RP Current Trends in Agriculture and Environmental Sciences
Vol. 1, No. 1 (July – September 2022) pp. 15 – 28
e-ISSN: 2583-6293

RESEARCH PLATEAU PUBLISHERS

Cite this article: Vijayshree, R.K. Lohchab, Bioremediation of chromium (Cr^{+6}) contamination using phytoextraction and biosorption, *RP Cur. Tr. Agri. Env. Sci.* **1** (2022) 15 – 28.

Original Research Article

Bioremediation of chromium (Cr^{+6}) contamination using phytoextraction and biosorption

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ARTICLE HISTORY
Received: 22 June 2022
Revised: 03 September 2022
Accepted: 04 September 2022
Published online: 06 September 2022

KEYWORDS
Bioremediation; chromium contamination; phytoextraction; biosorption.

ABSTRACT
Heavy metal contamination can be found in aqueous waste streams and soils from a variety of sectors, and because they are not biodegradable, they tend to accumulate in living things. Therefore, it is necessary to lower the pollutant concentration to a permitted level. There are numerous methods for reducing heavy metals, including electrodialysis, ion exchange, reverse osmosis, and ultra-filtration. These procedures are quite expensive and consume a lot of energy and additional chemicals. Therefore, a new effective and affordable approach for heavy metal removal is required. An developing approach called bioremediation makes use of the ability of both plants and microbes to change or absorb metals from soils and aqueous solutions. Using Brassica juncia and Pseudomonas sp. to biosorbent Cr from an aqueous solution, we applied this technology to remove Cr from soils in the current study. For the purpose of accumulating Cr^{+6} in the plant's roots and shoots, we employed Indian mustard. We discovered throughout the investigation that the roots of B. juncia eat the majority of the heavy metal, whereas the shoots only take up very small amounts. When the chelating chemical EDTA was used, the amount of Cr in the roots was substantially doubled, but no Cr reached the shoots (Undetectable). The amount of Cr^{+6} that Brassica without EDTA uptakes was very low at lower concentrations.

1. Introduction

Any metallic chemical element with a relatively high density that is dangerous or poisonous at low concentrations is referred to as a heavy metal. The Earth's crust naturally contains heavy metals. They sporadically get into our systems through food, water, and air. Some heavy metals are necessary for maintaining the body's metabolism as trace elements [1, 2]. However, they can cause toxicity at higher amounts. Because they have a propensity to bioaccumulate, heavy metals are hazardous. When a chemical's concentration in a biological organism rises over time relative to its concentration in the environment, this is referred to as bioaccumulation. Living organisms can accumulate compounds at any moment because they are absorbed and retained more quickly than they are digested or eliminated. Industrial and consumer waste, as well as acid rain, which breaks down soil and releases heavy metals into streams, lakes, rivers, and ground water, are all ways that heavy metals can get into water supplies.

The parent rocks are reflected and seen by the soil. The buildup of heavy metals in soils is caused by parent material, a natural source, and the varying concentrations of these metals in soil are caused by the parent material's nature. According to Katyal and Sharma [3], schist and sandstone, respectively, had the highest and lowest concentrations of Zn, Cu, Fe, and Mn. Nature-driven selenium toxicity has been reported from pockets of Punjab, where the selenium content of soil is as high as 2.41mg/kg. Reports of arsenic poisoning occurring due to drinking of geogenetically arsenic enriched groundwater in the various places of West Bangal. Generally sedimentary rocks have higher arsenic content than igneous and metamorphic rocks [4].

The acceptable limit of Cr^{+6} for potable and industrial discharge water is 0.05 and 0.1 mg/L, respectively, according to Indian norms [5, 6]. Utilizing distinct cultures of Pseudomonas species, research on the biosorption of chromium were conducted. A sorption of 84.5% of individual cultures was achieved under ideal circumstances. The findings of biosorption experiments on chromium solutions are discussed in this work. Processes like chemical precipitation, ion exchange, reverse osmosis, electro dialysis, ultrafiltration, and biosorption are frequently used to remove metal ions from aqueous streams. This method of heavy metal removal from water is commonly employed. It has long been the main technique for handling effluent from metal-containing industries. The procedure involves turning dissolved pollutants into insoluble particles, which makes it easier to remove the contaminant from the liquid phase later by using physical techniques like clarifying and filtration [7, 8]. Chemical precipitants are employed in a precipitation process to enlarge particle size through aggregation. The pH and alkalinity of the water determine how much chemical is needed during treatment. Usually, adding lime or sodium hydroxide during neutralisation causes heavy metals in water to precipitate. The

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outcomes of this procedure are, however, frequently far from satisfactory. Particularly in the presence of complexing agents, a full hydroxide precipitation does not occur [9 - 11].

According to research in the field of biosorption, it is a perfect substitute for decontaminating soils or effluents that include heavy metals. The adsorbate species is drawn to the adsorbent and bound there by various mechanisms as a result of the adsorbent's increased affinity for that species. The process keeps going until there is equilibrium between the adsorbate species and the portion of it that is still in the solution. (Ramachandra and others) Due to the fact that biosorption equilibrium has been reached, it can be moved in either direction: left for wastewater treatment or right for the removal and recovery of adsorbate [12]. The mechanism of metal microbe contact is shown in Figure 1.

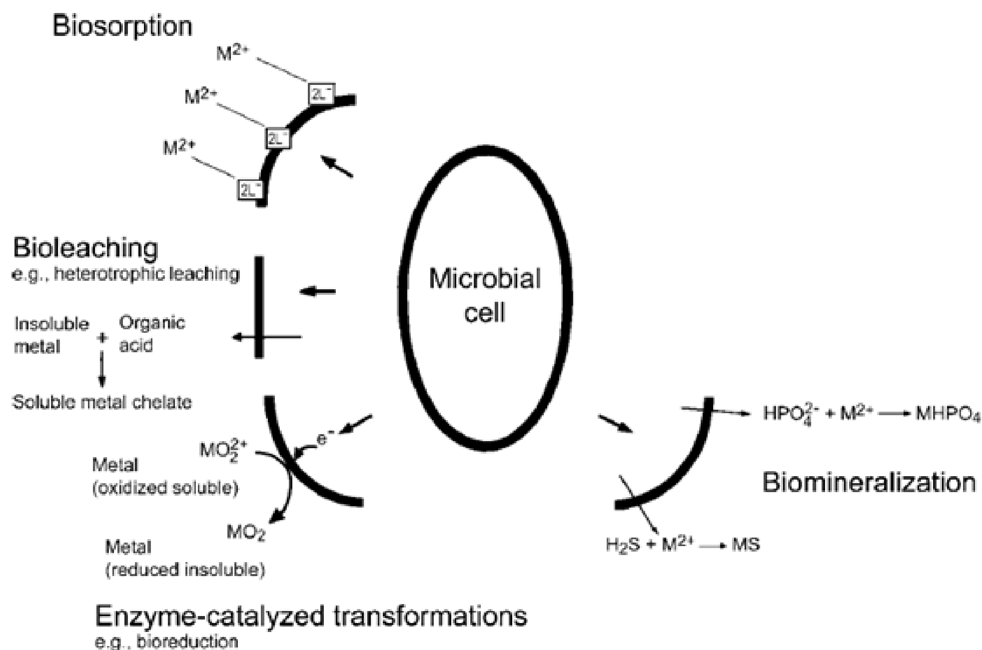

Figure 1. Mechanism of metal microbe interaction [13].

Microbes have the capacity to transfer metal ions into the cell or bind them at the cell surface for a variety of intracellular processes. Metals cannot be destroyed by bacteria during the bioremediation process, although they can change their chemical characteristics [14, 15]. In some cases the processes involve highly specific by their way of working for example the microbes used for biosensors first recognize the components and then detoxify the target metals [16]. On other hand the non specific mechanism microbes produce new mineral phase by which the metal can be entrapped within soils or sediments [17].

Bacterial metal binding is similar to ion exchange or, in some situations, adsorption. Depending on the bacterial strain and the environment, metal ions bind to the cells using a variety of physiological and chemical processes. Electrostatic contacts, van der Waals forces, covalent bonds, and extracellular precipitation redox interactions are some of the forces that hold metal ions to the cell surface [18]. Metal cations are attracted to the negatively charged regions of the bacterial cell wall and subsequently held by mineral nucleation [19]. Some bacterial isolates demonstrated the intracellular accumulation of metals by surface adsorption.

Biosorption is regarded as a less disruptive technique that can frequently be carried out locally, negating the need to transport hazardous materials to treatment facilities. Biomass that is both plentiful and waste can be used to make biosorbents. It has been shown that both living and non-living biomass can be used in the biosorptive process by which bacteria absorb metals. Biosorption is a step towards a perspective method due to its high absorption capacity and highly cost-effective raw material source. The removal of even very small amounts of heavy metal is thought to be possible using biosorption [13].

2. Objectives

Phytoextraction study:

(i) To collect the soil sample

(ii) To study the uptake of Cr^{+6} in roots and shoots of Brassica juncia (Indian Mustard).

Biosorption study:

(i) Collection of waste water and soil sample from any electroplating industry and slurry of the dairy farm.

(ii) Isolation of metal tolerant species of bacteria.

(iii) Removal of Cr^{+6} from synthetic solution on laboratory scale using isolated bacteria with the help of bioreactor.

3. Review of Literature

The most precious natural resource on Earth is water, which covers 70% of its surface. The existence of life on earth would not be possible without this essential molecule. Although this truth is well acknowledged, today's society

nevertheless faces a widespread issue with water resource pollution. Heavy metal contamination can be caused directly by effluent discharges from businesses, refineries, and waste treatment facilities as well as indirectly by contaminants found in soils, groundwater systems, and the atmosphere that reach water supplies by rain [20]. Due to their hazardous chemicals, which are often utilised in industrial processes and are widely dispersed in the environment, heavy metals are a serious problem. Long-term exposure as a result of the release of these heavy metal effluents can result in cancer, delayed neural damage, deformity in urban children, mutagenic alterations, neurological diseases, etc. A transition metal found in group VI-B of the periodic table is chromium (Cr). The most stable oxidation states of chromium in nature, trivalent chromium Cr^{+3} and hexavalent chromium Cr^{+6}, are the only ones that have ecological significance. Metal plating, anodizing, ink production, dyes, pigments, glass, ceramics, glues, tanning, wood preservation, textiles, and corrosion inhibitors in cooling water are the main sources of Cr. Both people and plants are severely impacted by the Cr^{+6} [21]. Lung cancer risk is increased by inhaling hexavalent chromium compounds on a regular basis (lungs are especially vulnerable, followed by fine capillaries in kidneys and intestine).

Some researchers [22 - 25] contend that hydroxyl radicals formed during the cell's hydrogen peroxide molecules' reoxidation of pentavalent chromium are to blame for the damage. Of all the chromates used in industry, zinc chromate is the most cancer-causing.

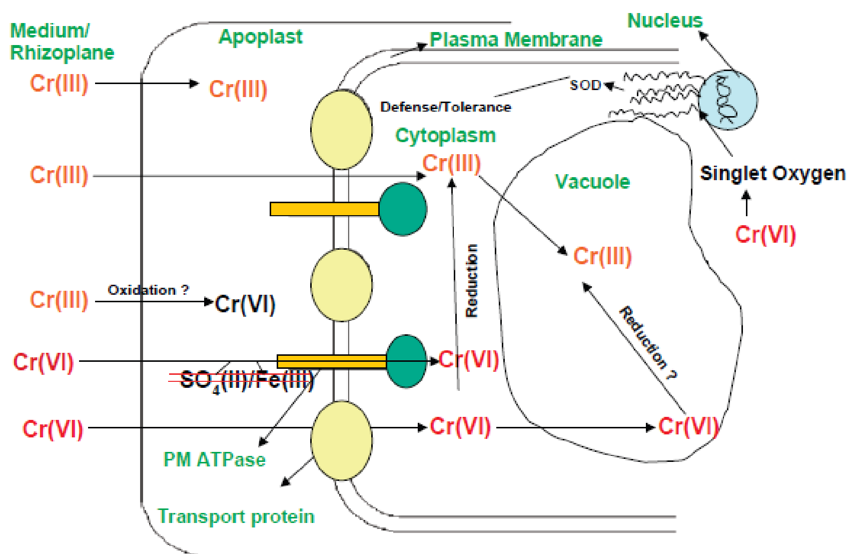

Figure 2. Hypothetical model of Cr transport and toxicity in plant roots [21].

Processes like chemical precipitation, ion exchange, reverse osmosis, electro dialysis, ultrafiltration, and biosorption are frequently used to remove metal ions from aqueous streams. Over the past 30 years, bioremediation has advanced from the lab to a fully commercialised process in many industrialised nations. The control of soil microbial communities capable of metabolising the pollutants is essential for a successful bioremediation plan. Biosorbents are used to assist in the bioremediation process. Bacteria, fungi, and algae are examples of microorganisms that can be employed as biosorbents.

Bacteria are everywhere (found in all possible habitats where life can exist). They are morphologically simpler and essentially monocellular. microorganisms that range in size from 2 microns in diameter to 10 microns in length. The stiff cell wall of every bacterial cell is made of peptidoglycan. A loose film of viscous slime or a capsule may surround the cell wall. Some bacteria use their flagella to move. Bacterial flagella are formed of a single strand of protein. Binary fission is asexual reproduction in bacteria. Conjugation, transformation, and transduction are three possible methods of genetic recombination. Mutations are primarily responsible for genetic diversity. There is no true sexual reproduction. The mode of nourishment used by bacteria can range from heterotrophic to autotrophic. In the available literature [26 – 37], it has been found that various researchers have reported on heavy metal ion impacts on the environment.

Latin word 'fungus' means mushrooms and the study of fungi is called Mycology. Fungi can be defined as thallus like, eukaryotic, spore bearing achlorophyllus plants where all cell walls consists of fungus cellulose or chitin or both. Anton de bang was the father of mycology. The body of multicellular fungi consists of slender, cottony filament called hyphae. The capability of filamentous fungi has a significant effect on uptake of heavy metals.

Numerous studies have also been done on heavy metal removal by fungus. Particularly the genera Rhizopus, Aspergillus, and Saccharomyces provide the benefits of having a large percentage of cell wall material with outstanding cadmium binding characteristics [39]. Common filamentous fungi are capable of sorbing heavy metals from aqueous solutions. To varied degrees, fungal biomass has been reported to sorb Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra, and U.

Algae are considered to be plant-like organisms that are typically aquatic and photosynthesizing but lack genuine roots, stems, leaves, and vascular tissue, as well as having basic reproductive systems. Aquatic, freshwater, and certain terrestrial ecosystems all contain algae. Large marine algae

make up seaweed. Their size can range from being minuscule in size, like diatoms, to being unicellular, colonial, or multicellular, up to being as large as trees, like kelp [40].

4. Materials and methods

To clean up Cr-contaminated soils and streams, a number of remediation approaches, including phytoremediation, have been proposed. Through phytoremediation, heavy metals from contaminated soils and streams are removed by live plants. After then, the plants are harvested and disposed of in a number of ways. Plants used in phytoremediation are distinguished by their high metal tolerance and highly effective metal uptake. One species that has been found to be able to absorb and accumulate metals including Cd, Cr, Cu, Ni, Pb, and Se into its above-ground sections is Indian mustard (Bressicca juncia). Heavy metals are more soluble in soil and more bioavailable to plants when they are chelated with EDTA, also known as ethylenediaminetetraacetic acid. Now, to fulfill the objectives of the present study the phytoremediation experiments were carried out as below:

(i) Phytoextraction of chromium by Indian mustard from chromium spiked sandy soil.
(ii) The effect of chelating agents i.e. EDTA on phytoextractability of Indian mustard.

In the winters of 2011, the phytoremediation experiment was carried out. The university campus' Energy Park, which has sandy soil, is where the soil samples were collected. The university's campus' Energy Park is where the soil was gathered. It was ground, air dried, and put through a stainless steel sieve with a mesh size of 2 mm. After then, plants were grown on this soil. To carry out soil sample characterisation, 150 gramme of soil sample were first taken, mixed thoroughly, and dried in an oven at 60°C for 24 hours. The standard procedure described below was used to evaluate the physio-chemical characteristics of the soil samples.

Moisture content of soil was determined by taking 5g of soil on Whatman’s filter paper. It was kept in oven at 105°C for 24 Hrs. After 24 Hrs dried sample was weighed and moisture content was determined. The pH was estimated by making thick slurry of soil sample and water in ratio of 1: 5. The 1g of soil was dissolved in 5 mL distill waster and was kept on magnetic stirrer for half an hour, then supernatant was taken for determining pH by glass electrode. 5g of the material was dissolved in a flask with 50 ml of distilled water (1: 10 w/v) to create a suspension. For thirty minutes, the contents were shaken over an orbital shaker. Standard KCl solution was used to calibrate the conductivity metre (0.01 M). The supernatant was then removed, and an EC metre was used to record the electric conductivity. In 100 mL of distilled water, 1g of dirt has been dissolved. It was shaken for 30 minutes. A small amount of Erichrome Black-T and the 1ml buffer solution were added. A standard EDTA (0.01N) solution was used to titrate the solution. The dry combustion method was used to determine the total organic carbon content. The pre-weighted crucible was used to weigh the 500 mg sample. The sample was heated to 550 degrees Celsius in a 35 muffle furnace for one hour. After allowing the furnace to cool, the generated ash was weighed. The formula shown below was used to determine the TOC.

$$\text{Ash \%} = \frac{\text{wt. of sample left after ignition} \times 100}{\text{wt. of sample taken}}$$

$$\text{Organic carbon (\%)} = \frac{100 - \text{ash percentage}}{1.724}$$

The micro-kjeldahl method was used to calculate total nitrogen. In a kjeldahl flask, place an appropriate volume of thoroughly mixed sample. Conical flask put on heated plate with 10 mL of digestion reagent (perchloric and sulphuric acid in a 1:3 ratio) added. After the sample seems clear, digestion should be continued for a further 30 minutes to confirm that all organic debris has completely decomposed or been destroyed. Depending on the flask's capacity, cooled and diluted to a volume of 150–300 ml with distilled water Turn on the heat and put the flask correctly in the distillation device. When the pH is just above 8.3, add 0.5 mL of the Phenolphthalein Reagent and then the sodium hydroxide-sodium thiosulfate Reagent. Collect the 200 mL distillate after it has been dissolved in 50 mL of boric acid. For calorimetric estimation, use pure boric acid to indicate boric for titrimatric estimate. After the distillation is finished, remove the flask containing the distillate and turn off the heat to prevent the solution from backwashing. Tirate the distillate with 0.02N H_2SO_4 until the indicator turned a light purple colour. Follow the procedures while carrying a blank and make the necessary corrections.

$$\text{TKN (mg/L)} = \frac{(A - B) \times 280}{V \text{ (ml sample)}}$$

where,
V = Volume of sample, ml
A = ml 0.02N H_2SO_4 required for sample.
B = ml 0.02N H_2SO_4 required for blank.
280 = 14 x 1000 x 0.02 N/100.

Took 5g of soil sample with 30ml of sodium acetate solution in a centrifuge tube and centrifuge for 5 minutes at 8000 rpm. Repeat the centrifugation with fresh sodium acetate. Shake the soil with 30ml of 95% ethanol for 5 minutes and discard the supernatant and again repeat 3 times. Finally extract the soil with three lots of 30 ml of NH_4OAC solution. Determine the Na concentration in the extract using flame photometer. CEC of soil sample was determined by the ammonium acetate method as:

$$\text{CEC (meq/100g) of soil or C mol(P-) /kg)} = \frac{X \times 2}{23}$$

where, X is the conc. of Na in the sample against reading from standard curve = X

The phytoextraction of chromium by Bressicca juncia(Indian mustard), was carried out as pot plantation in the Energy park of the university campus. The seeds of Indian mustard were colleted form Ram Dhan Singh farm of CCS HAU, Hisar, of the species of Bressicca juncia (Indian mustard) .Seeds were placed in a cool and dry place in the laboratory.

First of all the soil was mixed properly. This properly mixed soil was irrigated by distilled water and a dose of nutrients N, P, K, Fe, Zn, Mn and Cu@ 60, 25, 30, 10, 5, 5 &

2.5 mg/kg was added in solution form. This soil was kept as such for one week after that it was again mixed and irrigated with distilled water. Thus this soil was put into the pot @ 2 kg/pot. The experiment was set up in triplicate for control and different Cr dose i.e. 10, 25 and 50mg chromium/kg of soil. A set of triplicates of all doses of chromium contaminated and control was amended with EDTA of 2mM/kg of soil. The total 24 pots were set up, in each pot 2kg /pot of soil was added and 8-10 seeds were sown. After budding i.e. one week the different chromium doses were added to the pots in manner as discussed above. The EDTA was added to the triplicate of control and chromium contaminated pots before one week of harvesting i.e. 40 days. After 47 days the crop was harvested.

After harvesting, the plants' numerous components, including the roots, stem, leaves, flowers, and fruits, were separated. All of the components were carefully cleaned with distilled water before being air dried. After drying them in an oven set to 68–72°C, the initial and dried weights were recorded. The plant samples were combined, processed, and stored in the polythene bags using a stainless steel grinder. Plant samples were digested in a 9:1 v/v mixture of nitric acid and perchloric acid in order to measure the quantity of Cr in the samples. Following digestion, the volume was diluted to 25 ml using distilled water, then filtered and stored in thoroughly cleaned plastic bottles for the purpose of estimating the Cr content using AAS. Throughout the entire investigation, chromium contamination was measured using analytical grade standards. Atomic absorption spectrometer was used to measure the Cr^{+6} concentration (AAS). The standard curve for Cr^{+6} solutions containing 1 to 5 mg/L was produced. The following equation was used to calculate the concentration of in the solution:

$$\% \text{ Removal of } Cr^{+6} = \frac{C\text{ (initial)} - C\text{(final)}}{C\text{ (initial)}} \times 100$$

The metal uptake was calculated as follows:

$$Q = \frac{v\,(C_0 - C_f)}{M}$$

where,

Q is the metal uptake (mg/g)

C_0 and C_f are the initial and equilibrium metal concentration in the solution (mg/L), respectively.

v is the solution volume (L)

M is the mass of biosorbent (g)

There were three copies of each experiment performed. In order to address specific wastes, biotechnological approaches to the reduction of toxic metal pollution use the selective use and enhancement of these natural processes. Bio-sorption and bioaccumulation are the methods by which microorganisms interact with hazardous metals to enable their removal and recovery. By employing biosorbent bacterial inoculum (1 ml/50 ml) from their respective synthetic solutions, biosorption tests were conducted to remove Cr^{+6}. The procedure for biosorbing Cr^{+6} was as follows:

(i) Isolation of the bacterial strain from from contaminated sites.
(ii) Identification of selected pure strain.
(iii) Well maintained microbial strain was used with appropriate proportions for further experiments (Batch studies and bioreactor studies).

Firstly, we have collected the soil and slurry samples from an electroplating industry and a dairy farm, respectively. The soil and slurry samples were combined in a 1:1 weight-to-volume ratio. 5ml of distilled water was used to appropriately homogenise the samples. Now, using ten test tubes filled with 9 ml of distilled water each, we have serially diluted 1 ml of the prepared sample ten times. All of these dilutions were evenly distributed into petriplates made with 1 ml of agar growth media per plate and several plates for various dilutions. These plates were kept in an incubator for 72 hours at 32 degrees Celsius. All of the bacterial strains grew properly after 72 hours. Then, these bacterial strains were transplanted to brand-new petriplates with agar media. Then, by cultivating the bacterial strain in various doses of 10, 25, 50, and 100 ppm agar growth media, the tolerance of bacterial strains against Cr^{+6} was examined. Only 2 species out of all the species could tolerate Cr concentrations up to 100 ppm. These species were streaked 2-4 times more on Petri plates before being cultivated in nutrition broth (NB) media in conical flasks. Both species' development circumstances, including the growth curve, pH, temperature, contact time, and metal removal at various concentrations, were examined. The samples were then examined on a spectrophotometer each time. One species was obtained finally for further use based on maximum growth and removal to chromium, from growth conditions analysis. The final bacterial strain was further streaked for 3-4 times to obtain its pure culture. The pure cultured plates were then sent for identification to IMTEC, Chandigarth. The identified strain was prepared as standard inoculum in the NB media in 250 ml conical flask and maintained fresh by transferring to fresh NB media for each of the further experiments. Only twelve hrs old fresh media was used for inoculation in each experiment of batch and continuous mode. We performed all batch mode studies using the identified strain and observed how the optimization parameters (pH, temperature, contact duration, and metal removal at various concentrations) affected the bacteria's ability to grow and remove metal. The experiment was carried out in a lab using a synthetic solution with various Cr+6 concentrations (50, 100 and 250 ppm).

After the optimization study i.e. batch mode, we set up all the optimization parameters on the bioreactor and start the study in continuous mode. The continuous mode was proposed to check removal of Cr^{+6} concentrations from plant samples but the concentration obtained in plant samples was very low, so, the synthetic solution of Cr^{+6} was used for continuous mode of the study.

For this study metal media solution of desired concentration were used. In each experiment 1 litre metal media solution was made and inoculated with the required innoculum dose. The bioreactor parameters were set as obtained in the batch mode. The experiment was performed in continuation for 18 hrs. The samples were collected after each 3 hrs interval up to 18 hrs each time the samples were centrifuged and the supernatant was collected. The supernatant was analyzed for percentage removal of chromium on atomic absorption spectrophotometer (AAS). 1000 ppm solution of

potassium Dichromate ($K_2Cr_2O_7$) was used for different doses of bacterial isolates.

The bacterial species employed in the current investigation were isolated from soil and slurry samples using the serial dilution and pure plating methods. For the investigation, gram-negative bacteria were employed. These were separated from the soil that was contaminated with heavy metals in an industrial setting. For the experiment, the strain was kept in a nutrient medium with the proper ratios from Paonta Sahib's electroplating industry. Cultures were inoculated using customary sterile procedures. All glassware and the medium used for the microorganism were adequately sterilised by autoclaving at 1210°C for 30 minutes under a pressure of 15 lb/in^2.

The soil sample was taken from the electroplating industry's Paonta Sahib industrial complex (H P). Additionally, a sample of slurry was taken from the Kurukshetra Gaushala dairy farm on Delhi Road in Hisar. The species was regularly kept at 32 degrees Celsius on a nutritional agar medium that contained 20g/l, peptone 5g, sodium chloride 5g, agar 15g, beef extract 1g, yeast extract 1g, and pH 72. The necessary media composition was put to a 1 litre conical flask containing 1 litre of distilled water to create the selective media for bacteria. The media was autoclaved for 15 minutes at 15 psi pressure and 121°C to sterilise it. All glassware underwent oven sterilisation at a temperature of 120°C. The pour plate method was used to transfer approximately 10-15 ml of media into Petri plates with laminar flow. Bacterial cell cultures were prepared using nutrient broth medium.

Homogenized in sterile water and serially diluted were one gramme of the contaminated soil and one millilitre of the slurry sample. The Cr^{+6} integrated nutrient agar plates were created and inoculated with 0.1 ml of the diluted samples. These plates had concentrations of the chromium salt at 10, 25, 100, and 250 g/ml. cultivated for 24 hours at 32°C. Purification of isolated colonies was achieved through numerous further single colony transfers. Specifically, pure colonies were put into nutritional agar slants. The slants were incubated for 24 hours at 37°C. These were kept in the refrigerator at 4°C and used as the stock cultures. The characterisation and identification of pure bacterial isolates.

In triplicates, 50 ml of sterile nutrient broth was injected with a loopful of cells from the stock culture. The cells were then cultured at 32°C for 24 hours with intermittent shaking. Cells were collected after the incubation time by centrifugation at 4000 rpm. Serial dilutions were applied to the cultures throughout this process. Spread plate technique was used to inoculate an aliquot (0.1 ml) from each dilution into freshly made nutrient agar plates, which were then incubated at 32°C for 24 hours. With the aid of a UV spectrophotometer, different growth conditions for isolated isolates were investigated using the optical density approach. For the study, growth factors are tuned as described below.

In a 250 ml conical flask take 50 ml nutrient broth media. Autoclave it at 15 psi at 121°C. The inoculation was done in laminar flow with 1 ml inoculum. Then flask were put into in BOD incubator shaker at temperature of 32°C for incubation. Take absorbance at regular interval of time for 72 hrs with UV spectrophotometer at 600 nm wavelength. Take six 250 ml conical flasks and sterilized in oven. Take 50 ml nutrient broth media in each flask and autoclave it for sterilization. Add different amount (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) of inoculums in flasks in laminar flow. Take absorbance at 600 nm with UV spectrophotometer. The experiment was also repeated with the chromium concentrations of 10-50 ppm/l. Take five 250 ml conical flasks and sterilized in oven. Take 50 ml nutrient broth media in each flask and for sterilization autoclave all flasks. In laminar flow inoculation done by 1 ml inoculum adds in each flask. Take each flask on BOD incubator shaker at different temperature (25, 30, 32, 37, 40, 42°C) for incubation. Take absorbance at 600 nm with UV spectrophotometer. The experiment was also repeated with the chromium concentrations of 10-50 ppm/l. Take eight 250 ml conical flasks and sterilized in oven. Take about 50 ml nutrient broth media in each flask and adjust the pH with 1 N NaOH and 1 N HCl in the range of 2, 3, 4, 5, 6, 7, 8 and 9 and autoclave for sterilization. Add inoculums in each flask in laminar flow and then incubate all flasks in BOD incubator shaker for a time period of 12 hrs. After take absorbance at 600 nm by spectrophotometer and made graph between pH and absorbance. The experiment was also repeated with the chromium concentrations of 10-50 ppm/l. Make different concentrations of metal media solutions with nutrient broth media and autoclave it at 15 psi at 120°C. Adjust pH of metal media solutions with the help of base or acid (1 N HCl or NaOH). Inoculation done in laminar flow and add inoculums in all metal media solutions. Then incubate in BOD incubator shaker for a proper time period for sufficient growth. Take absorbance at 600 nm by UV spectrophotometer and make graph between absorbance and concentration.

All the batch biosorption experiments were carried out for optimization of different factors affecting the microbial growth and their metal removing capacities. The experiments were done at 37°C temperature and the Cr^{+6} concentration of 100 ppm. Batch experiments were carried out in the 250 ml conical flasks. The shaking incubator was used for the batch studies at 120 rpm. All optimization experiments were performed with 50 ml synthetic sample (prepared broth media) and metal solution was prepared in distilled water for growth of bacterial biomass. After desired contact period of 4 days (growing bacterial biomass), conical flasks were removed. The sample solutions were centrifuged at 4000 rpm at 25°C and the supernatant was filtered through Whatman filter paper no. 41 and filtrate of the solution was analyzed for Cr^{+6} concentration by Atomic Absorption Spectrophotometer.

The batch experiments were conducted to find out the effects of varying pH on Cr^{+6}removal from their respective synthetic solutions of constant concentration 100 mg /L at constant contact time of 18 hrs. pH was adjusted using 0.1N HCL and 0.1 N NaOH. After agitation, solution was centrifuged and supernatant was used for analysis of metal concentration by using AAS. The optimum metal ion concentration was found out at optimum pH with contact time of 18 hrs at various concentrations ranging from 10-200 ppm of Cr^{+6} metal solutions. After agitation, solution was centrifuged and supernatant was used for analysis of metal concentration by using AAS. The optimum pH and optimum metal ion concentration were used for experiment to find out the optimum biosorbent dose. The range of biosorbent dose was 0.5- 3.0 ml in 50 ml nutrient broth media. After agitation,

solution was centrifuged and supernatant was used for analysis of metal concentration by using AAS. Keeping optimum pH, optimum dose and optimum metal ion concentration, observations were taken with various intervals of time for finding exact contact time of the isolated bacteria. All above experiments were carried out in triplicates for better results and less errors.

A bioreactor was used to carry out the simultaneous, large-scale breakdown of Cr^{+6} utilising a colony of microorganisms. All process parameters in the bioreactor, including temperature, pH, DO, and the addition of anti foam, were automatically regulated. The bioreactor has a working volume of 3 litres. One litre of the metal-containing media was created and autoclaved. The bioreactor, which contained the 12 hour-old inoculum volume, automatically maintained the ideal conditions throughout the trial, including pH 7.5, temperature 37°C, and agitation 140 rpm. To evaluate the chromium degradation for 18 hours, sampling was done at regular intervals of time. The results of the treatment of chromium reduction show the differences in reduction of metal for different concentrations at large scale. That can be seen by plotting the graph between time and percentage removal of metal. The estimation of hexavelent chromium was done by the method discussed above.

5. Results and Discussion

The present study was divided in to two parts i.e. phytoextration of Cr^{+6} by Indian mustard and biosorption of Cr^{+6} by bacterial biomass isolated from Cr^{+6} contaminated soil. Because it produces better results in the rehabilitation of contaminated soil, the Brassica juncea is frequently employed for the removal of heavy metals. Indian mustard, or Brassica juncea, is a possible candidate plant for the phytoremediation of certain heavy metals. The biomass of Indian mustard cultivated on soils contaminated with Cr^{+6} increased at low levels of Cr, demonstrating that Cr can stimulate plant development at low levels. However, in both Cr-treated soils at high concentrations, there was a decrease in the above-ground biomass, indicating the toxicity of Cr (Han et al 2003). In this study we have also used the chelating agent EDTA to determine the phytoextractability of brassica juncia.

An experiment was setup for 47 days for phytoremediation of Cr^{+6} contaminated soil by Indian mustard at in the Energy park of the G.J. university campus. The experiment was carried out within pots. Soil used for experiment was properly processed and analyzed the various parameters as shown in Table 1. The soil is having neutral pH, 40% of organic carbon, 81 kg/ha nitrogen and 3.9 kg/ha the cation exchange capacity. The detailed study includes: chromium toxicity symptoms and Phytoextraction of Cr^{+6}. No toxicity symptoms were seen in the control group, with or without EDTA treatment, during the trial. In case of Cr^{+6} treated soil having a dose of Cr^{+6} 10mg/kg of soil, the growth of the plant was slightly inhibited, but no toxicity symptoms were appeared on leaves with or without EDTA treatment. When a dose of chromium @25 mg /kg of soil was grown, the plant shows the maximum growth whereas toxicity symptoms in the form of yellow and brown spots on leaves and margins of the leaves were yellow in colour. Due of Cr^{+6} high oxidation power, it has been hypothesised that the major source of its toxicity is membrane damage. Whereas, in case of Cr dose of 50 mg/kg of soil the plant germination was affected in addition to lack of plant growth with more pronounced toxicity symptoms as shown in case of chromium dose 25 mg/kg. Changes in the germination process as well as the growth of roots, stems, and leaves are among the toxic effects of Cr on plant growth and development. These changes may have an impact on yield and total dry matter output. The seeds of brassica were more ripe after the addition of EDTA.

After 47 days the plants were harvested. The above and below ground biomass of the plants (B. juncia). The biomass of stem in both cases (Initial and dry wt.) was maximum. If we compare the dry weight of stem in all chromium contaminated and non contaminated plants, we observed that the maximum weight was 4.283 gm without EDTA and 4.066 gm with EDTA. Plants using soil containing 25 mg/kg of chromium were able to achieve both weights. In plants with a soil chromium concentration of 50 mg/kg, the minimum weight was 0.190 gm with EDTA, whereas the minimum weights without EDTA are shown as 0.263 gm and 0.285 gm in plants with chromium concentrations of 10 mg/kg and 50 mg/kg of soil, respectively. It once more demonstrates how the given Cr concentrations impeded plant growth.

Many plants, including corn, sugar beets, and bush beans, have been found to be more hazardous to chromium Cr+6 than to chromium Cr^{+3} (Adriano, 1986). Therefore, to further optimise phytoremediation strategies, comparative investigations of Cr uptakes and its toxicity from both Cr^{+3} and Cr^{+6} polluted soils are necessary. This study set out to look at Indian mustard's phytoavailability in soils contaminated with Cr^{+6}. We observed that the maximum concentration of Cr^{+6} was accumulated in roots of all the plants in both case with or without EDTA. The maximum concentration 7.849 ppm was in roots of the plants having 50mg/kg of soil concentration of Cr^{+6} with amendment of EDTA. So, it shows the chelating effect of EDTA. Without EDTA the maximum concentration 4.187 ppm was also found in roots of the plants having 50mg/kg of soil concentration of Cr^{+6}. That shows the maximum amount of the metal concentration accumulated in roots. The minimum concentration shown as 0.291 ppm was in fruits of the plants having 50mg/kg of soil concentration of Cr^{+6} without EDTA. Most of shoots i.e. leaves, flowers and fruits were also showing the concentration accumulations less than that of roots with or without EDTA. According to Golovatyj et al. (1999), the distribution of Cr in crops had a stable character that was independent of the soil's characteristics and Cr concentration; the maximum amount of the element contaminant was always present in the roots and the least amount in the vegetative and reproductive organs. Only 0.1% of the Cr that had accumulated in beans was discovered in the seeds, compared to 98% in the roots [42]. The fact that Cr is immobilised in the vacuoles of the root cells, making it less toxic, may constitute a natural toxicity response of the plant and account for the large accumulation in the roots of the plants.

According to the explanation above, all of the Cr concentration was solely absorbed by the plant's roots with the aid of the chelating chemical EDTA, and it was not visible in any other areas. Initially during study it was planned to remove chromium from phytoremediated plant parts but the concentration of Cr was low in different parts of brassica

juncia (Cr phytoremediated plants). Thus the study was carried forwarded by using synthetic solution of chromium for its bio adsorption with the help of isolated bacterial species in bioreactor. The bacterial species were grown on different conc. of Cr $^{+6}$ i.e. 10 ppm, 25 ppm, 50 ppm, 100 ppm, and 250 ppm . Only 5 five species were tolerant to Cr^{+6} from which only one was having maximum number of colonies. Thus the strain has maximum no. of colonies was maintained for further studies. The isolated strain was streaked for 3-5 times to achieve the final pure culture. The pictures of the isolated and pure cultured plates are shown as slide 1 and slide 2, respectively. The strain of bacteria found on this pure cultured petri plate was identified as Pseudomonas sp. by IMTECH, Chandigarh. Batch studies were used to improve the effects of variables like pH, biosorbent dose, contact time, initial metal ion concentration, and temperature on biosorption. At a Cr^{+6} metal ion concentration of 100 ppm during the batch research, the maximum Cr removal observed was 84.78% after 13 hours of contact time at a pH of 7.5 and a temperature of 37°C. The sorption of chromium is significantly influenced by the pH of the solution. The chromate reduction experiment used a freshly made overnight culture that was incubated at 30°C with 200 rpm shaking. After a period of 12 hours of incubation, the cultures were harvested. The highest elimination of Cr +6 concentration was seen at pH 7.5, while Pseudomonas sp. performed chromium reduction in a pH range of 5–9. Results are presented in figure 7. The Cr^{+6} form is often mobile in soil and water systems and soluble over a broad pH range.

The incubation temperature has a significant impact on the ability of the bacterial strain to reduce Cr^{+6}. As shown in Figure 8, the Pseudomonas sp showed good decrease over the temperature range of 30-37°C, with a maximum at 37°C. At pH 7.5 and 100 mg/l of Cr^{+6} concentration solution in 50 ml volume, the effect of temperature on chromium reduction was investigated. Therefore, it was found that Pseudomonas sp. performed best at a temperature of 37°C, and Bae et al. [43] found that E. coli ATCC 33456 performed best at this temperature as well. Bacterial growth and chromium removal are affected by high temperatures (greater than 40°C) because cells lose their viability or metabolic activity [44]. At temperatures below 30°C, Cr^{+6} reduction was drastically reduced due to the reduction in the time needed to build up a critical biomass concentration lag, and at temperatures below 26°C, cell growth was suppressed.

In order to get the greatest Cr^{+6} reduction, it is critical to ascertain the inoculums' volume and age. The experiment was carried out using a culture flask in an incubator shaker for 13 hours at a fixed concentration of Cr^{+6} of 100 mg/l in a volume of 50 ml, an initial pH of 7.5, and a speed of 140 rpm. Figure 9 illustrates the impact of Cr^{+6} removal with inoculum dose. The inoculum dose of 3.0 ml resulted in the greatest amount of chromium elimination. Additionally, it was found that as inoculum volume increases, Cr^{+6} reduction likewise increases. This might be as a result of the lack of metal-binding sites as well as the blocking of binding sites by too much biomass.

The initial metal ion concentration has a significant impact on microbial reduction of Cr^{+6}. Figure 10 displays the findings for the Cr^{+6} removal by Pseudomonas sp at different initial metal ion concentrations from 10-200 ppm with constant temperature 37°C, pH-7.5, and inoculum volume of 3ml/50ml over a period of 13 hours. At a starting concentration of 100 ppm, the greatest clearance of Cr^{+6} was noted. The outcome demonstrated that the elimination of Cr^{+6} increases initially from 35-84.78% in 10 ppm to 100 ppm Cr^{+6} concentration, then reduces to 71.84 at 110 ppm, raises once more, and finally increases to 78.42% of the initial metal ion concentration of 190 ppm. Figure 10 illustrates the two phases of Cr^{+6} reduction that were observed: the initial phase of quicker degradation and the phase of slower degradation. The initial rapid uptake may have been caused by the abundance of Cr^{+6} species and the microorganisms' vacant metal binding sites. The reported uptake, however, may be explained by the saturation of metal binding sites. As the concentration of Cr^{+6} rises, microbial population growth slows. As the amount of heavy metal rose, the production of microbial biomass reduced.

The sorption capabilities of biomass at various time intervals can be determined through investigations at various contact times. With a continuous biosorbent dose of 3 ml/50 ml, a concentration of 100 mg/kg Cr^{+6}, a pH of 7.5, and a temperature of 37°C, removal by Pseudomonas sp. was conducted for various contact periods. Figure 11 depicts the greatest Cr^{+6} removal by Pseudomonas sp. during 13 hours of contact time. The bio mass growth is elevated for up to 9 to 13 hours during the Cr^{+6} degradation investigations, and subsequently it settles into equilibrium. After 14 hours, Pseudomonas sp's growth reached stationary phase.

After batch mode the continuous study was carried out in bioreactor for pilot scale. At Large scale degradation of synthetic solution of Cr^{+6} at its optimum conditions in a bioreactor (Lark innovative's 'HYGENE') was performed using the microbial strains of Cr^{+6} reducing bacteria Pseudomonas sp. In bioreactor Cr^{+6} removals by pseudomonas species was observed of varying initial metal ion concentration Cr^{+6} from 50-250 ppm and different time interval, i.e. 3-18 hrs. The optimized parameters were assigned to the bioreactor as pH7.5, temperature 37°C, biosorbent dose @ 3 ml/50 ml in the one lit. volume of metal media solution of desired concentration.. The samples were taken after each 3 hours, centrifuged and analyzed with the help of AAS for percentage removal. The results are shown in the Figure 12. The maximum chromium removal observed at initial metal ion concentration of 50 ppm, 100 ppm and 250 ppm were 58%, 60% and 57%, respectively at 13 hrs. of contact time. This removal was decreased from 84% removal of Cr^{+6} at batch mode to 60% in bioreactor mode. It is observed that a decrease in Cr^{+6} removal from batch mode to bioreactor study while all other parameters were constant. From results it is clear that maximum Cr^{+6} observed at initial metal ion concentration 100 ppm. This optimum dose and contact time for bioreactor study was 100 ppm and 13 hrs, respectively. It has been found that biomass growth or production peaked at the equilibrium time, then remained constant as incubation time rose. This bioreactor investigation led to the conclusion that this bacteria can grow and remove Cr, making it a strong choice for scaling up production as a biosorbent.

Table 1. The physiochemical characteristics of soil sample.

Parameter name	Value (Limits)
pH	7.0
Electrical conductivity (EC)	1545 µs
Total organic carbon	40%
Nitrogen	81.0 kg/ha
CEC	232 meq/100g of soil.
K_2O	151.7 kg/ha

Table 2. Various physiological and biochemical characteristics of isolated bacterial strain

Colony morphological tests	ER
Configuration	Circular
Margin	Entire
Elevation	Convex
Surface	Smooth
Pigment	Creamish yellow
Opacity	Opaque
Gram's reaction	-ve
Cell shape	Rods
Size (micro meter)	0.8-0.1
Arrangement	Scattered
Spore(s)	+ve
Motility	-

Table 3. Chromium (Cr^{+6}) removal at different pH

pH ranges	Percentage removal
5	42%
5.5	46%
6	48%
6.5	51%
7	51%
7.5	53%
8	42%
8.5	39%
9	29%

Table 4. Chromium (Cr^{+6}) removal at different Temperature ranges

Temperature in °C	Percentage removal
31	10
33	25
35	50
37	62
39	54
41	28

Table 5. Chromium (Cr^{+6}) removal at different innoculum volume

Inoculum volume	Percentage removal
0.5	51
1.0	53
1.5	51
2.0	54
2.5	54
3.0	57

Table 6. Chromium (Cr^{+6}) removal at different Varying Metal conc.

Metal conc. in ppm	Percentage removal
10	35.72%
20	51.68%
25	72.87%
30	70.03%

40	75.16%
50	77.21%
60	72.72%
70	74.47%
80	74.51%
90	79.71%
100	84.78%
110	71.84%
120	74.02%
130	74.12%
140	73.61%
150	73.88%
160	73.73%
170	74.00%
180	76.61%
190	78.42%
200	77.89%

Table 7. Chromium (Cr^{+6}) removal at different varying time intervals in hrs

Time in hrs	Percentage removal
3	56
6	57
9	57
12	57.6
13	60
15	43
18	43
21	45
24	44

Figure 3. Photograph of growth of Brassica juncia in control.

Figure 4. Photograph of growth of Brassica juncia in 10 ppm Cr^{+6}

Figure 5. Photograph of growth of Brassica juncia in 25 ppm Cr^{+6}

Figure 6. Photograph of growth of Brassica juncia in 50 ppm Cr^{+6}

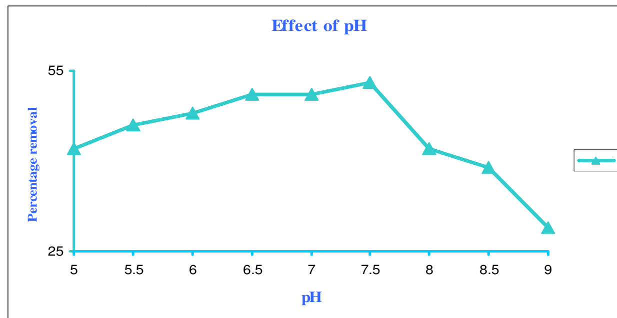

Figure 7. Chromium (Cr^{+6}) removal at different pH

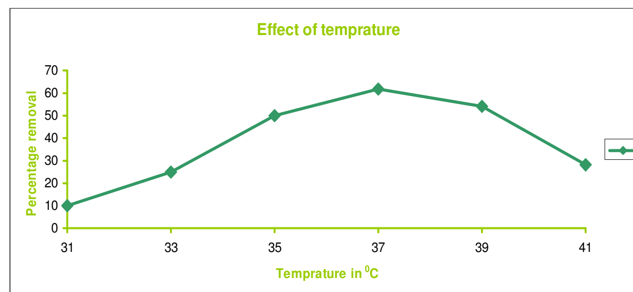

Figure 8. Chromium (Cr^{+6}) removal at different pH

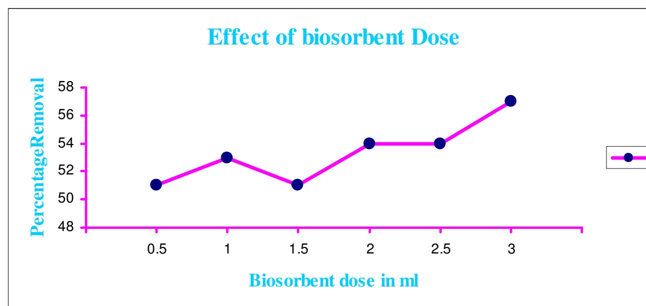

Figure 9. Chromium (Cr^{+6}) removal at different innoculum volume

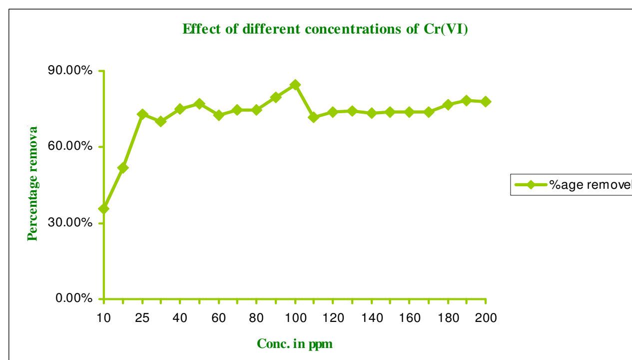

Figure 10. Chromium (Cr^{+6}) removal at different varying metal conc. in ppm

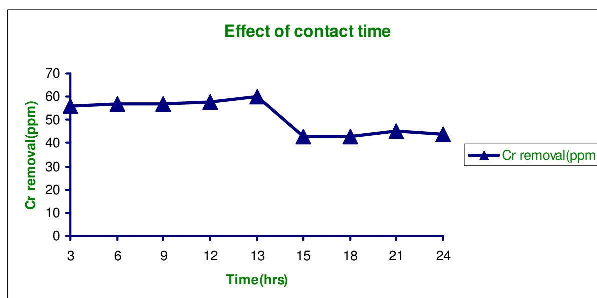

Figure 11. Chromium (Cr^{+6}) removal at different varying time.

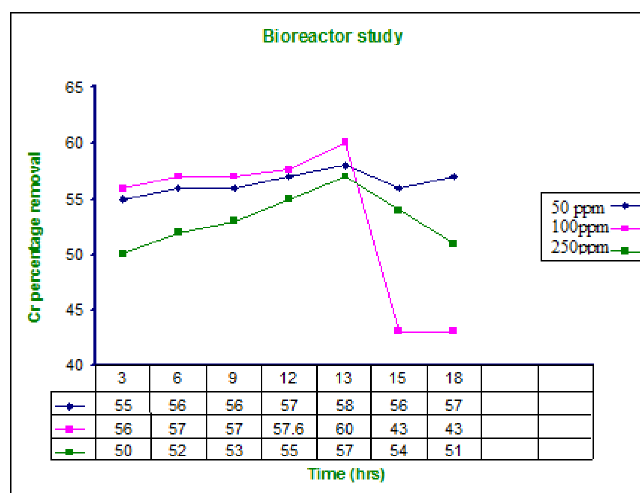

Figure 12. Chromium (Cr^{+6}) removal at different varying metal conc. in ppm with respect to time.

Table 8. Initial and dry weight (in gms) of Brassica juncia at different doses of Cr applied to soil

Conc. In ppm	Pots	Roots		Stem		Leaves		Flowers		Fruits	
		Initial weight	Dry weight	Initial weight	Dry weight	Initial weight	Dry weight	Initial weight	Dry weight	Initial weight	Dry weight
Cr0	P1	1.322	1.022	4.604	0.556	2.608	0.174	0.458	0.041	0.266	0037
	P2	2.237	0.460	6.958	1.755	1.788	0.370	1.740	0.184	1.505	0.198
	P3	4.222	0.635	11.232	2.169	3.285	0.421	2.184	0.188	2.190	0.247
Cr0 +EDTA	P1	4.288	1.536	16.404	3.364	7.848	1.954	3.146	0.330	2.571	0.323
	P2	5.078	0.700	7.877	0.743	7.734	0.595	0.980	0.096	0.067	0.006
	P3	3.402	0.464	6.296	0.742	5.447	0.800	1.365	0.121	0.153	0.031
Cr10	P1	3.005	0.369	11.063	1.890	6.471	1.093	0.500	0.228	2.736	0.331
	P2	2.041	1.057	3.206	0.263	4.656	0.482	0.506	0.048	n.a.	0.012
	P3	2.746	0.853	7.602	1.045	5.006	0.800	1.217	0.116	0.086	n.a.
Cr10 +EDTA	P1	3.787	0.872	14.771	2.082	11.458	1.160	2.099	0.055	1.093	0.117
	P2	2.156	0.836	5.323	1.055	2.303	0.394	1.077	0.112	0.701	0.090
	P3	2.325	0.670	12.308	1.497	7.898	1.029	1.577	0.156	n. a.	n.a.
Cr25	P1	8.131	2.951	15.727	4.283	3.558	1.060	2.787	0.406	3.768	0.490
	P2	5.367	1.830	17.661	2.779	8.107	2.144	2.856	0.342	2.645	0.350
	P3	3.322	1.323	14.772	3.222	4.453	1.121	1.911	0.186	2.509	0.370
Cr25 +EDTA	P1	0.997	0.758	4.471	4.066	7.280	1.764	3.898	0.286	1.901	0.243
	P2	6.092	2.216	15.824	2.474	4.247	0.893	3.594	0.292	1.710	0.228
	P3	2.444	0.960	6.887	3.624	1.937	0.671	1.582	0.231	0.345	0.074
Cr50	P1	3.862	0.805	13.568	1.535	1.054	0.937	1.753	0.191	2.276	0.260
	P2	3.406	1.013	12.230	2.119	9.038	1.703	2.107	0.236	2.151	0.273
	P3	3.921	0.290	1.333	0.285	1.800	0.393	0.323	0.032	n.a.	n.a.

Table 9. Chromium (Cr^{+6}) concentrations (in ppm.) in different parts of Brassica juncia.

Cr^{+6} Conc	Roots		Stem		Leaves		Flowers		Fruits	
	Without EDTA	With EDTA	Without EDTA	With EDTA	Without EDTA	With EDTA	Without EDTA	With EDTA	Without EDTA	With EDTA
0	ND	0.526	0.021	ND	0.036	ND	0.000	ND	ND	ND
10	1.568	2.061	1.034	ND	2.014	ND	0.846	ND	ND	ND
25	2.061	4.448	1.847	ND	1.467	ND	0.448	ND	ND	ND
50	4.187	7.846	2.518	ND	1.178	ND	1.649	ND	0.291	ND

6. Conclusions

The most suitable, economical, and environmentally benign method for removing microorganisms from chromium-contaminated locations is bioremediation. The majority of the Cr received by plants is stored in the roots. It is evident from the overall picture of Cr toxicity in plants that different species of Cr are hazardous to plants to varying degrees at various stages of their growth and development. Brassica Juncia had a low chromium concentration, so phytoremediation of chromium was not a possibility. In the contaminated settings, microbes interact with chromium in a variety of ways. One of the theories of successful bioremediation involves the reduction of Cr+6 by microorganisms. Pseudomonas sp. totally reduced the 100 mg/l Cr+6 in our sample within 24 hours. The utilisation of Cr-reducing bacteria may represent a very efficient method for the removal and detoxification of the toxic forms of Cr in areas where environmental contamination with Cr+6 is a major area of concern.

References

[1] I. Raskin, B.D. Ensley, *Phytoremediation of toxic metals: using plants to cleanup the environment,* John Wiley & Sons, New York (2000) pp. 352.

[2] D.J. Thiele, Metal detoxification in eukaryotic cells. Crisp Data Base of National Institute of Health, Washington DC (1995).

[3] J.C. Katyal, B.D. Sharma, Heavy metals and environmental quality, *Geoderma* **49** (1999) 165-179.

[4] S.K. Sanyal, Adoption of modern technologies: their impact on present agricultural situation and environment, *Annual technical* **4** (2000) 142-148.

[5] R.S. Lokhande, P.U. Singare, D.S. Pimple, Toxicity study of heavy metals pollutants in waste water effluent samples collected from Taloja Industrial Estate of Mumbai, India, *Research of Environment* **1** (2011) 13-19.

[6] A. Baral, R. Engelken, W. Stephens, J. Farris, R. Hannigan, Evaluation of aquatic toxicities of chromium and chromium-containing effluents in reference to chromium electroplating industries*, Arch. Environ. Contam. Toxicol.* **50** (2006) 496– 502.

[7] NEESA, Precipitation of Metals from Ground Water. Naval Energy and Environmental Support Activity, Port Hueneme, CA. NEESA Document Number 20.2-051.6 (1993).

[8] S.M. Nomanbhay, K. Palanisamy, Removal of heavy metal from industrial wastewater using chitosan coated oil palm shell charcoal, *Electron. J. Biotechnol. 8* (2005) 43-53.

[9] Y. Xu, T. Xu, Heavy metal complexes wastewater treatment with chelation precipitation, IEEE Xplore. 2789-2793. Bioinformatics and Biomedical Engineering. ICBBE 2008.The 2nd International Conference (2008).

[10] M.V. Vaca, R.L.P. Callejas, R. Gehr, B.J.N. Cisneros, P.J.J. Alvarez, Heavy metal removal with Mexican clinoptilolite: multi-component ionic exchange, *Water Res.* **35** (2001) 373-37.

[11] Z. Pawlak, S. Zak, L. Zablocki, Removal of hazardous metals from groundwater by reverse osmosis, *Pol. J. Environ. Stud.* **15** (2005) 579- 583.

[12] K. Chojnacka, Biosorption and bioaccumulation – the prospects for practical application, *Environ. International.* **36** (2010) 299–307.

[13] M. Gavrilescu, Removal of heavy metal from the environment by biosorption, *Eng. Life Sci.* **4** (2004) 219-232.

[14] H.L. Ehrlich, *Geomicrobiology*, Marcel Dekker, New York (1996).

[15] A.P. Summers, L.I. Sugarman, Cell-free mercury (II) reducing activity in a Plasmid bearing strain of Escherchia coli. *J. Bacteriol*. **119** (1974) 242-249.

[16] E.L. Andersson, Analysis of various bioreactor configurations for heavy metal removal using the fungus Penicillium ochro-chloron. M Sc Thesis, Worcester Polytechnic Institute. Worcester, MAS, USA (1999).

[17] A. Kogej, A. Pavko, Laboratory experiments of lead biosorption by self immobilized Rhizopus nigricans pellets in the batch stirred tank reactor, *Chem. Biochem. Eng. Q.* **15** (2001) 75-79.

[18] A. Blanco, Immonalization of nonviable cyanobacteria and their use for heavy metal adsorption from water, *Environmental Biotechnology and Cleaner Bioprocesses* **8** (2000) 135-151.

[19] J. Wase, C. Foster, *Biosorbents for Metal Ions*, Taylor and Francis, Bristol (1997).

[20] K. Vijayaraghavan, Y.S. Yun, Bacterial biosorbents and biosorption, *Biotechnol. Adv.* **26** (2008) 266–291.

[21] A.K. Shankera, C. Cervantesb, H. Loza-Taverac, S. Avudainayagam, Chromium toxicity in plants, *Environment International* **31** (2005) 739– 753.

[22] K.P. Shukla, N.K. Singh, S. Sharma, Bioremediation: Developments, Current Practices and Perspectives, *Genetic Engineering and Biotechnology Journal* **3** (2010) 1-20.

[23] E.P. Bruce, Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites (2001).

[24] F.X. Han, B.B.M. Sridhar, D.L. Monts, Y. Su, Phytoavailability and toxicity of trivalent and hexavalent chromium to Brassica juncea, *New Phytologist.* **162** (2004) 189–199.

[25] A.K. Gupta, S. Sinha, Chemical fractionation and heavy metal accumulation in the plant of Sesamum indicum (L.) var. T55 grown on soil amended with tannery sludge: Selection of single extractants, *Chemosphere* **64** (2006) 161–173.

[26] B. Voleskey, Biosorption for the next century. Biohydro-metallurgy symp. El Escorial, Spain (1999).

[27] F.A.O. Camargo, F.M. Bento, B.C. Okeke, W.T. Frankenberger, Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate, *J. Environ. Qual*. **32** (2003) 1228–1233.

[28] M.A. Polti, R.O. García, M.J. Amoroso, C.M. Abate, Bioremediation of chromium (VI) contaminated soil by Streptomyces sp. MC1, *Journal of Basic Microbiology* **49** (2009) 285–292.

[29] E. Parameswari, A. Lakshmanan, T. Thilagavathi, Chromate resistance and reduction by bacterial isolates, *Australian Journal of Basic and Applied Sciences* **3** (2009) 1363-1368.

[30] V. Raicevic, Z. Golic , B. Lalevic, L. Jovanovic, D. Kikovic, S.A. Mladenovic, Isolation of chromium resistant bacteria from a former bauxite mine area and their capacity for Cr (VI) reduction, *African Journal of Biotechnology* **9** (2010) 6727-6732.

[31] K. Sundar, R. Vidya, A. Mukherjee, N. Chandrasekaran, High chromium tolerant bacterial strains from Palar river basin: Impact of tannery pollution, *Research Journal of Environmental and Earth Sciences* **2** (2010) 112-117.

[32] F.R. Shakoori, S. Tabassum, A. Rehman, A.R. Shakoori, Isolation and characterization of Cr^{6+} reducing bacteria and their potential use in bioremediation of chromium containing wastewater, *Pakistan J. Zool.* **42** (2010) 651-658.

[33] A.P. Das, S. Mishra, Biodegradation of metallic carcinogen hexavalent chromium Cr (VI) by an indigenously isolated bacterial strain, *Journal of carcinogenesis* **9** (2010) 1-6.

[34] V. Kavitha, N. Radhakrishnan, A. Gnanamani, A.B. Mandal, Management of chromium induced oxidative stress by marine. *Bacillus licheniformis*, *Biology and Medicine* **3** (2011) 16-26.

[35] C. Mack, J.E. Burgess, J.R. Duncan, Membrane bioreactors for metal recovery from wastewater: A review **30** (2004) 521-532.

[36] R.K. Krishna, L. Philip, Bioremediation of Cr(VI) in contaminated soils, *Journal of Hazardous Materials B* **121** (2005) 109–117.

[37] R. Faryal, M. Yusuf, K. Munir, F. Tahir, A. Hameed, Enhancement of Cr^{6+} removal by Aspergillus Niger RH 19 using a biofermenter, *Pak. J. Bot*. **39** (2007) 1873-1881.

[38] F. Veligo, F. Beolchini, Removal of metals by biosorption: a review, *Hydrometallurgy* **44** (1997) 301-316.

[39] J. Panikar, X. Ge, R. Liu, G.H. Tang, Characteristic features of *Bacillus cereus* cell surfaces with biosorption of Pb(II) ions by AFM and FT-IR, *Colloid. Surface. Biointerfer.* **52** (1993) 89–95.

[40] P. Kotrba, M. Mackova, T. Macek, Microbial biosorption of metals, Springer Science (2011).

[41] K.P. Talaro, A. Talaro, *Foundations in Microbiology*, McGraw-Hill Collegue, Blacklick (2002).

[42] J. Huffman, H.W. Allaway, Chromium in plants: distribution in tissues, organelles, and extracts and availability of bean leaf Cr to animals, *J Agric Food Chem.* **21** (1973) 982–986.

[43] W.C. Bae, K.T. Gu, I.K. Kang, Y.J. Won, B.C. Jeong, Reduction of hexavalent chromium by Escherichia coli ATCC 33456 in batch and continuous cultures, *Journal of Microbiology* **12** (2000) 36-39.

[44] R.S. Laxman, S. More, Reduction of hexavalent chromium by streptomyces griseus, *Minerals Engineering* **15** (2007) 831–837.

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