Impact of Metals on the Biodegradation of Organic Pollutants

Todd R. Sandrin1 and Raina M. Maier2

1Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, Wisconsin, USA; 2Department of Soil, Water and Environmental Science, The University of Arizona, Tucson, Arizona, USA

Forty percent of hazardous waste sites in the United States are co-contaminated with organic and metal pollutants. Data from both aerobic and anaerobic systems demonstrate that biodegradation of the organic component can be reduced by metal toxicity. Metal bioavailability, determined primarily by medium composition/soil type and pH, governs the extent to which metals affect biodegradation. Failure to consider bioavailability rather than total metal likely accounts for much of the enormous variability among reports of inhibitory concentrations of metals. Metals appear to affect organic biodegradation through impacting both the physiology and ecology of organic degrading microorganisms. Recent approaches to increasing organic biodegradation in the presence of metals involve reduction of metal bioavailability and include the use of metal-resistant bacteria, treatment additives, and clay minerals. The addition of divalent cations and adjustment of pH are additional strategies currently under investigation. Key words: bioavailability, biodegradation, bioremediation, hazardous waste, heavy metals, inhibition, metal toxicity, pollutants. Environ Health Perspect 111:1093–1101 (2003). doi:10.1289/ehp.5840 available via http://dx.doi.org/[Online 4 March 2003]

Remediation of sites co-contaminated with organic and metal pollutants is a complex problem, as the two components often must be treated differently, yet 40% of the hazardous waste sites currently on the National Priority List of the U.S. Environmental Protection Agency (U.S. EPA) are co-contaminated (Sandrin et al. 2000). Metals most frequently found at U.S. EPA Superfund sites include arsenic, barium, cadmium, chromium, lead, mercury, nickel, and zinc. Common organic co-contaminants include petroleum, chlorinated solvents, pesticides, and herbicides. Biodegradation to innocuous end products (CO2, cell mass, water) is considered to be an environmentally sound and cost-effective process for removing organic contaminants (National Research Council 1994). In contrast, the nonbiodegradable metal component must either be removed or stabilized within the site. Removal involves a combination of steps that may include mobilization, separation and collection, off-site transport, and disposal. Stabilization of metals requires that the site be permanently changed in some way. Most drastic is vitrification, wherein contaminated soil is heated to form a glasslike substance (Staley 1995). Alternatively, the site may be capped or paved to prevent water from entering the site and transporting metal contaminants, or site conditions may be imposed (e.g., anaerobic conditions) that reduce the potential for metal mobilization and transport (Liu et al. 2001; Zoumis et al. 2001). In either case, metal removal or metal stabilization, treatment of the organic component by biodegradation is likely to be the first step in remediation of co-contaminated sites (Roane et al. 1996).

It is well documented that the presence of metals can inhibit a broad range of microbial processes including methane metabolism, growth, nitrogen and sulfur conversions, dehalogenation, and reductive processes in general. An exhaustive review of the impacts of metals on many of these processes is available (Baath 1989). However, the effects of metal toxicity on organic pollutant biodegradation in contaminated water and soil environments have not been adequately defined quantitatively or qualitatively. This is because metals may be present in a variety of different physical and chemical forms, namely, as separate-phase solids, soil-adsorbed species, colloidal solutions, soluble complexes, or ionic solutes. Related complications stem from the fact that the physical and chemical state of metals is affected by environmental conditions such as pH and ionic strength of the water phase as well as soil properties that include ion exchange capacity, clay type and content, and organic matter content.

In this review we discuss metal inhibition and toxicity in the context of the biodegradation of co-contaminant organic chemicals for which treatment is deemed necessary. Specifically, we address: a) the importance of the physical-chemical state of metals in relation to metal bioavailability and inhibition of microbial activity, b) the impact of metals on aerobic and anaerobic biodegradation processes, c) relationships between metal concentration and metal impacts on biodegradation, and d) how metal toxicity can be mitigated to allow effective biodegradation of targeted organic pollutants.

Metal Toxicity and Bioavailability

Metals exert their toxic effects on microorganisms through one or more mechanisms. An excellent review is available that describes modes of metal toxicity and the mechanisms by which microorganisms resist such toxicity (Nies 1999). Toxic metal cations may substitute for physiologically essential cations within an enzyme (e.g., Cd2+ may substitute for Zn2+), rendering the enzyme nonfunctional. Similarly, metal oxoanions, such as arsenate, may be used in place of structurally similar, essential nonmetal oxoanions, such as phosphate. In addition, metals impose oxidative stress on microorganisms (Kachur et al. 1998).

Metal toxicity is most commonly ascribed to the tight binding of metal ions to sulphydryl (–SH) groups of enzymes essential for microbial metabolism. In fact, the minimum inhibitory concentration (MIC) of a given metal to Escherichia coli tends to be related directly to the dissociation constant of the metal sulfide (Nies 1999). Metals may inhibit pollutant biodegradation through interaction with enzymes directly involved in biodegradation (e.g., pollutant-specific oxygenases) or through interaction with enzymes involved in general metabolism. In either case, inhibition is mediated by the ionic form of the metal (Angle and Chaney 1989). The implication is that metal toxicity is related to the concentration of ionic species rather than to the total or even total soluble metal concentration (which may include metal-organic complexes that are not capable of binding to enzymes). It follows, then, that the metal concentration of interest is that which is capable of binding to enzymes and interfering with microbial activity. It is this metal concentration that we define here as bioavailable metal. Although the concept of bioavailable metal is important, measurement of bioavailable metal is difficult because it varies depending on the environment and the type of organism exposed. The solution-phase metal concentration is therefore often used to approximate bioavailable metal, as discussed in the following sections.

Effect of medium and soil components on metal bioavailability. In their review of metal speciation (i.e., the distribution of different forms, or species, of a given metal),
bioavailability, and toxicity, Hughes and Poole (1991) stress the importance of understanding metal speciation in the test system. Unfortunately, few studies provide speciation information. As a result, an enormous range of metal concentrations has been reported to inhibit organic biodegradation (Tables 1 and 2). For instance, five orders of magnitude separate lowest reported concentrations of zinc that inhibit biodegradation.

The fact that not all of the studies cited in Tables 1 and 2 made exhaustive efforts to determine the lowest concentration of metal required to cause a reduction in biodegradation may explain, in part, the large differences observed. However, the large range of reported inhibitory concentrations is also due to differences in experimental protocols that

### Table 1. Reported metal concentrations that cause inhibition of aerobic biodegradation of organic contaminants.

| Metal | Organic | Lowest metal concentration reported to reduce biodegradation | Microbe studied | Environment | pH | Reference |
|-------|---------|-------------------------------------------------------------|-----------------|-------------|----|-----------|
| Cd²⁺ | 2,4-DME | 0.100 mg/L²                                                | Indigenous community | Sediment (microcosm) | 6.5 | Said and Lewis 1991 |
| Cd²⁺ | 2,4-DME | 0.629 mg/L²                                                | Indigenous community | Aufwuchs² (microcosm) | 5.6 | Said and Lewis 1991 |
| Cd²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 25.3–50.6 mg/L²   | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Cd²⁺ | 2,4-D | > 3 mg/L²                                           | Alcaligenes eutrophus JMP134 | Mineral salts medium | 6.0 | Roane et al. 2001 |
| Cd²⁺ | 2,4-D | 24 mg/L²                                           | Alcaligenes eutrophus JMP134 | Mineral salts medium containing cadmium-resistant isolate | 6.0 | Roane et al. 2001 |
| Cd²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | 0.080 mg/g⁴           | Alcaligenes eutrophus | Soil microcosms | 8.2 | Roane et al. 2001 |
| Cd²⁺ | 2,4-D | 0.080 mg/g⁴                          | Alcaligenes eutrophus | Field-scale bioreactors | 8.2 | Roane et al. 2001 |
| Cd²⁺ | PHEN | 1 mg/L²                                    | Indigenous community | Soil microcosms | 7.6 | Maslin and Maier 2000 |
| Cd²⁺ | PHEN | 1 mg/L²                                    | Burkholderia sp. | Dilute mineral salts medium containing 1.4 mM phosphate | 6.5 | Sandrin et al. 2000 |
| Cd²⁺ | TOL | 37 mg/L²                                    | Bacillus sp. | Mineral salts medium containing 36 mM phosphate | 5.9 | Amor et al. 2001 |
| Co²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 13.3–1330 mg/L²  | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Cr³⁺ | 2,4-DME | 0.177 mg/L²                                               | Indigenous community | Aufwuchs² (microcosm) | 6.1 | Said and Lewis 1991 |
| Cr³⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 131 mg/L²ₐ,b           | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Cu²⁺ | 2,4-DME | 0.076 mg/L²                                               | Indigenous community | Sediment (microcosm) | 6.1 | Said and Lewis 1991 |
| Cu²⁺ | 2,4-DME | 0.027 mg/L²                                               | Indigenous community | Aufwuchs² (microcosm) | 5.0 | Said and Lewis 1991 |
| Cu²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 14.3–71.6 mg/L²ₐ,b  | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Cu²⁺ | PHB | 8 mg/L²                                    | Acidovorax delafieldii | Agar plates containing 4.70 mM phosphate | 6.9 | Birch and Brandl 1996 |
| Cu²⁺ | Crude oil | 6.30 mg/L²                                               | Pseudomonas sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Cu²⁺ | Crude oil | 11.25 mg/L²                                             | Micrococcus sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Cu²⁺ | Pseudomonas sp. | 0.01 mg/L²                                              | Acinetobacter calcoaceticus, | Bioreactor medium containing 0.15 mM phosphate | 7.8 | Nakamura and Sawada 2000 |
| Cu²⁺ | Crude oil | 317.0 mg/L²                                             | Pseudomonas sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Hg²⁺ | 2,4-DME | 0.002 mg/L²                                               | Indigenous community | Aufwuchs² (microcosm) | 6.8 | Said and Lewis 1991 |
| Hg²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 45.2–226 mg/L²ₐ,b  | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Mn²⁺ | Crude oil | 317.0 mg/L²                                             | Pseudomonas sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Mn²⁺ | Crude oil | 28.2 mg/L²                                            | Micrococcus sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Ni²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | 5.18–10.3 mg/L²ₐ,b  | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Ni²⁺ | TOL | 20 mg/L²                                             | Bacillus sp. | Mineral salts medium containing 36 mM phosphate | 5.9 | Amor et al. 2001 |
| Pb²⁺ | Crude oil | 2.80 mg/L²                                             | Pseudomonas sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Pb²⁺ | Crude oil | 1.41 mg/L²                                             | Micrococcus sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Zn²⁺ | 2,4-DME | 0.006 mg/L²                                             | Indigenous community | Sediment (microcosm) | 6.4 | Said and Lewis 1991 |
| Zn²⁺ | 2,4-DME | 0.041 mg/L²                                             | Indigenous community | Aufwuchs² (microcosm) | 5.6 | Said and Lewis 1991 |
| Zn²⁺ | 4CP, 3CB, 2,4-D, XYL, IPB, NAPH, BP | < 29.5–376 mg/L²ₐ,b  | Alcaligenes spp., Pseudomonas spp., Moraxella sp. | Tris-buffered minimal medium plates | 7.0 | Springael et al. 1993 |
| Zn²⁺ | PH | 10 mg/L²                                             | Acinetobacter calcoaceticus, | Bioreactor medium containing 0.15 mM phosphate | 7.8 | Nakamura and Sawada 2000 |
| Zn²⁺ | Crude oil | 0.43 mg/L²                                             | Pseudomonas sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Zn²⁺ | Crude oil | 0.46 mg/L²                                             | Micrococcus sp. | Mineral salts medium containing 31 mM phosphate | 7.2 | Benka-Coker and Ekundayo 1998 |
| Zn²⁺ | TOL | 2.8 mg/L²                                             | Bacillus sp. | Mineral salts medium containing 36 mM phosphate | 5.9 | Amor et al. 2001 |

Abbreviations: BP, biphenyl; IPB, isopropylbenzene; MTC, maximum tolerated concentration; TOL, toluene; XYL, xylene.

*Value represents total metal added to system. *Value represents MIC calculated by multiplying MTC by a factor of 2.25. *Floating algal mats. *Value represents solution-phase concentration of metal present in system.
affected solution-phase metal concentrations. For example, many laboratory media contain metal-binding (e.g., yeast extract) and metal-precipitating (e.g., phosphate or sulfate salts) components that can reduce solution-phase metal concentrations (Hughes and Poole 1991; Poulsen et al. 1997). Medium pH also dramatically impacts solution-phase metal concentrations. As pH increases, metals tend to form insoluble metal oxides and phosphates, resulting in decreased solution-phase metal concentrations (Hahn and Kroonje 1973). Specifically, in media that contain phosphate, perhaps the most common buffer used in microbiology, even a small change in pH can decrease metal solubility, reducing solution-phase metal concentrations by several orders of magnitude. The effects of pH and phosphate concentration on the amount of cadmium in solution as predicted by a metal speciation modeling program (MINEQL+; Environmental Research Software, Hallowell, ME) are illustrated in Figure 1. At pH 7 the concentration of cadmium in solution is 88 mM in the absence of phosphate. As phosphate is increased to 0.13, 1.3, 13, and 130 mM, the solution-phase concentration of cadmium is reduced to 50, 17, 2, and 0.1 mM, respectively. In the studies summarized in Tables 1 and 2, pH varied from 5.0 to 8.2, and phosphate concentrations ranged from 0 to 50 mM. In addition, many studies used media rich in metal-binding components, whereas others did not. The variability of each of these factors hampers meaningful comparisons between studies and underscores the need for future studies to report solution-phase metal concentrations. In the soil environment, organic matter and clay mineral content are important factors that can reduce solution-phase metal concentrations. For example, only 0.01 mg solution-phase cadmium/L was required to inhibit trichloroaniline (TCA) dechlorination in a mineral-dominated soil, whereas 0.2 mg solution-phase cadmium/L was required for inhibition in an organic matter–dominated soil (Pardue et al. 1996). This increase in the amount of cadmium required to inhibit dechlorination was correlated to saturation of metal-binding sites on organic matter. Similarly, Said and Lewis (1991) reported that biodegradation of 2,4-dichlorophenoxyacetic acid methyl ester (2,4-DME) was much more sensitive to metal inhibition in aufwuchs (floating algal mats) than in sediments. The authors suggested that this was due to greater metal-binding capacity of sediments. Clay minerals have also been shown to reduce metal bioavailability. Clays with high cation exchange capacities (CECs), such as montmorillonite, appear to be most effective at reducing metal bioavailability and toxicity (Babich and Stotzky 1977a, 1977b, 1978). In fact, the large impact of clays on metal bioavailability has prompted investigation into the use of clays to reduce metal

![Figure 1. Effect of pH and phosphate concentration on the solution-phase cadmium ([Cd]²⁺) concentration in a mineral salts medium as predicted by MINEQL+ geochemical modeling software.](image-url)

### Table 2. Reported metal concentrations that cause inhibition of anaerobic biodegradation of organic contaminants.

| Metal | Organic | Lowest metal concentration reported to reduce biodegradation | Microbe(s) studied | Environment | pH | Reference |
|-------|---------|-------------------------------------------------------------|--------------------|--------------|----|-----------|
| Cd²⁺  | TCA     | 0.01 mg/L[^a]                                               | Indigenous community | Laboratory soil microcosms containing rice paddy and bottomland hardwood soils | 6.9–7.4 | Pardue et al. 1996 |
| Cd²⁺  | TCA     | 0.2 mg/L[^a]                                               | Indigenous community | Laboratory soil microcosms containing organic matter-rich soil | 6.8 | Pardue et al. 1996 |
| Cd²⁺  | 2CP, PH, BEN, 3CB | 0.5–1.0 mg/L[^b]                                           | Indigenous community | Sediment slurry in anaerobic growth medium | 7.0 | Kuo and Genther 1996 |
| Cd²⁺  | 2CP, 3CP | 20 mg/L[^b]                                               | Indigenous community | Microcosms containing contaminated sediment | 7.0 | Kong 1998 |
| Cd²⁺  | HCB     | 0.001 mg/g[^b]                                             | Indigenous community | Sediment slurry in anaerobic growth medium | 7.0 | Jackson and Pardue 1998 |
| Cd²⁺  | 2CP, PH, BEN, 3CB | 0.01–0.5 mg/L[^b]                                          | Indigenous community | Sediment slurry in anaerobic growth medium | 7.0 | Kuo and Genther 1996 |
| Cd²⁺  | 2CP, 3CP | 20 mg/L[^b]                                               | Indigenous community | Sediment slurry in anaerobic growth medium | 7.0 | Kuo and Genther 1996 |
| Cu²⁺  | 2CP, PH, BEN, 3CB | 0.1–1.0 mg/L[^b]                                           | Indigenous community | Sediment slurry in anaerobic growth medium | 7.0 | Kuo and Genther 1996 |
| Cu²⁺  | 2CP, 3CP | 20 mg/L[^b]                                               | Indigenous community | Soil slurry containing 50 mM phosphate buffer | 6.5 | Roberts et al. 1998 |
| Cu²⁺  | 4-ADNT  | 8 mg/g[^b]                                                | Indigenous community | Soil slurry containing 50 mM phosphate buffer | 6.5 | Roberts et al. 1998 |
| Hg²⁺  | 2CP, PH, BEN, 3CB | 0.1–1.0 mg/L[^b]                                           | Indigenous community | Aquatic sediment enrichment in anaerobic growth medium | 7.0 | Kuo and Genther 1996 |
| Pb²⁺  | HCB     | 0.001 mg/g[^b]                                             | Indigenous community | Microcosms containing contaminated sediment | NR | Jackson and Pardue 1998 |
| Pb²⁺  | 2,4-DANT, RDX | > 1 mg/g[^b]                                              | Indigenous community | Soil slurry containing 50 mM phosphate buffer | 6.5 | Roberts et al. 1998 |
| Zn²⁺  | PCP     | 2 mg/L[^b]                                                | Indigenous community | Anaerobic digester sludge in a liquid medium containing 0.5 mM phosphate | NR | Jin and Bhattacharya 1996 |
| Zn²⁺  | 2,4-DANT | 1.5 mg/g[^b]                                              | Indigenous community | Soil slurry containing 50 mM phosphate buffer | 6.5 | Roberts et al. 1998 |
| Zn²⁺  | NB      | 10 mg/L[^b]                                               | Indigenous community | Anaerobic enrichment cultures in serum bottles | NR | Majumdar et al. 1999 |
| Zn²⁺  | PCP     | 8.6 mg/L[^b]                                              | Indigenous community | Anaerobic enrichment cultures in serum bottles | NR | Majumdar et al. 1999 |

Abbreviations: 4-ADNT, 4-amino-2,6-dinitrotoluene; NB, nitrobenzene; NR, not reported; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine.

[^a]: Value represents solution-phase concentration of metal present in system.
[^b]: Value represents total metal added to system.
bioavailability and toxicity, as described later in this review.

In addition to organic matter and clay minerals, metals may interact with organic pollutants to affect bioavailable concentrations of metals. Although a dearth of information is currently available on this topic, one study has shown that salicylate, a common intermediate in the biodegradation of aromatic hydrocarbons, increased cadmium uptake and toxicity in E. coli (Rosner and Aumercier 1990). Additional research is needed to determine whether bioavailability and toxicity are affected similarly with other microorganisms, metals, and organic pollutants.

Several recent studies illustrate clearly the degree to which the composition and pH of media and soils affect metal concentrations. For example, only 1% of the total zinc added to acetate enrichment anaerobic cultures in the work of Majumdar et al. (1999) was in the aqueous phase. Similarly, Kong (1998) found that solution-phase metal concentrations in sediment slurries initially amended with 20 mg total metal/L were below detection limits of 0.03–0.04 mg/L. Amendments of 100 mg total metal/L yielded only 1 mg solution-phase cadmium/L and less than 0.12 mg solution-phase copper and chromium/L. Finally, Roberts et al. (1998) were unable to detect solution-phase lead (below 1 mg/L) in anaerobic soil-slurry bioreactors initially containing 10,000 mg total lead/kg.

**Measurement of bioavailable metal.** Reporting of bioavailable metal concentrations is a vital step in the process of standardizing experiments to determine the impact of metals on organic pollutant biodegradation. Bioavailable metal concentrations can be estimated from solution-phase metal concentrations using tools such as ion-selective electrodes and atomic absorption spectroscopy. There are also a number of promising tools in development that use biological systems to detect metal speciation and/or degradation equations. For instance, Amor et al. (2001) used a form of the Andrews equation (often used to describe microbial growth with inhibition) to model the effect of cadmium, zinc, and nickel on rates of alkylbenzene biodegradation

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S + S^2 / K_i},
\]

where \(\mu\) is the alkylbenzene biodegradation rate, \(\mu_{\text{max}}\) is the maximum alkylbenzene biodegradation rate, \(S\) is the alkylbenzene concentration, \(K_s\) is the alkylbenzene concentration that yields \(\mu_{\text{max}}\), and \(K_i\) is the metal inhibition constant.

None of these models currently incorporates metal speciation and bioavailability. The concern with the use of such models is that the data generated may only be meaningful for the medium or soil that was used to develop the model. For example, the medium used by Nakamura and Sawada (2000) was adjusted to a pH of 7.8 and contained 0.147 mM phosphate. Similarly, the medium used by Amor et al. (2001) was adjusted to a pH of 5.9 and contained 36 mM phosphate. In both media, much of the metal may precipitate. Thus, these models are likely to underpredict metal toxicity in systems that have a lower pH and/or less phosphate.

**Metal Inhibition of Microbiological Processes**

An extensive body of work is available on the effect of metals on general soil microbiological processes. The impact of metals on litter decomposition, methanogenesis, acidogenesis, nitrogen transformation, biomass generation, and enzymatic activity all have been studied (Babich and Stotzky 1985; Bardgett and Sagar 1994; Burkhart et al. 1993; Capone et al. 1983; Doelman and Haanstra 1979a,b; Hickey et al. 1989; Knight et al. 1997; Kouzelikatsiri et al. 1988; Lin 1993; Masakazu and Iyata 1995; Mosey 1976; Nandan et al. 1990; Pankhania and Robinson 1984; Rogers and Li 1985). Metals including copper, zinc, cadmium, chromium (III and VI), nickel, mercury, and lead are reported to inhibit each of these processes. However, addition of metals has also been observed to stimulate activity in some cases. For example, some metals, including mercury, lead, nickel, cadmium, and copper, stimulate methanogenesis in anoxic salt sediments (Capone et al. 1983). In addition, nickel (< 300 mg/L) was reported to stimulate acidogenesis (Lin 1993).

Studies on the effect of metals on organic pollutant biodegradation are not extensive but demonstrate that metals have the potential to inhibit pollutant biodegradation under both aerobic and anaerobic conditions. These studies are summarized in the sections that follow.

**Aerobic biodegradation.** Metals inhibit aerobic biodegradation of a variety of organic pollutants of concern (Table 1), including chlorinated phenols and benzoates (BENs), low molecular weight aromatics, and hydroxymethylbenzoates. Copper, cadmium, mercury, zinc, and chromium (III) inhibited biodegradation of 2,4-DME in lakewater samples inoculated with either a sediment or an aufwuch (floating algal mat) sample (Said and Lewis 1991). In the sediment samples, zinc was most toxic, with an MIC of 0.006 mg total zinc/L, whereas in samples of aufwuchs, mercury was most toxic, with an MIC of 0.002 mg total mercury/L. A pure culture study using a naphthalene (NAPH)-degrading *Burkholderia* sp. reported an MIC of 1 mg solution-phase cadmium/L (Sandrin et al. 2000). A comparable MIC was reported by Said and Lewis (1991) for cadmium (0.1 mg total cadmium/L for sediment samples and 0.629 mg total cadmium/L for aufwuch samples). The differences between these MICs are likely organism/community specific.

Springael et al. (1993) reported metal inhibition of pollutant biodegradation by several bacterial genera tested under pure culture conditions. In this case, the reported MICs were 2–4 orders of magnitude higher than those reported by Said and Lewis (1991) (Table 1). This large discrepancy is likely due to differences in test conditions. Agar media were used in this study, and it has been pointed out that colony growth may protect against metal toxicity and result in higher MICs.

Metal inhibition has also been observed in metal-contaminated soil systems. For example, cadmium added at levels of 60 mg total cadmium/kg was found to inhibit biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) in a soil system inoculated with the 2,4-D-degrading *Alcaligenes eutrophus* JMP134 (Roane et al. 2001; Roane and Pepper 1997). Experiments with an indigenous soil community (Maslin and Maier 2000) examined the
impact of cadmium on phenanthrene (PHEN) biodegradation in two desert soils over a 9-day period. Results showed a 5-day increase in lag period for PHEN degradation in the presence of 1 and 2 mg solution-phase cadmium/L and complete inhibition at 3 mg solution-phase cadmium/L. Note that in this soil system, 3 mg solution-phase cadmium/L was equivalent to 394 mg total cadmium/kg added to the soil.

Studies investigating the impact of metal toxicity on biodegradation are not limited to aromatic contaminants. The impact of copper toxicity on biodegradation of a biodegradable polymer, polyhydroxybutyrate (PHB), has been investigated (Birch and Brandl 1996). This compound is commonly used for medical, agricultural, and industrial purposes. In agriculture, the material is used both as film mulch and as a long-term delivery device for fertilizers. In both cases the material is expected to biodegrade after use. However, treatment of agricultural fields with sewage sludges, which often have high copper concentrations, can increase the soil copper content. The impact of copper toxicity on PHB biodegradation was determined by placing a PHB-containing agar overlay on a copper-containing agar. The plate was incubated at a slant so that copper diffusion into the overlay created a concentration gradient along the length of the plate. The plates were then inoculated with a PHB-degrading strain of Acidovorax delafieldii. The bioavailable concentration of copper along the gradient was estimated by measuring copper in filter paper that contacted the gradient. Using this novel method, the authors found that 8–15 mg bioavailable copper/L were required to inhibit PHB biodegradation.

Not all studies have investigated the impact of single metals on biodegradation of only a single, pure organic. Benka-Coker and Ekundayo (1998) investigated the impact of zinc, lead, copper, and manganese on crude oil biodegradation by a Microoccus sp. and a Pseudomonas sp. Biodegradation was reduced most by zinc (concentrations as low as 0.43 mg total zinc/L) and least by manganese (concentrations as low as 28.2 mg total manganese/L). Interestingly, combinations of metals were less toxic than some single metals. For instance, toxicity of 0.5 mg total zinc/L was mitigated by addition of 0.5 mg total copper, lead, and manganese/L.

An aerobic biodegradation. Anaerobic metabolism is an important and sometimes the sole process for biodegradation of highly halogenated organics such as trichloroethene and perchloroethene (Alexander 1999). Often, these solvents have been co-disposed with metals. For this reason several recent studies have addressed the impact of metal toxicity on the anaerobic biodegradation of organic pollutants (Table 2). Despite that anaerobic conditions are thought to largely reduce the solubility and mobility of many toxic metals, data from studies summarized below suggest that metal inhibition of biodegradation is significant in many of these systems.

Representative of solution studies, Kuo and Genthner (1996) investigated the impact of cadmium, copper, chromium, and mercury on dechlorination and biodegradation by an anaerobic bacterial consortium isolated from an aquatic sediment. This consortium was capable of completely degrading 2-chlorophenol (2CP), 3-chlorobenzoate (3CB), phenol (PH), and BEN. In general, the addition of low levels of metals (0.1–2.0 mg total metal/L) lengthened acclimation periods and decreased dechlorination and biodegradation rates. Biodegradation of 3CB was inhibited most by cadmium and chromium, biodegradation of BEN was most sensitive to copper, and biodegradation of PH was reduced most by mercury. Similarly, cadmium has been shown to reduce the rate of anaerobic pentachlorophenol (PCP) biodegradation (Kamashwaran and Crawford 2001). Kuo and Genthner (1996) point out that their results suggest that, in addition to adversely affecting degraders in a consortium, metals may affect nondegrading consortium members that play a vital but indirect role in the degradation process. For instance, members of the consortium that produce reducing equivalents for reductive dehalogenation or remove dechlorinated products from the system to allow further dehalogenation may be inhibited, thus reducing the overall rate and extent of biodegradation.

Such an indirect mode of toxicity has also been implicated in metal inhibition of anaerobic biodegradation of trinitrotoluene (TNT) metabolites (Roberts et al. 1998). In this study, copper, zinc, and lead did not affect establishment of anaerobic conditions in bioreactors containing soil slurries nor did these metals impact loss of the parent TNT compound. However, the subsequent removal of TNT degradation intermediates was reduced by each of the metals. For example, lead (total concentrations > 1,000 mg/kg) delayed degradation of a TNT biodegradation intermediate (2,4-diamino-6-nitrotoluene [2,4-DANT]) by as many as 9 days. Zinc (1,500 mg total zinc/kg) delayed degradation of the same intermediate by eight days. Copper (4,000 and 8,000 mg total copper/kg) completely inhibited removal of this intermediate. Clearly, when considering the impact of metals on organic biodegradation, the effects of metals on populations other than degraders of the parent compound must also be considered.

A small number of studies have been conducted in anaerobic soil and sediment systems. Work in soil systems suggests that soil type influences metal toxicity. For example, Purdie et al. (1996) examined the impact of cadmium on reductive dehalogenation of TCA in different soils. In microcosms containing two mineral-dominated soils, only 0.01 mg solution-phase cadmium/L was required to inhibit reductive dehalogenation. In microcosms containing an organic matter-dominated soil, more than an order of magnitude higher cadmium concentration (0.2 mg solution-phase cadmium/L) was required to inhibit dehalogenation. Furthermore, results showed that the dehalogenation pathway used was affected by the cadmium concentration. A single dehalogenation pathway was observed until the cadmium concentration neared the inhibitory concentration. At this point, a second degradation pathway was observed. This suggests that cadmium stress selected for a different dehalogenating population. Sediments have also been shown to mediate metal toxicity. The impact of metals on reductive dehalogenation of hexachlorobenzene (HCB) in a waste lagoon sediment co-contaminated with cadmium and lead has been investigated (Jackson and Purdie 1998). In this study, cadmium and lead inhibited reductive dehalogenation, but only when not bound to sediment material and present in the free, bioavailable form.

**Figure 2.** Effect of metal concentration on pattern of inhibition of organic pollutant biodegradation assuming: A, a direct relationship; B, additional pattern 1; and C, additional pattern 2.
Additional pattern 1: low metal concentrations stimulate biodegradation; high metal concentrations inhibit. A number of studies show a pattern of metal toxicity in which low metal concentrations stimulate activity until a maximum level of stimulation is reached, and thereafter, metal toxicity increases with increasing metal concentration (Figure 2B). It should be noted that all these studies used consortia not single isolates. Therefore, it is likely that this pattern is a result of differential toxicity effects, wherein a second population more sensitive to metal stress competes in some way with the population expressing the activity of interest. Inhibition of the second population reduces competition for resources needed by the first population. Supporting this pattern is evidence from Capone et al. (1983) that methanogenesis was stimulated by the addition of some metals. The authors suggested that this may have been due to differential inhibition of the methanogenic and nonmethanogenic microorganisms. Metals may have selected for a metal-resistant, methanogenic population, which reduced competition from a metal-sensitive, nonmethanogenic population. Similarly, Kuo and Gentner (1996) reported that the addition of some metals at low levels stimulated biodegradation. Hexavalent chromium (0.01 mg total chromium/L) increased the biodegradation rate of PH by 177% and that of BEN by 169% over controls containing no metals. Copper and cadmium (both at 0.01 mg total metal/L) increased the BEN biodegradation rate 185% and the 2CP biodegradation rate by 168%. Furthermore, 1–2 mg total mercury/L increased the biodegradation rates of 2CP and 3-chlorophenol (3CP) by 133–154%. Similar results have been reported (Hughes and Poole 1989; Sterritt and Lester 1980). These groups suggested the stimulatory effect may be due to metals reducing competition for reducing equivalents or nutrients between metal-resistant degraders and metal-sensitive nondegraders. As in the work of Roberts et al. (1998) and Capone et al. (1983), the impact of metals on microbiologically mediated processes in these studies may be due mainly to the effects of metals on a population other than the one carrying out the process of interest. The existence of this pattern of metal effects underscores the importance of considering not only the physiological impact of a toxic metal on a target-degrading population but also the ecological impact of the toxic metal.

Additional pattern 2: low metal concentrations inhibit biodegradation; high metal concentrations inhibit less. Some studies have shown that low metal concentrations of metals increasingly inhibit activity until a maximum level of inhibition is reached, and thereafter, metal toxicity decreases with increasing metal concentration (Figure 2C). The work reported by Said and Lewis (1991) generally shows that 2,4-DME biodegradation decreased as the metal concentration increased (Figure 2A). However, a closer examination of their data revealed that the maximal degradation rate of 2,4-DME ($V_{max}$) was significantly less in the presence of 10 µM cadmium (0.61 ± 0.03 µg 2,4-DME/L/min) than in the presence of 100 µM cadmium (0.74 ± 0.00 µg 2,4-DME/L/min). In a later study, a similar pattern of inhibition was observed as populations of 2,4-D degraders in a cadmium-contaminated soil were reported to be more resistant to cadmium toxicity at a higher concentration of cadmium (40 mg total cadmium/L) than at a lower concentration of cadmium (20 mg total cadmium/L) (Roane and Pepper 1997). These responses to metals might be explained by microbial community dynamics wherein high metal concentrations create selective pressure for metal-resistant, organic-degrading microorganisms. This selective pressure may have reduced competition from metal-sensitive, nondegrading microorganisms, thus increasing biodegradation at higher metal concentrations; however, this pattern has also been observed in a pure culture study of the effect of cadmium on NAPH biodegradation by a Burkholderia sp. (Sandrin et al. 2000). Whereas solution-phase cadmium concentrations from 1 to 50 mg/L increasingly inhibited NAPH biodegradation, the highest investigated concentration of solution-phase cadmium (100 mg/L) showed reduced inhibition of NAPH biodegradation. It is possible that at high cadmium concentrations, cadmium uptake was reduced. This hypothesis is supported by a study showing that the initial rate of cadmium uptake by E. coli K-12 was lower at a higher cadmium concentration (5.0 µM) than at a lower cadmium concentration (2.5 µM) (Laddaga and Silver 1985). It also remains possible that high metal concentrations may more rapidly induce a metal-resistance mechanism important in cadmium detoxification (e.g., an efflux pump) than low metal concentrations.

In summary, the existence of different patterns of response to metals complicates understanding and predicting metal toxicity in the environment. As demonstrated by the patterns described above, metals may impact both the physiology and ecology of pollutant-degrading microorganisms. For this reason models designed to predict the impact of metals on biodegradation may fail to do so accurately unless they incorporate both physiologic and ecologic impacts of metals on organic-degrading microorganisms.

**Approaches to Increasing Biodegradation in Co-contaminated Environments**

A review of the literature shows a number of possible approaches that can be used to lower metal bioavailability and/or increase microbial tolerance to metals. These include inoculation with metal-resistant microorganisms and addition of materials that reduce metal bioavailability, including calcium carbonate, phosphate, clay minerals, and surfactants.

**Metal-resistant bacteria.** Microorganisms exhibit several types of metal resistance (Ji and Silver 1995; Nies 1992, 1999; Nies and Silver 1995; Rosen 1996; Silver 1996; Silver and Phung 1996). For example, many microorganisms can mitigate the toxicity of some metals (e.g., divalent mercury and arsenate) through reduction by using the metals as electron acceptors. However, many toxic metals such as cadmium (redox potential, –824 mV) have redox potentials outside the aerobic physiological redox range (from –421 mV to +808 mV). Thus, their toxicity cannot be mitigated by reduction. Other mechanisms of metal resistance are common and include intra- and extracellular metal sequestration, metal reduction, metal efflux pumps, and production of metal chelators such as metallothioneins and biosurfactants. Microorganisms may be capable of acclimating to metal toxicity, as has been suggested for mercury (Liebert et al. 1991). Thus far, only one study has investigated inoculation with metal-resistant bacteria to enhance organic contaminant biodegradation in a co-contaminated system (Roane et al. 2001). In this study, soil microcosms were co-contaminated with 2,4-D (500 mg/kg) and cadmium (60 mg total cadmium/kg). Because this soil did not contain an active 2,4-D-degrading population, inoculation with A. eutrophus JMP134, a 2,4-D degrader, was required; however, JMP134 is sensitive to cadmium. For rapid degradation of 2,4-D to be achieved, it was necessary to inoculate with both JMP134 and a cadmium-resistant isolate, Pseudomonas H1, which accumulates cadmium intracellularly. These results suggest that in the presence of a toxic metal, inoculation with metal-resistant microorganisms that reduce bioavailable metal concentrations via sequestration will foster increased biodegradation.

**Treatment additives.** Treatment additives such as calcium carbonate, phosphate, cement, manganese oxide, and magnesium hydroxide can be added to metal-contaminated sites to reduce metal bioavailability and mobility (Hettiarachchi et al. 2000; Ruby et al. 1994; Traina and Lapereche 1999). Despite the well-documented ability of treatment additives to reduce metal mobility and solubility, only a single study has examined the impact of such reductions on metal toxicity to pollutant-degrading soil microorganisms. Jonioh et al. (1999) examined the effect of calcium carbonate on the toxicity of lead to microorganisms isolated from a military rifle range soil contaminated with lead and other heavy metals. Using the Microtox
Co-contaminated environments (Borgmann and Norwood 1995; Braide 1984; Ibim et al. 1992; Ogunde 1999). For this reason, the use of other chelating agents to reduce metal toxicity is of greater interest.

Makuluk et al. (1998) have shown that a commercially available chelating resin (Chelex 100; Biorad, Hercules, CA) and surfactant-modified clays reduced cadmium toxicity during biodegradation of NAP. Clays were modified by adsorbing a cationic surfactant to the clay surface to which various metal-binding ligands (e.g., palmitic acid) were attached via hydrophobic interactions. NAP biodegradation occurred at higher cadmium concentrations in the presence of either Chelex 100 or the modified clays than in controls containing either no clay or unmodified clay. The abilities of the resin and the modified clays to reduce cadmium toxicity were quantitatively related to the metal adsorption characteristics of the two chelating agents.

Microbially produced surfactants (biosurfactants) show promise for enhancing organic biodegradation in the presence of metals. Sandrin et al. (2000) showed that a rhamnolipid biosurfactant produced by *P. aeruginosa* reduced cadmium toxicity during NAP biodegradation by a *Burkholderia* sp. in solution studies. The mechanism by which the biosurfactant reduced cadmium toxicity appeared to involve a combination of rhamnolipid complexation of cadmium and rhamnolipid-induced lipopolysaccharide release from the outer membrane of the degrader (Al-Tahhan et al. 2000; Goldberg et al. 1983; Leive 1965). Maslin and Maier (2000) used the same biosurfactant to reduce cadmium toxicity during biodegradation of PHEN by indigenous populations in two soils co-contaminated with PHEN and cadmium. PHEN mineralization was increased from 7.5 to 35% in one soil and from 10 to 58% in the second soil in response to up to three applications of rhamnolipid. Repeated application was necessary because of biodegradation of rhamnolipid, which occurred in 2–3 weeks.

**Possible approaches: pH and divalent cations.** Two environmental factors that profoundly impact the toxicity of metals to microorganisms are pH (Babich and Stotzky 1977a, 1977c, 1983a, 1983c; Korkela and Pekkanen 1978) and cell yield was increased. Zinc had no effect with this approach, it may be feasible to reduce the pH of a metal and organic co-contaminated soil to first optimize organic biodegradation. After biodegradation of the organic contaminant had occurred, the pH of the soil could be further reduced to maximize metal leaching. Suggesting that this approach may be effective, Sandrin and Maier (2002) found that cadmium toxicity during NAP biodegradation could be reduced by lowering pH from 7 to 4.

**Divalent cations.** Divalent cations, such as zinc, have been reported to mitigate metal toxicity. Higham et al. (1985) showed that addition of 60 μM total zinc reduced toxicity of 3 mM total cadmium to *P. putida*. Specifically, the lag phase was reduced, and the growth rate and cell yield were increased. Zinc had no effect on cells grown without cadmium. Similarly, magnesium reduced toxicity of nickel to bacteria and yeast (Abelson and Aldous 1950), to filamentous fungi (Babich and Stotzky 1981, 1982a, 1983b, 1983c), and to a filamentous alga (Say and Whitton 1977). Calcium has been reported to reduce cadmium toxicity to an alga (Gippes and Coller 1982) and to reduce zinc toxicity to a cyanobacterium (Shehata and Whitton 1982) and algae (Harding and Whitton 1977; Rai et al. 1981). The protective effect of divalent cations
such as zinc against metal toxicity is not limited to microorganisms. Zinc has been implicated in protection from cadmium-induced formation of tumors (Gunn et al. 1963), sarcoma (Gunn et al. 1964), and lesion development in rats and mice (Gabbiani et al. 1976).

Despite the widespread demonstration of the protective effects of divalent cations such as zinc against metal toxicity, little is understood with regard to the mechanism of protection. However, cadmium uptake has been found to be very dependent on zinc concentration. In studies investigating uptake of $^{109}$Cd$^{2+}$, zinc was a competitive inhibitor of cadmium uptake and exhibited a $K_i$ of 4.6 µM (Laddaga and Silver 1985). A more detailed understanding of the mode of protection by divalent cations might lead to the development of strategies to bioremediate co-contaminated sites in which a relatively non-toxic divalent cation (e.g., calcium) is added to a site to induce metal resistance and enhance organic biodegradation. Sandrin (2000) investigated the ability of seven divalent cations (calcium, cobalt, copper, iron, magnesium, manganese, zinc) to reduce inhibition of NAPH biodegradation caused by 10 and 37.5 mg solution-phase cadmium/L. Addition of 90 mg total zinc/L to treatments containing 37.5 mg solution-phase cadmium/L cadmium eliminated a 48-hr cadmium-induced lag phase. The remaining cations had inhibitory or no effects on NAPH biodegradation. Additional research is required to ascertain whether less toxic cations can be used to elicit similar effects.

Conclusions

The timely and cost-effective remediation of metal and organic co-contaminated sites mandates an understanding of the extent and mechanisms by which toxic metals inhibit organic biodegradation. Past attempts to quantify the impact of metals on biodegradation are difficult to interpret because they have generally been based on total metal rather than solution-phase or bioavailable metal concentrations. This has resulted in reported inhibitory concentrations of metals that vary by as many as 5 orders of magnitude. A crucial first step will be to report consistently solution-phase or bioavailable metal concentrations in the future so that a legitimate comparison of biodegradation behavior in co-contaminated sites can be made. Currently, our best approximation is to measure and use solution-phase metal data. However, new methods of defining and determining bioavailable metal are rapidly being developed. Despite the enormous variance among reported inhibitory concentrations of metals, it remains clear that metals have the potential to inhibit organic biodegradation in both aerobic and anaerobic systems. The mechanisms by which metals inhibit biodegradation vary with the composition and complexity of the system under study and include both physio-logical and ecological components. A more thorough understanding of these systems taking into account various levels of complexity is needed to develop remediation bacteria to remediate the contamination of co-contaminated sites. That said, there already exist a number of approaches, including addition of metal-resistant microorganisms, pH adjustment, and additives that reduce metal bioavailability. However, field trials are needed to validate these approaches.

References

Abelson PH, Aldous E. 1950. Ion antagonism in microorganisms: interference of normal magnesium metabolism by nickel, cobalt, cadmium, zinc, and manganese. J Bacteriol 60:401–413.

Al-Tahhan R, Sandrin T, Bodour A, Maier R. 2000. Cell surface hydrophobicity of P. aeruginosa: effect of monorhamnolipid on fatty acid and Sphingosine content. Appl Environ Microbiol 66:3262–3268.

Alexander M. 1999. Biodegradation and Bioremediation. San Diego, CA:Academic Press.

Amor L, Kennes C, Viaiga MC. 2001. Kinetics of inhibition in the biodegradation of aromatic hydrocarbons in presence of heavy metals. Biosource Technol 78:181–185.

Anglo JD, Chaney RL. 1989. Cadmium resistance screening in nitrotoxic/bacteria-buffered minimal media. Appl Environ Microbiol 55:2101–2104.

Baath E. 1989. Effects of heavy metals in soil on microbial activity. Rev Environ Microbiol 1:339–369.

Baderoff RD, Sagar S. 1984. Effects of heavy metal contamination on the short term decomposition of $^{14}$CGlucose in a pasture soil. Soil Biol Biochem 26:727–733.

Birch L, Brand H. 1996. A rapid method for the determination of metal toxicity to the biodegradation of water insoluble polymers. Fresen J Anal Chem 354:760–762.

Blake DA, Blake RC, Khosravi-louei M, Pavlov AR. 1998. Immunomodulation for metals. Anal Chim Acta. 376:13–19.

Borgmann U, Norwood WP. 1995. EDTA toxicity and background concentrations of copper and zinc in Hyalella azteca. Can J Fish Aquat Sci 52:975–981.

Braide VB. 1984. Cadmium EDTA toxicity—renal excretion of endogenous trace metals and the effect of repletion on col-lagen degradation in the rat. Gen Pharmacol 15:37–41.

Burkhardt C, Insam H, Hutchinson TC, Rebh H. 1993. Impact of heavy metals on the degradative capabilities of soil bacterial communities. Biol Fertil Soils 16:154–156.

Capone DG, Reed KS, Kiene RP. 1983. Effects of metals on methanogenesis, sulfate reduction, carbon dioxide evolution, and microbial biomass in anoxic salt marsh sediments. Appl Environ Microbiol 45:1596–1591.

Collins YE, Stotzky G. 1992. Heavy metals alter the electrokinetic properties of bacteria, yeasts, and clay minerals. Appl Environ Microbiol 58:1192–1600.

Corbiser PS, Van Der Leele D, Borrermans B, Provost A, De Lorenzo V, Brown NL, et al. 1998. Whole cell and protein based biosensors for the detection of bioavailable heavy metals in environmental samples. Anal Chim Acta 387:235–244.

Doelman P, Haanstra L. 1979a. Effect of lead on soil respiration and dehydrogenase activity. Soil Biol Biochem 11:479–479b.

Doelman P, Haanstra L. 1979b. Effects of lead on the decomposition of organic matter. Soil Biol Biochem 11:481–485.

Forsberg CW. 1978. Effects of heavy metals and other trace ele-
ments on the fermentation activity of the rumen microflora and growth of functionally important rumen bacteria. Can J Microbiol 24:298–306.

Franklin NM, Staubler JL, Markich SJ, Lim RP. 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Cloroha ssp.). Aquat Toxicol 48:275–289.

Gabbiani G, Gregory A, Baic D. 1996. Cadmium induced selective lesions of sensory ganglia. J Neuropathol Exp Neurol 55:489–506.

Giff JP, Cellar BAW. 1982. Effect of some nutrient cations on uptake of cadmium by Chlorella pyrenoidosa. Aust J Mar Freshwater Res 33:379–387.

Goldberg SS, Gargas UR, Kiyosue I, Mares-Guia ML. 1982. Release of lipopolysaccharide (LPS) from cell surface of Trypanosoma cruzi by EDTA. Int J Parasitol 12:11–18.

Gunn SA, Gould TC, Anderson WIAD. 1983. Cadmium-induced interstitial cell tumors in rats and mice and their prevention by zinc. J Natl Cancer Inst 71:745–749.

Hettiarachchi GM, Pierzynski GM, Ransom MD. 2000. pH-depen-
tent toxicity of copper and uranium to a tropical freshwater alga (Cloroha ssp.). Aquat Toxicol 48:275–289.

Hettiarachchi GM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.

Hibbertschi CM, Pierzynski GM, Ransom MD. 2000. Depletion of essential elements by calcium disodium EDTA treatment and softening of hard water. J Water Supply Research Technol 49:371–380.
plasma membrane of bacterial cells. Microbiology 96:589–594.

Jackson WA, Pardue JT. 1998. Assessment of metal inhibition of reductive dechlorination of hexachlorobenzene at a Superfund site. Environ Toxicol Chem 17:141–144.

Ji DY, Silver S. 1995. Bacterial resistance mechanisms for heavy metals of environmental concern. J Ind Microbiol 14:61–75.

Jin PK, Bhattacharyya SK. 1996. Anaerobic removal of pentachlorophenol in presence of zinc. J Environ Eng 122:590–598.

Jonovich O, Obbard JP, Stanforth RR. 1999. Impact of treatment additives used to reduce lead solubility and microbial toxicity in contaminated soils. In: Bioremediation of Metals and Inorganic Compounds (Leeson A, Alleman BC, eds). Columbus, OH: Battelle Press, 7–12.

Kachur AV, Koch CJ, Biaglow JE. 1996. Mechanism of copper-catalyzed oxidation of glutathione. Free Radic Res 28:259–269.

Kamatharwam SR, Crawford DL. 2001. Anaerobic biodegradation of pentachlorophenol in mixtures containing cadmium by two physiologically distinct microbial enrichment cultures. J Ind Microbiol Biotechnol 27(1):111–17.

Kamel Z. 1986. Toxicity of cadmium to two Streptomyces species as affected by clay minerals. Plant Soil 93:193–205.

Khosrovian M, Pavlov AR, Flowers GC, Blake DA. 1998. Detection of heavy metals by immunassay optimization and validation of a rapid, portable assay for ironic cadium. Environ Sci Technol 32:137–142.

Knight BP, Mcgrath SP, Chaudri AM. 1997. Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. Appl Environ Microbiol 63:39–43.

Kong IC. 1993. Effect of heavy metals on acidogenesis in anaerobic sewage sludge. Environ Technol Lett 9:261–270.

Kong IC. 1998. Metal toxicity on the dechlorination of mono- and dichlorobenzene. Environ Microbiol 62:2317–2323.

Kong IC. 1998. Metal toxicity on the dechlorination of trichloroacetic acid. Environ Toxicol 15:1083–1088.

Pichtel J, Pichtel TM. 1997. Comparison of solvents for metal uptake by encapsulated cultures of Kluyvera aerogenes. Microb Ecol 21(2):139–149.

Koeppe F, Fekken TJ. 1978. The effect of pH and potassium on biotransformation and biodegradation of 2-chlorophenol in sewage sludge. Environ Technol Lett 9:261–270.

Kong NY. 1996. Metal toxicity on the dechlorination of trichloroacetic acid. Environ Toxicol 15:1083–1088.

Kong IC. 1998. Metal toxicity on the dechlorination of trichloroacetic acid. Environ Toxicol 15:1083–1088.