Research Paper

Biotransformation of hexavalent chromium as influenced by indigenous microbes in tannery effluent contaminated sites

Environment

KEYWORDS : Cr (VI), Biotransfomation, Pseudomonas sp., Trichoderma sp., Aspergillus sp.

Sunitha, R — Department of Environmental Science, Agriculture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India

S. K. Rajkishore — Department of Environmental Science, Agriculture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India

ABSTRACT

Majority of the tannery industries are located in Ambur, Vaniyambadi, Walajapet and Thirupattur areas of Vellore district, Tamil Nadu. The industries dispose the tannery waste contained chromium into soil and onto the Palar river basin. Large amounts of Cr is found in groundwater and as well as the soil ecosystem. Based on the basic survey on tanneries and its pollution in the Vellore district, our studies focused on bio-remediation of tannery contaminated soil. Cr resistant microbes were isolated from contaminated soil and characterization was done. The cultures were evaluated with hexavalent chromium reduction in soil. Three microbial cultures were identified from the contaminated soil (Pseudomonas fluorescens, Aspergillus niger and Trichoderma viride). The per cent reduction ranged from 39 to 79 by Pseudomonas fluorescens, 30.8 to 58.4 by Trichoderma viride and 50.3 to 83.4 by Aspergillus niger. Among the three native isolates, Aspergillus niger was found relatively superior to Pseudomonas fluorescens and Trichoderma viride in reducing the Cr VI in soil.

INTRODUCTION

As per 2001 census population of the surrounding towns of Ranipet adjacent to Palar river basin that is, Vaniyambadi, Ambur, Pernampattu, and Ranipettai are 103,841; 99,855; 41,323; 36,675, respectively, with 138 tanneries in Vaniyambadi, 83 tanneries in Ambur, 18 tanneries in Pernampattu, and 39 tanneries in Ranipettai. Due to mushrooming of tanneries day by day the study became imperative to check the flow of chromium and other heavy metals in the region. Although some of the tanning industries have been closed down which neither had their own effluent treatment plants nor were connected to a common effluent treatment facility, this hardly made any difference on the ground as tanneries had been discharging effluents for several decades.

A large number of small-scale tanneries located within the country do not have access to a common treatment plant and discharge their wastes in open fields or dump them in landfill sites. The use of sludge as a cheap manure in agricultural practices is also not uncommon in this region. These indiscrete methods of disposal contaminate the soil and water providing an easy pathway of Cr in the food chain. Plant uptake of chromium accounted for less than 1% of the chromium removed from the soil (Banks *et al.,* 2006). Once assimilated by plants, Cr (VI) is readily reduced to Cr (III) (Aldrich *et al.,* 2003). Overall, the addition of organic matter had the strongest influence on chromium mobility (Banks *et al.,* 2006). Although bioremediation of Cr (VI) to Cr (III) is a viable cleanup approach, it produces soluble organo-Cr (III) complexes in the environment which depends on the organic ligands (Park *et al.,* 2008).

Though many studies were carried out for the treatment of Cr (VI) contaminated water/wastewater, not much research has been carried out on the remediation of Cr (VI) contaminated soils either using *in situ* or *ex situ* bioremediation techniques. Turick *et al*. (1998) demonstrated the Cr (VI) reduction in a contaminated soil by indigenous microbial consortium under anaerobic condition. Under anaerobic conditions, indigenous microbes reduced 65% of Cr (VI) from contaminated soil with the addition of glucose. Tseng and Benefieldt (2002) studied the *in situ* bioremediation of Cr (VI) contaminated soil by supplying various carbon sources. Information available on the *ex situ* treatment of Cr (VI) contaminated soil is scarce. In the present study, development of a biological system for the treatment of Cr (VI) contaminated soils is described. The indigenous microbes were evaluated in an incubation study followed by the evaluation of biotransformation study.

MATERIALS AND METHODS

Isolation of microorganisms from contaminated sites

The population density of bacteria, fungi and actinomycetes were enumerated using serial dilution and plating technique (Parkinson *et al*., 1971). One gram soil was weighed and serially diluted up to 10^{-6} for bacteria with specific medium of Nutrient agar (Allen, 1953), 10^{-3} dilution for fungal population with Rose Bengal agar medium (Martin, 1950) and 10^{-2} dilution for Actinomycetes population with Kenknights agar medium (Rangaswami, 1966). The soil samples were collected from Cr contaminated sites. The beneficial microorganisms were isolated and purified for Cr detoxification studies. The microbial strains were characterized and identified by biochemical test. These microbes tolerated up to 1646 mg of total Cr kg^{-1} in soil.

Biochemical test for microbes

The soil samples were collected from chromium contaminated soil and the beneficial microbes were isolated. The population of *Pseudomonas fluorescens* in the soil was enumerated by serial dilution and plating technique. One gram of soil sample was serially diluted up to 10^{-5} dilution and one ml of 10^{-5} dilution was plated in a sterile petriplate using King's B agar medium. After incubation for two to three days, the colonies were counted and expressed as per g dry weight of the soil. This specific media was used for development of fluorescent colour (Plate 1). Starch hydrolysis test was carried out for *Pseudomonas fluorescens* culture by clear zone development (Gerhardt *et al.,* 1994). For fungus (*Aspergillus niger* and *Trichoderma viride*) spore counting method was followed (Harrigan and Mc cance, 1966).

Biotransformation of Cr (VI)

The effect of selective microbial culture was examined by conducting a closed incubation experiment in the laboratory. Bulk soil samples were collected at 0 to 30 cm depth from Eastern Block field (No. 37), TNAU, Coimbatore. The samples were air dried at 25^0C and sieved (< 2 mm). One hundred gram of air dried soil was spiked with different concentrations of Cr (VI) (0, 10, 25, 50, 75 and 100 µg Cr (VI) g^{-1} of soil, in plastic containers). The analytical grade $K_2Cr_2O_7$ was used as the source of Cr (VI). The Cr- spiked soil was inoculated with different microbial strains (*Pseudomonas fluorescens, Trichoderma viride* and *Aspergillus niger*) at a rate equivalent to 5 percent (w/w). The rate of microbial strain was fixed through a preliminary experiment and 5 % was found optimum to achieve a desirable level of Cr (VI) in soil. Required quantity of distilled water was added to achieve a final moisture content equivalent to field capacity. The plastic containers were covered with polythene bags containing small pin-sized holes to permit aeration. Three replicates of each treatment were prepared, randomly placed and incubated in the laboratory at $25^0C \pm 2^0C$ for 15 days. Based on weight loss distilled water was added to the container to maintain the moisture content throughout the incubation experiment. At the end of 15 days, soil samples were collected and determined for Cr (VI) by Diphenylcarbazide (DPC) method. Moisture factor was computed and applied to express the results as oven dry basis.

Cultures used for detoxification study:
1. *Pseudomonas fluorescens,* 2. *Trichoderma viride,* 3. *Aspergillus niger*

RESULTS AND DISCUSSION
Bioremediation of Cr contaminated soil generally aims to reduce the toxicity of Cr in soil, mainly by immobilization or mobilization that enhance the bioavailability and thus plant uptake. Various microbial strains are capable of reducing the toxicity of Cr in soil, and therefore they could form components of bioremediation. These microbial strains are essential for the success of the technology.

Screening of microbial strains for Cr (VI) reduction
The biotoxicity is determined by the Cr species that exist in soil. In Cr contaminated soil, Cr is predominantly present as Cr (III) which is non toxic. Though the oxidation of Cr (III) to Cr (VI) is rather uncommon, due to the presence of manganese mineral and a specific type of microorganisms capable of converting Cr (III) to Cr (VI), large amount of Cr (VI), a highly toxic form of Cr was found in Cr contaminated soil in Vellore district (Mahimairaja *et al.,* 2000). Therefore, the effect of microbial strains, viz., *Pseudomonas fluorescens, Trichoderma viride* and *Aspergillus niger* (@5 % concentration) on Cr (VI) reduction was examined. In a laboratory bioassay study, the microbial strains were exposed to varied concentration of Cr (VI) in soil. Some important characteristics of the soil were determined. The soil pH and EC were 7.80 and 0.36 dSm^{-1} respectively. The available N ($KMnO_4$-N), P ($NaHCO_3$-P) and K (NH_4OAc-K) were 72.8, 14.07 and 180 kg ha^{-1} respectively. The soil organic carbon (SOC) content was 0.98 % with the cation exchange capacity (CEC) of 14.50 cmol kg^{-1}.

In the *Pseudomonas fluorescens* treated soil, the initial concentration of Cr (VI) ranged from 9.62 to 96.1 mg kg^{-1}. Whereas, after 15 days it ranged from 2.0 to 58.2 mg kg^{-1}. Large amount of Cr (VI) was found reduced in soil due to the presence of *Pseudomonas fluorescens*. In the soil treated with *Trichoderma viride*, the initial concentration ranged from 9.62 to 95.35 mg kg^{-1}; whereas, after 15 days it was significantly reduced and ranged from 4 to 66 mg kg^{-1}. The microbial strain, *Aspergillus niger* was found very effective in reducing the concentration of Cr (VI). Initially, the Cr (VI) concentration in soil ranged from 9.0 to 96.5 mg kg^{-1}; whereas, after 15 days it was from 1.5 to 48.0 mg kg^{-1}. Significant reduction in Cr (VI) was observed due to the inoculation of *Aspergillus niger* (Table 1).

The microbial strains isolated from Cr contaminated soil were exposed to different concentration of Cr VI in soil, and their potential to reduce Cr VI was examined. The results showed that the three isolates differed markedly in their ability to reduce the Cr VI in soil. The per cent reduction ranged from 39 to 79 by *Pseudomonas fluorescens*, 30.8 to 58.4 by *Trichoderma viride* and 50.3 to 83.4 by *Aspergillus niger*. The per cent reduction was higher at lower concentration of Cr VI and it was found reduced markedly with an increase in the concentration of Cr VI (Fig. 1). Among the three native isolates, the *Aspergillus niger* was found relatively superior to *Pseudomonas fluorescens* and *Trichoderma viride* in reducing the Cr VI in soil. All the strains appeared to have tolerated up to 100 mg Cr VI kg^{-1} soil. Microorganisms are capable of reducing Cr (VI) to Cr (III) include bacteria (*Pseudomonas, Micrococcus, Enterobacter, Bacillus* and *Desulfomamaculum*), algae (Cervantes *et al*, 2001), yeast and fungi (*Trichoderma, Aspergillus, Phanerochates* and *Penicillium*) (Jatasingh and Philip, 2005). Megharaj *et al* (2003) also reported strains, which were isolated from Cr polluted soil, could tolerate up to 100 µg ml^{-1} of Cr VI. However, other studies have shown that even at low concentration the Cr VI was found toxic to many bacteria, e.g *Bacillus subtilis*, *Bacillus cerveces*, *Escherichia coli* and *Proteus vulgaris* (Shimada and Matsuchima, 1983; Sundar *et al*., 2010).

In Cr contaminated soil, presence of toxic Cr (VI) is inevitable. The reduction of Cr (VI) to less toxic Cr III is considered to be an important step in developing remediation technology for Cr contaminated soil. Therefore, laboratory study was conducted to isolate and screen microbial strains which are capable of reducing Cr (VI) to Cr (III). The results have shown the presence of both bacterial (*Pseudomonas fluorescens*) and fungal (*Trichoderma viride* and *Aspergillus niger*) strains in the Cr contaminated soil. Occurrence of substantial quantity of bacterial population at high concentration of Cr in contaminated soil has been reported (Megharaj *et al.,* 2003; Bopp *et al*., 1988; Losi and Frankenberger 1994). They appeared to have tolerated high concentration of Cr (total Cr 683 mg kg^{-1}). It is not known whether they could tolerate high concentration of Cr VI and have the potential to reduce Cr VI to Cr III in soil.

Mujeeb *et al*. (2007) reported that the rate of Cr VI reduction was dependant on the concentration of inoculum (*Pseudomonas sp*), as the increase in the inoculum concentration increased the reduction of Cr VI. They observed a maximum reduction of 1125 mg kg^{-1} with a 30 per cent inoculum (v/v) concentration, as against only 5 per cent concentration of inoculum used in the current study. The rate of Cr VI reduction was also dependant on several factors like pH, temperature and source of carbon and nutrients for the microbial strains (Parameswari *et al*., 2009; Megharaj *et al*., 2003).

The microbial reduction of Cr VI commonly occurs as a two- or three step process with Cr VI initially reduced to the short lived intermediates $Cr (V)^+$ and / or $Cr (IV)^+$ before further reduction to the thermodynamically stable end product, Cr III is formed. However, it is at present not clear as to whether the reduction of $Cr (V)^+$ to $Cr (IV)^+$ and Cr IV to Cr III was spontaneous or enzyme mediated. NADH, NADHP and electron from the endogenous reserve are implicated as electron donors in the Cr VI reduction process. The Cr reductase Chr R transiently reduces Cr VI with a one-electron shuttle to form $Cr (V)^+$, followed by two-electron transfer to generate Cr III (Cheung and Gu, 2006). Such enzymatic reduction of Cr VI has also been reported by several researchers.

The widespread occurrence of these bacterial (*Pseudomonas fluorescens*) and fungal (*Trichoderma viride* and *Aspergillus niger*) strains possessing Cr VI reducing abilities, offers great potential for *in situ* bioremediation of Cr contaminated soils in Vellore district.

CONCLUSION
The microbial cultures act as Cr reductase substance. Both bacterial (*P. fluorescens*) and fungal (*T. viride* and *A. niger*) strains were identified in the Cr contaminated soil. The three isolates differed markedly in their ability to reduce the Cr VI in soil. The per cent reduction ranged from 39 to 79 by *Pseudomonas fluorescens*, 30.8 to 58.4 by *Trichoderma viride* and 50.3 to 83.4 by *Aspergillus niger*. Among the three native isolates, the *Aspergillus niger* was found relatively superior to *Pseudomonas fluorescens* and *Trichoderma viride* in reducing the Cr VI in soil. All the strains appeared to have tolerated up to 100 mg Cr VI kg^{-1} soil. The present study reveals the capacity of bacterial cells to bind metallic ions in an eco friendly manner and it is clear that the bacterial cell may act as nucleation sites for the removal of metals from the environment.

Table 1. Effect of microbial cultures on hexavalent chromium (Cr^{6+}) detoxification at 15 days incubation

Treatments	Pseudomonas fluorescens		Trichoderma viride		Aspergillus niger	
	0^{th} day ($\mu g\ g^{-1}$)	15^{th} day ($\mu g\ g^{-1}$)	0^{th} day ($\mu g\ g^{-1}$)	15^{th} day ($\mu g\ g^{-1}$)	0^{th} day ($\mu g\ g^{-1}$)	15^{th} day ($\mu g\ g^{-1}$)
Control	0.00	0.00	0.00	0.00	0.00	0.00
100g soil + 10 $\mu g\ g^{-1}$ of $K_2Cr_2O_7$ salt	9.62	2.00	9.62	4.00	9.01	1.50
100g soil + 25 $\mu g\ g^{-1}$ of $K_2Cr_2O_7$ salt	22.23	10.60	24.04	13.00	24.42	6.40
100g soil + 50 $\mu g\ g^{-1}$ of $K_2Cr_2O_7$ salt	47.27	26.20	49.46	30.00	43.65	17.40
100g soil + 75 $\mu g\ g^{-1}$ of $K_2Cr_2O_7$ salt	72.12	42.20	72.12	44.00	71.31	34.00
100g soil + 100 $\mu g\ g^{-1}$ of $K_2Cr_2O_7$ salt	96.15	58.20	95.35	66.00	96.53	48.00
Un inoculated soil	0.00	0.00	0.00	0.00	0.00	0.00
Mean	35.34	19.89	35.80	23.86	34.99	15.33
SEd	4.918	2.892	4.938	3.509	4.876	2.308
CD(p=0.05)	10.549	6.203	10.592	7.528	10.459	4.951

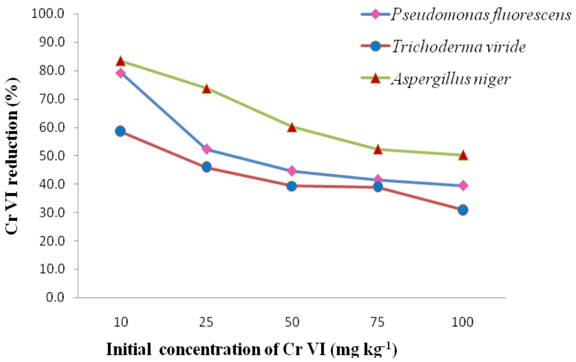

Fig 1. Percentage reduction of Cr (VI) by indigenous microbes

REFERENCE

• Aldrich, M. V., Gardea-Torresdey, J. L., et al (2003). Uptake and reduction of Cr (VI) to Cr (III) by mesquite (Prosopis spp.): chromate-plant interaction in hydroponics and solid media studied using XAS. Environ. Sci. Technol., 37(9): 1859 -1864. | • Allen. O.N. 1953. Experiments in Soil Bacteriology. Minneapolis, Minn, U.S.A: In Burgess Publ. Co., pp: 69-70. | • Bopp, H.L., Chakrabarty, A.M., Ehrlich, H.L. 1988. Chromate resistance plasmid in Pseudomonas fluorescens. J. Bacteriol., 155: 1105 - 1109. | • Cheung, K.H., Gu, J.D. 2006. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. J. Microbiol. Biotechnol., 16: 855 - 862. | • Flores, A., Perez, J. M. 1999. Cytotoxicity, apoptosis, and in vitro DNA damage induced by potassiumchromate. Toxicol. Appl. Pharmacol., 161(1): 75 - 81. | • Gerhardt, P., Murray, R. G. E., Wood, W.A. Krieg, N.R. 1994. Methods for general and molecular bacteriology, ASM, Washington DC, ISBN 1-5881-048-9. pp: 518. | • Harrigan, W.F., Mccance, M.E. 1966. Laboratory Methods in Microbiology. Academic Press, London. | • James, B. R. and R. J. Bartlett. 1983. Behaviour of chromium in soils. VII. Adsorption and reduction of hexavalent forms. J. Environ. Qual., 12: 177-181. | • Jeyasingh, J., Ligy Philip. 2005. Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. J. Hazard. Matl., B118: 113 - 120. | • Losi, M.E., Jr. Frankenberger, W.T. 1994. Chromium- resistant micro organism isolated from evaporation ponds of a metal processing plant. Water Air Soil Pollut : 405 - 413. | • M. K. Banks, Schwab, A. P., Henderson, C. 2006. Leaching and reduction of chromium in soil as affected by soil organic content and plants. Chemosphere, 62(2): 255 - 264. | • Mahimairaja, S., Sakthivel, S., Divakaran, J., Naidu, R., Ramasamy, K. 2000. Extent and severity of contamination around tanning industries in Vellore district. In :'Towards Better management of Soils Contaminated with Tannery Wastes. ACIAR Publication No 88: 75 - 82. | • Martin, J.P. 1950. Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci., 69: 215 - 232. | • Megharaj, M., Avudainayagam, S., Naidu, R. 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Curr. Microbiol., 47: 51 - 54. | • Mujeeb, U., Rahman, R., Shreen, G.U.L., Mohammad Zahoor, U.L. HAQ. 2007. Reduction of chromium (VI) by locally isolated Pseudomonas sp. Tuk. J. Biol., 31: 161-166. | • Parameswari, E., Lakshmanan, A., Thilagavathi, T. 2009. Chromate Resistance and Reduction by Bacterial isolates. Aust. J. Basic Appl. Sci., 3(2): 1363 - 1368. | • Parkinson, D., Gray, J.R.G., Williams, S.T. 1971. Methods for studying the ecology of soil microorganisms. Oxford Blackwell Scientific Publications. pp :. 116. | • Peitzsch, N., Eberz, G. Nies, D.H. 1998. Alcaligenes eutrophus as a bacterial chromate senso. Appl. Environ. Microbiol., 64(2): 453 - 458. | • Puzon, G. J., Tokala, R. K., Zhang, Yonge, H. D., Peyton, B.M., Xun, L. 2008. Mobility and recalcitrance of organo-chromium(III) complexes. Chemosphere, 70(11): 2054 - 2059. | • Rangaswami, G. 1966. Agricultural Microbiology. Asia Publishing House. London. | • Shimada, K., Matsuchima, K. 1983. Isolation of potassium chromate resistant bacterium and reduction of hexavalent chromium by the bacterium. Appl. Environ Micobiol., 67: 101 -106. | • Sundar, K., Vidya, R., Amitava Mukherjee, Chandrasekaran, N. 2010. High chromium tolerant bacterial strains from palar river basin: Impact of tannery pollution. Res. J. Environ. Earth Sci., 2(2): 112 - 117. | • Tseng, J.K., Bielefeldt, A.R. 2002. Low temperature Cr (VI) biotransformation in soil with varying electron acceptors. J. Environ. Qual., 31: 1831 - 1841. | • Turick, C.E., Graves, C., Apel, W.A. 1998. Bioremediation potential of Cr (VI) contaminated soil using indigeneous microorganisms, Biorem. J., 2: 1 - 6.