Terrestrial and Aquatic Environmental Toxicology ©2012 Global Science Books

Remediation of Chromium-Contaminated Soils Using *Pseudomonas aeruginosa* Strain BS2

Sanjeev K. Singh[1*] • Asha A. Juwarkar[1] • Ackmez Mudhoo[2] • Kirti V. Dubey[3]

[1] Eco-Restoration Division, National Environmental Engineering Research Institute (NEERI), Council of Scientific and Industrial Research, Nehru Marg, Nagpur-440020, India
[2] Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Mauritius, Réduit, Mauritius
[3] Sevadal Mahila Mahavidyalaya, Sakkardara Chowk, Nagpur-440009, India

Corresponding author: * **skr_singh@neeri.res.in**

ABSTRACT

The bioremediation of chromium-contaminated soil by a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* strain BS2 has been explored through column studies using uncontaminated soil spiked with toxic concentrations of heavy metals i.e. 1000 mg/kg chromium. Results on removal of chromium from the spiked soil by using di-rhamnolipid and tap water have shown a high potential of di-rhamnolipid in mobilization and decontamination of contaminated soil. Within 36 h of the leaching study, di-rhamnolipid facilitated the removal of chromium 10-13-fold compared to tap water. These results indicated that the specificity of this biosurfactant towards chromium was very high and 92% removal of chromium was observed. Hence, di-rhamnolipid selectively favours mobilization of chromium from contaminated soil. Biosurfactant specificity observed towards a specific metal will help the preferential elution of a specific contaminant using di-rhamnolipid. Leachates collected from chromium-spiked soil column treated with di-rhamnolipid solution had a lower pH (6.60-6.78) than leachates from heavy metal-spiked soil column treated with tap water (pH 6.90-7.25), which showed high dissolution of metal species from the spiked soil, and effective leaching of metals.

Keywords: bioremediation, biosurfactant, di-rhamnolipid, heavy metals, leachates
Abbreviations: CEC, cation exchange capacity; **EC**, electrical conductivity; **ICP-OES**, inductively coupled plasma-optical emission spectrometer; **MSM**, mineral salt medium

INTRODUCTION

Rapid developments and increase in mining and industrial activities have gradually redistributed many of the toxic metals from the earth's crust to the environment. This has substantially raised the chances of human exposure to these heavy metals, which increase in excess of their natural concentration, through ingestion, inhalation or skin contact. All metals that are mined are normally dissipated into the environment, thereby endangering the components of the ecosystem. As metals cannot be degraded further to non-toxic products (Khan *et al.* 2009), their deleterious effects tend to be permanent unless measures are taken to recover the metals economically from the contaminated site.

Metal contamination

Metal contamination of soil represents a potential environmental hazard in terms of toxicity to animals and inhibition of microbial processes (Babich and Stotsky 1985; Bayat and Sari 2010). According to an USEPA survey of 395 remedial action sites, it was found that heavy metals were the most prevalent class of contaminants (USEPA 1984). Soils are described as the sinks for metals. The latter being immobile in soil, accumulate in the topsoils, thus endangering crops and vegetables and microflora. Soil has complex functions which are beneficial to man and other living organisms. It acts as a filter, buffer, storage and transformation system and thus protecting the global ecosystem against the adverse effects of environmental pollution. In contaminated sites, heavy metal concentrations may be high enough to inhibit microbial activity (Ore *et al.* 2010; Sani *et al.* 2010). Soil microorganisms may be critical to plant growth because they encourage the development of stable soil structure, release required nutrients in inorganic forms by mineralization and produce growth regulating substances (Bronick and Lal 2005; Zaidi *et al.* 2009). Soil microorganisms also contribute to plant growth by immobilizing heavy metals in soil (Kuffner *et al.* 2008). Heavy metal contamination of soil, on the other hand, decreases microbial activity, microbial numbers, and microbially-mediated soil processes such as nitrification, denitrification and decomposition of organic matter (Doelman and Haanstra 1979; Chang and Broadbent 1981; Nordgren *et al.* 1988). Attempts to remediate metal contaminated soil have involved soil washing strategies or pump and treat strategies for subsurface environments (Geets *et al.* 2008). Washing strategies can be greatly enhanced by the use of an agent that can increase the desorption of the soil-bound metals and thereafter facilitate their transport through the soil matrix.

Biosurfactant-enhanced metal removal

An ideal complexing agent is one that is soluble in water, chemically stable under environmental conditions, not strongly bound to soil particles, and has a high affinity for complexing metals (Chang and Broadbent 1981). Chang and Broadbent (1981) described the use of water-soluble bacterial exopolymers to mobilize soil-bound metals in sand materials.

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extra-cellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, which are reducing surface and interfacial tension at the surface and interface respectively. They are structurally diverse group of surface active molecules synthesized by microorganisms (Muthusamy *et al.* 2008).

Received: 5 August, 2011. Accepted: 21 January, 2012.

Original Research Paper

Table 1 Physico-chemical and microbiological characteristics of uncontaminated soil and heavy metal spiked soil. Values shown are mean ± standard deviation calculated for triplicates.

Parameters	Uncontaminated soil	Metal-spiked soil	Method used
Physical properties			
Bulk density (g/cm^3)	1.16 ± 0.02	1.28 ± 0.03	Keen-Rackzowski box method (Ramesh *et al.* 2008; Thakur *et al.* 2011)
Water holding capacity (%)	60.20 ± 3.04	56.20 ± 4.23	Keen-Rackzowski box method (Ramesh *et al.* 2008; Thakur *et al.* 2011)
Porosity (%)	51.60 ± 4.02	49.10 ± 5.51	Keen-Rackzowski box method (Ramesh *et al.* 2008; Thakur *et al.* 2011)
Sand (%)	27 ± 3	28 ± 3	International Pipette Method
Silt (%)	26 ± 2	27 ± 3	International Pipette Method (Rasool *et al.* 2008)
Clay (%)	47 ± 5	45 ± 5	International Pipette Method (Rasool *et al.* 2008)
Textural Class	Clay	Clay	International Pipette Method (Rasool *et al.* 2008)
Chemical properties			
pH	7.70 ± 0.41	6.30 ± 0.53	Soil to distilled water ratio of 1:2 (Rousk *et al.* 2009)
EC (mS/cm)	0.19 ± 0.01	0.29 ± 0.01	Conductivity method (Adviento-Borbe *et al.* 2006)
Cation exchange capacity (meq/100 g)	73.27 ± 3.96	66.20 ± 4.24	Ammonium-sodium acetate method (Candela *et al.* 2007)
Organic Carbon (%)	0.45 ± 0.01	0.38 ± 0.01	Micro-titration method (Beck *et al.* 2008)
Nitrogen (%)	0.044 ± 0.002	0.030 ± 0.002	Kjeldahl method (Pavlova *et al.* 2009)
Phosphorous (%)	0.072 ± 0.008	0.052 ± 0.005	UV-Vis spectrophotometry (Sattayatewa *et al.* 2011)
Potassium (%)	0.157 ± 0.011	0.170 ± 0.010	Flame photometer (Hemkar *et al.* 2010)
Total heavy metals (mg/kg). Determined by inductively coupled plasma-optical emission spectrometer (Hamilton *et al.* 2008)			
Chromium	47.8 ± 3.6	940.5 ± 14.6	
Microbial properties (CFU/g). Determined by the pour-plate technique (Bauermeister *et al.* 2008)			
Bacteria	17×10^5	58×10^4	
Fungi	26×10^3	42×10^2	
Actinomycetes	43×10^3	18×10^1	
Azotobacter	23×10^3	17×10^1	
Rhizobium	21×10^3	16×10^2	

Recently, it has been shown that rhamnolipid biosurfactants can complex heavy metals and are improving effective in removing soil bound cadmium, zinc and lead (Tan *et al.* 1994; Herman *et al.* 1995; Dahrazma and Mulligan 2007; Wang and Mulligan 2009; Bondarenko *et al.* 2010). Microbially produced surfactants offer the advantage of being potentially less toxic and more biodegradable than some synthetic surfactants; the diversity of chemical forms produced by a variety of microbial species may allow the selection of microbial products with a high specificity for certain applications (Torrens *et al.* 1998). A promising technology currently being explored is soil flushing with pump and treat technologies for *in-situ* remediation. For *in-situ* remediation, it is important that the remediation process be as noninvasive and environmentally benign as possible if the end product is intended to be a healthy productive ecosystem (Maier *et al.* 2001). Unfortunately, the few reactants capable of mobilizing all metal contaminants are either toxic or destructive to the physical, chemical or biological structure of the soil. Anionic surfactants have also shown potential as soil washing agents due to their ability to solubilize metals within micelle (Ahn *et al.* 2009). They can be potentially as effective with some distinct advantages over the highly used synthetic surfactants including high specificity, biodegradability and biocompatibility (Cooper 1986). Due to the anionic nature of rhamnolipids, they are able to remove metals from soil and ions such as cadmium, copper, lanthanum, lead and zinc due to their complexation ability (Tan *et al.* 1994; Herman *et al.* 1995).

Biosurfactants, surfactin from *Bacillus subtilis*, rhamnolipids from *Pseudomonas aeruginosa* and sophorolipids from *Torulopsis bombicola* have been found to enhance the removal of metals from the sediments as well as soil (Van Bogaert *et al.* 2007; Wang *et al.* 2010). Although 80% of chromium can be removed from artificially contaminated soil, in field samples the results were more in the range of 20-80% as reported by Fraser (2000). In summary, rhamnolipids still being effective for heavy metal removal from laboratory scale systems, studies have not been performed sufficiently on a larger scale. Still more information is required to establish the nature of the biosurfactant-metal complexes. Stability constants were established by an ion-exchange resin technique (Gasper *et al.* 2007). Yet, extensive research is further needed to assess the potential effectiveness of biosurfactants for soil flushing of metal-contaminated soils.

In this paper, a column study was conducted to study the potential of the di-rhamnolipid biosurfactant produced from *P. aeruginosa* strain BS2 for the removal of chromium sorbed on soil matrix. The effect of metal contamination on soil characteristics and the assessment of the effects of using the latter biosurfactant during metal decontamination on soil biota have also been reported. This study focuses on chromium because chromium is of great concern as it has highly toxic and carcinogenic properties and also for its potential for the contamination of groundwater due to its greater mobility in soils and in the aquatic environment (Massar *et al.* 2007).

MATERIALS AND METHODS

Column studies were carried out at bench scale at the National Environmental Engineering Research Institute (NEERI), Nagpur, India to evaluate the effectiveness of the di-rhamnolipid biosurfactant *P. aeruginosa* strain BS2 towards the removal of chromium from soil-contaminated matrix.

Soils

Two soils were used for the column study. The uncontaminated soil collected from NEERI premises. For the second set, the uncontaminated soil was spiked with toxic concentrations of chromium at 1000 mg/kg using $K_2Cr_2O_7$, respectively to increase the concentration of the respective metal in the soil. Soil was shaken for 3 days on the rotating shaker, then spread on plastic trays for air-drying and sieved through 200-mm mesh. Regular grab sampling in triplicates and analysis of the chromium-spiked soils were carried out until stabilization was reached in 2 months. The soil used for the column study was analyzed for physico-chemical parameters. Microbes such as bacteria, fungi, actinomycetes and nitrogen fixing strains of *Rhizobium* and *Azotobacter* were analyzed by following standard methods for soil microbial populations and were expressed in terms of colony forming units (CFU/g) (Juwarkar and Jambhulkar 2008) (**Table 1**). All values have been reported as their respective mean with standard deviation calculated for a triplicate set of raw data.

P. aeruginosa strain BS2, a potential di-rhamnolipid biosurfactant producing culture that was isolated from an oily sludge and

Table 2 Details of the different treatments screened under column experiments.

Treatment No.	Column details	Washing solution
I	uncontaminated soil	tap water
II	heavy metals spiked soil	tap water
III	uncontaminated soil	0.1% di-rhamnolipid biosurfactant
IV	heavy metals spiked soil	0.1% di-rhamnolipid biosurfactant

was used for di-rhamnolipid production (Dubey and Juwarkar 2001). The di-rhamnolipid biosurfactant used in the present was produced from distillery wastewater (a no-cost complete nutrient medium for biosurfactant production) by *P. aeruginosa* having accession No. BS2 (assigned MTCC No. 5266 by Patent Collection Center of Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India). Di-rhamnolipids produced by the strain BS2 are highly efficient in regard to their surface-active properties.

Batch fermentation was carried out in Erlenmeyer flasks using sterile mineral salt medium (MSM) (Dubey and Juwarkar 2001). The MSM used contained (in distilled water): $NaNO_3$ - 2.5 g/L; K_2HPO_4, - 1.0 g/L KH_2PO_4 - 0.5 g/L; $MgSO_4$ - 0.5 g/L; KCl - 0.1 g/L, $FeSO_4$ - 0.01 g/L; $CaCl_2$ - 0.01 g/L; Glucose - 30 g/L. The pH was adjusted to 6.8-7.0 before sterilization. Sterile medium was inoculated with *P. aeruginosa* strain BS2 and incubated at 37°C, agitation 170 rpm and incubation period of 96 h. After 96 h of fermentation, biosurfactant was concentrated by foam fractionation method (Cooper *et al.* 1981). The rhamnolipid thus obtained was centrifuged and was passed through millipore filter and was quantified by measuring L-rhamnose by orcinol assay method (Chandrasekan *et al.* 1980). As a measure of quality control surface tension of rhamnolipid was measured and found to be 27 mN/m.

Column experiments

A column study was conducted to study the feasibility to remove the heavy metals from soil matrix using di-rhamnolipid biosurfactant. 50 g of heavy metals spiked soil was filled in 3 glass columns with internal diameter of 2.0 cm and length of 15 cm and into the fourth column of same dimensions; 50 g of uncontaminated soil was filled. From the top of the columns (I-IV), washing solutions were applied and the total volume of each washing solution was kept constant at 200 mL. The treatment details are depicted in **Table 2**.

Collection of the first leachate samples from all the four columns was possible within 2-3 h following start of the experiments. Thereafter, leachates were collected regularly after 12 h of time interval. Total leachate metals content were determined following a modification of the USEPA Method 3051 and analysed with inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 4100 DV, Perkin Elmer, USA). 0.1% biosurfactant solution and tap water were also similarly digested to determine the presence of heavy metal contaminants. The removal of the metals by the rhamnolipid was calculated from Equation 1.

$$\text{Removal}(\%) = \frac{[\text{Metal}(\text{mg}/\text{kg})]_{\text{removedbybiosurfactant}}}{[\text{Metal}(\text{mg}/\text{kg})]_{\text{initial,unspiked}}} \times 100 \quad (1).$$

RESULTS AND DISCUSSION

Characteristics of di-rhamnolipid biosurfactant

P. aeruginosa strain BS2 as a potential di-rhamnolipid biosurfactant producing culture was isolated from an oily sludge and used for further di-rhamnolipid production. The isolate *P. aeruginosa* having accession No. BS2 has been assigned MTCC No. 5266 by Patent Collection Center of Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The isolate was identified on the basis of the morphological, cultural and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (1984) and results are presented in **Table 3**.

Table 3 Morphological, biochemical and cultural characteristics of *P. aeruginosa strain* BS2 (MTCC No. 5266).

Morphological	Inferences
Gram staining	Gram negative
Motility	Highly motile
Size (μm)	2.0 × 0.6
Shape	Rods
Cultural	
Pigment production on	
Nutrient agar (Pyorubin pigment)	Positive
King's-A medium (Pyocyanin pigment)	Positive
King's-B medium (Fluorescien)	Positive
Haemolysis on blood agar	Positive
Biochemical	
Glucose	Positive
Fructose	Positive
Galactose	Positive
Lactose	Negative
Maltose	Negative
Mannitol	Positive
Rhamnose	Positive
Xylose	Positive
Enzyme production	
Catalase	Positive
Oxidase	Positive
Gelatinase	Positive
Lecithinase	Positive
Lipase	Positive
Caseinase	Positive
Amylase	Negative
Arginine dihydrolase	Positive
Growth on citrate	Positive
Growth on acetamide	Positive
Growth on succinate	Positive
Growth on lactate	Positive
Growth on hydrocarbons (decane, dodecane, tetradecane, hexadecane, crude oil and oil sludge)	Positive
Growth at 4°C	Negative
Growth at 41°C	Positive
Production of indole	Negative
Production of H_2S	Positive
G+C content (mole %)	64.97 ± 4.21

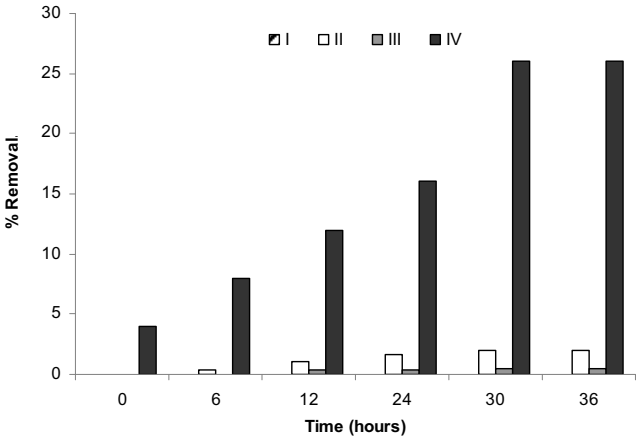

Fig. 1 Effect of time on removal of chromium from the heavy metal spiked soil using di-rhamnolipid biosurfactant (sample size, n=3).

Results presented in **Fig. 1** show that chromium removal efficiency is very low i.e. in the range of 0.4-2% when tap water is used for mobilization of heavy metals present in the spiked soil whereas the di-rhamnolipid biosurfactant effectively increased the removal of chromium from the spiked soil by 13 folds within 30 h. The first leachate sample collected from column IV, had 4% of chromium which steadily increased to 8% at 6 h, 12% at 12 h, 16% at 24 h and 26% at 30 and 36 h, respectively in subsequent leachate

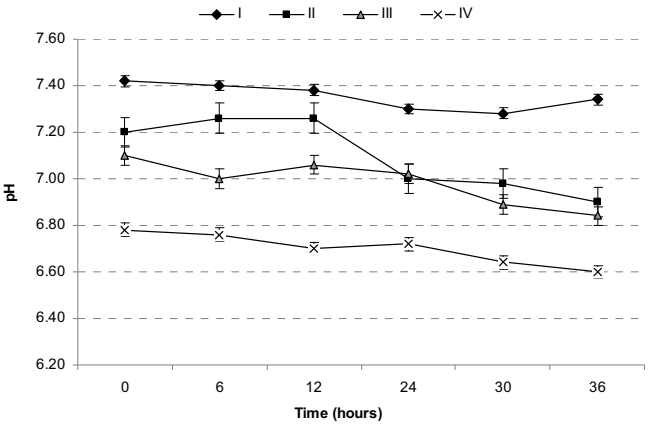

Fig. 2 Variation in the pH of the leachate collected from soil columns (n=3 for each data point).

samples collected. The di-rhamnolipid biosurfactant also removed chromium in the range of 0.4-0.5% from garden soil. In contrast to the di-rhamnolipid biosurfactant solution, tap water alone failed to remove chromium from garden soil. The total removal of chromium from heavy metal spiked soil was 92% when spiked soil was eluted with 0.1% di-rhamnolipid biosurfactant as compared to 1.80% when eluted with tap water within 36 h. Surface tension measurements of rhamnolipid before and after treatment of soil ensured that there was little loss of rhamnolipid due to adsorption to soil or degradation. Rhamnolipid at 0.1% removed up to 92% chromium from the spiked soil.

Variation in pH of the leachate

Leachates collected from soil columns I-IV were also monitored for the changes in pH along with the concentrations of heavy metals. pH gives an indication for the extent of metal removal because retention/mobilization mechanism is strongly pH dependent. Results presented in **Fig. 2** indicated that leachates collected from column having uncontaminated soil treated with tap water (column I) had pH above neutral ranging 7.28-7.42 which was almost equal to the pH of uncontaminated soil. Leachate collected from heavy metal spiked soil treated with tap water i.e. column II had comparatively lower pH ranging from 6.90-7.26, which was due to the treatment with tap water that removed the heavy metals in the range of 0.4-2.4%. Leachates from column III which contained uncontaminated soil treated with di-rhamnolipid had pH in the range 6.84-7.10 wherein the pH of 6.84 coincided with high concentrations of heavy metals *viz.* Pb (2%) and Cd (0.8%) in the leachate. These concentrations were higher than the concentrations of these metals present in the leachates from column I in which tap water was used as eluant instead of the di-rhamnolipid. In case of leachates collected from heavy metal spiked soil treated with biosurfactant solution (column IV), the pH range was the lowest at 6.60-6.78 indicating high dissolution of metal species from the simulated soil and effective leaching with the aid of di-rhamnolipid biosurfactant which amounted to 4-26%. The lowest pH of 6.60 recorded at 36 h of leachate collection coincided with the highest leaching of Pb (22%) and Cd (20%) each.

Results depicted in **Table 4** show that the pH, electrical conductivity (EC) and cation exchange capacity (CEC) of the uncontaminated soil before and after treatment with di-rhamnolipid biosurfactant varied from 7.70 to 8.02, 0.19 to 0.22 mS/cm and 73.27 to 743.4 mg/kg, respectively. Similarly, the pH, EC and CEC of the heavy metal spiked-soil before and after treatment with di-rhamnolipid biosurfactant changed from 6.30 to 6.80, 0.29 to 0.27 mS/cm and 66.20 to 723.4 mg/kg, respectively. Thus, only an appreciable change was seen in pH caused by accumulation or removal of cations. Similarly, the organic carbon and nutrient status with respect to nitrogen, phosphorous and potassium of the heavy metal-spiked soil before and after treatment with di-rhamnolipid biosurfactant varied from 0.38-0.42% and 0.030-0.040%; 0.052-0.075% and 0.170-0.240%, respec-

Table 4 Physico-chemical characteristics of uncontaminated soil and heavy metal spiked soil before and after treatment with di-rhamnolipid biosurfactant. Same methods of determination used as indicated in **Table 1**. Values shown are mean ± standard deviation calculated for triplicates.

Parameters	Different treatments under glass columns with specifications			
	Uncontaminated soil		Heavy metal-spiked soil	
	Before treatment	After treatment	Before treatment	After treatment
Bulk density (g/cm^3)	1.16 ± 0.02	1.18 ± 0.02	1.28 ± 0.03	1.24 ± 0.02
Maximum water holding capacity (%)	60.20 ± 3.04	62.50 ± 3.51	56.20 ± 4.23	55.20 ± 5.01
Porosity (%)	51.60 ± 4.02	52.10 ± 5.13	49.10 ± 5.51	50.10 ± 6.03
Sand (%)	27 ± 3	27 ± 2	28 ± 3	30 ± 3
Silt (%)	26 ± 2	27 ± 2	27 ± 3	28 ± 2
Clay (%)	47 ± 5	46 ± 4	45 ± 5	42 ± 4
Textural class	Clay	Clay	Clay	Clay
pH	7.70 ± 0.41	8.02 ± 0.32	6.30 ± 0.53	6.80 ± 0.63
EC (mS/cm)	0.19 ± 0.01	0.22 ± 0.02	0.29 ± 0.01	0.27 ± 0.02
Cation exchange capacity (meq/100g)	73.27 ± 3.96	74.34 ± 4.17	66.20 ± 4.24	72.34 ± 3.89
Organic carbon (%)	0.45 ± 0.01	0.46 ± 0.01	0.38 ± 0.01	0.42 ± 0.02
Nitrogen (%)	0.044 ± 0.002	0.049 ± 0.002	0.030 ± 0.002	0.040 ± 0.002
Phosphorous (%)	0.072 ± 0.008	0.079 ± 0.007	0.052 ± 0.005	0.075 ± 0.007
Potassium (%)	0.157 ± 0.011	0.168 ± 0.013	0.170 ± 0.010	0.240 ± 0.020
Chromium (mg/kg)	47.8 ± 3.6	29.8 ± 1.8	940.5 ± 14.6	80.0 ± 6.4

Table 5 Microbiological characteristics of uncontaminated and heavy metal spiked soil samples from glass columns after treatment with di-rhamnolipid biosurfactant solution

Glass Column with specifications of soil treatments	Bacteria (CFU/g)	Fungi (CFU/g)	Actinomycetes (CFU/g)	*Azotobacter* (CFU/g)	*Rhizobium* (CFU/g)
Control					
I. Uncontaminated soil treated with tap water	19×10^5	31×10^3	45×10^3	28×10^3	33×10^3
II. Heavy metal spiked soil treated with tap water	64×10^4	47×10^2	24×10^1	21×10^1	20×10^2
Experimental					
III. Uncontaminated Soil treated with 0.1% di-rhamnolipid biosurfactant	23×10^5	29×10^3	51×10^3	24×10^3	41×10^3
IV. Heavy metal spiked soil treated with 0.1% di-rhamnolipid biosurfactant	10×10^5	26×10^3	13×10^3	21×10^3	20×10^3

tively. The changes in organic carbon and nutrients status of the heavy metal spiked soil were due to the changes in pH from slightly acidic to neutral (Yan *et al.* 2008; Kumar *et al.* 2011). Thus, the treatment of heavy metal-spiked soil with 0.1% di-rhamnolipid biosurfactant solution enabled the soil to regain its lost fertility.

The results presented in **Table 5** show that the total heavy metal content with respect to chromium in the uncontaminated soil before and after treatment with di-rhamnolipid biosurfactant varied from 47.8 to 29.8 mg/kg, respectively. Similarly, the total heavy metal content with respect to chromium in the heavy metal spiked soil before and after treatment with di-rhamnolipid biosurfactant varied from 940.5 to 80.0 mg/kg, respectively. This indicated that the di-rhamnolipid biosurfactant had mediated the removal of chromium with high efficiency.

Effect of treatment of soils with di-rhamnolipid biosurfactant (0.1%) on soil microbiological characteristics

Data in **Table 5** also shows the microbiological characteristics of metal spiked-soil samples from glass columns before and after treatment with tap water and di-rhamnolipid biosurfactant solutions. High counts of different microbial groups such as bacteria; fungi, actinomycetes and nitrogen fixers were recorded for soil treated with plain tap water. The total counts of bacteria, fungi and actinomycetes in uncontaminated soil were 19×10^5, 31×10^3 and 45×10^3 CFU/g, respectively while the nitrogen fixers viz. *Azotobacter* and *Rhizobium* amounted to 28×10^3 and 33×10^3 CFU/g, respectively. These counts were nearly similar to the counts of different microbial groups estimated before running with tap water. Spiking of uncontaminated soil with toxic concentrations of chromium was found to drastically lower the counts of these micro floras. However, after treatment with plain tap water, there was a decrease in the concentration of chromium due to leaching which facilitated marginal improvements in the counts of different microbial groups. Results also showed that treatment of uncontaminated soil with 0.1% di-rhamnolipid biosurfactant solution did not affect the counts of bacteria, fungi, actinomycetes and nitrogen fixers. This indicated that 0.1% di-rhamnolipid can be safely used in the bioremediation of chromium-contaminated soil without disturbing the integrity of soil micro flora. Marked improvements in the counts of bacterial, fungi, actinomycetes and nitrogen fixers which was almost equal to the counts found in uncontaminated soil (control soil) was observed in the heavy metal spiked soil after treatment with 0.1% di-rhamnolipid biosurfactant solution (Whang *et al.* 2008; Thavasi *et al.* 2010). This was mainly attributed to di-rhamnolipid-mediated removal of the toxic heavy metals such as cadmium and lead from the spiked soils, which are normally otherwise inhibitory and toxic to the soil microflora. Thus, the treatment of heavy metal spiked soil with 0.1% di-rhamnolipid biosurfactant solution had no deleterious effects on soil biota, but instead enabled the soil to regain its lost fertility through the removal of heavy metals. Similar results were reported with regards to the effectiveness of biosurfactant-enhanced chromium removal by Massara *et al.* (2007) wherein rhamnolipids had the capability of extracting 25% portion of the stable form of chromium(III), from the kaolinite, under optimal conditions; and rhamnolipids had enhanced the removal of chromium(VI) compared to water by a factor of 2.

CONCLUSION

The column studies reported herein provide encouraging and promising results on the use of rhamnolipid biosurfactant to remove heavy metals. The results of soil washing demonstrated that approximately 92% of lead could be removed by the biosurfactant within 36 hours. The use of biosurfactant at 0.1% for the decontamination of metals off the soil showed no toxic effect on the soil microbial population. These results strongly advocate the potential applicability of the rhamnolipid biosurfactant isolated from the *P. aeroginosa* strain BS2 for heavy metal remediation. Based on the results of this study, it is expected that in a field study multiple washings may be needed to completely remove the metals off the soil. This necessitates further investigation.

ACKNOWLEDGEMENTS

The authors thank Dr. Sukumar Devotta, Director, NEERI for his kind encouragement and valuable suggestions during the preparation of this paper.

REFERENCES

Adviento-Borbe MAA, Doran JW, Drijber RA, Dobermann A (2006) Soil electrical conductivity and water content affect nitrous oxide and carbon dioxide emissions in intensively managed soils. *Journal of Environmental Quality* **35 (6)**, 1999-2010

Ahn CK, Park D, Woo SH, Park JM (2009) Removal of cationic heavy metal from aqueous solution by activated carbon impregnated with anionic surfactants. *Journal of Hazardous Materials* **164 (2-3)**, 1130-1136

Babich H, Stotsky G (1985) Heavy metal toxicity to microbe-mediated ecologic process - A review and potential application to regulatory policies. *Environmental Research* **36**, 111-137

Bauermeister LJ, Bowers JWJ, Townsend JW, McKee SR (2008) The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. *Poultry Science* **87**, 2390-2398

Bayat B, Sari B (2010) Comparative evaluation of microbial and chemical leaching processes for heavy metal removal from dewatered metal plating sludge. *Journal of Hazardous Materials* **174 (1-3)**, 763-769

Beck M, Dellwig O, Liebezeit G, Schnetger B, Brumsack HJ (2008) Spatial and seasonal variations of sulphate, dissolved organic carbon, and nutrients in deep pore waters of intertidal flat sediments. *Estuarine, Coastal and Shelf Science* **79 (2)**, 307-316

Bondarenko O, Rahman PKSM, Rahman TJ, Kahru A, Ivask A (2010) Effects of rhamnolipids from *Pseudomonas aeruginosa* DS10-129 on luminescent bacteria: Toxicity and modulation of cadmium bioavailability. *Microbial Ecology* **59 (3)**, 588-600

Bronick CJ, Lal R (2005) Soil structure and management: A review. *Geoderma* **124**, 3-22

Candela L, Álvarez-Benedí J, Condesso de Melo MT, Rao PSC (2007) Laboratory studies on glyphosate transport in soils of the Maresme area near Barcelona, Spain: Transport model parameter estimation. *Geoderma* **140 (1-2)**, 8-16

Chandrasekan EV, Bemiller JN (1980) Constituent analyses of glycosaminoglycans. *Methods Carbohydrate Chemistry* **8**, 89-96

Chang FH, Broadbent FE (1981) Influence of trace metals on carbon dioxide evolution from a yolo soil. *Soil Science* **132**, 416-421

Cooper DG, Macdonald CR, Dull SJB, Kosaric N (1981) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Applied and Environmental Microbiology* **42**, 408-412

Cooper DG (1986) Biosurfactants. *Microbiological Science* **3**, 145-149

Dahrazma B, Mulligan CN (2007) Investigation of the removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration. *Chemosphere* **69 (5)**, 705-711

Doelman P, Haanstra L (1979) Effect of lead on soil respiration and Dehydrogenase activity. *Soil Biology and Biochemistry* **11**, 475-479

Dubey KV, Juwarkar AA (2001) Distillery and curd whey waste as viable alternative to synthetic medium for biosurfactant production and pollution abatement of these wastes. *World Journal of Microbiology and Biotechnology* **17**, 61-99

Fraser L (2000) Innovations: Lipid lather removes metals. *Environmental Health Perspectives* **108**, A320

Gasper JD, Aiken GR, Ryan JN (2007) A critical review of three methods used for the measurement of mercury (Hg^{2+})-dissolved organic matter stability constants. *Applied Geochemistry* **22 (8)**, 1583-1597

Geets J, Vangronsveld J, Diels L, Taghavi S, van der Lelie D (2008) Microbial activities, monitoring and application as part of a management strategy for heavy metal-contaminated soil and ground water. *Developments in Soil Science* **32**, 521-559

Hemkar N, Sharma DC, Riyat M (2010) Alteration in chemical composition of red blood cells in iron deficiency anemia. *Journal of Clinical Medicine and Research* **2 (2)**, 12-14

Herman DC, Artiola JF, Miller RM (1995) Removal of Cd, Pb and Zn from soil by rhamnolipid biosurfactant. *Environmental Science and Technology* **29**, 2280-2285

Hamilton MA, Rode PW, Merchant ME, Sneddon J (2008) Determination and comparison of heavy metals in selected seafood, water, vegetation and sediments by inductively coupled plasma-optical emission spectrometry from an industrialized and pristine waterway in Southwest Louisiana. *Microchemical Journal* **88 (1)**, 52-55

Juwarkar AA, Jambhulkar HP (2008) Phytoremediation of coal mine spoil dump through integrated biotechnological approach. *Bioresource Technology* **99 (11)**, 4732-4741

Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters* **7 (1)**, 1-19

Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. *Plant and Soil* **304 (1-2)**, 35-44

Kumar RN, McCullough CD, Lund MA (2011) How does storage affect the quality and quantity of organic carbon in sewage for use in the bioremediation of acidic mine waters? *Ecological Engineering* **37 (8)**, 1205-1213

Maier RM, Neilson JW, Artiola JF, Jordan FL, Glenn EP, Descher SM (2001) Remediation of metal-contaminated soil and sludge using biosurfactant technology. *International Journal of Occupational Medicine and Environmental Health* **14 (3)**, 241-248

Massara H, Mulligan CN, Hadjinicolaou J (2007) Effect of rhamnolipids on chromium-contaminated kaolinite. *Soil and Sediment Contamination* **16 (1)**, 1-14

Muthusamy K, Subbuchettiar G, Thiengungal KR, Panchaksharam S (2008) Biosurfactants: Properties, commercial production and application. *Current Science* **94 (6)**, 736-747

Nordgren A, Baath E, Soderstorn B (1988) Evaluation of soil respiration characteristics to assess heavy metal effect on soil microorganism using glutamic acid as a substrat. *Soil Biology and Biochemistry* **20**, 949-954

Ore S, Mertens J, Brandt KK, Smolders E (2010) Copper toxicity to bioluminescent *Nitrosomonas europaea* in soil is explained by the free metal ion activity in pore water. *Environmental Science and Technology* **44 (23)**, 9201-9206

Pavlova AI, Dobrev DS, Ivanova PG (2009) Determination of total nitrogen content by different approaches in petroleum matrices. *Fuel* **88 (1)**, 27-30

Ramesh V, Korwar GR, Mandal UT, Prasad JVNS, Sharma KL, Ramakrishna Yezzu S, Kandula V (2008) Influence of fly ash mixtures on early tree growth and physicochemical properties of soil in semi-arid tropical Alfisols. *Agroforestry Systems* **73 (1)**, 13-22

Rasool R, Kukal SS, Hira GS (2008) Soil organic carbon and physical properties as affected by long-term application of FYM and inorganic fertilizers in maize–wheat system. *Soil and Tillage Research* **101 (1-2)**, 31-36

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH Effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* **75 (6)**, 1589-1596

Sani RK, Rastogi G, Moberly JG, Dohnalkova A, Ginn TR, Spycher N, Shende RV, Peyton BM (2010) The toxicity of lead to *Desulfovibrio desulfuricans* G20 in the presence of goethite and quartz. *Journal of Basic Microbiology* **50**, 160-170

Sattayatewa C, Arnaldos M, Pagilla K (2011) Measurement of organic nitrogen and phosphorus fractions at very low concentrations in wastewater Effluents. *Water Environment Research* **83 (8)**, 675-683

Tan H, Champion JT, Artiola JF, Brusseau ML, Miller RM (1994) Complexation of cadmium by a rhamnolipid biosurfactant. *Environmental Science and Technology* **28**, 2402-2406

Thakur PC, Apurva P, Sinha SK (2011) Comparative study of characteristics of biocompost produced by millipedes and earthworms. *Advances in Applied Science Research* **2 (3)**, 94-98

Thavasi R, Jayalakshmi S, Banat IM (2011) Effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, *Corynebacterium kutscheri* and *Pseudomonas aeruginosa*. *Bioresource Technology* **102 (2)**, 772-778

Torrens JL, Herman DC, Miller-Maier RM (1998) Biosurfactant (rhamnolipid) sorption and the impact on rhamnolipid-facilitated removal of cadmium from various soils under saturated flow conditions. *Environmental Science and Technology* **32**, 776-781

United States Environmental Protection Agency (USEPA) (1984) Summary Report: Remedial Response at Hazardous Waste Sites; EPA-540/2-84- 002a; U.S. EPA: Washington, DC, March 1984

Van Bogaert INA, Saerens K, De Muynck C, Develter D, Soetaert W, Vandamme EJ (2007) Microbial production and application of sophorolipids. *Applied Microbiology and Biotechnology* **76 (1)**, 23-34

Wang S, Mulligan CN (2009) Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals from mine tailings. *Process Biochemistry* **44 (3)**, 296-301

Wang XS, Li FY, He W, Miao HH (2010) Hg(II) removal from aqueous solutions by *Bacillus subtilis* biomass. *CLEAN – Soil, Air, Water* **38**, 44-48

Whang LM, Liu PWG, Ma CC, Cheng SS (2008) Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *Journal of Hazardous Materials* **151 (1)**, 155-163

Yan ND, Somers KM, Girard RE, Paterson AM, Keller W, Ramcharan CW, Rusak JA, Ingram R, Morgan GE, Gunn JM (2008) Long-term trends in zooplankton of Dorset, Ontario, lakes: the probable interactive effects of changes in pH, total phosphorus, dissolved organic carbon, and predators. *Canadian Journal of Fisheries and Aquatic Sciences* **65 (5)**, 862-877

Zaidi A, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiologica et Immunologica Hungarica* **56 (3)**, 263-284