

REMEDICATION OF METAL-CONTAMINATED SOIL AND SLUDGE USING BIOSURFACTANT TECHNOLOGY

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Abstract. Development of environmentally benign approaches to remediation of metal-contaminated soils and sewage sludges are needed to replace currently used techniques of either landfilling or metal extraction using caustic or toxic agents. We report results from four application technologies that use a metal-chelating biosurfactant, rhamnolipid, for removal of metals or metal-associated toxicity from metal-contaminated waste. The four applications include: 1) removal of metals from sewage sludge; 2) removal of metals from historically contaminated soils; 3) combined biosurfactant/phytoremediation of metal-contaminated soil; and 4) use of biosurfactant to facilitate biodegradation of the organic component of a metal-organic co-contaminated soil (in this case the biosurfactant reduces metal toxicity). These four technologies are nondestructive options for situations where the final goal is the removal of bioavailable and leachable metal contamination while maintaining a healthy ecosystem. Some of the approaches outlined may require multiple treatments or long treatment times which must be acceptable to site land-use plans and to the stakeholders involved. However, the end-product is a soil, sediment, or sludge available for a broad range of land use applications.

Key words:

Metals, Biosurfactant, Rhamnolipid, Remediation, Soil

INTRODUCTION

According to the 1999 U.S. Environmental Protection Agency (USEPA), Agency for Toxic Substances and Disease Registry Hazardous Substances List, five of the top 20 hazardous substances are metals including arsenic (#1), lead (#2), mercury (#3), cadmium (#7), and chromium (#16). Metal-contaminated sites vary according to location, the source of metal contamination, and the history and age of the metal contamination. Common sources of metal wastes include mining, nuclear materials processing, wastewater sludges, metal plating, and industrial manufacture of batteries, metal alloys, munitions,

electrical components, paints, preservatives and insecticides [1]. Thus far, the most common approach to cleaning metal-contaminated sites has been physical removal and landfilling. This is an expensive option which merely moves the contamination to another location. An alternative approach is *in situ* treatment of the site wherein either metal is removed or it is stabilized so that it cannot move off-site. 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.

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In any discussion of metal toxicity, it is the bioavailable (that amount that may actually impact human or ecological health) and potentially leachable (that amount that may move off-site and impact ground or surface water quality) metals that are important. Depending on metal type, disposal history, and soil type, metal bioavailability and leachability can differ greatly. Further, most sites contain more than one metal, some toxic and some benign (e.g., lead, iron, and zinc), which can greatly impact treatment of a target metal (e.g., lead). In considering alternative approaches to metal removal, metal stabilization is acceptable, however, removal has obvious advantages in that it permanently eliminates the associated health threat. We have previously studied and reported on the properties of the metal-chelating biosurfactant, rhamnolipid, that is produced by *Pseudomonas aeruginosa* [2–7]. Note that while there are a great number of metal chelators few of these materials are environmentally benign. While synthetic chemicals such as nitrilotriacetic (NTA), ethylenediamine-tetraacetic acid (EDTA), and diethyltri-amine-pentaacetic acid (DTPA) are extremely effective at metal complexation (Table 1), their use in the field for *in situ* removal is questionable because of their demonstrated toxicity effects. For example, NTA is a Class II carcinogen [8] and DTPA is a potential carcinogen. Both EDTA and DTPA are toxic as measured by invertebrate toxicity tests [9,10]. EDTA and NTA were shown to significantly reduce growth and cause leaf abscission in poplars being used to remediate cadmium-contaminated soil [11]. A further concern is biodegradability. EDTA, which has been buried with radioactive wastes through its use in decontamination, has been found in groundwater demonstrating limited biodegradability in the environment [12].

Herein we report results from four application technologies that use a metal-chelating biosurfactant for removal of metals or metal-associated toxicity from metal-contaminated waste. The four applications include: 1) removal of metals from sewage sludge; 2) removal of metals from historically contaminated soils; 3) combined biosurfactant/phytoremediation of metal-contaminated soil; and 4) use of biosurfactant to facilitate biodegradation of the organic component of a metal-organic co-con-

Table 1. Stability constants for various organic ligands with lead (Pb)*

Organic ligand	Naturally-occurring or synthetic	Stability constants** Lead
DTPA	synthetic	18.66
EDTA	synthetic	17.88
NTA	synthetic	11.34
Rhamnolipid	naturally-occurring	8.58
Oxalic acid	naturally-occurring	4.00
Citric acid	naturally-occurring	4.08
Acetic acid	naturally-occurring	2.15

* Data from Maier and Soberon-Chavez [4].

** Stability constants are expressed in log values. All stability constants are from Martell and Smith, [13] except for rhamnolipid [14] and SDS (Gage and Maier, unpublished).

taminated soil (in this case the biosurfactant reduces metal toxicity).

MATERIALS AND METHODS

Technology 1- Biosurfactant removal of metals from sewage sludge

Anaerobically-digested sludge was collected from the Ina Road Wastewater Treatment Facility, Tucson, AZ, USA. Five ml aliquots were placed in plastic centrifuge tubes to minimize sorption of metals to surfaces and then spiked with 0, 500, or 2000 mg/l copper in the form of $\text{Cu}(\text{NO}_3)_2$ and 0, 12.5, and 50 mM rhamnolipid (Jeneil Biosurfactant, Co., Saukville, WI, USA). The centrifuge tubes were shaken for 24 h and then centrifuged at 48,400 • g for 20 min to pellet the sludge solids. The supernatant was removed, placed into another centrifuge tube, acidified with 5 drops HNO_3 to precipitate the rhamnolipid, and refrigerated for 24 h. The tubes were centrifuged at 12,100 • g for 10 min. The supernatant was then filtered through a 0.2 μm filter and the copper content was determined using atomic adsorption spectroscopy (Instrument Laboratory Video 12 aa/ae spectrophotometer, Allied Analytical Systems, Waltham, MA, USA). All treatments were conducted in triplicate.

Technology 2 – Biosurfactant removal of metals from historically contaminated soils

Historically contaminated soils were obtained from the Lower Coeur d'Alene River system in Idaho and the

Camp Navajo Army Depot near Flagstaff, Arizona, USA. The source of contamination for the Idaho soil was mine waste from local silver, lead, and zinc mines and for the Camp Navajo soil was lead-based paint from buildings and lubricating oils from railroad cars. In addition, the soils experienced different weathering conditions. The Coeur d'Alene soils were taken from the flood plain of the Coeur d'Alene river while the Camp Navajo soils were taken from a well drained area well above the water table. Soil samples from both locations were air-dried, sieved (2 mm), mixed well and stored at room temperature in the dark. Batch soil washing experiments were conducted with both soils. Metal removal by 10 mM purified rhamnolipid [15] adjusted to pH 7.1 was compared to control removal by KNO_3 solution adjusted to the same ionic strength as the rhamnolipid solution and 50 mM $\text{Ca}(\text{NO}_3)_2$. The ionic strength of the rhamnolipid was maintained below 10 mM to minimize sorption of rhamnolipid to soil which has been correlated with increasing ionic strength [5]. For comparison, total toxicity characteristic leaching procedure (TCLP), and DTPA extractions were performed to characterize the fractions with which the metals were associated.

For batch soil washing experiments, triplicate 2.5 g soil samples of each soil type were placed in acid-washed 50 ml plastic centrifuge tubes with 10 ml extracting solution and incubated for 18–22 h on a rotary shaker at 200 rpm. Samples were centrifuged at $48,000 \cdot g$ for 20 min followed by filtration of the supernatant through a $0.2 \mu\text{m}$ cellulose acetate filter. The filtrates were analyzed for lead and iron by atomic absorption spectroscopy. For rhamnolipid, KNO_3 , and $\text{Ca}(\text{NO}_3)_2$, ten sequential extractions were performed by repeating the above procedure to evaluate the limiting factors in the process. For each sequential extraction, the supernatant was decanted, 10 ml of extracting solution was added to the soil pellet, and the tube returned to the shaker.

DTPA extractions were performed as described by Lindsay and Norvell [16]. Total and TCLP extractions were performed using the EPA Method 3051: microwave assisted digestion of sediments, sludges, soils and oils, and

the EPA Method 1311 applied procedure for assessment of hazardous wastes in soils-TCLP, respectively [17].

Technology 3 – Combined biosurfactant/phytoremediation of metal-contaminated soil

In a preliminary study we compared the effect of a 5 mmol/kg soil application of rhamnolipid and EDTA on the uptake of metals by 3–5 week old transplants of corn (*Zea mays* Mayo Tuxpeno) and a halophyte (*Atriplex nummularia*) from a historically contaminated soil. The soil is mine tailing waste classified as a loamy sand and contains the following metal contamination levels: Cu, 2.0%; Pb, 0.2%; and Zn, 0.1%. Since the soil was highly toxic to both plants, it was mixed 1:1 with a forest mulch prior to the experiment.

Corn and *Atriplex* seedlings were transplanted into the contaminated soil mixture and grown for a week under a daily watering regime to allow establishment of the root system. During the second week, the plants were watered to field capacity every second day with either 5 mM rhamnolipid, 5 mM EDTA or water alone for a total dose of 5 mmol chelator/kg soil. All treatments were conducted in triplicate. At the end of the experiment, the plants were harvested and separated into root and shoot material. The plants were washed in double distilled water and dried prior to milling to 20 mesh in a Wiley mill. Milled samples were ashed at 500°C for 5 h in an ash furnace and then further digested by boiling in 3 M HCl. All samples were filtered through #42 Whatman filter prior to analysis for copper using atomic absorption spectroscopy.

Technology 4 – Biosurfactant reduction of metal toxicity to enhance organic biodegradation in metal-organic co-contaminated soils

A toxic level of cadmium was added to two soils that were amended with ^{14}C -phenanthrene as described by Maslin and Maier [15]. Mineralization of the ^{14}C -phenanthrene was measured in the absence of rhamnolipid (the control system) and in the presence of rhamnolipid applied once at the beginning of the experiment or applied in pulses throughout the experiment.

RESULTS AND DISCUSSION

Technology 1 – Biosurfactant removal of metals from sewage sludge

In highly industrialized and also in mining areas, sewage sludges that contain elevated levels of toxic metals are generated. For example, sludges from 11 treatment plants in Canada (including Quebec and Ontario) and the United States (Delaware and Maryland) contained copper levels ranging between 147–3689 mg/kg [18]. Repeated application of metal-containing sludges to land can cause accumulation to toxic levels. Therefore, a study was performed to test whether rhamnolipid could remove metals from sludge material to be used as a treatment prior to land application. Preliminary results are shown in Fig. 1. Copper recovery rates were as high as 59.4% in solutions spiked with 2000 mg/L, copper and treated with 50 mM rhamnolipid. Solutions treated with 12.5 mM rhamnolipid recovered an average of 39.8% of the added copper. This was 21 and 14 times higher than copper recovery by the no rhamnolipid control (aqueous solution of similar ionic strength) which removed only 2.8% of the copper. For solutions spiked with 500 mg/L, Cu, and recovery rates were 5.2, 21.1, and 34.5% for the no rhamnolipid, 12.5 mM and 50 mM rhamnolipid treatments, respectively.

These results suggest that rhamnolipid-washing is an effective treatment to remove metals from sludges and perhaps other materials that are high in organic matter content. Future work will examine the use of rhamnolipid to recover multiple metals and the use of multiple washings to increase the amount of metal removed.

Technology 2 – Biosurfactant removal of metals from historically contaminated soils

In previous work done in a soil (Hayhook) spiked with cadmium, a single extraction removed approximately 35% of the metal [5]. Further metal washing experiments performed under saturated flow conditions showed up to 80% metal removal in various soils [5]. The Hayhook soil had similar chemical and physical properties to the Coeur d'Alene and Camp Navajo soils used in the present study.

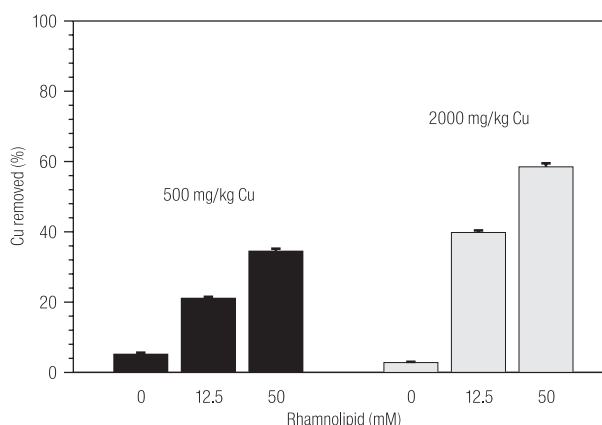


Fig. 1. Rhamnolipid-facilitated removal of copper from anaerobically-digested sewage sludge. Three treatments were tested (0, 12.5, and 50 mM rhamnolipid) in sludge samples spiked with two different copper concentrations (500 and 2000 mg/kg).

The major difference between the Hayhook soil and the two soils used in this study was that it was spiked with metal immediately prior to the experiment (designated here as recently contaminated). In contrast, the Coeur d'Alene and Camp Navajo soils are historically contaminated. A second difference between the soils is that the Hayhook soil was spiked with a single metal while the Coeur d'Alene and Camp Navajo soils contain multiple metals.

As shown in Fig. 2, rhamnolipid removed a total of 14 to 15% of the lead from each historically contaminated soil in 10 extractions. This is 140 to 350 times greater removal than for 10 extractions with KNO_3 or $\text{Ca}(\text{NO}_3)_2$ showing that rhamnolipid greatly enhanced metal removal. Even though the percent removal by rhamnolipid was similar for the two soils, the total mass of metal removed was quite different; 540 and 3660 $\mu\text{g/g}$ were removed from the Coeur d'Alene and Camp Navajo soils, respectively. This demonstrates that the rhamnolipid was not the limiting factor for lead removal at least from the Coeur d'Alene soil. In addition, it is interesting to note that the removal of iron was far less efficient than the removal of lead in both soils. These results indicate that rhamnolipid is capable of metal removal far in excess of the soluble portion removed by the control extractants, but the removal efficiency is dependent on soil type and the source of and type of metal contamination.

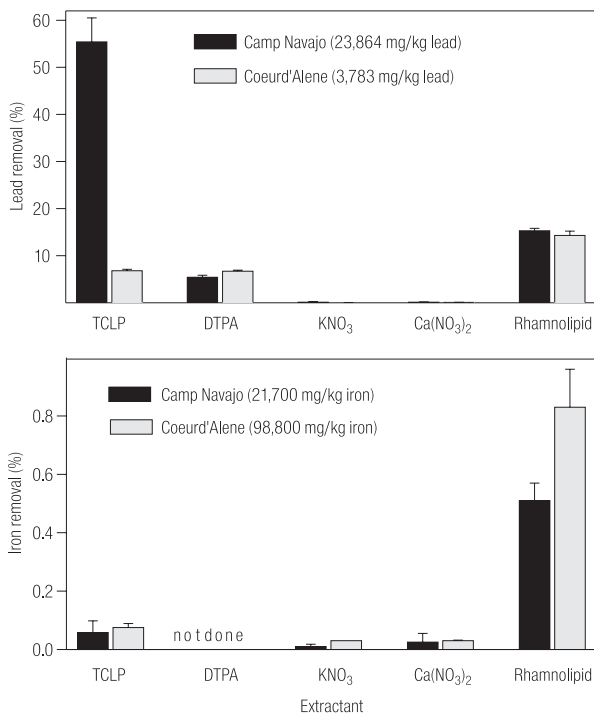


Fig. 2. Rhamnolipid-facilitated removal of lead (top graph) and iron (bottom graph) from two historically metal-contaminated soils. In this case 10 sequential extractions with 10 mM rhamnolipid was compared to 10 sequential extractions with KNO₃ (8.5 mM) and Ca(NO₃)₂ (50 mM), as well as to metal extracted by the DTPA soil test [16] and TCLP analysis [17].

The large difference in extraction efficiencies between the rhamnolipid, KNO₃, and Ca(NO₃)₂ extractants indicates that rhamnolipid removes more than the soluble fraction of lead. Work is ongoing to determine what metal fractions the Jeneil rhamnolipid can remove. Preliminary data from the DTPA test (designed by Lindsay and Norvell [16] to extract the readily available or exchangeable metals and not the carbonate bound metals) and TCLP test (an acid extraction which will dissolve carbonate bound metals), show that the two soils used in this study are very different. While the DTPA extractable metal was similar for both soils (6.7% for Coeur d'Alene and 5.4% for Camp Navajo), the TCLP extractable metal varied greatly with 54% lead removal from the Coeur d'Alene soil and 7% removal from the Camp Navajo soil. Rhamnolipid removed twice the DTPA extractable lead in both soils indicating that the exchangeable lead is removed, as well as some lead that is sequestered in other fractions. The TCLP results suggest that lead is sequestered primarily in

the carbonate fraction in the Camp Navajo soil, but not in the Coeur d'Alene soil. Overall this initial study indicates that rhamnolipid removed 8–10% of the nonreadily available lead associated with unique fractions in each of these soils but it is still not clear what fractions are most susceptible to rhamnolipid treatment.

TCLP metal levels are important because they are currently used by the U.S. EPA to set regulatory limits with respect to metal contamination in soil. Any soil containing a metal above the regulated TCLP level is considered a hazardous waste. For lead, the TCLP level is 5 mg/L. The TCLP levels measured for the soils used in this study were 12.8 mg/L for the Coeur d'Alene soil and 660 mg/L for the Camp Navajo soil, both far exceeding the regulatory limit.

The preliminary results reported here suggest that rhamnolipid is a useful soil washing extractant for recent, soluble, and exchangeable sources of metal contamination. Further research is needed to determine specific applications of rhamnolipid for remediation of historically contaminated soils. For example, rhamnolipid may be useful in historically contaminated soils with specific types of metals or metal species. In the case of the two soils tested here, the rhamnolipid lead extraction efficiency exceeded the TCLP level for the Coeur d'Alene soil, but not for the Camp Navajo soil.

Technology 3 – Combined biosurfactant/phytoremediation of metal-contaminated soil

Phytoremediation is the use of green plants to accumulate or stabilize toxic metal concentrations in contaminated soils. For the most part, heavy metal uptake by plants has been shown to be nonspecific, with the exception of uptake and regulation of essential trace metals such as calcium, iron, and zinc [16]. The use of synthetic chelators, EDTA, NTA and HBED (N,N'-di(2-hydroxybenzyl)ethylenediamine N,N'-diacetic acid), has been reported to enhance plant metal uptake up to 10-fold, but as for plants, chelator-facilitated plant metal uptake is also nonspecific [20–23].

Herein we present preliminary data from a study conducted to investigate the potential use of rhamnolipid to

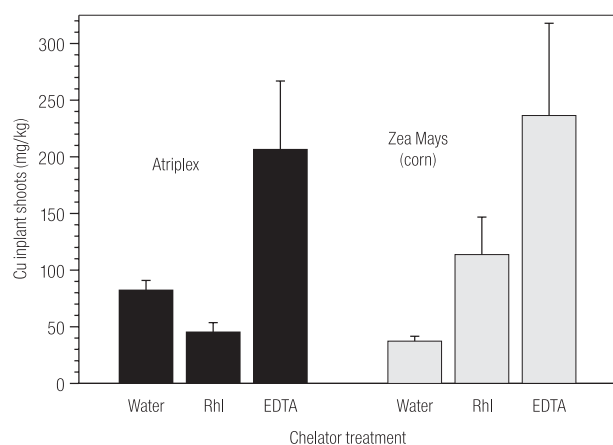


Fig. 3. The effect of rhamnolipid (5 mM) and EDTA (5 mM) on plant uptake of copper from a historically metal-contaminated soil.

facilitate the uptake of heavy metals by plants. In this study, the copper concentrations in the shoot material of both corn and *Atriplex* were determined after plants were grown in an aged contaminated soil and then amended with either water alone, rhamnolipid, or EDTA (both 5 mmol/kg soil). Interestingly, rhamnolipid results were plant specific. For corn, rhamnolipid enhanced shoot uptake of copper by 3-fold from 37 to 113 mg/kg. In contrast, for *Atriplex*, rhamnolipid decreased shoot uptake from 82 to 45 mg/kg. EDTA addition resulted in increased copper uptake for both plants (2.5 to 6-fold) in comparison to the control (Fig. 3).

These results indicate that the use of Jeneil rhamnolipid for phytoremediation will be governed by the plant chosen. First, rhamnolipid could be used with plants such as *Atriplex* to reduce heavy metal concentrations in shoot material, thereby decreasing exposure levels and the possibility of bioaccumulation in higher animals. Second, rhamnolipid could be used with plants such as corn as an environmentally compatible means to increase plant uptake of heavy metals through phytoextraction.

Technology 4 – Biosurfactant reduction of metal toxicity to enhance organic biodegradation in metal-organic co-contaminated soils

Sites contaminated with mixtures of metals and organic chemicals pose unique challenges in terms of remediation. To date, the main strategy applied in such sites, pump and

treat, has not been very effective for reaching remediation goals [24]. Bioremediation for removal of organic contaminants is now an established technique [25]. Recently interest has developed in applying bioremediation to sites contaminated with both metals and organics (co-contaminated sites). Ideally, in co-contaminated sites, treatments effective for concurrent removal of organics and metals need to be developed. However, since metals are not biodegradable, and since metal-induced inhibition of normal heterotrophic microbial activity has been well documented [26–29], in co-contaminated sites it may be necessary to use sequential or combined treatments that address the two contaminant types separately to achieve remediation goals.

To this end, we recently reported a series of experiments that were performed to investigate whether rhamnolipid could reduce the toxicity of a model metal, cadmium, to indigenous soil populations during the mineralization of phenanthrene. Two soils were tested, Brazito and Gila, each of which harbored an indigenous phenanthrene-degrading population. Results showed that cadmium inhibited phenanthrene mineralization in both soils at bioavailable cadmium concentrations as low as 3 $\mu\text{g/ml}$ (total cadmium = 394 $\mu\text{g/g}$). This inhibition was reduced by the addition of rhamnolipid. Since rhamnolipid was degraded by soil populations in approximately a 2-week period, a rhamnolipid pulsing strategy was used to maintain a constant level of rhamnolipid in the system. Using this strategy, phenanthrene mineralization levels comparable to the control (0 $\mu\text{g/ml}$ Cd/0 mM rhamnolipid) were achieved in the presence of toxic cadmium concentrations. For the Brazito soil, two 1 mM rhamnolipid pulses abrogated the toxic effects of 20 $\mu\text{g/ml}$ bioavailable Cd. For the Gila soil, four 1 mM rhamnolipid pulses abrogated the toxic effects of 10 $\mu\text{g/ml}$ bioavailable Cd [15]. This research demonstrated that pulsed application of rhamnolipid allows bioremediation of the organic contaminant component in sites that are co-contaminated with organics and metals. Further, since the rhamnolipid was biodegradable, no toxic residuals were left in the system after treatment.

CONCLUSIONS

Remediation of metal-contaminated soils or sediments is generally accomplished by physical removal to a landfill, or by *ex situ* destructive soil washing using caustic, acidic, or toxic agents. None of these options restores the contaminated material to a healthy state with unrestricted land use options. The technologies discussed in this paper are nondestructive options for situations where the final goal is the removal of bioavailable and leachable metal contamination while maintaining a healthy system. Some of the approaches outlined may require multiple treatments or long treatment times which must be acceptable to the site land-use plans and to the stakeholders involved. However, the end-product is a soil, sediment, or sludge suitable for a broad range of land uses.

Obviously, further research is needed to evaluate the cost, time, and *in situ* effectiveness of the strategies outlined here. This should include a performance assessment of achievable remediation endpoints and technology limitations in a variety of soils and sediments. However, it should also be pointed out that the data discussed here were from research concerning a single metal-chelator, the Jeneil rhamnolipid. It is likely that there are many other biological metal-chelating compounds that may have similar or superior properties to this rhamnolipid. For example, Mulligan et al. [30] have shown that surfactin, a biosurfactant produced by *Bacillus subtilis* is also effective at metal removal from soil containing elevated levels of copper and zinc, as well as hydrocarbons. Also, some microorganisms are known to produce siderophores and metallothioneins. These molecules that have metal-complexing abilities superior to Jeneil rhamnolipid were not studied here. The challenge will be to study these molecules in complex environmental systems and to produce them cost-effectively at levels required for remediation.

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