resist the toxic effects of heavy metal(loid)s is biofilm formation, which is formed by extracellular polymeric substances called exopolysaccharides (Armendariz et al., 2015; Fujishige et al., 2006). It may imply that bacteria (TY6 and K7Pb) may be carrying plant growth-promoting traits such as auxin production and phosphate solubilization, which are responsible for enhanced plant growth (Shafique et al., 2017).

Table 6.1: Summary of chlorophyll assessment in leaves

	TY6_As(III)			K7Pb_As(V)		
Sample	**C_ *a***	**C_ *b***	**TC**	**C_*a***	**C_ *b***	**TC**
Blank	0.81	1.50	2.05	0.83	1.81	2.62
A_Control	0.60	1.12	1.62	0.60	1.55	1.62
A_5	1.10	2.06	3.08	1.35	2.52	3.84
A_10	0.89	1.65	2.34	0.97	1.12	2.24
A_20	0.84	1.55	2.13	0.85	1.57	2.17
C_Control	0.49	0.91	1.36	0.49	0.93	1.41
A+C_5	0.49	0.92	1.37	0.51	0.95	1.43
A+C_10	0.56	1.04	1.43	0.56	1.04	1.50
A+C_20	0.56	1.02	1.37	0.58	1.07	1.47

C_*a = Chlorophyll *a*; C_*b* = Chlorophyll *b*; TC = Total Chlorophyll

6.7 Summary

This study was focused on arsenic and chromium removal from soil using bacteria, *Pseudomonas aeruginosa* strain K7Pb and *Proteus alimentorum* strain TY6. Arsenic extraction from soil was carried out by using $CaCl_2$, DW and AR, whereas chromium was extracted with NaOH, DW and AR. For arsenic, $CaCl_2$ was the most effective extracting agent with maximum efficiency of 61.82±2.47% and 64.18±3.21% for As(III) and As(V), respectively after 15 days. On the other hand, NaOH proved to be the most effective agent for Cr(VI) with a maximum removal efficiency of 54.54±2.73% and 56.06±2.80% with TY6 and K7Pb, respectively, after 15 days. The study revealed that both bacteria can be effectively used for the removal of arsenic and chromium from soil and may possibly help in plant growth.