

Bioremediation of Pb-Contaminated Soil Based on Microbially Induced Calcite Precipitation

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To remediate lead (Pb)-contaminated soils, it is proposed that microbially induced calcite precipitation (MICP) would provide the best alternative to other remediation technologies. In this study, Pb bioremediation in soils was investigated using the calcite-precipitating bacterium *Kocuria flava*. Results indicate that the Pb is primarily associated with the carbonate fraction in bioremediated soil samples. The bioavailability of Pb in contaminated soil was reduced so that the potential stress of Pb was alleviated. This research provides insight into the geochemistry occurring in the MICP-based Pb-remediated soils, which will help in remediation decisions.

Keywords: *Kocuria flava*, calcite, urease, dehydrogenase, bioremediation

Lead (Pb) has been recognized as one of the most emerging hazardous heavy metals among environmental pollutants. Hundreds and thousands of tons of lead are discharged from electric battery manufacturing, lead smelting, and mining activities, as well as internal combustion engines fueled with leaded petroleum and Pb-bearing products, forming the primary sources of Pb contamination [9]. Such irregular inputs of Pb result in high concentrations of Pb in soils [13]. In nature, lead exists with an oxidation state of 0 or +II; however, Pb²⁺ is the most reactive and common form of lead.

Since lead has been used widely for centuries, elevated levels of lead have been reported in soils worldwide [12]. Soil lead pollution is very serious in many cities of China. The reported soil Pb concentrations in cities including

Beijing, Changchun, Guiyang City, Wuhan, and Shenyang have found to be as high as 207, 205, 276, 480, and 15,127 mg/kg, respectively [7, 22, 24]. The soil of Urumqi, an economically quickly developing city in northwestern arid China, is known to be highly contaminated with lead. Even road dusts in Urumqi have an elevated concentration of Pb, up to 54 mg/kg [21]. On a worldwide scale, elevated levels of lead represented the greatest risk to human health. Lead toxicity usually begins with the nervous system in both adults and children. High levels of lead can cause damage to the brain, and kidney, and can cause anemia [10]. A novel technique for the removal of such toxic metals from soils is highly desired.

Conventional methods for heavy metal removal have several disadvantages, such as less effective metal-ion removal, high reagent requirements, high costs, the generation of toxic sludges, and the problem of the safe disposal of the materials [9, 16]. Bacterial methods for the removal of lead from contaminated sites provide an attractive alternative to physicochemical methods and have been employed for a long time [19]. Some previous studies focused on the bioremediation of soils contaminated with heavy metals (such as Pb, Cd, Cr, and so on) by phytoremediation [8, 11]; however, the limitation is that it can not be used successfully in the harsh and dry climate of arid area such as northwestern China. Biomineralization based on microbially induced calcite precipitation (MICP) provides a promising technique to remediate toxic metals from contaminated soils with additional advantages on current bioremediation techniques [1, 17].

In the present study, we investigated the role of *Kocuria flava* CR1, previously isolated from a mining area of Urumqi, China in our laboratory [1], in the remediation of Pb-contaminated soil. The bacterium was able to grow on nutrient agar media supplemented with 50 mM Pb²⁺ [Pb(NO₃)₂].

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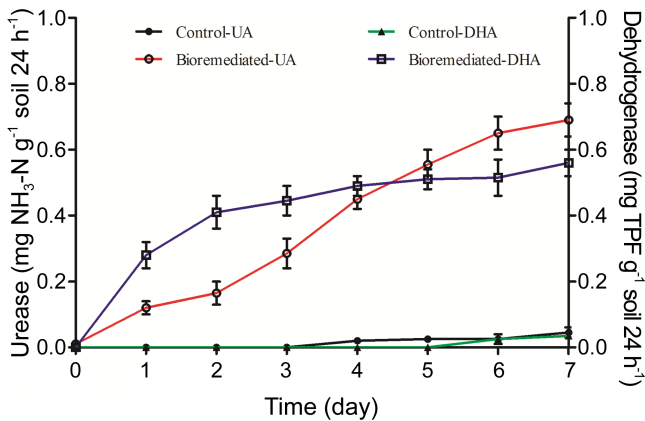


Fig. 1. Urease activity (UA), and dehydrogenase activity (DHA) in control and bioremediated Pb-contaminated soil samples (n = 3).

Soil was collected from a farmland of Urumqi, China. To perform bioremediation studies, the sterilized dried soil (200 g), supplemented with 100 mg/kg Pb, was mixed thoroughly with overnight grown *K. flava* CR1 (in 2:1 ratio, wet-weight basis) in a beaker and incubated at room temperature. Bacterial cells were grown in calcite-inducible nutrient broth media containing 2% urea and 25 mM CaCl₂ [2]. The experiments were terminated at the end of 7 days. Bacterial cells were not added in control experiments containing soil samples supplemented with 100 mg/kg Pb. The samples were subjected to analyze soil urease and dehydrogenase activities at regular time intervals as per the non-buffer method of Zantua and Bermner [23] and Casida *et al.* [6], respectively.

A significant amount of urease was produced by *K. flava* CR1 in the remediated soil samples (Fig. 1). Urease activity was continuously increased in bioremediated soil and found to be 0.7 mg NH₃-N/g at the end of 7 days. Urease is a key enzyme in the calcite precipitation process produced by certain microorganisms [3, 4, 18]. Dehydrogenase activity was also shown by *K. flava* CR1 in treated soil that was absent in control. Bioremediated soil samples produced 0.56 mg TPF g⁻¹ soil 24 h⁻¹ dehydrogenase at the end of 7 days. It is noteworthy that control soil samples produced negligible amount of urease and dehydrogenase activities (Fig. 1). Activities of enzymes such as urease and dehydrogenase are considered sensitive to heavy metal pollution and have been proposed as indicators for measuring the degree of soil sustainability [5].

Because the toxicity of metal was associated with metal bioavailability, the soils were analyzed for Pb fractions (soluble-exchangeable, carbonate-bound, Fe-Mn oxides-bound, organic-bound, and residual) according to the continual extract procedure of Tessier *et al.* [20]. The distribution coefficient γ_i of each Pb fraction showed the ratios of Pb content in the different fractions and was calculated by

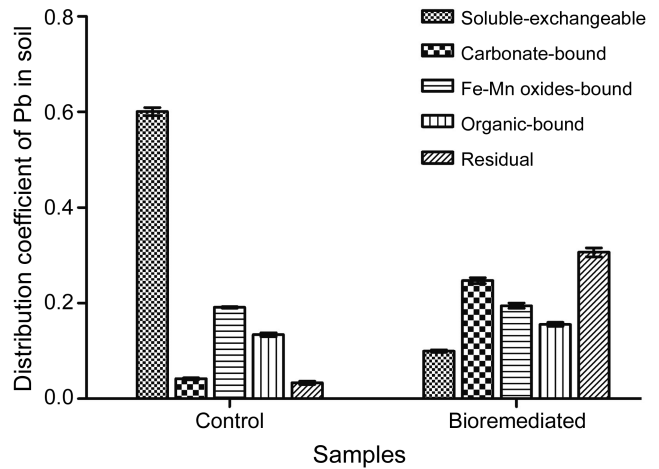


Fig. 2. Distribution coefficient (γ_i) of Pb in control and bioremediated soil samples by *K. flava* CR1 (n = 3).

$$\gamma_i = \frac{f_i}{f}$$

where f_i and f represent the content of each Pb fraction and the total amount of Pb in soils, respectively.

Compared with the control soils, the highest concentration of carbonate-bound Pb, organic-bound Pb, and residual Pb, and the lowest concentration of soluble-exchangeable Pb were found in bioremediated soil, which offered the evidence for the lowest mobility and bioavailability of Pb. The soluble-exchangeable Pb content in the control soil displayed the highest value (85.4 mg/kg) and then remarkably decreased (14.2 mg/kg) after bioremediation with *K. flava* CR1. After bioremediation, the concentration of carbonate-bound Pb in remediated soil increased significantly to 35.1 mg/kg from 5.9 mg/kg in the control soil.

The higher the distribution coefficient (γ_i) of each Pb fraction, the more the corresponding Pb fraction will be. The γ_i of soluble-exchangeable Pb was the highest in the control soil, which showed Pb existed mainly in the soluble-exchangeable form before remediation (Fig. 2). In bioremediated soil samples, the γ_i of soluble-exchangeable Pb reduced greatly, whereas carbonate-bound Pb increased significantly. These results revealed that the active Pb had been transformed into inactive Pb, indicating the alleviation of Pb contamination after bioremediation [13].

The results showed the least toxicity of Pb to living organisms, and the least stress from Pb on the environment for the significant reduction of active Pb in bioremediated soil, compared with those in the control (Fig. 2). Pb was chelated with the MICP product, which is the mechanism responsible for Pb immobilization. Pb uptake by calcite that formed as a result of ureolytic bacteria could be of considerable relevance because of its stability in a variety of geologic environments; therefore, calcite could represent an effective agent for Pb immobilization.

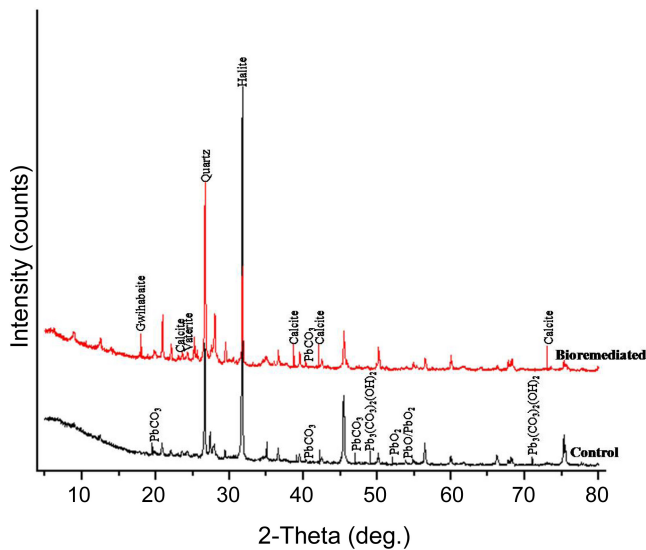


Fig. 3. XRD spectra conforming biomineralization products in soil samples induced by *K. flava* CR1 and comparison with the control.

The control and bioremediated soil samples were also analyzed by XRD using a Bruker D8 diffractometer [1]. The major lead species found in contaminated soils include lead carbonate (PbCO_3), lead oxides (PbO/PbO_2), and hydrocerussite ($\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$) [15]. The X-ray diffraction patterns of the reaction products of bioremediated lead-contaminated soils showed evidence of transformations of Pb minerals into geochemically stable calcite species. XRD analysis showed that the majority of carbonate deposits were present as calcite, besides vaterite in bioremediated soil (Fig. 3). Another important identified mineral was gwhihabite [$(\text{NH}_4, \text{K})\text{NO}_3$] in bioremediated soil samples, reported previously to have formed as a result of bacterial action [14]. It may be recalled that Pb oxyanions may substitute for the carbonate group in the calcite structure.

In conclusion, the results showed that incubating contaminated soil with *K. flava* CR1 could reduce the active Pb, alleviate the Pb stress, and stabilize the Pb-contaminated soil [13]. The omnipresence of MICP and the ability of its products to trap heavy metals may provide a new *in situ* remediation method for contaminated soil. This process can be widely used in the remediation of heavy metals, even in arid areas where phytoremediation cannot be successful. Moreover, MICP is not sensitive to redox potential, which makes this process highly effective in bioremediation [1]. The MICP process used in this study could not only immobilize Pb ions in soils, but also effectively improve soil microbial activities and their metabolic capacity. For the first time, MICP-based remediation has been reported to remediate Pb-contaminated soil.

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