

**INVESTIGATION OF THE USE OF RHAMNOLIPID
BIOSURFACTANT FOR CADMIUM
PHYTOEXTRACTION IN SOILS**

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Abstract

Phytoextraction is a technology to remove hazardous metals from soil which has developed rapidly in the last 20 years by using metal-tolerant hyperaccumulating plants or using plants with high biomass production and enhancing metal uptake using chelants. Although phytoextraction of metals is not a routine remediation approach, continued efforts have been made to refine this "green" technology in the hope that it could replace traditional remediation measures (e.g. excavation, soil washing, etc.), as soil handling less and costs are typically lower, leaving the soil fertile and able to support subsequent vegetation. Currently phytoextraction has not yet had significant success in field applications, due to difficulties in seeking environmental-friendly and effective chelants and low biomass production of many metal-tolerant plants. Conventional chelants enhance metal solubility in soil, but there are concerns over their use due to persistence in the environment and the potential for metal leaching to ground water. Therefore, there is a need to identify and evaluate chelants with potentially good metal-extraction efficiency and environmental compatibility. Recently, rhamnolipid, a biologically synthesized microbial product, has been used to remove metals from contaminated soils. In particular, rhamnolipid has been used to wash cadmium (Cd) from contaminated soils due to its strong affinity for Cd compared to many other metals. Unlike many conventional chelants, such as ethylenediaminetetraacetic acid (EDTA), rhamnolipid can form uncharged, lipophilic complexes with metal cations. There is some evidence that the uncharged or lipophilic metal rhamnolipid complexes are equally or more available than the free metal ions for plant uptake. Therefore, rhamnolipid could potentially be useful for Cd phytoextraction. This study therefore investigated the possibility of using rhamnolipid as a potential chelant to enhance the phytoextraction of Cd and its co-contaminant zinc (Zn). As well as cost and availability, the three key attributes of chelants that need to be considered in chelant-assisted phytoextraction are (i) persistence of the chelants in soil; (ii) adsorption behaviour of the chelants and its metal complexes; and (iii) toxicity of the chelants to plants.

Chelant biodegradation is of importance to phytoextraction efficiency. Persistence in the environment is not regarded as favourable and too rapid degradation results in poor enhancement of metal availability. Hence rhamnolipid biodegradation in soil was compared with conventional chelants, i.e. EDTA and citric acid, in Chapter 2. Rhamnolipid (applied at low – 2.0 mmol/kg soil and high – 10.0 mmol/kg soil concentrations) was found to be biodegradable in soil, but persisted long enough to enable metal mobilisation during phytoextraction.

Chapter 3 showed that soil sorption of rhamnolipid was concentration dependent, as was its ability to mobilise metal ions. In soil batch adsorption experiments, rhamnolipid was found to adsorb to soil when applied at low concentrations (< 1.7 mM applied concentration, equivalent to 8.5 mmol/kg soil), whereas when the concentration increased to 4.4 mM (22 mmol/kg soil), rhamnolipid adsorption to soil reduced and Cd desorption was significantly increased. This effect may have been due to the formation of larger rhamnolipid aggregates (e.g. vesicles) at the higher rhamnolipid concentrations. Determination of the octanol/water partition coefficients for metal-rhamnolipid complexes showed that rhamnolipid complexes change their morphology and hydrophilicity with concentration, which could be an alternative reason for the reduced rhamnolipid adsorption in soil. A solution concentration of rhamnolipid ranging from 4 – 5 mM was found to be most promising for Cd and Zn phytoextraction because of the low rhamnolipid sorption to soil.

High concentrations of applied rhamnolipid (4 – 5 mM, equivalent to 20 – 25 mmol/kg, as multiple weekly doses or applied as a single dose before harvest) were found to be the most suitable conditions selected for Cd and Zn mobilisation. However, rhamnolipid at those concentrations induced toxicity symptoms in maize (*Zea mays*). Therefore, the use of high concentrations (≥ 4 mM) was not suitable for phytoextraction. In a subsequent experiment, low concentrations of rhamnolipid (≤ 1.4 mmol/kg, equivalent to 1 mM in soil solution) were assessed for their ability to enhance Cd uptake by maize (*Zea mays*) and sunflower

(*Helianthus annuus*). Cadmium uptake was moderately improved ($P \leq 0.001$) following rhamnolipid application at 0.02 and 0.2 mmol/kg/week with no observable phytotoxicity. However, in general, rhamnolipid did not dramatically improve Cd and Zn uptake by the plant shoots.

An aseptic hydroponic experiment (Chapter 4) was undertaken to understand the effect of rhamnolipid on plant uptake of Cd and Zn at low concentrations (4 – 20 μM). At constant total Cd and Zn concentrations in solution and with increasing EDTA concentrations, plant uptake of Cd decreased, conforming to the free ion activity model. Rhamnolipid complexed Cd and Zn to a lesser extent than EDTA, but enabled a relatively constant Cd and Zn uptake in the root as complexation increased and free ion activities declined. This effect may have been due to the absorption of intact metal-rhamnolipid complexes. At normal Ca concentrations (2 mM, commonly found in soil solutions) in the uptake solutions, Cd translocation from roots to shoots was inhibited compared to low Ca concentrations (0.035 mM). This indicates that there will be no beneficial effect of rhamnolipid application on Cd phytoextraction in soil where Ca concentrations are several orders of magnitude higher than Cd.

The body of the work emphasizes the key criteria for selecting suitable chelants for phytoextraction. Rhamnolipid was deemed to be unsuitable for Cd phytoextraction.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jia Wen and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Date

List of Publications

Wen, J., Stacey, S. P., McLaughlin, M. J., Kirby, J. K., 2009. Biodegradation of rhamnolipid, EDTA and citric acid in cadmium and zinc contaminated soils. *Soil Biol. Biochem.* **41**, 2214-2221.

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Abbreviations

AEAA	N-(2-aminoethyl) aspartic acid
ANOVA	analysis of variance
ASV	anodic stripping voltammetry
ATSDR	Agency for Toxic Substances & Disease Registry
CA	citric acid
CEC	cation exchange capacity
DDC	diethyldithiocarbamate
DI	deionised water
DMT	Donnan membrane technique
DTPA	diethylenetriaminepentaacetic acid
EDDS	ethylenediaminedisuccinic acid
EDGA	ethylenediaminediglutamic acid
EDTA	ethylenediaminetetraacetic acid
EK	electrokinetics
EXAFS	synchrotron-based extended X-ray absorption fine structure
FIAM	free ion activity model
HAs	humic acids
HMWOAs	high molecular weight organic acids
IARC	International Agency for Research on Cancer
ICP-MS	inductive coupled plasma mass spectroscopy
ICP-OES	inductive coupled plasma optical emission spectroscopy
IDSA	iminodisuccinic acid
IUPAC	International Union of Pure and Applied Chemistry
LMWOAs	low molecular weight organic acids
LSC	liquid scintillation counting
MWHC	maximum water holding capacity
NTA	nitrilotriacetic acid
PC	phytochelatins
PIPES	piperazine-N,N'-bis(2-ethane-sulfonic acid)
PS	phytosiderophores
SEM/EDX	scanning electron microscopy with energy dispersive X-rays
SEPs	sequential extraction procedures
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction
XANES	X-ray absorption near edge spectroscopy