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Synergistic effects of microbes and plant for remediation of chromium contaminated soil

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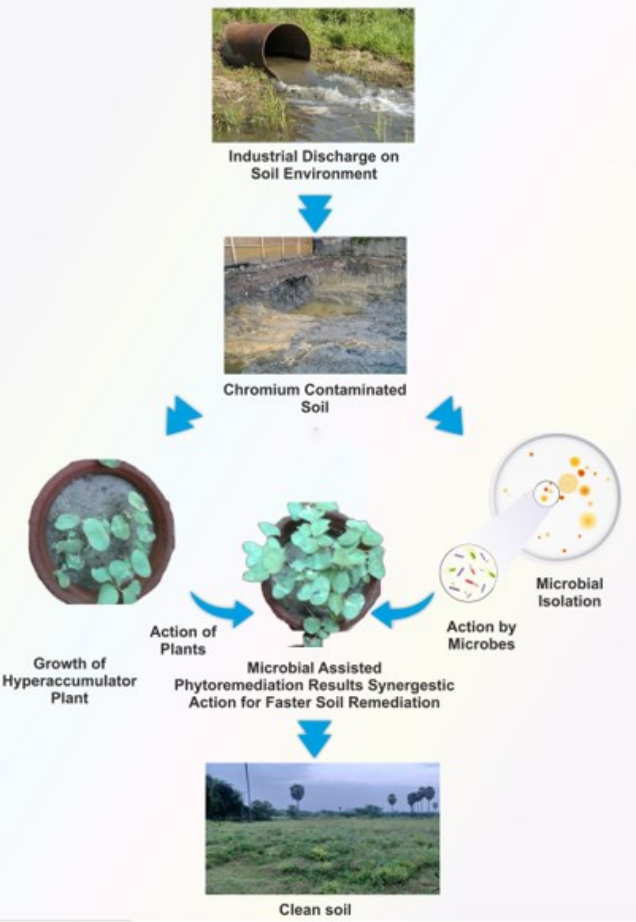
Graphical abstract



Abstract

The contamination of soil is a serious issue to the environment caused by the human activities in the form of improper disposal methods. There are several contaminated dumpsites in India where, hazardous and other wastes contain heavy metal were dumped historically, which subsequently results in the contamination of soil, surface and subsurface water, and land. One of such heavy metal pollutant is Chromium. There are many conventional methods available for reclaiming soil and water which are contaminated by chromium and other heavy metals. However, the biological or bioremediation technique is the most widely used method for in-situ application. In this experimental study, the synergistic action between the plants and bacteria for effective remediation of chromium contaminated site was studied. This study aims to address the capability of reduction of chromium (VI) by bacterial strain and to assess the synergistic linkage between the plants and the microbes. Under laboratory conditions, the bacteria that was isolated from the chromium-contaminated soil was able to withstand upto 500 mg/L of Cr (VI). By, 16S rRNA method, *Staphylococcus saprophyticus* bacterium was identified. The SDS PAGE analysis showed that the reductase enzyme was expressed during the chromium remediation process by *Staphylococcus saprophyticus*. The synergistic action between plants the selected plants like Napier grass, Cotton, Sorghum, Nerium and Jatropha and the identified *Staphylococcus saprophyticus* was studied. The research shown that the synergistic effects raise the chromium accumulation and enhance the plant biomass, root length, and shoot length. The higher accumulation was found in the roots than leaves. The accumulation was found to be in the order of Napier grass (229.955 mg/kg) > Cotton (179.98 mg/kg) > Nerium (116.353 mg/kg) > Jatropha (84.735 mg/kg) > Sorghum (61.71 mg/kg) in control plants. The combination of Napier grass and *Staphylococcus saprophyticus* shows the best combination for chromium reduction. In view of this the synergistic action of plants and bacteria helps in accumulation of higher concentration of chromium without causing any toxic effects to the plant. Apparently it enhances the efficiency of bioremediation process and can be successfully applied for the reclamation of chromium contaminated sites.

Keywords: Heavy metal, chromium, synergism, *staphylococcus saprophyticus*, bioremediation, phytoremediation

1. Introduction

As soil is the predominant natural resource for food production in agricultural revolution. Prevention of soil

degradation is the top most priority in the global sector. Due to the fast industrialization, the waste generation is also increasing accordingly. Globally, Tanneries are considered as one of the major source of chromium pollution to the environment. The waste may be of various forms, but the heavy metals is a critical problem for the environment. Through bioaccumulation, toxic heavy metals and metalloids can build up in the soil and have an impact on the local soil fertility, plants, and health hazards in the hierarchy of food chains. (Wuana & Okieimen 2011 and Upadhyay *et al.* 2017). The wastewater discharging into the environment without treatment cause serious threat to humans in developing countries where advanced treatment technologies are not affordable, this condition necessitates an eco friendly approach and a cost effective technology to remediate it.

The heavy metals which contaminate the soil are mostly due to the industrial application. The metal which is deposited in the soil can change into more mobile form that might migrate into the soil water posing a risk to plants, groundwater and other biota (Ermakov *et al.* 2018). For instance, a soil's infertility may result from a higher concentration of heavy metals like chromium. (Pajuelo *et al.* 2008) and subsequently reduces the yield of crop. Thus our study provides the treatment for chromium reduction in the contaminated soil.

Generally, Chromium is widely used in three types of industries namely metallurgical, chemical and refractory (Ayele and Godeto 2021) and one of the most commonly used in stainless steel industry for making anti corrosive steel. It is found in oxidation states between +2 and +6 Cr. (J and Ravisankar 2014). Though there are various forms of chromium, the most common one is trivalent chromium (Cr III) and hexavalent Chromium (Cr VI) (Thatoi *et al.* 2014). The carcinogenic and mutagenesis features of Cr (VI) make it even more dangerous than Cr (III) (Velez *et al.* 2017). It also causes changes in the morphology of gramme positive and gramme negative bacteria (Mishra and Bharagava 2016). The higher amount of chromium consumption in human may lead to reduction of hemoglobin. Various health risks due to chromium in human and animal are lung disorder, unfertility and cardiovascular disorders (Engwa *et al.* 2019). In plants, the presence of heavy metals has a significant impact on plant growth and negatively impacts seed germination, pigment degradation, nutritional imbalance, anti-toxicant depletion, and enzyme depletion. The Higher amount of chromium level attacks the chloroplast which destroys the photosynthesis process (Asati *et al.* 2016). Among the various methods available for chromium reduction (Ayele and Godeto 2021) phytoextraction (Lotfy and Mostafa 2014) and Bioremediation (J and Ravisankar 2014) are based on natural process which relies on the functions of bacteria, fungi and plants to reduce, remove and degrade the environmental pollutants which leads to restoring the contaminant sites to a clean non toxic environment. This study particularly made an attempt to found the synergistic linkage between plant and microbes.

Synergism is defined as an effect that results from the interaction of two or more agents, entities, or substances that is greater than the total of the effects of those agents, entities, or substances alone. Although plants and microbes has potential as a viable remediation strategy for persistent heavy metals, pollutants above the permissible limit may be harmful to both the plants and micro organisms related subsequently decreasing remediation. To increase the plant biomass in contaminated soils, the bacteria can be used to mitigate the stress of plant to enhance the growth and degrading the contamination. This is carried out by Endophytic, Rhizobacteria and Arbuscullar Mycorrhizal Fungus (AMF) (Peng *et al.* 2009). Plant and microbes combination is a well adaptive technology for the remediation of contaminated soil (Hansda *et al.* 2014). The microbe mostly of its native species which stimulates the growth of plant and degradation of pollutant in the soil can be a potential natural system to remediate the contaminated soil (Kamran *et al.* 2017 and Nayak *et al.* 2018). Thus the objectives of this present work are: a) To assess the capability of reduction of Cr (VI) by bacterial strain isolated from the soil obtained from the contaminated site. b) To analyze the phytoextraction efficiency of the hyperaccumulator plants. c) To analyse the removal efficiency of Cr (VI) on plant – microbe interaction for enhanced chromium accumulation in plants.

2. Materials and methods

2.1. Soil sampling and characterization

The soil samples were obtained from the contaminated sites of electroplating industry in Coimbatore, Tamil Nadu, India. The obtained samples were combined, allowed to air dry, and then sieved with a 2 mm sieve to remove the coarse particles. The physio-chemical parameters such as pH, electrical conductivity, nitrogen, potassium, phosphorus and organic matter were examined in the laboratory (IS code) (Shi *et al.* 2020). A triple acid digestion process was used to digest the dirt. For digestion, mixture of nitric acid, sulphuric acid and perchloric acid was mixed in the ration of 9:3:1, 1 g of soilwas mixed with 25 ml of triple acid digestion solution and digested overnight. The sample was then heated to 110°C till it turns white (Shi *et al.* 2020) and (Santiago and Santhamani 2010). Then the sample was filtered and diluted. The Chromium (IV) concentration present in the sample is determined using the Plasma-Optical emission spectroscopy (ICP-OES).

2.2. Isolation of bacteria

10 g of the contaminated soil was suspended in 100 mL of sterilized saline (0.85 percent NaCl solution) and shaken for 10 minutes. Then, the extraction was serially dilutedto 1000, 10,000 fold dilution and spread on Luria-Bertani Agar (LBA) plates amended with 50 mg/L of Cr (VI) (10 g tryptone, 10 g NaCl, 5 g yeast extract, and 20 g agar in 1 L deionized water, pH 7) in triplicates and incubated at 37 °C for 7 days to findout the presence of Cr (VI) utilizing bacteria in the soil (Wu *et al.* 2019). The isolated obtained from the plates were inoculated with 100 mg/L of Cr (VI) and incubated for 2 days. Then, the solutions were serially diluted and plated in LBA plates to access the isolated

capable of growing in increasing Cr(VI) concentration. This step was carries out in the incremental order of 100 mg/L starting with 200 mg/L to 500 mg/L of Cr (VI). The declination of microbial growth was observed which shows that the bacteria were resistant or tolerant to higher concentration of chromium was able to grow at increasing concentration. At 500mg/L, a white colony that was found in abundant was taken for the study. This experiment was repeated to check the reproducibility and was successful in each trial.

2.3. Identification of isolated species present in the polluted soil

Identification of the isolate was carried out using 16S rRNA sequencing. The 16S rRNA gene was amplified by PCR using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5' GGTTACCTTGTTACGACTT-3') after genomic DNA extraction. The BLASTN algorithm was used to compare the 16S rRNA gene sequences to known the sequences from NCBI database. These sequences were aligned with ClustalW, and a phylogenetic tree was built using Molecular Evolutionary Genetic Analysis' neighbour-joining method. MEGA 6 is a piece of software (Aslam *et al.* 2020). 1 PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, 20 mmol/L MgCl2 pH 8.3, 0.01 percent (w/v) gelatine), a combination of 0.2 mmol/L dNTPs/each, 2.5 U Taq DNA polymerases, 0.5 mmol/L from each primer, and 50 ng/L bacterial DNA template were used in a total volume of 50 Initial denaturation at 94°C for 3 minutes and 35 cycles; denaturation at 94°C for 1 minute; annealing at 48°C for 2 minutes, extension at 72°C, and final extension at 72°C for 7 minutes. EXOSAP-IT (Ambion, CA) was used to purify the PCR product, which was then sequenced forward and reverse using PCR primers (Mohamed *et al.* 2020).

2.4. Studies on the reduction of Cr. by the bacterial isolate

The diphenylcarbazide (DPC) method, which determined the reduction of hexavalent chromium concentration, was used to test a bacterial isolate's capacity to convert Cr (VI) into Chromium (III), a harmless form of the metal. The 24 hour-old growing culture was inoculated in 100 ml of LB broth with 50 mg/L of Cr (VI) and incubated in a rotating incubator at 30°C to calculate the chromium reduction. The experiments were carried out in duplicates.5 mL of the culture was removed in the aseptic conditionat regular intervals of 12 hrs, untill 3 days. The bacterila suspension wascentrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. The remaining concentration of Cr (VI) in the supernatant was assessed using a spectrophotometer to measure the absorbance of the Cr (VI)-DPC complex at 540 nm at various time intervals. The percentage of chromium reduction was determined by the formula given below. (Upadhyay *et al.* 2017)

$$\text{Cr(VI) Reduction in \%} = \frac{A-B}{B} \times 100$$

where, A- Absorbance of control, B- Absorbance of sample

2.4.1. Preparation of mass culture

Bulk production of the microbial isolate was needed for the study of synergistic activity of the bacteria along with the plants for treatment of chromium. This was done using mass bacterial culturing or mass production process. It is the process of production of higher quantity of required organism. This is done in a particular media and mixed with carrier before introducing into the soil. The media provides nutrition for the growth of microbe and carrier acts as substrate for the microbe. In this process, mass culturing was done in LB broth. The bacterial strain which was isolated from the contaminated soil has the capacity to grow at 500mg/l. The bacterial strain was prepared to 5L in the laboratory and used for the study.

2.5. Selection of hyper accumulator plant for the remediation of chromium

Cotton (*Gossypium arboreum*), Sorghum (*Sorghum bicolor*), Napier grass (*Pennisetum purpureum*), Nerium (*Nerium oleander*) and Jatropha (*Jatropha curcas*) plants were chosen to study the reduction of chromium in polluted soil based on the review of the literature, Cotton plant has the capacity of absorbing heavy metals. It is crucial to use transgenic cotton cultivars for Cr(IV) remediation in Cr-polluted areas since they are being rapidly grown in industrialized parts of the world (Lotfy and Mostafa 2014). Sorghum is a grass type plant classified as one of the hyperaccumulators as it accumulates higher concentration of Cr(IV) (Karimi 2013). The presence of cadmium increases the biomass of Napier grass drew the attention in phtoremediation. Very few researchers worked with Napier grass for heavy metal phytoremediation (Juel *et al.* 2021). It helps in hyper-accumulating higher concentration of various heavy metals. It has been found that, it is a good bioindicator of Zn and Cu. *N. oleander* acts as remover of Al, Ba, Cr, Fe and Pb. Jatropha has been found that it is very effective in removal of Cr(VI). *J. curcasto* has the capability to absorb zinc, copper, and chromium from sewage sludge and has the ability to phytoremediate cadmium and lead from polluted soil (L. Awotedu and O. Ogunbamowo 2019).

2.6. Experiment on synergistic action

2.6.1. preparation of seeds and planting

The seeds of cotton & sorghum were procured from Seed production institute, Ramanathapuram, Tamilandu, India. The seeds were cold treated at 10^0C for 3 days to break dormancy and synchronize germination. The surface of the seeds were sterilized by washing in 10% bleach for 15mins, then with sterile distilled water, then with 70% ethanol finally with distilled water. The seeds were cocultured with the bacterial strain. Co culturing is a technique of mixing of two types of cells in vitro to allow for synergistic or antagonistic interactions. The seeds were added to the 50ml of bacterial solutions and kept in incubator at 30^0 C overnight. Then the seeds were transferred to sterilized petri dish containing cotton where it was allowed for germination. After the germination, the seeds were transferred to the pots (Kamran *et al.* 2017).

2.6.2. Preparation of mass culture

Bulk production of the microbial isolate was required to study the synergistic activity of the bacteria along with the plants for treatment of chromium. This was done using mass bacterial culturing or mass production process. It is

the process of production of higher quantity of required organism. This is done in a particular media and mixed with carrier before introducing into the soil. The media provides nutrition for the growth of microbe and carrier acts as substrate for the microbe. In this process, mass culturing was done in LB broth. The bacteria strain that were recovered from the polluted soil have a 500mg/l growth rate. The bacterial strain was prepared to 5L in the laboratory and used for the study.

2.6.3. Plantation with bacterial cultured soil

The bacteria obtained through mass culture were mixed with the soil. A precultured bacterial strain was introduced into soil samples at a concentration of 0.5 mg/kg of soil. The study was carried out in triplicate (Nayak *et al.* 2018).

The germinated seeds of cotton & sorghum, seedlings of uniform shoot length and root length were planted in a control pot. This was placed in green house and watered regularly using distilled water. Similarly, the saplings of Napier grass, Nerium and Jatropha were planted. Compost was added as fertilizer once in a week to support the growth. The contaminated soil and bacterial biomass were incubated with the plants, which were then allowed to grow for 5 weeks before being harvested. Plant samples were collected after five weeks and overnight dried to determine the amount of heavy metals in the plants and soil. Atomic absorption spectroscopy (AAS) was used to measure the concentration of Cr(VI) after the dried samples were acid digested to extract the metals.) (Shi *et al.* 2020).

3. Results and discussion

3.1. Physico chemical characteristics of contaminated soil sample

The physico chemical characteristics of soil was examined where the pH of the soil was found to be 8.2 (alkaline). It resulted due to the soil's exposure to a number of metallic salts. The pH was within the limit for the growth of plant. The EC was 6.069 mS and it is higher in limit. Because there are more dissolved salts in the soil sample, the EC is higher. The texture of soil was found to be sandy. The presence of Phosphorous was higher (16966 mg/kg) in the soil, as it was widely used in the electroplating industry. Nitrogen and organic materials both made up 1% of the sample. The Potassium in the soil were <0.1% (Table 1).

Table 1. Physiochemical Analysis of contaminated soil

Sl.No	Parameters	Units	Values
1	Texture	-	Sandy soil
2	pH	-	8.2
3	Electrical conductivity	mS	6.069
4	Nitrogen	%	1
5	Potassium (as K)	%	< 0.1%
6	Phosphorous	mg/Kg	16966
7	Organic matter	%	1
Concentration of chromium and other Heavy metals			
1	Total chromium	mg/Kg	940
2	Zinc	mg/Kg	88.2
3	Iron	mg/Kg	413.15
4	Manganese	mg/Kg	277.7

To analyze the presence of chromium concentration, the sample was digested using triple acid and filtered. In view of that Chromium was found to be 940 mg/kg of soil which exceeds the permissible limits. The other heavy metals that were determined are Zinc, Iron and Manganese. The concentration of those heavy metals were tabulated (Table 3.1). Since the soil was taken from the electroplating industry, Chromium and other heavy metal concentrations were found to be higher. Chromium was used as Potassium dichromate salt in gold plating, H_2CrO_4 salt in chromium plating and in post treatment process. The other heavy metals like Zinc, Iron and Manganese were required for a post treatment process called as Phosphating. Heavy metal concentrations in the soil environment have increased as a result of the discharge of unneeded chromium and other heavy metals into the water and soil. Since the concentration level of these toxic heavy metals were higher in the contaminated soil, a remediation process is required, further more to enhance the degradation process the soil was treated using the plants and microbes which acts synergistically for the reclamation of the soil.

3.2. Identification of bacterial species for the reduction of chromium

The 16S rRNA analysis shows that the sequence of isolate was 99.9% similar to *Staphylococcus saprophyticus.* It belongs to the genus Streptococcus. The *Staphylococcus* genus are gram positive, non-motile cocci and facultative anaerobic. They grew in clusters and are pathogenic.

3.3. Efficiency of staphylococcus saprophyticus in Cr(VI) removal using bacterial strain

The bacterial strain been used to the decrease in heavy metal toxicity and accumulation in plants. The bacteria were inoculated in 100ml of LB media containing 500mg/L of Cr (VI). The reduction was detected by 1,5 – Di phenyl carbazide method. The bacteria showed the reduction of 31.68mg/L after 12hrs, then to 202.33mg/L after 24hrs, 164.26mg/L after 36hrs, 94.54mg/L after 48hrs, 52.79mg/L after 60hrs and 12.83mg/L after 72hrs respectively, a removal efficiency of 97.43% was obtained after 3 days of incubation. This was done under aerobic condition. Figure 1 shows the removal efficiency of chromium by the microbe over time. The removal efficiency increases as the time increases (Figure 1). From the process it was found that the bacteria *Staphylococcus saprophyticus* as the strain to remediate Cr (VI) and thus it was selected for the study.

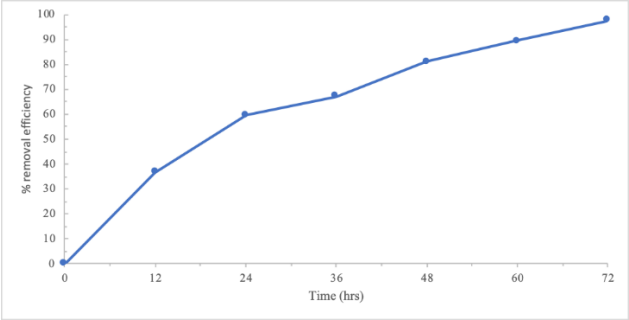


Figure 1. Removal efficiency of Cr (VI) by *Staphylococcus saprophyticus*

The degradation of Cr (VI) was studies for the bacterial isolate in controlled environment under aerobic condition. The conversion of Cr (VI) to Cr (III) was detected by 1,5 – Di phenyl carbazide method. Figure 1 shows the removal efficiency increases as time increases. 97.43% degradation efficiency observed within 72 hrs. 50% reduction was obtained within 24 hrs. This shows that the bacterial isolate from the contaminated soil is capable to treat Cr (VI) up to 500 mg/L.

3.4. Effects of synergistic action between plant and microbes

Chromium buildup was substantially less common in non-inoculated plants compared to control conditions, when Staphylococcus saprophyticus was administered to plants in a synergistic action. This action shows good results in the production of biomass in shoots and roots. the interaction between various plant species and Chromium accumulation in root of plant under control and treated were discussed below

3.4.1. Interaction between Napier grass and staphylococcus saprophyticus

The Napier grass was planted in the soil and grown for 5 weeks. At the first week the grass started to dry. After 1 week, new leaves were developed in both control and the treated plants. The grass planted in inoculated soil showed good biomass than in control. No damage or pest infection was observed in the grown grass. Chromium was shown to accumulate more in the roots of plants growing in soil mixed with *Staphylococcus saprophyticus* than in the leaves. The Accumulation in root was found to be 311.55mg/kg where as in control it was found to be 44.63mg/kg. The accumulation in shoot was found to be 148.36mg/kg and in control was found to be 29.7mg/kg. in Figure 2. The grass was found to be an effective plant that can be grown in contaminated sites and accumulation of heavy metals (Xia 2004 and Zhang *et al.* 2010).

3.4.2. Interaction between cotton and staphylococcus saprophyticus

Cotton was sown as seeds after co-culturing with *Staphylococcus saprophyticus*. The plant showed good survival growth. The seedlings showed growth after 4 days of planting in the soil. In inoculated soil, the plant fared better than in control soil. This is as a result of the heavy metal toxicity in the control soil. The fiber crops show higher accumulation of toxic metals within its part. The Accumulation within root was found to be 227.96mg/kg where as in control it was found to be 103.96mg/kg. The accumulation in shoot was found to be 133.84mg/kg and in control was found to be 54.6mg/kg. The cotton plants have the ability to accumulate heavy metals (Bailey *et al.* 1999).

3.4.3. Interaction between Nerium and Staphylococcus saprophyticus

The plant was able to grow for 1 week from plantation. Later it started to dry and almost died after 3 weeks. This is due to the higher concentration of chromium which was toxic to plant. The Accumulation in root was found to be 128.86mg/kg where as in control it was found to be 84.158mg/kg. The accumulation in shoot was found to be 103.89mg/kg and in control was found to be 74.05mg/kg.

3.4.4. Interactions between sorghum and staphylococcus saprophyticus

The sorghum seedlings showed the growth from the third day of germination. In comparison to the control, the inoculated soil had a higher rate of plant growth survival. The plant showed increase in biomass than in the control. The bacteria helped in increasing the biomass of plants. These plants developed mechanism to withstand higher concentration of heavy metals. It acts as phyto-accumulator, where plants can accumulate fewer amounts when compared to hyper accumulator plants (Karimi 2013). Accumulation in root was found to be 74.70mg/kg where as in control it was found to be 39.6mg/kg. The accumulation in shoot was found to be 48.72mg/kg and in control was found to be 12.6mg/kg.

3.4.5. Interactions between jatropha and staphylococcus saprophyticus

The plant showed growth till first week and later started drying. The same condition was observed in both the control and inoculated soil condition. This is due to the toxic nature of chromium to the plants (Mangkoedihardjo, Ratnawati and Alfianti 2008). The Accumulation in root was found to be 114.85mg/kg where as in control it was found to be 58.6mg/kg. The accumulation in shoot was found to be 54.62mg/kg and in control was found to be 16.5mg/kg. Figure 2 shows the concentration of chromium accumulated by the plants and Figure 3 shows the effect of heavy metal on the plants grown in control soil (without bacterial inoculum) and treated soil containing bacterial inoculum.

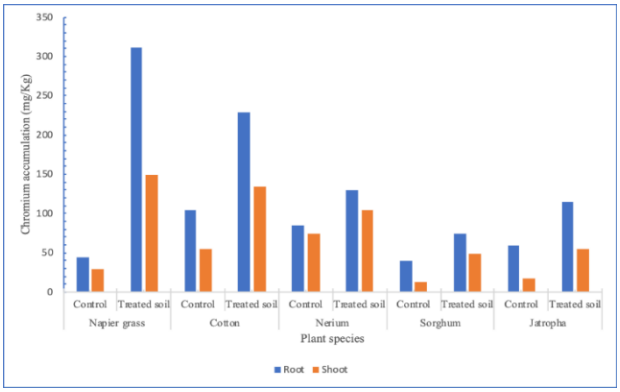


Figure 2. Chromium accumulation in root of plant under control and treated

Figure 3. Effect of contaminated soil on plants

3.4.6. Synergistic effect on other heavy metals

Additionally, the synergistic impact on the buildup of additional heavy metals was identified. Heavy metals like Zinc, Iron and Manganese were determined by AAS. The synergistic effect on the accumulation of other heavy metals was also determined. Heavy metals like Zinc, Iron and Manganese were determined by AAS. Napier grass and cotton accumulated higher concentration of Iron. Nerium had the capacity to increase the content of manganese in its body. This demonstrates that these plants are suitable for phytoremediation of heavy metal-contaminated locations since they were able to acquire other heavy metals. The accumulation of heavy metals by the plants is depicted in Figure 3.

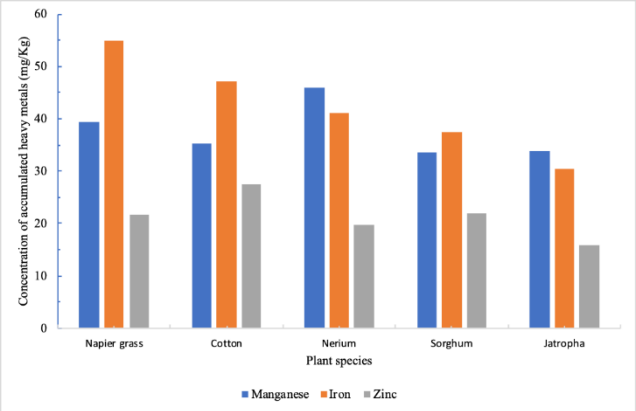


Figure 3. Heavy metal accumulation in plants

3.5. Removal of chromium by synergistic action

The plants along with the bacteria under synergistic action were able to accumulate higher concentration of chromium. The Cr (VI) was carried into plants by metabolic pathway and Cr (III) was taken to plants by non metabolic pathway: (R.A. Skeffington, P.R. shewry 1976). There was an active intake of Cr species was observed in Barley. This result was similar to the study. The uptake of Cr depends on Cr concentration, soil texture and plant species. The increase in accumulation of chromium by the plants involves one the following process. It might be caused by the creation of substances that promote growth, such as indole-3-acetic acid (IAA), siderophores that are found to increase plant growth in soils with elevated Cr levels (Davies 1937). The siderophores help plants accumulate more chromium by combining with divalent or trivalent metal ions (Polti *et al.* 2011). The release of organic exudates from plants helps in the higher accumulation of chromium from the soil. The production of acids or phosphates from plants and microbes also increases accumulation of chromium. By inoculating plants with plant-associated bacteria, it was discovered that as metal extraction increased, B. juncea, Brassica napus, Sorghum bicolor, and Solanum nigrum biomass output rose (Chen *et al.* 2010). The plants cultivated in the inoculated soil also showed an increase in biomass, root length, and shoot length. Bacteria have been shown in multiple studies to increase the root and shoot length of plants like zea mays. (Marta A. Poltia *et al.* 2011). From the study it was found that the root and shoot of Napier grass shows higher chromium accumulation with *Staphylococcus saprophyticus* and the least accumulation was found in Sorghum. The other interaction of plants and *Staphylococcus saprophyticus* were found in the order of Napier grass > Cotton> Nerium > Jatropha > Sorghum. Figure 4 shows the chromium accumulation in root and shoots system of the slected plant species.

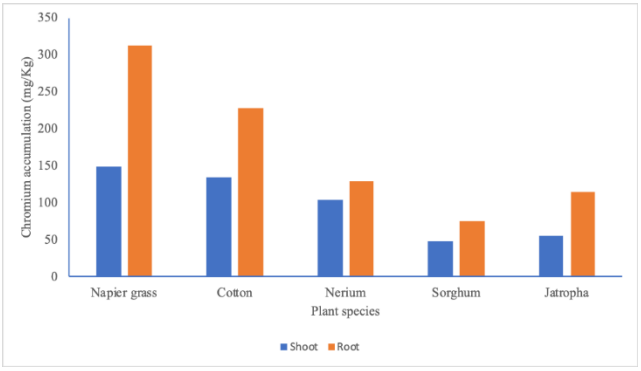


Figure 4. Comparative results of synergism in plant species

4. Conclusion

- This study showed that the synergistic action between the microbe and plants helps in the accumulation of higher concentration of chromium.
- The soil contained 940 mg/kg of chromium. The microorganism that could remove chromium from the environment was isolated from the soil.
- The 16S rRNA was performed and the bacterium was found to be *Staphylococcus saprophyticus*. The SDS PAGE was carried out which detected the expression of reductase enzyme that was responsible for the Cr (VI) conversion.
- The *Staphylococcus saprophyticus* was mass cultured and mixed with soil. The plant species like Napier grass, Cotton, Nerium, Sorghum and Jatropha were planted to the soil. The plants were able to accumulate higher concentration of chromium in its parts.
- In control plants, the plants were also able to demonstrate an increase in biomass. The higher accumulation was found in the roots.
- This was because the plants immobilize chromium and absorbs it into the root and stored in the vacuoles, The Cr was taken by dependent and independent mechanism in leaves and shoots and stored in the xylem of plants. The higher accumulation was found in roots, this was because of the plants immobilize chromium and absorbs it into the root and stored in vacuoles.
- The plants roots and leaves were also able to accumulate other heavy metals like Manganese, iron and zinc in it.
- The production of IAA and other organic substance increases the accumulation by the interaction between bacteria and plants, which caused the metals to mobilize and enhance the accumulation in plants, was the primary cause of

this rise in accumulation. The suitable combinations of plant and S. saprophyticus was found to be Napier grass Cotton> Nerium > Jatropha > Sorghum.

- The synergistic strategy is a successful one in bioremediating chromium contaminated soil with economic and without affecting the cycles of ecosystem. The ultimate aim of synergism is to convert Cr(VI) into Cr(III).
- This study shows that the best and most economical strategy for cleaning up chromium-contaminated sites by the synergistic action of plants and microorganisms.

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