S.N. Singh • R.D. Tripathi
Environmental Bioremediation Technologies

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(Eds.)

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With 58 Figures, 1 in colour

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Foreword

Environmental contamination from both natural and anthropogenic sources is, today, a major environmental concern due to pervasiveness and persistence of many toxicants. It is considered as an inevitable evil of our progress and modernization. To decontaminate the soils, sediments and waters, polluted by anthropogenic activities, the scientists and technologists have evolved different technologies over the years. Although we have to pay high cost for physical and chemical environmental technologies, but they are not eco-friendly and safe. Hence, it was deeply realized to develop viable technologies employing microbes and plants to remediate not only metallic residues and radionuclides, but also the xenobiotic compounds like PCBs, PAHs, PCPs, petroleum sludge and the military wastes. No doubt, the scientists have also got some success in this endeavour and as the result, many companies are in place today to promote the sale of plant or microbe-based technologies to deal with specific environmental contamination challenges. Besides, these technologies are self-driven and do not disturb the sites in cleaning process.

In order to give a boost to this technology, I would like to appreciate the sincere efforts of my colleagues, Dr. S.N. Singh and Dr. R.D. Tripathi, both senior scientists of Ecotoxicology and Bioremediation Group of our institute, to publish this volume which contains latest information on the various aspects of bioremediation to deal with specific environmental contaminants. I hope this book will serve as a ready reckoner to the new researchers and also help the scientists working in this area in identifying the gaps for research. I consider this book a value addition to the scientific knowledge on bioremediation – an emerging and promising technology of today.

Rakesh Tuli
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Preface

Environmental bioremediation is an emerging technology because conventional methods to clean up the environment are cost-intensive and eco-unfriendly. In this technology, we employ from micro-organisms to higher plants to treat hazardous organic and metallic residues or by-products which enter into soils and sediments from various processes associated with domestic, municipal, agricultural, industrial and military activities. Hazardous materials may render harm to humans, livestock, wildlife, crops or native plants through handling, ingestion, application to land or other distributions of the contaminated materials into the environment.

No doubt, naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time. However, metallic residues can not be degraded in composting, but may be converted into organic combinations that have less bioavailability than mineral combinations of the heavy metals. In addition, microbes can transform the oxidation states of several toxic metals and increase their bioavailability in the rhizosphere to be taken up by metal hyperaccumulating plants. This technology is termed as phytoremediation and has received a lot of attention in recent years due to its cost effectiveness solar driven and high efficiency. In addition, biotechnology provides us tools to accelerate the phytoremediation process through either over expression of genes responsible for the sequestration of metals in plants or gene transfer from low biomass accumulating metal hyperaccumulator plants to high biomass yielding non-accumulating plants.

To address this problem, we present before you an edited volume which focuses on different aspects of environmental bioremediation, such as (i) Accumulation, detoxification and bioremediation of heavy metals and radionuclides by plants and microbes (ii) Biotechnological approaches to enhance phytoremediation efficiency (iii) Bioremediation of petroleum sludge and polycyclic aromatic hydrocarbons (PAHs) (vi) Fungal-based treatment of textile wastewater and PCP-contaminated soil (v) Use of aquatic macrophytes in metal and nutrient removal (vi) Application of biofilms in porus media: mathematical modeling and numerical simulation (vii) Phytomonitoring and phytoremediation of air pollutants and (viii) Nanotechnology for bioremediation of heavy metals. These aspects have been dealt with in 21 chapters contributed by the leading workers, drawn from world over, in their own fields.

In this endeavour, we, the editors were not alone, but assisted by many people. We thank Director, NBRI, Dr. Rakesh Tuli, for his kind support and

encouragement to this task. Besides, we would like to acknowledge all the contributors who responded to our request and contributed their chapters enthusiastically, containing the latest information on the relevant aspects. We also record our appreciation to all those, more particularly Dr. Todd R. Sandrin, USA, who helped us in editing the some of the manuscripts for value addition. The services rendered by our own research workers, Dr. Amitosh Verma, Dr. Sanjay Dwivedi, Dr. Larisha Tyagi, Dr. Vinay Singh Baghel, Mrs. Seema Mishra, Mr. Sudhakar Srivastava, Ms. Ragini Singh, Mrs. Babita Kumari, Mrs. Sudha Dwivedi, Ms. Sadhana Tiwari, Mr. Rishabh Kr. Tripathi and Mr. Deepak Pandey were remarkable and appreciable. Mr. Dilip Kumar Chakraborty deserves special thanks for his relentless efforts for computer work to prepare the manuscript on camera ready format.

Lastly, the editors acknowledge their family members for their inspiration, endurance and moral support during this period.

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Bioremediation of Organic and Metal Co-contaminated Environments: Effects of Metal Toxicity, Speciation, and Bioavailability on Biodegradation

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1. Introduction

Forty percent of the hazardous waste sites on the U. S. Environmental Protection Agency's National Priority List (NPL) are co-contaminated with metal and organic pollutants (Sandrin et al. 2000). Metals most frequently found at Superfund sites include arsenic, barium, cadmium, chromium, lead, mercury, nickel and zinc. Common organic co-contaminants include petroleum, chlorinated solvents, pesticides and herbicides. Conventional approaches to removing the organic pollutants at these sites, such as pump and treat, are costly and often ineffective (NRC 1994). Bioremediation is a viable alternative to conventional technologies, but metal toxicity at co-contaminated sites may limit its utility. Many studies report that metals inhibit general microbial activity (e.g., litter decomposition, methanogenesis, acidogenesis, nitrogen transformation), but a few have specifically investigated the impact of metals on organic pollutant biodegradation. The fact, that metals affect a myriad of microbial activities suggests that metals have the potential to affect the biodegradation of organics in co-contaminated environments. In some studies, metals have no impact or have a stimulatory effect on microbial activity. Thus, the effect of metals on organic pollutant biodegradation remains poorly characterized. This review discusses: 1) the toxicity of metals to microorganisms, 2) the roles metal speciation and bioavailability play in governing the extent to which metals affect organic pollutant biodegradation, 3) reported effects of metals on aerobic and anaerobic biodegradation, 4) patterns in which metals affect biodegradation, and 5) approaches to increasing organic biodegradation in co-contaminated systems.

2. Metal Toxicity to Microorganisms

An understanding of mechanisms of metal toxicity is essential in anticipating to what extent, metals will inhibit pollutant biodegradation by a particular population of microorganisms. A lucid and comprehensive understanding of modes of metal toxicity may lead to the development of novel technologies to mitigate metal toxicity in metal and organic co-contaminated environments. Mechanisms of metal toxicity to microorganisms have been studied extensively, and several excellent reviews are now available (Nies 1992; Rouch et al. 1995a; Ji and Silver 1995; Silver and Phung 1996; Rosen 1996; Silver 1996; Nies 1999). Despite this sizable body of work, the precise mechanisms of the toxicity of many metals remain unclear. Hence Nies so astutely observed in his review of microbial metal toxicity and resistance, “We are just beginning to understand the metabolism of heavy metals” (Nies 1999).

2.1 Metal Chemistry

Incompletely filled d-orbitals allow metals to form complex compounds with organic ligands, such as the proteins (Nies 1999), nucleic acids, and cell wall materials of microorganisms (Toth and Tomasovicova 1989). This binding is beneficial in the case of some metals such as calcium, magnesium, manganese, copper, and zinc. These metals serve as enzyme co-factors in complex biochemical processes; however, at high concentrations, the same essential metals can form non-specific complexes with organic ligands. This leads to toxicity. In addition, some metals, such as mercury, cadmium, and silver, form such strong complexes with organic ligands that they are rarely used in biochemical process (Nies and Silver 1995). For example, only one enzyme, carbonic anhydrase utilized by a marine diatom, is known to use cadmium as a cofactor (Lane and Morel 2000; Lane et al. 2005).

Metals bind to functional groups of biological molecules with varying affinities and can be classified as either hard or soft. Hard metals (e.g., sodium, potassium, magnesium, calcium, manganese and iron) are small cations that are not readily polarizable, while soft metals (e.g., copper, lead, cadmium, mercury, and silver) are larger cations that are very polarizable due to their large number of electrons (Hughes and Poole 1991). Hard metals prefer to bind to ligands containing oxygen, such as carboxylic acid, sulfate, and phosphate functional groups. In contrast, soft metals preferentially bind to ligands containing sulfur, such as the sulfhydryl ($-SH_2$) groups found in proteins.

2.2 Heavy Metal Uptake

Of course, for a metal to bind to an essential protein, nucleic acid or membrane component, the metal must first be taken up by the cell. Differentiating between

toxic and non-toxic metals is a complex cellular process. The structures of many metals, toxic and non-toxic, are remarkably similar. For instance, manganese, iron, cobalt, nickel, copper and zinc have ionic diameters which vary by less than 14% (from 138-160 pm) (CRC 1991). In addition, each of these cations is divalent. Serving as further disguise, some metals can coordinate with oxygen in such a way as to resemble common innocuous molecules. Arsenate (AsO_4^{3-}) resembles phosphate (PO_4^{3-}), while chromate (CrO_4^{2-}) is remarkably similar to sulfate (SO_4^{2-}). Evolution has endowed microorganisms with effective mechanisms to distinguish between toxic and non-toxic metals. Two general types of uptake mechanisms have been described: 1) selective, substrate-specific uptake systems that are slow and require considerable energy (ATP) and 2) substrate-non-specific, fast systems that transport metals using a chemiosmotic gradient rather than ATP (Nies and Silver 1995). Fast, nonspecific uptake systems are constitutively expressed, while slower, specific, energy-consuming uptake systems are inducible (Nies and Silver 1995).

An example of a fast, non-specific uptake system is the magnesium uptake system, CorA, found in Gram negative bacteria, archaea and baker's yeast. This system is responsible for the uptake of a variety of cations in addition to magnesium, including nickel, cobalt, zinc, and manganese. Two common fast transport systems that heavy metals often exploit to enter cells are Pit (phosphate inorganic transport) and the sulfate transport system. Arsenate is able to enter via Pit, while chromate can infiltrate cells via the sulfate transport system (Nies 1999). Slow, specific metal uptake systems include the P-type ATPases that transport zinc, manganese, cadmium, magnesium, calcium, potassium, copper, lead and silver (Fagan and Saier 1994).

2.3 Interaction of Heavy Metals with Cellular Components

Even highly evolved, substrate-specific uptake mechanisms may not prevent entry of a toxic metal into a cell. Once inside, metal cations can interact with various cellular components including cell membranes, proteins, and nucleic acids. Interactions of metals with these cellular components have been linked to toxicity (Toth and Tomasovicova 1989). Baath (1989) reported that copper and zinc disrupt the cell membrane. Furthermore, an early step in metal uptake may be binding of the metal to the cell surface. The outer membrane of Gram negative bacteria effectively complexes metals including sodium, calcium, magnesium, strontium, nickel, manganese, lead, and iron. In addition, the thin layer of peptidoglycan of Gram negative bacteria can bind metals, albeit not nearly as effectively as the thick layer of peptidoglycan of Gram positive bacteria which contain teichoic acid, a potent metal chelator (Beveridge and Doyle 1989).

The ability of cell surfaces to complex metals lies in their net negative charge at normal growth pH. In Gram negative bacteria, the net negative charge of the cell surface results from the phosphate and carboxyl groups of

lipopolysaccharide molecules (Goldberg et al. 1983; Volesky 1990), while the negative 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, thus rendering the cell more susceptible to the toxic effects of the metal (Rai et al. 1996).

Interactions of metals with cellular proteins are more commonly implicated in causing toxicity than interactions of metals with membranes. Toxic metals readily bind to sulfhydryl groups of proteins. As mentioned above, soft cations, such as cadmium and lead, preferentially bind sulfur-containing ligands over oxygen-containing ones. This binding affects the structure and function of the protein. Interestingly, the dissociation constants of soft metals complexed to sulfhydryl groups correlate well with the minimum inhibitory concentration (MIC) of the same metals. This illustrates the importance of the ability of a metal to bind to proteins in determining its toxicity (Nies 1999).

2.4 Substitution for Essential Metabolites

If both hard and soft cations are present, soft cations will replace hard cations on ligands. This can lead to substitution of an essential metabolite by a toxic metal. The resemblance of some deleterious heavy metals to essential metals not only allows them to enter the cell, but also to exert their toxic effects via substitution. For example, chromate is often mistakenly used as sulfate, arsenate is mistaken for phosphate, cadmium is used as an enzyme co-factor instead of zinc or calcium, nickel and cobalt replace iron, and zinc is commonly mistaken for magnesium. All of these mistaken identities result in the construction of an unstable, inhibited, or non-functional enzyme or other biological molecule (Nies and Silver 1995; Nies 1999).

2.5 Heavy Metal Induced Oxidative Stress

The toxicity of heavy metals to Gram negative bacteria is due, in part, to oxidative stress (Kachur et al. 1998). Metal cations may bind two glutathione molecules, forming a bis-glutathione molecule that reacts with diatomic oxygen to yield oxidized bis-glutathione, the metal cation, and hydrogen peroxide. The oxidized bis-glutathione must be reduced using NADPH; however, the metal cation released in the process is once again free to re-initiate this process and continue imposing considerable oxidative stress on the cell (Nies 1999).

3. Metal Speciation and Bioavailability

Despite the substantial information concerning mechanisms of metal toxicity, meaningful quantitative data on responses of pollutant-degrading

microorganisms to metals is still lacking. This is largely due to the fact that making comparisons between concentrations of metals that inhibit biodegradation reported by different studies is exceedingly difficult. For example, five orders of magnitude separate literature reports of concentrations of zinc that inhibit biodegradation (Table 1). While it should be noted that not all studies attempted to pinpoint the lowest concentration that inhibits biodegradation, many disparities likely result from variations in metal bioavailability between studies.

Most commonly, metal inhibition of biodegradation has been related to the total metal concentration in a system. This may not be the most appropriate predictor of metal toxicity, as suggested by the wide range of total metal concentrations reported to inhibit biodegradation (Table 1). The concentration of the most bioavailable form (i.e., species) of the metal (commonly held to be the free, ionic, solution-phase metal species) is likely a better indicator of the extent to which a metal will inhibit biodegradation. In media commonly used to study metal toxicity, metals exist in a number of different species in addition to the free, ionic species. Depending on medium characteristics described below, metals can exist as free ions (possibly with different oxidation states), hydroxo-complexes, or be complexed to organic or inorganic ligands (Hughes and Poole 1991; Twiss et al. 2001). The distribution of these different metal forms is referred to as metal speciation.

3.1 Factors Affecting Metal Speciation and Toxicity

It is well-established that different metal species vary in their biological reactivity (Hughes and Poole 1991; Traina and Laperche 1999; Twiss et al. 2001; Behra et al. 2002). Certain metal species are more likely than others to associate with biochemically active sites (e.g., enzymes) and initiate biological responses. In this review, we define bioavailability as the ability of a metal species to access these sites. In the case of organic-degrading microbes, interactions of metals with enzymes results in the inactivation of enzymes necessary for biodegradation (e.g., monoxygenases, dioxygenases) or of enzymes used in the general metabolism (Nies 1999; Baldrian et al. 2000; Sandrin and Maier 2003). There is still some debate as to which metal species are most bioavailable. Currently, though, there is a considerable amount of evidence suggesting that free, ionic, solution-phase metal species are most bioavailable (Angle and Chaney 1989; Traina and Laperche 1999; Behra, et al. 2002). Despite being highly bioavailable, the free ionic metal concentration may represent only a small fraction of the total metal species distribution in a solid or aqueous medium. For these reasons, it is of paramount importance to understand what properties of metal toxicity test systems impact metal speciation and metal bioavailability. Two of the most important of these properties are medium chemical composition and pH.

Table 1. Reported metal concentrations that inhibit aerobic (A) and anaerobic (B) biodegradation and/or transformation of organic pollutants

A.

Metal	Organic	Lowest metal conc. reported to reduce biodegradation	Microbe(s) Studied	Environment	pH	Reference
Cd^{2+}	2,4-D	0.060 mg/g[a]	*Alcaligenes eutrophus* JMP134	soil microcosms	8.2	Roane et al. (2001)
Cd^{2+}	2,4-D	0.060 mg/g[a]	*Alcaligenes eutrophus* JMP134	field-scale bioreactors	8.2	Roane et al. (2001)
Cd^{2+}	2,4-DME	0.100 mg/l[a]	indigenous community	sediment (microcosm)	6.5	Said and Lewis (1991)
Cd^{2+}	2,4-DME	0.629 mg/l[a]	indigenous community	aufwuchs[e] (microcosm)	5.6	Said and Lewis (1991)
Cd^{2+}	PHEN	1 mg/l[b]	indigenous community	soil microcosms	7.6	Maslin and Maier (2000)
Cd^{2+}	NAPH	1 mg/l[b]	*Burkholderia* sp.	dilute mineral salts medium containing 1.4 mM phosphate	6.5	Sandrin et al. (2000)
Cd^{2+}	diesel fuel	1.1 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Cd^{2+}	2,4-D	>3 mg/l[a]	*Alcaligenes eutrophus* JMP134	mineral salts medium	6.0	Roane et al. (2001)
Cd^{2+}	2,4-D	24 mg/l[a]	*Alcaligenes eutrophus* JMP134	mineral salts medium containing cadmium resistant isolate	6.0	Roane et al. (2001)

Cd^{2+}	4CP, 3CB, 2,4D, XYL, IPB, NAPH, BP	<25.3 - 50.6 mg/l[a,c]	*Alcaligenes* spp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Cd^{2+}	TOL	37 mg/l[a]	*Bacillus* sp.	mineral salts medium containing 36 mM phosphate	5.9	Amor et al. (2001)
Cd^{2+}	EDTA	562 mg/l[d]	Enrichment culture	MOPS-buffered minimal medium	7.0	Thomas et al. (1998)
Co^{2+}	4CP,3CB,2,4D, XYL, IPB, NAPH, BP	<13.3 - 1,330 mg/l[a, c]	*Alcaligenes* spp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Co^{2+}	NTA	116.9 mg/l[d]	enrichment culture	PIPES-buffered mineral salts medium	7.0	White and Knowles (2003)
Co^{2+}	EDTA	292 mg/l[d]	Enrichment culture	MOPS-buffered minimal medium	7.0	Thomas et al. (1998)
Cr^{3+}	2,4-DME	0.177 mg/l[a]	indigenous	aufwuchs[e] (microcosm)	6.1	Said and Lewis (1991)
Cr^{6+}	diesel fuel	2.32 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Cr^{6+}	4CP, 3CB, 2,4D, XYL, IPB, NAPH, BP	<131 mg/l[a c]	*Alcaligenes* spp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Cu^{2+}	PH	0.01 mg/l[a]	*Acinetobacter calcoaceticus* AH strain	bioreactor medium containing 0.15 mM phosphate	7.8	Nakamura and Sawada (2000)
Cu^{2+}	2,4-DME	0.027 mg/l[a]	indigenous community	aufwuchs[e] (microcosm)	5.0	Said and Lewis (1991)
Cu^{2+}	2,4-DME	0.076 mg/l[a]	indigenous community	sediment (microcosm)	6.1	Said and Lewis (1991)

Cu^{2+}	NTA	3.18 mg/l[d]	*Chelatobacter heintzii* ATCC 29600	PIPES-buffered mineral salts medium	7.0	White and Knowles (2000)
Cu^{2+}	crude oil	6.30 mg/l[a]	*Pseudomonas* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Cu^{2+}	diesel fuel	6.35 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Cu^{2+}	PHB	8 mg/l[b]	*Acidovorax delafieldii*	agar plates containing 4.70 mM phosphate	6.9	Birch and Brandl (1996)
Cu^{2+}	crude oil	11.25 mg/l[a]	*Micrococcus* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Cu^{2+}	4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP	<14.3 -71.6 mg/l[a,c]	*Alcaligenes* sp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Cu^{2+}	NTA	127.1 mg/l[d]	enrichment culture	PIPES-buffered mineral salts medium	7.0	White and Knowles (2003)
Cu^{2+}	NTA	127.1 mg/l[d]	*Mesorhizobium* sp. NCIMB 13524	PIPES and phosphate-buffered mineral salts media	7.0	White and Knowles (2003)
Cu^{2+}	EDTA	318 mg/l[d]	Enrichment culture	MOPS-buffered minimal medium	7.0	Thomas et al. (1998)
Hg^{2+}	2,4-DME	0.002 mg/l[a]	indigenous community	aufwuchs[e] (microcosm)	6.8	Said and Lewis (1991)
Hg^{2+}	diesel fuel	4 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Hg^{2+}	4 CP, 3 CB, 2,4-D, XYL, IPB, NAPH, BP	<45.2 - 226 mg/l[a,c]	*Alcaligenes* sp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)

Mn^{2+}	crude oil	28.2 mg/l[a]	*Micrococcus* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Mn^{2+}	crude oil	317.0 mg/l[a]	*Pseudomonas* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Ni^{2+}	4 CP, 3 CB, 2,4-D, XYL, IPB, NAPH, BP	5.18 - 10.3 mg/l[a,c]	*Alcaligenes* sp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Ni^{2+}	diesel fuel	5.9 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Ni^{2+}	TOL	20 mg/l[a]	*Bacillus* sp.	mineral salts medium containing 36 mM phosphate	5.9	Amor et al. (2001)
Ni^{2+}	NTA	117.4 mg/l[d]	*Mesorhizobium* sp. NCIMB 13524	PIPES and phosphate-buffered mineral salts media	7.0	White and Knowles (2003)
Ni^{2+}	EDTA	293 mg/l[d]	Enrichment culture	MOPS-buffered minimal medium	7.0	Thomas et al. (1998)
Pb^{2+}	crude oil	1.41 mg/l[a]	*Micrococcus* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Pb^{2+}	crude oil	2.80 mg/l[a]	*Pseudomonas* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Pb^{2+}	diesel fuel	41.4 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Zn^{2+}	2,4-DME	0.006 mg/l[a]	indigenous community	sediment (microcosm)	6.4	Said and Lewis (1991)
Zn^{2+}	2,4-DME	0.041 mg/l[a]	indigenous community	aufwuchs[e] (microcosm)	5.6	Said and Lewis (1991)

Zn^{2+}	crude oil	0.43 mg/l[a]	*Pseudomonas* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Zn^{2+}	crude oil	0.46 mg/l[a]	*Micrococcus* sp.	mineral salts medium containing 31 mM phosphate	7.2	Benka-Coker and Ekundayo (1998)
Zn^{2+}	TOL	2.8 mg/l[a]	*Bacillus* sp.	mineral salts medium containing 36 mM phosphate	5.9	Amor et al. (2001)
Zn^{2+}	pH	10 mg/l[a]	*Acinetobacter calcoaceticus* AH strain	bioreactor medium containing 0.15 mM phosphate	7.8	Nakamura and Sawada (2000)
Zn^{2+}	4 CP, 3 CB, 2,4-D, XYL, IPB, NAPH, BP	<29.5 - 736 mg/l[a,c]	*Alcaligenes* sp., *Pseudomonas* spp., *Moraxella* sp.	Tris-buffered minimal medium plates	7.0	Springael et al. (1993)
Zn^{2+}	diesel fuel	65.4 mg/l[a]	Enrichment culture	MES-buffered mineral salts medium containing 0.33 mM phosphate	6.8	Riis et al. (2002)
Zn^{2+}	NTA	130.8 mg/l[d]	*Mesorhizobium* sp. NCIMB 13524	PIPES-buffered mineral salts media	7.0	White and Knowles (2003)

Abbreviations:
2,4D, 2,4-dichlorophenoxy acetic acid; 2,4-DME, 2,4-dichlorophenoxy acetic acid methyl ester; BP, biphenyl; CB, chlorobenzoate; CP, chlorophenol; EDTA, ethylenediaminetetraacetic acid; IPB, isopropylbenzene; MES, morpholinoethane sulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; NAPH, naphthalene; NTA, nitrilotriacetic acid; PH, phenol; PHB, poly (3-hydroxybutyrate); PHEN, phenanthrene; PIPES, Piperazine-N,N'-bis(2-ethanesulfonic acid); TOL, toluene; XYL, xylene

[a] value represents total metal added to system

[b] value represents solution phase concentration of metal present in system

[c] value represents Minimum Inhibitory Concentration (MIC) calculated by multiplying Maximum Tolerated Concentration (MTC) by a factor of 2.25. MIC = MTC*2.25.

[d] metal was complexed to a biodegradable organic (NTA or EDTA)

[e] floating algal mats

B.

Metal	Organic	Lowest metal conc. reported to reduce biodegradation	Microbe(s) Studied	Environment	pH	Reference
Cd^{2+}	HCB	0.001 mg/g[a]	indigenous community	microcosms containing contaminated sediment	NR	Jackson and Pardue (1998)
Cd^{2+}	TCA	0.01 mg/l[b]	indigenous community	laboratory soil microcosms containing rice paddy and bottomland hardwood soils	6.9-7.4	Pardue et al. (1996)
Cd^{2+}	TCA	0.2 mg/l[b]	indigenous community	laboratory soil microcosms containing organic matter-rich soil	6.8	Pardue et al. (1996)
Cd^{2+}	2CP, PH, BEN, 3CB	0.5-1.0 mg/l[a]	indigenous community	aqueous sediment enrichment in anaerobic growth medium	7.0	Kuo and Genthner (1996)
Cd^{2+}	TCE	5 mg/l[a]	*Burkholderia picketti* PK01	mineral salts medium containing 44 mM phosphate; denitrifying conditions	NR	Degraffenreid and Shreve (1998)
Cd^{2+}	2CP, 3CP	20 mg/l[a]	indigenous community	sediment slurry	7.0	Kong (1998)
Cr^{6+}	2CP, PH, BEN, 3CB	0.01-0.5 mg/l[a]	indigenous community	aqueous sediment enrichment in anaerobic growth medium	7.0	Kuo and Genthner (1996)
Cr^{6+}	2CP, 3CP	20 mg/l[a]	indigenous community	sediment slurry	7.0	Kong (1998)
Cr^{c}	OD	5,000 µg/g[a]	indigenous community	clay-containing sediment slurry	6.5	DeLaune et al. (1998)
Cu^{2+}	2CP, PH, BEN, 3CB	0.1-1.0 mg/l[a]	indigenous community	aqueous sediment enrichment in anaerobic growth medium	7.0	Kuo and Genthner (1996)
Cu^{2+}	2,4-DANT, RDX	4 mg/g[a]	indigenous community	soil slurry containing 50 mM phosphate buffer	6.5	Roberts et al. (1998)

Cu^{2+}	4-ADNT	8 mg/g[a]	indigenous community	soil slurry containing 50 mM phosphate buffer	6.5	Roberts et al. (1998)
Cu^{2+}	2CP, 3CP	20 mg/l[a]	indigenous community	sediment slurry	7.0	Kong (1998)
Hg^{2+}	2CP, PH, BEN, 3CB	0.1-1.0 mg/l[a]	indigenous community	aqueous sediment enrichment in anaerobic growth medium	7.0	Kuo and Genthner (1996)
Pb^{2+}	HCB	0.001 mg/g[a]	indigenous community	microcosms containing contaminated sediment	NR	Jackson and Pardue (1998)
Pb^{2+}	2,4-DANT, RDX	>1 mg/g[a]	indigenous community	soil slurry containing 50 mM phosphate buffer	6.5	Roberts et al. (1998)
Zn^{2+}	2,4-DANT	1.5 mg/g[a]	indigenous community	soil slurry containing 50 mM phosphate buffer	6.5	Roberts et al. (1998)
Zn^{2+}	PCP	2 mg/l[a]	indigenous community	anaerobic digester sludge in a liquid medium containing 0.6 mM phosphate	NR	Jin and Bhattacharya (1996)
Zn^{2+}	PCP	8.6 mg/l[a]	indigenous community	anaerobic enrichment cultures in serum bottles	NR	Majumdar et al. (1999)
Zn^{2+}	NB	10 mg/l[a]	indigenous community	anaerobic enrichment cultures in serum bottles	NR	Majumdar et al. (1999)

Abbreviations:
2,4-DANT, 2,4-diamino-6-nitrotoluene; 4-ADNT, 4-amino-2,6-dinitrotoluene; BEN, benzoate; CB, chlorobenzoate; CP, chlorophenol; HCB, hexachlorobenzene; NB, nitrobenzene; NR, not reported; OD, octadecane; PCP, pentachlorophenol; PH, phenol; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; TCA, trichloroaniline; TCE, trichloroethylene.

[a] value represents total metal added to system

[b] value represents solution phase concentration of metal present in system

[c] oxidation state not specified