

# ENVIRONMENTAL BIOTECHNOLOGIES FOR BIOREMEDIATION OF CONTAMINATED LANDS AND SOIL BY MICROBES, PLANTS AND EARTHWORMS

*Rajiv K. Sinha*  
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Environmental Remediation  
Technologies, Regulations and Safety

Novinka



**ENVIRONMENTAL REMEDIATION TECHNOLOGIES,  
REGULATIONS AND SAFETY**

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**Nova Science Publishers, Inc.**  
*New York*

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Additional color graphics may be available in the e-book version of this book.

### LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

Sinha, Rajiv K. (Rajiv Kumar)

Microremediation, phytoremediation, and vermiremediation biotechnologies for contaminated lands and soil / authors, Rajiv K. Sinha, Dalsukh Valani, and Shweta Sinha.

p. cm.

Includes bibliographical references and index.

ISBN 978-1-61668-148-7 (softcover)

1. Soil remediation. 2. Phytoremediation. 3. In situ bioremediation. 4. Soil microbiology. 5. Vermicomposting. I. Valani, Dalsukh. II. Sinha, Shweta. III. Title.

TD878.S56 2010

628.5'5--dc22

2010004373

*Published by Nova Science Publishers, Inc., + New York*



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## ABSTRACT

Bioremediation is a soft bioengineering technique to clean up contaminated lands & soils using microbes, plants and earthworms. It is also a technique to stabilize the eroded lands and prevent soil erosion. Microbes are adapted to thrive in 'adverse conditions' of high acidity / alkalinity / toxicity and high temperature. Under favorable conditions of growth microbes can biodegrade / biotransform the complex hazardous organic chemicals into simpler and harmless ones. Environmentalists are viewing microbes such as yeast, bacteria, algae, diatoms and actinomycetes as an 'eco-friendly nano-factories' for metal remediation. Some bacteria can also ingest the most toxic 'cyanide' from water. After the use of 'super bug' in cleaning up oil spills, there have been several successful stories of microbial technique in clean-up of contaminated lands and soils. The Microbiological Resource Centers (MIRCENS) in Egypt is examining the use of microbes in degrading persistent pesticides pollutants.

Plants involved in bioremediation are adapted to thrive in very harsh environmental conditions of soil and water; absorb, tolerate, transfer, assimilate, degrade and stabilize highly toxic materials (heavy metals, radionuclides and organics such as solvents, crude oil, pesticides, explosives, chlorinated compounds and polyaromatic hydrocarbons) from the polluted soil and water. The organic pollutants may ideally be degraded to simpler compounds like carbon dioxide ( $\text{CO}_2$ ) and water ( $\text{H}_2\text{O}$ ). More than 400 plant species ranging from herbs to trees have now been identified which are hyperaccumulators of toxic metals. They can accumulate 100 - 500 times higher levels of metal concentrations in their above-ground parts. The brake fern (*Pteris vittata*) can reduce the concentration of 200 ppm of arsenic to nearly 100-fold (10 ppm) within 24 hours.

Earthworms in general (especially *E. fetida*) are highly resistant to many chemical contaminants including heavy metals and organic pollutants in soil. They have been found to remove heavy metals,



pesticides and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons (PAH) from the soil and have been used for land recovery, reclamation and rehabilitation of sub-optimal soils such as poor mineral soils, polder soils, open cast mining sites, closed landfill sites and cutover peats. Worms have been shown to bio-accumulate even the most toxic 'dioxin' in their tissues and concentrate it on average 14.5 fold. They can bio-accumulate heavy metals cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn) and zinc (Zn), particularly extremely high amounts of Zn and Cd. Cadmium levels up to 100 mg per kg dry weight have been found in tissues.

**Keywords:** Transgenic (Genetically Engineered) Plants Combating Soil Pollution; Phytoextraction; Phytovolatilization; Phytostabilization; Phytostimulation; Rhizofiltration; Phytotransformation; Phytodegradation; Excluder Plants; Hyper-accumulator Plants Species; Symbiotic Engineering; Biotransformation; Microbial Detoxification of Metals; Microbial Destruction of Toxic Organics & Hazardous Wastes; Plant – Assisted Microbial Destruction of Toxic Compounds; Earthworms Bio-accumulate & Bio-degrade & Bio-transform Toxic Chemicals; Earthworms Immobilize Soil Contaminants.



## INTRODUCTION

Large tract of arable land is being chemically contaminated due to mining activities, heavy use of agro-chemicals in farmlands, landfill disposal of toxic wastes and other developmental activities like oil and gas drilling. No farmland of world especially in the developing nations are free of toxic pesticides, mainly aldrin, chlordane, dieldrin, endrin, heptachlor, mirex and toxaphene. According

to National Environment Protection Council there are over 80,000 contaminated sites in Australia- 30,000 in NSW, another 30,000 in Queensland, 10,000 in Victoria, 4000 in South Australia, another 4000 in Western Australia, 1000 in Northern Territory and 500 each in Tasmania and the Australian Capital Territory (ACT). There are 40,000 contaminated sites in US; 55,000 in just six European countries and 7,800 in New Zealand. There are about 3 million contaminated sites in the Asia-Pacific. These also include the abandoned mine sites along with the closed landfills. The contaminated sites mostly contain heavy metals cadmium (Cd), lead (Pb), mercury (Hg), zinc (Zn) etc. and chlorinated compounds like the PCBs and DDT. Cleaning them up mechanically by excavating the huge mass of contaminated soils and disposing them in secured landfills will require billions of dollars. There is also great risk of their leaching underground (aggravated by heavy rains) and contaminating the groundwater. Contaminated soils and waters pose major environmental, agricultural and human health problems worldwide.

Traditionally, remediation of chemically contaminated soils involves 'off-site' management by excavating and subsequent disposal by burial in secured landfills. This method of remediation is very costly affair and merely shifts the contamination problem elsewhere. Additionally, this involves great risk of environmental hazard while the contaminated soils are being transported and 'migration of contaminants' from landfills into adjacent lands and water bodies by leaching. Soil washing for removing inorganic contaminants from soil is another alternative to landfill burial, but this technique produce a



‘residue’ with very high metal contents which requires further treatment or burial.

Since the late 1980s, after the chemical and mechanical treatments of lands and water bodies and thermal treatment (incineration) of hazardous wastes proved economically and environmentally unsustainable, focus shifted towards the biological methods which are cost-effective as well as environmentally sustainable and also socially acceptable. Bioremediation is a soft bioengineering technique to clean up contaminated lands / sites using microbes (bacteria or fungus), plants (terrestrial and aquatic) and earthworms. It is also a technique to stabilize the eroded lands and prevent soil erosion. Bioremediation works carried out by the microorganisms are called ‘micro-remediation’ while those performed by plants are called ‘phyto-remediation’. Earthworms have also been found to perform some environmental cleaning jobs and is termed as ‘vermi-remediation’.



# CONTENTS

<b>Abstract</b>		<b>vii</b>
<b>Introduction</b>		<b>ix</b>
<b>Chapter 1</b>	<b>Microbial Remediation of Polluted Lands and Soils by Adapted Microbes</b>	<b>1</b>
	<i>Microbial removal of heavy metals</i>	<i>1</i>
	<i>Microbial detoxification of heavy metals</i>	<i>5</i>
	<i>Microbial destruction of toxic organics</i>	<i>8</i>
	<i>Microbial destruction of PAHs</i>	<i>11</i>
	<i>Microbial destruction of hazardous wastes</i>	<i>16</i>
	<i>Plant-assisted microbial degradation (of toxic materials)</i>	<i>17</i>
	<i>Genetically engineered bacteria for destruction of hazardous chemicals</i>	<i>18</i>
	<i>Microbial biosensors</i>	<i>19</i>
<b>Chapter 2</b>	<b>Phytoremediation of Polluted Lands and Soils by Adapted Plants</b>	<b>23</b>
	<i>Chemical and radioactive contaminants suitable for removal by phytoremediation</i>	<i>24</i>
	<i>Environmentally adapted plants for phytoremediation</i>	<i>28</i>
	<i>Hyperaccumulator plants for metals (removal from soil)</i>	<i>29</i>
	<i>Detoxification of heavy metals by plants</i>	<i>37</i>
	<i>Fate of chemicals in plants shoots and roots</i>	<i>39</i>
	<i>Phytodetoxification of toxic cyanide (from mining wastes)</i>	<i>40</i>



	<i>‘Wonder Plants’ for phytoremediation of contaminated lands</i>	42
	<i>Genetically engineered ‘transgenic plants’ for improved phytoremediation</i>	48
	<i>‘Symbiotic Engineering’ (Plants &amp; Microbes)</i>	53
<b>Chapter 3</b>	<b>Vermiremediation of Polluted Lands and Soils by Earthworms: Charles Darwin’s ‘Unheralded Soldiers of Mankind Working Day &amp; Night Under the Soil’</b>	<b>55</b>
	<i>Earthworms resistant to chemicals</i>	60
	<i>Earthworms involved in soil remediation</i>	61
	<i>Removal of heavy metals (by earthworms)</i>	65
	<i>Removal of PAHs (by earthworms)</i>	68
	<i>Removal of petroleum hydrocarbons (by earthworms)</i>	70
	<i>Removal of agrochemicals (by earthworms)</i>	72
	<i>Removal of PCBs (by earthworms)</i>	73
	<i>Removal of endocrine disrupting chemicals (EDCs) (by earthworms)</i>	74
	<i>Earthworms vermicompost as bioadsorbent of soil pollutants</i>	84
	<i>Earthworms converts ‘wasteland’ into ‘wonderland’</i>	86
	<b>Acknowledgments</b>	<b>91</b>
	<b>References and Additional Readings</b>	<b>93</b>
	<b>Index</b>	<b>113</b>



## *Chapter 1*

# **MICROBIAL REMEDIATION OF POLLUTED LANDS AND SOILS BY ADAPTED MICROBES**

Microbes are adapted to thrive in 'adverse conditions' of high acidity / alkalinity / toxicity and high temperature. They can develop 'biological resistance' against any toxic substance in the environment due to special 'jumping genes'. Hence while a number of them may be killed due to high toxicity, some resistant microbes survive and are cultured for further use. Under favorable conditions of growth e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen, microbes can biodegrade / biotransform the complex hazardous organic chemicals into simpler and harmless ones. After the use of 'super bug' in cleaning up oil spills, there has been several successful stories of microbial technique in clean-up of contaminated lands and soils. (USGS, 1997). The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in degrading persistent pesticides pollutants. (UNEP Reports, 1996-2006).

Whilst microbial remediation (bioremediation) is a well established technology for the removal of organic soil contaminants, the use of microorganisms to transform inorganic contaminants like heavy metals is still being investigated. Environmentalists are viewing microbes as an 'eco-friendly nano-factories' for metal remediation through biotechnological applications employing microbes, such as yeast, bacteria, algae, diatoms and actinomycetes.

There are bacteria which can also ingest the most toxic 'cyanide' from water.



## **MICROBIAL BIOREMEDIATION OF HEAVY METALS**

Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions. Other metals have no biological role e.g. cadmium (Cd), lead (Pb), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Some of them e.g.  $\text{Cd}^{2+}$ ,  $\text{Ag}^{2+}$ ,  $\text{Hg}^{2+}$  tend to bind the SH groups of enzymes and inhibit their activity (Turpeinen, 2002).

Soil contamination by heavy metals may repress or even kill parts of the microbial community in soil. Interaction of metals with cellular proteins / enzymes are more commonly implicated in causing toxicity than interaction with membranes. Binding affects the structure and function of proteins and enzymes.

## **METAL TOLERANCE AND RESISTANCE BY MICROBES**

It is generally assumed that the exposure to metals leads to the establishment of a tolerant / resistant microbial population. Microbial 'resistance' is defined as the ability of a micro-organism to survive toxic effects of metal exposure by means of a detoxification mechanism produced in direct response to the 'heavy metal species' concerned. Microbial 'tolerance' is defined as the ability of a micro-organism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity.

Soil micro-organisms have been shown to bio-accumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil.

Micro-organisms employ a variety of mechanisms to resist and cope with toxic metals. The principal mechanism of resistance of inorganic metals by microbes are metal oxidation, metal reduction, methylation, demethylation, enzymatic reduction, metal-organic complexation, metal ligand degradation, intracellular and extracellular metal sequestration, metal efflux pumps, exclusion by permeability barrier and production of metal chelators such as metallothioneins and biosurfactants.



## PROCESS AND MECHANISM OF MICROBIAL REMEDIATION OF CONTAMINATED SOIL AND WATER

Microbial remediation of toxic metals occurs in two ways:

1. Direct reduction by the activity of the bacterial enzyme 'metal reductase'. It is applied for groundwater decontamination, using bioreactors (pump & treat) and also for soils after excavation (pulping or heaping and inoculation with appropriate microbial consortium). These techniques are *ex-situ* methods, and very expensive and has low metal extraction efficiencies.
2. Indirect reduction by biologically produced hydrogen sulfide ( $H_2S$ ) by sulfate reducing bacteria to reduce and precipitate the metals. This is an *in-situ* method, and an environmentally sound & inexpensive alternative to pump & treat (for contaminated groundwater) or excavate & treat (for contaminated soils). Microbial growth is induced in sub-surface zones by injecting substrates. The migrating metals are intercepted and immobilized by precipitation with biologically produced  $H_2S$ .

There are at least three major microbial processes that influence the bioremediation of metals and these are:

### 1. Biosorption & Bioaccumulation

Biosorption is sequestration of the positively charged heavy metal ions (cations) to the negatively charged microbial cell membranes and polysachharides secreted in most of the bacteria on the outer surfaces through slime and capsule formation. From the surface the metals are transported into the cell cytoplasm through the cell membrane with the aid of transporter proteins and get bioaccumulated.



## 2. Biologically Catalysed Immobilization

Inside the microbial cells, metal ions gets fixed to Iron (Fe)-Oxides and into organic colloids and becomes immobilised. This is achieved by enzymatic reduction by microbes (described below).

## 3. Biologically Catalysed Solubilization

Metal reducing bacteria enzymatically reduce and also under appropriate conditions, solubilize oxide minerals. Such dissolution reaction have been shown to release cadmium (Cd), nickel (Ni) and zinc (Zn) into solution during reduction of goethite (a form of Fe-oxide) by anaerobic bacterium *Closteridium* spp.

Microorganisms do not actually biodegrade inorganic metals, but changes (bio-transform) their oxidation state. This can lead to an increase in solubility (and subsequent removal by leaching), or precipitation and reduction in bioavailability. Metallic residues (heavy metals) may be converted into ‘metal-organic combinations’ that have less bioavailability (to pass into human food chain) than the ‘metal-mineral combinations’ of the heavy metals. Microbes transform the oxidation states of several toxic metals and increase their bioavailability in the rhizosphere (root zone) thus facilitating their absorption and removal by hyper-accumulating plants by phytoremediation.

Many divalent metal cations like  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  are very similar in structure. Also, the structure of oxyanions, such as chromate, resembles that of sulphate. Evolution has endowed micro-organisms with effective mechanisms to distinguish between similar metal ions and between toxic and non-toxic metals. Microbes have solved this problem by developing two types of uptake mechanisms and systems for metal ions:

1. Selective, substrate-specific uptake system that are slow and require considerable cellular energy (ATP) and is only produced by the cell in times of need;
2. Substrate-non-specific rapid system, that transport metals using a chemiosmotic gradient across the cytoplasmic membrane of the bacteria rather than using ATP. (Nies, 1999).

Even highly evolved, substrate-specific uptake mechanisms may not prevent entry of toxic metals in cells. Once inside, metal cations can interact



with various cellular components including cell membranes, proteins and nucleic acids. Incompletely filled d-orbitals allow metals to form complex compounds with organic ligands, such as the proteins, nucleic acids & cell wall materials of micro-organisms. The ability of microbial cell surfaces to form complex with metals lies in their net negative charge at normal growth pH. The outer membrane of Gram negative bacteria effectively complexes metals including magnesium (Mg), nickel (Ni), strontium (Sr), manganese (Mn), lead (Pb), iron (Fe), sodium (Na) and calcium (Ca). In Gram –eve bacteria the, the net –eve charge results from the phosphates and carboxyl groups of lipopolysaccharide molecules, while the –eve charge in Gram positive bacteria results largely from teichoic acid. A more negative cell surface charge may more effectively attract and bind toxic metal cations. Toxic metals readily binds to sulfhydryl group of proteins. (Nies, 1999; Sandrin & Hoffman, 2006).

The resemblance of some toxic heavy metals to essential metabolite (minerals) allows them to readily enter into the microbial cells. Thus, chromate (Cr) is often mistakenly taken up in place of sulphate (S), arsenate (As) is mistaken for phosphate (P), cadmium (Cd) is used as an enzyme co-factor instead of zinc (Zn) or calcium (Ca), nickel (Ni) and cobalt (Co) is mistaken for iron (Fe), and zinc (Zn) is very commonly mistaken for magnesium (Mg). (Nies, 1999; Sandrin & Hoffman, 2006).

## MICROBIAL DETOXIFICATION OF METALS

One or more of the resistance mechanisms allows microbes to function in metal contaminated environments and detoxify them. Micro-organisms can detoxify metals by ‘valence transformation’, by ‘extracellular chemical precipitation’ or by ‘volatilization’.

Generally, microbial transformations & detoxifications of metals occur either by redox conversions (reduction) of inorganic forms or conversions from inorganic to organic forms and *vice versa*. Most toxic heavy metals are less soluble and less toxic when in ‘reduced state’ than in ‘oxidised state’. Reduction of metals can occur through dissimilatory metal reduction, where microbes utilize metals as terminal electron acceptor for anaerobic respiration.

To date arsenic (As), chromium (Cr), mercury (Hg), uranium (U) and selenium (Se) have responded well to detoxification by microbial reduction. *Oscillatoria* spp. (a blue-green algae), *Chlorella vulgaris* & *Chlamydomonas*



spp. (green algae), *Arthrobacter*, *Agrobacter*, *Enterobacter* & *Pseudomonas aeruginosa* are some metal reducing microbes. (Ramasamy et, al., 2006).

## 1. Microbial Reduction of Chromium (VI) to Cr (III):

Chromium is widely used in many industrial & developmental activities, such as in leather & tannery industries, electroplating, steel and automobile manufacturing, production of paint pigments and dyes, refractory and in wood preservation. Its world production is in the order of 10,000,000 tons per year. It is a hazardous contaminant and is a serious threat to human health as it readily spreads beyond the site of initial contamination through aquatic ecosystems and groundwater. (Viti & Giovannetti, 2006).

Chromium in environment is able to exist in several oxidation states, ranging from Cr (II) to Cr (VI), but in soils the most stable & common forms are trivalent Cr (III) and hexavalent Cr (VI) species. The trivalent & hexavalent forms can inter-convert. Cr (III) is essential for animal and human nutrition. Utmost consideration is given to Cr (VI) because it is water-soluble, highly toxic and mutagenic to most organisms and carcinogenic for humans. It is also involved in causing birth defects and the decrease of reproductive health.

A wide range of microbes have been found to have chromium (Cr) tolerance, resistance and reducing ability. The blue-green algae *Nostoc* have been reported to exist in a soil chronically polluted by chromium (about 5000 mg/kg of soil) from leather tannery. Other microbes tolerating / resisting Cr (IV) levels are *Arthrobacter crystallopoites* (500 mg/L), *Pseudomonas* spp. CRB 5 (520 mg/L), *Bacillus maroccanus* ChrA21 (1040 mg/L), *Corynebacterium hoagii* Chr B20 (1144 mg/L), *Bacillus cereus* ES04 (1500 mg/L). (Viti & Giovannetti, 2006). Anaerobic sulfate reducing and methanogenic bacteria possess inherent abilities to sorb more than 90 % of chromium to its cell biomass. Microbe reduce the highly soluble chromate ions to Cr (III), which under appropriate conditions precipitates as  $\text{Cr(OH)}_3$ . Organic matter (carbon sources) of the soil plays an important role in the reduction of Cr (VI) to Cr (III) by creating reducing conditions, such as increasing activities of soil microbes and by acting as an electron donor, & also by indirectly lowering the oxygen level of the soil (due to increased microbial respiration). Carbon sources, such as organic acids, manure, molasses, have been proposed to improve Cr (VI) reduction, that otherwise is very slow.