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Biodegradation of Chromium Contaminated Soil by Some Bacterial Species

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Abstract: ***Chromium (Cr) is one of the most common heavy metals affecting the soil quality which is introduced into the environment from industries such as leather tanning, electroplating and inorganic pigment (green, orange and yellow) production. The leather industry is the major source for the environmental influx of Cr. Cleaning up of the Cr contaminated sites is a challenging task. Bioremediation is an emerging area that could be considered for the remediation of contaminated sites because of its cost effectiveness and aesthetic advantages. Microbial degradation of heavy metals is one of the major practices in natural decontamination process. In the present study heavy metals degradation were analyzed using isolated bacterial strains. Bacterial strains were isolated from the industrial affected contaminated soil sample. The selected three bacterial strains were named as SS5, MR2, and MR3. These organisms were identified based on the cultural, morphology and biochemical characteristics and results of SS5 (Pseudomonas fluorescence), MR2 (Bacillus cereus) and MR3 (Bacillus decolorationis). The heavy metals degradation of isolated bacterial strains was analyzed based on the growth of heavy metals containing medium. The heavy metals degrading ability of isolated strains were analyzed at 48 hrs after inoculation. The effect of heavy metals on the growth of pigeon pea (Cajanus cajan L.) were determined by pot culture experiment and analyzed the germinating and growth ability in different experimental groups showed that Pseudomonas fluorescence and Bacillus cereus have more efficient than Bacillus decolorationis .***

Keywords: Heavy metals, Biodegradation, Germination, Pigeon pea (*Cajanus Cajan* L.).

1. Introduction

During the last few decades, the Mandideep, Madhya Pradesh India, has undergone rapid social and economic development. It could face public health and ecological problems if heavy metal loads exceed a critical value. Little information is available on heavy metal concentrations on soils of Mandideep. A geographical information technology was used to select representative sampling sites and to present a distribution of the metals in the soils. The impact of pollution in the Mandideep of overcrowded cities and from industrial effluents and automobile exhausts has reached a disturbing magnitude and is arousing public awareness. However, in recent years, a few of these countries have achieved significant strides in their quest for rapid economic growth through industrialization. Thus, a number of factories, usually sited haphazardly, have developed. Population explosion and the increased use of automobiles have become very common in urban areas.

Bioremediation is the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state. The main principle of this technique is to remove pollutants from the natural environment or convert the pollutants to a less harmful product using the indigenous microbiological community of the contaminated environment [1]. Interest in the microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to clean up contaminated environments. These bioremediation and biotransformation methods use the naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclide's and heavy metals.

The long term effects of genotoxic compound in contaminated soil can adversely defects the health of many living organisms including human beings, by exposing them through diverse pathways, such as ingestion of plants that uptake soil pollutants and also leaching of compound from contaminated soil to ground and surface water used as drinking water [2]. The contaminated areas can be cleaned-up by the emerging science and technology of bioremediation. According to EPA [3], bioremediation is defined as a managed of spontaneous process in which microbiological process are used to degrade, break down or transform hazardous contaminants to less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from environment media. Microorganisms used chemical contaminants as an energy source using their metabolic process throughout the microbiological process. Factors such as nutrient availability, nitrogen, phosphorus, moisture content, pH, temperature of soil matrix are necessary for microbial biodegradation activity and cell growth. However, the excessive amounts inorganic nutrients in soil cause microbial inhibition [4].

Heavy metal (HM) pollution is a major environmental problem [5] that reduces crop production and food quality. Unlike organic contaminants, metals are not degradable and thus remain in the environment for long periods of time; when present at high concentrations, metals can negatively affect plant metabolism [6].

Industrial wastes are major source of heavy metals pollution in India due to inadequate wastewater treatment system. Heavy metals are harmful to humans and animals, tending to accumulate in the food chain. Tanneries and distilleries are important source of chromium (Cr), copper (Cu), manganese (Mn), iron (Fe), nickel (Ni), cadmium (Cd), lead (Pb), and zinc (Zn) pollution in the environment [7, 8]Chandra et al.

2004a, 2004b). In addition, mining metallurgical activities, smelting of metal ores and fertilizers have contributed to high level of heavy metal concentrations in the environment [9].

Physical or chemical treatment method for decontamination of soils by heavy metals often have some limitations and are also not sustainable in the long run. On the other hand, microorganism driven methods offer an attractive in terms of low operational cost and efficient treatment. [10]

Although many heavy metals are naturally occurring, some however, have shown potential health hazards especially in high concentrations in human and plant cells. Heavy metals such as cadmium, lead, and others like copper and zinc are potentially toxic and pose great threat to food safety and human health even in minute concentrations [11]

Numerous soil contaminants, including PAHs, have been studied with respect to their sorption/desorption behaviors in soil, and various studies have also identified the microbial strains capable of degrading these compounds and have elucidated the biodegradation pathways involved. In addition to knowledge about the biodegradation and the transport of the contaminants in the soil, it is also necessary to know about microbial transport through the soil matrix in order to predict the fate of these contaminants and to monitor the progress of soil bioremediation, especially in-situ bioremediation [12].

Chromium is one of the most toxic heavy metals which deteriorate the environment. Chromium is used on a large scale in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, Cr chemicals production, and pulp and paper production. The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40% of the total industrial use [13]. Chandra *et al.* (1997) estimated that in India alone about 2600 to 4200 tonnes of elemental Cr escape into the environment annually from the tanning industries, with a Cr concentration ranging between 2000 and 5000 mg L−1 in the effluent compared to the recommended permissible limit of 2 mg L−1. Typical concentrations in natural soils are 1–1000 mg /kg soil [14].

In our previous studies, we have studied the feasibility of remediation of soils contaminated with Cr using different floriculture plant species, i.e., tuberose (*Polianthes tuberosa*) [15] Ramana et al., 2012), chrysanthemum, calendula, aster and dahlia [16]. From these studies, it was found that majority of the plant species could tolerate at the most 10–15 mg Cr/kg soil. However, the contaminated sites would have very high levels of Cr and at times would even be unfit for cultivation of the crops.

Recently, more studies have described the capabilities of microorganisms especially *Pseudomonas spp.* to biodegrade petroleum hydrocarbons. *Pseudomonas* spp. is widely known as a model microorganism for studying hydrocarbons degradation. [17] previously tested on the biodegradation of total aliphatic and aromatic with a sample collected from oil contaminated site in Pakistan by means of bacterial consortium (*Pseudomonas, Alcaligens, Psychrobacter*, *Bacillus, Micricoccus*, and *Staphylococcussp*). The acquired results indicated that total aliphatic and aromatic degradation were up to 94.84% and 93.75% respectively after 24 days of incubation.

2. Materials and Methods

2.1 Soil Collection & Treatment-

Soil samples were first collected randomly from industrial contaminated soil areas nearby Pharmaceutical industry, Tractor manufacturing industry, Food industry, and Leather industry of Mandideep District Raisen of Madhya Pradesh, India. Soil samples were collected in sterilized polythene bags and immediately bought to the laboratory. Soil samples were air - dried in a circulating air in the oven at 30°C to a constant weight and then passed through a 2 mm sieve and stored in dry labelled plastic and taken to the laboratory for pretreatment and analyses under frozen condition (4°C) to prevent any microbial activity.

Soil samples for chromium were digested according to the procedure described by Sharidah (1999). The dried soil samples were digested with 10 ml di-acid mixture (9 HNO3: 4 HClO4) and the concentration of Cr was determined with Atomic Absorption Spectrophotometer (Perkin Elmer). Standard solutions prepared by appropriate dilution of the stock solution 1000 µg/mL were used to calibrate the device by means of the standard curve method. The detection limit for all analyzed heavy metals was 0.015 mg. / kg. The accuracy of the results obtained in this study was assessed by preparing blank solutions the same manner as employed for the digested soil samples. The blank solutions were checked and found to be uncontaminated. The data was analyzed statistically and the treatment means were compared using LSD technique at 5 % probability appropriate for CRBD [18] Gomez and Gomez, 1984).

2.2 Isolation and identification of microbes from soil samples-

The microbial strains were isolated from the collected soil samples by serial dilution technique. Selective isolation of bacterial spp. was performed by spreading the samples on their individual media. Individual distinct colonies were further undergone repeated sub-culturing. Selected colonies identified by using morphological, cultural and biochemical characteristics according to Bergey's manual of systemic bacteriology classification.

2.3 Screening of Cr degradation-

Different concentrations of chromium prepared by dissolving required amount of potassium chromate ($K_2Cr_2O_7$), control, 5, 10, 25 and 50 ppm concentrations were selected for the experiment. Standard nutrient broth was prepared and autoclaved at 121°C for 15 minutes and was cooled in a water bath. In 250 ml Erlenmeyer flasks, 50 ml nutrient broth was taken along with the above mentioned concentration of chromium. Under aseptic conditions, the three chosen microorganisms were inoculated individually into these

flasks with 0.1ml cells. The flasks were incubated at shaking incubator 140 rpm, 37°C temperatures. Uninoculated control flasks were also maintained in the same manner. After 48 hours, samples were taken from each flask and centrifuged at 8,000 rpm for fifteen minutes. The supernatants were analysed with AAS for chromium concentration adopting standard methods. Percentage reduction in chromium concentration was calculated for each chromium concentration based on the initial and final readings.

3. Analysis of Morphometric parameters-

3.1 Pot Culture Experiment-

Effects of Cr on the growth of *Cajanus cajan* plant were analyzed by pot culture experiment. The following treatment was made for this study. 1. Control (sterile soil + seeds). 2. Effect of Cr (sterile soil + Cr + seeds). 3. Effect of microbes (sterile soil+ Cr + microbes + seeds).

The soil for the experiment was collected from 0 to 15 cm depth from the nearby agricultural field. The soil was ground, dried under shade and passed through 4 mm sieve and analyzed for various physico-chemical characteristics. The general characteristics of the soil were: pH 7.8; EC 2.2 dS m^1 and organic carbon 4.1 g kg^{-1}. The soil was transferred to 5 kg plastic pots. A stock solution of 1,000 ppm Cr was prepared by dissolving 2.83 g $K_2Cr_2O_7$ in 1,000 ml distilled water. The soil in the pot was then treated with aqueous solution of $K_2Cr_2O_7$ so as get 25 mg Cr (VI) kg^{-1} soil. The soil was then subjected to wetting and drying cycles for 1 month and later the soil was taken out and mixed uniformly. *Cajanus cajan* seed were collected from local market and were sowed in each pot. The experiment was conducted in a completely randomized block design and was replicated five times. The plants were harvested at 1 month stage and separated into roots, shoots and the data on plant height & root length (cm), and Dry weight of shoot & root (g/plant) were recorded.

3.2 Percentage of Germination and Agronomical Parameters of Plant-

The percentage of seed germination was calculated from the each treated pot after 10 days from the sowing. Plants were collected for agronomical characterization from each treated pot after 1 month from the sowing date of seed. The length of the root and shoot was measured individually for plant and expressed in cm. Dry weight of shoot & root (g/plant), were recorded. [19]

4. Results & Discussion

4.1 Present Cr concentration in soil-

Present concentration of Cr in soil samples was found maximum in leather industry (256 mg/kg) followed by tractor manufacturing industry (105 mg/kg), Pharmaceutical industry (65 mg/kg) and food industry (45 mg/kg) found low level of chromium concentration. (Table 1)

4.2 Isolation of bacteria

Bacterial strains were isolated from the Cr contaminated soil sample by using serial dilution and using Nutrient agar medium respectively. Among this study 42 bacterial colonies were noted in 10^{-6} and 10^{-5} dilution.

4.3 Identification of bacterial strains

The selected three bacterial strains were named as SS5, MR2 and MR3. These organisms were identified based on the cultural, morphology and biochemical characteristics (Table 2 & 3) and results of SS5, MR2 and MR3 were compared with Bergey s manual of systemic bacteriology classification.

4.4 Cr degrading Ability of selective strains-

P. fluorescence, B. cereus and *B. decolorationis* were inoculated into nutrient broth containing chromium at varying concentrations (control, 5, 10, 25 and 50 ppm). A control tube was also inoculated which lacked chromium. The bacterial strain *P. fluorescence,* were found maximum removed 87%, 73%, 65% and 60% of chromium from medium in 48 hours starting with the initial concentration of 5 ppm/L, 10 ppm/L, 25 ppm/L and 50 ppm/L respectively. Followed by *Bacillus cereus* and *Bacillus decolorationis* were showed low level of chromium degradation. (Table 4).

Percentage removal of chromium was similar observation made by Basu *et al* (2014) who reported 97% removal of chromium *Bacillus subtilis* starting with an initial concentration of 2.5 mg/L. The strain was isolated from wetlands Basu *et al.* (2014). Results of the present study for chromium removal indicate that the isolates could tolerate to chromium. Percentage reduction in chromium concentration was calculated for each chromium concentration based on the initial and final readings. This is due to the fact that as the volume of inoculum was constant relatively less biomass was available for chromium removal from the media, in case of higher concentrations. Raghuraman *et al.* (2013) has also reported that the higher reduction of chromium for lower initial concentrations by *P. aeruginosa* and *P. fluorescence.*

4.5 Analysis of seed germination and plant growth ability-

The Cr degrading ability of isolates and the effect of Cr on the growth of *Cajanus cajan* also analyzed by pot culture experiments. The seed germinating and plant growth ability were analysed in different experimental groups using *Pseudomonas fluorescence, Bacillus cereus* and *Bacillus decolorationis*. *Pseudomonas fluorescence* (95%), *Bacillus cereus (90%)* have showed maximum seed germinating and plant growth ability noted compared to *Bacillus decolorationis* (84%) inoculated strain. The length of the root and shoot was measured individually for plant and expressed in cm. Fresh weigh of shoot and root (g/plant), Dry weight of shoot & root (g/plant) were recorded. (Table-5)

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Author Profile

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Table 1: Analysis of present Cr concentration in different soil samples

S.No.	Soil Samples	Cr conc. ppm (mg/kg)
1.	Pharmaceutical industry	65ppm
2.	Tractor manufacturing industry	105 ppm
3.	Food industry	45 ppm
4.	Leather industry	256 ppm

Table 2: Morphological characterization of isolated bacterial species

S.No.	Morphological characteristics	Isolated bacterial colonies		
		SS5 (*P. fluorescence*)	MR2 (*B. cereus*)	MR3 (*B. decolorationis*)
1.	Colour of the colony	White	White	White
2.	Shape of the cell	Rod	Rod	Rod
3.	Gram s Staining	Negative	Positive	Positive
4.	Motility	Motile	Motile	Motile

Table 3: Biochemical characterization of isolated bacterial species

S.No	Biochemical characterization	Isolated bacterial colonies		
		SS5 (*P. fluorescence*)	MR2 (*B. cereus*)	MR3 (*B. decolorationis*)
1.	Indole	Negative	Negative	Negative
2.	Methyl red	Negative	Negative	Negative
3.	Voges-proskauer test	Negative	Positive	Negative
4.	Citrate utilization test	Positive	Positive	Negative
6.	Urease hydrolysis	Variable	Negative	Negative
7.	Oxidase	Positive	Negative	Positive
8.	Catalase	Positive	Positive	Positive

Table 4: Degradation of Chromium ability by isolated bacterial strains

S.No.	Test organisms	1st day in Cr conc. ppm (Initial)	48 hrs in Cr conc. ppm (final)	% of Cr degradation
1.	SS5 (*P. fluorescence*)	5	0.65	87%,
		10	2.7	73%,
		25	8.75	65%
		50	20	60%
2.	MR2 (*B. cereus*)	5	1.25	75%
		10	3.8	62%
		25	11.25	55%
		50	26	48%
3.	MR3 (*B. decolorationis*)	5	1.45	71%
		10	4.2	58%
		25	12	52%
		50	28	44%

Table 5: Germination and plant growth characterization of *Cajanus cajan* for 1 month of sowing.

S.No.	*Plant Growth Parameters*	*Control+ Seed*	*Cr + Seed*	*Cr + (seed) SS5*	*Cr + (seed) MR2*	*Cr + (seed) MR3*
1.	Germination	85	68	95	90	84
2.	Plant height (cm)	28.58	18.35	26.56	23.76	21.00
3.	Root length (cm)	10.28	7.85	9.87	8.88	8.14
4.	Fresh weight of shoot (g/plant)	0.829	0.563	0.785	0.665	0.623
5.	Fresh weight of root (g/plant)	0.155	0.098	0.135	0.124	0.112
6.	Dry weight of shoot (g/plant)	0.27	0.17	0.24	0.23	0.21
7.	Dry weight of root (g/plant)	0.04	0.02	0.03	0.03	0.02

SS5 -(*P. fluorescence*), MR2 - (*B. cereus*), MR3 - (*B. decolorationis*), Cr-Chromium.

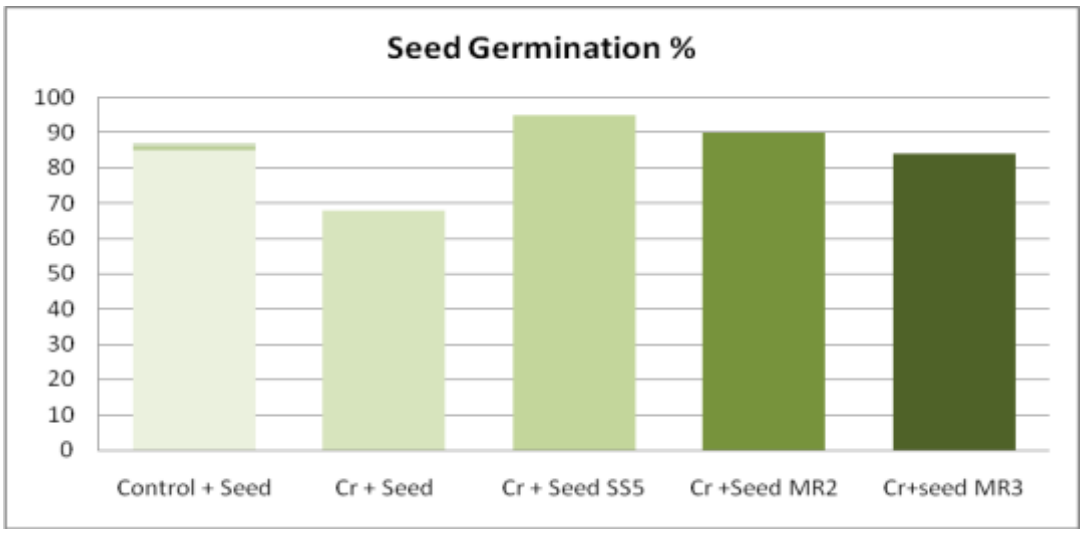

Figure 1: Graphical representation of seed germination percent

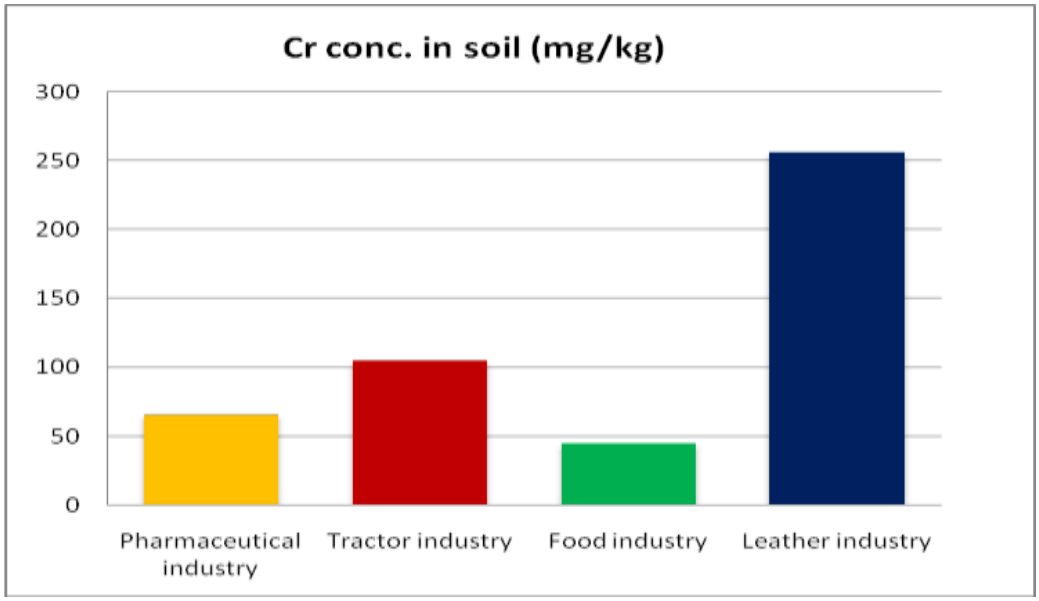

Figure 2: Graphical representation of present Cr conc. (mg/kg) in different soil